Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

In the Faculty of Humanities

The Relationship between Elevated Night-Time Glucocorticoid Activity and Dreaming: A Perspective on Sleep-Dependent Memory Consolidation

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February 2014

Declaration

I hereby declare that this dissertation is my own unaided work, both in concept and execution. To the best of my knowledge and belief this dissertation contains no material written by another person, except where due acknowledgement has been made in the text. Neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university.

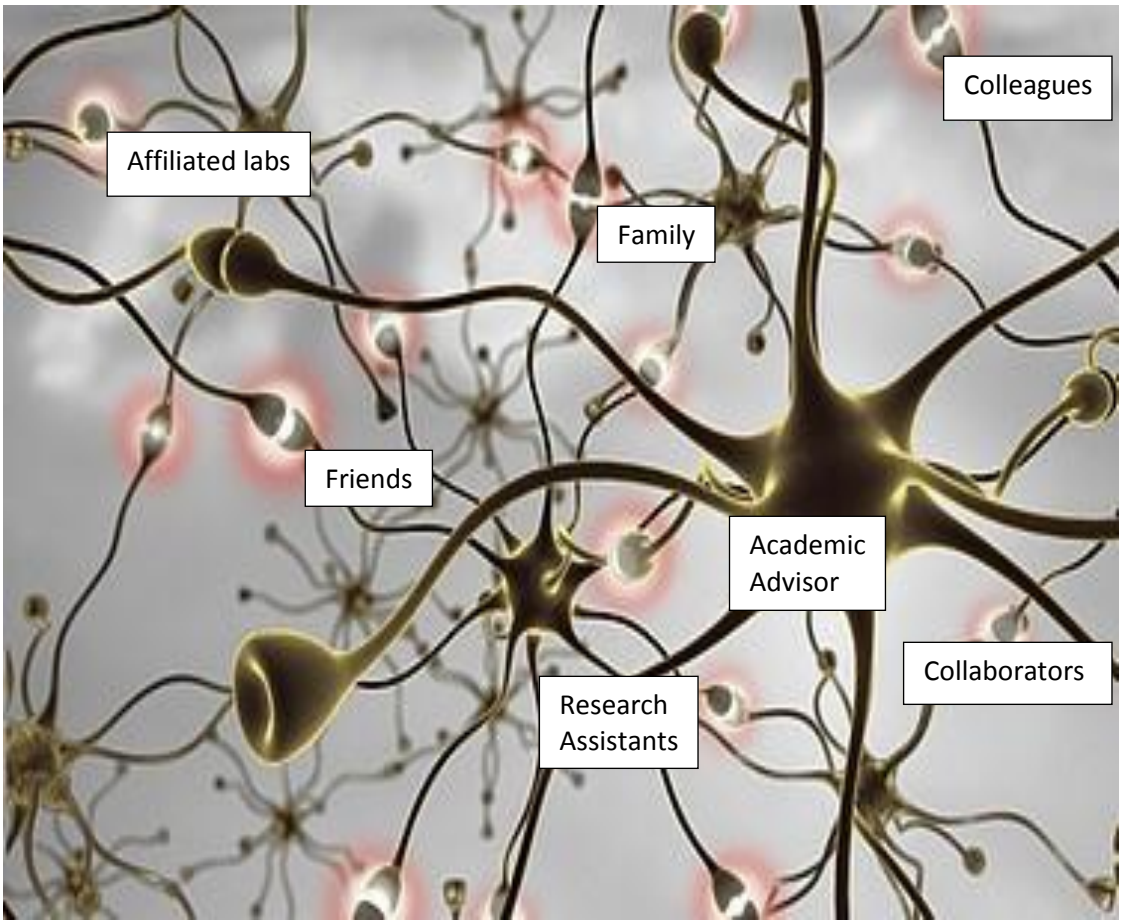
Signed by candidate

Ridwana Timol (Ms) Date: 15/02/16

Acknowledgements

Zulu proverb: *Umuntu Ngumuntu Ngabantu*: "A person is a person because of people."

I could not have done this without you…



Completion of this PhD dissertation would not have been possible without the support and encouragement of many. To them, I dedicate this:

Supervision:
Kevin G.F. Thomas, I don't know how you committed to this for so long so rigorously, with so much dedication…thank you for being an exceptional mentor.

Resources, training and facilities:
The Applied Cognitive Science & Experimental Neuropsychology Team (ACSENT) laboratory: The whole gang for exceptional professional development training & peer support.
The Sleep Disorder Centre at the Life Vincent Pallotti Hospital: Marlene Gounder & Alta Dorse, without whom sleep testing would not have taken place. Marlene, you taught me everything!
The UCT-SSEM Thembu Sleep Sciences laboratory dream team: Gosia, Mariza, Michelle & Danyal.
Panorama Mediclinic: Jan Top, Sleep Technologist.
Consulting neuroanatomist: Dr. Christopher Warton from the department of Human Biology, University of Cape Town.
Imaging technique consultants: Dr. Ernesta Meintjies and her research team from the Division of Biomedical Engineering, Department of Human Biology, Faculty of Health Sciences, University of Cape Town.
Cape Universities Brain Imaging Centre (CUBIC): Dr Stephan du Plessis and Dr Bruce Spottiswoode.
Centre for High Performance Computing (CHPC), Rosebank, Cape Town.

Assistance with project conceptualization and design:
Dr. Hawarden and Dr. Richard Raine from the UCT Lung Institute
Dr. Barak Morgan from the Department of Biomedical Engineering at UCT

Research assistants, my heroes:

Anthony Hodge	Mariza van Wyk	Alexa Heekes
Hussain Boodhoo	Siobhan McCreesh	Sasha Heekes

Family and friends, thank you for supporting the dream, I know it was heavy at times:

Maa	Mom	Grant	Adiilah
Sham	Sali	Dai	Karima
Tonton Hos	Mamou	Tonton Haroun	Gory
Bilou	Sam	Saniou	Solly
Sanaa	Niya	Ismael	Melike
Heike	Leigh		

Abbreviations

ACTH	-	adrenocorticotropic hormone
AMT	-	Autobiographical Memory Test
BDI II	-	Beck Depression Inventory-Second Edition
BNT	-	Boston Naming Test
CNS	-	central nervous system
CRH	-	corticotrophin releasing hormone
EC	-	eczema controls
ECG	-	electrocardiography
EEG	-	electroencephalography
EMG	-	electromyography
EOG	-	electrooculography
FSIQ	-	Full Scale IQ
FTT	-	Finger-Tapping Task
GC	-	glucocorticoid collection
GH	-	growth hormone
GHRH	-	growth hormone-releasing hormone
GR	-	glucocorticoid receptors
HC	-	healthy controls
HPA axis	-	hypothalamic-pituitary-adrenal axis
ICV	-	intracranial volume
LM	-	logical memory
LMI	-	logical memory immediate recall
LMII	-	logical memory delayed recall
LTP	-	long-term potentiation
MA	-	mild asthma

MINI	-	Mini International Neuropsychiatric Interview
MR	-	mineral corticoid
MRI	-	Magnetic resonance imaging
MS	-	moderate- to- severe
NREM	-	non- rapid eye movement
PIQ	-	Performance IQ
PSG	-	polysomnograph
PTDIS	-	progressive temporal delay into stage
PTSD	-	post-traumatic stress disorder
RAVLT	-	Rey Auditory Verbal Learning Test
REM	-	rapid eye movement
SE	-	sleep efficiency
SOL	-	sleep onset latency
SWA	-	slow-wave activity
SWS	-	slow-wave sleep
TAS-20	-	Toronto Alexithymia Scale
UA	-	untreated asthma
VPA	-	Verbal Paired Associates
WAIS-R	-	Wechsler Adult Intelligence Scale-Revised
WASI	-	Wechsler Abbreviated Scale of Intelligence
WASO	-	wake after sleep onset

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ABSTRACT

Background. The consolidation of episodic memory is particularly vulnerable to fluctuations in glucocorticoid levels, both during wakefulness and during sleep. Corticosteroid exposure is associated with changes in endogenous glucocorticoid activity, sleep disruption, episodic memory impairment, and reduced hippocampal volume. This dissertation had two primary aims. The first was to explore the relationship between corticosteroid exposure and sleep-dependent memory processes, including dreaming, with special focus on associations between corticosteroid exposure and (a) night-time glucocorticoid activity and (b) sleep organization. The second was to explore the neuroanatomical foundation for these relationships in young adults with asthma. To achieve these aims, I conducted three studies.

Methods. Study 1 (N = 68) used a cross-sectional, matched-sample, quasi-experimental design to compare night-time salivary cortisol levels, memory performance pre- and post-sleep, sleep organization (measured using polysomnography), and dreaming in groups of asthmatics and non-asthmatics with varying degrees of corticosteroid exposure. Study 2 (N = 23) used a double-blind, randomized placebo-control true experimental design to test, in healthy young adults, the effects of a single 25 mg dose of prednisone on the same outcome measures. Study 3 (N = 19) used a quasi-experimental design to compare hippocampal volume of moderate-to-high corticosteroid-exposed asthmatics with that of matched healthy controls. That study also examined the relationship between (a) night-time cortisol levels and hippocampal volume, (b) night-time cortisol levels and declarative memory performance, (c) hippocampal volume and declarative memory performance. All participants were English-speaking university students, aged 18-39 years.

Results. Studies 1 and 2 showed that, relative to healthy controls, night-time glucocorticoid activity was elevated and sleep organization was disrupted in corticosteroid-exposed individuals. Furthermore, there were significant inverse associations between glucocorticoid activity and (a) the organization of slow wave sleep (SWS) and rapid-eye movement (REM) sleep, (b) performance on both declarative and procedural memory tasks, and (c) the episodic memory content of dreams. There were significant positive associations between (a) the proportions and the organization of SWS and REM sleep and performance on measures of both declarative and procedural memory, and (b) the organization of REM sleep and the episodic content of dreams. Study 3 data analyses detected significantly smaller hippocampal volume in asthmatics relative to controls. Severity of asthma was inversely related to left hippocampal volume, but corticosteroid exposure alone was not. Furthermore, a smaller hippocampus was associated with better memory performance among healthy controls, but not among asthmatics.

Conclusions. The association between the organization of SWS and REM sleep and performance on measures of both declarative and procedural memory lends support to the sequential hypothesis of sleep-dependent memory processing. The current findings also suggest that glucocorticoid activity is associated with (a) dream content, (b) the organization of SWS and REM sleep, and (c) post-sleep memory performance after sleep, and that these relationships may intersect. Although asthmatics did not display memory deficits or aberrant dreaming patterns, their hippocampal volume data, patterns of night-time cortisol, and sleep disruptions suggest further investigation is warranted into the implications of subtle HPA-axis dysfunction and consequent atypical brain development on cognitive function and quality of life in asthmatics, whether exposed to corticosteroid treatment or otherwise.

CHAPTER ONE:

GENERAL INTRODUCTION

The micro- and macrostructure of sleep have been investigated extensively to establish if, and to understand why, this offline ensemble of variations in consciousness contributes uniquely to aspects of learning and memory. Currently, the general consensus appears to be that sleep provides an optimal cellular, molecular, electrophysiological, and behavioral environment for the processing of information after its acquisition during waking (Ambrosini & Giuditta, 2001; Born, Rasch, & Gais, 2006; Born & Wilhelm, 2012; Diekelmann & Born, 2010; Ribeiro & Nicholelis, 2004; Stickgold & Walker, 2007; Walker & Stickgold, 2010). Furthermore, a significant body of literature posits that dream content is a gauge of the level and the type of information processing that is enabled during sleep (e.g., Antrobus, 1977, 1978, 1990, 1991, 1993; Cicogna & Bosinelli, 2001; Cicogna, Cavallero, & Bosinelli, 1991; DeGracia & LaBerge, 1998; Hobson, Pace-Schott, & Stickgold, 2000; Hobson & Stickgold, 1994; Johnson, 2004; Kavanau, 1996, 2001; Nielsen, 2004; Ribeiro & Nicholelis, 2004; Stickgold, Hobson, Fosse, & Fosse, 2001).

Dreams replaying real-life waking events are rare. A relatively large body of literature makes it clear that there is no isomorphic relationship between the content of dreams and episodes of waking experience (for a review, see Fosse, Fosse, Hobson, & Stickgold, 2003). It is incontestable, however, that waking experiences are sometimes incorporated into dreams. Furthermore, most remembered dreams, although containing some element of the dreamer's waking experiences (e.g., a familiar face, person, place, or preoccupation), express novelty at various levels (Fosse et al., 2003). One way to account for this situation is to posit that the difference between waking episodes and dream scenarios reflects the manner in which memories are consolidated during sleep. During dreaming, we have access to

incomplete information, or to information out of its original context, and we are unable to apply waking logic to reconstruct our perceptions according to the consensual standards of normal and plausible experience (Muzur, 2005). Most of the time during sleep, units of memories, as opposed to entire episodes, are reactivated. This partial reactivation is reflected in dreams as the meshing of memory elements that do not necessarily belong together, or that were, at least, not encountered in the same combination during waking (Levin & Nielsen, 2007; Paller & Voss, 2004). For example, we have all experienced incongruity in dreams: your house is transposed into a country you visited recently, you revisit primary school as an adult, or you have a conversation with your mother who, in your dream, looks like the neighbor with whom you had a conversation this morning.

It appears, then, that memory consolidation during sleep does not involve a simple reproduction of memories in the form that they were originally perceived and acquired (Levin & Nielsen, 2007; Paller & Voss, 2004; Walker & Stickgold, 2010). In fact, Paller et al. (2004) argue that, in general, episodic memories are *reconstructed* during consolidation. Walker et al. (2010) describe this reconstruction as constituting of (a) *unitization* of information, where units of information are broken down into chunks, (b) *assimilation*, where new information is integrated into existing schemas of semantically-related concepts, and lastly (c) *abstraction*, where implicit rule underlying the structure and nature of the information at hand is extracted and understood. In other words, the consolidation of autobiographical episodes involves a reorganization of information, where new connections between episodes of experience are established in a way that best serves survival. The nature and direction of these new connections are guided by individual motivations that are not always easily traced (Paller et al., 2004; Levin & Nielsen, 2007; Walker et al., 2010). This process of deconstruction and reconstruction, and the simultaneous strengthening of relevant and weakening of irrelevant aspects of a new experience, relies on certain

quantitative and qualitative aspects of sleep (Ambrosini & Giuditta, 2001; Stickgold & Walker, 2007). These aspects of sleep refer to the optimal proportions and adequate circadian organization of different stages of sleep, and they are regulated, at least in part, by activity of the hypothalamic-pituitary-adrenal (HPA) axis (Buckley & Schatzberg, 2005; Friess, Wiedemann, Steiger, & Holsboer, 1995; Steiger, 2003, 2007). Hence, HPA-axis deregulations should disrupt the organization of sleep, and should thereby disrupt sleep-dependent functions, such as the related processes of memory consolidation and dreaming. Some interesting questions that remain in this literature are (a) whether the extent of episodic memory incorporation in dreams is related to one's general ability to benefit from sleep-dependent consolidation of episodic memory, and (b) if the same processes that contribute to the reactivation and assimilation of waking episodic memories during sleep (e.g., cortisol quiescent period during early sleep and ascending levels during late sleep, and the concentration of slow-wave sleep (SWS) during early sleep and the intensification or lengthening of REM during late sleep) aid the successful inclusion of episodic elements in dreams.

The rest of this chapter is organized as follows: First, I describe the role of the HPA-axis in regulating sleep and sleep-dependent memory consolidation. Second, I discuss two models of memory consolidation during sleep to illustrate how specific disruptions in sleep, brought on by HPA-axis dysfunctions, can predict specific disruptions in memory consolidation. Third, I discuss the relationship between the ultradian and circadian features of sleep and dream content to illustrate how those relationships reflect those between memory consolidation processes and sleep, hence arguing that dreaming emerges, at least in part, from the memory consolidation process. Fourth, I discuss how the HPA-axis might shape dreaming patterns through its impact on sleep. Fifth and finally, I describe my research rationale and objectives.

The series of studies described here involves adults with asthma because they often present with disruptions of the HPA axis (defined in the literature as suppressed adrenal function measured by lower levels of cortisol secretion, or delayed morning acrophase of cortisol relative to healthy controls, or both; Fei, Liu, Zhang, & Zhou, 2004; Heim, Ehlert, Hanker, & Hellhammer, 1999; Kraft, Pak, & Martin, 1998; Landstra, Boezen, Postma, & van Aalderen, 1999; Peebles et al., 2000; Ritz, Ayala, Trueba, Vance, & Auchus, 2011; Sutherland, Ellison, Kraft, & Martin, 2003; Sutherland, 2003, 2005), of sleep (Cukic, Lovre, & Dragisic, 2011; Daniel, Boergers, Kopel, & Koinis-Mitchell, 2012; Kales, Beall, Bajor, Jacobson, & Kales, 1968; Klink & Quan, 1985; Montplaisir, Walsh, & Malo, 1982; Redline et al., 2004; Smaldone, Honig, & Byrne, 2007), of memory (Bender, Lerner, & Poland, 1991; Brown et al., 2003, 2004; Stores, Ellis, Wiggs, Crawford, & Thomson, 1998; Suess, Stump, Chai, & Kalisker, 1986), and of dreaming (Klink & Quan, 1985; Monday, Montplaisir, & Malo, 1987; Montplaisir et al., 1982; Montplaisir, Monday, Walsh, & Malo, 1983; Nielsen et al., 1997). Despite the co-incidence of these conceptually-related phenomena in a disorder that affects millions of individuals around the world, the relationship between sleep, sleep-related functions, and the HPA axis remains unexplored in asthmatics. The same co-incidence of phenomena is well documented in the elderly (e.g., Van Cauter, Plat, Leproult, & Copinschi, 1998; Van Cauter, Leproult, & Plat, 2000; Vgontzas et al., 2003) and in patients with Cushing's disease (e.g., Born & Fehm., 1998; Martignoni et al., 1992; Mauri et al., 1993; Schteingart et al., 1980); both of these populations have cortisol profiles that are similar to asthmatics. I therefore discuss the research conducted on those populations to draw inferences about asthmatics.

Sleep: The Role of the HPA-Axis

HPA-axis activity is at its minimum during the first half of the sleep night, when endogenous cortisol is at its nadir. The transition from early to late sleep is akin to a sudden shift from a state of relaxation to one of stress, marked by heightened autonomic activation and arousal and a parallel rise in cortisol levels. During the second half of sleep, glucocorticoid receptors become increasingly activated, the delta to sigma ratio declines, and electroencephalographic (EEG) activity in general increases in frequency (Antonijevic, Murck, Frieboes, Holsboer, & Steiger, 1999; Buckley & Schatzberg, 2005; Plihal, Pietrowsky, & Born, 1999).

Six decades of electrophysiological observations form the basis of our knowledge of the structure of sleep, be it normal or aberrant (Antrobus & Bertini, 1992; Aserinsky & Kleitman, 1957; Dement & Kleitman, 1957; Rechtschaffen & Kales, 1968; Kryger, Roth, & Dement, 2011). However, our understanding of the processes that regulate sleep architecture, in terms of predictable transitions from one variation in consciousness to the next, is derived largely from neuroendocrine studies tracking the biological correlates of electrophysiological events during sleep (Steiger, 2003, 2007).

The electrophysiological correlates of sleep reflect a continuous, cyclical progression from one phase to another. Conventional classification has it that this progression can actually be divided into five stages (Rechtschaffen & Kales, 1968). Stage 1 (sleep onset) is characterized by a predominance of theta waves and slow eye movements, with a decrease in metabolic activity. Stage 2 is marked by the same predominance of theta waves, along with further slowing of vital functions such as heart rate and blood pressure. In addition, sleep spindles, which reflect the oscillatory cortico-thalamic activity signaling true sleep, area signature EEG feature of stage 2 sleep.

Stages 3 and 4 together constitute SWS, and are characterized by an increasing prevalence of delta waves and minimal physiological activity (e.g.,breathing becomes slower and deeper). This deep sleep is often followed immediately by a brief ascent to the lighter NREM stage 2 sleep, before a shift to rapid-eye movement (REM) sleep, which is characterized by the presence of all wave frequencies (alpha, beta, theta, and delta) with a relative predominance of theta activity (Johnson, 2004). REM sleep is also called *paradoxical sleep* because it is so dissimilar to the restful nature typically attributed to sleep: During REM sleep, metabolic activity (e.g., cerebral blood flow and glucose consumption) increases sharply relative to the other sleep stages (Scheen, Byrne, Plat, Leproult, & Van Cauter, 1996; Madsen & Vordstrup, 1991.

A typical five-stage sleep cycle lasts between 60 and 90 minutes. The structure of each cycle changes as sleep progresses throughout the night, however. Specifically, periods of SWS become shorter and shorter with each successive cycle, whereas REM stages increase in length (Muzur, 2005).

The timing and distribution of the electrophysiological constituents of sleep architecture suggest a gradual progression from the non-REM or NREM to REM modes of consciousness, as opposed to abrupt shifts into and out of mutually exclusive states. For instance, although delta EEG shows abrupt shifting patterns at the onset and offset of REM, some REM-on neurons display gradual, oscillatory activation before REM EEG can be clearly demarcated visually (for a review, see Nielsen, 2004).

The evolving structure of sleep throughout the night is modulated by the interactions between, and the independent actions of, neuropeptides and steroids. For instance, the preponderance of SWS in the early cycles of sleep versus an ascending preponderance of REM sleep during the latter half of sleep coincides with the hypothalamo-pituitary actions of

somatotrophic growth hormone-releasing hormone (GHRH), on the one hand, and of adrenocortical corticotropin-releasing hormone (CRH), on the other.

Growth hormone (GH) levels are high during the first half of sleep; adrenocorticotropic hormone (ACTH) and cortisol levels are high during the second half of sleep. The peak of GH precedes sleep onset, and there is a close temporal relationship between GH highs and SWS. Furthermore, increasing GH release by administering GHRH to animals (Ehlers, Reed, & Henriksen, 1986; Obál et al., 1988) and to humans (Kerkhofs et al., 1993; Marshall, Derad, Starsburger, Fehm, & Born, 1999; Perras, Marshall, Köhler, Born, & Fehm, 1999; Steiger et al., 1992) prolongs SWS. Additionally, the fall in GH production associated with normal aging coincides with the sharp decrement in SWS observed beginning at the fifth decade of human life (Van Cauter et al., 1998, 2000; van Coeverden et al., 1991).

The second half of sleep is marked by increasing levels of CRH, which in turn results in increasing levels of ACTH and cortisol. Cortisol then down-regulates CRH in a negative feedback loop. In humans, the ascending slope of CRH appears to cause the suppression of SWS as the night progresses. Conversely, during the second half of the night, the descending slope of CRH, brought on by the inhibitory, homeostatic action of cortisol, contributes to the lengthening of REM sleep. When cortisol is infused during early sleep, SWS increases. However, it does not increase through the direct action of peripheral cortisol, but through its inhibitory central nervous system (CNS) action on CRH. Thus, although late sleep is characterized by relatively high cortisol levels, SWS is suppressed because CRH levels are on the rise (for reviews, see Buckley & Schatzberg, 2005; Steiger, 2003).

Deregulation of the HPA axis during sleep usually translates as a hyperactivity of the neuroendocrine system, its negative feedback loop failing to down-regulate its activity. Such dysfunction during sleep is marked by simultaneous elevations in CRH, ACTH, and night-time cortisol during what should normally be their quiescent period (i.e., early sleep; Buckley

& Schatzberg, 2005). High night-time cortisol "ages" sleep in the sense that it reproduces the changes in sleep architecture that accompany normal aging. These changes include sleep that remains mostly shallow throughout the night because it is (a) frequently disrupted by intermittent periods of wakefulness, and (b) characterized by a significant reduction of SWS and REM sleep (Van Cauter et al., 1998, 2000; Vgontzas et al., 2003).

However, studies describing the action of steroids and neuropeptides during sleep, and the ways in which they interact to produce conventional sleep architecture, have not reported consistent results. Current understanding of the role of the HPA axis in regulating sleep is complicated by factors relating to between-study methodological differences including subject variables, choice of which HPA-axis agent is investigated, and manipulation of variables, all of which modulate the relationship between the HPA axis and sleep. These factors include (a) individual differences in age and sex, (b) the route of action that corticosteroids and neuropeptides take (i.e., whether they have CNS or peripheral effects), (c) whether HPA-axis deregulation is caused by the action of exogenous corticosteroids, or by an imbalance in endogenous levels of corticosteroids, or by both, and (d) the chronicity of that deregulation.

Factors modulating the relationship between sleep and the HPA-axis. Below, I discuss how each of the four factors mentioned above modulates the action of corticosteroids and neuropeptides on sleep architecture.

***Demographic factors: Age and sex*.** There are age-related differences in the effects of neuropeptides on sleep. For instance, it appears that exogenously administered CRH has little effect on the sleep architecture of young adult men, but significantly affects depth of sleep in middle-aged men (Born, Späth-Schwalbe, Schwakenhofer, Kern, & Fehm, 1989; Vgontzas et al., 2001). In their study, Vgontzas et al. (2001) showed that middle-aged men (M = 45.1 ± 4.9 years) experienced longer sleep onset latencies, significant fragmentation of sleep, and

significant reduction in SWS on a CRH-infusion night compared to baseline sleep nights. Furthermore, their average responses to CRH with regard to these sleep parameters was significantly greater than those of younger men (M = 22.7 ±2.8 years). The authors argued that older adults tend to be at a double disadvantage: They have been exposed to more life stressors, and they have less efficient HPA-axis responses. In other words, an age-related increase in lifetime stress exposure is compounded by the fact that the transition from young adulthood to middle-age is associated with growing vulnerability to the arousing properties of CRH and to general imbalances in HPA-axis functioning, a phenomenon otherwise referred to as *reduced HPA-axis resiliency* (Seeman & Robbins, 1994).

Furthermore, there is a well-documented sexual dimorphism in the secretion of GH during sleep: In men, there is a surge observed around sleep onset, whereas in women, GH is released in smaller bursts, sporadically, before sleep onset and again during the second half of sleep. Additionally, in women there appears to be a synergistic relationship between GHRH and CRH regulation of sleep, whereas in men there appears to be an antagonistic relationship between GHRH and CRH regulation of sleep (Steiger, 2003). More specifically, whereas CRH lightens and fragments sleep in both sexes, the administration of GHRH consolidates sleep and enhances NREM sleep in men, but fragments sleep and reduces SWS and stage 2 sleep in women (Antonijevic, Murck, Frieboes, Barthelmes, & Steiger, 2000). This synergistic relationship between the hypothalamic-pituitary-somatotropic (HPS) and HPA-axes in women is likely to explain why women secrete higher levels of cortisol during sleep: Both GHRH and CRH lead to the secretion of cortisol (Antonijevic et al., 1999; Steiger, 2007).

CNS versus peripheral effects of steroids and neuropeptides. The accumulated experimental data indicates that distinguishing between CNS and peripheral effects of steroids and neuropeptides helps explain why the regulation of sleep by the HPA-axis is so

complex (Müller-Preuss, Wiesner, Lu, Deussing, & Kimura, 2005; Steiger et al., 1991, 1993). For instance, cortisol exerts its effects on sleep through the action of mineral corticoid (MR) and glucocorticoid (GR) receptors in the brain (i.e., via a CNS route), whereas ACTH exerts effects on sleep by influencing glucocorticoid activity (i.e., via a peripheral route; Steiger, 2007).

The bioavailability and route of action of HPA-axis agents influence the relationship between the HPA-axis and sleep. The different modes of experimental manipulation of these agents illustrates this fact. For instance, Born et al. (1989) attempted to chart the dynamics underlying the HPA-axis regulation of sleep by comparing the effects of continuous infusions of cortisol, ACTH, and CRH during sleep. Continuous intravenous infusions of ACTH and cortisol both increased sleep fragmentation: In participants who received these manipulations, there were frequent awakenings after sleep onset, increased stage 1 sleep, and suppressed REM sleep. Furthermore, whereas cortisol increased SWS in a predictable manner, ACTH had no effect on delta sleep. Regarding SWS, the effects of cortisol appear to bypass the peripheral actions of the ACTH negative feedback loop and are more likely mediated through (a) concomitant changes in CRH activity, (b) classical receptor affinity mechanisms for the mineralocorticoids contained in natural cortisol, and/or (c) the action of cortisol metabolites. In contrast to the Born et al. (1989) findings, Steiger et al. (1991) found that pulsatile intravenous infusions of the ACTH synthetic analogue ebiratide resulted in CNS activation without alterations in REM sleep, GH, or cortisol. In the former case, the influence of the continuously infused ACTH on cortisol and CRH translated into significant changes in REM sleep. In the latter case, the pulsatile infusion of ebiratide did not have an effect on sleep because it exerted its effects directly and exclusively on the CNS, without affecting cortisol levels.

Endogenous versus exogenous corticosteroids: The role of receptors. Endogenous and exogenous corticosteroids can have different effects on sleep depending on their respective receptor binding affinities and through the effect of their interactions on the HPAaxis.

Here, I use the terms *endogenous corticosteroids* and *natural corticosteroids* interchangeably. This even though hydrocortisone, which is a natural corticosteroid, can and has been administered pharmacologically. My decision to use the terms interchangeably is based on the fact that hydrocortisone is used experimentally to mimic the effects of naturally-occurring or endogenous elevations of cortisol.

I use the term *exogenous corticosteroids* to refer to synthetic corticosteroids. Synthetic corticosteroids bind mostly to GRs (hence the term *glucocorticoid*, which is employed in reference to most synthetic corticosteroids used as anti-inflammatory agents; examples here are prednisone and dexamethasone), whereas cortisol binds to both GRs and MRs. However, under ordinary circumstances cortisol binds most effectively to MRs; it only binds to GRs under conditions of stress, when its circulating levels are very high (Born, de Kloet, Wenz, Kern, & Fehm, 1991; Buckley & Schatzberg, 2005; Heffelfinger & Newcomer, 2001; Wagner & Born, 2008; Wagner, Degirmenci, Drosopoulos, Perras, & Born, 2005). Hence, the action of MRs predominates when the HPA axis is not exerted: 90% of MRs, and only 50% of GRs, are occupied under baseline conditions. The activity of GRs increases under pathological conditions, with this increase proportional to the magnitude of the stress experienced (Keenan & Kuhn, 1999).

Whereas there appears to be a preferential influence of MR activity on the regulation of SWS, REM sleep seems to be more susceptible to fluctuations in GR activity. MR activity during sleep predominates when cortisol is at its nadir (i.e., during the first half of sleep). GR activity, on the other hand, increases as cortisol levels rise (i.e., during the second half of

sleep; cortisol reaches peak levels in the morning, after awakening; Besedovsky, Born, & Lange, 2012; Buckley & Schatzberg, 2005). The fact that early sleep is rich in SWS whereas late sleep is rich in REM sleep is therefore not surprising. Because exogenous corticosteroids bind mostly to GRs, as mentioned above, they are more susceptible to affecting REM sleep than they are SWS.

However, our understanding of corticosteroid action has evolved beyond the dichotomous receptor action perspective since the discovery of *heterodimer*s, which is the molecular co-expression of MRs and GRs (Arriza et al., 1987). MRs and GRs are now known to be co-expressed in certain cells and to act synergistically at those sites, which include the hippocampus (Arriza, Simerly, Swanson, & Evans, 1988; de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998; for a review see Nishi, 2011).

MR-GR heterodimers carry action signatures that are distinct from both MR-MR or GR-GR homodimers, and heterodimerization occurs preferentially to homodimerization at various sites because heterodimers bind to DNA more effectively (Trapp & Holsboer, 1996; Trapp, Rupprecht, Castrén, Reul, & Holsboer, 1994). For instance, at low basal concentrations of cortisol, heterodimers are preferentially recruited, whereas at high concentrations of the hormone glucocorticoid homodimers become more active (Trapp & Holsboer, 1996).

Acknowledging heterodimerization may facilitate more accurate predictions of the nature and direction of a given corticosteroid action. In other words, the degree of corticosteroid activation would depend on both the allostatic load at hand (because heterodimers and homodimers operate at different thresholds), and on the site of action targeted (because they are not distributed equivalently across brain regions). The clinical, *in vivo*, implications of the heterodimerization of corticosteroid receptors are not yet known.

However, the complex interactions between the two receptors support the idea that the regulation of behaviour, including sleep, by the HPA-axis is modulated by numerous factors. For instance, the effects of exogenous corticosteroids on SWS are not straightforward; the mechanisms underlying that relationship are still poorly understood. One indirect route of action may be through the suppression of cortisol. In other words, in order for exogenous corticosteroids to suppress SWS, they need to suppress endogenous cortisol effectively. Direct support for this explanation derives from a study using metyrapone, an agent used to inhibit the synthesis of cortisol. The inhibition of cortisol by metyrapone during sleep is associated with significantly reduced amounts of SWS (Wagner et al., 2005). Indirect support can be derived from studies comparing the effects of endogenous versus exogenous corticosteroids on sleep. For instance, Fehm et al. (1986) found that whereas hydrocortisone, which metabolizes into cortisol, increases SWS (specifically stage 4), dexamethasone reduces SWS (presumably by suppressing or down-regulating endogenous cortisol). Of note here, however, other studies using dexamethasone or fluocortolone have not replicated this finding (Born, Zwick, Roth, Fehm-Wolfsdorf, & Fehm, 1987; Plihal et al., 1999).

Inhibition of basal cortisol alone may not be sufficient for the suppression of SWS by exogenous corticosteroids. Another condition necessary for the disruption of SWS may be reduced MR activity (Born et al., 1991). Plihal et al. (1999) demonstrated that cortisol infusion (about 8-12 mg for approximately 2 hours) had no effect on percentage SWS, but that blocking MR activity during early sleep using canrenoate suppressed SWS by as much as 17.5%. Of note in their study was the fact that canrenoate action also increased cortisol. The authors argued that this pattern of data (an increase in cortisol without a change in SWS) implies that it is not free circulating cortisol that has an effect on SWS, but rather the ratio of MR activation that accompanies the quiescent phases of cortisol secretion.

In support of this argument, there is a clearly demonstrated co-incidence between elevated night-time cortisol and reduced SWS in older adults (Van Cauter et al., 1998, 2000; Vgontzas et al., 2003) and in hypercortisolic individuals (e.g., patients with Cushing's syndrome; Steiger, 2007). The decline in MRs that accompanies aging probably accounts for the suppression of SWS in the elderly (Bohlhalter, Murck, Holsboer, & Steiger, 1997). In the case of patients with Cushing's syndrome, MRs are overactive but the lack of normal HPA-axis inhibition during early sleep may be the cause behind SWS suppression (Born & Fehm, 1998). Therefore, it appears that *both* the amount and the balance of corticosteroid receptor activity during sleep impact on sleep architecture.

For instance, altering MR activity alone by suppressing cortisol does not always lead to changes in SWS. Administering MR agonists such as aldosterone and fluocortolone (Born et al., 1987) or MR antagonists such as spironolactone (Steiger et al., 1993) has negligible effects on the electrophysiology of sleep.

Chronicity of HPA-axis deregulation. One traditional view on the relationship between cortisol and stress stipulates that the organism produces excess cortisol to counter excess stress in an attempt to homeostatically restore the body's defences (Selye, 1936). However, more recent studies have revealed that in humans, exposure to extreme or chronic stressors, and to stress-related illnesses, is instead correlated with hypocortisolism (Heim, Ehlert, & Hellhammer, 2000). In this regard, post-traumatic stress disorder (PTSD), depression, rheumatoid arthritis, and asthma, amongst others, have received special attention (Chikanza, Petrou, Chrousos, Kingsley, & Panayi, 1992; Demitrack et al., 1991; Heim, Ehlert, Hanker, & Hellhammer, 1998; Hellhammer, 1990; Kruger & Spiecker, 1994). The pattern of corticosteroid response to stress appears to differ depending on whether the stressful situation is immediate, readily reversible, and requires quick action, or whether it is

prolonged, persistent, and requires adaption where escape is not possible (Dhabhar & McEwen, 1997).

The Spectrum of Stress Hypothesis (Dhabhar & McEwen, 1997) suggests there are different levels of stress that necessitate different physiological responses. This evolutionary argument proposes that, under conditions of *eustress* (acute, short-lived stress), the organism will enhance its protective mechanisms and fight to restore balance by increasing the action of corticosteroids. In contrast, the organism will inhibit its defences when faced with *distress* (persistent, prolonged stress), possibly in an attempt to conserve energy by inhibiting the action of corticosteroids. Hence, organisms employ different survival strategies, which are associated with contrasting corticosteroid responses, based on the chronicity of stress.

Empirical support for this theory emerged from Dhabhar and McEwen's (1997) observation (in rats) of an increase of corticosteroid response following exposure to acute stress, but an attenuation of increase in plasma corticosterone when exposure to stress was prolonged. Furthermore, they found that besides causing a decrease in the absolute amount of blood plasma corticosteroid, chronic stress (3-4 weeks) reversed the circadian corticosteroid rhythm. Specifically, significant increases in corticosterone levels were noted during periods of sleep, when they are supposed to be at their nadir. This circadian profile of corticosteroid secretion parallels that of asthmatics: low levels of cortisol early in the morning, normal-to-low levels on average, and relatively elevated evening levels (Ball, Anderson, Minto, & Halonen, 2006; Fei et al., 2004; Fujitaka et al., 2000; Masharani et al., 2005; Schleimer, 2000).

Such reversal in the diurnal secretion of cortisol (characterized by relatively low levels during the day and elevated levels at night) has been associated with well-characterized sleep disturbances in patients with Cushing's syndrome and with depression. These disturbances include sleep fragmentation, disinhibited and denser REM sleep, short REM

latencies, and reduced or displaced SWS from the first to the second half of sleep. These symptoms are reversed when cortisol and ACTH levels are restored through pharmacological or surgical interventions, to their normal ranges (Steiger, 2007). The effects of elevated night-time cortisol in asthmatics have not been systematically investigated as they have in the other two patient populations, despite the similarities in their cortisol secretion patterns.

Similarly to the effects of reversals in the night-time cortisol secretion patterns in patients with Cushing's disease, experimentally elevating cortisol during sleep fragments sleep and reduces REM sleep in healthy young adults, in the elderly, and in individuals with depression. In contrast, infusing cortisol increases SWS (Bohlhalter et al., 1997; Born et al., 1991; Friess, Bardeleben, Wiedemann, Lauer, & Holsboer, 1994; Schmidt et al., 2000). With regards to synthetic glucocorticoids, an acute dose of a synthetic glucocorticoid suppresses endogenous cortisol and enhances GR activation. The enhanced GR activation alone suppresses REM sleep; the suppression of SWS depends on multiple factors, as discussed above.

Less is known about the effects of prolonged exposure to synthetic corticosteroids on sleep. The (limited) extant evidence indicates some similarities to acute exposure effects. For instance, in patients with multiple sclerosis, 10 days of treatment with prednisone induces depression-like changes in sleep architecture (reduced REM sleep latency, increased REM sleep density and reduced SWS; Antonijevic & Steiger, 2003). However, in patients making long-term use of corticosteroid treatment (e.g., patients diagnosed with asthma), little to nothing is known about the relationship between corticosteroid use and sleep architecture. Data from Addison's disease patients, who are characterized as extremely hypocortisolic, is limited to a handful of studies. The rarity and severity of the illness limits its extensive investigation, especially when studies involve high logistical demands from participants (as sleep studies almost invariably do). Existing findings include that treatment with

hydrocortisone restores the quantity and organization of REM sleep and reduces sleep fragmentation in Addison's patients (García-Borreguero et al., 2000; Gillin, Jacobs, Snyder, & Henkin, 1974).

The effects of exogenous corticosteroid treatment in that context are that they are likely to restore some of the vital functions of cortisol. Therefore, the relationship between corticosteroid treatment and sleep architecture in Addison's disease may not be generalizable to other disorders of the HPA axis, with more modest scopes of dysfunction. For instance, in a condition such as asthma, corticosteroids are prescribed to alleviate airway inflammation and not to restore cortisol to a level that is beyond a life-threatening range.

In sum, healthy sleep architecture is associated with the inhibition of the HPA-axis. Various factors induce disinhibition of the HPA-axis during sleep, resulting in conditions (e.g., as in normal aging, and in disorders such as asthma) that are associated with shallow, fragmented sleep, disturbances of REM sleep, and, sometimes, SWS suppression. Importantly for the current research, the dialectical relationship between sleep and corticosteroids has an impact on the quality of sleep-dependent memory consolidation.

Impact of HPA-Axis Deregulation on Sleep-Dependent Memory Processing

Chronic hypercortisolemia has been associated with reduced hippocampal volume and with impaired performance on hippocampal-dependent memory tasks (Lupien et al., 1998, 2013; Sapolsky, Uno, Rebert, & Finch, 1990; Sheline et al., 1996; Starkman, Gebarski, Berent, & Schteingart, 1992; Watanabe et al., 1992a, 1992b). Acute elevation of cortisol has been associated with similar but reversible memory impairments (e.g., Buchanan, Tranel, & Adolphs, 2006; de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; de Quervain et al., 2003; Elzinga & Roelofs, 2005; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Kuhlmann, Piel, & Wolf, 2005; Newcomer et al., 1999; Payne et al., 2007; Young, Drevets,

Schulkin, & Erickson, 2011) and with impaired hippocampal and medial temporal lobe functioning (de Quervain et al., 2003; Lovallo, Robinson, Glahn, & Fox, 2010). Interestingly, the suppression of endogenous cortisol, induced by the actions of exogenous corticosteroids, has also been associated with declarative memory impairment in healthy adults and in various patient populations, including asthmatics (Bender et al., 1991; Brown et al., 2004; Coluccia et al., 2008; Keenan, Jacobson, Soleymani, Mayes, & Yaldoo, 1996; Newcomer, Craft, Hershey, Askins, & Bardgett, 1994; Schmidt, Fox, Goldberg, Smith, & Schulkin, 1999; Wolkowitz, Reus, Canick, Levin, & Lupien, 1997).

Evidence from this literature suggests that corticosteroids affect declarative memory at all three levels of processing: encoding, consolidation, and retrieval (Ferguson & Sapolsky, 2007; see Fietta & Fietta, 2007; Het, Ramlow, & Wolf, 2005; Kuhlmann et al., 2005; and Roozendaal, 2000, for reviews).Therefore, if hippocampal-dependent memories are consolidated during sleep, and corticosteroids affect the processing of these memories, then one might predict that fluctuations in corticosteroid activity during sleep would affect sleep-dependent memory consolidation, both directly and through their effects on sleep.
Evidence also suggests that HPA-axis hyperactivity is associated with disruptions in sleep architecture, and that the latter help perpetuate the cognitive symptoms frequently associated with such hyperactivity (for a review, see Harand et al., 2012; Wagner et al., 2006). For instance, in the elderly, research suggests that changes in sleep are precursors to impairment in declarative memory (e.g., Backhaus et al., 2007) and to elevated night-time cortisol (for reviews, see Van Cauter et al., 1998, 2000).

In their reviews, Van Cauter et al. (1998, 2000) argue that sleep disruptions precede the changes in cortisol patterns observed in the elderly. Neurobiological and lifestyle changes precipitate these sleep disruptions. Such changes include enhanced somatostatin control (which inhibits GH release) and reduced photic and non-photic exposure to circadian-

regulating cues such as changes in daytime activity and reduced cognitive stimulation (Bohlhalter et al., 1997; Conte, Carobbi, Errico, & Ficca, 2012). An accumulation of disrupted, fragmented, and lightened sleep leads to elevation of night-time cortisol, which in turn compromises the HPA -axis. This proposed direction of the relationship is supported by studies demonstrating that (a) total or partial sleep deprivation among healthy young adults results in a circadian cortisol pattern that mimics that observed in the elderly (Vgontzas et al., 2001), and (b) in the course of normal aging, REM suppression, which is attributed to elevated cortisol, only appears about two decades after disruptions in SWS (Van Cauter et al., 1998).

Conversely, in patients with Cushing's syndrome, changes in cortisol appear to precede sleep disruptions and memory impairment. Studies of patients with Cushing's syndrome reveal impairment of working memory and of both verbal and visual long-term memory (Forget, La Croix, Somma, & Cohen, 2000; Martigoni et al, 1992; Mauri et al., 1993; Starkman et al., 1986; Whelan et al., 1980), as well as abnormal sleep architecture similar to that found in depression (i.e., shortened REM latency, and greater REM density and sleep fragmentation; Shipley, Schteingart, Tandon, & Starkman, 1992). These symptoms of memory impairment and sleep disruption are reversible: When cortisol is restored to normal levels (either surgically, by removing carcinogenic adrenal tissue, or pharmacologically, by using an antineoplastic drug with anti-adrenocorticoid properties such as mitotane), memory performance improves and sleep is less disrupted (Martignoni et al., 1992; Mauri et al., 1993; Schteingart et al., 1980).

Whether sleep disruptions precede hypercortisolism or vice-versa, the two phenomena appear to perpetuate one another. Synergistically or additively, hypercortisolism and compromised depth and continuity of sleep disrupt memory consolidation.

As mentioned earlier, poor episodic memory, aberrant HPA-axis functioning, hippocampal insults, and disrupted sleep all co-exist in patients with Cushing's syndrome, in the elderly, and in patients with asthma. Although these phenomena have been linked to one another in the first two populations, their relationship remains unexplored among asthmatics. The hippocampus and related limbic structures contain a large concentration of corticosteroid receptors (de Kloet, 1991; Jacobson & Sapolsky, 1991; Sapolsky, Krey, & McEwen, 1986; Wagner & Born, 2008). The HPA-axis and the hippocampus exert reciprocal effects onto one another through the combined actions of MRs and GRs. Cortisol regulates the balance of MR to GR activation within the hippocampus in order to facilitate memory processing. In turn, the hippocampus down-regulates the secretion of cortisol through negative feedback mechanisms to maintain it at an optimal level. Pathological levels of cortisol disrupt key hippocampal functions, including down-regulating HPA-axis activity (maintaining a hypercortisolic state) and processing episodic memories.

The effective consolidation of episodic memory during sleep depends on a minimal activation of the GRs; this is the point where the relationships between memory processing and early versus late sleep and cortisol intersect. Although I have argued that treating MR versus GR effects dichotomously is not always helpful (especially in the hippocampus where these receptors are most likely to function optimally as heterodimers), the balance between MR and GR action does impact on cognition in predictable ways.

The suppression of GR activity during SWS facilitates efficient communication between the hippocampus and the neocortex, and therefore facilitates episodic memory consolidation. When GRs are overexpressed during sleep, such as when exogenous corticosteroids like prednisone or dexamethasone are administered, excessive GR activation inhibits the transport of glucose to hippocampal neurons and glial cells. This action curbs hippocampal glutamatergic neurotransmission and blocks output from CA1 neurons whose

axons carry information from the hippocampus to the cortex (Born & Wagner, 2009; Born et al., 2006; Plihal & Born, 1999; Wagner & Born, 2007). The intake of exogenous corticosteroids thus disrupts the fine balance between GR and MR activation that is necessary for the optimal consolidation of episodic memory.

The following sections attempt to clarify whether sleep mediates the relationship between, on the one hand, the night-time functioning of the HPA-axis (as assessed by the level of circulating cortisol during sleep) and, on the other hand, offline memory processes.

To do so, I discuss the following:

(a) Ultradian versus circadian influences on sleep-dependent memory consolidation. I present two competing models of sleep-dependent memory consolidation, followed by an attempt at reconciling these perspectives;

(b) The role of cortisol in regulating both ultradian and circadian rhythms. I posit that cortisol is a modulator for the kind of memory consolidation that is facilitated at various times during sleep;

(c) Sleep mentation as an aspect of sleep-dependent memory processing. The quality and distribution of dreams encountered during different ultradian or circadian phases of sleep are framed as the by-product, at least partially, of the degree and the type of memory consolidation facilitated during those times; and

(d) The proposal that, if HPA-axis activity affects sleep and sleep-dependent memory processing, which is an integral part of dreaming, then changes in HPA-axis activity will affect the quality of dreams.

Models of Sleep-Dependent Memory Consolidation

The dual-process model of memory consolidation. The specificity of sleep-dependent memory facilitated by SWS or REM sleep forms the basis of this model. The model states that (a) hippocampal-dependent memory systems, such as episodic memory, rely on brain states (e.g., those present during SWS) where information flows from efferent hippocampal-to-cortical pathways, whereas (b) hippocampal-independent memory systems either do not benefit from sleep (e.g., semantic memory) or might be consolidated when information flows from the neocortex to the hippocampus, as it does during REM sleep (e.g., procedural memory; Brualla, Romero, Serrano, & Valdizan, 1998; Conway & Smith, 1994; Gais & Born, 2004; Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Maquet, 2001; Peigneux et al., 2003; Perrin et al., 1999; Plihal & Born, 1997, 1999; Smith, 1995, 1996). Some researchers (e.g., Born & Wilhelm, 2012; Muzur, 2005) describe SWS as an off-line period or state of consciousness that facilitates hippocampal-cortical replay of previously encountered events. Muzur (2005) argues that the slow, deep activity experienced during SWS has the function of focusing attention on the processing and storage of relevant information, with no additional cognitive resources remaining to attend to peripheral stimuli. The relative silence during SWS is marked by minimal electrophysiological interference or stimulus noise, and underlies the reconstruction and re-experience of an event in order to consolidate it into long-term memory (Born et al., 2006; Muzur, 2005; Payne & Nadel, 2004; Plihal & Born, 1999).[1]

A significant body of empirical evidence supports this position. This line of research demonstrates a causal link between the neurophysiological correlates of SWS and the consolidation of memory during sleep (Marshall et al., 2004, 2006). One such neurophysiological correlate of SWS is slow-wave activity (SWA), which refers to slow oscillations of high amplitude waves, cycling at 0.75 Hz a second, originating from the

[1] Therefore, throughout the thesis, I make reference to standardized verbal declarative tests in comparison to procedural memory tests but I refer to episodic memory as a distinct counterpart to declarative semantic memory, especially when referring to the memory content of dreams.

prefrontal cortex. SWA is involved in the selection, reactivation, transfer, and reorganization of temporarily stored information to long-term storage. SWA integrates sharp wave-ripple activity from the hippocampus and sleep spindles from the thalamus in an oscillating, synchronised concert, thus promoting a state of information transfer from deep limbic and subcortical structures to the cortex. In this way, information encoded during wakefulness is filtered and subsequently consolidated during sleep.

SWA is enhanced if information that is salient to the individual's survival and future plans is encountered. SWA, in turn, enhances the quality and stability of information deemed relevant (Born & Wilhelm, 2012).[2]

Overall, SWA has proven to be the most consistent and compelling correlate of sleep-dependent memory processing. Hence, the dual-process model is supported, at least in part, by the role of SWA in the consolidation of episodic memory. However, ambiguity with regard to the role of REM sleep in the process of memory consolidation exposes the model's weakness.

Studies investigating the dual-process model have used various approaches, including split-night, selective deprivation, and circadian-cancellation protocols, to tease out NREM versus REM stage effects on learning (for reviews, see Ficca & Salzarulo, 2004; Rauchs, Desgranges, Foret, & Eustaches, 2005). The results of such investigations are not consistent. First, REM sleep deprivation does not always result in decrements in procedural memory performance (Moroni et al., 2008; Smith & MacNeill, 1994). Second, although there are some clear causal links between SWS and the consolidation of *neutral* declarative memories, the consolidation of *emotional* declarative memories mostly involves REM sleep (Nishida, Pearsall, Buckner, & Walker, 2009; Payne, 2010; Payne & Kensinger, 2010; Wagner, Gais,

[2]Note: Other electrophysiological phenomena, outside of SWS, such as PGO bursts in REM sleep, or immediate early gene (IEG) expression also assist in the consolidation of memories (Ambrosini & Giuditta, 2001; Ribeiro & Nicholelis, 2004).

& Born, 2001, 2006; Walker, 2009). Third, performance on certain motor learning tasks benefits more greatly from stage 2 sleep than from REM sleep (Brière, Forest, Lussier, & Godbout, 2000; Fogel, Jacob, & Smith, 2001; Fogel, Smith, & Cote, 2007; Tamaki, Matsuoka, Nittono, & Hori, 2008; Walker, Brakefield, Hobson, Morgan, & Stickgold, 2002). Fourth, performance on certain procedural memory tasks benefits from both SWS and REM sleep (Gais et al., 2000; Mednick et al., 2003; Stickgold, James, & Hobson, 2000). Fifth, aspects of performance on motor sequence tasks benefit from NREM sleep, including SWS (Huber et al., 2004; Moroni et al., 2008; Robertson et al., 2004)

At least some of this inconsistency can be attributed to methodological difficulty in isolating the effects on memory consolidation of a single sleep stage. REM deprivation, for instance, brings about changes in the entire architecture of sleep cycles and of the evolution of sleep throughout the night (Ficca & Salzarulo, 2004; Rauchs et al., 2005). Another aspect of the problem lies in the multifaceted nature of performance on memory tasks: Different features of declarative memory tasks (e.g., the gist of an event versus its emotional relevance) and of procedural memory tasks (e.g., motor dexterity versus sensory discrimination) may depend on different stages of sleep. Some theorists have suggested that these difficulties underscore the very nature of memory consolidation during sleep and of the complementary rather than antagonistic functions of NREM and REM sleep (Ficca & Salzarulo, 2004; Stickgold & Walker, 2007). In other words, the benefits for memory of NREM and REM sleep, although different, may be cumulative. Different stages of memory consolidation may rely on the different physiology of NREM versus REM sleep stages, but the process as a whole may require all the stages, in their natural order, to be complete. For instance, Rauchs et al. (2004) used a partial sleep-deprivation paradigm to assess whether SWS or REM or both were associated with performance on the three dimensions of episodic memory (i.e., factual, spatial, and temporal components). They found that whereas REM sleep benefitted

recall for the 'where' aspect of an episode, SWS benefitted the 'when' aspect. Hence, they concluded that SWS and REM play complementary roles in the consolidation of episodic memories.

The sequential hypothesis of memory consolidation. This model is an alternative to the dual-process model. It proposes that the cycling of NREM to REM stages and back is functionally relevant to different stages of memory processing, irrespective of the type of information being consolidated (Ficca & Salzarulo, 2004; Giuditta et al., 1995). In other words, the smooth transition from NREM to REM is essential for memory consolidation because these two broad sleep states fulfil distinct yet co-dependent roles that together ensure complete and effective processing of encoded information (Ambrosini & Giuditta, 2001; Born & Wagner, 2004; Fischer, Drosopoulos, Tsen, & Born, 2006; Peigneux et al., 2001; Poldrack & Rodriguez, 2003, 2004; Rasch, Born, & Gais, 2006; Ribeiro & Nicholelis, 2004; Stickgold et al., 2000; Walker & Stickgold, 2010).

According to the sequential hypothesis model, during sleep memory traces are processed in stages, so that NREM and REM sleep contribute to consolidation in stepwise progression. SWS consolidates individual units of a new episode and strengthens their representations separately, whereas REM sleep integrates the new information into a meaningful whole by linking the novel experience to similar or related existing references (taken from older memories and semantic knowledge). This process serves to constantly expand and re-organize the individual's experiences into associative networks, thus refreshing knowledge of the world and refining experiences (Walker et al., 2010).

Support for the sequential hypothesis model comes from three sources. First, as noted earlier, there is an inconsistent body of evidence on the role of individual sleep states on specific subtypes of memory. Many studies have failed to isolate stage-specific effects; these failures are not solely accounted for by methodological differences (Schabus, 2009). For

instance, Gais et al. (2004) found a positive association between early SWS-rich sleep and performance on a visual texture discrimination task, whereas Stickgold et al. (2000) found that the combination of the first, early SWS period and the last, late REM sleep period was the best predictor of overnight skill enhancement on the same procedural memory task. The latter finding makes sense if one considers that complex memory tasks, such as those requiring the coordination of input from various brain regions, including the cerebellum and the visual and motor cortices, may require different dimensions of consolidation that cannot be fulfilled by a single sleep state (Rasch et al., 2006). Some researchers argue that there are explicit and implicit components to most encoded memories, and that consolidation during sleep attends to each of these systems separately (Born et al., 2004; Fischer, Drosopoulos, Tsen, & Born, 2006; Peigneux et al., 2001; Poldrack & Rodriguez, 2003, 2004). Hence, it is not surprising that studies often fail to establish a connection between a single sleep stage and overnight gains in performance on a memory task.

The second source of support for the sequential hypothesis model emerges from a few studies demonstrating that performance on declarative memory tasks is related to the length (Mazzoni et al., 1999) and to the number (Ficca, Lombardo, Rossi, & Salzarulo, 2000) of NREM-REM cycles, as opposed to the percentage of SWS or REM sleep. Furthermore, Stickgold et al. (2000) demonstrated that a whole night's sleep offered greater benefits to performance on a procedural memory task than periods of SWS or REM sleep separately. The third source of support for the sequential hypothesis model emerges from a few studies demonstrating the role of REM sleep in consolidating aspects of episodic memory. For instance, REM sleep tends to enhance the consolidation of emotionally-valenced episodic memories (Empson & Clarke, 1970; Tilley & Empson, 1978; Rauchs et al., 2004; Wagner et al., 2001, 2006; for a review see Walker, 2009). Furthermore, sleep cycles with a greater

proportion of REM to NREM are associated with better consolidation of the temporal order of episodic memories (Griessenberger et al., 2012).

In summary, evidence supporting the sequential hypothesis model suggests that NREM and REM sleep perform complementary functions by consolidating different aspects of the same memories. In other words, effective memory processing during sleep depends on the frequency and integrity of entire cycles, as opposed to the separate functioning of distinct stages of sleep. Perhaps this notion can be understood better if one considers the notion of replay of waking events during sleep. Electrophysiological, molecular, cellular, and neuroimaging data reveal that waking events are reactivated during SWS, and that REM sleep potentiates the reactivated networks (Born & Wilhelm, 2012; Ribeiro & Nicholelis, 2004; Stickgold & Walker, 2007). After memory traces are encoded, the neural patterns that represent these traces undergo consolidation in stages (which are consistent regardless of whether consolidation occurs during wake or sleep): First, they are reactivated or replayed, and this reactivation renders the memory trace vulnerable to change or corruption (in other words, it becomes destabilised). During sleep, this initial reactivation process occurs, presumably, during NREM stages. If the memory trace is not strengthened at this critical stage through long-term potentiation (LTP), or preserved from interference by competing information, it degrades. However, if the memory trace is successfully potentiated it is said to be reconsolidated and to be committed to long-term storage (Ribeiro & Nicholelis, 2004; Stickgold & Walker, 2007). During sleep, LTP (and hence reconsolidation) occurs mostly during REM sleep. These reactivation and potentiation processes rely on neuronal reverberation and plasticity respectively; both of these are necessary and neither alone is sufficient for memory consolidation in any given state of consciousness (Hebb, 1949; Jackson, Johnson, & Redish, 2006; Nádasdy, Hirase, Czurkò, Csicsvari, & Buzsáki, 1999; Ribeiro & Nicholelis, 2004; Wilson & McNaughton, 1994).

Taken together, recent evidence in the area of sleep-dependent memory consolidation seems to suggest that (a) SWS and REM sleep play distinct but complementary roles with regard to the offline processing of waking experiences, (b) sleep-dependent learning is directly proportional to the extent and nature of these experiences, and (c) the structure and number of NREM-REM cycles are potentially more relevant to effective consolidation than absolute percentages of each stage (for a review, see Cartwright, 2004).

Now, if dreams reflect the offline processing of waking events and preoccupations, and if one accepts that this offline processing depends on non-fragmented transitions from NREM-to-REM sleep with a circadian-dependent evolution of NREM-to-REM sleep ratio throughout the night, it is reasonable to expect that any change in the normal structure of sleep cycles (e.g., change brought about by a deregulation of the HPA axis) should influence the content of sleep mentation.

Sleep Mentation: The Nature and Sources of Dreams

Antrobus's (1991), (personal communication entitled *Theories of dreaming*, 1998) cortical activation theory of dreaming, Nielsen's (2000) theory of *covert* REM, and Cicogna and Bosinelli's (2001) dream generation model all acknowledge the dual influences of ultradian and circadian processes on dreaming. Despite their different positions regarding (a) the exact mechanisms underlying sleep physiology, and (b) the nature of the relationships between those mechanisms and sleep mentation, these theories all propose that the same processes that support cognitive activity during sleep support dreaming. Specifically, all of these dream theories associate cognitive activity during sleep to post-encoding memory processing (i.e., memory consolidation). Because aspects of memory consolidation occur during both NREM and REM sleep (see Rausch et al., 2005, for a review), within these theoretical frameworks the strong implication is that sleep mentation occurs throughout sleep.

Furthermore, then, the quantitative and qualitative differences found in dreams reflect, at least in part, the degree of memory consolidation facilitated during different sleep stages.

Cicogna and Bosinelli's (2001) clearly formulated model of dream generation, which is a refinement of Foulkes' (1985) conceptualization of the dreaming process provides some useful perspectives on the nature of memory sources in dreams. The foundation of Foulkes' model is three-fold. It involves (a) bottom-up mnemonic activation, (b) top-down planning and organization of the activated information, and (c) a conscious elaboration of the material into a narrative containing imagery, language, and emotions. Cicogna and Bosinelli elaborated on this model by further defining the nature of the mnemonic material and the process through which memories might get activated during sleep mentation. They did so using Schacter and Tulving's (1994) conceptualization of long-term memory. Hence, assuming that long-term memory consists of four subsystems (viz., procedural, perceptual representation, semantic, and episodic), Cicogna and Bosinelli's dream generator model posits that, at any point in time, depending on the internal neurophysiological environment or state of consciousness in which the person finds himself, memories belonging to these systems can be activated either simultaneously or sequentially, and can be retrieved entirely independently from one another. The bizarre nature of some dreams illustrates this concept: For instance, in NREM dreams, dreamers sometimes report the distinct knowledge of a person being present without any visual representation of that person being available to accompany that impression. In this case, the model might argue that this sort of dreaming is representative of the activation of semantic memories in the absence of any perceptual representation. On the other hand, in REM dreams, the model might interpret the very vivid experience of a collage of images that are connected in a manner not plausible by waking standards as resulting from the activation of episodic memories in the absence of semantic involvement.

From this theoretical perspective, the way dreams differ depends on the level of engagement with mnemonic information. The latter, in turn, depends on the brain regions that are active (and the relative degree to which each is) at the time that the dream is (presumably) being experienced. This framework places NREM and REM and conceptual/thought-like dreams and perceptual/vivid dreams on a continuum, because greater cortical activation is required for the generation of imagery. Consequently, by this model, factors that influence cerebral activation during sleep, such as HPA-axis activity, should influence the kind of sleep mentation encountered.

Ultradian versus circadian influences on dreaming: The role of the HPA axis. The HPA-axis plays a vital role in the regulation of biological rhythms; these rhythms include the organism's pattern of activity and rest, and consequently, the architecture of sleep and sleep-dependent processes such as dreaming. This sub-section discusses the relative influence on dreaming of sleep stage overtime of night. Because cortisol level is one of the three markers of circadian synchrony or desynchrony, together with melatonin and core body temperature (Benloucif et al., 2005; Klerman, Gershengorn, Duffy, & Kronauer, 2002), the aim of this discussion is to evaluate the role of cortisol in shaping dream experiences, especially with regard to the memory content of dreams.

NREM and REM dreams. Available evidence on the distinct, yet complementary, memory functions fulfilled by NREM (SWS) and REM sleep can be extended to explain the content of dreams. First, the distinct memory consolidation processes enabled by SWS and REM sleep sometimes reflect the differences in dream reports obtained from NREM awakenings versus those obtained from REM awakenings. The differences between NREM and REM dreams with regard to their lengths, perceptual and emotional features, continuity with waking experiences, and memory content have been investigated and documented

extensively (for reviews, see, e.g., Baylor & Cavallero, 2001; Domhoff, 2003; Fosse et al., 2003; Foulkes, 1966, 1985; Hobson et al., 2000; Nielsen, 2000, 2004; Stickgold et al., 2001). Although the extent and the validity of the differences between NREM and REM dreams are highly debated and depend largely on the methodology used to study them, there are certain differences that are commonly reported (for reviews, see Domhoff, 2003; Nielsen, 2004). For instance, NREM dreams are characterized more by a thought-like nature rather than by visual imagery; after such dreams, the dreamer wakes up feeling that she has been pondering over a matter the entire night, despite being asleep. Moreover, NREM dreams tend to bear a strong connection with recent experiences; their sources can be traced back to waking events easily. Hence, one may draw a parallel between memory replay during sleep and the nature of NREM dreams (Nielsen, 2000, 2004; Ribeiro & Nicholelis, 2004).

In contrast, REM dreams are characterized by a mixture of loosely related recent and remote experiences that form seemingly original and often bizarre scenarios. Those scenarios are frequently defined by the emotions that are currently autobiographically salient to the dreamer. The nature of the contents of REM dreams is consistent with the direction of information transfer during REM sleep: The flow is from neocortical to hippocampal circuits (Payne & Nadel, 2004).

This direction of information flow may explain the greater presence of remote memories during REM-rich sleep (Roffwarg et al., 1978; Verdone, 1963, 1965). It possibly underlies the role that REM sleep plays in assimilating new information within existing, and relevant, memory networks (Payne & Nadel, 2004; Walker et al., 2010). REM dreams are characterized by certain features which illustrate this function, for example the striking disobedience of the laws of physics (e.g., characters can find themselves transposed from one place to another within a matter of seconds), the meshing of old experiences with recent ones

in a single timeframe, and sharing seemingly novel or counterintuitive interactions with people who are no longer alive or present in the dreamer's life.

Other aspects pertaining to impressions of improved or exaggerated or idealised abilities (Payne & Nadel, 2004), such as being able to fly or to speak a relatively unfamiliar foreign language fluently, may in fact reflect the potentiating stage of sleep-dependent memory consolidation, specifically of procedural learning, which benefits from REM sleep (Brualla et al., 1998; Conway & Smith, 1994; Gais & Born, 2004; Karni et al., 1994; Maquet, 2001; Peigneux et al., 2003; Perrin et al., 1999; Plihal & Born, 1997, 1999; Smith, 1995, 1996). Hence, what appears to be incoherence on the surface is, arguably, a reflection of the complexity of the associative networks that are being activated, and of the product of shifts in consciousness.

Nielsen (1997, 2000, 2004) suggests that these shifts in consciousness from NREM to REM sleep are more like evolutions; they occur progressively. Similarly, some evidence suggests that NREM and REM dreams evolve towards one another along a continuum, instead of shifting abruptly from one quality of dreaming to another. Nielsen (2004) refers to this continuum as the "sinusoidal" nature of dreams.

***Early-night and late-night dreams*.** There is much debate as to whether different components of dreaming (e.g., access to memories, choice of imagery content, visual acuity, intensity of emotion, and continuity of dream scenarios with waking events) are modulated by ultradian processes (REM versus NREM sleep states), by circadian processes, or by both. Most evidence on the factors affecting the presence, extent, and quality of dreams suggests two things: (a) there are quantitative as well as qualitative differences between REM and NREM dreams, and (b) dreams are further influenced by circadian factors that are superimposed upon ultradian factors. In other words, dreams occurring during REM are different to those occurring during NREM, and dreams occurring during the first half of

normal sleep are different to dreams occurring during the early hours of the morning. Hence, it appears that ultradian and circadian factors influence dreams both independently and in an additive manner (Nielsen, 2004; Ribeiro & Nicholelis, 2004).

Memory access during dreaming reflects these joint ultradian and circadian influences on dreaming. Research suggests that NREM dreams preferentially access episodic and self-referential memories, whereas REM dreams tend to draw on a combination of semantic and episodic material. In fact, REM dreams tend to draw more extensively on semantic knowledge and on older memories than on recently-acquired episodic memories (Baylor & Cavallero, 2001; Cicogna et al., 1991; Schwartz, 2003).

Furthermore, dreams with greater episodic content predominate during the first few hours of sleep, whereas dreams with greater idiosyncratic content, interlaced with disjointed remote memories, predominate during the second half of the night. At first glance, this pattern of dreaming seems to support a greater ultradian influence on dream content; as noted earlier, NREM SWS is concentrated during early sleep, but REM sleep increases in length and density and the night progresses. However, evidence suggests that memory access in dreams differs across REM periods, especially between REM1 and REM2. Specifically, REM1 dreams bear stronger thematic continuity with recent waking experiences and preoccupations than later REM stages (for a review, see Schredl, 2003).Therefore, sleep stage alone cannot explain dream content. The cycle from which the dream is extracted appears to also predict its content.

It is debatable whether or not early-night NREM dreams are more similar to early-night REM dreams than they are to late-night NREM dreams (Nielsen, 2004; Wamsley, Hirota, Tucker, Smith, & Antrobus, 2007). The various methodological approaches used to collect and study dream reports affect the measurement of variance that is explained by ultradian versus circadian factors. For instance, using the progressive temporal delay into

stage (PTDIS) protocol for collecting dream reports (e.g., performing the awakening 5 minutes into the first REM, 10 minutes into the second REM, and 15 minutes into the third REM) might confound circadian effects with ultradian effects because qualities that make sleep mentation more "dreamlike" (e.g., vividness of dream imagery) are gradually enhanced as the sleep stage progresses (Nielsen, 2004). Therefore, if later dreams appear longer and more vivid it would be difficult to determine whether this is due to awakenings triggered after longer durations into REM sleep, later at night, relative to awakenings provoked much sooner into REM sleep, earlier at night, or if it is due to an actual circadian oscillator.

A better approach in determining if there is a circadian influence on a certain feature of dreaming may be to (a) collect more than one dream in one night, and (b) collect those dreams from the same sleep stage, while (c) using a constant time delay interval to perform the awakenings. The PTDIS may be more appropriate if the aim is to compare NREM and REM dreams. To do so effectively, the effects of the previous stage might be controlled by altering the temporal delay into stage awakening, with the alteration being proportional to the ratio of NREM to REM in each evolving sleep cycle (Nielsen, 2004).

Another methodological hurdle that may affect the measurement of ultradian versus circadian influences on dreaming is the correct estimation of the circadian nadir in forced circadian desynchrony protocols. The more that different stage dreams are all collected during the same circadian phase (i.e., during either the descending or the ascending phase, without any overlap), the less prominent the typical NREM versus REM dream differences appear to be. For instance, estimates of variance of visual imagery accounted for by ultradian versus circadian factors shrink from as much as .70 (Antrobus, Kondo, Reinsel, & Fein, 1995) to as little as .40 (Wamsley et al., 2004) following more accurate chartings of circadian phase.

The content of dreams: The role of cortisol. Night-time is marked by low cortisol levels that persist at sleep onset and that reach their nadir at around midnight. This cortisol trough often coincides with the longest period of SWS that occurs during the first complete sleep cycle. Empirical studies suggest that episodic memory consolidation is more efficient during the first half of sleep, when sleepers experience 80% of their SWS (Plihal & Born, 1997, 1999). During the second half of an 8-hour sleep cycle (approximately 3-4 hours after sleep onset), cortisol starts rising and continues to rise in parallel with the ascending leg of the circadian slope. This state of progressive arousal occurs simultaneously with (a) higher incidence of dream recall, and (b) increased intensity and vividness of dreams (Nielsen, 2004; Payne & Nadel, 2004). In other words, the longest REM stage and the longest, most vivid dreams are reported precisely during the period when cortisol starts peaking, during the last hour of sleep.

It is therefore important to investigate the implications of the way in which circadian alterations correlate with dream quality. As mentioned above, one of the approaches used to study the effects of the circadian processes on dreaming is to induce circadian desynchrony experimentally. For instance, Takeuchi et al. (2002) deprived their healthy participants of REM sleep in order to induce REM rebound and early REM onset, and to demonstrate the relationship between short REM latencies and dream disturbances like sleep paralysis (e.g., Takeuchi et al., 2002).

A more indirect approach involves investigating the dreaming patterns in either (a) non-pathological conditions that cause phase advances in circadian rhythms (e.g., normal aging, or jet lag from westward transmeridian travel), or (b) patient populations prone to circadian desynchrony (e.g., asthmatics, or patients with PTSD and/or depression).
In the elderly and in the case of jetlag, circadian oscillators tend to be triggered prematurely (Yoon et al., 2003), and sleep in older adults and in travelers is associated with short REM

latencies (Snyder, 1983), and with an increase in the incidence of sleep paralysis and intense nightmares during early sleep (Wing, Chiu, Leung, & Ng, 1999).

Studies on the dreaming patterns of patients with PTSD and depression have highlighted a relationship between abnormal night-time cortisol secretion and "a phase advance in dreaming" (Nielsen, 2004, p. 415). This phase advance in dreaming is characterized by intense, negative, and vivid dreams at the beginning of the night. As the night progresses, there is a waning of the dream-like quality of sleep mentation, instead of the normal intensification of the dream narratives reported as the night progresses (Nielsen, 2004; Hefez, Metz, & Lavie, 1987; van der Kolk et al., 1984). This phase advance in dreaming is accompanied by (a) a phase advance in the evolution of the sleep architecture, with sleep anomalies such as reduced REM latency and increased REM density, (b) a reversal in autonomic responsivity, with greater responsivity found during the first as opposed to the second half of the night, and (c) HPA-axis dysfunction, with circadian phase-specific hypocortisolemia. This concert of changes suggests either a reversal or an acceleration of the ascending slope of night-time circadian rhythms. This change impacts on the physiology of sleep and on patterns of dreaming, shifting their normal intensification from the second to the first half of sleep (see Nielsen, 2004 for a review).

As mentioned earlier, asthma is a condition associated with hypo-activation of the HPA axis and with aberrant circadian cortisol profiles. Moreover, individuals with asthma often present with abnormal dreaming patterns (Klink & Quan, 1985; Monday et al., 1987; Montplaisir et al., 1983; Nielsen et al., 1997). Therefore, the abnormal cortisol profiles and dreaming patterns observed in asthmatics make them a suitable population in which to study the effects of circadian desynchrony on dreaming. However, the dreams of asthmatics have been examined only in the context of disordered breathing and/or acquired personality

pathologies such as alexithymia; they have not been examined in the context of HPA-axis dysfunctions, unlike those of patients with PTSD and/or major depression.

Aims and Rationale

The overarching aim of this research was to explore and describe the relationship between corticosteroid exposure and sleep-dependent memory consolidation. The studies described here used the content of dreams as a gauge for memory consolidation during sleep. The mild circadian desynchrony observed in asthmatics has been linked to reports of fatigue and daytime sleepiness and, in some cases, to impaired everyday functioning (Smolensky, Reinberg Martin, & Haus, 1999; Stores, Ellis, Wiggs, Crawford, Thomson, 1998; Stuck et al., 2003; Yigla, Tov, Solomonov, Rubin, & Harlev, 2003). I therefore designed Study 1 to investigate how cortisol desynchrony is associated with sleep quality, and with the operation of sleep-dependent mental processes, such as memory consolidation and dreaming, among adult asthmatics. Furthermore, I explored this association in asthmatics who were and who were not undergoing corticosteroid treatment.

I then designed Study 2 to test the effects of a single 25 mg dose of prednisone on memory, sleep organization, and dreaming in healthy young adults. The aim behind the design of these two studies was to determine if the chronic effects of inhaled corticosteroids (Study 1) are comparable to those of the acute effects oral corticosteroids (Study 2). In other words, I investigated whether chronic exposure to relatively small doses of synthetic inhaled corticosteroids (between an average of 100mg to 1000mg of Budesonide or equivalent, daily) had comparable effects on sleep and sleep-dependent cognition to those of a once-off, mild dose of a commonly prescribed oral synthetic corticosteroid (prednisone).

High doses of inhaled corticosteroids are often prescribed by physicians despite evidence that they reach their ceiling of efficiency at moderate doses (Guleria & Mohan,

2007; Holt et al., 2001; Powell & Gibson, 2003). These high doses are prescribed because these medications are less potent than oral forms, with fewer side effects. However, even with limited systemic effects, inhaled corticosteroids can suppress HPA-axis function. Therefore, if the prolonged use of inhaled corticosteroids has clinically significant effects on sleep, memory, and dreaming, there may be important implications for the treatment of asthma.

As mentioned earlier, sustained hypercortisolemia or prolonged exposure to glucocorticoids are both associated with episodic memory impairment and with reduced hippocampal volume (Lupien et al., 1998; Sapolsky et al., 1990; Sheline et al., 1996; Starkman et al., 1992; Watanabe et al., 1992a, 1992b). Although asthmatics do not present with hypercortisolemia as such, I wanted to investigate whether their cortisol circadian desynchrony would be associated with structural changes in the hippocampus because that brain structure is involved in the regulation of cortisol, and as mentioned above, is particularly vulnerable to the action of corticosteroids. Hence, Study 3 compares hippocampal volume between the Moderate-to-Severe Asthma and the Healthy Control participants of Study 1.

This dissertation represents, to my knowledge, the first integrated research program that compares the relationship between altered circadian cortisol and memory and altered corcadian cortisol and sleep, in conditions where individuals are (a) exposed versus not exposed to corticsteroids, (b) chronically versus acutely exposed to corticosteroids, while (c) investigating the involvement of hippocampal volume in mediating the memory effects, and (d) including dream content and dream distribution as a gauge of the offline (i.e., during sleep) consolidation of episodic memory when glucocorticoid activity is elevated.

The rest of the dissertation is organized thus: Chapters Two, Three, and Four provide the full details of Studies 1, 2, and 3, respectively. Chapter Five discusses the results of all three

studies and of their complementary contributions in achieving the broad aims described above.

CHAPTER TWO

STUDY 1: CHRONICALLY ELEVATED NIGHT-TIME CORTISOL AND SLEEP-DEPENDENT MEMORY CONSOLIDATION

Introduction

Chapter 1, the General Introduction, presented an argument for the importance of studying dreaming in the context of sleep-dependent memory processing. If what we dream about affects how we remember our experiences, gives us the appropriate cognitive and emotional resources to manage waking reality, and builds on our perceptions of the self in the world, then it is worth knowing if and how a substance used by millions of individuals, that is corticosteroids, impacts on sleep mentation. Typically, asthmatics are treated with corticosteroids, either acutely or chronically, using oral or inhaled forms. Additionally, their endogenous levels of corticosteroids are often particular. Specifically, they have elevated night-time cortisol but delayed morning cortisol acrophase (Ball et al., 2006; Fei et al., 2004; Fujitaka et al., 2000; Haen et al., 1991; Masharani et al., 2005; Schleimer, 2000). The effects of this alteration in circadian cortisol secretion pattern on sleep architecture, sleep-dependent memory consolidation, and sleep mentation are poorly understood. Given the complex relationships between sleep architecture, the HPA-axis, memory consolidation, and dreaming, it seems likely that a clinical population, that is asthmatics, presenting with abnormalities in all these areas may provide valuable insight into their inter-dependency. Furthermore, the role of corticosteroid treatment in mediating the relationship between aberrant night-time cortisol and cognitive functioning has not been explored. This is of particular relevance given the ongoing debate about the over-dosage of corticosteroids for the treatment of asthma in common medical practice (Cates & Lasserson, 2010; Cave, Arlett, & Lee, 1999; Colice, 2004; Dekhuijzen & Honour, 2000; Kelly & Nelson, 2003; Lalloo et al., 2007; Shepherd et al., 2008; Stafford, Finkelstein, Haver, & Cockburn, 2003).

Asthma has a strong hereditary component, and can manifest either in the absence or presence of certain environmental triggers. Onset is usually in childhood or adolescence, although the illness can develop at any time during the lifespan. There is no cure for asthma; the general approach to treatment centers around symptom management, usually by controlling the underlying inflammatory process of the syndrome (Akinbami et al., 2012; Colice, 2004; Lalloo et al., 2007; Leff, 1997; Shepherd et al., 2008; von Mutius & Drazen, 2012).

Asthmatics are a population of particular interest in this research because the treatment of asthma by traditional medicine depends primarily on the regular use of glucocorticoids. In asthma, chronic inflammation of the airways leads to recurrent episodes of wheezing, tight-chestedness, breathlessness, and coughing (Barnes & Pederson, 1993; Colice, 2004; Kelly & Nelson, 2003; Kraft, Vianna, Martin, & Leung, 1999; Lalloo et al., 2007; Shepherd et al., 2008; Sher et al., 1994). Many asthmatics experience symptom exacerbation, or have asthma attacks, in the evening. Nocturnal symptoms, which often indicate greater asthma severity, frequently fragment sleep and lead to distressed awakenings. The mechanism of these attacks leads to airway obstruction, which can be reversed either spontaneously or with medication (Kraft et al., 1999).

The kinds of medication used to control the symptoms of asthma may be classed, broadly, as either reliever medications or preventer medications. *Reliever medications* contain short-acting beta-agonists, anti-cholinergics, and systemic corticosteroids. *Preventer medications* (also known as *controller medications*) operate over a longer time span, and include corticosteroids, cramolyn sodium, nedocromil, long-acting beta-agonists, methylxanthines, leukotriene modifiers, and immunoglobulin E antibody blocker (omalizumab). Of these active agents, corticosteroids relieve airway inflammation most effectively and consistently, and are the gold standard for the long-term management of

asthma (Dahl, 2006). International guidelines recommend using low doses of inhaled corticosteroids for mild, persistent asthma, and using medium doses for moderate-to-severe asthma. High doses are recommended only for patients with poorly-controlled, persistent asthma that does not respond to treatment at medium doses (Dahl, 2006; Global Initiative for Asthma [GINA], 2012; Lalloo et al., 2007).

Inhaled corticosteroids aid breathing by dilating airways, and by relieving airway inflammation, airflow obstruction, and bronchial hyper responsiveness (Cave et al., 1999; Dahl, 2006; Masharani et al., 2005; Schleimer, 2000). The use of glucocorticoids in the treatment of asthma highlights the function of cortisol in combating the stress imposed on the organism by inflammatory processes, and in assisting with the efficient functioning of immune processes in attacking foreign, harmful agents and in repairing damage at the cellular level (Cave et al., 1999). In the case of asthma, endogenous cortisol seems to be unable to perform these functions adequately and, therefore, exogenous administration becomes necessary.

Asthma and cortisol. This section describes the particularities of corticosteroid activity in asthma, both as part of the disease process and in response to corticosteroid treatment.

***Diurnal cortisol patterns in asthma*.** An extensive body of research has investigated the prevalence of altered endogenous cortisol in asthmatics. Before reviewing this literature, however, I note here that many methodological obstacles have prevented the description of a clear relationship between the asthmatic condition and diurnal cortisol rhythm. These obstacles arise mostly because of the heterogeneity of the population of asthmatic patients. The considerable range in demographic, diagnostic, and treatment profiles of asthmatics complicates our understanding of the interaction between asthma and cortisol (Wlodarczyk et al., 2008). Because patients vary on factors such as severity, illness management, and age,

studies investigating HPA-axis function in asthmatics have found conflicting results with regard to (a) profiling diurnal endogenous cortisol and, (b) measuring the effects of exogenous corticosteroids on endogenous cortisol.

Some studies have found specific changes in the diurnal cortisol profile of asthmatics. These studies describe mild asthmatics and moderate-to-severe asthmatics as displaying (a) flattened cortisol secretion curves during sleep, and (b) a delay in the morning acrophase that is proportional to asthma severity (Fei et al., 2004; Fujitaka et al., 2000; Haen et al., 1991). These findings have not been replicated consistently, however. For instance, Haen et al. (1991) found that asthmatics had *higher* cortisol levels than healthy controls, and a few studies (e.g., Barnes, Fitzgerald, Brown, & Dollery, 1980; Zsefler et al., 1991) failed to demonstrate a significant relationship between asthma symptoms and circadian cortisol levels altogether.

Although there is no absolute consensus, the burden of evidence slants in favor of the notion that asthmatics generally appear to have significantly lower levels of endogenous cortisol than healthy controls, although they often retain a normal diurnal cortisol secretion profile (Heim et al., 1999; Landstra et al., 1999; Kraft et al., 1998; Peebles et al., 2000; Ritz et al., 2011; Sutherland, 2005; Sutherland et al., 2003).

It is not clear whether this alteration in the pattern of cortisol secretion is an adaptation to the asthmatic condition (which in itself constitutes a chronic form of physiological stress), or whether it is a pathogenetic feature of asthma that is present from the onset of the illness. In their review of stress-related psychological and bodily disorders, Heim et al. (2000) argue that hypocortisolism precedes stress-related illness in genetically-predisposed individuals or in individuals chronically exposed to environmental stressors. The persistent lack of cortisol in response to constant stress disinhibits immunosuppressive, inflammatory processes associated with conditions such as asthma.

A few studies have reported that, even though average diurnal cortisol levels may not differ hugely from those in healthy controls, asthmatic subjects display an absence of cortisol surge following awakening (Ball et al., 2006). In healthy individuals, the morning cortisol acrophase is superimposed on and separate from the gradual circadian increase in cortisol that occurs as the night wanes (Buckley & Schatzberg, 2005). Reports of asthmatic patients showing reduced cortisol surge in the morning and reduced cortisol responsiveness to stress (Heim et al., 2000) are reminiscent of the documented alterations in circadian cortisol rhythm observed in normal aging (Clow, 2004). Asthmatics and the elderly experience the same kind of flattened circadian rhythm marked by an attenuated difference between morning acrophase and evening levels of cortisol, most often accounted for by abnormally elevated night-time cortisol (Beluche, Carrière, Ritchie, & Ancelin, 2010; Fiocco, Wan, Weekes, Pim, & Lupien, 2006). Even where endogenous levels of cortisol per se are not significantly decreased in asthmatics, most studies reveal suppressed adrenal function. It appears that in asthmatics, the HPA axis does not release enough cortisol relative to the amount of stress and inflammation encountered by the body (Cave et al., 1999).

A recent study investigating the relationship between acute psychosocial stress, cortisol, and airway inflammation found that asthmatics show lower levels of baseline endogenous cortisol than healthy controls, even after controlling for use of inhaled corticosteroids (Ritz et al., 2011). Furthermore, the authors found a significant relationship between the degree of airway inflammation and the bioavailability of cortisol. Asthmatic participants who had higher levels of endogenous cortisol coped better with stress-induced inflammation.

Studies investigating the cortisol secretion patterns of steroid-free asthmatics (Fei et al., 2004; Fujitaka et al., 2000) may shed some light on endogenous diurnal cortisol rhythms of individuals with asthma. Fei et al. (2004) reported that circadian salivary melatonin and

salivary cortisol were significantly different in asthmatics compared to healthy control participants. Overall, asthmatics had lower melatonin and cortisol levels; moderate-to-severe asthmatics presented with an inverse circadian cortisol profile, with low levels in the morning and elevated levels at night.

Furthermore, there is evidence of reduced glucocorticoid-receptor binding affinity during the early hours of the morning among asthmatics who are not on any form of inhaled or oral corticosteroids and who experience nocturnal symptoms of asthma. This observation is not replicated among asthmatics without nocturnal symptoms, however (Kraft et al., 1999). This pattern of data may explain why, in some individuals with poorly-controlled asthma, cortisol levels may be elevated at night. It may also explain why the cortisol profiling of asthmatics is inconsistent. In fact, the studies (e.g., Barnes et al., 1980, Haen et al., 1991) cited above as observing average normal-to-elevated cortisol levels among asthmatics are those basing their investigations on asthma characterized by nocturnal symptoms. Sutherland (2005) argues that night-time symptoms are not merely a gauge of asthma severity and control but that nocturnal asthma may represent a separable category of illness, with unique genetic and physiological features (Sutherland, 2005; Sutherland et al., 2003).

In a study supporting the argument that nocturnal asthma is distinct from other forms of asthma, Sutherland et al. (2003) compared the circadian cortisol profiles of asthmatics with nocturnal asthma, asthmatics without nocturnal asthma, and healthy controls. Their participants had a corticosteroid washout period of a minimum of 2 months to isolate the effects of exogenous corticosteroids from endogenous cortisol patterns. Results revealed that mild asthmatics without nocturnal asthma experienced higher early-night and lower late-night cortisol levels, as well as a significantly greater 24-hour area under the curve, relative to healthy controls. Nocturnal asthmatics, on the other hand, experienced a 90-minute phase advance in their peak and trough of circadian cortisol, thus behaving quite differently to the

other asthmatics. These findings illustrate the difficulty in charting the 'typical' or 'average' cortisol profile of asthmatics, and suggest that different asthma phenotypes present with distinct forms of HPA-axis deregulation (Kraft et al., 1999; for a review see Sutherland, 2005).

Effects of chronic exogenous corticosteroid administration on endogenous cortisol. Exogenous corticosteroids are used to assist in relieving the symptoms (e.g., in asthma, airway inflammation and bronchoconstriction) of somewhat deficient HPA-axis functioning and of an overly sensitized immune system. The irony here, however, is that exogenous corticosteroids also exacerbate adrenal insufficiency in asthmatics (Barnes & Pedersen, 1993; for a review see Cave et al., 1999; Dahl, 2006; Dekhuijzen & Honour, 2000; Masharani et al., 2005; Tayab et al., 2007), and suppress HPA-axis functioning in healthy adults, even after a single administration of high-dose inhaled corticosteroids (Grahnen, Eckernas, Brundin, & Ling-Andersson, 1994; Grove et al., 1994; Gupta & Bhatia, 2008). This suppression of the HPA axis leads to relative decreases in free circulating endogenous cortisol (Gupta & Bhatia, 2008).

Exogenous corticosteroids affect endogenous cortisol by down-regulating the production of adrenocorticotropic hormone (ACTH). ACTH regulates the production of glucocorticoids by the adrenal cortex. It is secreted in bursts, every 30-120 minutes, throughout the 24-hour day/night cycle. ACTH is, in turn, regulated by the corticotrophin releasing hormone (CRH), which controls the amount of ACTH released at any one time. Together, ACTH and CRH shape the diurnal rhythm of endogenous cortisol. At excessive levels, cortisol suppresses ACTH production via a negative feedback loop. Exogenous corticosteroids have a similar effect on ACTH; they suppress it through the same feedback loop as that of endogenous cortisol. If the adrenal glands are suppressed to the extent that

they are not secreting sufficient cortisol, this action of exogenous corticosteroids on ACTH can lead to adrenal insufficiency (Dahl, 2006; Gupta & Bhatia, 2008; Kelly & Nelson, 2003).

This line of evidence suggests that HPA-axis suppression and low endogenous cortisol levels among individuals with asthma are not merely a product of the pathophysiology of asthma, but can be exacerbated or maintained through exposure to corticosteroids (see Kelly & Nelson, 2003 for a review). The degree of exogenous corticosteroid exposure necessary for adrenal suppression is a contentious issue, but, generally, the evidence seems to support the view that HPA-axis suppression depends on (a) endogenous characteristics (e.g., the baseline health of the HPA axis), (b) other individual characteristics (e.g., age, and duration of illness), and (c) characteristics of the exogenous corticosteroid administrated (Cave et al., 1999; Dekhuijzen & Honour, 2000; Henzen et al., 2000; Martin et al., 2002; Skoner, Gentile, & Angelini, 2010; Smith & Hodson, 1983; Szefler et al., 2002).[3]

A few studies have compared the effects of varying inhaled-corticosteroid exposure (no exposure versus recommended low dose versus high dose exposure) on the endogenous cortisol profile of asthmatics. The evidence suggests that those asthmatics not treated with corticosteroids display a reversal in the diurnal pattern of cortisol, with significantly lower levels in the morning than in the evening (Fei et al., 2004; Fujitaka et al., 2000). This pattern of secretion is in direct contrast to the diurnal pattern seen in healthy adults, where cortisol levels wane as the day progresses (e.g. for reviews see Buckley & Schatzberg, 2005; Dahlgren, 2006; Friess et al., 1995; Steiger, 2003, 2007).

Furthermore, steroid exposure accentuates this reversal in diurnal cortisol patterns. Masharani et al. (2005) observed that steroid-exposed asthmatics had significantly lower

[3]However, even if normal HPA-axis function is preserved, corticosteroid-induced cortisol suppression can occur (Clark, Grove, Cargill, & Lipworth 1996).

morning cortisol and a tendency towards higher evening cortisol than non-steroid exposed asthmatics.

Additionally, there is a dose and potency effect in how a particular corticosteroid affects cortisol acrophase and cortisol trough, or the total area under the curve of 24-hour cortisol secretion, with the higher-dose and potency corticosteroids being associated with the lowest morning and highest evening cortisol levels (Masharani et al., 2005; Skoner et al., 2010). In sum, high doses of a corticosteroid affect cortisol levels more than do low doses of the same corticosteroid, and, in equivalent doses, more potent corticosteroids have a greater suppressive effect on cortisol than less potent corticosteroids.

The variability of corticosteroid treatment regimens (in terms of type, dose, and mode of delivery of medication) is related to the severity of the asthmatic condition. The knock-on effect here is that the effect of corticosteroids on endogenous cortisol is dose- and potency-dependent. Oral forms of corticosteroids have a greater impact on the HPA-axis than inhaled forms because their systemic absorption guarantees greater bioavailability of the drug. However, although administered topically, some systemic absorption does occur with inhaled corticosteroids. The lipophilicity of an inhaled corticosteroid predicts the extent of its suppressive effect on the HPA-axis, which explains why there is so much variability in how inhaled corticosteroids affect endogenous cortisol (see, e.g., Fardon, Lee, Haggart, McFarlane, & Lipworth, 2004; Tayab et al., 2007).

Taken together, the studies reviewed above suggest that asthmatics have an abnormal diurnal endogenous corticosteroid profile that can be exacerbated by using exogenous corticosteroids. This relationship is a complex one, however, because whether corticosteroid-based therapy is supportive or suppressive depends hugely on the disease and demographic profile of the patient, and on the type and dose of inhaled corticosteroid used.

Cortisol, sleep, and asthma. A few studies have examined the relationship between night-time cortisol and inflammatory processes in asthma, and the role of exogenous corticosteroid treatment in mediating this relationship (Barnes et al., 1980; Petrovsky, McNair, & Harrison, 1998; Szefler et al., 2002). Neither of these studies has, however, investigated the effects of glucocorticoid activity, whether endogenous or exogenous, on the sleep architecture of asthmatics. Otherwise stated, research has not described a firm link between poor sleep and elevated night-time cortisol, and between poor sleep and asthma-related corticosteroid treatment.

Most sleep research involving asthmatics has focused either on understanding the mechanisms underlying nocturnal asthma attacks or on the effects of sleep-disordered breathing on quality of sleep and on quality of life. For instance, in the case of nocturnal asthma, polysomnographic data reveals that nocturnal bronchoconstriction may be related to the physiology of REM sleep. Several authors have argued that breathing difficulties are prevalent during REM sleep because of the loss of or irregularity in airway muscle tone an epiphenomenon of this stage of sleep (Barnes et al., 1980; Haxhiu, Rust, Brooks, & Prabha, 2006; Klink & Quan, 1987; Montplaisir et al., 1982, 1983; Rhind, Connaughton, McFie, Douglas, & Flenley, 1985; Shapiro, Catterall, Montgomery, Raab, & Douglas, 1986). However, there is evidence suggesting that asthma attacks occur as frequently in NREM as in REM sleep, and that they are not associated with any particular stage of sleep (Kales et al., 1968, 1970; Montplaisir et al., 1982, 1983; Malo et al., 1985).

Survey data reveal a high prevalence of sleep complaints among individuals with obstructive airway diseases, including asthma. These complaints include difficulty in initiating and in maintaining sleep, reduced sleep efficiency, excessive daytime sleepiness, and nightmares (Klink & Quan, 1987). The few studies that have attempted to characterize

sleep objectively in individuals with asthma have revealed inconsistent differences with regard to the distribution of, and the proportion of time spent in, each stage.

For instance, whereas Kales et al. (1968) observed that individuals with asthma experienced significantly less stage 4 SWS, but similar proportions of REM sleep, compared to healthy controls, Montplaisir et al. (1982) observed that the diagnosis of asthma was related to an alteration in the distribution of REM sleep, but not of SWS. The asthmatic participants in the Montplaisir et al. (1982) study experienced, relative to healthy controls, significantly less REM sleep during the first third of sleep. The root of these conflicting findings may lie in the different samples studied, or in the method used to characterize sleep architecture. Whereas Kales et al. (1968) included participants who were prescribed oral corticosteroid treatment, Montplaisir et al.'s (1982) participants were either on short-acting beta agonist medication alone or on combined treatment regimens involving inhaled corticosteroids and beta-agonists. As noted above, the potency and the degree of exposure to corticosteroids moderate disruptions of sleep architecture.

Regarding characterization of sleep parameters, Kales et al. (1968) explain that although percentage REM did not differ between the groups, the adults with asthma did experience less REM than healthy controls in terms of absolute number of minutes spent in REM. What does appear to be consistent across these studies, however, is that asthmatics experience more periods of wakefulness after sleep onset than controls, and hence experience reduced sleep efficiency. Therefore, it appears that asthma is associated with less time spent asleep, with the deeper stages of sleep being most affected by this fragmentation; this pattern fits Hofman's (1994) definition of poor sleep.

Memory performance of adults with asthma. Interest in the neurocognitive profile of asthmatics stems from the debilitating nature of the disorder. Specifically, illness-induced fatigue and sleep deprivation appear to interfere with everyday social and occupational

functioning. However, there have, to date, been only superficial explorations of the relationship between sleep disruption and cognitive impairment in asthma. Of particular note is that no mechanism of action has yet been posited linking steroid-based treatment, sleep architecture, and memory performance in asthma. This, despite the established effects of sleep deprivation on memory (for reviews see e.g., Ambrosini & Giuditta, 2001; Born et al., 2006; Gais et al., 2000; Ficca & Salzarulo, 2004; Harand et al., 2012; Ribeiro & Nicholelis, 2004; Stickgold et al., 2007), and the role of glucocorticoids in mediating that relationship, at least partially (e.g., Backhaus et al., 2007; Gais et al., 2000; Genzel, Dresler, Wehrle, Grözinger, & Steiger, 2009; Harand et al., 2012; Rajaraman, Gribok, Wesensten, Balkin, & Reifman, 2009).

In a sample of children with asthma aged between 5 and 16 years, Stores et al. (1998) found that delayed story recall performance improved significantly as asthma symptoms and sleep efficiency improved following a change in treatment regimen. Similar research on the cognitive effects of glucocorticoid therapy on adult asthmatics is sparse. Most studies (e.g., Brown et al., 2003, 2004) focus on the effects of oral corticosteroids during short-to-intermediate courses of treatment. For instance, Brown et al. (2003) studied the effects of lamotrigine, a glutamate-release inhibitor used for its anticonvulsant and mood stabilizing properties, on the psychiatric and cognitive symptoms of 5 adult patients (3 asthmatics) undergoing corticosteroid therapy (M = 19.8 mg/day, SD = 12.9) for a period of at least 6 months.[4] The authors reported significant and marked improvements over baseline in terms of performance on the Rey Auditory Verbal Learning Test (RAVLT; Rosenberg et al., 1984). In a similar study, Brown et al. (2004) compared the cognitive performance of patients with

[4]Corticosteroid-induced CNS effects are believed to act synergistically with glutamate activity and hence glutamate antagonists are believed to have a prophylactic effect on corticosteroid-induced psychiatric disturbances and cognitive impairment.

chronic inflammatory diseases (including asthma) who were on oral glucocorticoid therapy against that of matched controls not on glucocorticoid therapy. They reported that patients performed significantly more poorly on the RAVLT. To date, no study has investigated the effects of inhaled corticosteroids on memory performance in adult asthmatics.

A lot more is known about the neurocognitive profile of children with asthma. In children, the typical CNS effects associated with the use of inhaled corticosteroids include transient psychosis, aggression, hyperactive behavior, insomnia, and impaired concentration (Barnes & Pedersen, 1993). Systematic investigations of the effects of glucocorticoid treatment on specific cognitive domains have, however, generated inconsistent findings. There is some evidence demonstrating the negative effects of oral corticosteroid treatment on declarative memory performance (Bender et al., 1991; Suess et al., 1986), and there is also some evidence suggesting that psychosocial factors mediate the relationship between corticosteroid treatment and memory deficits (Bender et al., 1991).

Suess et al. (1986) found that asthmatic children and adolescents aged between 9 and 18 years, on combined theophylline and low-dose prednisone treatment, performed significantly more poorly on visual retention and verbal-associate learning tasks than (a) asthmatic counterparts on theophylline only, and (b) healthy controls. The authors reported that these steroid-induced effects on memory were apparent only a few hours after treatment, but were not sustained 24-48 hours later. Bender et al. (1991) investigated the impact of daily corticosteroid treatment (prednisone) on the memory performance of children with asthma who had been hospitalized due to severe exacerbations of their symptoms. They reported a steroid-specific effect on verbal memory performance on days where the children were on high steroid treatment (M = 61.4 mg, SD = 23.0) versus days where they were on low steroid treatment (M = 6.97, SD = 6.73). Specifically, children tended to perform more poorly on days when they were on high steroid treatment relative to days when they were on low steroid

treatment. This effect was independent of dose-order effects, attention levels, IQ, asthma severity scores, socioeconomic status, sex, and age.

Regarding the mediating role of psychosocial and socioeconomic factors on the relationship between corticosteroids and memory performance, Bender et al. (1991) found that psychosocial adjustment was a strong predictor of verbal memory performance. Children from dysfunctional family environments and/or those displaying problems with conduct and management of emotions appeared most vulnerable to steroid-induced memory impairment.

Asthma and dreaming. The limited literature on dreaming and asthma indicates more frequent reports of nightmares (Klink & Quan, 1987), poor dream recall rates, and shorter dream reports by asthmatics relative to non-asthmatic, healthy controls (Monday et al., 1987; Montplaisir et al., 1983; Nielsen et al., 1997). Some studies have reported that, relative to healthy adults, adult asthmatics experience a high incidence of "white dreams" (Monday et al., 1987; Montplaisir et al., 1983; Nielsen et al., 1997).[5]

The phenomenon of poor dream recall in asthmatic patients has been described as a possible secondary characteristic of asthma, linked to either (a) repression of so-called conflictual material related to physical discomfort during sleep (Montplaisir et al., 1983), (b) the prevalence of a lack of emotional awareness (i.e., alexithymia) amongst asthmatics as a confounding variable (see Nielsen et al., 1997 for an overview), or (c) the effects of hypoxemic symptoms on memory (Montplaisir et al., 1983). However, none of these explanations consider the interplay between disrupted sleep architecture in asthma and sleep-dependent memory consolidation as having the potential for affecting the quality of sleep

[5]The term "white dream" refers to a situation where an individual reports having had an emotionally charged dream but either lacks the words to describe the dream or is unable to clearly remember the dream scenario accompanying these strong emotions (Montplaisir et al., 1983).

mentation generated by asthmatics.[6] The limited findings on the dreaming patterns of asthmatics are discussed below.

Like sleep, dreaming in asthma has been investigated in the context of nocturnal attacks (Malo et al., 1985; Monday et al., 1987; Montplaisir et al., 1983). Montplaisir et al. (1983) found that although there is no difference in the *frequency* of dreams in asthmatics, they appear to struggle to recall the *content* of their dreams. In that study, significantly more asthmatics, relative to healthy controls, met the criteria for "black or white dreams" (Montplaisir et al., 1983, p. 90). In other words, they reported a feeling of coming out of a dream without the ability to recall any content for these dream impressions. Furthermore, Montplaisir et al. (1983) found that the dreams with content reported by the asthmatic participants consisted of significantly fewer, and shorter, sentences relative to those reported by controls. Poor dream recall ability among asthmatics has been reported elsewhere, in studies that have used subjective prospective measures (Nielsen et al., 1997) and objective REM awakenings (Monday et al., 1987; Ouellet et al., 1994).

Monday et al. (1987) tested potential mechanisms underlying the apparent retrieval challenge that asthmatics seem to face when attempting to recall their dreams. One of these potential mechanisms was hypoxemic events during sleep. However, their study failed to demonstrate an association between the presence of hypoxemia and dream recall. Hence, the authors proposed a psychoanalytic explanation instead, suggesting that recall of content is repressed because of the distressing nature of some asthmatic dreams. For example, in psychotherapeutic settings, asthmatics often report dream scenarios centered around choking sensations or feelings of being "strangled" or "drowned", which seem related to the

[6]Interest in the dreams of asthmatics is dated and appears to have given way to interest in other patient populations with affective deficits (e.g., depression and PTSD) as opposed to internal medicine patients. However, asthma as a condition is interesting because it presents with various mutations in circadian biorhythms (e.g., alterations in cortisol secretion patterns, variations in sleep distribution and cohesion) without always being co-morbid with psychiatric disorders that, in and of themselves, modulate dream content.

breathlessness and tight-chestedness they experience during asthma attacks (Levitan, 1983; Warnes, 1976; Warnes & Finkelstein, 1971).

In addition to, or as a result of, their anxieties around nocturnal asthma attacks, many asthmatics tend to develop alexithymia, a syndrome that features a dominant mode of externally-oriented thinking, a tendency towards *pensée operatoire* which is an overemphasis on the minor details of one's life at the expense its central issues, detachment from emotional processes which translates into a lack of awareness of one's emotions and a difficulty in expressing emotions (De Gennaro & Ferrara, 2003; Parker, Bauermann, & Smith, 2000; Taylor, Bagby, & Luminet, 2000). This personality profile has been linked to the process of repression of dream content described above. Individuals with asthma, who often live with the fear of death and the lack of control over vital bodily functions such as breathing, develop alexithymic traits as an adaptive/pathological response to these stressful and perhaps even traumatic experiences (Monday et al., 1987; Nielsen et al., 1997). This proposal is corroborated by evidence showing a high incidence of alexithymia among asthmatics (Feiguine, Hulihan, & Kinsman, 1982).

However, studies investigating the association between asthma and alexithymia (e.g., Malo et al., 1985; Monday et al., 1987; Montplaisir et al., 1983; Nielsen et al., 1997) do not provide systematic comparisons between asthmatic and healthy control participants on the relationship between dreaming and alexithymia. It is therefore not possible, within the context of those investigations, to isolate the relationship between asthma status and dreaming from the relationship between alexithymia and dreaming. Furthermore, those studies have not examined the relationship between general memory performance and integrity and dream recall within the population specifically. Lastly, those investigations focused on how the personality trait may have influenced dream content and recall psychodynamically, but did not consider the relationship between the personality

characteristic and sleep. For instance, Bazydlo, Lumley, and Roehrs (2001) found that alexithymia in healthy adults was related to REM instability, either through fragmentation by stage 1 intrusions or through arousals. The authors theorized that the mechanism of action underlying the relationship between poor dream recall and alexithymia is REM fragmentation.

There appears to be a co-occurrence of differences in the distribution of REM sleep, differences in sleep integrity, and differences in dream recall between individuals with asthma and healthy controls. One proposal linking these differences is that the constant fragmentation of sleep in asthma leaves the dreamer with elusive impressions of mentation and in-between states of wakefulness and sleep. This situation, then, explains the high incidence of white dreams in asthmatics, with or without alexithymia. Furthermore, the specific fragmentation of REM sleep, leading to unstable and poorly-intensified REM sleep, could explain the difficulty asthmatics encounter in developing elaborate dreams, with a strong perceptual quality, that can be remembered adequately.

Rationale, Specific Aims, and Hypotheses

Overall, the aims of this study were to: (a) investigate the relationship between chronic corticosteroid exposure and (i) the operation of different memory subsystems and (ii) endogenous night-time cortisol concentrations, (b) explore the relationships between elevated night-time cortisol and (i) sleep organization and (ii) sleep-dependent memory processes, (c) establish whether the nature and extent of memory consolidation taking place during a particular sleep night predicts the content of dreams on that night, and (d) test the role of cortisol as a mediator in the relationship between dreaming and sleep-dependent memory consolidation.

To explore these associations, I recruited samples of demographically-matched (a) asthmatic adults, (b) healthy controls, and (c) eczema controls. Briefly stated, my design included comparisons of (a) adult asthmatics with healthy adults, (b) asthmatics on corticosteroid treatment with asthmatics not exposed to corticosteroids, (c) asthmatics on high doses of inhaled corticosteroids to those on low-dose treatment regimens, and (d) all corticosteroid-exposed groups to all corticosteroid-free groups.

The sets of hypotheses I tested were the following: The first set of predictions relates to baseline memory performance. I hypothesize that participants suffering from atopic diseases (asthma and eczema) would not perform as well as healthy control participants on memory tasks measuring (a) the encoding and retrieval of verbal episodic and autobiographical memories, (b) short-term auditory attention span, and (c) working memory performance. Furthermore, I predicted that those participants undergoing chronic corticosteroid treatment (i.e., mild and moderate-to-severe asthmatics and eczematics) would perform more poorly than participants not exposed to corticosteroids (untreated asthmatics and healthy controls) on those tasks. Lastly, I predicted that asthmatics with a higher degree of corticosteroid exposure (moderate-to-severe asthmatics) would perform relatively worse than asthmatics with a lower degree of corticosteroid exposure (mild asthmatics). In contrast, I predicted that performance on semantic and procedural memory tasks would not vary according to group membership.

The second set of predictions relates to cortisol. I predicted a significant main effect of group status on average night-time cortisol levels, with the following order of means: Moderate-to-Severe Asthma > Mild Asthma = Eczema Control > Untreated Asthma > Healthy Control. That is to say, I predicted that all participants with asthma, irrespective of their treatment regimen and illness severity profile, would present with levels of night-time cortisol that are elevated relative to those seen in healthy controls. Furthermore, I predicted

that asthmatics on corticosteroid treatment would display more elevated night-time cortisol than asthmatics not treated with any corticosteroids, with the higher exposure group having the most elevated cortisol levels. A related prediction here is that both asthma severity and corticosteroid exposure are positively associated with levels of night-time cortisol.
In addition, I predicted a Group x Time interaction effect. Specifically, regarding cortisol level across an 8-hour sleep observation period, I hypothesized that (a) for healthy controls, it would be low at the beginning of sleep and would rise progressively as the night progresses, whereas (b) for all patients, it would be relatively elevated at the end of the first two sleep cycles, and would then decline gradually until reaching normal early-morning levels by the end of the last sleep cycle preceding awakening.

The third set of hypotheses relates to sleep. Because of the (predicted) cortisol patterns among the patients, I predicted that those participants would experience a relatively poorer quality of sleep, with the following order of means: Healthy Control > Untreated Asthma > Mild Asthma = Eczema Control > Moderate-to-Severe Asthma. Poor quality of sleep was defined as long sleep onset latency, sleep fragmentation (a high percentage of WASO and low sleep efficiency), proportionally more stage 1, less SWS and less REM sleep (Hofman, 1994)

The fourth set of predictions pertains to the relationship between corticosteroids, sleep organization, and sleep-dependent memory consolidation. I predicted that corticosteroid-exposed participants (i.e., participants in the Mild Asthma, Moderate-to-Severe Asthma, and Eczema Control groups) would show no improvement in their morning memory performance (declarative and procedural) relative to that on the previous night. In contrast, I predicted that the performance of non-exposed participants (i.e., those in the Untreated Asthma and Healthy Control groups) would reflect the presumed consolidating effect of sleep on memory (i.e., there would be significant improvements from pre-sleep to post-sleep).

Furthermore, I predicted that the proportion and the distribution of both SWS and REM would mediate the relationship between cortisol level and performance on verbal episodic memory tests, such that elevated cortisol during the night would either suppress these stages of sleep or disrupt their normal circadian organization, or both. In turn, these disruptions would hinder memory performance in the morning. Specifically, I hypothesized that (a) average night-time cortisol will correlate negatively with post-sleep measures of declarative memory, and (b) that the relationship between night-time cortisol and sleep-dependent declarative memory performance will be mediated by SWS and REM sleep.

The fifth set of predictions pertains to the relationship between dreaming and asthma. I predicted that (a) all asthmatic participants would recall fewer dreams. If recalled, I predicted that (b) their dreams would have impoverished textures, as measured by the subjective qualia variables (bizarreness, visual vividness, and emotional intensity) and would be more thought-like in nature. I further predicted that (c) sleep efficiency, percentage REM, and REM intensification would predict dream recall scores, with greater efficiency and intensification being associated with better recall.

Regarding the factors affecting dreaming among the asthmatic participants, I predicted that asthmatics would (d) have higher alexithymia scores, (e) report a greater number of white dreams compared to healthy control participants, and that (i) alexithymia, (ii) the severity of asthma, (iii) corticosteroid exposure, and (iv) post-sleep performance on verbal episodic memory tasks would be inversely associated with the incidence of white dreams.

The sixth set of predictions pertains to the relationship between dreaming and sleep organization. Regarding the relationship between REM distribution and dream content, the normal intensification of REM from early to late sleep would predict the presence of more content, whether episodic content or presence of more unfamiliar and creative elements in

dreams. Regarding the relationship between the distribution of SWS and dream content, more SWS during early sleep relative to late sleep would predict more episodic content. In contrast, the distribution of SWS would not impact on dream content categorized as unfamiliar, novel, and creative.

I predicted that the reduced REM sleep and the differential organization of SWS and REM sleep among asthmatics will result in (a) those participants experiencing less episodic content in dreams, relative to controls, and (b) the absence of a circadian influence on the distribution of dreams. In other words, I predicted that, for asthmatics, *residue of the day* dreams would not be concentrated during the first REM period, *laboratory-related* dreams would not be concentrated during the second REM period, and *idiosyncratic* dreams would not be concentrated during the last REM period. The prediction was that this distribution of dreams would stand in contrast to that of healthy controls.

The seventh set of predictions pertains to the relationship between cortisol, dream content, and sleep organization. Regarding (a) the relationship between cortisol and dream content, I predicted that average night-time cortisol levels would be inversely related to average episodic content but positively related to average originality content. Regarding (b) the relationship between cortisol and sleep distribution, I predicted that elevated night-time cortisol would be inversely related to both SWS distribution and REM intensification scores. Regarding (c) the relationship between sleep organization and the memory content of dreams, I predicted that (i) the normal intensification of REM from early to late sleep would predict more episodic content, as well as the presence of unfamiliar and creative elements in dreams, and (ii) that more SWS during early sleep relative to late sleep would predict more episodic content. I predicted that, in contrast, the distribution of SWS would not impact on originality content. Lastly, I predicted that the intensification of REM sleep and the distribution of SWS would mediate the relationship between cortisol and the episodic content of dreams.

The eighth and final set of predictions pertains to the relationship between cortisol, episodic memory consolidation, and waking inclusions in dreams. I predicted that low average cortisol would be associated with (a) better recall on episodic memory tasks in the morning, and (b) more episodic dream content. I further predicted that (c) an individual's ability to consolidate episodic memories during sleep (measured indirectly through the average amount of episodic content present in his/her dreams) would mediate the relationship between his/her average night-time cortisol level and memory performance post-sleep.

Methods

Design and setting. The study described here was conducted at the Vincent Pallotti Private Hospital sleep laboratory, as part of collaboration between the University of Cape Town's Department of Psychology and the hospital's sleep team. The main predictor variable was group status, with five levels: (a) Mild Asthma, (b) Moderate-to-Severe Asthma, (c) Untreated Asthma, (d) Eczema Control, and (e) Healthy Control. I included an Eczema Control group to account for the possible effects of asthma in and of itself as a chronic disease, and not related to alterations in cortisol levels, on sleep, dreaming, and cognitive function. The standard prescribed and most widely adopted treatment for eczema involves the intermittent and often chronic use topical corticosteroids (Chang, Keen, & Gershwin, 2007; Delescluse & van der Endt, 1996; Hoare, Li Wan Po, & Williams, 2000; Tschen & Bucko, 1998).

The outcome measures can be categorized, broadly, as (a) night-time cortisol, (b) sleep architecture, (c) dream content, and (d) memory performance. With the exception of certain dream variables, all outcome measures were treated as continuous variables.

Participants. I used non-probability, quota sampling to recruit from a population of university students. An advertisement (see Appendix A) was circulated through the university

intranet site, and was posted on faculty and campus library notice boards. The advertisement was posted electronically once every semester for two consecutive years, and hard copies were posted once a month, starting September 2009 and ending July 2011.

Potential participants volunteered by responding to the advertisement. Figure 1 presents a flow chart of participant attrition through the different stages of the study. As the Figure shows, there were 300 respondents. Of that number, 183 were excluded immediately from their email responses to the experimenter because they reported (a) co-morbidity of atopic diseases (i.e., asthma and eczema), (b) having had childhood asthma that had become asymptomatic in adult years, and/or (c) being a smoker. I contacted 117 of the remaining respondents via email or telephone to schedule in-person screening interviews. As the Figure shows, of the 117 potential participants who were screened, 78 met the eligibility criteria described below. Figure 1 also provides details on the exclusion of the other 39 potential participants.

Of the 78 people who met the eligibility criteria, 6 (1 man, 5 women) withdrew their consent for participation 3 days prior to scheduled testing during a routine telephonic reminder. All cited the reason for their withdrawal as being the time commitment the study required of them. In addition, a 21-year-old woman, assigned to the Moderate-to-Severe Asthma group, scored 19 on the Beck Depression Inventory-Second Edition (BDI-II; Beck, Steer, & Brown, 1996) on the night of the experiment. That person's participation was terminated immediately, and she was referred to the university's student counseling service. Hence, 71 participants started the formal experimental procedures. One of them (a 28-year-old woman assigned to the Untreated Asthma group) withdrew from the study before the placement of electrodes as she became anxious about the inconvenience of having electrodes in her hair. Additionally, I excluded from final analyses the data from 2 participants who completed all experimental procedures. One dataset (from a 25-year-old man assigned to the

Healthy Control group) was excluded because the participant did not fall asleep. The other dataset (from a 19-year-old man assigned to the Healthy Control group) was excluded because the participant had a cortisol profile that fell within the Cushingoid range (38.95 nmol/L at the first collection time). One week after testing the participant, I submitted an additional midnight salivary cortisol test to control for assay analysis error; the test results remained within the Cushingoid range (10.43 nmol/L), excluding the possibility of assay error.

Hence, the final sample that provided the data reported and analyzed below consisted of 68 English-speaking participants (36 women, 32 men) between the ages of 18 and 39 years (M = 21.17, SD = 4.11). Group assignment was as follows: Mild Asthma (n = 14), Moderate-to-Severe Asthma (n = 14), Untreated Asthma (n = 14), Eczema Control (n = 13), and Healthy Control (n = 13).

Eligibility criteria. To be assigned to one of the asthma groups or to the Eczema Control group, the individual's condition had to have been physician-diagnosed. Any person diagnosed with both asthma and eczema was excluded from participation. An important inclusion criterion in the recruitment and categorization of asthmatic participants was the stability of the dose of corticosteroids they reported using. That is, a participant was required to have been on the same dose and medication for at least 3 months. Regarding potential Eczema Control participants, they were required to have used corticosteroid treatment within 1 month of study participation. To be assigned to the Healthy Control group, individuals were required to be in good health, to have no history of childhood asthma or eczema, to have no illness known to impact on the HPA-axis, and to have used no corticosteroids within 12 months of study enrolment. All of this information regarding the potential participant's use of corticosteroids and diagnosis of asthma or eczema was obtained via self-report, during the in-person screening. The sub-sections below outline other specific eligibility criteria.

Age. I only recruited participants aged between 18 and 39 years. The reasons for this restriction are that age affects (a) the circadian rhythm of cortisol (Clow, 2004), (b) sleep architecture (Kales et al., 1970), and (c) the pattern, quality, and content of dreams (Foulkes, 1982; Kales et al., 1968). In addition, hippocampal neurodegeneration and loss of function is associated with normal aging (Lupien et al., 1994, 1998; McEwen, 1999).

General intellectual functioning. At in-person screening, I assessed IQ formally to control for any between-subject differences that could influence performance on the administered cognitive tasks. Any potential participant with an IQ score below 85 (i.e., below the norm-defined "average" range) was excluded.

Psychiatric co-morbidity. At in-person screening, I administered psychiatric screening interviews and self-report questionnaires. Any individual experiencing current or chronic psychiatric disturbance associated with altered endogenous cortisol levels (e.g., any affective disorder) was excluded. For instance, depression is related to hypocortisolism, and is frequently co-morbid with chronic illness (see, e.g., Heim et al., 1999).

Exogenous or endogenous factors altering the balance of female reproductive hormones. Potential female participants were excluded if they reported being pregnant during the in-person screening interview, or if they had been using oral or any other form of hormone-based contraceptives. The reason for this exclusion criterion is that certain changes in the balance of female reproductive hormones have a significant impact on cortisol levels (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kuhlmann et al., 2005). Furthermore, female participants were tested during their late luteal phase (days 21 to 27 of a 28-day menstrual cycle; Lenton, Landgren, & Sexton, 1984), when endogenous levels of circulating cortisol in women are comparable to those in men (Kirschbaum et al., 1999). At in-person screening, each female participant was required to report the date of her last menses

in order to establish, roughly, when the next late luteal phase would occur and to thus schedule a date for testing. Women reporting irregular menstrual cycles were excluded.

Other respiratory disorders or illnesses. At in-person screening, potential participants completed a questionnaire detailing their current health status and medical history (see Appendix B). Those who reported current experience of any pulmonary disease other than asthma (e.g., influenza) were excluded from participation. Participants who reported suffering from colds or flu on the night of testing had their testing re-scheduled. This happened on two occasions (one male participant, 21 years of age, from the mild asthma group and one female participant, 18 years of age, from the healthy control group); participants showed symptoms such as sneezing and coughing and reported having a cold upon probing. They were driven back home and testing was postponed. I called them 1 week later to enquire about their health and scheduled testing for the following weekend in the case of the male and for one month later in the case of the female (recall that she needed to be tested during her late luteal phase).

Certain specific, non-steroidal asthma medication. Asthmatic individuals using the leukotriene receptor antagonist Montelukast Sodium, commercialized as *Singulair*®, as part of their treatment regimen were excluded from participation. Post-marketing studies report "bad or vivid dreams", "memory problems", "sleep walking", and "trouble sleeping" as common side effects (*Singulair® patient information*, 2005; p.3). Furthermore, leukotriene receptor antagonists may confound the relationship between inhaled corticosteroids and endogenous cortisol due to their own effects on the HPA-axis (Shader et al., 1999).

Insufficient asthma symptoms. Potential asthma-group participants who did not experience all three cardinal symptoms of asthma (shortness of breath, wheezing, and tight chest) within the year prior to the screening interview were excluded.

Disrupted sleeping patterns. Potential participants who self-reported, at in-person screening, erratic sleeping patterns or sleep difficulties unrelated to nocturnal asthma

symptoms (e.g., difficulty falling asleep, frequent disruptions during sleep, and premature morning awakening), or any formally diagnosed sleep disorder (e.g., sleep apnea), were excluded.

Smoking. Smokers were excluded from this study because smoking results in heightened cortisol stress responses (Badrick, Kirschbaum, & Kumari, 2007) and alters sleep architecture. With specific regard to the latter, smoking delays sleep onset, reduces SWS, increases stage 1 sleep, and reduces total sleep time (Zhang, Samet, Caffo, & Punjabi, 2006).

Materials and instruments. I used the questionnaires, tests, and electrophysiological apparatus described below to gather necessary information across the five stages of the study (screening, preparation for testing, pre-sleep testing, sleep monitoring, and post-sleep testing).

Screening measures. These included a general sociodemographic and health questionnaire, a psychiatric screening interview and self-report depression questionnaire, and a brief measure of general intellectual functioning.

Sociodemographic and health questionnaire. This questionnaire (see Appendix B) gathered general biographical (e.g., age, sex, level of education) and health-related (e.g., presence of any chronic illness, or current pulmonary or respiratory conditions other than asthma) information. It also enquired about the use of psychoactive substances, including tobacco and alcohol. Participants with asthma were asked to provide details about their symptoms and about their asthma medication for the most recent 12-month period. Female participants were asked to report the date of the start of their last period, which is the first day of the menstrual cycle.

The questionnaire also included an asthma severity classification scheme I devised based on the guidelines described by GINA (2012) and by Lalloo et al. (2007). These guidelines facilitate evaluation of the extent of corticosteroid exposure across participants

who use of different forms of inhaled corticosteroids. The guidelines do so by providing a classification system that takes into account the equipotency of different classes of inhaled corticosteroids (Wlodarczyk et al., 2008).

Hence, the classification scheme used here was based on (a) the manifestation of three cardinal symptoms (viz., wheezing, tight chest, and shortness of breath) as well as coughing; although the latter is not essential to the diagnosis of asthma, it features as a widely-accepted symptom that relates to severity, (b) the chronicity of the symptoms, (c) exposure to corticosteroids as form of treatment, and (d) the level of exposure to corticosteroids (type, dose, and frequency).

There are various approaches to evaluating asthma across different clinical institutions and countries (Barnes & Sharp, 1999; Colice, 2004; Lalloo et al., 2007). Furthermore, there is great variability in the implementation of diagnostic guidelines by healthcare providers. For instance, Colice (2004) argues that measures of airway inflammation are more reliable in determining severity than traditional measures focusing on lung function and symptomatology only. I therefore used degree of exposure to corticosteroids, in conjunction with subjective reporting of symptoms, as an indirect measure of airway inflammation and, hence, of severity.

I used the four criteria listed above to generate a combined score classifying participants as experiencing either mild or moderate-to-severe asthma. A severity score was calculated by (a) multiplying the number of symptoms by the frequency at which they were reported (i.e. daily, weekly, monthly, or yearly), (b) multiplying the dose of inhaled corticosteroid taken by the frequency at which it was taken (i.e. daily, weekly, monthly or yearly) and adding scores (a) and (b). The untreated asthma group included participants with varying asthma symptomatology who were not using any form of corticosteroid-based treatment. I did not consider the use of rescue, reliever beta-agonists, or of other non-steroid

medication, in classifying participants. Symptom presentation averaged across 1 year was used to determine chronicity (see Table 1).

Table 1
Combined Classification Scheme for Asthma Severity (N = 41)

	Asthma severity		
Variable	Untreated (n = 10)	Mild (n = 14)	Moderate-to-Severe (n = 13)
Asthma symptoms	Daily to yearly	Daily to yearly	Daily to monthly
Corticosteroid exposure	None	$200 \leq \mu \leq 500$	≥ 500
Severity scores			
Range	3-16	5-14	15-26
M (*SD*)	7.43 (3.65)	10.86 (2.35)	17.46 (2.67)

Note. Doses presented here reflect average daily Budesonide measures only; equivalent doses for Fluticasone are assumed. The variable *Asthma symptoms* refers to the number and chronicity of asthma symptoms reported; the variable *Corticosteroid exposure* refers to the dose, potency and chronicity of corticosteroids used. Severity scores for the untreated asthma group were generated from the asthma symptoms variable only.

Mini International Neuropsychiatric Interview. The MINI (English version 5.0.0; Sheehan et al., 1998) is a brief (approximately 15-min) structured diagnostic interview that assesses the major DSM-IV Axis I psychiatric disorders. It has good psychometric properties, and can be administered by a clinician or by a lay interviewer who has undergone the appropriate training (Sheehan et al., 1998).

Beck Depression Inventory-Second Edition. The BDI-II (Beck et al., 1996) is a standardized 21-item self-report questionnaire that assesses current presence and severity of depression in adults. It is used in clinical settings and as a research tool, and has achieved adequate reliability and validity (Beck et al., 1996; Beck, Steer, & Brown, 2005; Kendall, Hollon, Beck, Hammen, & Ingram, 1987). Potential participants who scored 14 or more (the cut-off score for minimal depression; Beck et al., 1996) were excluded.

Wechsler Abbreviated Scale of Intelligence. The WASI (Wechsler, 1999) is a standardized measure of general intellectual functioning that is used widely in both research and clinical settings (Axelrod, 2002). I used it here to ensure there were no major between-group differences in terms of general cognitive ability.

For the sake of conciseness, given the already extensive and elaborate nature of the 2-hour screening process, I did not administer the entire WASI (i.e., the set of subtests that allow an

estimate of Verbal IQ, alongside Block Design and Matrix Reasoning, to obtain an estimate of FSIQ) so as not to tire the potential participants. I administered the Block Design and Matrix Reasoning subtests of the WASI to measure Performance IQ (PIQ), which is considered a reliable estimate of Full Scale IQ (FSIQ). The test administration manual reports a correlation of .87 between PIQ and FSIQ.

Measures used at pre-sleep testing. These included measures of alexithymia and memory performance.

Toronto Alexithymia Scale. The TAS-20 (Bagby, Parker, & Taylor, 1994) is a standardized self-report questionnaire. The respondent rates each of 20 statements on a 5-point Likert-type scale rating from 1 (*strongly disagree*) to 5 (*strongly agree*). Statements refer to the ability to demonstrate an awareness of feelings, the ability to communicate those feelings to others, the ability to daydream, the prevalence of somatic complaints, and the tendency to focus on external events over inner experiences. The TAS-20 has achieved adequate reliability (internal consistency coefficient, $\alpha = .086$ for all 20 items, based on a sample of 1933 adults) and good construct validity (confirmatory factor analysis, goodness of fit index, $r = .98$; Parker, Taylor, & Bagby, 2003). Several studies (e.g., Brown, Fukuhara, & Feiguine, 1981; Chugg, Barton, Antic, & Crockett, 2009; Feldman, Lehrer, & Hochron, 2002; Montplaisir et al., XX; Nielsen et al., 1996; Serrano et al., 2006) have used the TAS-20 to investigate the relationship between asthma and alexithymia, and one study (Nielsen et al., 1997) has used it in an attempt to investigate the role of alexithymia in explaining poor dream recall by asthmatics.

Verbal Paired Associates. I used Uttl, Graf, and Richter's (2002) 15-item verbal paired associates test (VPA-15) to assess retrieval and consolidation (following sleep) of episodic memory material. This test is a variant of the VPA subtest of the Wechsler Memory Scale-Third Edition (WMS-III; Wechsler, 1997). The list of 15 word pairs contains four

related/easy pairs (e.g., *fruit-apple*) and 11 unrelated/difficult pairs (e.g., *bank-milk*). The four related/easy word pairs and four of the unrelated/difficult pairs are taken from the WMS-Revised (Wechsler, 1987). I chose the VPA-15 over the WMS-III VPA because the former produces fewer ceiling effects (Uttl et al., 2002).

In the VPA-15, participants are presented with a series of paired words and are instructed to memorize them. The words are read aloud by the experimenter, one pair at a time, with 3 seconds between each pair. Thereafter, the experimenter reads the first word of each pair and asks the participant to recall its partner. The first section of the test (VPA I) features two such trials, one administered immediately after the other. On each learning trial, the list is presented using the relevant study order provided by Uttl et al. (2002, p. 573). At each cued recall, the order in which cues are presented is random (and again based on Uttl et al., 2002). On each trial, the study order differed from the recall order.

WMS-III Logical Memory subtest. This subtest provided another means to assess retrieval and consolidation (following sleep) of episodic memory material. Here, the examiner reads two stories to the participant. Story A is read once and Story B twice. After each reading, the examiner asks the participant to give an account of all the elements that s/he can remember from the story, in as much detail as possible. Lezak describes the test as being "the purest measure of episodic memory compared to a word list learning task and a visuospatial task because of its relatively low association with non memory measures" (p. 447). It has good test-retest reliability (.88 for LMI and .79 for LMII, averaged across all age groups; Wechsler, 1997) and convergent validity as measured by its moderate-to-strong, positive correlations with tests measures of similar constructs such as with the WAIS III verbal IQ (VIQ) and full scale IQ (FSIQ), with correlation coefficient estimates ranging between .54 and .60.(Wechsler, 1997) and other learning tests such as the California Verbal

Learning Test (CVLT-9) with significant ($p < .0001$) correlation coefficients ranging between .35 and .49 (Woodard, Goldstein, Roberts, & McGuire, 1999).

Autobiographical Memory Test. The AMT (Williams & Broadbent, 1986) assesses participants' ability to retrieve specific autobiographical memories in response to a series of cue words with different emotional valences (positive, negative, and neutral). The test has been assessed for construct validity, which proved to be high with the confirmatory factor analyses revealing comparative fit index (CIF) values ranging between .96 and 1.0, χ^2 = NS, indicating that all of the word cues together effectively measure a single construct, that is the ability to retrieve specific autobiographical memory (Griffith et al., 2009).

The version of the AMT used here drew closely on the original AMT paradigm in terms of the number and types of words used. Hence, I used a total of 15 cue words: 5 positively-valenced, 5 negatively-valenced, and 5 neutral. The cue words were selected from a sample used by Brittlebank, Scott, Williams, & Ferrier (1993), and were matched for frequency of occurrence using Kucera-Francis (1967) ratings. In addition, a review of studies by William et al. (1996) on the effect of imageability on the specificity of autobiographical memory revealed that words that are more effective in provoking mental images help generate more specific memories. Therefore, the positive and negative words were also chosen on the basis of their high emotionality ratings (Brittlebank et al., 1993). The positive words were *happy, relieved, joy, devoted,* and *tender*. The negative words were *failure, guilty, hopeless, rejected,* and *sad*. The neutral words were *rhythm, shoes, tree, uncle,* and *library*.

WAIS-III Information subtest. This subtest from the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III; Wechsler, 1997) assessed semantic memory. The subtest consists of 28 questions which are aimed at evaluating an individual's general knowledge in areas such as art, science, and history, and consequently their ability to memorize facts, their

level of engagement with environment and culture, their intellectual curiosity, mastery of mother tongue and familiarity with other languages, and the quality of the education they received. The test has an adequate degree of construct validity in its ability to assess semantic memory (Kaufman & Lichtenberger, 2005). Like most of the WAIS III subtests, the internal consistency of the test (split-half analysis) is estimated to lie between .90 and .91 depending on the age group (ages 10 to 44 years considered), the average stability of the test for all age groups (test-retest reliability) is .94. Information correlates highly with the factor "verbal comprehension" and a "*g*" factor (general intelligence), with coefficients .81 and .75, respectively (Wechsler, 1997).

Boston Naming Test. The second edition of the BNT (Kaplan, Goodglass & Weintraub, 2001) also assessed semantic memory. The BNT tests the participant's knowledge of names for commonly as well as rarely encountered objects. Picture naming tests are commonly used to effectively test the integrity of semantic memory among patients with dementia of the Alzheimer's type (Chertkow & Bub, 1990). In their review, Strauss, Sherman, and Spreen (2006) report that the BNT has good internal consistency (σ coefficient value between .78 and .96). Despite the effects of age, IQ, and English language proficiency on performance, the BNT has good criterion validity as a naming test (e.g. $.76 \leq r \leq .86$ between BNT and the Visual Naming Test of the Multilingual Aphasia Examination).
The test consists of 60 line drawings of objects, presented one by one to the participant. The pictures are arranged in order of familiarity and difficulty, with the least frequently encountered objects presented last. At first, the participant is required to name to picture without any cueing. If s/he fails to do so, a semantic cue is provided. If the participant still struggles to retrieve the name of the object, then a phonetic cue is offered. Lastly, if the participant fails to retrieve the name upon cueing then the interviewer presents the participant with a choice of four names, both verbally and visually, including the correct one. A point is

awarded for correct answers given spontaneously and for answers provided after semantic cuing.

WAIS-III Digit Span subtest. This WAIS-III subtest assessed short-term auditory attention span and working memory (reliability: test-retest: .83, split-half: .90; specificity of variance: 50%; Kaufman & Lichtenberger, 2005). The forward component of the test consists of sequences two to eight digits long, while the backward component is two to seven digits long. Sequences are presented in ascending order of length; each is presented twice and verbally. For the forward component of the test, the patient/participant is required to repeat the sequence of digits verbatim, immediately after presentation. For the backward component, s/he is required to do some mental double-tracking, which is to hold the sequence in mind while reversing the order of the digits. Forward digit span is a test of attention span, whereas the backward digit span taps into working memory. Performance on the former component is a relatively stable in healthy adults; sub-optimal performance is often indicative of left-hemisphere damage or visual field defects. The latter component is more sensitive to diffuse damage as encountered in neurodegenerative conditions such as Alzheimer's disease (Lezak, 2004).

Finger-Tapping Task. The FTT (Walker et al., 2002) is a procedural motor-skill computer-based task, programmed using E-Prime software (version 1.1, 2002). Following previous studies (Kuriyama, Stickgold, & Walker, 2004; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002; Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005), I used the FTT to assess the effects of sleep on procedural memory performance. Participants are required to type a 5-digit sequence using their non-dominant hand. The sequence, which features single digits that are not in numeric order, appears on top of the computer monitor at all times to avoid reliance on working memory. Participants are instructed to type the

sequence repeatedly, as accurately and as quickly as possible, for 30 seconds. Here, I administered 12 such trials, with a 30-second fixation period separating each from the next.

Apparatus used to monitor sleep, measure cortisol, and collect dream reports. The study took place at the Vincent Pallotti Private Hospital's Sleep Disorder Centre. The sleep laboratory consists of three rooms: a monitoring room flanked by two patient rooms. The latter are both fully partitioned from the monitoring room with ground-to-ceiling concrete walls. Each partition contains a 700 x 500 mm glass pane through which researchers, seated in the monitoring room, can observe the patient/participant. Visibility and lighting from one room to the other is controlled by blinds on each side.

Monitoring sleep. The sleep laboratory uses the NeuroFax EEG9000 32-channel polysomnograph (PSG) from Nihon Kohden, and the Polysmith Online version 6 software for staging. I used a bipolar EEG montage, recording from left and right central, frontal, parietal, and occipital sites using a recommended derivation of adequate sleep staging montage (F3-C3, C3-P3, P3-O1, F4-C4, C4-P4, P4-O2), horizontal and vertical EOG, EMG from chin electrodes, and ECG (Terzano et al., 2001), with standardized filters for recording to ensure good signal integrity from individual channels: 0.5-35 Hz for the EEG and EOG leads, 10-70 Hz for the EMG leads, and 1-70 Hz for the ECG leads. This particular montage was selected as it best fit the demands of the different sleep studies run by our research laboratory, requiring analysis of subsidiary processes underlying REM and non-REM sleep. Electrodes were placed according to the International 10-20 System (Jasper, 1958). Sleep stages N1, N2, N3, and R were classified in 30-s epochs according to AASM guidelines (Berry et al., 2012). Signals were verified by performing a standard biocalibration procedure on every test night (Rechtschaffen & Kales, 1968).

I used online visual scoring in real time to identify the first two REM sleep cycles. Each participant was woken up twice, after approximately 2.5 minutes into clearly

demarcated REM sleep. During the awakenings (timed period of ≤ 10 min), I went into the patient rooms and interviewed participants about dreaming, recorded their responses, asked them to fill out a brief questionnaire if they reported having been dreaming, and collected cortisol samples.

Collecting salivary cortisol. Salivary cortisol is a reliable estimate of free circulating cortisol, and can be measured non-invasively (Hucklebridge et al., 2000; Kirschbaum & Hellhammer, 1994; Wlodarczyk et al., 2008). There are three main means of collecting saliva samples: (1) passive collection into a sterile container, (2) Salivette devices (Sarstedt, Inc., Rommelsdorf, Germany), and (3) Sorbette devices® (‘eyespear’; Salimetrics LLC, Pennsylvania, USA), which consists of a cellulose-cotton tip on a plastic stick. I deemed Salivettes the safer instrument for collection of salivary cortisol in the present study. The basis of this choice is described in Appendix C.

Collecting dream reports. If a dream was recalled, I ensured that the process of collection was as brief as possible. I aimed to have lights off within approximately 5 minutes following awakening. Although long awakening periods lead to unnecessary NREM sleep deprivation, brief, infrequent awakenings do not disrupt the general architecture of sleep; sleep regulatory processes appear to be relatively robust (Endo et al., 1998; Grözinger, Kögel, & Röschke, 2002).

I first collected open-ended, verbal dream reports which I audio-recorded using the Edirol R-09 24bit Wave/MP3 digital voice recorder by ROLAND, a portable stereo recording device that utilizes an SD memory card as a recording medium and is USB downloadable. These dream reports were later transcribed by two psychology postgraduate, male research assistants who were blind to the identity of the participants and to the aim of the experiment. Then, I asked the participants to fill out a dream inventory which I had devised (see Appendix D). Its purpose was to allow participants to provide subjective evaluations on

various aspects of the dream they had just reported. I verbally provided the definition of each variable to the participants before they filled out the dream inventory to ensure that participants understood how to interpret terms contained in it, providing them with examples, and instructing them on how to rate each variable using the numerical scale of 0 to 10.

The inventory incorporated both (a) parameters describing the qualia or texture of dreams, and (b) ratings of the memory sources of dreams. Specifically, the dream inventory facilitated the subjective evaluation of these six dream outcome variables: (a) *episodic content*, that is, the presence of familiar people, places, and events in a dream; (b) *original content*, that is, the presence of unfamiliar people, places, and events in a dream; (c) *bizarreness*, that is, the extent to which the dream scenario was implausible by waking standards; (d) *visual imagery*, that is, the degree to which visual elements were vivid in the dream; (e) *thought-like*, that is, the degree to which the dreamer could recall thought processes as being part of the dream, either through him/her thinking during the dream or if the dream felt more like a thought than a visual scenario; and (f) *emotional intensity*, that is, the degree to which the dream triggered emotions.

Procedure. As noted above, the study proceeded across five stages: screening, preparation for the sleep night, pre-sleep testing, sleep monitoring, and post-sleep testing. Before these study procedures began, I contacted potential participants via email and telephone. If they met basic requirements (e.g., had asthma or eczema or were healthy with no history of any chronic disease, were non-smokers, did not take any form of hormonal contraceptives, and had no sleep complaints), I scheduled an in-person screening session at their convenience. The one-on-one screening sessions took place in an office in the UCT Department of Psychology. Figure 1 shows the structure of the experimental protocol.

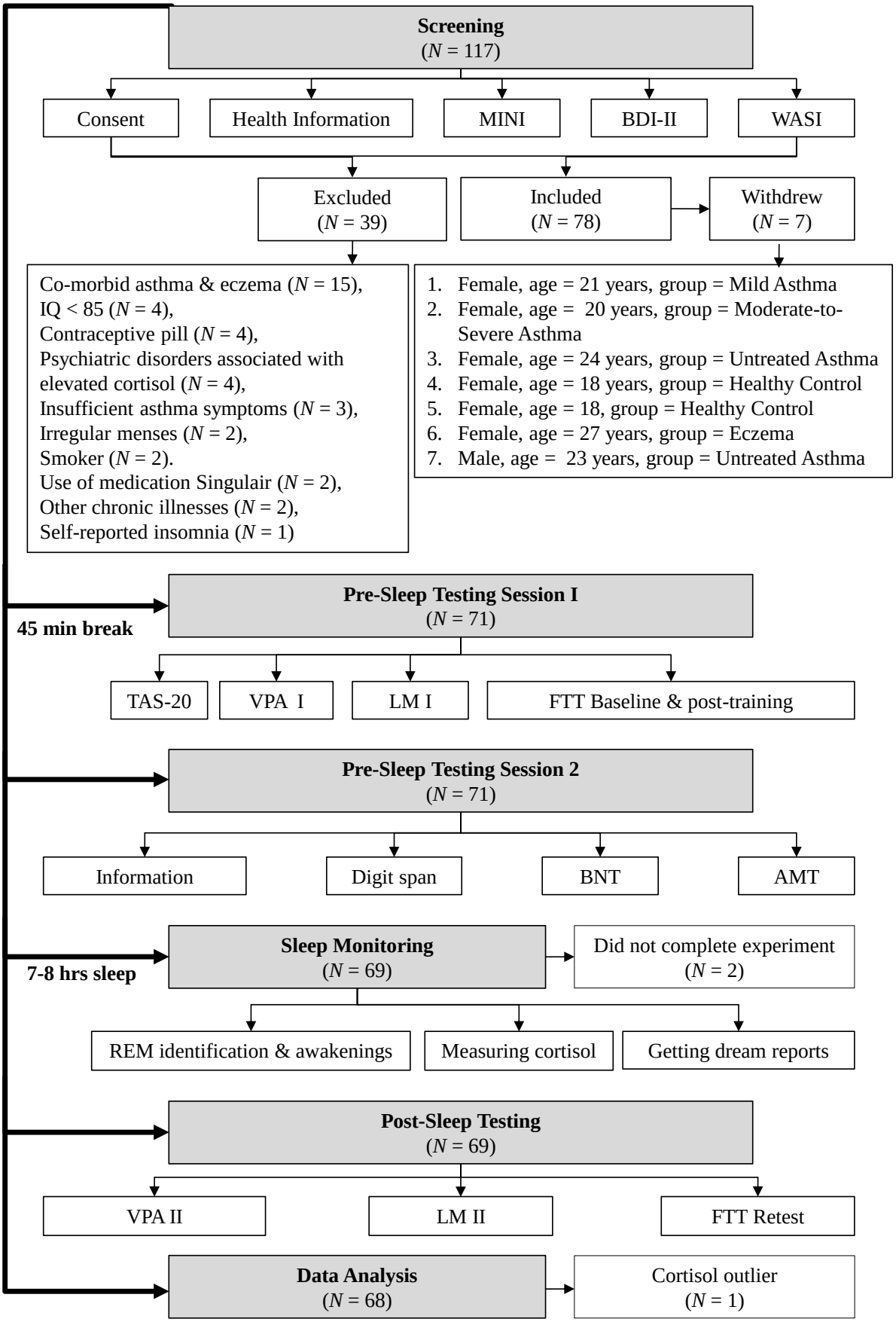

Figure 1. Diagram showing the experimental procedure, and participant attrition.

Stage 1: Screening. I established the eligibility of each potential participant by conducting a 90-minute in-person screening session. Immediately upon arrival, participants read and signed an informed consent document (see Appendix E). They then completed the sociodemographic and health information questionnaire (see Appendix B) and a medical indemnity form (see Appendix F). I then administered, in order, the MINI, the BDI-II, and the WASI subtests.

If the participant met the eligibility criteria held within these screening instruments, I arranged a date for sleep testing. Male participants were scheduled for testing within 1 week of the date of the screening session. Regarding female participants, I calculated their next luteal phase from the date of their last period, which they reported on the health information questionnaire. I then scheduled testing within 3 days from the beginning of their anticipated luteal phase. Three days before testing and on the day of testing, I contacted female participants to ensure that they had not menstruated before the predicted date; if they did, I postponed testing for the following month. This happened twice: One woman, in the Health Control group, was aged 18 years, and the other, in the Untreated Asthma group, was aged 19 years).

At the end of the screening session, participants were asked to keep to a consistent bedtime schedule (i.e., to be asleep before midnight), and to get a minimum of 7 hours of sleep for at least 3 days leading up to testing. (This requested was not verified). I made this request because sleep deprivation on one night can result in elevated cortisol levels on the following night (Leproult, Copinschi, Buxton, & van Cauter, 1997). Other instructions included being asked to, on the day of testing, carry out routine daily activities and to not nap during the day. Daytime naps can delay sleep onset (Ancoli-Israel & Martin, 2006). Participants were also instructed to abstain from alcohol consumption for at least 24 hours, and from caffeine for at least 7 hours, prior to sleep testing. Alcohol and caffeine

consumption lead to elevated cortisol levels (Lovallo et al., 2000, 2005). I called participants 3 days before and on the day of testing to remind them of these instructions. When participants mentioned having been sleep deprived because of their university work, I rescheduled their testing. This situation arose three times. These participants included three male students, two from the Healthy Control group (ages = 22 and 24 years, respectively) and one from the Moderate-to-Severe group (age = 18 years).

Stage 2: Preparation for the sleep night. Participants arrived at the sleep laboratory between 19h30 and 20h00. A single participant was tested on each test night, and each participant spent a single night at the sleep laboratory.

Participants were not allowed to consume any foods or beverages (except water) once the experiment had commenced. They were also made to brush their teeth at least 2 hours prior to scheduled bedtime, in order to avoid micro-vascular leakage into the saliva samples. As an added precaution, they were requested to rinse their mouth thoroughly after brushing their teeth.

Participants in the asthma groups brought along their usual asthma medication (inhaler and/or cortisol tablet and antihistamine), as well as emergency medication (asthma reliever or rescue inhaler). They kept these by their bedside at all times. The experimenter also kept emergency medication (Salbutamol beta-agonist, marketed as Ventolin®) as a precautionary measure.

Shortly after arrival at the sleep laboratory, the participant filled out the BDI-II questionnaire. I repeated administration of the BDI-II on the night of testing because sometimes (especially in the case of female participants) there was a gap of several weeks between the date of screening and the date of testing, and the BDI-II measures current depression within a 2-week time frame. Hence, the repeat administration on the night of sleep testing sought to exclude the possibility of current depression; if the participant scored 19

points or higher, testing was cancelled and the participant excluded. This event happened once, in the case of a 19-year-old woman assigned to the Eczema Control group, during the course of the study.

Stage 3: Pre-sleep testing. This stage of the procedure was divided into two sessions. During the first session, I administered, in order, the TAS-20, VPA I, LM I, and the FTT. This session lasted approximately 15 minutes. The participant was then left alone to settle in the bedroom, and the second session resumed 1 hour after the beginning of the first. During the second session, I administered, in order, the WAIS-III Information and Digit Span subtests, the BNT, and the AMT.[7]

Stage 4: Sleep monitoring. Below I detail the separate aspects of this stage of the study procedure, including participant preparation and REM awakenings.

Participant preparation. At the conclusion of Stage 3, I prepared participants for sleep monitoring. Participants slept for 7-8 hours (lights out was between 22h30 and 23h30, and waking happened between 06h30 and 07h30). Before turning the lights off, I warned participants that there would be two REM awakenings during the night; I did so because abrupt and unexpected awakenings have been reported to cause greater cortisol responses than expected awakenings (Hucklebridge, Clow, Rahman, & Evans, 1999).

REM awakenings. Participants were awakened after identification of the first REM period (REM1). This awakening typically happened between 60 and 90 minutes after sleep onset. I entered the patient room and called out to the participant using his/her first name. I then switched on a dim, yellow light (<5 lx) that was just bright enough to enable visibility during the saliva collection procedure. Clow (2004) reports that bright lighting causes a rapid and sharp cortisol surge similar to the morning acrophase; the aim of the REM awakenings in

[7]Stage 3 was divided into two sessions for the sake of consistency with Study 2, which measured the effects of acute exposure to corticosteroids.

this study was to measure cortisol at the level at which it occurs during sleep. On signs of awakening, I inserted a Salivette into the participant's mouth and asked him/her to chew on the cotton swab for 1 minute. I timed the procedure, recorded the exact time at which the sample was collected, and stored the Salivette in a nearby freezer.

Shortly after saliva collection, I instructed the participant to provide me with a detailed account of anything that had been going through their mind before I awoke them. If they had something to report, I first asked them to sit up. Then, I switched on an audio-recording device and recorded their account, verbatim, without interrupting, prompting them for clarification only when their voice was not audible. Lastly, I asked participants to fill out the dream inventory. If no dream was recalled, I switched the lights off and allowed the participant to go back to sleep immediately.

This awakening procedure was repeated once more during the course of the night (i.e., at REM2, between 3 and 3.5 hours after sleep onset). Finally, participants were awakened after 7-8 hours of sleep recording, again during a REM period (REM3). The second and third dream reports were collected following the same procedure as at the REM1 awakening.
The last saliva sample was collected when the participant was awakened at the end of the sleep recording. The sample was taken within 10 minutes of awakening so as to avoid measuring the morning cortisol acrophase, which occurs between 15 and 60 minutes post-awakening in healthy adults (Clow, 2004; Wust et al., 2000).
I concluded the sleep testing stage by removing the electrodes and instructing the participant to wash, dress, and join me in the monitoring room.

Stage 5: Post-sleep testing. In the monitoring room, the participant completed the (a) VPA II, a single cued-recall trial of the VPA-15, with the list of cue words presented in an order different to those presented at VPA I, (b) the LM II, the delayed free recall trials for

each Logical Memory story and (c) a three-trial version of the FTT. The study protocol ended at this point. The participant was compensated for his/her time, debriefed, and dismissed.

Ethical considerations. All study procedures were approved by the Research Ethics Committees of the University of Cape Town's Department of Psychology and Faculty of Health Sciences.

Informed consent, voluntary participation, and deception. Participants were provided with elaborate written and verbal information about the study and gave their informed consent before being formally enrolled into the study. The information supplied included details about the study procedures, its risks and benefits, assurance that the tests would not harm them in any way, and that they would be compensated for their time. Additionally, the consent form (see Appendix E) secured their right to withdraw their participation at any stage of the study, without penalty.

Participants were informed that the aim of the study was to investigate the quality of learning and sleep in adult asthma; the specific hypotheses of the study were not disclosed to them. Participants were debriefed fully after study completion.

Risks and benefits. Although participants did not benefit directly from the study, they were given information on the relationship between asthma, asthma management, and sleep and learning. Participants were given a copy of their hypnograms and a brief presentation on sleep architecture. Additionally, they were compensated with R150 for their time, and students enrolled in Psychology courses were awarded course credit.

Participant safety protocol. A medical indemnity form (see Appendix F) provided participants with the assurance that the researcher and her affiliated research team would take responsibility for their safety and wellbeing for the duration of the study. All necessary precautions were taken and a safety protocol was put in place, with the collaboration of the

Vincent Pallotti Private Hospital nursing staff and of their emergency department, to ensure that participants could be assisted adequately in the event of an asthma attack.

My aim was to conduct the study within an environment fully equipped to carry out rescue interventions in the event of a participant's asthma exacerbation. The hospital sleep laboratory is equipped with an emergency panic button that served to alert the experimenter and the nurses on duty to a participant's needs in case of distress. The 24-hour emergency service in place at the hospital was made available to the research team, at their cost, in case of medical emergency. The evening nurses' station was situated about 5 meters away from the sleep laboratory. The team at that station consisted of staff trained and ready for emergency interventions. Furthermore, it is important to note that the sleep laboratory specializes in dealing with sleep apnea patients. It therefore had all the procedures and equipment in place to relieve patients from breathing difficulties.

Finally, the entire research team was obligated to undergo Basic Life Skills (BLS) for Health Care Providers training. This training included sessions on cardiopulmonary resuscitation (CPR). The training program is an accredited course (American Heart Association, 2005) coordinated by the hospital, and is generally offered to resident staff members. No participant experienced asthma attacks or any other medical distress during the course of the experiment.

Data Management and Statistical Analyses

This section consists of two parts: The first part, *deriving predictor and outcome variables*, describes the operationalization of the four groups of outcome variables (memory, cortisol, sleep and dreaming) whose predictor variable was asthma group status. The memory, cortisol, sleep variables also acted as predictor variables to one another and to the dreaming outcome variables. The second part of this section, *statistical analyses*, describes

the analytical approach used to investigate the relationships between the predictor and the outcome variables.

Deriving predictor and outcome variables.

Variables assessing memory and attention performance. The various cognitive tests described below assessed verbal episodic memory, autobiographical memory, semantic memory, procedural memory, working memory, and short-term auditory attention span.

Verbal episodic memory. I measured immediate cued recall (VPAI) versus sleep-delay cued recall (VPAII) using the VPA-15 word list and immediate free recall (LMI) and sleep-delay free recall (LMII) using the LM short story recall test.

Cued recall. I provided the first word of each word pair and the participant had to recall the second word. I derived a recall score based on the number of correct responses the participant provided. There were 15 word pairs in the list, with 1 point allocated for each word that was correctly paired, resulting in a maximum score of 15 points. I scored VPAI (immediate recall) and VPAII (delayed recall) performances in the same manner

For immediate recall, I read the list of word-pairs twice to the participant while I presented the list only once to test delayed recall. However, the first trial of the VPAI was considered a practice session, and hence the score on that trial was not considered in the final data analysis. Instead, the score on the second trial alone constituted the VPAI score. The difference in scores between the 2 trials provides a measure of encoding efficiency.
The difference in scores between VPAI and VPAII, calculated by subtracting VPAI scores from VPAII scores, dividing the difference in scores by VPAI scores and multiplying by 100, constituted the VPA Retention score. It measured the percentage retention (improvement or deterioration in recall) between the immediate and the delayed recall sessions.

Free recall. To measure performance on the LM test, I allocated one point to each unit of information (as pre-determined on the score sheet) that the participant successfully

recalled about Story A and then Story B. LMI (immediate recall) scores consisted of the total number of story units remembered from the reading of Story A, the first reading of Story B, and the second reading of Story B.

Autobiographical memory. Two independent trained raters (me and a research assistant) evaluated the audio-recorded memories retrieved by the participants and were both blind to the identity and group status of the participants. The research assistant in question was a female postgraduate student, member of the UCT Sleep Research team. There was excellent inter-rater reliability, as measured by a two-way ANOVA (N = 24 sets of 15 words), mixed effects model Intraclass Correlation Coefficient (ICC) test, with single measures Cronbach α = .89 and the average measures α coefficient being = .94, p< .001.

On presentation of each word cue, participants had 30 seconds to retrieve an autobiographical memory relating to that word. I allocated a score of 2 to each memory categorized as "specific" and 0 to a failure to report a memory associated to the word cue presented or any response categorized as a "categorical" or an "extended" memory or a "semantic associate" as opposed to a specific event involving a personal experience. Upon failure to retrieve a specific memory, I reiterated the instructions to participants and they were given a second opportunity. I allocated a score of 1 to success on the second 30-second trial. There were 15 word cues and hence a maximum possible score of 30 points.

Semantic memory. Two test scores measured semantic memory: the number of pictures correctly identified by their proper names, without semantic or phonetic cueing, on the BNT, and the number of questions correctly answered on the Information subtest of WAIS-III.

Procedural memory. The FTT included three measures: (a) baseline (the first of 12 trials), (b) performance after training (average of last 3 of 12 trials), and (c) performance after sleep (average of 3 trials). For each of the three, I derived an accuracy score (number of

errors made per sequence divided by the total number of sequences typed, multiplied by 100) and a speed score (absolute number of completed sequences).

Working memory. I assessed working memory by using the backward component of the Digit Span test and score as the number of sequences correctly reversed (min = 0, max = 10).

Short-term auditory attention span. Short-term auditory attention was assessed through the forward component of the Digit Span test and scored as the number of sequences repeated correctly (min = 0, max = 12).

Cortisol. I collected three salivary samples during the course of the sleep study, one at the beginning of each awakening (i.e., at REM1, REM2, and REM3). I averaged the total volume of cortisol obtained for each participant to generate an average night-time cortisol level.

Three participants had only two cortisol data points, and therefore their cortisol data were excluded from all cortisol-related analyses. In the case of the first participant (an 18-year-old man in the Moderate-to-Severe Asthma group), the experimenter could not reliably identify REM sleep until 03h52, and hence performed only two awakenings. The second of those participants (a 22-year-old man in the Moderate-to-Severe Asthma group) had a very long REM latency (294.50 minutes after sleep onset), and thus only two REM awakenings were performed for him. The third of these participants (a 20-year-old man in the Mild Asthma group) provided insufficient saliva for cortisol assay on the second of his three awakenings.

Sleep architecture. The sleep quality variables analyzed in this study were *sleep onset latency* (*SO*; the number of minutes from Lights Off to the apparition of the first 30-minute epoch of any sleep stage), *REM latency* (the number of minutes from SO to the first 30-second epoch of REM sleep), the percentages of the following stages of sleep relative to the

total amount of recorded sleep: *slow wave sleep* (*SWS*); *rapid eye movement* (*REM*) *sleep*, *stage 1* and *stage 2* sleep; the percentage of *wake after sleep onset* (WASO) relative to the total amount of sleep, and *sleep efficiency* (*SE*), which is the proportion of total sleep measured relative to the total time spent in bed.

I scored the eight sleep variables according to the criteria set out by the AASM (2007). All sleep records were assigned a code reflecting the order in which each participant was tested. Jan Top (JT), the resident sleep technologist of the Panorama MediClinic, and Marlene Gounder (MG), the sleep technologist at the Vincent Pallotti Hospital Sleep Disorder Centre, provided comprehensive training on sleep testing and scoring to all the members of the UCT Sleep Research Team, including me, as part of a collaborative effort between the laboratories. JT and MG also validated 25% of the records I scored to ensure reliable scoring of the data. All scorers were blind to the participants' group memberships. Agreement is based on epoch-by-epoch comparison performed by the *Polysmith* analysis and scoring software version 6.0, which converts Cohen's kappa (*k*) values into percentages. I achieved an inter-rater reliability of 85.06% ($k = .79$) and 87.27% ($k = .81$) with JT and MG, respectively. According to Landis and Koch's (1977) benchmarks for the evaluation of *k*, these values reflect substantial ($.61 \leq k \leq .80$) and almost perfect ($.81 \leq k \leq 1.00$) inter-rater reliability, respectively. Furthermore, a study conducted by the European Sleep Research Society measuring the inter-rater reliability for sleep stage scoring revealed that the new AASM scoring rules generated an 82% (or $k = .76$) rate of agreement among expert raters (Danker-Hopfe et al., 2009). Taken together, these criteria indicate that I achieved at least adequate inter-rater reliability with the two other scorers.

I took two steps to test differences in the circadian distribution of sleep across the five groups: First, I measured the distribution of SWS and REM sleep by dividing sleep into two distinct halves, comparing the first half (Early Sleep) with the second (Late Sleep). I defined

Early Sleep as the period starting from SO to the half-way cut-off point; I defined Late Sleep as the period starting from the half-way cut-off point to when lights were switched on in the morning.

Second, I defined the difference between Early and Late REM sleep as *REM Intensification* (percentage REM during Late Sleep minus percentage REM during Early Sleep) and the difference between Early and Late SWS as *SWS Distribution* (percentage SWS during Early sleep minus percentage SWS during Late sleep).

Dreaming. The sections below describe the objective and subjective measurement of the episodic memory content outcome variable, the objectively-evaluated dream categories as well as dream themes, and lastly, the subjectively-rated dream qualia variables including bizarreness, vividness of imagery, emotional intensity and thought-like quality.

Two members of the UCT Sleep Research team (both female postgraduate students) and I rated the dream reports using transcripts of the audio-taped dream reports. We were blind to the source of the dream reports; these had been coded numerically by an independent research assistant. Raters scored the reports for their episodic content, recall category, and theme cluster, independently, after establishing set parameters for analysis. The three raters scored the same 25% (N = 74) of all the dream reports (Study 1 and Study 2 combined) for inter-rater consistency analysis. Inter-rater reliability, as measured by a mixed effects model Intraclass Correlation Coefficient (ICC) test, was excellent with single measures Cronbach's α = .92 and the average measures α = .97. The remaining reports (N = 222) were divided equally among the three researchers.

Objective episodic content score. The presence of episodic content was determined by noting if the participant mentioned in his/her report that the dream scenario related to a past or ongoing experience, or if s/he made clear references to information encountered in the context of the experiment prior to sleeping (e.g., the stories or words that s/he had been asked

to memorize). It is important to note, however, that episodic content hardly appears in isolation of semantic content; nonetheless, the aim here was to specifically identify autobiographical elements within the dream scenario. See Appendix D for the coding schedule of dream variables.

In order to do so, raters had to give a score of 0 to 10 for references to (a) characters familiar to the dreamer, (b) places recognized from waking reality, and (c) the inclusion of waking events into the dream. The aggregate of those three scores constituted the objective episodic score. A rating of 0 indicated that element was absent, scores from 1-5 indicated that the element rated was somewhat present, and a score between 6 and10 indicated that the element in question was present to a remarkable degree. In addition, the raters used N/A for instances where it was not possible to rate the familiarity of an element objectively, due to the lack information contained within the dream report. N/A was assigned a negative score (-1) to distinguish it from "0" which signifies the opposite of "familiar" in this context.

Objectively rated dream recall category. The raters evaluated each dream report for the type of recall it represented. They were instructed to classify each report as either: (a) an instance where the participant reported not having dreamt or not recalling any dreams (*No Recall*; assigned a score of 0), (b) a report where the participant was convinced that s/he had dreamt but could not recall the specific contents of the dream (*White Dreams*; assigned a score of 1), or (c) a report that featured dreams with recall of specific content (assigned a score of 2).

The scores on each participant's three dream reports were totaled, and the sum was designated as the Total Recall Score. Here, the minimum possible score was 0 (indicating a situation where no dreams were recalled at each awakening) and the maximum possible score was 6 (indicating a situation where dreaming with recall of specific content was present at all three awakenings).

Objectively-rated dream themes. The dreams with content were subsequently divided into three broad thematic categories: (a) *residue of the day,* referring to dreams containing pieces of current events and current preoccupations, (b) *laboratory-related dreams*, referring to clear dream narratives involving the experimental situation, and (c) *idiosyncratic dreams*, referring to intricate dream narratives with vivid visual imagery that the participant could not trace to any personal experience. In healthy adult dreams, the distribution of these themes follows a circadian pattern; the first type is typically encountered during the first REM stage, the second type is typically encountered during subsequent REM stages of the first half of the night, and the third type is typically encountered during the last REM stages of the night.

Subjective evaluations of dreams. I applied the same scoring approach I used to calculate the objective episodic score to determine the subjective episodic score. I aggregated the points from the first four items (familiar person, familiar place, familiar experience-current, familiar experience- past) of the inventory to generate an episodic score out of 10 points. The rest of the inventory consisted of four items that each measured one of the four qualia variables mentioned previously (bizarreness, visual vividness, emotional intensity, and thought-like nature). Each item generated a score between 0 and 10 and whichever score the participant allocated to the qualia variable was retained for analysis as indicating the degree to which that variable was present in the dream.

Statistical analyses. All analyses were performed using SPSS. The analysis began with an exploration of the data and the testing of assumptions that underlie inferential statistical analysis. This exploration gave an initial picture of the performance of all the participants, and of possible differences between the five groups. I used Shapiro-Wilk tests and Levene's statistic to assess normality of data distribution and homogeneity of variance, respectively. In some instances, I also considered measures of skewness and kurtosis to validate the results of the Shapiro-Wilk tests, as those are known to be very sensitive with

large samples. Furthermore, I calculated variance ratios (David, Hartley, & Pearson, 1954) to validate Levene's statistic and to thus ensure that any significant value was truly indicative of a violation in homogeneity of variance for the group comparisons at hand.

I applied log-10 transformations where required and ran parametric analyses on the transformed data. Significance was set at α value of .05. Depending on whether homogeneity of variance and sample sizes were equal or not, I performed Bonferroni or Games-Howell corrections for post-hoc analyses constituting of multiple sets of comparisons. When sphericity was violated, I used the Greenhouse-Geisser correction for sphericity estimates < .75; I applied the Huynh-Feldt correction when sphericity estimates were > .75 (Girden, 1992).

I identified and excluded binary outliers by using standardized residuals and Cook's distance methods before each analysis. I investigated any case with a standardized residual bigger than 3.29 and excluded it if its Cook's distance value was large (i.e., close to 1). If the outcome variable contained more than 1% of absolute values greater than 2.58, then I considered the variable for transformation and re-assessed its distribution after transformation.

After these initial steps, I used the following approaches to test my hypotheses: First, I ran a series of one-way ANOVAs to test between-group differences on (a) memory measures administered a single time during the experiment, that is short-term auditory attention span and working memory, semantic and autobiographical memory tests, (b) sleep parameters such as sleep onset latency, REM Latency and percentage sleep efficiency. Additionally, two ANOVAs tested between-group differences in parameters of sleep organization (i.e., the outcome variables SWS Distribution and REM Intensification).

Second, I ran a series of mixed-model ANOVAs sought to detect whether there were significant between-group differences in (a) patterns of night-time cortisol secretion across

the night, (b) the distribution of sleep stages across the night with proportions of stages 1, 2, SWS, REM sleep and WASO with 2 levels of variations each (Early sleep versus Late sleep), and (c) overnight gains in memory performance (episodic and procedural).

Third, I ran a series of simple linear and repeated linear contrasts, as well as Games-Howell-corrected post-hoc analyses, on all of the eight sleep outcome variables to determine (c) which patient group differed most from the Healthy Control group, and (d) whether there were differences between the corticosteroid-exposed groups (Mild Asthma, Moderate-to-Severe Asthma, and Eczema Control) and the non-corticosteroid groups (Untreated Asthma and Healthy Control).

Fourth, I ran an ANCOVAs with Group as the main predictor variable and Sex as a covariate to test (a) whether the asthma groups would have lower dream recall scores, relative to healthy and eczema controls, and I ran a series of mixed-model ANOVAs, with Group as the between-group factor and Time as the within-group factor, to explore (b) differences on outcome measures describing the quality of the dream experiences across the night.
Then, I used linear regression to test whether (c) sleep efficiency, percentage REM, and REM Intensification predicted total dream recall scores.

Fifth, regarding the factors affecting dreaming among the asthmatic participants, I ran (a) a one-way ANOVA to test whether the asthmatic participants scored higher on the TAS-20 scale relative to the control participants, and (b) a multinomial logistic regression analysis with Dream Recall Status (No Recall versus White Dream versus Recall with Content) as the dependent variable, Group (5 levels) and Time (REM1 versus REM2 versus REM3) as the predictors, to test whether there is a higher incidence of white dreams among asthmatic individuals. Additionally, I then ran three separate simple regression analyses to test whether (i) alexithymia or (ii) asthma severity scores, (iii) corticosteroid exposure scores, or (iv) post-

sleep performance on verbal episodic memory tasks (VPA II & LM II) predicted the incidence of white dreams.

Sixth, I used a series of linear regression equations to test whether REM intensification and SWS Distribution scores, respectively, predicted the following memory content outcome variables:

(i) Average Objective Episodic Content score

(ii) Average Subjective Episodic Content score

(iii) Average Originality score

Seventh, I ran two ANCOVAs with Group as the predictor variable, Sex as the covariate, and average objective episodic score and average subjective episodic score as the respective outcome variables to test whether the asthmatic participants differed from the healthy control participants on the memory content of their dreams.

Eighth, I ran a multinomial logistic regression to determine whether the three categories of dreams (*residue of the day* versus *laboratory-related* versus *idiosyncratic*) were distributed equally across the three awakenings (REM1 versus REM2 versus REM3) for members of the five groups. The model tested included a full factorial which tested the main effects of Time (3 levels) and of Group (5 levels) on the relative proportions of dream categories (3 levels), and of Time x Group interactions on dream categories.

Ninth, I used simple regression modeling and bivariate correlations to analyze the relationships between (a) corticosteroid exposure and cortisol, (b) asthma severity and cortisol, (c) cortisol and sleep, (d) sleep and memory, (e) sleep and dreaming, (f) dreaming and memory, and (g) cortisol and dreaming.

Tenth, I used mediational analyses to test triadic models which may explain how cortisol, sleep, memory and dreaming fit into a coherent framework of processes. To do so, I tested the following set of predictions: (a) individuals making chronic use of corticosteroids

(viz., those participants in the Mild Asthma, Moderate-to-Severe Asthma, and Eczema Control groups) will have elevated night-time cortisol levels relative to healthy control participants; (b) elevated night-time cortisol will be associated with poor quality of sleep, particularly by being negatively correlated with percentage SWS and percentage REM; (c) poor quality of sleep will disrupt effective consolidation of declarative and procedural memory; and (d) poor sleep-dependent memory consolidation will affect the content of dreams reported (i.e., individuals with poor declarative memory performance will report less episodic content in their dreams).

Results

Sample characteristics. The five groups were well matched in terms of age, sex, lack of depressive symptomatology, and general intellectual functioning (see Table 2). Regarding BDI-II scores, the study's eligibility criteria were successful in excluding from participation any individual whose score fell with the range conventionally described as "clinically depressed."

Table 2
Sociodemographic, IQ, and Psychiatric Characteristics of the Current Sample (N = 68)

		Group							
		Asthma			Control				
Variable	Range/ratio	Mild (*n* = 14)	Moderate-to-Severe (*n* = 14)	Untreated (*n* = 14)	Eczema (*n* = 13)	Healthy (*n* = 13)	*F/H/χ²*	*p*	ESE
Age	18-39	21.36 (5.43)	22.86 (5.42)	21.14 (4.13)	20.69 (3.04)	20.33 (1.97)	0.62	.65	.04
Sex (F:M)	36:31	6:8	6:8	9:5	8:5	7:6	2.26	.73	.18
Depression	0-13	3.43 (3.61)	5.71 (5.30)	4.00 (3.16)	4.54 (3.77)	3.75 (3.89)	0.73	.57	.04
PIQ	87-140	112.21 (8.91)	115.86 (9.76)	107.71 (17.38)	111.77 (13.19)	107.64 (14.29)	3.23	.52	.06

Note. Means are presented with standard deviations in parentheses. Depression scores here refer to BDI-II scores. PIQ = Performance IQ; ESE = effect size estimate (in this case, for Sex, Cramer's *V* statistic was reported as an estimate of effect size and partial η^2 for the remaining variables). For the between-group comparisons, degrees of freedom were (4, 67) for analyses of Age and BDI-II data, and (4, 66) for analyses of the IQ data. One participant's IQ scores were not included in the analyses following the participant's request that her IQ information not be used. One-way ANOVAs were used for Age and Depression, Kruskal- Wallis (*H*) test for PIQ, and Pearson's Chi Square test (χ^2) for Sex.

Table 3
Between-group comparisons: Memory performance variables (N = 68)

	Group							
	Asthma			Control				
Variable	Mild (n = 14)	Moderate-to-Severe (n = 14)	Untreated (n = 14)	Eczema (n = 13)	Healthy (n = 13)	F	p	ESE
Episodic memory								
VPAI	12.64 (2.98)	12.36 (2.50)	10.43 (3.50)	11.69 (3.40)	11.83 (2.59)	1.12	.36	.07
VPAII	11.92 (3.20)	12.07 (2.76)	10.15 (3.36)	12.33 (2.90)	11.75 (2.30)	0.72	.58	.04
VPA Retention %	95.18 (8.62)	101.65 (11.57)	101.33 (8.09)	104.04 (12.24)	100.76 (16.33)	1.24	.31	.07
LMI	50.07 (8.22)	47.79 (11.79)	47.14 (10.07)	45.69 (8.58)	53.62 (9.35)	1.33	.27	.08
Learning Slope[a]	6.07 (2.02)	5.15 (2.61)	6.08 (2.18)	7.92 (1.93)	4.50 (3.32)	3.35	.02*	.19
LMII[a]	32.08 (5.77)	32.09 (8.80)	31.31 (7.47)	29.58 (5.66)	34.00 (6.06)	0.95	.44	.06
LM Retention %	88.15 (14.90)	93.00 (11.15)	91.92 (9.52)	87.03 (14.63)	93.08 (13.44)	0.50	.74	.03
AMT	26.79 (3.22)	26.07 (2.24)	24.57 (4.24)	25.00 (4.08)	27.85 (1.82)	1.97	.11	.12
Semantic memory								
WAIS-III Information	20.50 (3.28)	20.43 (3.37)	19.71 (5.82)	19.69 (4.11)	20.15 (3.13)	0.10	.98	.01
BNT	50.93 (6.56)	51.57 (7.99)	45.86 (12.52)	50.85 (7.31)	47.92 (7.82)	0.98	.43	.06
Working memory								
WAIS-III DS Backward[b]	7.36 (2.41)	7.64 (2.34)	8.07 (3.27)	7.54 (2.22)	8.42 (1.93)	0.39	.82	.02
Auditory attention span								
WAIS-III DS Forward[b]	11.07(2.34)	11.00(1.71)	10.64 (1.87)	10.92 (2.40)	11.50 (2.54)	0.26	.90	.02

Note. Means are presented with standard deviations in parentheses. VPA = Verbal Paired Associates; LM = Logical Memory; AMT = Autobiographical Memory Test (the outcome variable here is number of specific memories recalled); WAIS-III = Wechsler Adult Intelligence Scale-Third Edition; BNT = Boston Naming Test; DS = Digit Span; ESE = effect size estimate (in this case, partial η^2).[a]Two data points missing because of experimenter error (one male participant, age 21, from the untreated asthma group and one female participant, age 20, from the eczema control group) .[b]One data set (male participant from the healthy control group) is missing due to experimenter error. *p< .05.**p< .01.***p< .001.

Hypothesis 1: The effect(s) of chronic exposure to inhaled corticosteroids on memory performance. The relevant data here are displayed in Table 3.

Verbal episodic memory. In terms of baseline episodic memory performance (i.e., scores on the VPAI and LMI outcome variables), there were no significant between-group differences. However, there was a statistically significant between-group difference in Learning Slope across the various groups. The results of planned contrasts (simple and repeated) revealed that the differences lay between the Eczematics and the Healthy Control participants, $t(22) = 3.42$, $p = .001$, Cohen's $d = -1.32$, and between the Eczematics and the Moderate-to-Severe asthmatics, $t(23) = -2.76$, $p = .007$, Cohen's $d = -1.24$, with the Eczematics scoring higher in both cases.

With regards to autobiographical memory, although the Healthy Control group retrieved more specific memories than all the other groups, there were no significant differences across the groups in their performance on the AMT task. This analysis was performed on ranked transformed data since the analysis run on the raw data generated results which violated Levene's test of homogeneity of variance. However, both analyses generated consistent results (ANOVA results on raw AMT scores: $F(4, 61) = 2.01$, $p = .11$, partial $\eta^2 = .12$).

Semantic memory. There was no effect of Group on the Information subtest of the WAIS-III, or on the BNT performance.

Working memory. Although, the corticosteroid-exposed groups scored one point lower on average on the backward digit subtest, the ANOVA test revealed no significant between-subject differences across the five groups on that sub-test of the Digit Span test. Furthermore, the regression analysis indicated that corticosteroid-exposure score did not predict performance on the backward recall component of the Digit Span test, $R^2 = .06$, $F(2, 25) = 1.53$, $p = .228$.

Short-auditory attention span. There were no significant differences across the groups in terms of forward digit span scores.

Table 4
Night-time Cortisol Levels across Groups during Three Awakenings (N = 63)

		Group					
	Time	Mild Asthma (*n* = 13)	Moderate-to-Severe Asthma (*n* = 12)	Untreated Asthma (*n* = 13)	Healthy Control (*n* = 13)	Eczema Control (*n* = 12)	Whole Sample
Cortisol	REM1	2.61(1.69)	1.70(0.95)	2.79(2.30)	1.33(1.12)	2.86(1.61)	2.26(1.69)
	REM2	2.98(1.74)	2.12(1.43)	2.46(1.91)	1.83(1.50)	3.55(2.06)	2.58(1.79)
	REM3	8.70(4.60)	7.34(7.07)	9.82(5.40)	6.83(4.65)	13.32(8.50)	9.17(6.39)
	Average	4.76(2.31)	3.72(2.91)	5.02(2.25)	3.82(3.02)	6.58(3.55)	4.77(2.93)

Note. Means are presented with standard deviations in parentheses. REM1, REM2, and REM3 refer to the first, second, and third REM sleep awakenings, respectively, during which times salivary cortisol was collected. Units of cortisol concentration are reported in *nmol/l*. The data of 3 participants were excluded from these analyses because they had less than 3 cortisol data points. The data of 2 others were excluded because they were identified as outliers who were unduly influencing the model.

Hypothesis 2: The pattern of night-time cortisol across groups.

The following hypotheses were tested using a mixed-model repeated ANOVA on log-transformed data, followed by simple planned contrasts. Table 4 provides descriptive statistics for the average level of cortisol at each REM awakening, for each of the five groups and for the sample as a whole. Mauchly's test indicated that the assumption for sphericity had been violated ($\chi^2(2) = 23.35$, $p < .05$), therefore degrees of freedom were corrected using the Huyn-Feldt correction ($\varepsilon = .809$). The results of this correction were confidently accepted despite its reputation for being liberal since the uncorrected *F* values as well as the Greenhouse-Geisser corrected *F* values generated the same conclusions with respect to significance. Furthermore, the results of the multivariate analysis, which does not make assumptions about sphericity, confirm the conclusions drawn from the mixed-model ANOVA and Levene's tests revealed that there was homogeneity of variance at each level of the within-subject factor comparison.

First, there was a significant main effect of Group status with regards to night-time cortisol, $F(4, 58) = 2.76$, $p = .036$, partial $\eta^2 = .16$. Specifically, the patient groups had higher night-time cortisol across all three collection time points (REM1, REM2, and REM3) than the Healthy Control group. Simple planned contrasts revealed that for all the patient group participants, with the exception of the Moderate-to-Severe asthmatics, cortisol levels differed from the levels observed for the Healthy Control group participants, with these differences being significant for the Mild Asthma group versus the Healthy Control group and the Eczema Control group versus the Healthy Control group. Table 5 presents the results of the contrasts for this effect.

The analysis also revealed that there was a main effect of Time on night-time cortisol for the sample as a whole, $F(1.58, 91.86) = 111.67$, $p < .001$, partial $\eta^2 = .66$. There was however, no significant Group x Time interaction effect, $F(6.34, 91.86) = .37$, $p = .904$,

partial η^2 = .03. It would appear that generally, all the groups followed a trend whereby cortisol at REM1<REM2<REM3, as is illustrated in figure 2.

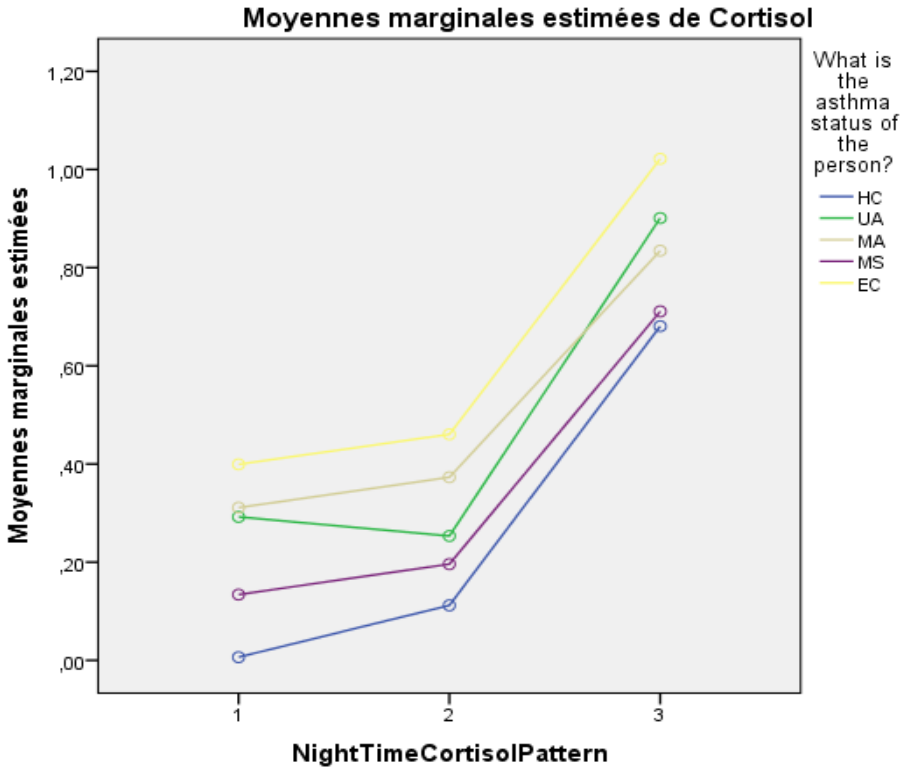

Figure 2. Night-time cortisol trends for each group

Second, except for the Eczema participants who had significantly higher cortisol levels than the Healthy Control participants, the corticosteroid-exposed groups did not have higher cortisol levels than the non-corticosteroid exposed groups. In fact, the Untreated asthmatics had a significantly higher average night-time cortisol level compared to the Moderate-to-Severe asthmatics.

The same post-hoc tests described above revealed that the high exposure group (i.e., Moderate-to-Severe Asthma) did not display higher night-time cortisol than the low exposure group (i.e., Mild Asthma). In fact, the mean cortisol levels during REM1 and REM2 were higher for the Mild group than for the Moderate-to-Severe group. Table 5 shows the results of linear contrasts and post-hoc tests for all between-group comparisons.

Third, asthma severity was significantly associated with night-time cortisol but the direction of the relationships was opposite to what was predicted. Asthma severity scores were inversely associated with (a) average night-time cortisol, (b) REM1 cortisol, (c) REM3 cortisol, but not with (d) REM2 cortisol. However, corticosteroid exposure alone was not associated with average night-time cortisol and any cortisol measures. Furthermore, asthma severity scores were not associated with corticosteroid exposure scores. Table 6 provides the details of these correlations.

Table 5

Between- group Comparisons: Average Cortisol Level at each Awakening (N = 63)

	Night-time cortisol											
	REM1			REM2			REM3			Average		
Group Comparison	*t*	*P*	ESE	*t*	*p*	ESE	*T*	*p*	ESE	*t*	*p*	ESE
Untreated Asthma vs. Healthy Control[a]	-2.02	.0275*	-0.81	-.95	.177	-0.37	-1.38	.0905	-0.59	1.83	0.72	-0.45
Mild Asthma vs. Healthy Control[a]	-2.34	.01*	-0.89	-1.80	.0425	-0.70	-0.91	.1855	-0.40	2.03	.046*	-0.35
Moderate-to-Severe Asthma vs. Healthy Control[a]	-0.00	.167	-0.04	-0.54	.2965	-0.20	-0.16	.4315	-0.09	0.68	.505	0.03
Eczema Control vs. Healthy Control[a]	-3.49	.001*	-1.10	-2.41	.012*	-0.95	-2.10	.0235	-0.95	3.01	.004*	-0.84
Mild Asthma vs. Untreated Asthma[b]	-0.13	.449	0.09	-0.84	.2035	-0.28	0.45	.327	0.22	-0.20	.839	0.11
Moderate-to-Severe Asthma vs. Untreated Asthma[b]	1.08	.1455	0.62	0.38	.3545	0.20	1.28	.107	0.39	1.76	.046*	0.50
Eczema Control vs. Untreated Asthma[b]	-0.83	.417	-0.03	-1.47	.155	-0.55	-0.88	.386	-0.49	-0.96	.1725	-0.52
Mild Asthma vs. Moderate-to-Severe Asthma[c]	1.31	.101	0.66	1.20	.121	0.54	0.78	.2225	0.23	0.83	.191	0.40

Note. Post-hoc *t*-tests tested the predictions that at REM1, REM2, and REM3: [a]All the patient groups would have higher cortisol levels relative to the healthy control group, *$p < .0125$, [b]that the corticosteroid-exposed groups would have higher night-time cortisol than the non-exposed groups, *$p < .017$, and [c]that the higher-exposure group would have higher cortisol than the lower-exposure group, *$p < .05$. Simple and linear contrasts were used for the average night-time cortisol group comparisons, *$p < .05$. ESE = effect size estimate; in this case, Cohen's *d*. The data of 3 participants were excluded from these analyses because they had less than 3 cortisol data points. The data of 2 others were excluded because they were identified as outliers who were unduly influencing the model.

Table 6

The *Relationships between Asthma Severity, Exogenous Corticosteroid Exposure and Endogenous Cortisol.*

	Cortisol					
	REM1	REM2	REM3	Average	Asthma severity	Corticosteroid exposure
Asthma severity	-.21*	-.09	-.27*	-.30*	1.00	.20
Corticosteroid exposure	-.09	.08	-.05	-.03	.20	1.00

Note. The figures presented in the table are Kendall's tau (τ) coefficient correlation statistics. Significance level was set at < .05*. The degrees of freedom were 35, 23, and 26 for the relationships between asthma severity and cortisol outcome variables, corticosteroid exposure and cortisol outcome variables and between asthma severity and corticosteroid exposure, respectively.

Hypothesis 3: Differences in sleep architecture across treatment groups.

The conditions for transformation were met for SOL, REM Latency, and percentage of stage 2 sleep and I used log-10 transformations for these. The transformations were successful in normalizing the respective distributions. Consequently, parametric analyses were performed on the transformed data. Percentage SWS, REM , stage 1 sleep, sleep efficiency, and WASO were normally distributed and hence parametric analyses were performed on the raw data for those variables.

Analyses of variance performed on the log-transformed sleep measures revealed no significant differences in time taken to fall asleep (SOL), time taken to achieve the first REM sleep stage (REM Latency). Analyses of variance on the raw measures reveal no significant differences in sleep efficiency and WASO.

Mixed-model repeated measures ANOVAs revealed that stage 1 sleep differed significantly across groups. There was no main effect of Time on stage 1, $F(1, 59) = 0.75$, $p = .391$, partial$\eta^2 = .01$, nor was there a Group x Time interaction, $F(4, 59) = 1.23$, $p = .306$, partial$\eta^2 = .08$. Descriptive statistics revealed that the groups were very varied in their distribution of stage 1 sleep with Untreated Asthmatics experiencing the most stage 1 sleep, while the Moderate-to-Severe Asthmatics experienced the least stage 1 sleep, with the difference between these two groups being significant, $t(24) = 2.88$, $p = .004$, Cohen's $d = 1.13$.

Post-hoc between-group analyses were run for Early Stage 1 and Late Stage 1 separately, using log-transformed data. The results of these analyses revealed that the between-group differences were significant during Late sleep and lay between the Untreated Asthma participants and (a) the Healthy Control participants, $t(23) = -2.82$, $p = .005$, Cohen's $d = -.62$, (b) the Mild asthmatics, $t(25) = 2.55$, $p = .0085$, Cohen's $d = 0.77$, and, (c) between the Untreated Asthma participants and the Moderate-to-Severe asthmatics, $t(23) = 3.40$, $p =$

.0015, Cohen's d = 0.91. There were no significant between-group differences for Early stage 1 sleep.

On the other hand, the main effect of Time of stage 2 sleep was significant, with all the participants generally experiencing more stage 2 during Late sleep than during Early sleep, $F(1, 60) = 16.06$, $p < .001$, partial$\eta^2 = .21$. There was no significant Group effect on the percentage of stage 2 sleep, $F(4, 60) = 1.02$, $p = .402$, partial$\eta^2 = .06$. There was also no Time x Group interaction, $F(4, 60) = 1.92$, $p = .119$, partial$\eta^2 = .11$. However, post-hoc *t*-tests were still performed owing to noted differences in means between the corticosteroid-exposed asthmatics and the healthy control participants for Early sleep Stage 2. See means plot in figure 3.

These tests revealed that the patient participants experienced more stage 2 sleep than the healthy controls during the first half of the night, with the difference in means being significant for the Mild Asthma versus Healthy Control comparison after Bonferroni correction, $t(24) = -3.10$, $p = .0025$, Cohen's $d = -1.22$.

Similarly, there was a significant main effect of Time on WASO, with all the participants experiencing more awakenings during Late sleep than during Early sleep, $F(1, 56) = 82.74$, $p < .001$, partial$\eta^2 = .60$. There were however no Group, $F(4, 56) = 0.38$, $p = .824$, partial$\eta^2 = .03$, nor any Time x Group interaction effect, $F(4, 56) = 0.51$, $p = .729$, partial$\eta^2 = .04$.

Essentially, the two mixed-model repeated measures ANOVAs revealed significant main effects of Time (Early Sleep versus Late Sleep) for the distribution of REM sleep, $F(1, 60) = 282.89$, $p < .001$, partial $\eta^2 = .83$, with Late Sleep containing a greater percentage of REM sleep, and the distribution of SWS, $F(1, 60) = 103.18$, $p < .001$, partial $\eta^2 = .63$, with Early Sleep containing a greater percentage of SWS for the sample as a whole. There was additionally, a significant Group effect on the percentage of REM sleep experienced, $F(4, 60)$

= 2.52, p = .050, partial η^2 = .14. On the other hand, there was no Group effect on the total percentage of SWS experienced by the participants during the whole night, $F(4, 60) = 1.52$, p = .208, partial η^2 = .09. However, when I analyzed the percentage of total SWS for the whole night across the five groups, even though the analysis of variance did not reveal a significant effect on the whole model, $F(4, 60) = 2.19$, p = .081, partial η^2 = .13, the results of simple linear contrasts comparing each patient group with the Healthy Control group revealed that the Untreated Asthma participants experienced less SWS, $t(24) = -2.58p$ = .012, as did the Mild Asthma participants, $t(24) = -2.16$, p = .034.

A series of post-hoc comparisons, using Games-Howell's correction, reveal that the Moderate-to-Severe asthmatics, p = .040, 95% CI [0.17, 9.26] , and the Eczema Controls, p = .050, 95% CI [0.003, 8.74] , experienced significantly smaller proportions of REM sleep to total sleep, compared to Healthy Controls.

Further, there was no Group x Time interaction effect on percentage REM sleep, $F(4, 60) = 1.15$, p = .34, partial η^2 = .07, implying that all the groups experienced more REM during Late Sleep than during Early Sleep and that the size of the gap between Early REM and Late REM for each group was comparable. This result is corroborated by the fact that the five groups did not differ significantly with regards to their average REM intensification scores, $F(4, 61) = 1.25$, p =.299, partial η^2 = .08, which measured the difference between the total percentage of REM sleep experienced during Early Sleep and the total percentage of REM sleep experienced during Late Sleep.

On the other hand, there was a significant Group x Time interaction effect on percentage SWS, $F(4, 60) = 2.68$, p = .04, partial η^2 = .15.Post-hoc t-test analyses indicated that that Untreated asthmatics experienced markedly less SWS during Early Sleep, $t(24)$= 2.14, p = .0215, Cohen's d = .85, but an equal proportion of SWS during Late Sleep, $t(24)$= 0.01, p = .49, Cohen's d = .01, thus experiencing a smaller decrement in their SWS during the

course of the night, relative to healthy controls. Moreover, the Eczematics experienced significantly less SWS during Early Sleep, $t(23)= 2.17$, $p = .020$, Cohen's $d = .87$, but significantly more SWS during Late Sleep, $t(23)= -2.59$, $p = .008$, Cohen's $d = -1.04$, when compared to healthy controls. However, except for the difference in means between the Eczema Control and the Healthy Control groups for Late SWS, these differences were not significant as per Bonferroni corrections which require a p value of .0125 in these cases (α value of .05 divided by the number of comparisons, i.e. 4: HC vs. UA, HC vs. MA, HC vs. MS, & HC vs. EC).

Additional analyses. The lack of significance may be due to a weakness in power and this is in part supported by the fact that SWS Distribution, which measures the percentage difference between Early SWS and Late SWS, showed a significant difference across groups, $F(4, 61) = 278.71$, $p = .037$, partial $\eta^2 = .15$, specifically between the Eczematics and the Healthy controls, $p = .026$, 95% CI [1.25, 24.91], as indicated by Games-Howell post-hoc tests.

Table 7 shows descriptive statistics for sleep outcome variables for the whole night, as well as Early Sleep to Late Sleep.

Table 7
Between-group Comparisons: Sleep outcome variables (N = 67)

	Group				
Variable	Mild Asthma (n=14)	Moderate-to-severe Asthma (n=12)	Untreated Asthma (n=14)	Eczema Control (n=13)	Healthy Control (n=12)
Sleep latency	11.79(8.29)	12.13(6.07)	10.54(11.86)	12.00(10.93)	11.75(9.95)
Sleep efficiency	85.72(6.39)	81.19(10.61)	83.23(9.88)	84.92(9.29)	84.50(7.78)
WASO %	13.95(6.68)	18.71(10.58)	17.32(9.58)	15.14(9.23)	15.37(7.79)
*§Stage 1 NREM %					
Whole night	8.78(4.12)	6.37(4.66)	11.71(4.76)	10.23(7.04)	8.93(4.96)
First half	9.66(6.09)	6.36(5.21)	10.54(3.88)	11.07(9.38)	10.38(5.51)
Second half	7.93(3.24)	6.70(4.63)	12.34(7.48)	9.46(6.57)	7.52(7.96)
*Stage 2 NREM %					
Whole night	46.78(10.69)	48.23(11.55)	52.54(12.92)	46.29(9.60)	42.72(8.34)
First half	47.59(9.80)	44.23(13.53)	38.01(9.58)	42.76(13.66)	36.00(9.17)
Second half	46.04(16.98)	52.54(12.92)	46.59(8.41)	50.16(8.80)	50.04(12.83)
SWS%					
Whole night	11.56(4.17)	12.13(4.17)	10.76(6.06)	14.05(4.32)	15.70(5.18)
First half	17.70(5.94)	18.60(9.75)	16.20(10.42)	17.18(8.02)	23.90(7.41)
Second half	4.25(5.78)	6.64(5.53)	4.21(4.18)	10.60(6.22)	4.42(6.03)
*SWS Distribution	13.45(8.02)	11.04(11.84)	11.99(10.26)	6.58(11.79)	19.66(7.80)
*REM %					
Whole night	18.62(6.37)	14.43(3.74)	18.46(5.95)	14.12(2.98)	19.83(3.36)
First half	6.05(3.65)	2.88(3.00)	6.76(6.07)	4.99(3.24)	5.73(5.89)
Second half	32.39(16.83)	24.64(6.01)	29.22(9.90)	23.25(5.09)	31.25(8.04)
REM Intensification	26.34(16.02)	20.09(8.52)	22.46(11.00)	18.25(5.24)	25.52(11.68)
REM latency	96.64(42.80)	148.71(95.23)	141.04(59.40)	115.38(46.97)	101.38(36.54)

Note. Means are presented with standard deviations in parentheses. One data set was lost due to equipment failure. *Refers to any significant effect in post-hoc pairwise comparisons between patient groups and the healthy control group. § Refers to any significant effect in post-hoc pairwise comparisons between corticosteroid-exposed groups and the untreated asthma group. Significance was set at $p < .0125$.

Hypothesis 4: Overnight gains in memory performance: The role of cortisol.

Sleep-dependent enhancement of declarative memory performance. The mixed-model repeated measures ANOVA revealed no significant main effect of Group, $F(4, 61) = 0.97$, $p = .431$, partial $\eta^2 = .06$, or of Time, $F(1, 61) = 0.15$, $p = .705$, partial $\eta^2 = .002$, for the VPA- 15 task. There was also no Group x Time interaction effects, $F(4, 61) = 1.04$, $p = .396$, partial $\eta^2 = .06$. In other words, the groups did not perform differently from one another either pre- or post-sleep, and Sleep Delay had no differential effect on any Group, in fact Sleep had no effect on performance.

With regards to the VPA Retention Score and the LM Retention Score, the one-way ANOVAs revealed no significant between-group differences with regards to the percentage of word pairs retained (performed on log-transformed data), or to the percentage of story elements remembered between pre- and post-sleep. Furthermore, sleep appeared to have a generally decremental effect on story recall as all the average retention scores were below 100 percent. See Table 3 (p. 110) above for details of analyses.

Sleep-dependent enhancement of procedural memory. Table 8 shows descriptive statistics for the procedural memory outcome variables (FTT Speed and FTT Accuracy) for each group. The *F* statistics for the within-subject effects reported here underwent Huyn-Feldt corrections due to a violation of sphericity. The results of the mixed-model ANOVA were accepted because they were consistent with the results of the multivariate tests generated automatically by SPSS. The planned contrasts were deemed reliable since Levene's tests for each level of comparison were non-significant.

The results of a mixed-model repeated measures ANOVA revealed a significant main effect of Time (Baseline versus Post-training versus Post-sleep) on Speed, $F(1.71, 102.78) = 75.22$, $p < .001$, partial $\eta^2 = .56$, and of Sex on Speed, $F(1, 60) = 7.47$, $p = .008$, partial $\eta^2 = .11$, but no main effect of Group, $F(4, 60) = .58$, $p = .675$, partial $\eta^2 = .04$.Therefore having

asthma did not affect one's speed of performance on the FTT. Furthermore, there was no Time x Group ($F(6.85, 102.78) = 1.13$, $p = .35$, partial $\eta^2 = .07$) interaction. In other words, for all participants, performance improved from baseline to post-training and from post-training to post-sleep. The increment from baseline to post-training was statistically significant, $F(1, 60) = 99.13$, $p < .001$, partial $\eta^2 = .62$, whereas the benefit of sleep on speed of performance after the participant had been trained on the task, was not demonstrated, $F(1, 60) = 2.43$, $p = .124$, partial $\eta^2 = .04$.

However, there was a significant Time x Sex ($F(1.71, 102.78) = 3.26$, $p = .050$, partial $\eta^2 = .05$) interaction. Planned contrasts revealed that male participants benefitted more from training than did their female counterparts, $F(1, 60) = 4.50$, $p = .038$, partial $\eta^2 = .07$.

For the Accuracy measure of the FTT, running the analyses on the raw data violated various assumptions: Equality of covariance matrices, sphericity and homogeneity of variance at the different levels of comparison on the within-subject variable, Time of assessment. Therefore, a mixed-model ANOVA was run on rank-transformed data, which resolved all of these problems. The results of the analyses revealed that there were no significant main or interaction effects on measures of Accuracy. Time had no effect on Accuracy, $F(2.00, 120) = 2.21$, $p = .114$, partial $\eta^2 = .04$, nor did Group status, $F(8, 120.00) = 1.00$, $p = .443$, partial $\eta^2 = .06$, and Sex, $F(2.00, 120.00) = 2.37$, $p = .097$, partial $\eta^2 = .04$.

Table 8
Between-group Comparisons: Procedural memory outcome variables (N = 68)

	Group				
Variable	Mild Asthma (*n*=14)	Moderate-to-severe Asthma (*n*=14)	Untreated Asthma (*n*=14)	Eczema Control (*n*=13)	Healthy Control (*n*=13)
FTT Speed					
Baseline	8.00(3.70)	10.50(4.75)	8.14(3.06)	9.38(3.33)	10.23(4.57)
Post-training	61.86(21.37)	60.50(19.57)	51.29(9.56)	55.46(16.39)	55.77(14.38)
Post-sleep	65.50(21.18)	66.93(21.06)	57.50(10.65)	67.08(20.49)	55.92(20.44)
FTT Accuracy					
Baseline	77.68(17.98)	83.84(13.09)	85.15(13.53)	83.49(15.09)	89.84(10.60)
Post-training	93.01(4.55)	93.71(5.11)	92.65(5.73)	90.66(7.31)	91.97(6.77)
Post-sleep	95.42(2.07)	92.33(11.97)	94.36(4.93)	95.19(3.80)	87.44(25.38)

Note. Means are presented with standard deviations in parentheses.

The distribution of sleep & declarative memory performance: The role of cortisol. I correlated both average night-time cortisol levels and exogenous corticosteroid exposure with post-sleep performances on declarative memory tests (VPAII, LMII, VPA Retention & LM Retention). All analyses involving endogenous cortisol used log-transformed data as mentioned earlier. Average night-time cortisol was significantly and inversely associated with percentage LM Retention scores, $r(63) = -.24$, $p = .028$.

Furthermore, a series of simple regression analyses testing the relationships between sleep measures (percentage REM, percentage SWS, REM Intensification, SWS Distribution & sleep efficiency) and LM Retention revealed no significant relationships with the exception of REM Intensification and LM Retention. Appendix G provides the details of these regression analyses. I therefore attempted a mediational analysis testing REM Intensification as the mediator in the relationship between cortisol and LM Retention but it was not completed as some of the assumptions were not met to test a triadic relationship between sleep, memory consolidation, and cortisol.

The mediational model used (Baron & Kenny, 1986) to test the relationship between REM Intensification, sleep-dependent episodic memory consolidation, and cortisol rested on the fulfillment of four assumptions:

Step 1. Direct relationship c: The linear relationship between average night-time cortisol and percentage LM Retention scores approached significance, $R^2 = .057$, $F(1, 62) = 3.82$, $p = .055$.

Step 2. Indirect relationship a: However, average night-time cortisol did not predict REM Intensification, $R^2 = .011$, $F(1, 60) = 0.69$, $p = .408$

Step 3. Indirect relationship b: REM Intensification predicted percentage LM Retention scores, $R^2 = .064$, $F(1, 62) = 4.24$, $p = .044$.

Since relationship a in Step 2 was not upheld, Step 4 or the mediational relationship c' was not tested.

The distribution of sleep & procedural memory: The role of cortisol. With regards to procedural memory, although REM sleep predicted Speed on the FTT post-sleep (relationship a), $R^2 = .07$, $F(1, 63) = 4.89$, $p = .031$, a regression analysis indicated that average night-time cortisol did not, $R^2 = .007$, $F(1, 59) = 0.43$, $p = .514$ (relationship b). Therefore it is unlikely that cortisol plays a part in the relationship between REM sleep and procedural memory.

Table 9
Dream qualia: Between-group comparisons (N = 19)

	Group							
Variable	Mild Asthma (n = 2)	Moderate-to-severe Asthma (n = 3)	Untreated Asthma (n = 4)	Eczema Control (n = 5)	Healthy Control (n = 5)	F	p	ESE
Bizarreness	2.00(1.95)	3.22(1.29)	3.83(1.38)	2.80(1.23)	2.73(1.23)	0.18	.945	.05
Vivid Imagery	5.17(2.05)	6.11(1.67)	7.08(1.45)	4.47(1.30)	5.60(1.30)	0.49	.746	.12
Thought-like quality	5.17(2.04)	5.89(1.67)	6.00(1.44)	4.93(1.29)	4.60(1.29)	0.18	.943	.05
Emotional Intensity	3.50(1.90)	6.22(1.60)	5.08(1.34)	3.73(1.20)	3.33(1.20)	0.73	.585	.17

Note. Degrees of freedom were (4, 14). Means are expressed with standard errors in parentheses. ESE here refers to partial η^2.

Table 10
Regression Analyses Between Sleep Variables and Dream Recall Scores.

Regression	R^2, AdjustedR^2	F	p value
Sleep efficiency predicts Total Recall	.002, -.014	0.13	.723
Percentage REM sleep predicts Total Recall	.002, -.013	0.15	.702
REM Intensification predicts Total Recall	.095, .080	6.69	.012*

Note. Degrees of freedom were (1, 65) for the above analyses. Significance level was set at *p< .05.

Table 11
Predictors of White Dreams: Regression analyses

Regression	R^2, Adjusted R^2	F	p value
Alexithymia predicts white dreams	.035, .020	2.30	.134
Asthma severity predicts white dreams	.005, -.020	0.21	.650
Corticosteroid exposure predicts white dreams	.004, -.036	0.09	.765

Note. Degrees of freedom were (1, 65) for the above analyses. Significance level was set at *p < .05.

Hypothesis 5: Dreaming and asthma.

a. The hypothesis stating that asthmatics will demonstrate poorer dream recall than control participants was not supported. An ANCOVA revealed that neither Group, $F(4, 63) = 1.11$, $p = .361$, partial $\eta^2 = .07$, nor Sex, $F(1, 63) = 2.85$, $p = .096$, partial $\eta^2 = .04$, had a significant effect on total dream recall.

b. The series of mixed-model ANOVAs revealed no statistically significant main effects of Time on any of the outcome measures describing the subjective experiences of the quality of the dreams reported, that is (i) bizarreness, $F(2.00, 13.00) = 1.28$, $p = .309$, partial $\eta^2 = .17$, (ii) vividness of visual imagery, $F(2.00, 13.00) = 1.67$, $p = .226$, partial $\eta^2 = .21$, (iii) thought-like quality, $F(2.00, 13.00) = 0.17$, $p = .848$, partial $\eta^2 = .03$, and (iv) emotional intensity, $F(2.00, 28.00) = 0.37$, $p = .696$, partial $\eta^2 = .03$. The results of multivariate analysis are reported for the effect of Time on bizarreness, vividness of visual imagery, and thought-like quality instead of the repeated-measures ANOVA results as the ratings at REM3 violate Levene's test of homogeneity of variance for bizarreness and vividness of visual imagery, making the within-subject comparisons unreliable. The thought-like quality measure violated Box's matrix of co-variance. With regards to Group, there were no significant main effects of Group influencing the subjective experience of dreams along those parameters. Table 9 provides details of these analyses.

c. While sleep efficiency and percent REM did not predict total recall scores, REM intensification scores significantly predicted dream recall scores. However, the relationship is opposite to what was expected, such that high REM intensification scores appear to be correlated with low recall scores, with a beta value of -.052. This inverse relationship is clearly illustrated through a Pearson's correlation, $r(66) = -.27$, $p = .014$. See table 10 for details of the regression analyses.

d. There were no statistically significant differences in alexithymia scores between participants with asthma or eczema and healthy controls, $F(4, 61), = 0.46$, $p = .766$, partial $\eta^2 = .03$.

e. The results of the multinomial analysis indicated a main effect of Group on the incidence of white dreams, with Mild Asthmatics having significantly more white dreams than dreams with content, $b = 2.03$, Wald $\chi^2(1) = 5.51$, and $p = .019$, and the odds ratio = 7.60. There was also a significant effect of Time where participants were more likely to experience white dreams than dreams with content at REM1, $b = 1.52$, Wald $\chi^2(1) = 6.09$, and $p = .014$, and the odds ratio = 4.58. White dreams accounted for 18 percent of all the dream reports collected in this study (27 out of a total of 150) and Mild asthmatics reported 30 per cent of those white dreams, in contrast to Healthy controls who reported a mere 19 percent of those white dreams and eczematics who reported 7 percent. The other asthma groups each reported 22 per cent of all white dreams.

Additional analyses. A series of simple linear regression analyses demonstrated that none of the following factors were associated with the incidence of white dreams: (i) alexithymia, (ii) asthma severity, and (iii) corticosteroid exposure. However, sleep-dependent memory performance (VPAII scores) was significantly correlated with the incidence of white dreams, $rs(66) = .34$, $p = .003$. See Table 11 for the details of the regression analyses testing the hypothesized predictors of white dreams.

Hypothesis 6: The relationship between corticosteroid exposure and dreaming.

Sleep organization and the content of dreams. With regards the prediction that REM intensification leads to less episodic content and more original content, a series of linear regression analyses supported the hypothesis for the most part. REM intensification predicted a lack of episodic content when the objective episodic content score was used as the outcome variable. However, REM intensification did not predict the subjectively rated episodic score.

Finally, REM intensification predicted the presence of original content in dreams. On the other hand, the distribution of SWS distribution score was not associated with any of the memory content measures. Table 12 presents the statistics for the regression analyses on the relationship between the degree of memory content in dreams and the distribution of REM sleep, and the distribution of SWS.

Table 12
Sleep Organization and Memory Content of Dreams: Regression analyses

Regression	R^2, Adjusted R^2	F	p value
REM Intensification predicts episodic content	.085, .69	5.27	.025
SWS Distribution predicts episodic content	.004, -.013	0.24	.625
REM Intensification predicts original content	.162, .149	11.20	.001
SWS Distribution predicts original content	.004, -.014	0.23	.637

Note. Degrees of freedom were (1, 57) for the above analyses. Significance level was set at *p< .05.

Asthma and the episodic content of dreams. Two ANCOVAs revealed that neither the main predictor Group, $F(4, 53) = 0.46$, $p = .765$, partial $\eta^2 = .03$, nor the co-variate Sex, $F(1, 53) = 0.80$, $p = .375$, partial $\eta^2 = .02$, affected episodic dream content significantly, whether objectively or subjectively rated.

Asthma and the distribution of dreams. A multinomial logistic regression analysis revealed no main Group, $\chi^2(8) = 12.13$, $p = .15$, or Time, $\chi^2(4) = 0.65$, $p = .96$, effect on the distribution of dream categories for the model as a whole, with a Cox and Snell R^2 value of .10. In other words, asthmatics, eczematics and healthy participants did not differ with regards to the type of dreams they were likely to encounter, and there was also no significant difference in the proportion of *Residue of the Day* versus *Laboratory-related* versus *Idiosyncratic* dreams at REM1 versus REM2 versus REM3. Interaction effects were not computed and the likelihood ratio for the final model was $\chi^2(12) = 13.18$, $p = .36$.

Hypothesis 7: Night-time cortisol and dream content: The role of sleep organization.

There was (a) a significant moderate and inverse correlation between average night-time cortisol levels and objective average episodic scores ($rs(55) = -.29$, $p = .017$) whereas the correlations between (b) night-time cortisol and the subjective average episodic scores ($rs(55) = .01$, $p = .452$) and (c) night-time cortisol and originality scores ($rs(55) = .05$, $p = .469$) were not significant.

I did not perform the mediation analyses testing the relationship between cortisol, REM Intensification/SWS Distribution and the memory content of dreams since analyses above (hypothesis 6) indicated that REM Intensification was not predicted by cortisol and that SWS Distribution did not predict the memory content of dreams. Therefore, no triadic relationship could be tested.

Additional analyses. When analyzed separately, the correlation between average night-time cortisol and objective average episodic content score was significant only for the Moderate-to-Severe group. Appendix H provides the details of the correlations for each group.

Hypothesis 8: Sleep-dependent memory consolidation and dream content: The role of cortisol.

The mediational model used (Baron & Kenny, 1986) to test the relationship between the percentage of retention of episodic information post-sleep, the presence of episodic content in dreams, and cortisol rested on four assumptions:

Step 1. Direct relationship c: Average night-time cortisol predicted percentage LM retention non-linearly, $R^2 = .07$, $F(1, 63) = 4.26$, $p = .029$, and linearalising the data through a log transformation generated an effect which bordered on significance, $R^2 =$, $F(1, 63) = 3.85$, $p = .055$.

Step 2. Indirect relation a: Average night-time cortisol predicted objective episodic dream content following a non-linear, inverse model, $R^2 = 1.00$, $F(1, 57) = 6.19$, $p = .016$. Up to a

certain level of cortisol, the higher the cortisol the lower the episodic dream scores tended to be and as from a particular level (~ 10.00 nmol/L), the relationship plateaued. Rank transformations were applied to all the variables in order to run a linear regression. The linear relationship between average night-time cortisol and objective episodic dream content was characterized by an R^2 value of .07, $F(1, 57) = 3.95$, and a significance value of $p = .052$. As was expected, the linear model was not ideal to represent the relationship between cortisol and episodic dream content ($R\Delta = .05$).

Step 3. Indirect relation b: The presence of episodic memory content in dreams predicted LM Retention scores. Specifically, a high performance on the post-sleep episodic task was associated with a greater incidence of episodic content in dreams, $R^2 = .10$, $F(1, 57) = 6.22$, $p = .016$.

Step 4. The degree of episodic content in dreams will mediate the relationship between night-time cortisol and the percentage of information retained in the morning relative to the amount of information recalled on the previous night (*i.e.*, LM Retention).

In light of the difficulties encountered with Steps 1 & 2, Step 4 or the mediational relationship c' was not tested.

Discussion

The effect(s) of chronic exposure to inhaled corticosteroids on memory.

Hypothesis 1. There was no sizable memory deficit noted among the patient groups on any of the memory tests. On the other hand, some between-group effects were observed on the LM task at the level of encoding. The patient groups all tended to encode less information on the very first story recall trial, but compensated sizeably after rehearsal such that their performances approximated normal levels. This pattern of encoding affected the eczematics in particular.

Participants with eczema encoded significantly fewer story units on their first recall trials compared to healthy as well as moderate-to-severe asthmatics. Conversely, on their second trials, they performed as well as or better than the Healthy Control and the Moderate-to-Severe asthma participants. This pattern of performance resulted in steeper learning slopes for the eczematics. Evidence in the literature suggests that cortisol affects memory at the pre-learning, encoding stage (Kirschbaum et al., 1996; Newcomer et al., 1999). Therefore, the current findings may be associated with factors specific to the eczematics tested other than their cortisol profiles or their exposure to corticosteroid treatment, the nature of which could not be investigated in this study.

As predicted, asthma or corticosteroid-exposure status was not related to performance on semantic memory tasks and despite the existing body of evidence on the effects of corticosteroid exposure on working memory, no such relationship was observed in the current study. Although healthy participants performed slightly better than the participants with asthma and eczema on all subtests of the digit span test, the advantage in performance was not statistically significant. Furthermore, analyses did not detect a clear relationship between a participant's degree of corticosteroid exposure and his or her working memory performance. The participants in this study were generally exposed to low or moderate doses of topical steroids. It is possible that at this degree of exposure and at such low levels of systemic absorption, corticosteroids do not effectively hinder working memory function. This argument is expounded on in the General Discussion chapter, where the results from Study 1 are compared to those of Study 2, which tests the effects of a moderate dose of prednisone on the same outcome variables as in Study 1.

The pattern of night-time cortisol across groups: Hypothesis 2. The absolute levels of cortisol differed significantly across the various groups, with the asthmatics and the eczematics experiencing higher levels of cortisol at each awakening. However, there was no

interaction between group status and time of cortisol collection to indicate any circadian flattening. The night-time cortisol measures appeared to follow a normal circadian pattern for the sample as a whole, with average cortisol levels increasing with each new awakening, with a subtle rise from REM1 to REM2 and a sharp and significant rise between REM2 and REM3.

Some studies have reported that while the area under the cortisol curve indicated different absolute levels of cortisol among asthmatics compared to healthy individuals, the secretory patterns observed were not necessarily reversed and different (Heim et al., 1999; Landstra et al., 1999; Kraft et al., 1998; Peebles et al., 2000; Ritz et al., 2011; Sutherland, 2005; Sutherland et al., 2003), while others have clearly demonstrated differences in the diurnal secretion of cortisol (Fei et al., 2004; Fujitaka et al., 2000; Haen et al., 1991; Masharani et al., 2005). Given that cortisol is known to be secreted in short, frequent bursts (Buckley & Schatzberg, 2005), the current study may have been limited in its ability to chart reliable secretion trends with only three collection time points. However, although cortisol levels increased for all groups as the night progressed, the elevated night-time cortisol among the patient participants is indicative of a disruption in HPA-axis functioning.

Planned contrasts revealed that the mild asthma and the eczema participants were the ones who had significantly higher average night-time cortisol levels compared to healthy control participants. The literature suggests that even patients not treated chronically with corticosteroids show patterns of circadian flattening or reversal of cortisol secretion, but that those disruptions of the HPA-axis are more pronounced among corticosteroid-exposed individuals (Kraft et al., 1999). Indeed, although the untreated asthmatics had relatively higher cortisol levels than healthy participants, this difference was not significant. Paradoxically and contrary to predictions, the moderate-to-severe participants had on average, very similar cortisol levels as the healthy control participants and significantly lower

average night-time cortisol than untreated asthmatics. Similarly to the present findings, Masharani et al. (2005) found that cortisol levels in the morning and at night were lowest among individuals exposed to the most potent inhaled corticosteroids compared to those on less potent ones. However, their study did not include a healthy control group and thus limits further comparison from being made. It is difficult to argue for cortisol suppression by the use of exogenous corticosteroids in the case of moderate-to-severe asthmatics as their cortisol levels approximate those of Healthy Control participants'. Perhaps these odd findings can be explained by the criteria I used to determine the severity of asthma among the corticosteroid-exposed asthmatics. One major factor that distinguished Mild Asthmatics from Moderate-to-Severe Asthmatics was that the former individuals only used their medication intermittently, whereas the latter generally took inhaled corticosteroids daily. It may be that the rigorous daily intake of medication reflects a more diligent management of the asthmatic condition, resulting in better-controlled asthma and therefore, less compromised HPA-axis activity. The inflammatory processes which characterize asthma, such as high levels of cytokine activity and of histamine are known to being inversely related to endogenous cortisol secretion (Petrovsky et al., 1998).

What both the Mild Asthmatics and the Eczema Control participants in this study share is the trend of corticosteroid usage: Both groups of individuals are defined by their transient use of topical (inhaled or cutaneous) corticosteroids. These individuals reported using their treatment only when they were symptomatic, during an average of a quarter of a year. The Mild Asthmatics included in the sample experienced asthma exacerbations at the change of seasons and the eczematics experienced cutaneous flare ups during periods of stress, especially around the two university semester exam periods. All of them were tested while they were undergoing corticosteroid treatment as a requirement of the study. It is therefore possible, that a stable longer-duration course of low-to-moderate doses of

corticosteroids, as those utilized by the asthmatics categorized in this study as Moderate-to-Severe, is more supportive than disruptive to the functioning of the HPA-axis. The elevations in night-time cortisol sometimes observed among asthmatics perhaps resolve when inflammatory processes are well-controlled and this would partly explain inconsistencies across studies. It would be interesting to examine (a) the patterns of night-time cortisol among asthmatics for a prolonged period and (b) the effects on long-duration courses of oral corticosteroids, which are more potent than topical forms, on endogenous cortisol secretion patterns.

With regards to the cortisol secretion patterns observed among the Eczema Control participants, the literature suggests that predisposition to atopy in general (dermatological e.g. eczema or respiratory, e.g. asthma) is associated with altered HPA-axis functioning (Buske-Kirschbaum et al., 1996, 1997). Infants with a family history of atopy show elevated cortisol responses to stress before developing eczema (Buske-Kirschbaum, Fischbach, Rauh, Hanker & Hellhammer, 2004) and babies with higher evening cortisol are more likely to develop eczema at age 2 (Stenius et al., 2011). Like asthmatics, individuals with eczema do not show signs of hypercortisolism (based on average 24-hour cortisol) but they do demonstrate pathological secretion patterns. For instance, Rupprect et al. (1995) observed a hypersecretion of cortisol during the early morning hours (00 00 to 06 00), compensated by a reciprocal decrease in secretion during the first half of the day, post-awakening (07 00 to 12 00) in patients with eczema compared to healthy controls. Furthermore, studies with adults and children show that treatment with topical corticosteroid creams (Fluticasone cream 0.05% and betamethasone 17-valerate 0.01%) in patients with eczema does not suppress endogenous cortisol (Friedlander, Hebert, & Allen, 2002; Munro, 1976; Tschen & Bucko, 1998). These findings imply that evening cortisol may therefore remain elevated in eczematics, whether they are being treated with topical corticosteroids or not. The current findings are consistent

with the existing evidence in that the eczema participants showed the highest levels of night-time cortisol compared to the other participants, and significantly elevated night-time cortisol relative to the healthy participants.

The relationship between chronic exogenous corticosteroid use and endogenous cortisol. The asthma severity scores, which were based on the degree of corticosteroid exposure and the chronicity of symptoms, were moderately and inversely related to cortisol levels at REM1 and REM3 and to average night-time cortisol. Greater asthma severity was associated with lower levels of average night-time cortisol: a finding consistent with the fact that the participants with the highest severity scores were the Moderate-to-Severe Asthmatics who also displayed lower cortisol levels than their other asthmatic counterparts. However, corticosteroid exposure per se was not associated with endogenous cortisol levels. These results are difficult to interpret given that all of the asthmatics, as well as the eczematics, exhibited higher average night-time cortisol and higher cortisol levels at the different times during sleep while the Moderate-to-Severe Asthmatics exhibited cortisol levels similar to those of healthy participants. Low cortisol levels cannot signify both normal HPA-axis functioning and dysfunction. In order to attempt disentangling these seemingly contradictory findings, I performed correlations for each group separately. These analyses revealed that for untreated asthmatics, a high asthma severity score was associated with high cortisol levels. This may imply an inverted-U relationship between asthma severity and cortisol.

Differences in sleep architecture across treatment groups: Hypothesis 3. Asthma participants, regardless of which group they belonged to, experienced significantly less REM sleep in proportion to other stages of sleep. Percentage REM sleep was especially compromised among the Moderate-to-Severe Asthma and the Eczema participants relative to the healthy controls. In contrast, the mild and the untreated asthmatics experienced near-

normal proportions of REM sleep. Hence, the suppression of REM sleep was significant only for asthmatics with the highest and most regular corticosteroid exposure and for the eczematics in this sample.

Although REM sleep was suppressed among asthmatics, on average, all participants experienced more REM sleep during the second half of the night than they did during the first half. In other words, the gross distribution of REM sleep between Early and Late sleep was not related to asthma status or corticosteroid-exposure. To corroborate this fact, the REM intensification score, which measured the difference between the proportions of Late REM and Early REM, did not differ significantly across the groups. In contrast, Montplaisir et al. (1982) found differences in the distribution of REM sleep between asthmatic participants and healthy controls during Early sleep where asthmatics spent a significantly smaller proportion of time in REM sleep during the first third of the night.

This discrepancy between the studies may be rooted in differences in the profiles of the asthmatics investigated. Two thirds of the participants in the Montplaisir et al. (1982) study suffered from nocturnal asthma attacks during sleep testing. When treated for their nocturnal symptoms and re-tested, the difference in REM distribution was no longer present. As mentioned in the introduction section of this chapter, there is some evidence to suggest that nocturnal asthma may constitute a distinct pathophysiological category with a unique HPA-axis profile (Sutherland, 2005; Sutherland et al., 2003). In contrast to the Montplaisir et al. (1982) asthmatics, none of the participants in my study experienced nocturnal symptoms and all were in a clinically stable state. It is therefore difficult to compare the findings of these studies in light of differences in patient profiles.

Another factor which may have contributed to the REM-specific differences in findings between the current study and the two older studies is the fact that medication was withheld a few hours preceding sleep testing in the latter studies. The present study did not

require participants to discontinue the use of corticosteroids because the aim was precisely to observe memory, sleep and dreaming processes as they occurred in the context of inhaled steroid treatment. Furthermore, a meta-analysis conducted by Wlodarczyk and colleagues (2008) suggests that the inhaled corticosteroid washout period has no significant interaction with cortisol level. Therefore, not asking participants to withhold medication served the purpose of capturing the effects of chronic corticosteroid exposure on sleep and cortisol.
In their characterization of the sleep disruptions present in adult asthmatics, Kales and colleagues (1968) did not observe REM suppression, nor did they observe any differences between corticosteroid-exposed and corticosteroid-free asthmatics with regards to sleep organization. The authors argue that if time spent asleep was taken into consideration, it would reveal that their participants with asthma spent less time in REM sleep. However, they did not formally undertake this analysis. On the other hand, they found a significant suppression of SWS, specifically of stage 4 sleep, and lower total sleep time among asthmatics relative to healthy participants.

Contrarily to Kales et al. (1968) but similarly to Montplaisir et al. (1982), I did not observe significant differences across the groups with regards to the total percentage of SWS experienced by participants according to the omnibus *F* test analysis. However, planned contrast analyses indicated that untreated and mild asthmatic participants experienced marginally but significantly less SWS than healthy controls. The bulk of evidence on the effects of glucocorticoids on sleep suggests that the action of glucocorticoid activity on SWS is not as pronounced or straightforward as its action on REM sleep, when proportions of these sleep stages are considered (Bohlhalter et al., 1997; Born et al., 1987, 1991, 1998; Plihal et al., 1999; Steiger et al., 1993; Wagner et al., 2005). This would explain why the overall relationship is masked even while some asthmatics demonstrate clear tendencies towards experiencing less SWS.

On the other hand, differences in terms of the distribution of SWS from Early to Late sleep across the groups were more notable: The asthmatics and the eczematics experienced a proportionally greater percentage of SWS than the healthy participants during the second half of the night. Illustrating the difference in the organization of delta sleep further, I observed a significant difference in the SWS Distribution scores (Early percentage SWS minus Late percentage SWS) across the groups. The difference in the distribution of SWS tended to indicate a certain difficulty to maintain delta sleep during Early sleep by Untreated Asthmatics and Eczematics in particular. While Eczematics managed to compensate for this difficulty by experiencing significantly more SWS than their healthy counterparts during Late Sleep, Untreated Asthmatics did not.

The Untreated Asthma group consisted of individuals who were not undergoing corticosteroid-based treatment and often not undergoing any form of treatment at all for their asthma. It is possible that participants in this group were characterised by a lack of awareness about the condition and its treatment options. Asthmatics are notorious for their lack of compliance to treatment regimens. Furthermore, non-compliance in taking asthma medication is reported as being one of the causes of poorly-controlled asthma, characterised by the exacerbation of symptoms (for reviews see Colice, 2004; Ingersoll & Cohen, 2008; Opolski & Wilson, 2005), which can in turn disrupt sleep. The evidence in the literature on the sleep disruptions associated with nocturnal asthma symptoms supports the view that controlling inflammatory processes, especially at night, is central to normalising sleep architecture among individuals with asthma (Kales & Kales, 1976; Kales et al., 1968; Klink & Quan, 1987; Monday et al., 1987; Montplaisir et al., 1982, 1983; Shapiro et al., 1986), especially since indices of high inflammation at night is associated with elevated cortisol (Barnes et al., 1980; Petrovsky et al., 1998; Szefler et al., 2002). It is possible that the selection criteria for untreated asthma identified individuals who were least compliant. Similarly, it is likely that

the eczema participants in the current study were experiencing heightened levels of inflammation since they were tested during periods where they had to make use of their corticosteroid treatment.

The differences in the amount of REM sleep and in the organization of SWS observed among the patient participants ought to affect the general organization of their sleep. Previous studies have reported that asthmatics have more fragmented sleep than what is considered healthy and spent less time asleep (Kales et al., 1968, 1970; Montplaisir et al., 1982; Shapiro et al., 1986). I found no evidence of greater sleep fragmentation among the patient groups, whether corticosteroid-exposed or not. Measures of sleep continuity (i.e. sleep efficiency and WASO) did not reveal any disadvantage relating to corticosteroid-exposure or asthma status. I did however observe differences in the light stages of sleep. The untreated asthmatics experienced more stage 1 sleep than the other asthmatics and the healthy participants whereas the corticosteroid-exposed asthmatics experienced more stage 2 sleep during Early sleep than healthy participants but this effect was not significant. Similarly, Kales et al. (1968) had observed a non-significant reciprocal increase in Stage 2 sleep among their asthmatic participants. It can thus be inferred that, instead of fragmenting sleep per se, asthma and corticosteroid exposure in this sample were associated with a lightening of sleep.

With regards to what is known on sleep architecture in eczema, Tantam, Kalucy and Brown (1982) found that patients with eczema experienced less REM sleep when compared to healthy controls and that this alteration in their sleep architecture was related to higher alexithymia scores on the Sifneos scale (1972). I observed the greatest REM sleep suppression among my participants with eczema. However, the eczematics in the current study did not show a higher incidence of alexithymia nor did they have higher scores on the TAS-20. Of note, the association observed in the Tantam et al. (1982) study between REM sleep and alexithymia is based on the observation of only three cases of eczematics with the

highest alexithymia scores out of a total sample of six patients. It is therefore more likely that the hypersecretion of cortisol during sleep, together with the sporadic use of corticosteroids, among eczematics may have contributed to the suppression of REM sleep among eczematics.

Children with eczema report significantly greater sleep disruptions compared to their healthy counterparts, with frequent awakenings and general sleeplessness (Camfferman et al., 2010; Lewis-Jones, 2006; Reid & Jones, 1995). These disruptions have been associated to itching and are thought to be limited to periods of flare ups (Reid & Jones, 1995). Bender, Ballard, Canono, Murphy, and Leung (2008) observed reduced sleep efficiency with the absence of other differences in sleep architecture among individuals with atopic dermatitis. A few studies have reported a higher incidence of scratching during stage 1 sleep (Aoki, Kushimoto, Hishikawa, & Savin, 1991), with treatment-induced reductions in stage 1 sleep being associated relief from nocturnal symptoms (Savin, Paterson, Adam & Oswald, 1979). I did not observe significantly greater sleep fragmentation in the adult eczematics tested but there was a non-significant trend towards a greater proportion of stage 1 sleep when compared to healthy controls.

Sleep-dependent memory consolidation and night-time cortisol: Hypothesis 4.

***Sleep-dependent enhancement of declarative memory performance*.** With regards to sleep-dependent consolidation of episodic memory, no significant differences within or across the groups were observed. Sleep did not improve recall of word pairs or stories encoded on the previous night and performance on the verbal paired associates and logical memory tests reached their ceiling for the sample as a whole. Therefore, sleep-dependent consolidation could not be demonstrated through these particular tasks.

Sleep-dependent enhancement of procedural memory. Procedural memory performance benefitted from both training and from sleep for the entire sample, with equivalent gains observed in all the groups. However, only the effect of training had a

significant impact on performance; the effect of sleep could not be demonstrated. Furthermore, training improved speed of performance on the FTT but not accuracy. Walker et al. (2002) also reported an increase in speed on the FTT, "without a loss in accuracy" (p.205) after sleep. In other words, similarly to the present results, they also observed that sleep did not reduce the number of errors made but it increased the speed of processing on the task. Lastly, male participants performed better than female participants on the FTT and benefitted even more from training than did their female counterparts. Male individuals are known to perform faster on psycho-motor tasks (Blatter et al. 2006; Thomas & French, 1985), especially when computer-generated (Grantcharov, Bardram, Funch-Jensen, & Rosenberg, 2003). Controlling for the effect of sex still did not reveal any significant relationship between group status and performance.

The distribution of sleep & declarative memory performance: The role of cortisol. The relationship between cortisol, sleep and episodic memory presented with a more complex picture. Sleep efficiency, percentage REM, and percentage SWS did not predict the consolidation of declarative memories. The lack of involvement of these sleep outcome measures in the consolidation of declarative memory may appear paradoxical since LM Retention % is operationalized specifically to assess consolidation after a retention period (independently of encoding or retrieval ability) and that the retention period in the current study was equated with a 7 to 8-hour sleep interval. However, the lack of a relationship between individual sleep parameters and memory consolidation does not exclude a relationship between the organization of sleep and memory.

As a matter of fact, REM Intensification scores predicted percentage LM Retention. These results seem to suggest that although declarative memory is clearly being consolidated during sleep, the quantity of isolated sleep stages or the total amount of sleep alone does not encapsulate the dynamic between sleep mechanisms and effective memory processing. On

the other hand, the organization of sleep, of REM sleep in particular, appears related to the effectiveness of sleep-dependent memory consolidation. According to the sequential hypothesis, it is the difficulty in achieving complete cycles of sleep and in having cycles which evolve in their structure (i.e. each new cycle consisting of less SWS and more REM sleep) more than the ability to experience enough of one particular stage of sleep that may disrupt sleep-dependent cognition (Ambrosini & Giuditta, 2001; Ficca et al., 2000, 2004; Giuditta et al., 1995; Mazzoni et al., 1999; Stickgold et al., 2000).

Although night-time cortisol levels were inversely related to post-sleep performance on the LM story recall task, the relationship was not predictive. Furthermore, although night-time cortisol predicted the distribution of SWS, there was no association between average night-time cortisol and REM Intensification. In fact, night-time cortisol levels were not associated with REM sleep efficiency, general sleep efficiency or sleep continuity. Therefore, the current study could not establish a mediating role for cortisol in the relationship between sleep and memory consolidation.

The distribution of sleep & procedural memory: The role of cortisol. On the other hand, with regards to the relationship between specific sleep stages and procedural memory, I did not find any relationship between stage 2 sleep and performance on the FTT contrarily to Walker et al. (2002). However, my study confirmed the well-established link between REM sleep and procedural learning (Gais et al., 2000; Karni et al., 1994; Stickgold et al., 2000a, 2000b). Percentage REM sleep predicted speed of performance on the procedural memory task. However, also consistent with existing evidence, cortisol did not mediate the relationship between REM sleep and procedural memory (Plihal et al., 1999; Born et al., 2004, 2009; Wagner et al., 2008).

Dreaming and asthma: Hypothesis 5. The current study did not find differences in the qualia of the dreams (i.e., subjective impressions of the dreams) reported by asthmatics

compared to healthy controls. In other words, individuals with asthma reported having dreams as vivid, with as many thoughts, as emotional, and as bizarre as their healthy counterparts, even when their dreams lacked autobiographical elements. Nor did the total number of dreams reported by asthmatics differ significantly from the number of dreams reported by healthy individuals. I did however find that asthmatic participants experienced proportionally higher numbers of white dreams than dreams with content when compared to their healthy or eczema patient counterparts. In particular, Mild asthmatics experienced significantly more white dreams than all the other participants.

Neither the symptom profile nor the treatment protocol of asthmatics, that is neither asthma severity nor corticosteroid exposure alone, predicted the incidence of white dreams. In addition, contrarily to previous findings, the link between white dreams and alexithymia was not established in this study. Alexithymia was not related to the frequency nor the depth of recall, even when sex was taken into account, nor was it more prevalent among asthmatics than among healthy controls. It is important to note that the studies which found associations between asthma, alexithymia and dream content either did not include the participation of healthy controls or selected asthmatics on the basis of a high alexithymic score. Alexithymia was not prevalent among the asthmatic participants in this study, with a single participant in the entire sample falling within the high alexithymic range and seven others falling within the middle alexithymic range, only three of whom were asthmatics.

As mentioned in the Introduction section of this chapter, the experience of white dreams may be a mnestic issue more than personality issue: Good recall of episodic information may go hand in hand with good recall of dream content. However, in the current study, performance on the word-paired associates' task predicted the incidence of white dreams but inversely. The poorer the dream recall, the better the performance on the verbal episodic memory test on the following morning was likely to be. Memory recall is adaptive:

Individuals recall material that they consider more important (Anderson & Schooler, 2000; Nairne, Thompson, Pandeirada, 2007; Shohami & Adcock, 2010). It is possible that the test material may have interfered with the ease of remembering the content of dreams among mild asthmatics, if participants felt that doing well on the test on the following morning was important, more so than remembering dreams.

Another explanation for the difficulty in recalling the content of dreams may be linked to the disruptions noted in organization of sleep of participants with asthma. The patient groups experienced lighter sleep, significantly less REM and less or differentially-distributed SWS. The difficulty in sustaining deeper stages of sleep in individuals with asthma is likely to have resulted in shorter or incomplete sleep cycles. These differences in sleep organization may not have been sufficient to disrupt performance on the declarative memory tasks, given the confounding effects of other factors such as IQ and task complexity, but they may explain the general poverty of content noted in dreams.

To corroborate the proposed association between the shallowness of sleep in asthma and the poverty of dream content reported by asthmatics, I found that REM intensification was inversely associated with total recall score. This finding can imply one of two things: First, that longer periods of REM are associated with poor dream recall or that second, the near absence of REM sleep during Early sleep is associated with poor dream recall during that half of sleep.

The first possibility is unlikely since it would require an inverse relationship between percentage REM and total recall, which I did not observe. REM Intensification was measured by subtracting Early sleep REM percentage from Late sleep REM percentage, and total recall was calculated from summing up recall scores from all three REM awakenings performed in the study. A high REM Intensification score implies high REM percentage during Late sleep and low REM percentage during Early sleep. It is possible that below a certain level, Early

REM percentage would be associated with inadequate dream recall during Early sleep and consequently with the total recall score.

Sleep organization and the memory content of dreams: Hypothesis 6. With regards to the role of sleep on the type or extent of memory sources encountered in dreams, I observed that REM intensification was associated with less episodic content and more original content. In other words, REM sleep appeared conducive to the inclusion of novel and creative elements in dreams, at the expense of familiar ones. However, the association between SWS and memory sources of dreams could not be established. The dreams analysed in this study were derived exclusively from REM awakenings, albeit at different points on the circadian slope. I had anticipated that if an individual had cycled through sufficient SWS during Early sleep, that the amount of SWS would predict the effective consolidation of episodic events and that this would be reflected in subsequent dreams regardless of the stage of sleep the dreams were generated from. However, it would appear that REM sleep, whether encountered in Early sleep or Late sleep, is associated with dream content so that it departs from simple replay of waking events to a greater reconstructive and elaborate associative process of integrating new experiences into our broad existing web of experiences.

With regards to the relationship between asthma or corticosteroid exposure and the memory content of dreams, the current study did not find any differences in terms of the amount of familiar (episodic) and unfamiliar (original) content across the groups. Relative to controls, asthmatic participants, on average, scored similar points with regards to the degree of episodic and original content present in their dreams. Furthermore, there were no differences in the proportions of dreams reflecting waking continuity between asthmatics and non-asthmatics. However, significantly more dreams reported by asthmatics were white dreams. In other words, asthmatics were more likely to report being convinced that they had experienced a dream for which they could recall little or no content, dreams which therefore

could not be rated for waking continuity. Given the strong relationship between REM sleep and the memory content of dreams, and the fact that asthmatics participants experienced significantly less REM sleep, one can speculate that the lower frequency of dreams with content among asthmatics is associated with the shallowness of sleep experienced by these participants.

With regards to the circadian influence on the distribution of dreams, the data did not reveal any circadian evolution in the waking-continuity of dream themes from REM1 to REM3, through REM2 for the sample as a whole. In other words, participants encountered as many dreams categorized as *residue of the day* and *laboratory-related* dreams as dreams with *idiosyncratic* themes at each awakening. Contrarily to what I expected, even in healthy controls, early REM dreams were not associated with a greater incidence of dreams with themes of waking continuity. The relevance of this finding is discussed in the General Discussion chapter of this dissertation (chapter Five).

Cortisol and the memory content of dreams: Hypothesis 7. Although there were no differences in terms of the memory content of dreams between asthmatics and non-asthmatics or between corticosteroid-exposed and non-exposed participants, the episodic content of dreams was inversely related to average night-time cortisol. Consistent with the hypothesis, high cortisol levels were associated with low episodic content. Furthermore, when the relationship was analysed separately for participants of each group, it was significant solely among the Moderate-to-Severe asthmatics. Elevated night-time cortisol was very strongly and negatively correlated with objective average episodic dream content for participants in that group. This relationship between cortisol and the episodic content of dreams was independent of REM sleep or SWS organization. Although REM Intensification scores predicted episodic content, REM Intensification in the current study could not be associated to levels of night-time cortisol. On the other hand, while cortisol predicted the

distribution of SWS, SWS Distribution scores were not associated with dream content variables.

Sleep-dependent memory consolidation and dream content: The role of cortisol: Hypothesis 8. Despite clear evidence of the associations between cortisol and the memory content of dreams and between the memory content of dreams and sleep-dependent consolidation of episodic memory, the current study was unable to successfully demonstrate a triadic relationship binding these variables. The mediating role of corticosteroids in the relationship between sleep and the consolidation of episodic memory was obscured by the non-linearity of the relationships and by the small size of the effect sought.

For instance, the amount of episodic elements recalled in dreams predicted delayed episodic memory recall following the sleep interval: A high degree of waking inclusions in dreams was associated with a high LM retention score or a high score on the LM II subtest. Evidence suggests that the presence of episodic content in dreams will predict better consolidation of memory after sleep, regardless of its proximity to the material tested (Wamsley et al., 2010).

In parallel, high cortisol levels were associated with both lower LM II and LM Retention scores and less episodic memory inclusions in dreams. However, the role of sleep as a mediator could not be established in this model: Although positively correlated to episodic memory performance post-sleep, REM Intensification was not predicted by cortisol.

On the other hand, cortisol predicted the distribution of SWS but SWS bore no relationship with performance on declarative memory tasks. These missing links potentially indirectly support the sequential hypothesis of sleep-dependent memory consolidation which suggests that the integrity of a sleep cycle and the evolving ratio of SWS to REM sleep predict the effectiveness of sleep-dependent cognition, and that subtle fluctuations in

hormone secretion during sleep may affect the evolution of cycles relative to one another, more so than sleep stages.

Limitations and Directions for Future Research

The current study endeavored to investigate the relationship between chronic corticosteroid treatment, sleep, memory and dreaming in a population of young adult asthmatics. No study had previously explored the relationship between inhaled corticosteroid use and sleep architecture, memory performance, and dream recall. The main prediction of Study 1 was that even topical corticosteroid exposure would be associated with disruptions in HPA-axis function during sleep, in a dose-response manner. Specifically, I expected to observe that greater corticosteroid exposure would be associated with relatively elevated night-time cortisol during sleep, a period known to be characterized by a cortisol trough.

Further, Study 1 aimed to demonstrate that this disruption in HPA-axis functioning may be related to sleep organization and, consequently, to sleep-dependent mentation, specifically relating to the processing of hippocampal-dependent information learnt before bedtime. This sleep mentation was accessed indirectly by comparing performance on declarative memory performance before and after sleep, and by analyzing dream contents for episodic memory inclusions. Although some patterns emerged linking night-time cortisol with sleep organization, memory performance, and dream content, no clear-cut linear relationship was established that could trace out a coherent network of relationships between exposure to exogenous corticosteroids by some asthmatics, elevations in night-time cortisol, disrupted sleep, disrupted memory performance, and/or impoverished dream content.

For instance, although asthma severity was associated with cortisol, the relationship was inverse implying that asthmatics categorized as more severe had lower night-time cortisol. Furthermore, although elevated cortisol predicted disorganization of SWS it did not

predict disorganization of REM sleep, which in turn was the only sleep parameter which predicted performance of declarative memory tasks. On the other hand, there was a definite relationship that was revealed between performance on declarative memory tasks and the extent of waking inclusions found in dreams and that the intensification of REM sleep and the level of cortisol were associated to both. However, it is the relationship between sleep and cortisol in relation to sleep-dependent mentation which could not be clarified.

One factor potentially confounding the triadic relationship between night-time cortisol, sleep organization, and sleep-dependent mentation, if it exists, is the method of group stratification used in this study. I assumed that the degree of corticosteroid exposure, defined by dose, chronicity and potency, would determine the degree of deviation from the norm in terms of night-time cortisol secretion; the norm here being the secretion pattern expressed by healthy controls. However, as the literature indicates, individuals respond in very diverse ways to corticosteroids. Factors such as the state of their HPA-axis functioning at baseline, the degree of respiratory inflammation, their immune system, lifestyle, personality characteristics, sex and race are but a few factors which impact on how much, if any, HPA-axis suppression they will experience when exposed to the same dose and type of corticosteroid than another asthmatic. The first three factors are physiological aspects of their asthmatic condition which cannot be fully accessed through self-reports of their symptoms nor assumed by the potency of the corticosteroids prescribed to them. Physicians are known to differ greatly with their approach to prescribing corticosteroid treatment to asthmatics, in ways that are not consistently related to lung function. Lung function tests are also apparently not entirely reliable measures of airway inflammation (Colice, 2004).

Future studies should consider the impact of these inter-individual factors by running the investigation in two phases: First, by stratifying individuals using direct HPA-axis suppression tests such as the dexamethasone-suppression test, independently of their

corticosteroid exposure and illness symptom profile. Second, by comparing the sleep organization, memory performance and dreaming patterns of HPA-axis suppressed individuals versus non-suppressed individuals. I had assumed that corticosteroid exposure itself would be associated with sleep and sleep-dependent cognition but at this level of exposure the relationship was not established. The means plots below provide an illustration of how the various groups in Study 1 were positioned relative to one another on certain measures of sleep organization, cortisol and declarative memory performance. It is clear from those plots that the groups do not fit the theoretical linear hierarchy that was being tested.

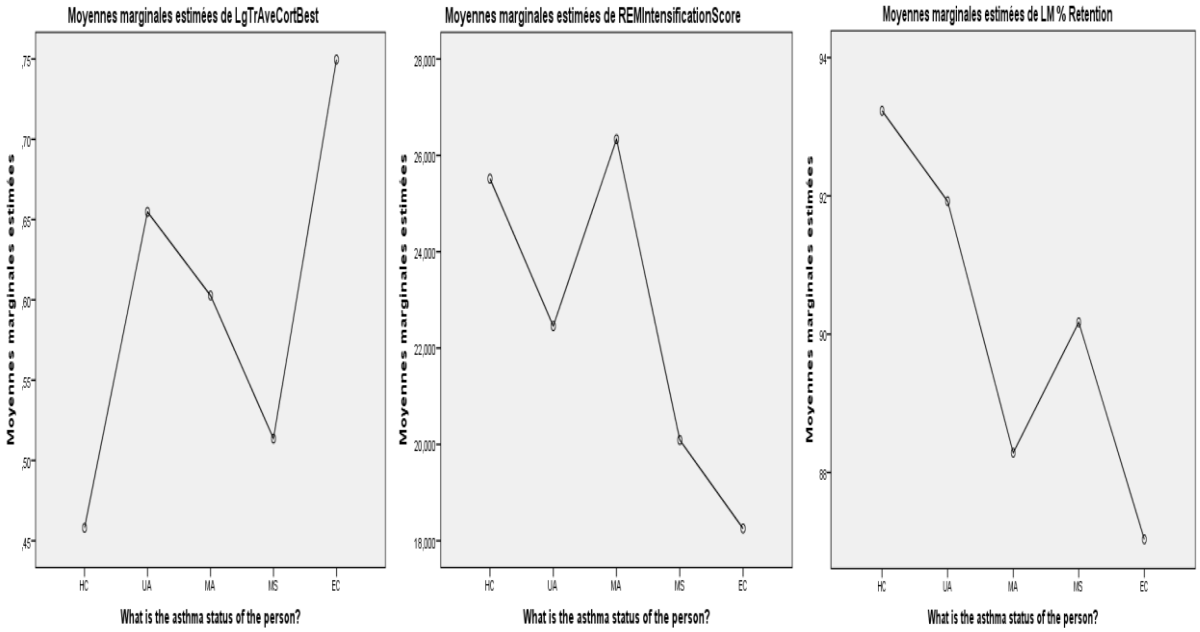

Figure 3. Means plots for cortisol, distribution of REM sleep, and LM Retention scores across all five groups of participants.

CHAPTER THREE:

STUDY 2: ACUTE EXPOSURE TO PREDNISONE BEFORE BEDTIME AND SLEEP-DEPENDENT MEMORY CONSOLIDATION

Introduction

Chapter 2 investigated the relationship between chronic exposure to corticosteroids and memory performance during wakefulness, and as mediated by sleep. In chapter 3, I attempted to analyse similar relationships, this time involving an acute exposure to corticosteroids instead. The Introduction section of this chapter first discusses the effects of acute corticosteroid exposure on memory performance and on sleep separately, and then discusses the effects of acute corticosteroid exposure on sleep-dependent memory consolidation processes. The core objective behind the design of Study 2 was to enable comparisons between chronic and acute corticosteroid exposure conditions. To that effect, the protocol adopted in Study 2, as detailed in the Method's section of this chapter, closely replicates that of Study 1. The remainder of the chapter includes the Results section which presents the outcome of Study 2 analyses, and the Discussion section which first relates the current findings to existing evidence and second, frames the current findings within the context of the broader project by comparing them to those of Study 1.

The acute effects of corticosteroids on memory. A conventional view in the literature is that elevated levels of corticosteroid activity affect memory performance negatively (see, e.g., Born et al., 2006, 2009; Ferguson & Sapolsky, 2007; Fietta & Fietta, 2007; Het et al., 2005; Kuhlmann et al., 2005; Plihal et al., 1999; Roozendaal, 2000; Wagner et al., 2007). In particular, previous studies have noted performance impairments on episodic, spatial, and working memory tasks, but not on semantic or non-declarative procedural tasks, under conditions of increased corticosteroid activity. The distribution of glucocorticoid receptors (GR) in the brain accounts for the selectivity of corticosteroid-induced memory

impairment (e.g., Arnsten, 2009; de Kloet, 2004; Elzinga & Roelofs, 2005; Het et al., 2005; Kuhlmann et al., 2005; Luethi, Meier, & Sandi, 2009; Lupien et al., 1998; Roozendaal, 2000; Wolf, 2003, 2006). These receptors are concentrated in the medial temporal lobes (MTLs) and in the prefrontal cortex (PFC). Therefore, memory systems that rely heavily on hippocampal function, for instance, are affected more strongly by fluctuations in corticosteroid levels than memory systems that do not. For example, Keenan et al. (1996) found that patients undergoing corticosteroid-based therapy performed poorly on a declarative memory task, relative to patients not taking corticosteroids, while performance on a verbal implicit memory (i.e., hippocampal-independent) task between the groups did not differ. These findings also indicate that the observed glucocorticoid-mediated effects on memory are independent of both disease-specific effects and of any secondary, diffuse cognitive impairment that may be observed with illness. Consistent with these findings, Newcomer et al. (1999) also found that the cortisol-mediated effects on memory that they observed were restricted to performance on the verbal, declarative memory task only and did not extend to performance on the nonverbal spatial memory, attention, and executive function tasks. These studies are discussed in more detail further under the section titled “the effect of exogenous corticosteroids on memory”.

The sections below describe and compare the effects of endogenous corticosteroids and exogenous corticosteroids on memory performance. It becomes apparent that most studies that have reported glucocorticoid-induced impairment of hippocampal-dependent memory performance, particularly of episodic memory, have used psychosocial stress protocols which induce elevations of endogenous cortisol levels. There exist very few studies that have studied the effects of exogenously-administered glucocorticoids on memory performance.

The effects of elevating cortisol through stress on memory. Kirschbaum et al. (1996) found that elevating endogenous cortisol using an experimental psychosocial stress protocol called the *Trier Social Stress Test* (TSST; Kirschbaum & Hellhammer, 1994) had a significant impact on declarative memory performance. They found that the amplitude of the cortisol response to stress was inversely related to the number of words recalled on a cued verbal recall task involving word list learning (r = -.70; variance explained = 49%). The stress protocol, which involves deceiving participants into believing that they are being evaluated by a panel of judges on their public speaking skills and on their ability to solve mental arithmetic problems, results in elevations of cortisol equivalent to the range observed during moderate psychosocial stress. Its validity has been demonstrated in a large number of studies using different population groups (Buchanan et al., 2006; for a review, see Kirschbaum & Hellhammer, 1994; Kirschbaum et al., 1996).

De Kloet, Oitzl and Joëls (1999) argue that studies observing the deleterious effects of corticosteroids on memory do so because of their designs, which introduce an unanticipated and out-of-context stressful physiological environment. It is this 'stress out-of-context' effect that mediates the deleterious effects observed, the physiological mechanism of action being stress-induced adrenergic sympathetic activation (as measured, for instance, by increased heart rate and blood pressure) in conjunction with elevation of cortisol. In other words, theoretical and empirical works suggest, as one explanation, that negative glucocorticoid effects on memory necessitate concurrent adrenergic activation (Elzinga & Roelofs, 2005). However, the experience of threat is not sufficient to determine, the effects of glucocorticoids on cognitive performance. Other subject-specific and drug-specific factors modulate the effects of glucocorticoid activity on memory.

For instance, a few psychosocial stress studies (Elzinga & Roelofs, 2005; Okuda, Roozendaal, & McGaugh, 2004; Rimmele, Domes, Mathiak, & Hautzinger, 2003;

Roozendaal et al., 1999; 2004) have demonstrated that conditions are necessary for declarative memory impairment to be elicited by endogenous elevations of cortisol should not only involve stress, in addition, the individuals tested must be cortisol responders (recommended classification: a cortisol increase of $\geq$ 1.5 nmol/l or 15.5% from baseline; Miller, Plessow, Kirschbaum, Stalder, 2013). In fact, the results of some studies have revealed that the inefficacy of a psychosocial stress protocol in inducing significant memory impairment is associated more strongly with inter-individual variations in cortisol response thresholds than with the extent of autonomic response induced by the stressor. That is to say, these studies show that elevating cortisol at the point of learning, without the element of stress, significantly impairs hippocampal-dependent declarative memory performance among cortisol responders. Cortisol non-responders, on the other hand, do not show any decrement in learning, whether or not they experienced stress-induced autonomic arousal (Buchanan et al., 2006; Kirschbaum et al., 1996).

The effect of exogenous corticosteroids on memory. The suggestion, from psychosocial stress studies, that there needs be a co-occurrence of (a) sympathetic activation and of (b) a cortisol response as a necessity for inducing memory impairment has been challenged by pharmacological manipulation studies (e.g., Buss, Wolf, Witt, & Hellhammer, 2004; Newcomer et al., 1994, 1999; Young et al., 2011) or studies observing the effects of synthetic corticosteroids on memory (Keenan et al., 1996). Although scarce, research on the acute effects of synthetic corticosteroids on memory, has contributed largely to our understanding of the relationship between corticosteroid action and memory performance. Evidence from a handful of studies suggests that whether or not corticosteroids have a detrimental effect on declarative memory depends on four additional factors namely, (c) the length of exposure, with repeated exposures being more effective than single exposures (Keenan et al., 1996; Newcomer et al., 1994, 1999), (d) the type, dose, and potency of the

corticosteroid administered, (Newcomer et al., 1994, 1999; Young et al., 2011), (e) the timing of corticosteroid administration in terms of the stage of memory processing (Coluccia et al., 2008; de Quervain et al., 2000), and (f) the emotional valence of the information to be recalled (Buchanan & Lovallo, 2001; Kuhlmann, Kirschbaum, & Wolf, 2005; Kuhlmann et al., 2005).

Regarding (c) the length of corticosteroid exposure, Keenan et al. (1996) conducted a prospective study examining the effects of exogenous corticosteroid treatment (prednisone; doses ranging from 5 mg to 80 mg) on explicit versus implicit verbal memory performance (paragraph recall task versus word priming task) in seven middle-aged and older adults (between 29 to 54 years of age) with varying medical profiles. The participants were recruited if they were identified as (i) requiring prednisone for a period of at least 12 weeks, for medical conditions with no CNS involvement, and (ii) not having been exposed to corticosteroids for a period of at least 6 months. The study included seven control participants, matched on demographic and medical profiles. Results indicated that, although between-group differences in performance on the declarative memory task appeared to emerge as early as 1 week into treatment, these did not achieve statistical significance (p = .061). However, by week 12, there were marked between-group differences in performance on the verbal declarative memory test, with the prednisone-treated group performing significantly more poorly (p = .019).

Further regarding the length of corticosteroid exposure, Newcomer et al. (1999) found that the pharmacological elevation of cortisol impaired verbal declarative memory (immediate and delayed recall) only after relatively prolonged exposure, to a relatively high dose of corticosteroids. Also regarding the (d) degree of corticosteroid exposure, the study compared the memory performance of a low-dose to a high-dose corticosteroid-exposed group of participants and that of both exposure groups to placebo controls, at baseline (Day

0), 24 hours after treatment (Day 1) and 4 days after treatment (Day 4), and after a 6-day washout period (Day 10). The authors observed reversible memory impairment on Day 4, in the high-dose condition (160 mg/d of hydrocortisone) only. Memory impairment was not observed on Day 1 or on Day 10, and was not observed at all in a low-dose condition (40 mg/d of hydrocortisone). Similarly, Young and colleagues (2011) observed that healthy adults who were exposed to a high dose of hydrocortisone (0.45 mg per kg, infused intravenously) retrieved a proportionally greater number of categorical memories relative to specific autobiographical memories, when compared to participants exposed to a moderate dose of hydrocortisone (0.15 mg per kg, infused intravenously) or to a placebo, both of the latter conditions having no effect on performance.

The acute or cumulative dose-response effect of corticosteroids on episodic memory is not uni-directional. There is well-documented evidence describing an inverted-U relationship between corticosteroid dose and performance on tasks of declarative memory: very low, as well as very high, doses disrupt performance (Lupien & McEwen, 1997). At certain optimal levels, however, glucocorticoids can have a positive impact on memory performance by enhancing consolidation (De Quervain et al., 2000; Kuhlmann et al., 2005; Roozendaal, 2000, 2002). Animal research indicates that the angles of slopes and the position of the tipping point of the inverted-U are determined by task complexity and concomitant adrenergic activation. In other words, the stress levels induced by a task moderate the amount of corticosteroid required to achieve an effect: The more threatening and/or difficult an experience or task is perceived to be, the lower the threshold is for glucocorticoid-mediated impairment on performance (Roozendaal, 2000).

Exogenous corticosteroids exert suppressive effects on endogenous cortisol. Several studies provide data supporting the argument that it is via this mechanism that exogenous corticosteroids disrupt memory performance (Bender et al., 1991; Brown et al., 2004;

Coluccia et al., 2008; Keenan et al., 1996; Newcomer et al., 1994; Schmidt et al., 1999; Wolkowitz et al., 1997). In essence, exposure to exogenous corticosteroids such as dexamethasone and prednisone produces an internal environment whereby glucocorticoid receptor activity is heightened, while free, circulating cortisol level is low. Based on the literature, it is therefore reasonable to infer that synthetic corticosteroids will produce an impairing effect on memory performance if the exposure is potent enough to suppress HPA-axis functioning. That is potentially why single exposures of low-to-moderate potency (10-20 mg of prednisone or 40-80 mg of hydrocotisone; van Rensburg, 2011) often do not result in significant memory disturbances. In support of this argument, an earlier study by Newcomer and colleagues (1994) revealed that repeated exposure to a low dose of dexamethasone (0.5mg on Day 1, and 1 mg on Days 2, 3, & 4) led to a significant decrement in performance on a verbal, declarative memory task on Day 4, compared to baseline. No such decrement was observed on Day 1, compared to baseline. Concurrently, the authors observed that plasma cortisol was suppressed while plasma dexamethasone doubled in quantity on Day 4 compared to Day 1. Furthermore, they found that higher plasma cortisol was associated with better scores on the paragraph recall task in the dexamethasone-exposed group only. They observed no such relationship between endogenous cortisol and memory in their placebo group; in other words, cortisol level was not associated with memory performance in participants not exposed to dexamethasone. In summary, these findings indicate that below a certain threshold, low cortisol induced by increased glucocorticoid-receptor occupation by an exogenous glucocorticoid, is associated with poor memory performance.

Over and above (c) length and (d) degree of exposure, some evidence in the animal (e.g., Lupien et al., 1997; Sandi, 1998; de Kloet et al., 1999; McGaugh et al., 2002) and in the human literature (De Quervain et al., 2000; Kuhlmann et al., 2005; Roozendaal, 2000, 2002) indicates that the same dose of glucocorticoids that can disrupt encoding or retrieval may

have a positive, or no, effect on consolidation. The fifth factor influencing the effect of corticosteroid exposure on memory performance relates to (e) the stage of learning at which corticosteroid exposure occurs. Performance on declarative memory tasks relies on three stages of processing: the encoding or acquisition of the new information, the consolidation of that information (i.e., the committing of information to long-term memory), and the retrieval of that information when it is needed and called back to the surface. It is not obvious, from the studies described above, which aspect of memory processing is affected by elevated glucocorticoid levels. In an attempt to isolate the stage at which elevating cortisol impairs memory, de Quervain et al. (2000) administered 25 mg of cortisone to 36 participants (n = 18 women) (i) 23 hours after learning and 1 hour before retrieval, (ii)1 hour before learning, and (iii) immediately after learning and 1 hour before retrieval. They found that verbal declarative memory was impaired only in the 24-hour delayed recall condition (i.e., the first condition). This piece of data isolates the effect of cortisol elevation to retrieval; there were no negative effects on memory performance in condition (ii), which tested effects on encoding, or in condition (iii), which tested effects on consolidation.

The authors argue that the pre-learning cortisol effects observed in other studies (e.g. Kirschbaum et al., 1996; Newcomer et al., 1999) may be explained by the designs of those studies. Within those designs, cortisol was elevated throughout all stages of memory processing, making it difficult to isolate the memory-impairing effects on any one stage of the process. In a later study (Coluccia et al., 2008) by the same laboratory, the experiment was replicated using 5 mg of prednisone instead (the equivalent dose to the 25 mg of cortisone used in the de Quervain et al., 2000 study). In support of the earlier findings, Coluccia and colleagues (2008) observed a significant impairment in performance in the prednisone group only. Furthermore, the authors found that a single dose of glucocorticoid produced impairment in performance on a word-paired associates' task (free recall) when it

was administered before delayed retention-testing, and not before encoding. These studies also indicate that the effects of exogenous glucocorticoids on memory are direct; the fact that immediate recall was not impaired suggests that these effects are not simply mediated by diffuse glucocorticoid effects on attention and arousal.

Sixth and last, besides the stage of memory processing most affected by the action of glucocorticoids, (f) the relevance of the information presented to the individual determines if and how well it is committed to memory. For instance, it is now well established in the literature that emotionally-charged experiences (i.e., those that are meaningful in terms of the identity and survival of the individual) are more likely to be remembered (Buchanan & Lovallo, 2001; Empson & Clarke, 1970; Kuhlmann et al., 2005; Rauchs et al., 2004; Tilley et al., 1978; Wagner et al., 2001, 2006; Walker, 2009). Hence, it is not altogether surprising that empirical evidence suggests that processes with influence on the efficacy of learning, such as glucocorticoid activity, affect emotionally-arousing material more than neutral material (Buchanan & Lovallo, 2001). For example, Kuhlmann et al. (2005b- the one with Piel- check if same in the 2005a kirschbaum one) observed that elevations of cortisol, induced by a psychosocial stressor, disrupted the recall of words with an emotional valence (positive or negative) but did not affect the recall of neutral words.

Chapter One discusses how the action of glucocorticoids on memory may also be mediated by sleep. The following sections discuss how acute exposure to glucocorticoid affects the structure of sleep, and how these effects may in turn affect memory performance.

The acute effects of glucocorticoids on sleep. Gillin, Jacobs, Fram, and Snyder (1972) devised the first experiment that systematically tested the objective effects of corticosteroids on sleep in healthy humans. They used a mixed design, including comparisons between drug versus placebo conditions among the same individuals, and between-group comparisons for low- (5 mg), medium- (20 mg) and high-dose (60 mg) prednisone exposure

conditions to test for dose-response effects. Their results suggested a drug-induced dose-response effect on sleep architecture, with the high-dose prednisone group experiencing significantly longer REM latencies, lower percentage REM sleep, and higher percentage stage 2 sleep than the low-dose and placebo groups. Although there were no other between-group differences in terms of sleep architecture, this pattern of data led the researchers to conclude that the effects of exogenous corticosteroids on sleep architecture differ from those of stress- or mood-induced elevations of endogenous corticosteroids (the latter typically have negative effects on sleep onset latency, sleep efficiency, delta sleep, and REM sleep).

Effects of exogenous versus endogenous glucocorticoids on sleep. Besides the respective effects of synthetic versus natural corticosteroids in relation to placebo conditions, there are significant differences between the two different types of corticosteroid treatments themselves on sleep. A few studies subsequently proceeded to test these differences between exogenous and endogenous corticosteroids, in an attempt to elucidate the mechanism(s) of action through which corticosteroids affect sleep in adult humans. The general consensus is that both endogenous and exogenous corticosteroids have a suppressive effect on REM sleep, but that a distinction emerges with respect to SWS. Cortisol, which is a naturally-occurring corticosteroid, tends to increase SWS (Born et al., 1987; Fehm et al., 1986), whereas synthetic corticosteroids, such as prednisone, dexamethasone or fluocortolone, tend to have no effect (Born et al., 1987; Gillin et al., 1972) or a suppressive effect on delta sleep (Fehm et al., 1986).

For instance, Fehm et al. (1986) found significant differences in the direction of the effect of dexamethasone versus hydrocortisone on stages 3, and 4 latencies and percentage SWS during the first half of the night. Dexamethasone lengthened delta sleep latencies, fragmented sleep, and reduced the percentage of SWS, whereas hydrocortisone achieved the exact opposite. The common effects of the two types of corticosteroids (i.e., the increase in

sleep fragmentation or WASO and the significant decrease in REM sleep) were restricted to the second half of the night.

Studies investigating the effects of corticosteroid exposure on sleep sometimes report increases in stage 2 sleep, but these changes generally fail to achieve statistical significance (e.g. Born et al., 1987; Fehm et al., 1986). For instance, Born et al. (1987) compared the effects of a natural glucocorticoid to a natural MR agonist and to a synthetic glucocorticoid. They found that none of the steroids affected stage 2 sleep. Data on the effect of glucocorticoids on stage 1 sleep, and on the quantity of intermittent wakefulness that occurs after initial sleep onset, are mixed: Some studies note significant increases in these sleep parameters (e.g., Plihal, et al., 1999; Vgontzas et al., 2003), whereas others fail to observe an effect when compared to placebo, control conditions (e.g., Born et al., 1987; Fehm, 1986).

One explanation for the differential effects of synthetic corticosteroids and cortisol might be the difference in potency across various groups of corticosteroids (Fehm et al., 1986). For instance, 25 mg of prednisone is equivalent to 3.75 mg of dexamethasone and 100 mg of hydrocortisone; that is, the ratio of potency of prednisone to hydrocortisone is 4:1, and of dexamethasone to hydrocortisone is 25:1. Therefore, if the effects of glucocorticoids affect SWS in an inverted-U manner as it does memory, then non-equivalent doses are likely to have different effects on delta sleep.

However, even when dose and potency are controlled for, the different classes of corticosteroids still seem to exert different effects on sleep. For instance, both works by Born et al. (1987) and Fehm et al. (1986) showed that synthetic or exogenous corticosteroids and natural or endogenous cortisol have diverging effects on the latency, the total amount and percentage of SWS, specifically on stage 4. For instance, hydrocortisone was associated with a significantly shorter SWS onset latency and a significantly greater amount and percentage of SWS relative to the placebo condition. However, the effect of fluocortolone (a synthetic

glucocorticoid) on SWS, although similar in its directionality, was marginal and statistically not significant.

Another explanation for the varying effects of synthetic versus natural corticosteroids on sleep is the difference in receptor binding affinity across these different corticosteroids. Synthetic corticosteroids bind mostly to GRs, whereas endogenous corticosteroids bind to both GRs and MRs. Cortisol is known to bind most effectively to MR (Type I) corticoid receptors, and only binds to GR (Type II) receptors when it is present at very high levels (Heffelfinger & Newcomer, 2001). The action of MRs is predominant during the first half of the night, when endogenous cortisol is at its nadir (Buckley & Schatzberg, 2005). It would therefore be reasonable to expect the greatest impact of an acute elevation of cortisol just before or during sleep to be most pronounced during the first few hours of sleep, which is where most delta sleep is concentrated. On the other hand, exogenous corticosteroids (e.g., prednisone) bind mostly to GRs, whose activity predominates during the REM-rich second half of sleep (Besedovsky et al., 2012; Buckley & Schatzberg, 2005; Wagner & Born, 2008).

Furthermore, Born and colleagues (1987) found that GR action alone modulated the electrophysiology of sleep, with MRs having only negligible effects. However, this finding is not equivocal, and as discussed in the General Introduction, a considerable portion of corticosteroid activity is heterodimeric (i.e., involves the combined action of GRs and MRs). In two separate but complementary studies, Plihal and Born (1999) found that (a) cortisol infusion (between 8-12 mg over a period of 2 hours) produced no effect on percentage SWS, but that (b) blocking MR activity during early sleep using canrenoate suppressed SWS by 15%. Furthermore, canrenoate action increased endogenous cortisol levels, which suggests that it is not free-circulating cortisol itself that has an effect on SWS.

Effects of corticosteroids on sleep-dependent memory processing. The relationship between acute corticosteroid activity, sleep, and memory processing can be studied

experimentally by either focusing on the effects of free circulating steroids, or by manipulating receptor activity through selective administration of MR and GR agonists and antagonists during sleep. In their first of a series of studies on sleep-dependent memory processing, Plihal and Born (1997) employed the first approach. From their findings, they drew one association between SWS and declarative memory, and another between REM sleep and procedural memory, by demonstrating that declarative memory was enhanced during early, SWS-rich sleep, while procedural memory benefitted more from REM-rich, late sleep. By inference, then, any process hindering SWS should primarily affect the consolidation of declarative memory, whereas any process hindering REM should primarily affect the consolidation of procedural memory. Since glucocorticoids influence the amount and distribution of SWS and REM, Plihal and Born postulated that the quiescent level of cortisol during SWS is one process that supports the enhancing properties of that stage of sleep for declarative memory. However, they observed that salivary cortisol was significantly lower during the early night relative to the late night testing period in both their wake and their sleep conditions. This finding would suggest dissociation between the effects of cortisol and of sleep on memory, thus failing to support the proposition that cortisol mediates the relationship between sleep and memory consolidation.

In a subsequent series of pioneering studies on the subject, the same team of researchers and their colleagues tested this proposition more directly by manipulating the levels of circulating cortisol and the receptor-ratio activity during retention sleep (i.e., period between the encoding of learnt material and retrieval performance comprising mostly of sleep). The timing of the interventions and the duration of the effects (i.e., after encoding had taken place, while ensuring that cortisol levels had returned to baseline before retrieval) were structured to isolate the process of memory consolidation from the other two stages of memory processing, and to thus test whether or not glucocorticoid action affects sleep-

dependent memory consolidation. While they observed a definite effect of altering the normal circadian glucocorticoid activity on declarative memory, the isomorphism between SWS-rich sleep and declarative memory was not supported by the evidence. That is to say, SWS activity did not appear to mediate the relationship between glucocorticoid activity and declarative memory performance during sleep (Born & Wagner, 2004; Plihal & Born, 1999; Plihal et al.,, 1999).

Plihal and Born (1999) demonstrated that verbal episodic memory was most impaired when cortisol was elevated during early sleep rather than during late sleep, whereas procedural memory was not affected at any point. However, in all of their experiments, Plihal and colleagues (Plihal & Born, 1999; Plihal et al., 1999) found no glucocorticoid effects on SWS whether cortisol was elevated by infusion of hydrocortisone continuously throughout retention sleep, or by the administration of canrenoate, or when it was suppressed following the administration of dexamethasone. The dissociation between glucocorticoid action on declarative memory versus its effect on SWS implies a direct effect of glucocorticoids on hippocampal functioning during sleep, that is, an effect independent of glucocorticoid effects on delta activity (Born & Wagner, 2004). It would appear, then, that alternating ratios of MR and GR receptor activity during early versus late sleep modulate memory processing directly, independently of their influence, or lack thereof, on stages of sleep.

Classically, findings from sleep studies suggest that the concomitant high MR activation, together with suppressed GR activation during early sleep, promote the consolidation of declarative memory for neutral information. The combined results from early studies using MR antagonists and GR agonists to validate this theory indicate that it is unlikely MRs make a contribution to cortisol-mediated memory consolidation. For instance, Plihal and colleagues (1999a, 1999b) showed that blocking MR action by administering the MR antagonist, canrenoate, intravenously to adult participants had no effect on retrieval of

either declarative or procedural memory material learnt prior to sleep. On the other hand, administration of the GR agonist dexamethasone significantly impaired declarative memory retrieval but did not affect procedural memory. These results support the conclusion that suppression of GRs during early sleep, more so than the activation of MRs, encourages the consolidation of declarative memory during that period of sleep.

However, a more recent study on the influence of the MR: GR ratio on memory consolidation during sleep, has revealed that MR blockade by metyrapone is associated with impairment on a declarative memory task (Wagner et al., 2005). The study revealed that participants performed more poorly on an episodic memory task involving neutral content, under the metyrapone-treatment condition compared to the placebo condition. Exposure to metyrapone was also associated with an attenuation of the typical rise of cortisol during late sleep and to reduced SWS. These latter findings are replicated elsewhere (Neylan et al., 2003). It is likely that this inconsistent evidence regarding the role of MRs in the process of memory consolidation rest on an issue of potency.

As opposed to the early study that used canrenoate and that failed to induce SWS suppression (Plihal et al., 1999a), the MR antagonist used in later studies induced such suppression. Hence, there is the suggestion that there is higher potency of the more recent interventions. Recall that it is possible that the effects of MRs on memory have to be mediated by SWS. In other words, unless MRs are suppressed sufficiently to induce a marked reduction in cortisol and subsequently impact on the quantity of delta activity, fluctuations in their level of activity do not influence memory consolidation. It is also possible that MRs act directly on memory processes. The higher affinity of these receptors for cortisol renders the range of their action more stable; only a marked fluctuation in their activity will result in an effect on performance. The latter view is supported by evidence (a) of the effects of spironolactone on working and long-term verbal memory (findings discussed previously) and

(b) that MR activation supports LTP (Cornelissen, Fagard, Coeckelberghs, & Vanhees, L. 2011).

The behavior of MRs in the glucocorticoid-memory relationship lends support to the theory proposing that glucocorticoids affect memory consolidation in an inverted-U manner. Suppressing cortisol below its optimal, effective point, such that MRs are under-occupied, hinders memory. Similarly, raising cortisol to a level where GRs are hyperactive hinders effective memory processing. Both of these actions are often achieved experimentally through the administration of moderate-to-high doses of exogenous corticosteroids such as dexamethasone and prednisone.

In summary, two mechanisms of action have been postulated to explain the relationship between acute corticosteroid activity, sleep, and memory consolidation. The first proposed mechanism of action is that corticosteroid activity affects memory consolidation during sleep by altering sleep architecture (more specifically, by suppressing SWS or REM). The second proposed mechanism of action is that sleep-dependent memory consolidation is modulated by the ratio of MR and GR activation at any given point during sleep. The evidence in the literature favors the second theory over the first, given that there are empirical demonstrations of glucocorticoid-induced influences on sleep-dependent learning in the absence of changes in sleep architecture. Having said that, the varying balance of MR: GR activity during SWS and REM sleep assists in the consolidation of different aspects of memory. The remainder of this section describes the separate, and yet complementary, effects of corticosteroid activity and sleep architecture on memory.

Thus far, the leading theory describing how cortisol affects memory consolidation states that the hormone starts disrupting processing when it is raised to a level high enough to recruit hippocampal GR receptors (Born et al., 1992; Buckley & Schatzberg, 2005; Heffelfinger & Newcomer, 2001; Wagner et al., 2005, 2008). During early sleep, optimal

levels of MR activation and GR suppression are necessary to support the consolidation of neutral, recent experiences such as learning new words, a recipe, or a formula (Plihal et al., 1999; Plihal & Born, 1999; Born & Wagner, 2004). During late sleep, the rise in GR activation moderates the emotional tone of an experience. In other words, an episodic memory is stripped of its emotional intensity, therefore facilitating rational functioning on the following day (Vandekerckhove & Cluydts, 2010; Wagner et al., 2005, 2008; Walker, 2009). Therefore, in contrast to the traditional dichotomy that associates declarative memory with delta sleep and procedural memory with REM sleep (Plihal & Born, 1997), it appears that both SWS and REM sleep consolidate, and regulate consolidation of, aspects of declarative memory.

Typically, the elevation of cortisol during late, REM-rich sleep is believed to moderate amygdalar activity and to thereby assist in the stripping of the emotional tone from the episodic phenomena associated to it, especially when the emotional tone is negative (Vandekerckhove et al., 2010; Wagner et al., 2005; Walker, 2010). Wagner et al. (2005) draw a parallel between low basal cortisol levels and the overwhelming combination of over-consolidation of emotional declarative memories but the under-consolidation of neutral declarative memories in PTSD to illustrate the relationship between cortisol and sleep stages in assisting memory consolidation. The deduction is that rising cortisol during REM sleep actually limits consolidation of emotional (and especially aversive) content. Related supporting evidence comes from studies of major depressive disorder: In depression, high cortisol during early sleep and relatively lower cortisol during late sleep is associated with a high incidence of nightmares, persistence of negative affect, and anxiety (e.g. Antonijevic & Steiger, 2003; Shipley et al., 1992; Steiger, 2007).

The electrophysiological (SWA, spindle activity), molecular (glucose transport from glia to hippocampal CA1 neurons), and cellular (glutamatergic neuronal excitation; long-term

potentiation) processes that are inherent to SWS and REM sleep consolidate memory through the processes of reactivation (including the reactivation of memory trace in hippocampus, in the amygdala and the neocortex, as well as the transfer of information from limbic system to neocortex) and assimilation (transfer of information from the neocortex to the hippocampus), respectively. Imbalances in MR: GR ratios disrupt memory consolidation by disrupting these processes during these SWS and REM sleep even without reducing the "quantity" of each sleep stage. In other words, abnormal glucocorticoid activity during sleep has neurotoxic effects that impair the *function* of sleep without necessarily affecting the gross macrostructure of sleep. Impairment of sleep-dependent learning thus precedes disruptions in sleep architecture, with the latter being an indicator of the severity of the glucocorticoid effect.

Rationale, Specific Aims, and Hypotheses

The aim of this study was to investigate the effects of increasing glucocorticoid activity during sleep by administering a combined MR and GR agonist, namely prednisone, to healthy young adults. The outcome measures included (a) performance on tasks tapping into different memory subsystems, (b) differences in performance on declarative versus procedural memory tasks before and after sleep, (c) sleep stage distribution, and (d) dreaming.

In contrast to hydrocortisone, prednisone is an exogenous, synthetic corticosteroid that is metabolized into prednisolone within 1 hour of being ingested. It is an intermediate-acting glucocorticoid, with a longer half-life and a lower percentage of MRs than hydrocortisone, and is generally administered orally. Both types of glucocorticoids reduce percentage REM but hydrocortisone alone increases SWS (specifically stage 4; Born et al., 1987; Fehm et al., 1986; Gillin et al., 1972). Exogenous corticosteroids reduce SWS by suppressing or down-regulating endogenous cortisol (Fehm et al., 1986).

Besides its use in the pioneering study on the effects of exogenous corticosteroids on sleep by Gillin and colleagues (1972), much less is known about prednisone's specific actions on cognition and sleep, especially when compared to the actions of dexamethasone. However, prednisone remains the most widely prescribed glucocorticoid to treat a host of conditions, ranging from cancer through adverse reactions to certain medications to inflammatory conditions such as rheumatoid arthritis and asthma. In the case of asthma and arthritis, prednisone is used primarily to treat acute symptom exacerbations of the chronic illnesses, and is occasionally prescribed as a maintenance drug in severe cases. This widespread use makes it particularly important to study the specific effects of this glucocorticoid on sleep-dependent cognition. Furthermore, the pioneering study on the effects of glucocorticoids on sleep architecture (Gillin et al., 1972) used prednisone. Given what we know of the specificity of effects across glucocorticoids, it is necessary to verify and build on existing knowledge by adopting the same agent.

Hence, the administration of prednisone here sought to induce an acute circadian reversal of the distribution and structure of sleep, and to compare the subsequent effects to those of the circadian reversal noted in asthmatics (a reversal resulting from chronic exposure to synthetic corticosteroids). Although there is an existing body of evidence on the effects of exogenous corticosteroids on sleep architecture and sleep-dependent memory processing (see, e.g., Born et al., 1987, 2006, 2009; Fehm et al., 1986; Gillin et al., 1972; Plihal et al, 1999; Wagner et al., 2007), no investigation has yet examined how these effects affect the content of dreams or how the content of dreams may be related to this relationship.

The study design allowed testing of eight sets of predictions. The first set of predictions stated that prednisone-treated participants would perform more poorly than placebo-control participants on (a) the Autobiographical Memory Test, because of the potential effects of raised glucocorticoid activity on the successful retrieval of episodic

autobiographical information, (b) the Digit Span test, because of the effects of elevated glucocorticoid levels on short-term auditory attention span (Digit Span Forward component) and on working memory performance (Digit Span backward component). In contrast, performance on (c) semantic memory tasks will not be affected by the intake of prednisone. Furthermore, I predicted that (d) there will be no between-group differences on baseline measures of the verbal episodic memory tasks administered before sleep (VPA I and LM I) as those were administered immediately upon ingestion of either prednisone or placebo and were completed within 15 minutes post-ingestion, a period deemed too short to have any impact of memory performance.

The second set of predictions stated that, while glucocorticoid levels would be low in the beginning of sleep and would rise progressively as the night progresses for the placebo-control participants, the ingestion of prednisone approximately 2 hours before bedtime would cause glucocorticoid activity to be raised from sleep onset to the half-mark of an 8-hour sleep period among prednisone-treated participants, after which it would decline gradually to reach normal early morning levels.

As a result, the third set of predictions stated that, relative to placebo-control participants, prednisone-treated participants would experience (a) longer sleep onset and REM latencies, greater sleep fragmentation (i.e., %WASO) and reduced sleep efficiency, reduced percentage REM sleep and SWS, and would (b) have smaller differences between the distribution of SWS and REM sleep during early and late sleep, also translating as lower SWS distribution and lower REM intensification scores. I made no predictions about the distribution of stages 1 and 2 sleep, and simply explored the data for potential between-group differences.

The fourth set of predictions stated that, relative to placebo-control participants, prednisone-treated participants would perform less well on measures of post-sleep (a)

declarative memory performance and (b) procedural memory performance. Furthermore, the prednisone-treated group would show less improvement, or even experience decrements, between their post- sleep and pre-sleep performances on memory tasks (both declarative & procedural). In contrast, Placebo-control participants would experience improvements in their post-sleep memory performances, relative to their pre-sleep performances.

The fifth set of predictions stated that the proportion as well as the distribution of both SWS and REM would mediate the relationship between glucocorticoid levels and declarative memory performance. In other words, glucocorticoid elevations during the night would suppress these stages of sleep and would disrupt their normal circadian organization, and these disruptions would in turn hinder memory performance. Hypothesis set 5 also predicts that elevated levels of glucocorticoids will be associated with a greater percentage of light sleep (percentage stage 1 and percentage stage 2), and greater sleep fragmentation (reduced percentage sleep efficiency and increased percentage of WASO). However, given that these effects are smaller and less consistent in the literature, I do not expect these sleep outcome measures to mediate the relationship between glucocorticoid levels and memory performance.

The sixth set of predictions stated that, because of REM suppression, prednisone-treated participants would perform more poorly on the procedural memory retest session (post-sleep) than on the post-training session (pre-sleep). Previous studies have demonstrated that, in contrast to declarative memory, procedural memory is not affected by fluctuations in glucocorticoid levels. However, procedural memory is affected by disruptions in sleep, in particular by disruptions in REM or at stage 2. I therefore also predicted that post-sleep performance on the Finger Tapping Task (FTT), for all participants, would not be associated with glucocorticoid levels but would instead be associated with percentage REM sleep and percentage stage 2 sleep.

The seventh set of predictions stated that the fragmentation and lightening of sleep by prednisone would cause prednisone-treated participants to (a) recall fewer dreams. If recalled, I predicted (b) that their dreams would have impoverished textures, such as measured by the subjective qualia variables (bizarreness, visual vividness, and emotional intensity) and would be more thought-like in nature.

The eighth and final set of predictions stated that the flattening of the descending slope of sleep by the intake of prednisone would (a) be associated with reduced episodic content in dreams, and that (b) in terms of broad dream theme categories (1. residue of the day, 2. experiment-related, or 3. idiosyncratic), prednisone-treated participants would experience proportionally fewer dreams with waking continuity themes (types 1 & 2) to dreams with idiosyncratic themes.

Methods

Design and setting. Study 2 was set in the same location as Study 1 (i.e., at the Vincent Pallotti Hospital sleep laboratory). I used a double-blind, randomized placebo-controlled experimental design to assess the effects of prednisone on sleep-dependent memory consolidation. The main predictor variable in this study was the treatment status of the participant, with two levels of variation: prednisone or placebo. The outcome measures were clustered into the following four categories: (a) glucocorticoid level (measured at three time points), (b) memory performance (declarative, procedural, and working memory), (c) sleep quality variables (sleep onset latency, REM latency, sleep efficiency, REM sleep percentage, SWS percentage, stage 1, stage 2 and WASO percentages), and (d) dream quality and content. All outcome measures were treated as continuous variables, with the exception of certain dream variables.

The rest of this section (a) details the operationalization of the main predictor variable by describing the process of participant selection and stratification, (b) describes the materials and apparatus used in the study, (c) defines the outcome variables, and (d) details the approach to data management and analysis.

Sample and participant selection. Participants were recruited from a population of university students using non-probabilistic convenience sampling. Potential participants responded to an electronic advert circulated on a university intranet site. They were then contacted for screening and were selected for participation if they met a specific set of criteria. In total, 56 potential participants were screened, and 28 met the criteria for participation. Figure 4 details the flow of participants through the different stages of the experiment and summarizes the reasons for exclusion of the 28 remaining individuals. Two of the 28 participants eligible for testing decided to withdraw from the study due to the time commitment required of them. Hence, the final sample of participants was 26 English-speaking individuals between the ages 18 and 39 years (M = 22.00, SD = 5.29). They were assigned randomly to two demographically-matched (specifically with regard to age, sex, race, and IQ) groups: The prednisone-treated group and the Placebo-control group (n = 13 each). The data from one of these participants data were excluded from analysis after she failed to fall asleep during the sleep-testing night (Group = Placebo, sex = female, age = 19 years). Of the 25 participants who completed the study, the data from one were excluded from final analyses because she was an outlier (Group = prednisone, sex = female, age = 21 years) on the cortisol measures collected at REM2 and REM3. Outliers were identified and dealt with following the same protocol as in Study 1. Finally, then, the data from 24 participants (n = 12 per group) were subject to the final analyses.

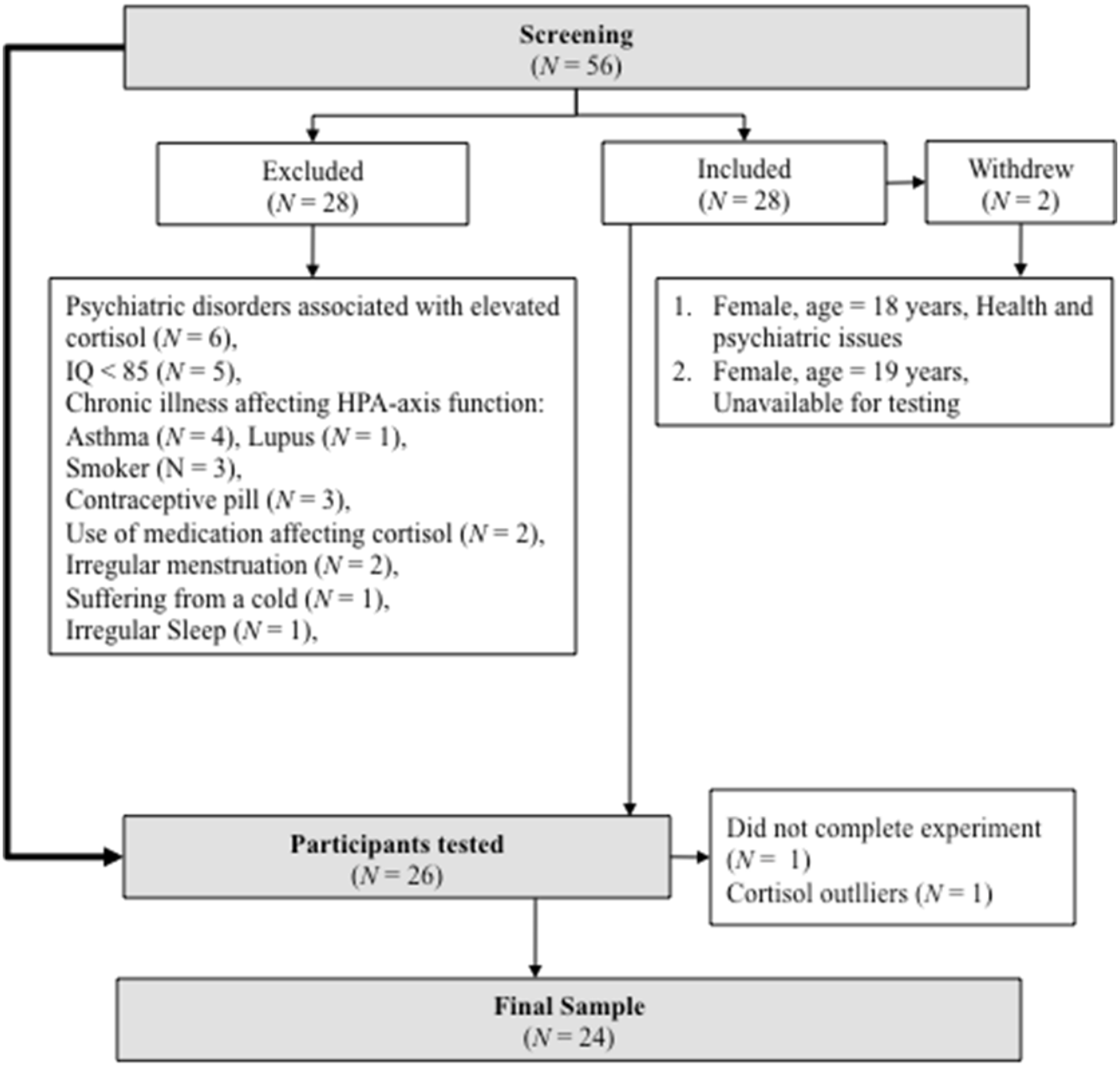

Figure 4. Flow chart of participant attrition.

Exclusion criteria. There were in total seven categories of exclusion criteria, all of which were similar to those of Study 1, with the exception of categories dealing specifically with asthma. In addition, the *health status* category included considerations on medical conditions where the use of corticosteroids is contra-indicated.

Age. Individuals outside the age range of 18-45 years were excluded. The reasons for this restriction is that older adults are known to have (a) altered cortisol circadian rhythms

(Clow, 2004), (b) hippocampal atrophy (Lupien et al., 1994, 1998; McEwen, 1999), (c) altered sleep cycles, and (d) different quality of dreams (Kales et al., 1968).

General intellectual functioning. At in-person screening, I assessed IQ formally to control for any between-subject differences that could influence performance on the administered cognitive tasks. Any potential participant with an IQ score below 85 (i.e., below the norm-defined "average" range) was excluded.

Psychiatric co-morbidity. At in-person screening, I administered psychiatric screening interviews and self-report questionnaires. Any individual experiencing current or chronic psychiatric disturbance associated with altered endogenous cortisol levels (e.g., any affective disorder) was excluded. For instance, depression is related to hypocortisolism, and is frequently co-morbid with chronic illness (see, e.g., Heim et al., 1999).

Health status. Individuals with a self-reported history of the following conditions were excluded from the study: peptic ulcer, osteoporosis, congestive heart failure, diabetes mellitus, chronic renal failure and uremia, quiescent tuberculosis, glaucoma, hypertension, myasthenia gravis, malaria, and thromboembolic disorders. High levels of glucocorticoids are known to exacerbate these conditions (Gibbin, 2000). In addition, individuals with any acute (e.g., common cold or influenza) or any other chronic medical condition not listed above (e.g., asthma) or any neurologic disorder (e.g., epilepsy) were excluded.

Smoking. Smokers were excluded from participation as smoking has been reported to result in heightened cortisol stress responses (Badrick et al., 2007).

Disrupted sleep. Any person reporting difficulty falling asleep, frequent disruptions during sleep, premature morning awakening, erratic sleeping patterns, or any formally diagnosed sleeping disorder was excluded from participation.

Exogenous or endogenous factors altering the balance of female reproductive hormones. For the sake of cross-sex comparisons, I adopted the same approach as in Study 1

with regard to selecting female participants. Hence, only women with regular menstrual cycles and those not using any form of hormone-based contraceptives were considered for participation. Female participants were tested during their luteal phase, in keeping with findings that identify this phase as a period of elevated cortisol approximating average male levels (Kuhlmann et al., 2005).

Materials and instruments. The same screening and testing materials and instruments used in Study 1 were used in the present study. The Sociodemographic and Health Information questionnaire was adapted to suit the requirements of Study 2, however (see Appendix I). The focus of the questionnaire in Study 1 was to determine the participant's asthma history, symptomatology, and treatment regimen. In contrast, the focus of the questionnaire in Study 2 was to isolate any condition that (a) could be exacerbated by the intake of cortisone, or (b) is associated with deregulations of the HPA-axis. In addition, the current experiment included the administration of oral cortisone in the form of prednisone capsules.

Assessing eligibility via the Sociodemographic and Health Information questionnaire. Participants were required to provide demographic information such as their sex, date of birth, and years of schooling. They were then asked to report if they smoked tobacco. Any degree of smoking was considered a factor for exclusion. The participants were also asked about their use of psychoactive substances. Current and chronic use of recreational drugs resulted in exclusion. Consumption of alcohol was investigated using the MINI, and participants indicating abuse and/or dependency were excluded.

The health information section of the questionnaire included a list of disorders known to either require or be exacerbated by glucocorticoid treatment. Potential participants were required to disclose if they had suffered from any of these conditions at any stage in their lifetime. Further, the questionnaire enquired about (a) sleeping habits, subjective evaluations

of quality of sleep, and any formally diagnosed sleep disorder, (b) chronic illnesses, (c) current medical conditions such as seasonal colds and flu, (d) history of any surgical interventions, and, if female, (e) menstrual cycle.

Prednisone preparation and placebo equivalent capsules. Prednisone is a synthetic, oral form of corticosteroid with short-acting glucocorticoid properties. Each participant in the Prednisone group was given 25 mg of prednisone orally. The biological half-life of prednisone ranges from 8 to 12 hours (Goodman & Gilman, 1980; Gupta & Bhatia, 2008), with peak effects experienced 1 to 2 hours after administration (Gibbin, 2000). The 25-mg dose has been identified in the literature as a minimum dose with physiological effects mimicking mild stress (de Quervain et al., 2003; Henzen et al., 2000). Placebo-control participants were given a lactose preparation. Both the prednisone and the lactose preparation were ground into a fine white powder and encapsulated to look identical (prepared and dispensed by RAUSA- KEM Pharmacy, Tygerberg Hospital).

Procedure. Study 2 followed the identical protocol as Study 1, with the single difference that participants were given prednisone or a placebo equivalent.

The first participant of each sex was given the opportunity to choose from two batches of capsules (one denoted by a circle and the other by a square). Thereafter, capsules were systematically alternated for men and women. This procedure was followed to ensure that the Prednisone and Placebo groups had an equal representation of both sexes.

The experiment began with the participant ingesting the chosen capsule. The time at which the capsule was taken was recorded. Pre-sleep testing was divided into two sessions. During Session 1, participants ingested either prednisone or the placebo, and the researcher then administered the VPA I list, the LM I task, and the FTT (training session). Session 1 lasted approximately 15 minutes.

At the end of Session 1, the participant was left to settle in the patient room. Session 2 resumed exactly 1 hour after s/he had taken the capsule. It included the WAIS-III Information and Digit Span subtests, the BNT, and the AMT. The time gap between the two sessions was required because of the time it takes for prednisone to manifest its peak effects, which is between 1 and 2 hours after administration (Gibbin, 2000).

Regarding the measurement of levels of free circulating corticosteroids, it is important to note that there is a degree of cross-reactivity between cortisol and prednisolone. Prednisolone's elimination half-life occurs within 4 to 5 hours of administration. Therefore, analysis of saliva samples taken within 8 to 10 hours of prednisone administration is likely to detect circulating prednisolone together with cortisol (Coluccia et al., 2008; Czock, Keller, Rasche, & Haussler, 2005). However, in Study 2, the salivary measures were taken to verify that the administration of prednisone before bedtime had enduring effects during the first two cycles of sleep, and had tapered off by the end of the sleep period. The purpose was not to evaluate the levels of cortisol versus prednisolone, or the effects of prednisone on endogenous cortisol. For this reason, corticosteroids measurements from the prednisone-treated individuals are henceforward referred to as "glucocorticoid" level, while the corticosteroids measurements from the placebo controls are referred to as "cortisol".

Ethical considerations. All study procedures were approved (reference number: 166/2007) by the Research Ethics Committees of the University of Cape Town's Department of Psychology and Faculty of Health Sciences (see Appendix J).

Informed consent, voluntary participation, and deception. Participants were provided with elaborate written and verbal information about the study and gave their informed consent before being formally enrolled in the study. The information supplied included details about the study procedures, its risks and benefits, and assurance that the tests would not harm them in anyway and that they would be compensated for their time.

Additionally, the consent form (see Appendix K) secured their right to withdraw participation at any stage of the study, without penalty.

The participants did not know beforehand if they were being given prednisone or a placebo because I used a double-blind design for group assignment. However, they were informed of the 50% possibility of being given prednisone during the screening process.

Risks and benefits. Although participants did not benefit directly from the study, they did receive information on the relationship between corticosteroids and sleep and learning. Participants were given a copy of their hypnograms and a brief presentation on sleep architecture.

Participant safety protocol. A medical indemnity form gave participants the assurance that the researcher and her affiliated research team would take responsibility for their safety and wellbeing for the entire duration of the study.

Any participant with a medical condition that prohibited the use of corticosteroids was systematically excluded. The dose of prednisone (25 mg) used in this study is documented as carrying no harmful or long-term effects on health and functioning (de Quervain et al., 2003). prednisone is metabolized into prednisolone, which is in turn effectively metabolized by the liver and passed out in urine (http://www.medsafe.govt.nz/profs/datasheet/p/prednisonetab.pdf). Nevertheless, a safety protocol was put in place, with the collaboration of the Vincent Pallotti Hospital nursing staff and Emergency Room, to ensure that participants could be assisted adequately in the event of an adverse reaction to prednisone. No such incident took place. Participants were driven home in the morning, after they had completed the experiment, in case they experienced lingering effects of the medication.

Data management and statistical analyses. As in Study 1, the analysis began with an exploration of the data, using the same tests and general approach to establish normality of

distribution, homogeneity of variance, and identification of significant outliers. I normalized the glucocorticoid data using rank transformations because they were not normally distributed and violated the assumption of homogeneity of variance. Formal inferential statistical analyses then proceeded following the steps described below.

First, I tested Hypothesis Set 1 (i.e., predictions regarding the effects of prednisone on (a) autobiographical memory (AMT), (b) working memory and short-term auditory attention span (WAIS-III Digit Span), (c) semantic memory (BNT and WAIS-III Information), and (d) baseline verbal episodic memory (VPA I and LM I) by using Student's *t*-tests, or Mann-Whitney *U* tests as the non-parametric equivalent where necessary.

Second, I used a series of mixed-model ANOVAs to test Hypothesis Set 2, testing glucocorticoid levels across the night, with three levels of variation (REM1 versus REM2 versus REM3), with Group (prednisone-treated versus placebo control) as the independent, between-group factor.

Third, for Hypothesis Set 3, I used Student's *t*-tests, or Mann-Whitney *U* tests as the non-parametric equivalent to test predictions regarding the effects of prednisone on sleep parameters such as sleep onset latency, REM onset latency, and percentage sleep efficiency (Hypothesis 3.a.). Then, I used a series of mixed-model ANOVAs to test the distribution of sleep stages across the night with proportions of stages 1, 2, SWS, REM sleep, and WASO with two levels of variations each (early sleep versus late sleep), with Group (prednisone-treated versus placebo control) as the independent, between-group factor.

Fourth, for Hypothesis Set 4, I used two series of mixed-model ANOVAs to test performance on the declarative memory task (VPA-15), with two levels of variation (immediate recall/pre-sleep versus delayed recall/post-sleep), and performance on the procedural memory task (FTT), with three levels of variation (pre-sleep baseline versus pre-

sleep post-training versus post-sleep), with Group (prednisone-treated versus placebo control) as the independent, between-group factor.

Fifth, to test certain triadic models, that is Hypothesis Set 5 and Hypothesis Set 6, I first ran three series of exploratory correlations to establish which glucocorticoid measures correlated with the sleep parameters and memory performance measures mentioned in the rationale, aims and hypothesis section. I ran non-parametric correlations (Spearman's *rho*) in equations involving glucocorticoids levels as these data were not distributed normally.

In series 1, I correlated all of these glucocorticoid measures with sleep parameters and predicted that they would correlate negatively with (i) % REM sleep, (ii) REM Intensification, (iii) % SWS sleep, (iv) SWS Distribution, (v) sleep efficiency, but positively with (vi) WASO, (vii) % stage 1 sleep, and (viii) stage 2 sleep.

In series 2, I tested relationships between glucocorticoid levels and post-sleep memory performance, and predicted that they would all correlate negatively with (i) VPA II, (ii) VPA Retention, (iii) LM II, and (vi) LM Retention. I correlated glucocorticoid measures and post-sleep procedural memory measures (vii) FTT Speed retest and (viii) FTT Accuracy retest, but made no predictions about the strength or direction of those relationships.

In series 3, I correlated percentage REM, percentage SWS, REM Intensification, SWS Distribution, and stage 2 with VPA II, LM II, FTT Speed retest, and FTT Accuracy retest. I predicted that each of the five sleep measures would correlate positively with both of the episodic memory measures and with both of the procedural memory measures.

Then, as a second step, I tested the following triadic models using mediational analyses to test whether sleep mediated the relationship between glucocorticoid levels and (a) performance on declarative memory tasks (Hypothesis Set 5), and (b) the enhancement of procedural learning (Hypothesis Set 6), in the morning. I ran a series of simple linear regression analyses in each case, based on the following assumptions:

To test Hypothesis Set 5, I predicted that (a) higher glucocorticoids levels during sleep would predict poorer declarative memory performance after sleep, (b) higher glucocorticoid levels during sleep would predict lower percentage SWS, REM sleep, sleep efficiency, and lower REM Intensification and SWS Distribution scores, but higher percentage stage 1, stage 2 sleep and WASO (c) that higher percentage of SWS, REM, and sleep efficiency and higher REM Intensification and SWS Distribution scores would in turn predict delayed, post-sleep declarative memory recall, and (d) the sleep parameters measuring the proportion of REM and SWS (% REM & % SWS) as well as those measuring their organization (SWS Distribution & REM Intensification scores) would mediate the relationship between glucocorticoid levels and memory performance.

To test Hypothesis Set 6, I predicted that (a) glucocorticoid levels during sleep would not be associated with performance on the FTT post-sleep measures, (b) poverty in REM sleep, in the proportion of stage 2, and in sleep efficiency would predict poor performance on the procedural task in the morning, and therefore that the association between these sleep parameters and performance on procedural memory tasks the following morning would be independent of glucocorticoid activity.

For Study 2, I treated Rate of Recall, that is whether the participant recalled a dream or not during each of three awakenings, as a dichotomous categorical variable where an awakening could generate one of two responses: Recall, that is when a dream was clearly remembered and No Recall, that is when no dream scenario came to mind. Similarly, a dream category could either fall under the theme of Waking Continuity, which referred to any dream with clear references to recently experienced events (including of the experiment situation), or under the theme of Idiosyncratic, which like in Study 1 represented dream scenarios with creatively associated content, with little direct or obvious connection to contextualized waking experiences. I decided to collapse Residue of the Day and Laboratory-related dreams

into a single category, namely Waking Continuity, owing to the small number of dreams that were reported. Therefore, to test Hypotheses 7a and 8b, I analyzed Rate of Recall and Dream Category using 2x2 chi-square tests with Group as the dependent variable.

To test Hypotheses 7b and 8a, I used Student's *t*-test to analyze between-group differences on the *thought-like* qualia variable of the dreams reported and on all three measures of memory content (*objective average episodic content*, *subjective episodic content*, and *original content scores*). I used Mann-Whitney *U* tests for the rest of the subjective ratings of the dream qualia variables (*bizarreness*, *visual vividness*, and *emotional intensity*) as these data were not normally distributed.

I could not analyze the progression of the dreams in terms of memory content, category, or qualia across the three different awakenings because there were too few cases to run reliable within-subject comparisons.

Results

Sample characteristics. The two groups were well matched in terms of age, distribution of sex and race, and general intellectual functioning (see Table 13). The data in the table also show that the groups were well matched with regard to (lack of) depressive symptomatology, with no individual participant falling within the range that the BDI-II describes as clinically depressed. The latter was expected, given the study's eligibility criteria.

Table 13
Demographic Details of Study Participants (N = 24)

Variable	Group: Prednisone (*n* = 12)	Group: Placebo (*n* = 12)	t / χ^2	p
Age (years)	22.00 (4.41)	22.00 (6.25)	0.00	1.00
Sex (F:M)	5:7	6:6	0.17	.50
Race (B:C:I:W)	7:0:2:3	3:4:1:4	5.83	.12
WASI PIQ	105.08 (11.27)	114.50 (15.72)	1.69	.11
BDI-II	4.83 (3.30)	3.92 (3.12)	-0.70	.49

Note. For the variables *Age*, *WASI PIQ*, *WASI FSIQ*, and *BDI-II*, means are presented with standard deviations in parentheses. Regarding the variable *Race*, the designations refer to Apartheid-era South African population groupings of Black, Coloured, Indian, and White. WASI = Wechsler Abbreviated Scale of Intelligence; PIQ = performance IQ. The *t*-test values were calculated with 22 degrees of freedom.

Testing Hypothesis Set 1: The effects of prednisone on memory performance.

This section details between-group comparisons on measures of verbal episodic memory, short-term auditory attention span and working memory, and of semantic memory.

Verbal episodic memory. Table 14 shows descriptive statistics and between-group comparisons for performance on all of the pre-sleep declarative memory tests. I report the between-group differences in post-sleep performances and the differences between pre- and post-sleep performances under Hypothesis set 4. As can be seen, there were no significant between-group differences in baseline memory performance.

Table 14

Between-group Comparisons: Pre-sleep declarative memory outcome variables (N = 24)

	Group				
Variable	Prednisone (*n*=12)	Placebo (*n*=12)	*t*	*p*	ESE
Word-paired associates task					
VPA I	10.75 (2.38)	11.67 (3.14)	0.81	.42	0.33
Story recall task					
LM I	46.25 (5.74)	48.92 (10.25)	0.79	.44	0.32
Learning Slope	6.08 (2.47)	5.08 (3.29)	0.84	.41	-0.34
Autobiographical Memory Test					
No. of specific memories	6.33 (3.11)	25.17 (3.90)	-0.81	.21	-0.33

Note. Means are presented with standard deviations in parentheses. VPA I = refers to Verbal Paired Associates score I measuring the immediate, pre-sleep, cued recall score on the word-paired associates task. LM I = refers to Logical Memory score I measuring the immediate, pre-sleep free recall score on the story recall task. The *t*-test values were calculated with 22 degrees of freedom. *p* values were set at .05.

Short-term auditory attention and working memory. As Table 15 shows, there were no between-group differences on a measure of auditory attention span (the Digit Span Forward subtest), but there was a significant difference (in favour of the Placebo group) on a measure of working memory (the Digit Span Backward subtest). That between-group difference was associated with the large effect size.

Table 15

Between-group Comparisons: Auditory attention and working memory variables (N = 24)

Note. Means are presented with standard deviations in parentheses. The *t*-test values were calculated with 22 degrees of freedom. *p* values were set at .05. ESE here refers to *r* effect size estimates.

	Group				
Variable	Prednisone (*n*=12)	Placebo (*n*=12)	*t*	*p*	ESE
Digit Span Forward	10.38 (2.57)	10.50 (2.68)	0.00	.50	0.05
Digit Span Backward	6.85 (1.52)	8.5 (2.61)	1.80	.04	0.77

Semantic memory. As Table 16 shows, there were no between-group differences on the measures of semantic memory.

Table 16

Between-group Comparisons: Semantic memory variables (N = 24)

	Group				
Variable	Prednisone (*n*=12)	Placebo (*n*=12)	*t/U*	*P*	ESE
Boston Naming Test	46.25 (10.91)	48.25 (9.07)	65.50	.72	0.20
WAIS-III Information	17.83 (4.39)	17.83 (4.49)	0.00	1.00	0.00

Note. Means are presented with standard deviations in parentheses. A Mann-Whitney *U* test and *t*-test were performed for the Boston Naming Test and the Information test, respectively. ESE here refers to *r* effect size estimates. The *p*-values presented here are two-tailed.

Testing Hypothesis Set 2: Levels of circulating glucocorticoids during sleep. I tested this set of hypotheses by first using a mixed-model repeated-measures ANOVA on the rank-transformed cortisol data, and following that with two separate repeated-measures ANOVAs examining the data from each group separately. Table 17 presents the relevant descriptive statistics.

Regarding the overall set of data, Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(2) = 17.50$, $p < .001$. Hence, I used the Huynh-Feldt corrected degrees of freedom ($\varepsilon = .652$). The results of this correction were confidently accepted, despite its reputation for being liberal, because the uncorrected *F* values as well as the Greenhouse-Geisser corrected *F* values generated the same conclusions with respect to statistical significance. Furthermore, the results of the multivariate analysis confirm the conclusions drawn from the mixed-model ANOVA. Multivariate analyses do not make assumptions about sphericity. Lastly, Levene's tests revealed that there was homogeneity of variance at each level of the within-subject factor comparison.

Table 17
Night-time Glucocorticoid Levels for each Group at Three Awakenings (N = 18)

	Group	
Measurement point	Prednisone (*n* = 9)	Placebo (*n* = 9)
REM1	33.63 (11.88)	2.29 (2.25)
REM2	26.18 (9.95)	2.79 (2.77)
REM3	5.91 (4.75)	10.50 (8.14)
Average	21.91 (7.40)	5.19 (4.17)

Note. Means are presented with standard deviations in parentheses. REM1, REM2, and REM3 refer to the first, second, and third REM sleep awakenings, respectively, at which times salivary cortisol was collected. The data from 6 participants (3 in each group) were excluded from these analyses because each had fewer than three cortisol data points.

The mixed-model repeated-measures ANOVA detected a significant main effect of Group, $F(1, 16) = 8.90$, $p = .009$, partial $\eta^2 = .36$, with the Prednisone group averaging higher glucocorticoid levels. Although that analysis did not detect a main effect of Time, $F(1.30, 20.86) = 0.82$, $p = .405$, partial $\eta^2 = .05$, it did detect a significant Group x Time interaction effect, $F(1.30, 20.86) = 20.15$, $p < .001$, partial $\eta^2 = .56$.

The two separate repeated-measures ANOVAs revealed that, in the Placebo group, cortisol levels increased as the night progressed, $F(1.23, 9.84) = 12.57$, $p = .004$, partial $\eta^2 = .61$, as one would predict under normal circumstances. In contrast, glucocorticoid levels decreased across the night in the Prednisone group, $F(1.32, 10.57) = 7.92$, $p = .013$, partial $\eta^2 = .50$, as the effects of the medication tapered off.

Planned simple linear contrasts revealed that for both groups, these within-group differences in glucocorticoid level were significant when comparing REM1 and REM3, and when comparing REM2 and REM3, with only marginal differences between REM1 and REM2 levels. Figure 5 depicts the glucocorticoid levels at REM1, REM2, and REM3 for the

two groups. The main effect of Time in the mixed-model ANOVA was likely neutralized by the almost exact mirror opposite trends observed in the two groups.

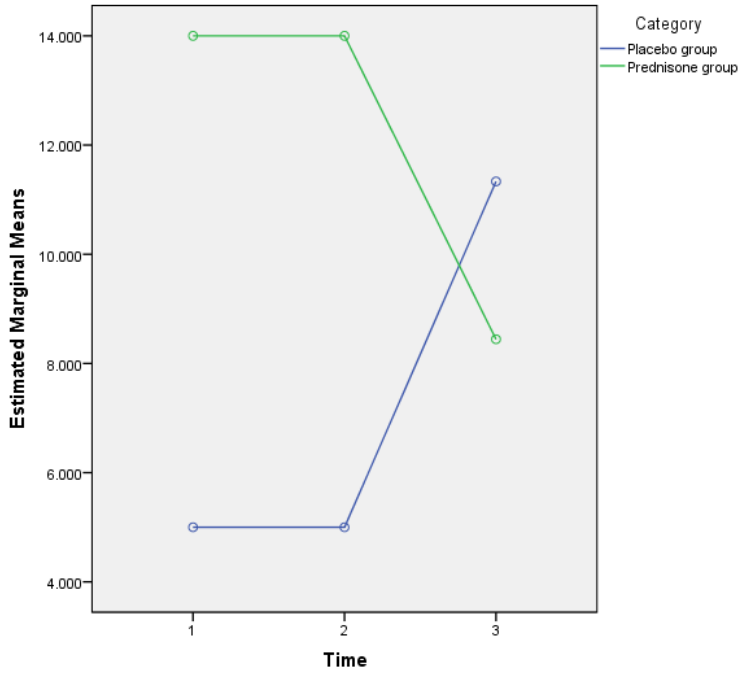

Figure 5. Night-time glucocorticoid levels in each group (estimated marginal means).

Testing Hypothesis Set 3: Prednisone and sleep architecture. The sleep onset and the whole night sleep stage variables met the criteria for normal distribution and homogeneity of variance. Consequently, I performed parametric analyses on the raw data for these variables. For the early versus late sleep comparisons, the variables percentage SWS, percentage REM sleep, percentage stages 1 and 2 sleep all met the criteria for equality of covariance matrices, and all met the assumption of homogeneity of variances between each level of within-group comparison. Regarding the WASO variable, I performed analyses on log-transformed data due to violations of equality of the covariance matrix and the significance of Levene's test of homogeneity of variance. The transformation was successful in addressing these violations of assumptions underlying parametric analyses. Table 18 provides the relevant descriptive statistics.

Table 18
Descriptive Statistics: Sleep outcome variables (N = 23)

	Group	
Variable	Prednisone (*n*=11)	Placebo (*n*=12)
Sleep Latency	12.68 (13.25)	12.75 (11.15)
REM latency	123.18 (63.23)	124.83 (72.22)
Sleep Efficiency	77.15 (10.43)	86.38 (5.90)
WASO %		
Whole Night	22.75 (10.20)	13.60 (5.88)
First Half	32.50 (14.86)	22.90 (11.76)
Second Half	17.81 (17.98)	6.40 (4.50)
Stage 1 NREM%		
Whole Night	8.04 (5.60)	8.07 (3.40)
First Half	9.97 (7.03)	8.94 (5.51)
Second Half	8.29 (4.22)	6.71 (4.10)
Stage 2 NREM%		
Whole Night	45.75 (11.43)	45.35 (8.07)
First Half	37.63 (14.47)	39.39 (18.79)
Second Half	48.26 (21.38)	49.28 (11.63)
SWS %		
Whole Night	9.57 (7.29)	12.64 (7.13)
First Half	11.81 (7.72)	19.73 (9.26)
Second Half	6.57 (6.82)	4.93 (5.29)
REM %		
Whole Night	13.38 (4.93)	21.68 (6.52)
First Half	6.39 (5.90)	9.01 (6.17)
Second Half	19.64 (6.52)	32.65 (9.40)
SWS Distribution Score	5.24 (5.11)	14.80 (7.83)
REM Intensification Score	13.25 (6.94)	23.64 (9.23)

Note. Means are presented with standard deviations in parentheses. All figures are presented as percentages. One data set was lost due to equipment malfunction (Group: Prednisone, sex = F, age = 19 years).

The independent-samples *t*-tests detected no significant between-group differences in terms of sleep onset latency, $t(21) = 0.13$, $p = .50$, Cohen's $d = 0.01$, or in terms of REM latency, $t(21) = 0.58$, $p = .477$, Cohen's $d = 0.02$. However, the analysis detected a significant between-group difference with regard to sleep efficiency, $t(21) = 2.65$, $p = .01$, Cohen's $d = 1.09$, with participants in the Prednisone group experiencing, on average, significantly lower percentage sleep efficiency than those in the Placebo group.

Regarding the WASO variable (i.e., percentage of time spent awake after first falling asleep), a mixed-model repeated-measures ANOVA detected a significant main effect of

Time, $F(1.00, 21.00) = 41.35$, $p < .001$, partial $\eta^2 = .67$. This analysis, and the associated descriptive statistics, suggests that all participants experienced a greater percentage of wakefulness during Early sleep than during Late sleep. The analysis also detected a significant main effect of Group, $F(1, 21) = 5.35$, $p = .031$, partial $\eta^2 = .21$; on average, participants in the Prednisone group experienced significantly more WASO than those in the Placebo group. The analysis detected no significant Time x Group interaction effect, $F(1.00, 21.00) = 1.90$, $p = .183$, partial $\eta^2 = .09$.

Regarding percentage of stage 1 sleep observed, a mixed-model repeated-measures ANOVA detected no main effect of Group, $F(1, 21) = 0.47$, $p = .50$, partial $\eta^2 = .02$, or of Time (Early sleep versus Late sleep), $F(1.00, 21.00) = 2.90$, $p = .103$, partial $\eta^2 = .12$. The analysis also detected no significant Group x Time interaction, $F(1.00, 21.00) = 0.06$, $p = .813$, partial $\eta^2 = .003$.

Regarding percentage of stage 2 sleep observed, a mixed-model repeated measures ANOVA detected a significant main effect of Time, $F(1.00, 21.00) = 8.53$, $p = .008$, partial $\eta^2 = .29$. This analysis, and the associated descriptive statistics, suggests that all participants experienced more stage 2 sleep during the second half of the night (Late sleep) than during the first (Early sleep). The analysis detected no significant main effect of Group, $F(1, 21) = 0.07$, $p = .791$, partial $\eta^2 = .003$, and no significant Time x Group interaction, $F(1.00, 21.00) = 0.01$, $p = .917$, partial $\eta^2 = .001$.

Regarding the distribution of SWS across the sample, a mixed-model repeated-measures ANOVA detected a significant main effect of Time, $F(1.00, 21.00) = 48.12$, $p < .001$, partial $\eta^2 = .71$, with early sleep containing a much greater percentage of SWS than late sleep. The analysis detected no significant main effect of Group, $F(1, 21) = 1.23$, $p = .280$, partial $\eta^2 = .06$, but it did detect a significant Group x Time interaction effect, $F(1, 21.00) = 10.95$, $p = .004$, partial $\eta^2 = .35$.

Regarding the distribution of REM sleep across the sample, a mixed-model repeated measures ANOVA detected a significant main effect of Time, $F(1.00, 21.00) = 115.62$, $p < .001$, partial $\eta^2 = .85$, with late sleep containing a much greater percentage of REM than early sleep. The analysis also detected a significant main effect of Group, $F(1, 21) = 10.12$, $p = .004$, partial $\eta^2 = .33$, with the Placebo group, on average, experiencing more REM than the Prednisone group. Finally, the analysis also detected a significant Group x Time interaction effect, $F(1, 21.00) = 9.18$, $p = .006$, partial $\eta^2 = .30$

Between-group comparisons examining differences in SWS Distribution and REM Intensification scores revealed that Prednisone group participants had, on average and relative to placebo group participants, (a) significantly less SWS during early sleep (i.e., when SWS is usually most prominent), $t(20) = 3.31$, $p = .004$, Cohen's $d = 0.69$, and (b) significantly less REM during late sleep (i.e., when REM sleep is usually most prominent), $t(21) = 3.03$, $p = .006$, Cohen's $d = 1.61$.

Testing Hypothesis Set 4: Overnight gains in memory performance. The analyses described under this heading either use a repeated-measures design, and therefore only include tests with comparable pre-sleep, baseline performances and post-sleep, re-test performances, or include measures of delayed retention where sleep constituted the long retention period.

Verbal declarative memory. There were no between-group differences in performances on the VPA II the LM II tasks. Furthermore, VPA Retention and LM Retention scores did not differ significantly between the two groups, indicating that ingesting prednisone before sleep did not affect performance on those verbal declarative memory tasks more than ingesting the placebo did. Table 19 provides the details for these analyses. Of note, the average percentage retention scores for both tests, in both groups, lie below 100%, indicating a decrement rather than an enhancement in performance post-sleep. In the case of

the word-paired associates' task, this decrement is significant for the sample as a whole, as indicated by the results of the mixed model ANOVA. The results of that analysis revealed a significant main effect of Time on VPA-15 performance, $F(1.00, 22.00) = 5.99$, $p = .023$, partial $\eta^2 = .21$, with performance declining from pre- to post-sleep measurement. The analysis detected no significant main effect of Group, $F(1, 22) = 0.73$, $p = .401$, partial $\eta^2 = .03$, and no significant Group x Time interaction, $F(1, 22.00) = 0.94$, $p = .763$, partial $\eta^2 = .004$.

Table 19
Between-group Comparisons: Post-sleep declarative memory outcome variables (N = 24).

	Group				
Variables	Prednisone (*n* = 12)	Placebo (*n* = 12)	*t*	*p*	EESE
Word-paired associates task					
VPA II	10.00 (2.70)	11.08 (3.42)	0.86	.20	0.37
VPA Retention %	93.21 (13.95)	94.50 (13.20)	0.23	.41	0.09
Story recall task					
LM II	29.33 (5.90)	30.92 (6.45)	0.63	.27	0.26
LM Retention %	84.25 (9.33)	86.25 (10.24)	0.50	.31	0.20

Note. Means are presented with standard deviations in parentheses. VPA II = refers to Verbal Paired Associates score II measuring the delayed, post-sleep, cued recall score on the word-paired associates task. LM II = refers to Logical Memory score II measuring the delayed, post-sleep free recall score on the story recall task. The *t*-test values were calculated with 22 degrees of freedom. *p* values were set at .05.

Procedural memory. The *F* statistics for the within-subject effects reported for the Speed outcome variable underwent Huynh-Feldt corrections due to a violation of sphericity. Sphericity was assumed for the Accuracy measure. Log and square-root transformations were unsuccessful in addressing issues of (a) covariance of matrices on the measure of FTT Speed, at all three within-group levels combined ($p = .040$), and (b) both covariance of matrices (.001) and of homogeneity of variances on the measure of FTT Accuracy, at all within-group levels combined. Excluding outliers (details below) resolved some of the homogeneity of variance problems, but did not resolve issues regarding covariance. Ranked transformations were the only type of transformation that successfully eliminated these violations.

However, while using ranked transformations in a mixed-design ANOVA worked for the cortisol data (i.e., the results garnered using these transformations were consistent with the results of all the other analyses run on differently-transformed data), using ranked data for the FTT did not. The results differed from those obtained when analyses were run using differently-transformed data and from those obtained using the Friedman's ANOVA test. Therefore, I decided to accept the results of the mixed-model ANOVA run on the raw data, especially because those results were consistent with results from both the Friedman's ANOVA test and the multivariate tests generated automatically by SPSS. Multivariate analyses do not rely on the assumptions of independence of covariance or homogeneity of variances.

Regarding the FTT Speed outcome variable, a mixed-model repeated-measures ANOVA, excluding the data from 1 outlier (n = 1; Group: Prednisone, Age = 22 years, Sex = M), detected significant main effects of Time, $F(1.83, 34.69) = 182.25$, $p < .001$, partial η^2 = .91, and of Group, $F(1, 19) = 7.65$, $p = .012$, partial $\eta^2 = .29$. Speed increased significantly from baseline to subsequent test points and the Placebo group typed a significantly greater number of sequences compared to the Prednisone group.

The analysis did not detect a significant main effect of Sex, however, $F(1, 19) = 0.71$, $p = .410$, partial $\eta^2 = .04$.

In terms of interactions, the analysis detected a significant Time x Group interaction, $F(1.83, 34.69) = 6.28$, $p = .006$, partial $\eta^2 = .25$. However, there were no significant Time x Sex, $F(1.83, 34.69) = 0.38$, $p = .669$, partial $\eta^2 = .02$, Group x Sex, $F(1, 19) = 0.85$, $p = .367$, partial $\eta^2 = .04$, or Time x Category x Sex interactions, $F(1.83, 34.69) = 0.54$, $p = .573$, partial $\eta^2 = .03$.

To investigate the Group main effect in detail, I ran post-hoc Mann-Whitney tests for baseline, post-training and post-sleep performances between the two groups. The analyses

revealed that baseline Speed did not differ between prednisone-treated participants and placebo controls, $U = 53.00$, $p = .45$, $r = -.17$. However, prednisone-treated participants performed significantly more poorly than placebo controls on both post-training. $U = 28.50$, $p = .02$, $r = -.48$, and post-sleep FTT Speed measures, $U = 27.50$, $p = .02$, $r = -.49$.

To investigate the Time x Group interaction more closely, I ran post-hoc Wilcoxon signed-ranked tests for each group separately. The analyses revealed differing performance trends within each group. In the Placebo group, Speed increased significantly from the average baseline score to the average post-training score, $Z = -3.06$, $p < .001$, $r = -.91$, and from the average post-training score to the average post-sleep score, $Z = -1.83$, $p = .037$, $r = -.37$. In contrast, in the Prednisone group, while Speed increased significantly from the average baseline score to the average post-training score, $Z = -2.94$, $p < .001$, $r = -.90$, there was no further significant increase between the average post-training score and the average post-sleep score, $Z = -0.80$, $p = .23$, $r = -.17$.

Regarding the FTT Accuracy outcome variable, a mixed-model repeated-measures ANOVA, excluding the data from 2 outliers (Group: Prednisone, Age = 22 years, Sex = M and Group: Placebo control, Age = 21 years, Sex = F), detected a significant main effect of Time, $F(2, 36) = 9.51$, $p < .001$, partial $\eta^2 = .35$. On the other hand, there were no significant effects of Group, $F(1, 18) = 0.64$, $p = .43$, partial $\eta^2 = .04$, nor of Sex, $F(1, 18) = ,47$, $p = .50$, partial $\eta^2 = .03$ on Accuracy. In terms of interactions, the analysis detected a significant Time x Group interaction effect, $F(2, 36) = 5.84$, $p = .006$, partial $\eta^2 = .25$. However, there were no significant Time x Sex, $F(2, 36) = 1.03$, $p = .368$, partial $\eta^2 = .05$, Group x Sex, $F(1, 18) = 0.42$, $p = .53$, partial $\eta^2 = .02$, or Time x Group x Sex interaction effects, $F(2, 36) = 1.14$, $p = .33$, partial $\eta^2 = .06$.

To investigate the Time x Group interaction more closely, I ran post-hoc Wilcox on signed-ranked tests for each group separately. In the Placebo group, Accuracy increased

significantly from the average baseline score to the average post-training score, $Z = -2.85$, $p < .001$, $r = -.58$. In contrast, there was no significant increase in Accuracy between the average baseline score and the average post-training score, $Z = -1.07$, $p = .16$, $r = -.23$, in the Prednisone group.

There were no significant differences between the average post-training Accuracy scores and the average post-sleep Accuracy scores in both groups. Placebo group: $Z = -0.89$, $p = .207$, $r = -.18$, and Prednisone group: $Z = -1.51$, $p = .07$, $r = -.32$. Table 20 presents descriptive statistics for all of the FTT measures.

Table 20
Procedural Memory Outcome Variables (Speed N = 23; Accuracy N = 22)

	Group	
	Prednisone	Placebo
Variable		
FTT Speed	(*n* = 11)	(*n* = 12)
Baseline	6.64 (2.62)	7.83 (3.38)
Post-training	46.91 (13.77)	63.83 (17.56)
Post-sleep	46.64 (16.02)	66.92 (18.76)
FTT Accuracy %	(*n* = 12)	(*n* = 12)
Baseline	92.93 (9.55)	76.89 (19.17)
Post-training	90.75 (2.84)	96.03 (1.81)
Post-sleep	92.22 (6.34)	95.70 (1.32)

Note. Means are presented with standard deviations in parentheses. FTT Speed = finger tapping task outcome variable measuring the total number of sequences completed within a fixed timeframe. FTT Accuracy = finger tapping task outcome variable measuring the percentage of correct sequences completed within a fixed timeframe. The analyses were performed on 23 participants for Speed and on 22 participants for Accuracy, excluding outliers.

Testing Hypothesis Set 5: The relationship between glucocorticoid levels, sleep and post-sleep declarative memory performance.

Step 1, Series 1. I used Spearman's non-parametric analyses to correlate glucocorticoid levels with outcome measures of light sleep (percentage stage 1 and percentage stage 2 sleep), of deep sleep (percentage SWS and SWS Distribution), of REM sleep (percentage REM and REM Intensification), and of sleep fragmentation (percentage

sleep efficiency and WASO) across the entire sample, and then for each of the two groups separately. Table 21 provides the details of these correlations. In the whole sample, glucocorticoid levels were inversely associated with percentage REM sleep, and to REM Intensification and SWS Distribution scores. Also in the whole sample, glucocorticoid levels were positively associated with WASO. In the Placebo group, cortisol was positively associated with percentage SWS. In the Prednisone group, glucocorticoid levels were positively associated with the light stages of sleep and inversely associated with REM Intensification scores.

Table 21
Relationship between Night-time Glucocorticoid Levels and Sleep Parameters

		Glucocorticoid level			
Sleep parameters		GC1	GC2	GC3	Mean GC
%SWS					
	Whole sample	-.20	-.17	-.06	-.26
	Prednisone	-.37	-.32	-.37	-.47
	Placebo	.37	.42	.23	.25
%REM					
	Whole sample	-.58**	-.45*	.08	-.55**
	Prednisone	-.25	.13	-.12	-.20
	Placebo	-.03	.08	.07	.13
SWS Distribution					
	Whole sample	-.48*	-.47*	.42*	-.38
	Prednisone	-.13	-.05	.40	-.03
	Placebo	-.15	.15	.37	.23
REM Intensification					
	Whole sample	-.57**	-.54*	.42*	-.48*
	Prednisone	-.58*	-.70*	< .001	-.43
	Placebo	.22	.38	.50	.45
%Stage 1					
	Whole sample	.01	.07	-.002	.04
	Prednisone	.18	.08	-.19	.16
	Placebo	-.07	.35	.07	.02
%Stage 2					
	Whole sample	.12	.01	.36	.16
	Prednisone	.60*	.27	.47	.58*
	Placebo	-.05	-.20	.10	<.001
%Total efficiency					
	Whole sample	-.50*	-.46*	.36	-.41*
	Prednisone	.02	.10	<.001	-.03
	Placebo	-.14	-.03	.28	.20
%WASO					
	Whole sample	.49*	.46*	-.35	.41*
	Prednisone	-.03	-.11	-.01	.03
	Placebo	.14	.03	-.28	-.20

Note. The values represented in this table refer to Spearman's non-parametric correlation coefficients *rs*. GC refers to glucocorticoid collection and GC1, GC2, and GC3 refer to the first, second, and third glucocorticoid collections, respectively. The data from 6 participants are missing due to insufficient amounts of saliva samples collected for those individuals at one or more of the three collection times. I ran the analyses for the whole sample (N = 18) and for the Prednisone group (n = 9) and the Placebo group (n = 9), separately. Significance level was set at p <.05.
p < .05. ** p < .01.

Step 1, Series 2. I used Spearman's non-parametric analyses to correlate glucocorticoid levels with post-sleep performance on (a) declarative memory tests (VPAII, LMII, VPA Retention, and LM Retention) across the entire sample, and then within each group separately. Table 22 shows the results of those correlational analyses. In the Placebo group, night-time cortisol was significantly and inversely associated with LM II, and LM Retention. In the Prednisone group, glucocorticoid levels were not significantly associated with any of the post-sleep memory measures. In the sample as a whole, glucocorticoid level at REM2 was significantly and negatively correlated with LM II scores.

Table 22
Night-time Glucocorticoid Levels and Post-sleep Performance on Memory Tasks.

		Glucocorticoid level			
		GC1	GC2	GC3	Mean GC
Memory measures					
VPAII	Whole sample	-.07	-.07	.33	.02
	Prednisone	.27	.34	.11	.15
	Placebo	.29	.16	.48	.46
VPA Retention					
	Whole sample	.12	.01	-.05	.12
	Prednisone	.48	.21	-.14	.18
	Placebo	-.07	-.29	.08	.04
LMII					
	Whole sample	-.40*	-.34	-.20	-.37
	Prednisone	-.49	-.30	-.10	-.35
	Placebo	-.71*	-.63*	-.45	-.47
LM Retention					
	Whole sample	-.22	-.24	-.31	-.23
	Prednisone	.10	-.09	-.18	.01
	Placebo	-.54	-.42	-.55	-.50
FTT Speed Retest					
	Whole sample	-.68**	-.59**	.03	-.67**
	Prednisone	-.26	-.14	-.31	-.24
	Placebo	-.36	-.07	-.27	-.37
FTT Accuracy Retest					
	Whole sample	-.44*	-.56*	-.15	-.51*
	Prednisone	-.29	-.41	-.10	-.41
	Placebo	-.46	-.62	-.50	-.60

Note. The values represented in this table refer to Spearman's non-parametric correlation coefficients *rs*. GC refers to glucocorticoid collection and GC1, GC2, and GC3 refer to the first, second, and third glucocorticoid collections, respectively. The data from 6 participants are missing due to insufficient amounts of saliva samples collected for those individuals at one or more of the three collection times. For FTT, the data set of 1 Prednisone group participant was excluded for being an outlier. I ran the analyses for the whole sample (N = 18) and for the Prednisone group (n = 8) and the Placebo group (n = 9), separately. Significance level was set at p <.05. *p <.05. **p <.01.

Step 1, Series 3. I used Spearman's non-parametric analyses to correlate sleep measures (stage 1, stage 2, percentage REM, percentage SWS, REM Intensification, SWS

Distribution & sleep efficiency) and declarative memory measures. As Table 23 shows, total sleep efficiency was significantly and positively elated to VPA II for the whole sample. With regards to REM sleep, there were significant relationships (both positive and negative) between percentage REM and REM Intensification and declarative memory performance in the Prednisone group only. SWS Distribution was positively and significantly correlated with VPA II for the whole sample.

Table 23

The Relationships between Sleep Parameters & Memory Performance in the morning (N = 23).

		Sleep parameters							
Memory tasks		%SWS	%REM	SWS Distribution	REM Intensification	%Stage 1	%Stage 2	%Total efficiency	%WASO
VPAII	Whole sample	.12	-.13	.36*	-.18	-.02	.34	.40*	-.39
	Prednisone	.16	-.45	.49	-.56*	-.39	.34	.34	-.30
	Placebo	.09	-.35	.27	-.17	.27	.39	.27	-.27
VPA Retention									
	Whole sample	.21	-.09	.19	-.12	-.18	.21	.19	-.19
	Prednisone	.32	-.22	-.06	-.29	-.36	.14	.14	-.15
	Placebo	.12	-.16	.08	-.23	.08	.22	.22	-.22
LMII									
	Whole sample	-.004	.14	.06	.10	-.07	.07	.05	-.05
	Prednisone	.33	.27	-.04	.72**	.17	-.07	.12	-.12
	Placebo	-.46	.03	.11	-.32	-.21	.22	.11	-.11
LM Retention									
	Whole sample	-.07	.01	-.07	.05	.29	-.07	-.02	.03
	Prednisone	-.26	-.60*	-.28	.01	.45	.06	-.21	.25
	Placebo	-.03	.19	-.07	.01	.12	-.12	.12	-.12
FTTSpeedRetest									
	Whole sample	.38*	.20	.40*	.13	.07	.11	.28	-.27
	Prednisone	.58*	.27	.22	-.16	-.31	-.18	.12	-.07
	Placebo	.14	-.55*	.42	-.12	.35	.51*	-.02	.02
FTTAccuracyRetest									
	Whole sample	-.32	.17	.26	.19	.21	.10	.21	-.22
	Prednisone	-.33	-.04	.29	.14	.13	-.01	-.04	.04
	Placebo	-.66*	-.34	-.17	-.14	.09	.38	-.13	.13

*Note. N = 23 for most correlations but FTTSpeed *sleep measures, N =22 one data point missing for FTT measure and one missing for sleep.*

Step 2. Although the analyses described above established that glucocorticoid levels at REM1 were associated with LM II scores, I did not attempt a mediational analysis (referred as step 2 in the method's section) testing REM Intensification as the mediator in the relationship between glucocorticoid levels at REM1 and LM II, as REM Intensification was not associated with LM II score in the whole-sample analysis.

Of note, in the Prednisone group, percentage REM sleep was negatively correlated with LM Retention. Although I did not explore this finding further owing to the small group sample size, I discuss the potential implications of this finding.

Testing Hypothesis Set 6: The relationship between glucocorticoid levels, sleep and post-sleep procedural memory performance. Contrary to what was expected, FTT Speed and FTT Accuracy after sleep correlated negatively with average glucocorticoid levels and glucocorticoid levels at REM1and REM2 for the sample as a whole. The correlations did not reach statistical significance when the two groups were analyzed separately. Furthermore, in the whole-sample analysis, FTT Speed correlated positively with percentage SWS and SWS Distribution but not with measures of REM or stage 2 sleep. Similarly, in the Prednisone group, FTT Speed correlated positively with percentage SWS but not with REM or stage 2 sleep. Interestingly, the data for the Placebo followed the opposite trend: the association Speed and SWS measures did not achieve statistical significance. However, FTT Speed was positively and significantly correlated with both percentage REM and stage 2 sleep, as predicted (see Table 23 above for details). I therefore decided to test SWS parameters, and not REM or stage 2 as suggested in previous literature, as the sleep mediators in the relationship between glucocorticoids and procedural memory performance.[8]

[8] Although, the hypothesis was confirmed for the placebo group, the size of that sub-sample (n = 12) was too small to meet the requirements for a mediational analysis.

In a further step, regression analysis did not detect a significant linear relationship between percentage SWS and FTT Speed post-sleep, even after using transformations in the model (see Appendix L). Hence, the only mediational relationship I proceeded to test was that between night-time glucocorticoid levels, SWS Distribution, and procedural memory performance post-sleep in the whole sample. The mediational model used to test whether sleep architecture (in this case, SWS Distribution) mediated the relationship between glucocorticoid levels and memory performance (in this case, speed of performance on the FTT post-sleep retest measure) rested on four assumptions (Baron & Kenny, 1986):

Step 1. Direct relationship c: Average glucocorticoid levels predicted FTT Speed post-sleep, R^2= .44, $F(1, 12) = 10.92$, $p = .005$, $\beta = -0.93$.

Step 2. Indirect relation a: Average glucocorticoid levels predicted SWS Distribution, R^2= .27, $F(1, 15) = 5.55$, $p = .032$, $\beta = -.0.65$.

Step 3. Indirect relation b: SWS Distribution predicted FTT Speed post-sleep, R^2= .29, $F(1, 19) = 5.79$, $p = .026$, $\beta = 0.50$.

Step 4. Mediational relationship c': SWS Distribution weakened the strength of the relationship between average glucocorticoid levels and FTT Speed post-sleep, R^2= .49, $F(1, 13) = 1.43$, $p = .254$.

Because all four criteria of the mediation model were met, I concluded that, in this particular sample as a whole, SWS Distribution did in fact mediate the relationship between average glucocorticoid levels and procedural memory.

Of note, as can be seen in Table 23 above, the relationships between (a) procedural memory performance and sleep and between (b) procedural memory and glucocorticoid levels were very different for the group, compared with the Placebo group. In the Placebo group, FTT Speed post-sleep was positively correlated with percentage REM and with percentage stage 2 sleep, whereas FTT Accuracy post-sleep was negatively correlated with

percentage SWS. Glucocorticoid levels were inversely related to post-sleep memory performance. In the Prednisone group, FTT Speed post-sleep was positively correlated with percentage SWS, FTT Accuracy post-sleep was not significantly correlated with any sleep parameter, and there were no associations between glucocorticoid levels and post-sleep memory performance. I did not explore these group-specific relationships further owing to the small sample sizes from which they were generated, but their potential implications will be discussed.

Testing Hypothesis 7a: Prednisone and dream recall. Through the night and across groups, I performed a total of 66 awakenings, of which 21 resulted in reports of dreaming. Although awakenings generated more *no recall* responses from prednisone-treated participants than from Placebo-treated participants, a chi-square analysis detected no significant between-group differences in terms of the ratio of *recall* versus *no recall*. Table 24 provides details of the chi-square test performed.

Testing Hypothesis 7b: Prednisone and the qualia of dreams. Hypothesis 7 stated that prednisone-treated participants would on average, score lower on measures of dream *bizarreness*, *visual vividness*, and *emotional intensity* but higher on the *thought-like* measure. This hypothesis was partially-confirmed. Regarding the subjective evaluations of the dreams reported, there were no between-group differences on self-ratings of bizarreness, thought-like quality, and vividness of the visual imagery. However, participants in the Prednisone group rated their dreams significantly higher on emotional intensity. Table 24 provides the details of these comparisons.

Testing Hypothesis 8a: Prednisone and the episodic content of dreams. The hypothesis stating that prednisone-treated participants would have less episodic content in their dreams was disconfirmed. Table 24 provides details of the independent-samples *t*-tests performed on the measures relating to the memory content of dreams. In sum, there were no

significant between-group differences on any of those measures, be they subjective or objective.

Testing Hypothesis 8b: Effects of a single dose of prednisone before bedtime on the type of dreams reported. The hypothesis stating that prednisone-treated participants would experience proportionally fewer dreams with waking continuity than dreams with idiosyncratic themes was disconfirmed.

Table 24 provides details of the chi-square analysis testing this hypothesis. Participants in the Placebo group reported twice the number of dreams with waking continuity themes than did participants in the prednisone group. In contrast, there were no between-group differences in terms of the number of idiosyncratic dreams. Overall, there was no significant between-group difference in the distribution of dreams with waking continuity versus dreams with idiosyncratic themes.

Table 24
Between-group comparisons of dream variables.

Dream variable		Group: Prednisone (*n* = 12)	Group: Placebo (*n* = 12)	*N*	$\chi^2/t/U$	*df*	*p*	*ESE*
Recall rate (%)								
	Recall vs. No recall	30: 70	33: 67	66	0.07	1	.500	0.03
Dream category								
	Waking Continuity vs. Idiosyncratic	20: 80	45: 55	21	1.53	1	.221	0.27
Memory content								
	Objective average episodic content	8.78(1.65)	6.72(2.39)	13	-1.68	11	.060	-1.00
	Subjective average episodic content	4.99(1.81)	5.04(3.16)	14	0.36	12	.486	0.02
	Average original content	3.66(2.65)	1.99(1.77)	14	-1.43	12	.090	-0.74
Qualia								
	Bizarreness	3.00(3.49)	4.33(2.93)	24	49.50	22	.103	0.41
	Thought-like	6.55(3.88)	6.15(2.97)	24	59.50	22	.248	-0.12
	Visual vividness	6.27(3.07)	5.23(2.92)	24	-0.85	22	.202	-0.35
	Emotional Intensity	6.82(3.74)	3.77(3.42)	24	36.00	22	.019	-0.85

Note. Percentages are presented for the categorical recall and dream category variables. Means are represented with standard deviations in parentheses for the memory content and the qualia variables. Of the 24 dreams reported, 3 dream reports could not be rated objectively due to the poor quality of the recordings. Odd ratios were calculated for the dream recall rate and dream category analyses and the numbers represent the likelihood of a prednisone-treated (a) recalling dreams and (b) having dreams with Waking continuity themes relative to placebo-control participant. Fisher's Exact test statistic is reported for (b). *ESE* is represented as Cramer's *V* for dream recall and category variables and as Cohen's *d* for the other variables.

Discussion

Effects of prednisone on memory: Hypothesis set 1. The aim of the first set of hypotheses was to test the effect of ingesting 25 mg of prednisone on subsequent memory performance, independently of any effects of sleep. The section discusses the effects of prednisone on verbal episodic memory, short-term auditory attention span, working memory, semantic memory and procedural memory.

Verbal episodic memory. The hypothesis stating that "prednisone-treated participants would perform more poorly than placebo-control participants on the Autobiographical Memory Test, because of the potential effects of raised glucocorticoid activity on the successful retrieval of episodic autobiographical information" was not confirmed. Participants in the Prednisone group did not retrieve fewer autobiographical memories than their placebo-control counterparts 90 minutes after ingesting 25 mg of prednisone. These results are consistent with previous findings which also did not detect a significant difference in the ability to retrieve specific memories between corticosteroid exposure and non-exposure conditions. For instance, Young et al. (2011) found no effect of glucocorticoid dose (placebo vs. moderate dose vs high dose of infused hydrocortisone) on the number, the specificity (specific vs. categorical), or the valence of memories retrieved. Similarly, Schlosser et al. (2010) did not find any significant main effects of treatment (hydrocortisone vs placebo) nor of group (participants diagnosed with major depression disorder vs. healthy controls) on the number of specific memories retrieved. However, both these studies detected significant interaction effects, either with regards to dose of corticosteroid exposure and specificity of memory (Young et al., 2011) or with treatment and group (Schlosser et al., 2010).

With regard to dose and specificity of memory retrieved, Young et al. (2011) found an interaction effect between the dose of glucocorticoid and the proportion of specific to categorical memories retrieved. They found that the high-dose exposure (mean total dose =

31.8 mg, S.D = 8.74) was associated with the retrieval of a greater number of memories which lacked specificity, that relative to both moderate-dose exposure (mean total dose = 10.9 mg, S.D = 2.05) and placebo conditions. This finding is consistent with the literature reviewed in the introduction section of the current chapter, which states that up to a certain threshold, the higher the glucocorticoid activation the more likely the declarative memory impairment.

With regards to treatment and group interaction effects, Schlosser et al. (2010) found that under the placebo condition, participants suffering from major depressive disorder (MDD) retrieved fewer specific memories than did healthy controls. Ingesting hydrocortisone impaired the performance of healthy controls, while not affecting that of MDD participants. In other words, MDD participants performed the same whether they ingested hydrocortisone or a placebo. The authors argue that hydrocortisone exposure did not bring about a further reduction in the number of specific memories which the MDD participants retrieved on average, because MDD patients are believed to have underactive glucocorticoid receptors. That is, the HPA-axis response to fluctuations in cortisol levels is blunted in individuals suffering from major depression.

The effects described above can only be detected within a within-subject framework of analysis, not applicable to the current study. Similarly, Buss et al. (2004) found a significant difference between their hydrocortisone-exposure condition versus their placebo condition using a within-subject design. The same participants retrieved fewer specific memories 60 minutes following exposure to 10 mg of hydrocortisone. The current study did not expose participants to varying doses of prednisone, nor did it compare the response of a patient sample to that of healthy controls under different exposure conditions. My study only compared the performance of healthy adults who had ingested prednisone (a single dose) to that of other healthy adults who had ingested a placebo.

On the other hand, hypothesis 1d which stated there would be "no between-group differences on baseline measures of the verbal declarative memory tasks administered before sleep (VPA I and LM I)" was confirmed. As mentioned previously, the VPA I and the LM I were administered immediately upon ingestion of either prednisone or placebo and retrieval was tested within 15 minutes post-ingestion. This short space of time is not sufficient to have affected any of the three stages of memory processing (encoding, consolidation, and retrieval). The literature identifies a length of 60 minutes as the minimum time for changes in glucocorticoid activity to impact on cognitive performance (de Quervain et al., 2000; Kirschbaum et al., 1996); this also coincides with prednisone's biological half-life (http://www.medsafe.govt.nz/profs/datasheet/p/prednisonetab.pdf).

Working memory and short-term auditory attention span. The performance of participants in the Prednisone group on the Digit Span – Backward subtest was significantly poorer than that of participants in the Placebo group. There were, however, no between-group differences on the Digit Span – Forward subtest.

The above findings stand in contrast to previous findings on the effects of glucocorticoids on working memory. For instance, Elzinga and Roelofs (2005) found cortisol-related effects on what they termed the maintenance sub-component of working memory (i.e., what they regard is measured by the digit-forward component of the test), but not on the manipulation sub-component (digit span backwards). However, I argue that the results of their study and this one are not directly comparable for two reasons.

First, Elzinga and Roelofs measured the effects of stress-induced elevations in endogenous cortisol on attention and working memory, whereas the current study measured the effects of an acute dose of an exogenous glucocorticoid on performance in the same cognitive domain. Second, Elzinga and Roelofs did not find differences between the performance of controls and that of stressed individuals on either component of the task.

Instead, they found an interaction effect within the stress group: Cortisol responders performed more poorly than stressed non-responders on the forward recall component. The authors argue that the decline in performance on the Digit Span forward component of the test in some participants was the result of the combined effects of autonomic arousal induced by performance anxiety and of the effects of a cortisol response on attention span, and less of attributable to an increase in GR activity alone, especially since memory was unaffected when cortisol was elevated after adrenergic activation had returned to baseline.

As mentioned previously, GR hyperactivity is associated with memory (including working memory) impairment (e.g., Born et al., 2006, 2009; Buchanan et al., 2001; Coluccia et al., 2008; de Quervain et al., 2000; Elzinga & Roelofs, 2005; Keenan et al., 1996; Kuhlmann et al., 2005, Newcomer et al., 1994, 1999; Plihal et al., 1999; Young et al., 2011; Wagner et al., 2007). The fact that working memory (backward digit span) was impaired in my study and not in the Elzinga and Roelofs study (2005) may be due to the fact that my participants were under the influence of more potent glucocorticoid activity. Cortisol, as mentioned previously, has a higher affinity for MRs, and it would take an elevation of 100 mg of cortisol to achieve the glucocorticoid potency of 25 mg of prednisone or its metabolite, prednisolone. Such an increment is highly unlikely, and has not been reported, following a TSST protocol. Furthermore, following from the argument in the previous paragraph, it is likely that impairment on simple tasks assessing short-term attention span would require some stressed-induced autonomic arousal, an element not manipulated in the current design.

Semantic memory. As predicted, the performance of participants in the Prednisone group on semantic memory tasks 1 hour after ingesting the drug did not differ significantly from that of participants in the Placebo group. In fact, average performance on the WAIS-III Information subtest was almost identical.

Levels of circulating glucocorticoids during sleep: Hypothesis set 2. Saliva samples collected at REM1 and REM2 indicated high levels of circulating glucocorticoid among prednisone-treated participants, while cortisol was low among Placebo controls at those times. In contrast, the salivary cortisol levels observed after spontaneous morning awakening (REM3) were comparable to those observed in the Placebo group. Taken together, these data suggest that, as predicted, prednisolone was active for the first 2 REM periods and had cleared by the end of sleep testing.

Also as predicted, in the Placebo group cortisol levels were low, relative to waking levels, during the first two sleep cycles and increased significantly during the last cycle. Therefore, the aim of reversing the normal pattern of glucocorticoid activity during sleep among prednisone-treated participants was successfully achieved. This achievement, then, provided the basis for subsequent between-group comparisons on parameters of sleep and of dreaming.

Prednisone and sleep architecture: Hypothesis set 3. Sleep onset latency and REM onset latency were not affected by the glucocorticoid treatment (i.e., there were no between-group differences in terms of time to fall asleep and time to enter the first REM sleep stage). In contrast to this finding, Gillin et al. (1972) found delayed sleep onset latency and delayed REM onset latency in some of their prednisone-treated participants: only in the high-dose (60 mg) group and not in the low-dose (20 mg) group. In the current study, the prednisone-treated participants ingested 25 mg of prednisone, an amount comparable to that ingested by Gillin et al.'s low-dose group. It appears, then, that a much higher dose than used in the current study is required to delay the onset of sleep and of REM.

There were also no significant between-group differences in the percentage and the distribution of stages 1 and 2 of sleep. Again, Gillin et al. (1972) found a significantly greater proportion of stage 2 sleep only among the high-dose participants. Furthermore, a close

inspection of studies examining glucocorticoid-related changes in the light stages of sleep reveals inconsistent findings. For instance, Born and colleagues (1987, 1989) found significant increases in stage 1 sleep (amount in minutes and percentage of total sleep) following administration of both cortisol and fluocortolone. Other studies did not find any statistically significant effect of cortisol or dexamethasone on stage 1 sleep (e.g., Bohlhalter et al., 1997; Fehm et al., 1986; Plihal & Born, 1999).

Regarding WASO, however, prednisone-treated participants experienced significantly more awakenings, and consequently experienced significantly lower sleep efficiency. Therefore, one might suggest that, if everything else is held constant, ingesting prednisone before bedtime induces fragmentation of sleep. This suggestion is consistent with the existing evidence (Fehm et al., 1986; Vgontzas et al., 2003).

More specifically with regard to relatively compromised sleep efficiency in the Prednisone group, the proportion of REM sleep was significantly suppressed for those participants. Furthermore, participants in the Prednisone group experienced, relative to those in the Placebo group, significantly less REM intensification from Early to Late sleep. Once a young adult has slept sufficiently, relative to the length of time s(he) has remained awake before falling asleep, the length of REM sleep s(he) experiences increases (Caskadon & Dement, 2011; Dauvilliers & Billiard, 2004; Nielsen, 2004). That is typically why most REM sleep is concentrated during the second half of the night, after the homeostatic urge to fall asleep wears off and metabolic and HPA axis activity start to increase, priming the person for morning wakefulness. However, the ascending slope of our circadian rhythm lasts for three to four hours before most humans are ready to wake up and resume their waking activities (Dauvilliers & Billiard, 2004; Nielsen, 2004). Therefore, one can argue that one of the functions of REM sleep is to sustain a state of sufficient arousal just below the threshold of wakefulness. It is also established that the marked rise in glucocorticoid activity during the

second half of sleep coincides with the occurrence of longer and denser REM sleep periods. It is possible that, beyond a certain level of activity, glucocorticoids induce wakefulness instead of supporting REM sleep. This would happen at the end of every normal nocturnal sleep period in healthy adult humans when cortisol reaches its peak. When glucocorticoid activity is enhanced beyond normal levels during sleep, the process towards wakefulness seizes to be gradual and instead results in chopped periods of sleep with consciousness fluctuating between wakefulness and light sleep.

The suppression of REM sleep, together with the positive correlation between sleep efficiency and percentage REM sleep in the current study, and the glucocorticoid-related suppression of REM sleep, together with sleep fragmentation and the lightening of sleep reported elsewhere (Bohlhalter et al., 1997; Born et al., 1987, 1989; Fehm et al., 1986; Gillin et al., 1972; Plihal & Born, 1999; Van Cauter et al., 1998, 2000; Vgontzas et al., 2003), offers some support to the notion that the relationship between glucocorticoid action and REM sleep may be rooted in the regulation of arousal during sleep. Theoretically, this phenomenon is believed to be tied to the incidence of dreaming, whereby autobiographical experiences and more general information are processed internally, without any engagement with the external environment, as a protective mechanism for sleep (Solms, 1997).

In addition, although participants in the Prednisone group did not experience a significantly smaller proportion of SWS compared to those in the Placebo group, the slope of their SWS distribution from Early to Late sleep was significantly flatter than that of controls, who experienced a strong concentration of SWS during Early sleep relative to Late sleep. Otherwise stated, prednisone-treated participants experienced similar proportions of SWS during the two halves of the night. Furthermore, the percentage of SWS during Early sleep was lower among prednisone-treated participants, and this between-group difference approached statistical significance ($p = .06$). The lack of SWS concentration during Early

sleep among prednisone-treated participants, without a significantly lower total percentage of SWS, is consistent with existing reports that exposure to synthetic corticosteroids can delay SWS latency, with limited effect on absolute quantities or percentage of SWS (Born et al., 1987; Fehm et al., 1986; Gillin et al., 1972). To summarize, similarly to earlier findings (Born et al., 1987; Fehm et al., 1986; Gillin et al., 1972), both REM sleep and SWS were affected in the current study but to varying degrees. REM sleep appeared more vulnerable to glucocorticoid effects than SWS. While prednisone effectively suppressed REM sleep, it mostly affected the organization of SWS.

Overnight gains in memory performance: Hypothesis set 4. With regards to the effect of glucocorticoids on the benefits of sleep on declarative memory performance, the hypothesis that prednisone-treated participants would perform worse than Placebo controls after a period of sleep, was not demonstrated. The pattern of VPA-15 test performance of participants in the Prednisone group was not statistically different to that of participants in the Placebo group. All participants demonstrated a slight but significant decrement in scores from baseline, on the word-paired associates' task (average of approximately 1 word-pair). In other words, participants in both groups performed slightly but significantly more poorly on re-testing, post-sleep, compared to their baseline performance prior to sleep. It is possible that sub-optimal quality of sleep, associated with laboratory conditions, affected the performance of participants, regardless of group membership. This effect on general sleep architecture may have masked prednisone-induced effects on declarative memory through REM suppression. In other words, despite the fact that the Prednisone group experienced significantly less REM sleep, their performance on the VPA task did not differ from that of the Placebo group.

With regards to the Logical Memory test, the absence of a detrimental effect of prednisone on story recall performance mirrors an observation made by Newcomer et al.

(1994). Those researchers found no effect on story recall performance following overnight exposure to dexamethasone. They demonstrated, instead, that extended (but not single) overnight exposure was required before the effects of the glucocorticoid became apparent. Of note here is that the cumulative dose of glucocorticoid used by Newcomer et al. (a total of 3.5 mg of dexamethasone over 4 days) was almost equivalent in terms of potency to the once-off dose used in the current study (25 mg of prednisone, equivalent to 3.75mg of dexamethasone in one day). It is therefore possible that repeated exposure, at lower doses that add up to 3.5 mg in total, has a greater impact on cognition than a single acute exposure at a moderate dose. As mentioned in the Introduction section, evidence reveals that the effects of glucocorticoids on cognition are not apparent after initial exposure, but become significant following repeated exposure (Keenan et al., 1996; Newcomer et al., 1999).

The timing of treatment is another important factor to consider when interpreting these results. Significant declines in declarative memory performance following glucocorticoid exposure have been observed when that exposure occurs prior to encoding or prior to retrieval (de Quervain et al., 2000). It appears that the effect of glucocorticoids on memory consolidation is more challenging to demonstrate; most studies find glucocorticoid effects at either or both the encoding stage (Kirschbaum et al., 1996; Newcomer et al., 1994) or the retrieval stage (de Quervain et al., 2000) of memory processing, with the study by Plihal and Born (1999) being the exception in finding glucocorticoid effects on consolidation processes.

With regards to the effects of glucocorticoid exposure on procedural memory processes, the pattern of FTT performance of participants in the Prednisone group was different to that of participants in the Placebo group mostly on the measure of Speed. Although, as anticipated, both groups of participants performed similarly at baseline in terms of Speed, the post-training performance of prednisone-treated participants was significantly

poorer than that of the placebo-controls. Although their speed of performance benefitted from training, the increment in their scores was significantly inferior to that of participants in the Placebo group. Furthermore, although participants in the Placebo group showed, on average, continued improvements in FTT Speed at post-sleep testing, there was no such enhancement among prednisone-treated participants (their speed of performance remained unchanged from post-training to post-sleep).

With regards to Accuracy, performance improved upon each re-testing (post-training and post-sleep) phase for the sample as a whole. Furthermore, participants in the two groups did not differ with regards to their average levels of accuracy on the task. When considered separately, the Placebo group demonstrated, on average, significant increases in accuracy from baseline to post-training, while the Prednisone group did not. Among prednisone-treated participants, FTT Accuracy remained stable across the three measurement points. Accuracy from post-training to post-sleep did not improve in both groups. One might deduce that taking prednisone hinders the positive effects of training on accuracy, however, the increment in scores from baseline to post-training in the Placebo group are likely the result of extreme values in the data set. Cook's distance statistics revealed that the impact of these extreme values did not alter overall between-group analyses in the mixed-model ANOVA I used. I therefore decided not to exclude these extreme cases from the analyses owing to my small sample size but instead to consider post-hoc, group-specific test results with caution.

The relationship between glucocorticoid levels, sleep and post-sleep declarative memory performance: Hypothesis set 5. This set of hypotheses stated that (a) night-time glucocorticoid levels would be negatively correlated with percentage SWS, percentage REM sleep, SWS Distribution, REM Intensification, and sleep efficiency, (b) night-time glucocorticoid levels would be negatively with post-sleep measures of verbal declarative memory (i.e. VPA II, VPA Retention, LM II & LM Retention), that (c) percentage SWS,

percentage REM sleep, SWS Distribution and REM Intensification would be positively correlated with post-sleep measures of verbal declarative memory (i.e. VPA II, VPA Retention, LM II & LM Retention), and finally that (d) percentage SWS, percentage REM sleep, SWS Distribution and REM Intensification would mediate the negative relationship between glucocorticoid levels and performance on post-sleep declarative memory tasks. In addition, the hypothesis stated that glucocorticoid level would be positively correlated with percentage stage 1 sleep, percentage stage 2 sleep, and percentage WASO. This set of hypothesis was partially-confirmed.

The portion of Hypothesis 5 regarding the relationship between glucocorticoid level and sleep was partially-confirmed. In the sample as a whole, higher glucocorticoid activity during sleep was in fact associated with smaller proportions of REM sleep, reduced concentration of SWS during Early sleep, reduced intensification of REM sleep from Early to Late sleep, and reduced sleep efficiency. Night-time glucocorticoid level was however, not significantly related to the proportion of SWS. However, examining the two groups separately, pointed to different relationships between sleep and glucocorticoid activity: Among prednisone-treated participants, high glucocorticoid levels during sleep were associated with lower REM intensification. On the other hand, the single significant association observed between free, circulating endogenous cortisol and sleep among participants in the Placebo group was a positive relationship at REM1 between cortisol and percentage SWS. Unlike those presented in previous studies (e.g., Born et al., 1997; Fehm et al., 1986; Gillin et al., 1972), the current findings are too modest to support any strong claims about the differential effects of synthetic versus endogenous glucocorticoids on sleep. However, the current findings are consistent with the extant literature (Born et al., 1987; Fehm et al., 1986; Gillin et al., 1972) in suggesting that whereas synthetic glucocorticoids

tend to fragment and to lighten sleep, and to suppress REM sleep, endogenous cortisol tends to enhance SWS.

The portion of Hypothesis 5 regarding the a priori prediction that glucocorticoid level would be negatively correlated with post sleep measures was also partially-confirmed. Specifically, for the sample as a whole, there was a significant negative correlation between glucocorticoid levels at REM2 and LM II scores. Within the Placebo group, there were significant negative correlations between VPA II, LM II, VPA Retention, and LM Retention, on the one hand, and average, REM1, REM2, and REM3 glucocorticoid levels, on the other. Within the Prednisone group, however, there were no significant relationships between any of the glucocorticoid measures and any of the delayed recall measures.

The portion of Hypothesis 5 which stated that percentage SWS, percentage REM sleep, SWS Distribution and REM Intensification would correlate negatively with VPA II, VPA Retention, LM II & LM Retention was only partially-confirmed. Only SWS Distribution was significantly and positively correlated with VPA II for the sample as a whole. Contrary to what was expected, percentage SWS, was not correlated with post-sleep performance on any of the declarative memory measures, whether in whole sample analyses or in separate-group analyses. This finding suggests that there might be a greater association between the *organization* of SWS and declarative memory than between the *amount* of SWS and declarative memory. This interpretation is consistent with theories proposing that the circadian organization of sleep stages have a greater impact on sleep-dependent memory processing than does the quantity of these sleep stages, in isolation from one another and from the rest of sleep architecture (Cicogna & Bosinelli, 2001).

Examining these relationships for the groups separately revealed that correlations between sleep and memory measures (a) did not achieve statistical significance in the Placebo group, and (b) were conflicting in the Prednisone group. For instance, in the Prednisone

group, while REM Intensification was significantly positively correlated with LM II (delayed story recall tested post-sleep), it was significantly negatively correlated with VPA II (delayed recall of word pairs tested post-sleep). Furthermore, percentage REM was significantly and negatively correlated LM Retention (difference in scores between pre-sleep, immediate and post-sleep, delayed story recall). One may speculate that given REM was significantly suppressed among prednisone-treated participants, the different information acquired before sleep likely competed to get consolidated (Ellenbogen et al., 2006; Lipinska, Timol, Thomas & Kaminer, 2014; Liu, Faraguna, Cirelli, Tononi, & Gao, 2010). This point is elaborated on in Chapter Five.

The portion of Hypothesis 5 which stated that percentage SWS, percentage REM sleep, SWS Distribution and REM Intensification would mediate the negative relationship between glucocorticoid levels and performance on post-sleep declarative memory tasks was not tested. The original rationale behind the choice of the current design was that exposure to corticosteroids before sleep would suppress the action of endogenous cortisol among prednisone-treated participants, hence disrupting effective consolidation of memory acquired before sleep directly by increasing GR activity at the expense of MR activity at that point of the circadian phase, and indirectly by suppressing REM and fragmenting sleep in that group. The current data succeeds in revealing various relationships between REM sleep and glucocorticoid activity, glucocorticoid activity and declarative memory performance, and declarative memory performance and REM sleep. Furthermore, the data demonstrates how the nature of each of those relationships is different for the corticosteroid-exposed (prednisone) group versus the non-exposed (placebo) group. However, in doing so, the data is split into very small samples of diverging trends (Prednisone group, $n = 11$ and placebo group, $n = 12$) which are not adequate for conducting mediation analyses. A larger sample of participants would be required to adequately test these relationships in the context of

corticosteroid exposure versus normal circadian glucocorticoid activity. A paper by Fritz and MacKinnon (2007) indicates that samples as large as 158 are required to achieve a power of .8 in Baron & Kenny (1986) mediation analyses, for a partial mediation (deemed more realistic in social science research), with small-to-medium effect sizes for each of Baron & Kenny's (1986) causal-steps 1, 2, 3 of 4. In hindsight, and with the benefit of the experience and wisdom gained in running this study, the limitations of a small sample size mean that a within-group design could have been more effective in (a) demonstrating the relationship between sleep architecture, free-circulating, normal night-time levels of corticosteroids and memory performance, and (b) testing the relationship between the introduction of exogenous corticosteroids on sleep and memory performance.

Lastly, consistent with the a priori prediction, glucocorticoid level was significantly positively related to sleep fragmentation as measured by percentage WASO. With regards to the light stages of sleep, glucocorticoid level was not significantly correlated with either percentage stage 1 sleep or with percentage stage 2 sleep for the sample as a whole or in the Placebo group. However, glucocorticoid levels were significantly and positively correlated with both percentage stage 1 sleep and percentage stage 2 sleep in the Prednisone group. This finding is consistent with literature suggesting that heightened glucocorticoid activity lightens sleep (Born et al., 1987, 1989; Gillin et al., 1972; Plihal et al., 1999; Vgontzas et al., 2003).

The relationship between glucocorticoid level, sleep and post-sleep procedural memory performance: Hypothesis set 6. This set of predictions was partially-confirmed. First, the prediction that, there would be no association between procedural memory and glucocorticoid levels was disconfirmed. In contrast to the a priori prediction, performance on the procedural memory task (both FTT Speed and FTT Accuracy measures) was significantly negatively correlated with glucocorticoid activity (specifically with mean glucocorticoid levels, and with night-time glucocorticoid levels at REM1, and REM2).

Second, the prediction that performance on the procedural memory task would be positively correlated with REM sleep and stage 2 sleep was partially-confirmed. There were significant moderate and positive correlations between FTT Speed and both percentage REM sleep and percentage stage 2 sleep in the Placebo group only. No such relationship achieved statistical significance in the Prednisone group or in the sample as a whole. However, interestingly, performance on the procedural memory task, for the sample as a whole and for the prednisone-treated participants, was positively correlated with percentage SWS. In addition, for the sample as a whole, there was a significant positive association between FTT Speed and SWS Distribution and that correlation was even stronger than between FTT Speed and percentage SWS.

With regards to the negative correlation found between accuracy measures and SWS among placebo participants, I can only once again speculate that speed of performance was enhanced at the expense of accuracy, given that familiarity with the operation of the task was improved with practice and potential sleep effects but since the task of itself was of no adaptive value to participants, accuracy was not prioritized (Anderson et al., 2000; Nairne et al., 2007; Shohami et al., 2010). These findings remain to being further investigated in much larger samples, however.

These unpredicted findings linking night-time glucocorticoid levels to procedural memory and to the distribution of SWS sleep are hopefully explained by the mediational analysis I consequently chose to run. The rationale behind that decision was that the literature suggests that performance on memory tasks can be affected negatively if the effects of an exogenous corticosteroid are sufficient to (a) disrupt the MR: GR activity ratio during early sleep, and/or (b) reduce the relative concentration of SWS during that same period (Bohlhalter et al, 1997; Born et al., 1991; Plihal et al., 1999; Wagner et al., 2005).

Indeed, the mediational analysis testing if SWS Distribution mediated the relationship between glucocorticoid levels and procedural memory performance revealed that there was a complete mediating relationship linking these variables. In other words, it appears as though the relationship observed between glucocorticoid levels and post-sleep FTT Speed is entirely driven by the changes in the organization of SWS. As mentioned earlier, the heightened glucocorticoid activity induced by taking 25mg of prednisone before bedtime, had a negative effect on SWS among prednisone-treated participants by reducing its relative concentration in Early sleep. As mentioned earlier, exposure to synthetic corticosteroids can delay SWS latency (Born et al., 1987; Fehm et al., 1986; Gillin et al., 1972). In the current sample, it seems that this disruption in the organization of SWS may have in turn affected performance on the procedural memory task.

Traditionally, REM sleep has been associated with the consolidation of procedural memory, while SWS has been associated with the consolidation of declarative memory (Brualla et al., 1998; Conway & Smith, 1994; Gais et al., 2004; Karni et al., 1994; Maquet, 2001; Peigneux et al., 2003; Perrin et al., 1999; Plihal et al., 1997, 1999; Smith, 1995, 1996). However, as discussed in Chapter One, a significant body of recent evidence suggests that the organization of REM sleep and of SWS during the night, in relation to one another, is more predictive of memory performance (declarative or implicit) than the discrete proportion of each stage is (Brière et al., 2000; Fogel et al., 2001, 2007; Gais, Plihal, Wagner, & Born 2000; Huber et al., 2004; Mednick et al., 2003; Moroni et al., 2008; Nishida et al., 2009; Payne, 2010; Payne et al., 2010; Robertson et al., 2004; Stickgold et al., 2000; Tamaki et al., 2008; Wagner et al., 2001, 2006; Walker et al., 2002, 2009). In particular, the current findings are consistent with empirical evidence from previous studies demonstrating significant positive associations between early-night SWS and performance on procedural memory tasks (Gais & Born, 2004; Walker et al., 2010).

The effect of prednisone on dream recall and qualia: Hypothesis 7. Regarding the effects of prednisone on sleep mentation, the Prednisone-group participants did not recall fewer dreams than the placebo controls. Prednisone-group participants accounted for 48 % of all dream reports recorded. The between-group difference in the ratio of *Recall* to *No recall* in each group was not statistically significant. In fact, the rate of recall for the two groups was very similar, with 33% of awakening resulting in successful recall among placebo controls, versus 30 % among Prednisone-group participants.

On the other hand, in terms of how participants rated the quality of the dreams that they did recall, participants in the Prednisone group rated theirs as significantly more emotionally intense than did those in the Placebo group. This finding is not surprising, given widespread reports of the affective impact of corticosteroid-based therapies on patients. For instance, short-term prednisone therapy has been associated with depressive and anxiety symptoms (see Brown & Chandler, 2001; Warrington & Bostwick, 2006 for reviews). Specifically, studies have found that treatment with corticosteroids (e.g., for immunologic or allergic illnesses) is associated with the experience of negative emotions linked to fear and sadness (Brown, Beard, Frol, & Rush, 2006; Groeneweg, Karst, de Kloet, & Joëls, 2011; Korte, 2001; McReynolds et al., 2010; Reus & Wolkowits, 2001; Roozendaal et al., 2009; Schmidt et al., 1999). For example, in one double-blind, placebo-controlled study, participants who were administered an acute high dose of prednisone (160 mg) on 4 consecutive days reported an increase in negative emotions that was significantly different to the trend observed among placebo controls (Schmidt et al., 1999). In the same study, exposure to prednisone was associated with increased beta and gamma activity in the right frontal lobes. On the basis of these data, the authors argued that prednisone inhibits the modulation of negative emotions. It is therefore possible that the experience of more intense emotions associated with corticosteroid-based treatment is expressed in dreams as well.

The dream inventory used in this study did not include a measure of emotional valence; it only allowed participants to rate the emotional intensity of their dreams. I therefore cannot infer that prednisone-treated participants experienced more negatively-toned dreams; all one can gather from these data is that they experienced more emotionally-charged dreams. Future research should investigate affect in the context of the relationship between corticosteroid exposure and dreaming, especially because an effect on subjective emotional experience during sleep mentation was observed here at an exposure level far inferior (25 mg versus 160 mg) to that used in the Schmidt et al. (1999) study during wakefulness.

In terms of the rest of the qualia variables, there were no between-group differences in terms of participant self-ratings of how bizarre, thought-like, or visually vivid dreams were.

Prednisone and the episodic content of dreams: Hypothesis set 8. On average, there were no between-group differences in terms of the episodic content of dreams/waking inclusions (i.e., whether dreams contained references to recent or remote waking experiences, including isolated fragments of lived experiences such as perceiving familiar places and people). With respect to a general level of preoccupation with previously experienced events, independently of the degree of episodic elements in dreams, I compared the proportion of dreams with waking continuity versus idiosyncratic themes to assess whether or not the groups differed. Although the prednisone-treated participants reported dreams in the Waking Continuity category only 20% of the time, in contrast to 45% percent for Placebo controls, this difference did not achieve statistical significance. The medium effect size suggests that the failure to detect a significant between-group difference could be attributed to the small sample size of dream reports; there were very small number of cases per cell in the chi-square analysis.

Although no between-group differences emerged in terms of waking inclusions in dreams, analyzing the relationship between glucocorticoid activity and dream content for the

two groups separately revealed some interesting associations. For instance, episodic content was inversely related to glucocorticoid levels at REM2, while original content was positively related to glucocorticoid levels at REM1 and at REM2 in the Prednisone group only. The analyses detected no such significant associations in the Placebo group. This pattern of data suggests that increased glucocorticoid activity during sleep may have a suppressive effect on the manifestation of episodic memory content in dreams. A parallel can be drawn between the current findings and findings from Study 1 on the association between night-time glucocorticoid level and dream content: The data in Study 1 detected a strong, significant negative relationship between both REM2 cortisol and average night-time cortisol level and episodic memory dream content.

In summary, although hypothesis 8 was not statistically confirmed, administration of a synthetic glucocorticoid appeared to inhibit the inclusion of waking experiences in dreams and to increase the presence of idiosyncratic and novel elements in dreams at the expense of dream content with waking continuity. On average, prednisone-treated participants tended to report dreams with less episodic content when compared to placebo controls, with between-group differences achieving a large effect size (Cohen's d = -1.01) and approaching significance (p = .06). In addition, during the times when prednisone was likely still active (i.e. at REM1 and REM2) among prednisone-treated participants, elevated night-time glucocorticoid levels were associated on the one hand, with lower episodic and familiar content in dreams and on the other hand, with higher dream content qualified as original and unfamiliar. However, when the effects of the synthetic glucocorticoid began to dissipate (i.e. at REM3), these significant relationships between glucocorticoid levels and familiar versus original content were no longer detected.

Because of the small sample size and the small number of dreams collected per participant at each collection point, this study was limited in its ability to adequately

demonstrate within-group differences in the evolution of dreaming across the night. However, the data suggest that there may well be interesting relationships between glucocorticoid activity and sleep mentation, and theory suggests that these relationships may be connected to processes that modulate memory reactivation and assimilation during sleep. Although the effects of corticosteroids on dreaming were not tested directly via a within-group study design which would monitor the inclusion of specific waking experiences before and after drug manipulation, these exploratory data set the stage for further investigation into the effects of corticosteroid exposure on the important offline cognitive and emotional processing that takes place during sleep.

Future studies should analyze both cognitive and affective aspects of dreams in the context of corticosteroid exposure, and should investigate the perceptual qualities of dreams and include measures of report length in assessing their overall quality. This latter recommendation stems from the anecdotal observation that, during this study, prednisone-treated participants tended to report very brief dreams, and tended to describe them as fleeting impressions, dominated by thoughts rather than images. However, they did not rate their dreams on measures of visual vividness and of thought-like nature any differently to Placebo controls, and because the length of the dream reports was not systematically measured, these anecdotes need to be investigated systematically before making any inferences about their veracity and their relationship with corticosteroid exposure.

CHAPTER FOUR:

STUDY 3: THE ROLE OF CORTISOL IN MEDIATING THE RELATIONSHIP BETWEEN HIPPOCAMPAL VOLUME AND DECLARATIVE MEMORY IN YOUNG ADULTS WITH ASTHMA

Introduction

Previous chapters have described the mechanisms of action through which glucocorticoids affect hippocampal structure and functioning. Briefly, glucocorticoids perform a wide range of functions that, taken together, contribute to neuronal plasticity and activity (McEwen & Gianaros, 2011; Sousa & Almeida, 2012; Vyas et al., 2016). At optimal levels, glucocorticoids (a) help regulate glucose transport to, and protein synthesis within, neurons and glial cells, (b) aid dendritic branching, (c) control the amount of myelin surrounding neurons, (d) modulate neurotransmitter activity, such as serotonin reuptake, and (e) enhance neuronal excitability via agonistic effects on glutamate. Because the actions of glucocorticoids affect neurophysiological function in an inverted-U manner, either excessive or deficient amounts are neurotoxic because they lead to a failure to regulate all of the above functions. The integrity and health of cells in regions with significant concentrations of MR and GR receptors (e.g., the hippocampal formation) are particularly vulnerable to glucocorticoid imbalance (Belanoff, Gross, Yagher, & Schatzberg, 2001; de Kloet, 1991; Jacobson & Sapolsky, 1991; Sapolsky et al., 1986; Wagner et al., 2008). By inference, individuals with deficient HPA-axis functioning and/or who are chronically-exposed to glucocorticoids (e.g., asthmatics) might experience impaired performance on hippocampal-dependent memory tasks.

As noted in Chapter 2, asthmatics use exogenous corticosteroids to relieve airway inflammation. However, chronic use of high-dose inhaled corticosteroids (ICS) can

exacerbate adrenal insufficiency in asthmatics, leading to either (a) relative decreases in salivary free-circulating, endogenous cortisol, or (b) partial reversals of the normal circadian cortisol secretion rhythm (i.e., higher-than-normal night-time levels, delayed cortisol acrophase in the morning, and a relatively flat secretion curve during the day). These endogenous changes, together with the independent effects of synthetic corticosteroids, are associated with hippocampal atrophy in the elderly (e.g., Bruehl et al., 2009; Lupien et al., 1998; Sindi et al., 2013) and with hippocampal-dependent, reversible memory impairment in adults in general (e.g., Buchanan et al., 2006; de Quervain et al., 2000; 2003, Elzinga & Roelofs., 2005; Kirschbaum et al., 1996; Kuhlmann et al., 2005; Newcomer et al., 1999; Payne et al., 2007; Young et al., 2011). Furthermore, two other areas of research that lend support to the probability of a causal relationship between hippocampal damage or dysfunction and glucocorticoid imbalance in humans are (a) the field of aging and memory, and (b) studies examining hippocampal functioning in patients with a dysfunctional HPA axis (e.g., those diagnosed with Cushing's syndrome, major depressive disorder (MDD), and post-traumatic stress disorder [PTSD]). Despite the existence of these separate, but related, lines of research, few studies have investigated the relationship between glucocorticoid activity and the integrity of the hippocampus (viz., its structure and hippocampal-dependent memory function) in asthmatics. Therefore, I will draw on the evidence from the aforementioned research areas to infer the potential detrimental effects of prolonged glucocorticoid exposure on the hippocampus in adults with asthma.

The rest of this introductory section is structured as follows: First it reviews the available literature on the effects of glucocorticoid exposure on cognitive functioning in asthmatic populations. Second, it discusses findings, derived from the aging literature, on the relationship between HPA-axis activity, hippocampal volume, and memory. Third, it discusses findings, derived the literature on other disorders of the HPA-axis, on the

relationship between HPA-axis activity, hippocampal volume, and memory. Fourth and last, it describes some empirical evidence, taken from (a) neuroimaging studies and (b) laboratory-based animal studies which use stress paradigms, in a discussion of the mechanism of action underlying the relationship between glucocorticoids and the hippocampus.

Effects of glucocorticoid exposure on cognitive functioning in asthma. There is ample evidence suggesting that, in healthy human adults, glucocorticoid exposure has detrimental effects on memory performance. These detrimental effects can manifest in the form of (a) decreased signal-to-noise ratio on word recognition tasks (as demonstrated by a greater incidence of commission errors; Wolkovitz et al., 1997), or (b) decreased recall rate on paragraph recall tests (Newcomer et al., 1994, 1999). When these effects appear varies: They are sometimes observed after single glucocorticoid administrations (De Quervain; 2000; Wolkowitz et al., 1997), but at other times require repeated administrations over the course of several days (Newcomer et al., 1994, 1999). These timing differences are accounted for by the different types or dosages of glucocorticoid used, and/or the different stages of declarative memory processing (e.g., encoding, consolidation, recall) tested. Mostly, however, these findings describe the effects of acute or sub-acute exposure to glucocorticoids. Literature on the effects of chronic exposure to glucocorticoids is lacking.

As mentioned in Study 2, the few studies that have investigated the effects of corticosteroids on memory in adults, focused on the effects of short-to-intermediate courses of oral corticosteroids treatment (e.g., Brown et al., 2003, 2004; Keenan et al., 1996). Research on the effects of prolonged, inhaled glucocorticoid therapy on memory performance in asthmatic, is lacking. To my knowledge, a single study looked at the specific relationship between glucocorticoid treatment and declarative memory in asthmatic children. Bender et al. (1991) found that treatment with prednisone impairs verbal declarative memory in a dose-response manner: Children in high-dose, but not low-dose, groups perform significantly more

poorly than non-prednisone treated controls. Similarly, only one study (Brown et al., 2004) has attempted to investigate the relationship between prolonged corticosteroid exposure, deficient HPA-axis functioning, and hippocampal-dependent memory function in adults. Brown and colleagues (2004) measured cognitive performance, including that on tests of declarative memory, as well as hippocampal volume and metabolism, in patients experiencing various inflammatory disorders, including asthma. They found that patients treated with prednisone performed more poorly on a verbal declarative memory task (the Rey Auditory Verbal Learning Test; Rosenberg et al., 1984), had smaller left and right hippocampi, showed evidence of reduced neuronal viability and significant signs of reduced neuronal proliferation (as measured by N-acetyl aspartate and choline metabolite ratios, respectively) when compared to matched controls with similar medical profiles. They found no between-group differences in IQ and in other cognitive domains, supporting the likelihood of cognitive impairment specific to glucocorticoid-mediated hippocampal damage.

However, Brown et al. (2004) found no direct relationship between prednisone-exposure variables (viz., dose administered, or duration of use) and hippocampal volume. Based on the data from the aforementioned previous studies, it is possible to infer that that prolonged exposure to prednisone has an effect on endogenous cortisol, which in turn affects hippocampal volume and memory. However, the study design did not include any direct measures of HPA-axis function (e.g., levels of endogenous cortisol). Furthermore, as the literature suggests, there is a large degree of inter-individual variability with regard to the extent of suppressive effects of exogenous corticosteroids on endogenous cortisol (Fardon et al., 2004; Tayab et al., 2007), which could explain the absence of a direct relationship between the degree of exposure to prednisone and hippocampal volume.

In the context of the current research, the Brown et al. (2004) study is a limited reference for comparison given that it is not focused solely on asthmatics, that many of the

participants have mixed medical profiles, that the effects of mood on cognition are not controlled for, and that the design of the study does not isolate the effects of glucocorticoids on cognition from the effects of other medication. For instance, the diverse patient profiles and treatment regimes may have contributed, over and above the effects of glucocorticoids, to the neurotoxic effects on hippocampal cells and, consequently, to the observed declarative memory impairments. For instance, participants in the prednisone-exposed group experienced a variety of inflammatory illnesses, including asthma, with unverified severity of illness in each case. Furthermore, the prednisone-treated individuals were using various combinations of medication in conjunction with prednisone, had undergone varying lengths of treatment with varying doses of prednisone, and were not always matched with adequate controls (e.g., one participant not on prednisone treatment reported co-morbid rheumatoid arthritis and asthma). Lastly, the Brown et al. (2004) study included participants with steroid-related mood disorders:

> "Psychiatric diagnoses in the corticosteroid-treated group consisted of prednisone-induced mood disorder with depressive features (past) (*n* = 5), prednisone-induced mood disorder with depressive features (current) (*n* = 3), prednisone-induced mood disorder with mixed features (current) (*n* = 1), prednisone- induced anxiety disorder with panic attacks (past) (*n* = 1), and panic disorder with agoraphobia (current) (*n* = 1)." p. 540-541

Furthermore, the Brown study has not been replicated, despite having been conducted more than one decade ago. Research that investigates the relationship between glucocorticoid action, hippocampal integrity, and hippocampal-dependent memory function specifically in asthmatics is needed.

In summary, the literature reviewed above suggests (a) there is a relationship between cortisol, hippocampal volume, and declarative memory and (b) that that relationship might be modulated by the chronic use of glucocorticoid treatment, but (c) that the latter link remains to be firmly established in asthmatics.

Cortisol, hippocampal volume, and memory: The impact of age. The field of cognitive aging is one area of research where the triadic relationship between high endogenous cortisol levels, hippocampal atrophy, and cognitive deficit is addressed extensively. Research examining changing memory performance in older adults has consistently demonstrated that individuals with higher cortisol levels (a) tend to have smaller hippocampi and (b) perform more poorly on verbal declarative memory tasks, relative to age-matched counterparts with lower levels of endogenous cortisol (Lupien, 1994; Lupien et al., 1998; MacLullich et al., 2005; McAuley et al., 2009; McEwen, 1999; O'Brien et al., 2004; Sapolsky et al., 1986). The data from these studies are compelling, even if they do not always confirm a mediating role for cortisol in the relationship between hippocampal volume and memory deficit (e.g., O'Brien et al., 2004). For example, MacLullich and colleagues (2005) observed a negative relationship between endogenous cortisol and declarative memory performance in healthy older men, but were unable to establish a relationship between HPA-axis functioning and hippocampal volume.

Taken together, however, these studies suggest that the direction of relationships between endogenous cortisol and hippocampal volume, and between hippocampal volume and memory performance, have been relatively well-established in older adults. In younger, non-clinical populations, evidence suggests that the association between cortisol (either basal levels or response post-challenge) and hippocampal volume is either non-significant or positive (Pruessner et al., 2007; Sindi et al., 2014), and that there might be a positive association between hippocampal volume and memory performance may be positive (Pruessner et al., 2007).

Two pieces of evidence suggest that a closer examination of this issue might be warranted. First studies comparing children with adults with PTSD indicate that children with PTSD either have similar or significantly larger hippocampi than healthy controls, whereas

adults with PTSD have smaller hippocampal volumes relative to their healthy counterparts (De Bellis, 2001; Tupler & De Bellis, 2006; Woon & Hedges, 2008). Second, cortisol secretion tends to follow different patterns in the two groups: Children with PTSD often show higher levels of cortisol than controls (e.g. De Bellis, 2001; Carrion et al., 2002; Pervanidou et al., 2007), whereas adults with PTSD tend to show lower levels than controls (e.g. De Bellis, 2001; Miller, Chen, & Zhou, 2007; Santa Ana et al., 2006). These findings support the possibility that there might be age-related differences in the relationship between HPA-axis activity and hippocampal volume, and that such differences might exist in both healthy and clinical populations. One explanation for the existence of such age-related differences is that high levels of cortisol during early developmental stages affect neuronal differentiation and impedes the process of pruning, leaving exposed children and young adults with larger, less efficient hippocampi (Pruessner et al., 2007). In other words, greater volumes in young populations may be indicative of pathology, whereas smaller volumes in adults might relate to pathology and to degree of atrophy (Tupler & De Bellis, 2006).

However, age is not the sole factor moderating the effects of cortisol on hippocampal volume and declarative memory. The stress associated with testing conditions in experiments may have an influence, over and above that of age, on whether or not one detects a relationship between cortisol and hippocampal volume and function. In their recent study, Sindi and colleagues (2014) demonstrated a negative association between hippocampal volume and cortisol response (area under the curve from pre- to post-testing) among both younger and older adults who were tested in environments unfamiliar to them. In contrast, no such relationship was observed in participants, both older and younger, who were tested in familiar and non-stressful environments. The study also demonstrated that smaller hippocampal volume is associated with greater stress-reactivity (i.e., greater cortisol response) in both young and older adults.

Greater stress reactivity, which is a marker of hyperactive HPA-axis functioning, is inherent to chronic medical illnesses, such as Cushing's syndrome, and psychiatric disorders such as MDD and PTSD. Research on the cognitive effects of abnormal HPA-axis activity in these three patient populations reveals that, in each, hippocampal damage and hippocampal-dependent memory impairment can be observed. However, the role of cortisol in the relationship between hippocampal volume, stress, and memory performance is complex, at least as far as these populations are concerned.

Cortisol, hippocampal volume, and memory: Impact of HPA-axis disorders. In humans, chronically elevated levels of endogenous cortisol, such as in patients with Cushing's syndrome and in the elderly are associated with smaller hippocampal volume and impaired declarative memory performance (Huang et al., 2009; Lupien et al., 1998; Sapolsky, 2000; Starkman et al., 1992). Specifically, Starkman and colleagues (1992) found a positive relationship between declarative memory function and hippocampal volumes. They characterized the hippocampal volumes they measured as 'smaller-than-normal' (using the volumetric data of one healthy control participant as benchmark) in their patient sample, and found that for those patients with smaller-than-normal hippocampi, the smaller the hippocampus, the worse the memory performance was. They were also able to relate the anatomical deficits to elevated endogenous cortisol levels. However, the study was unable to demonstrate, cohesively, the relationship between glucocorticoid hyperactivity (i.e., through an increase in the level of cortisol), hippocampal damage, and memory dysfunction. First, only a single healthy control participant was scanned for comparison, and therefore the notion of hippocampal "damage" among the patients in the study can only be inferred with great caution. Second, the authors did not investigate directly the relationship between memory performance and cortisol levels.

Research on HPA-axis activity, hippocampal volume, and memory in patients with depression involves similar limitations. For example, studies have found that patients with MDD present with significantly smaller hippocampal volumes and with declarative memory deficits, but that neither hippocampal volume nor memory deficits were associated with markers of HPA-axis function (e.g., basal cortisol levels, cortisol awakening response defined as the marked increase of cortisol within the first hour of morning awakening (CAR; Pruessner et al., 2007), or the dexamethasone suppression test; Gerritsen et al., 2011; . et al, 1996; Vythilingam et al., 2004). For instance, Dedovic et al. (2010) found a co-incidence of smaller hippocampal volumes and a lower CAR among individuals with a high-risk profile for depression, but they did not test if there was an association between the two. In the Sheline et al. (1996) study, participants were deliberately selected to be outside periods of current depressive episodes in order to measure the chronic effects of HPA-axis dysfunction on hippocampal volume in the context of depression. On the basis of the obtained data, the authors speculated that the effects of glucocorticoids on hippocampal structure persist even during intermittent periods of adequate HPA-axis functioning, and long after cortisol levels have returned to normal.

In the case of PTSD, although research indicates a co-incidence of hippocampal damage, memory impairment, and elevated cortisol, the directionality of these relationships is unclear. For instance, twin studies have suggested that a small hippocampus could explain an individual's inability to down-regulate the HPA axis adequately after stress, and may therefore be a risk factor for the development of PTSD, rather than being a consequence of the psychiatric disorder (Conrad, 2008; Gilbertson et al., 2002; Rooij et al., 2015). However, the dearth of longitudinal research in these areas makes it difficult to establish the directionality of the relationship.

Cortisol and the hippocampus: The nature of the relationship. Despite all the evidence clearly suggesting a relationship between cortisol, hippocampal volume, and memory function in humans (e.g., Brown et al., 2004; Coluccia et al., 2008; Lupien et al., 1994, 1998; McEwen, 1999; Qin, Hermans, van Marle, & Fernàndez, 2012; Starkman et al., 1992), establishing a clear model for this relationship is challenging. The above review has discussed some of the reasons for this difficulty. To recap, these include (a) differences among the various populations investigated (i.e., subject-specific variables, such as age and pathology), and (b) the protocols used to test the relationship (i.e., design-specific variables, such as the test environment, correlational design strategies, and failure to assess the cortisol-hippocampus-memory relationship as a triad). Nevertheless, as mentioned in Chapter 1, there is some evidence to suggest a mediating role for glucocorticoids in the relationship between hippocampal volume and memory performance. For instance, normalising cortisol either surgically or pharmacologically reverses the functional hippocampal deficits observed in patients with Cushing's syndrome (Martignoni et al., 1992; Mauri et al., 1993; Schteingart et al., 1980).

Furthermore, neuroimaging studies can provide more direct evidence for causal relationships between neurotoxic levels of glucocorticoid exposure and hippocampal structure and functioning. For instance, Lovallo and colleagues (2010) used functional magnetic resonance imaging (fMRI) to measure the degree and distribution of impact of high levels of cortisol on metabolic activity (measured by blood-oxygen-dependent (BOLD) contrast) in the brains of healthy adults. They found that a single administration of hydrocortisone significantly reduced metabolic activity in the hippocampal, parahippocampal, and amygdalar regions within 20-25 minutes of injection.

Similarly, animal literature investigating the impact of glucocorticoid hyperactivity on hippocampal cells can provide useful insights as to the nature of the relationship between

glucocorticoid action and hippocampal integrity. Specifically, these studies have implicated altered glucocorticoid-receptor activity and/or glucocorticoid-mediated disruption of glucose transport to hippocampal cells as an underlying mechanism in the relationship between stress and the structural integrity and optimal functioning of the hippocampus. There are also indications that over-activation of glucocorticoids during stress has neurotoxic effects on hippocampal cells and that such repeated insults leave an "imprint" (Conrad, 2008, p.6), rendering hippocampal cells vulnerable to damage even when HPA-axis responses to stress become blunted over time (see Conrad (2008) and McEwen (1999), for reviews). Moreover, the literature indicates that using stress paradigms to enhance glucocorticoid action or exogenously administering glucocorticoids has similar neuroanatomical and behavioral effects.

For instance, in rats, repeated exposure to stress leads to hippocampal-dependent behavioural dysfunction such as increased immobility and vigilance, reduced exploration of the environment, and reduced ability to learn adaptive escape and avoidance strategies relative to controls, all likely attributable to reversible cell degeneration in the CA3 region (e.g., Conrad, Galea, Kuroda, & McEwen, 1996; Leverenz et al., 1999; Mahlberg & Duman, 2003; Mirescu et al., 2006; Mueller & Tracy, 2008; Vyas, Mitra, Rao, & Chattarji, 2002). Similarly, in primates, prolonged exposure to high concentrations of glucocorticoids has various forms of neurotoxic effects in the CA2 and CA3 regions of the hippocampus (Sapolsky et al., 1990; Tata & Anderson, 2010; Uno et al., 1994).

Given that asthmatics present with compromised HPA-axis function and make chronic use of glucocorticoid-based treatment, and that learning and memory difficulties have been reported in children with asthma, I set out to investigate if adult asthmatics also present with reduced hippocampal volume in a way similar to that seen in the elderly, and in patients with Cushing's syndrome, MDD, and PTSD.

Specific Aims, Rationale, and Hypotheses

Although much is known about the neuroanatomical and behavioral effects of oral and injected glucocorticoids on the hippocampus, little is known about the effects of inhaled corticosteroids on the development of the hippocampus in young adults with asthma. Investigation of these effects is especially pertinent because (a) many asthmatics make daily use of potent inhaled corticosteroids as their first line of treatment, (b) inhaled corticosteroids can have neurotoxic effects and can further suppress HPA-axis function, and (c) these individuals undergo various stages of important neurocognitive development while using ICS.

Hence, the aim of the present study was to investigate whether chronic exposure to inhaled GR agonists would be associated with hippocampal volume, whether through neurotoxic mechanisms (examined by considering the degree of exposure to inhaled corticosteroids) and/or through effects on endogenous cortisol secretion (examined by measuring the levels of cortisol during sleep). The study also investigated whether hippocampal volume mediated the relationship between night-time cortisol levels and declarative memory performance. The study therefore tested the following predictions:

1. Individuals with asthma will have smaller hippocampal volumes than matched healthy controls.
2. Hippocampal volume will correlate positively with performance on verbal declarative memory tasks in the Asthma group, but negatively in the Healthy Control group.
3. Night-time cortisol levels will correlate negatively with performance on verbal declarative memory tasks in both the Asthma and Healthy Control groups.

4. Hippocampal volume will be inversely associated with (a) asthma severity score[9] and (b) average night-time cortisol in both the Asthma and Healthy Control groups.
5. Asthmatics will have higher cortisol levels than healthy controls.
6. Hippocampal volume will mediate the relationship between cortisol level and verbal declarative memory performance.

Methods

Design and setting. The study was conducted at the Cross-University Brain Imaging Centre (CUBIC), located on the Tygerberg campus of the University of Stellenbosch. A correlational design tested the relationship between corticosteroid-treated asthma and hippocampal volume. The main predictor variable in this study was group status, with two levels of variation: asthma and healthy control. A secondary predictor variable was average night-time cortisol level. The group categorization was based on stratification performed in Study 1, and the cortisol measures used were also collected in Study 1.The outcome measures included (a) left hippocampal volume, (b) right hippocampal volume, and (c) total hippocampal volume. All outcome measures were treated as continuous variables.

Sample and participation selection. The objective of the current study was to compare hippocampal volumes of the moderate-to-severe asthma participants (n = 13) with those of the healthy control participants (n = 12) from Study 2. Of these 25 participants, one moderate-to-severe asthma group and one healthy control group participant had titanium dental implants and two healthy control group participants had relocated between the time of completing Study 2 participation and recruitment for Study 3. Hence, 21 participants (n = 11

[9] Asthma severity score was based on (a) the frequency and number of asthma symptoms reported and (b) by the degree and potency of ICS the participant was exposed to. Exposure to ICS alone was not chosen in this analysis as, the asthma classification scheme used in Study 1 was such that moderate-to-severe asthmatics fell within a very homogenous range of ICS exposure when compared to mild asthmatics (i.e., scores ranged between 6 and 7 points, with one participant scoring 8 points). See Appendix M for the classification scheme and the severity and ICS exposure scores of participants with asthma.

in the Asthma group, and *n* = 10 in the Healthy Control group) were scanned. Of those 21, one participant's data set was excluded owing to the poor quality of their scan caused by excessive movement in the scanner (this participant was a 21-year-old female in the Asthma group, with a measured PIQ of 102). Another participant's data set did not survive to final analysis because she was identified as a true outlier based on the results of standardised residuals (> 1.96) and Cook's Distance (.53; this participant was a 37-year-old female in the Asthma group, with a measured PIQ of 105). Hence, the final data set for statistical analysis featured a sample size of 19 participants (*n* = 9 in the Asthma group, *n* = 10 in the control group). Figure 6 presents a flowchart of participant attrition through the different stages of the experiment.

Exclusion criteria. Medical conditions such as pregnancy, having certain foreign objects or metal implants in one's body, and psychological disorders such as claustrophobia or anxiety about the procedure render scanning unsuitable for certain individuals. These conditions were screened for using self-report questionnaires. Furthermore, these participants had previously been screened for psychiatric disorders prior to being enrolled in Study 1.

Figure 6. Flow-chart showing participant attrition.

Materials and procedure.

Safety screening and participant preparation. Participants completed a magnetic resonance (MR) hazard checklist to screen for any implants such as pacemakers, dentures, and aneurism clips that may affect and be affected by imaging. They also completed a participant information questionnaire adapted from the safety screening form for MR procedures from the American College of Radiology (ACR) White Paper on MR safety

(Kanal et al., 2002), which screens for any medical or psychological conditions contraindicated for neuroimaging.

sMRI image acquisition. A 3.0 T Siemens Allegra head scanner (Siemens Medical Systems, Erlangen, Germany) was used for data acquisition. High resolution T1-weighted 3D anatomical scans were acquired using a sagittal multi-echo MPRAGE sequence with the following parameters: resolution = 1x1x1mm^3, slices = 160, TR = 2530 ms, TE1 = 1.98ms, TE2 = 3.66 ms, TE3 = 5.34 ms, TE4 = 7.02 ms, TI = 1100 ms, flip angle = 7 degrees, FOVread = 256, base resolution = 256, phase resolution = 75% and bandwidth = 651 Hz/pixel. Total scan time for each participant was approximately 9 minutes.

Automated analyses. I used the Freesurfer image analysis suite, version 4.5 (documented and free for download at http://surfer.nmr.mgh.harvard.edu/) for the automated parcelation and segmentation of all brain regions. In this study, the main focus was the assessment of left and right hippocampal volumes. Reconstructions were run on an Intel Nehalem cluster at the Centre for High Performance Computing (CHPC) in Rosebank, Cape Town (http://www.chpc.ac.za/).

The automated processing of cortical reconstruction and volumetric segmentation that are of particular relevance to the current study includes automated Talairach transformation, segmentation of the subcortical deep gray matter volumetric structures such as the hippocampi (Fischl et al., 2002, 2004a), intensity normalization (Sled et al., 1998), tessellation of the gray matter/white matter boundary, and automated topology correction (Fischl, Liu & Dale, 2001; Segonne et al., 2007). The test-retest reliability of the software's morphometric procedures are reported to be good across scanner manufacturers and different field strengths (Han et al., 2006; Reuter et al., 2012). Furthermore, the maps generated are sensitive enough to detect sub-millimeter differences between groups, and are therefore ideal when no gross pathological differences are anticipated. More extensive accounts of the

Freesurfer technical procedures can be found elsewhere (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; Fischl & Dale, 2000; Fischl, Sereno, & Dale, 1999; Fischl, Sereno, Tootell, & Dale, 1999; Fischl et al., 2001, 2002, 2004a, 2004b; Han et al., 2006; Jovicich et al., 2006; Segonne et al., 2004).

I used TKMedit, an interface part of the Freesurfer suite that enables the review of data by displaying anatomical volumes in different orientations, to verify all reconstructions individually. The inspection was overseen by an independent consultant, who specializes in neuroimaging research, from CUBIC. This consultant was blind to the group status of the participants who had produced the images, and was unaware of the study hypotheses.

Manual analyses. I used Brain Voyager QX, version 2.0 (Brain Innovation B.V., Maastricht, The Netherlands) to prepare the structural images for manual tracing. Images were iso-voxeled, rotated into the AC-PC plane, and normalised to the standard Talairach anatomical brain template for the purpose of between-group analyses. They were subsequently exported to the Analyze format for manual tracing using MultiTracer, version 1.0 (Laboratory of Neuro-Imaging [LONI], UCLA). Images were magnified four times and traced, on a Lenovo tablet equipped with an LED screen, using a stylus pen.

Tracing was done in consultation with Dr. Christopher Warton, a neuroanatomist at the University of Cape Town who specializes in manual tracing of subcortical structures. The hippocampal structures were traced on both the sagittal and the coronal planes to optimize viewing accuracy, although only the coronal tracings were used to calculate volumes. I chose to retain and analyse the coronal tracings only because evidence suggests that accuracy is maximised by using slices oriented perpendicular to the long axis of the hippocampus (Sheline et al., 1996).

The whole hippocampus was traced and defined as including the head, body, and tail, the dentate gyrus, and part of the subiculum. I used the alveus to delineate the hippocampus

from the amygdala, drawing within the inner boundary of the alveus to exclude it, and I used the CSF and the lateral ventricle as additional landmarks, where they were visible. I also excluded the fimbria and the fornices using the upper medial extremity of the white matter of the temporal lobes as boundary. On the posterior slices, I drew a line at a 90º angle to the surface to delineate the hippocampal formation from the parahippocampal gyrus.

Dr. Warton and I traced hippocampi independently. We were both blind to participant group status. In total, we each traced 40 hippocampi (two for each of the 20 participants retained for the final analyses). I used the first 25 per cent of our tracings (N = 10) to run a two-way ANOVA , mixed-effects model Intraclass Correlation Coefficient (ICC) test to evaluate the degree of agreement between us two raters. The analysis detected excellent inter-rater reliability, with a single measures' Cronbach α value of .94 and an average measures α coefficient of .97, $p < .001$ in both cases. Dr. Warton's tracings were used in the final analyses.

Data management and statistical analyses.

Head-size correction procedure. Hippocampal volumes were corrected for intracranial volume (ICV), as the size of hippocampi is known to show considerable variation in proportion ICV (Buckner et al., 2004; Geuze, Vermetten & Bremner, 2005; Synek & Reuben, 1976; Zatz & Jernigan, 1983). Furthermore, previous research suggests that as the strength of the relationship between gray matter structures and ICV increases, so does the benefit of using corrected values as opposed to raw measures with regards to improving the criterion validity of the region of interest (Mathalon, Sullivan, Rawles, & Pfefferbaum, 1993; Sanfilipo, Benedict, Zivadinov, & Bakshi, 2004).

ICV was calculated using the automatic segmentation tools in Freesurfer. Rather than manually segmenting brain regions to obtain a measure of ICV (a process that is time-consuming and labor intensive and that requires proficiency in tracing various

neuroanatomical regions), I used the automatically-generated ICV measure to correct for head size for the manual analyses as well. Automated measures of ICV are considered to be as effective as manually-generated ICV in analyses involving manually-traced structures (Buckner et al., 2004).

I used the proportion approach to correct for head size. I divided the left, right, and total hippocampal volumes of each participant by their ICV to obtain quantitative indices (referred to hereafter as indexed hippocampal volume), which I used instead of the raw hippocampal volume measurements. I chose the proportion method of correction for head size as opposed to the residual method for the sake of consistency, to facilitate the comparison of my results with the Starkman et al. (1992) study, which investigates the cortisol-hippocampal volume-declarative memory triad in a patient population characterized by dysfunction of the HPA-axis, and being closest in terms of theoretical rationale and study design to the current study. My choice was also motivated by the fact that little is known about brain development in adults with asthma, and the proportion method allows for some insight into the development of the hippocampus in relation to the rest of the brain; the residual method, in contrast, isolates the development of hippocampus by removing the effect of head size entirely (see Mathalon et al., 1993; and Sanfilipo et al., 2004, for a debate on the different merits of the two approaches).

Establishing the distribution of the data set. As in Study 1 and Study 2, the analysis began with an exploration of the data. I used the same tests and the same general approach as in Study 1 and Study 2 to examine normality of distribution and homogeneity of variance, and to identify significant outliers. I used the criteria suggested by Bollen and Jackman (1990) to identify influential cases in my data set. Then, automated volumes were correlated with the manually traced volumes to assess the degree of proximity of the two methods of volumetric analysis. All analyses used a 95% confidence level, with the threshold for

statistical significance set at $p < .05$. Partial eta square (η^2) was calculated as a measure of effect size for all analyses.

Testing Hypothesis 1. To test the hypothesis that individuals with asthma will have smaller hippocampal volumes than matched healthy controls, I ran separate analyses of covariance for the left, right, and total hippocampal volume indices. Sex predicted hippocampal volume significantly (left hippocampus and sex: $R^2 = .65$, $F(1, 17) = 31.47$, $p < .001$; right hippocampus and sex: $R^2 = .64$, $F(1, 17) = 29.53$, $p < .001$). I consequently ran two ANCOVAs, with group status (two levels: asthmatic vs. healthy control) as the independent variable, mean indexed hippocampal volume (left or right) as the dependent variable, and sex as covariate.

As a supplementary analysis, I ran a mixed-model ANOVA to explore any potential differences in hippocampal volume laterality between the two groups, with the prediction that there would be no difference. I used Group as the main predictor variable, Sex as the covariate, and left versus right hippocampal volume as the within-subject factor in the analysis.

Testing Hypotheses 2-4. These hypotheses state that (2) hippocampal volume will correlate positively with performance on declarative memory tasks in the Asthma group but negatively in the Healthy Control group, (3) night-time cortisol will correlate negatively with performance on declarative memory tests in both asthma and control groups, and that (4) hippocampal volume will be inversely associated with (a) degree of exposure to ICS in the asthma group, and (b) average night-time cortisol in both asthma and control groups. To test each of these predictions, I ran a series of bivariate correlations to test for the strength and direction of the relationships between cortisol, hippocampal volume, corticosteroid exposure, and episodic memory outcome measures.

Testing Hypothesis 5. This hypothesis states that asthmatics will have higher cortisol levels than controls. To test this prediction, I ran an independent-samples *t*-test test to examine between-group differences in average cortisol levels.

Testing Hypothesis 6. To test the hypothesis that hippocampal volume will mediate the relationship between cortisol level and declarative memory performance, I first had to establish whether the relationships between cortisol and hippocampal volume, hippocampal volume and memory, and cortisol and memory were connected linearly. I did so by running simple linear regression analyses. Each of the four regression analyses constituted an assumption, necessary to my mediational model:

1. Night-time cortisol was associated with declarative memory performance.
2. Night-time cortisol was associated with hippocampal volume.
3. Hippocampal volume was associated with declarative memory performance
4. The association between cortisol and memory was weakened by hippocampal volume, thus pointing towards a potential mediatory relationship.

Because manually-traced regions of interest are considered the gold standard (i.e., they are assumed to generate more accurate volumes than the automated measures), all of the analyses were performed using the manually-traced hippocampal volumes (indexed) only. Please see Appendix O for correlations performed using the partialled residuals method.

Supplementary analyses. Lastly, I ran a series of correlations, as supplementary analyses: between IQ measures and asthma severity score, to exclude the possibility that asthma predicts intellectual potential; between hippocampal volume and VPA scores corrected for IQ, to control for the effects of IQ which proved to predict performance on the VPA task; and between raw hippocampal volumes and cortisol, to illustrate that the method of head-size correction can impact on the nature of linear relationships between these variables. These analyses are reported in Appendix N.

Ethical and safety considerations. All study procedures were approved by the Research Ethics Committees of the University of Cape Town's Department of Psychology and Faculty of Health Sciences.

Participants were provided with detailed written and verbal information about the study during the screening process, which took place in a research laboratory in the Department of Psychology at the University of Cape Town between 7 and 30 days prior to testing. Furthermore, they gave their informed consent before being formally enrolled in the study. The information supplied included details about the study procedures, the risks involved with exposure to the strong magnetic field, the potential benefits of the study, and that they would be compensated for their time. Additionally, the consent form secured their right to withdraw their participation at any stage of the study. A medical indemnity form gave them the assurance that the research team would take responsibility for their safety and wellbeing for the duration of the study. CUBIC is a well-equipped imaging center with highly qualified technical and support staff members who ensure strict adherence to all MR safety protocols. Before entering the scanner, participants removed all personal clothing and jewelry items and changed into hospital robes to avoid the presence of any metal object infiltrating Zone IV of the MR site.

Some contrasting agents are known to cause allergic reactions to individuals with asthma, but no such agents were used in this study. This fact was emphasized to participants. Furthermore, individuals with asthma were required to bring their asthma reliever inhaler to CUBIC.

Scanning was interrupted if a participant felt uncomfortable during the experiment, even if s/he had not reported anxiety about the procedure beforehand. To facilitate communication about the presence of such discomfort, there was audio contact between the experimenter or MR technologist and the participant during scanning. One participant (group

= Healthy Control, sex = female, age = 20 years old) expressed anxiety during the procedure, and scanning was halted so that she could be calmed. We resumed scanning after the participant assured us that she was no longer anxious and that she wanted to complete her involvement in the study.

Results

This section comprises three major parts. First, it provides details of the sample's sociodemographic and cognitive characteristics. Second, it presents analyses comparing the automated and manually-generated measures of hippocampal volume. Third, it presents the analyses that tested Hypotheses 1-6.

Sample characteristics. Table 1 provides details of the final sample's sociodemographic and cognitive characteristics. There were no between-group differences with regard to age at assessment. Most participants (77%) in the Asthma group were men, whereas most (60%) in the Healthy Control group were women. There were, however, no significant between-group differences in sex distribution.

Table 25
Sample Demographic Characteristics (N =19)

	Group					
	Asthma	Healthy Control				
Variable	(*n* = 9)	(*n* = 10)	*t* / χ^2	*P*	ESE	Range
Age (years)	22.78 (3.67)	21.90 (2.08)	-0.65	.52	.02	19-31
Sex (F:M)	2:7	6:4	2.77	.12	.38	----
WASI PIQ	118.11 (10.41)	105.70 (13.46)	-2.23	.04*	.23	92-131

Note. WASI = Wechsler Abbreviated Scale of Intelligence; PIQ = Performance IQ. For the variables *Age* and *PIQ*, means are presented with standard deviations in parentheses. Degrees of freedom for the *t*-tests were 18. The *p*-values for the *Age* and the IQ variables are 2-tailed. To test sex distribution across groups, I calculated a one-tailed Fisher's Exact test. ESE = effect size estimate (in this case, Cramer's *V* for the test of sex distribution and partial η^2 for the remaining variables).
*$p < .05$.

Regarding IQ scores, there were no significant between-group differences in FSIQ, although there was a strong tendency toward higher scores in the Asthma group. Participants in that group did obtain, on average, significantly higher Performance IQ (PIQ) scores than those in the Healthy Control group. However, PIQ was strongly correlated with Sex, $r_{pb}(17) = .63$, $p = .002$, with men ($M = 118.55$, $SD = 9.57$) scoring significantly higher than women ($M = 102.20$, $SD = 12.02$), $t(17) = 3.32$, $p = .004$, partial $\eta^2 = .39$. Hence, one potential reason for the between-group inequity in PIQ might be the differing representations of the sexes across groups. In fact, when sex was controlled for in a one-way ANCOVA, between-group differences in PIQ were no longer significant, $F(1, 17) = 1.96$, $p = .184$, partial $\eta^2 = .11$.

To further exclude the possibility that the between-group difference in PIQ was a true reflection of group membership (i.e., that it predicted asthma status), I ran a mediational analysis to evaluate the size of the effect of PIQ in the relationship between group status and hippocampal volume in the current data set. That analysis revealed that, although both PIQ and asthma status predicted hippocampal volume, the effects of those two predictors on the outcome were additive, not mediational. As Table 26 indicates, when each of the two predictors was added to the equation, the model explained incrementally greater proportions

of the variance in hippocampal volume (compare the adjusted R^2 values at modelling steps 1, 3, and 4). In other words, between-group differences in PIQ did not mediate the effect of group status on hippocampal volume; instead, there appears to be some independence to the contribution of each.

The significant between-group difference in PIQ might have been a consequence of the small sample size. That is, individual outlying values (e.g., a PIQ of 131 points in the Asthma group and a PIQ of 92 points in the control group) are likely to have had a disproportionate effect on the results of t-test. In support of this argument is the fact that there were no significant between-group differences in PIQ in the Study 2, which featured a larger sample and of which Study 3 participants were a sub-sample.

As a final corroboration of the fact that the presence of asthma was not, in the population, associated with higher levels of general intellectual functioning, I correlated severity of asthma with PIQ scores and found no significant results, $r_{pb}(9) = -.34$, $p = .11$. Therefore, one might conclude that there is a low probability of an association between having asthma and also having a higher level of general cognitive functioning.

Because of the results of these preliminary analyses, all subsequent analyses investigating between-group differences in hippocampal volume used group as the main predictor variable, sex as a covariate, and left/right/total indexed hippocampal volume as the outcome measure. Sex and PIQ could not both be included in the model as covariates because of their strong positive association. the ofI chose to include sex as a covariate rather than PIQ because (a) it explained a significantly greater proportion of the variance in hippocampal volume (see Table 27 below), and (b) the analyses described above suggested strongly that between-group differences in PIQ were, by and large, accounted for by between-group differences in sex distribution and by the negative impact of having a small sample size.

Table 26

Mediational Analysis: Is Performance IQ a mediator of the relationship between group status and hippocampal volume? (N = 19)

Modelling Step	Adjusted R^2	*F*	*p*
Step 1:			
Group status and left hippocampal volume	.31	9.13	.008**
Group status and right hippocampal volume	.23	6.30	.022*
Group status and total hippocampal volume	.29	8.22	.011*
Step 2:			
Group status and Performance IQ	.18	4.97	.040*
Step 3:			
Left hippocampal volume and Performance IQ	.47	17.07	.001**
Right hippocampal volume and Performance IQ	.47	16.95	.001**
Total hippocampal volume and Performance IQ	.51	19.49	< .001***
Step 4:			
Group (main predictor), Performance IQ (mediator), and left hippocampal volume (outcome variable)	.53	9.07	.008**
Group (main predictor), Performance IQ (mediator), and right hippocampal volume (outcome variable)	.49	9.54	.007**
Group (main predictor), Performance IQ (mediator), and total hippocampal volume (outcome variable)	.55	10.87	.005**

Note. Degrees of freedom were (1, 17) for the above analyses.

*$p < .05$. **$p < .01$.

Table 27

Linear Regression Models: Sex as a Predictor of Hippocampal Volume (N = 19)

Outcome variable	Adjusted R^2	*F*	*P*
Left hippocampal volume	.61	29.53	< .001***
Right hippocampal volume	.63	31.47	< .001***
Total hippocampal volume	.67	37.07	< .001***

Note. Degrees of freedom were (1, 17) for each model.

***$p < .001$.

Comparison between manual and automated measures. The literature suggests that there is a considerable gap between volume estimates obtained from automated analyses and those obtained from manual tracings, with Freesurfer-generated volumes being approximately 35% larger (Tae, Kim, Lee, Nam, & Kim, 2008). To investigate whether this pattern of data would hold for the current sample, I ran three separate, Intraclass Correlation Coefficient (ICC) tests (type: two-way mixed model with measures of consistency) to evaluate the level of agreement between the current manually- and automatically-generated measures of hippocampal volume. Table 28 presents details of those analyses.

The table reveals that, in the current sample, the similarity in volume measurements obtained using manual tracings versus automated calculations for each individual hippocampus, stands between 49 and 65 percent, leaving a huge margin of disagreement. On the other hand, if we analyze the average agreement for the entire set of (i) left hippocampi, (ii) right hippocampi, and (iii) total hippocampal volume, the percentage agreement figures were 66, 79 and 74 percent, respectively. In other words, on average, differences between manual and automated measures were 34% for left, 21% for right, and 27% for total hippocampal volume, which is consistent with the degree of disagreement reported by Tae et al. (2008) in their comparison of the two methods. In their paper, they suggest that very fine differences in delineating neuroanatomical structures might account for such differences in volume estimates. It also suggests that manual tracings offer the possibility of better accuracy. Therefore, although I present between-group analyses using both automated and manually-generate volume estimates, the conclusions I draw are based on data from the latter.

Table 28
Intraclass correlation between manual and automated hippocampal volume estimates (N = 19)

	Intraclass Correlation Coefficient tests			
Raw hippocampal volume	Measures	Intraclass correlation	*F*	*p*
Left	Single	.49	2.93	.014*
	Average	.66	2.93	.014*
Right	Single	.65	4.75	.001**
	Average	.79	4.75	.001**
Total	Single	.58	3.80	.003**
	Average	.74	3.80	.003**

Note. Intraclass correlation statistics are presented as Cronbach α estimates. Single measures analyses refer to differences between each pair of tracings performed by the 2 raters, while average measures analyses refer to the average difference in tracings across the entire data set. Degrees of freedom were (1, 18) for the above analyses. *p* was set at a significant value of $p < .05$.

*$p < .05$. **$p < .01$

Hypothesis 1: Influence of group status on hippocampal volume. Hypothesis 1 stated that individuals with asthma will have smaller hippocampal volumes than matched healthy controls. Before presenting the between-group analyses for hippocampal volume, it is important to note that there were no between-group differences with regard to intracranial volume (see Table 29), indicating that any between-group differences in hippocampal volume cannot be attributed to differences in head size.

As Table 29 shows, all current measures of corrected hippocampal volume, whether automated or manual, indicated that, on average, asthmatics in the current sample had smaller left, right, and total hippocampal volumes than healthy controls. However, only two of these between-group differences reached the threshold for statistical significance: manually-traced left hippocampal volume, and manually-traced total corrected hippocampal volume.

Table 29
Hippocampal Volume: Differences between asthmatics and healthy controls (N = 19).

	Group				
	Asthma	Healthy Control			
Variable	(*n* = 9)	(*n* = 10)	*F*	*p*	ESE
Intracranial volume	1682.44 (151.53)	1484.87 (194.88)	2.51	.134	.14
Manual tracing					
Left hippocampal volume	.0016 (.0001)	.0019 (.0002)	5.73	.029*	.26
Right hippocampal volume	.0017 (.0001)	.0020 (.0003)	2.83	.112	.15
Total hippocampal volume	.0033 (.0003)	.0039 (.0005)	5.09	.038*	.24
Automated tracing					
Left hippocampal volume	.0025 (.0002)	.0027 (.0002)	2.07	.169	.12
Right hippocampal volume	.0026 (.0002)	.0028 (.0002)	2.66	.123	.14
Total hippocampal volume	.0051 (.0003)	.0055 (.0004)	2.69	.121	.14

Note. Means are presented, with standard deviations in parentheses. Average intracranial volume is presented in cubic millimetres and the average hippocampal volume indices and standard deviations are presented to decimal places. All *p* values were generated from univariate analyses using Sex as covariate, with degrees of freedom (1, 17). ESE = effect size estimate; in this case, partial η^2.
*$p < .05$

Supplementary analyses. As Table 29 shows, across both groups, and on average, right hippocampal volume was greater than left hippocampal volume. However, results from the mixed-model ANOVA, described below, suggest that this difference did not reach statistical significance. The variable Group (Asthma vs. healthy control) was the main predictor variable, Sex the covariate, and left versus right hippocampal volume (Lateralization effect) were the within-subject factor.

Automated tracings. For the sample as a whole, the analysis detected no significant difference between left and right hippocampal volumes, $F(1, 16) = 0.41$, $p = .534$, partial $\eta^2 = .03$. In other words, the main effect of lateralization was not significant.

There was no significant main effect of Group on hippocampal volume, $F(1, 16) = 2.79$, $p = .114$, partial $\eta^2 = .15$. There was also no significant Group x Lateralization interaction effect, $F(1, 16) = 0.01$, $p = .91$, partial $\eta^2 = .001$.

Although there was a significant main effect of Sex on hippocampal volume with men having bigger hippocampi than women, $F(1, 16) = 8.17$, $p = .011$, partial $\eta^2 = .34$, there was no significant Sex x Lateralization interaction effect, $F(1, 16) = 2.32$, $p = .147$, partial $\eta^2 = .13$.

Manual tracings. For the sample as a whole, the analysis detected no significant difference between left and right hippocampal volumes, $F(1, 16) = 0.24$, $p = .88$, partial $\eta^2 = .001$. Once again, the main effect of lateralization was not significant.

There was a significant main effect of Group on hippocampal volume, $F(1, 16) = 5.05$, $p = .039$, partial $\eta^2 = .24$. However, there was no significant Group x Lateralization interaction effect, $F(1, 16) = 0.12$, $p = .738$, partial $\eta^2 = .01$.

Similarly, although there was a significant effect of Sex on hippocampal volume, $F(1, 16) = 28.89$, $p < .001$, partial $\eta^2 = .64$, there was no Sex x Lateralization interaction effect, $F(1, 16) = 1.23$, $p = .305$ and partial $\eta^2 = .07$.

Hypothesis 2: Relationship between memory performance and hippocampal volume. This hypothesis stated that, hippocampal volume would correlate positively with performance on verbal declarative memory tasks in the Asthma group but negatively in the Healthy Control group. As Table 30 shows, the statistical analyses confirmed this hypothesis, at least partially: In the Healthy Control group, indexed hippocampal volume (left, right and total) was indeed inversely and significantly correlated with VPA I and VPA II. However, within the same group, right and total hippocampal volume measures were positively and significantly correlated with Learning Slope. Similar significant relationships were detected for the sample as a whole: Larger hippocampal (left, right, and total) to intracranial volume ratio was indeed associated with poorer performance on both the immediate and delayed recall VPA-15 tasks. Furthermore, larger right and total hippocampal to intracranial volume ratio was significantly positively associated with LM Learning Slope scores. However, the current data set did not detect any significant relationship between hippocampal volume and verbal declarative memory in the Asthma group, hence disconfirming the part of the hypothesis predicting positive correlations between hippocampal volume and verbal declarative measures in that group. See table 30 for details.

Hypothesis 3: Relationship between memory performance and night-time cortisol levels. This hypothesis stated that, across both groups, night-time cortisol levels will correlate negatively with performance on verbal declarative memory tasks. As Table 31 shows, this hypothesis was also partially confirmed. Although the analyses detected no significant associations between average night-time cortisol levels and VPA-15 immediate and delayed recall scores, it did detect significant, moderate, and negative associations between average night-time cortisol levels and (a) VPA retention scores (p = .022), and (b) LM retention scores (p = .012). It appears, then, that night-time cortisol was related to

changes in performance from pre- to post-sleep, rather than with the absolute performance scores at each test session.

Analysing the correlation between night-time cortisol and declarative memory performance for each group separately revealed that night-time cortisol was inversely and significantly correlated with VPA Retention scores for asthmatics (p = .018) but not for healthy (p = .113) participants. There were no other significant relationships detected when the groups were analysed separately, although the inverse relationship between night-time cortisol and LM Retention bordered significance for healthy participants (p = .054).

Hypothesis 4: Relationship between HPA-axis functioning and hippocampal volume. Hypothesis 4(a) stated that hippocampal volume will be inversely associated with asthma severity. This hypothesis was partially confirmed. There was a significant negative association between asthma severity scores and left indexed hippocampal volume, τ = -.53, p = .030. There were moderate, negative, but non-significant, correlations between asthma severity scores and right (τ = -.40, p = .076) and total (τ = -.44, p = .061) hippocampal volumes. This set of results reflects the fact that left hippocampal volume, but not right or total hippocampal volume, was significantly smaller among participants in the Asthma group. Hypothesis 4(b) stated that, across both groups, hippocampal volume will be inversely associated with average night-time cortisol levels. As Table 30 shows, this hypothesis was disconfirmed. The analyses detected no significant associations between average night-time cortisol and any of the proportional hippocampal volume measures.

Table 30

Relationship between Hippocampal Volume and (a) Declarative Memory Performance, and (b) Night-Time Cortisol Levels (whole sample: N = 19, Asthma group: N = 9, Healthy Control group: N = 10)

		VPA-15			Logical Memory					
		Immediate Recall	Delayed Recall	Retention	Immediate Recall	Delayed Recall	Learning	Retention	AMT	Cortisol
Hippocampal volume										
Right										
	Whole sample	-.69**[a]	-.47*[g]	.31	.03	.23	.46*[m]	.17	.08	-.01
	Asthma group	-.25	.07	.42	.49	.45	-.07	-.18	-.18	-.08
	Healthy Control	-.77**[b]	-.64*[h]	.25	-.50	.08	.67*[n]	.27	-.29	-.10
Left										
	Whole sample	-.69**[c]	-.51*[i]	.25	.04	.17	.30	.18	.20	-.13
	Asthma group	-.08	-.04	.18	.33	.29	-.05	-.09	-.33	-.29
	Healthy Control	-.88***[d]	-.74**[j]	.26	-.45	-.01	.50	.24	-.02	-.09
Total										
	Whole sample	-.71***[e]	-.49*[k]	.31	.04	.22	.42*°	.20	.13	-.24
	Asthma group	-.22	.04	.34	.44	.38	.03	-.19	-.26	-.22
	Healthy Control	-.82**[f]	-.67*[l]	.27	-.48	.09	.63*[p]	.32	-.19	-.14

Note. All values are for Pearson's *r* correlation coefficient. VPA = Verbal Paired Associates; AMT = Autobiographical Memory Test. The variable *Cortisol* represents the average across all night-time collections, in nmol/l.

[a]$p = .001$, [b]$p = .005$, [c]$p < .001$, [d]$p < .001$, [e]$p < .001$, [f]$p = .002$, [g]$p = .020$, [h]$p = .023$,
[i]$p = .013$, [j]$p = .008$, [k]$p = .016$, [l]$p = .017$, [m]$p = .025$, [n]$p = .017$,
°$p = .038$, [p]$p = .026$.

*$p < .05$. **$p < .01$. ***$p < .001$.

Table 31

Relationship between Night-Time Cortisol Levels and Declarative Memory Performance (whole sample: N = 19, Asthma group: N = 9, Healthy Control group: N = 10)

	VPA-15			Logical Memory				
	Imm. Recall	Del. Recall	Retention	Imm. Recall	Del. Recall	Learning Slope	Retention	AMT
Cortisol								
Whole sample	.24	-.01	-.35*	-.02	-.26	.06	-.38*	-.24
Asthma group	.13	-.36	-.70*	-.21	-.38	-.14	-.52	.54
Healthy Control group	.16	-.13	-.42	.14	-.37	-.05	-.54	.19

Note. All values are for Pearson's *r* correlation coefficient. VPA = Verbal Paired Associates; AMT = Autobiographical Memory Test. The variable *Cortisol* represents the average across all night-time collections, in nmol/l.

*$p < .05$.

Hypothesis 5: Relationship between asthma and cortisol. This hypothesis stated that asthmatics will have significantly higher cortisol levels than controls. On average, participants in the Asthma group (M = 3.87, SD = 3.45) had higher night-time cortisol levels than those in the Healthy Control group (M = 2.86, $S.D$ = 1.67). However, an independent-samples t-test detected no significant between-group differences, N = 18, $t(16)$ = -0.82 , p = .21, d = -.37.[10] Hence the hypothesis was disconfirmed.

Hypothesis 6: Triadic relationship between hippocampal volume, cortisol, and declarative memory. This hypothesis stated that "hippocampal volume will mediate the relationship between cortisol level and verbal declarative memory performance." Listed below is the set of associations necessary to demonstrate a mediational relationship between hippocampal volume, average night-time cortisol, and declarative memory performance:

Step 1. Cortisol must be significantly associated with memory.

Step 2. Cortisol must be significantly associated with hippocampal volume.

Step 3. Hippocampal volume must be significantly associated with memory.

Step 4. The association between cortisol and memory must be weakened significantly by the effect of hippocampal volume on both cortisol and memory.

Tables 30 and 31, which detail the analyses performed for Hypotheses 2-4, indicate that although the required relationship described at Step 1 was observed, average night-time cortisol levels did not correlate with the same declarative memory outcome measures that hippocampal volumes correlated with. Furthermore, the required relationship described at Step 2 was not observed. Hence, the full mediational analysis (i.e., Step 4), was not conducted, and the hypothesis was disconfirmed.

[10] One data set from the Asthma group was excluded because the participant was missing one of three cortisol data points.

Discussion

The current study aimed to investigate the relationship between exposure to inhaled corticosteroids and the size of the hippocampus in young adults with asthma. Specifically, I examined whether, in a sample of 9 asthmatic individuals characterized by daily use of moderate-to-high inhaled corticosteroids and 10 demographically matched healthy controls (both subgroups of the Study 1 sample), chronic exposure to inhaled glucocorticoid receptor agonists was associated with hippocampal volume. The study also investigated whether hippocampal volume mediated the relationship between night-time cortisol levels and declarative memory performance. These investigations comprised the testing of six separate hypotheses. The status of each hypothesis is considered in turn below.

Hypothesis 1 stated that individuals with asthma will have smaller hippocampal volumes than healthy controls. This hypothesis was partially confirmed. Both left and right head-size corrected hippocampal volumes were smaller in asthmatics, by 16% and 15% respectively, but only the differences in left hippocampal volume achieved statistical significance.[11] These findings are consistent with the observation made by Brown and colleagues (2004) that individuals with inflammatory disorders undergoing corticosteroid treatment, including asthmatics, had smaller left hippocampi compared to patients who had similar illness profiles but who were not on corticosteroid treatment. In the present sample, as in several previous studies (e.g., Brown et al., 2004; Mervaala et. al, 2000; Niemann, Hammers, Coenen, Thron & Klosterkötter, 2000; Starkman et al., 1992), right hippocampal volume tended to be larger than left hippocampal volume. However, the between-hemisphere difference was neither statistically significant nor affected by group status.

The pattern of data detected in the present study (i.e., significantly smaller left, but not right, hippocampal volume in asthmatics) is also consistent with literature suggesting that the

[11]Unsurprisingly, total hippocampal volume (i.e., left + right volume) was also significantly smaller in asthmatic participants compared to healthy controls. There is limited value in further interpretation of this difference given the aggregate nature of the variable.

left hippocampus is more vulnerable than the right hippocampus to the effects of chronic stress and various forms of psychological trauma (Anacker et al, 2013; Bremner et al., 1997; Kühn & Gallinat, 2013; Mervaala et al., 2000; Stein, 1997; Vythilingam et al., 2002; Winter & Irle, 2004; Zhang et al., 2011). This consistency echoes a neurobiological similarity noted earlier in the dissertation: The chronic experience of stress and trauma dysregulates HPA-axis functioning in much the same way as the chronic use of inhaled corticosteroids affects that system. Indeed, in the current sample, asthma severity scores, which are based on (a) the average weekly severity and chronicity of asthmatic symptoms which have manifested themselves during the course of 12 months, and (b) the extent of exposure to corticosteroids (chronicity as well as potency) during that same period, correlated negatively with indexed left hippocampal volume. The nature of this relationship implies that the burden of the chronic inflammatory disease and/or of the associated long-term use of more potent inhaled corticosteroids may be detrimental to hippocampal structures (and, hence, may have negative consequences for hippocampal-dependent functions such as declarative memory processing).

Analyses testing Hypothesis 4(a) examined, directly, the relationship between hippocampal volume and exposure to inhaled corticosteroids. Those analyses did not, however, detect any significant relationships, thus disconfirming the hypothesis that, within the asthma group, hippocampal volume would be inversely associated with degree of exposure to inhaled corticosteroids. In other words, the dose and frequency of inhaled corticosteroid usage (as measured by the corticosteroid-exposure score), independently of the severity and chronicity of asthma symptoms, did not correlate with any of the hippocampal volume measures.

This finding is consistent with that of Brown et al. (2004), who found no relationship between the dose and the duration of prednisone and hippocampal volume. Of particular interest is that these authors did not detect the sought-after association even though oral

corticosteroids such as prednisone are more potent than inhaled corticosteroids. The implication for the present study, therefore, is that the current negative finding is not necessarily attributable to threats to internal validity (e.g., design factors, such as the small sample size, and potential confounding variables, such as between-subject variability in baseline HPA-axis functioning).

Therefore, a possible explanation for the absence, in the current sample, of a statistically significant relationship between exposure to corticosteroids and hippocampal volume is that there is, in fact, no significant relationship between the two. Why, then, have Brown et al. (2004) and the current study found structural damage to the hippocampus in asthmatics who make chronic use of corticosteroids? One proposal is that inflammatory processes, such as raised levels of the IL-6 cytokine, affect hippocampal integrity (Bruehl et al., 2009). There is evidence suggesting associations of IL-6 with hippocampal volume and function; for instance, levels of that cytokine are associated with hippocampal-dependent memory performance in women with breast cancer (Kesler et al., 2013; see also Marsland et al., 2008; Siegers & Fardell, 2011). The IL-6 cytokine is known to be elevated in asthmatics, with particular involvement in mediating airway inflammation and lung function (Neveu et al., 2010; Rincon & Irvin, 2012). IL-6 is also involved in the regulation of glucocorticoids in response to psychological and physiological stress, but its actions on the brain can be dissociated from those of glucocorticoids (for a review, see Kronfol & Remick, 2000). It is therefore possible that the observed hippocampal damage in asthmatics is independent of the action of corticosteroids, and is instead associated with inflammatory processes (some of which underlie the very pathogenesis of asthma).

Another possibility is that hypoxic events associated with asthma attacks lead to neuronal atrophy or actual cell death in the hippocampus. There is evidence in the literature attributing hippocampal volume shrinkage to hypoxia (*e.g.*, Hopkinsa, Myers, Shohamy,

Grossman, & Gluck, 2004; Yonelinas et al., 2002), and there is also evidence demonstrating that many asthmatics experience hypoxic episodes (e.g., Montplaisir et al., 1983; Vogtel, Myriam; Michels, Alexandra, 2010). It is therefore reasonable to infer that, for at least some asthmatics with severe and poorly-controlled asthma, hypoxia may lead to dendritic shrinkage or neuronal death in the hippocampus, independently of the action of glucocorticoids.

Regardless of the source of hippocampal damage in asthmatics, it is indisputable that HPA-axis functioning is compromised in that population. This compromise results from chronic exposure to (a) illness-related physiological and psychological stress, and (b) corticosteroid-based therapies that attempt to counter the inflammatory processes underlying the pathogenesis of asthma (Ball et al., 2006; Barnes & Pedersen, 1993; Cave et al., 1999; Dahl, 2006; Dekhuijzen & Honour, 2000; Heim et al., 1999; Henzen et al., 2000; Kraft et al., 1998; Landstra et al., 1999; Martin et al., 2002; Masharani et al., 2005; Peebles et al., 2000; Ritz et al, 2011; Skoner et al., 2010; Smith et al., 1983; Sutherland, 2005; Sutherland et al., 2003; Szefler et al., 2002; Tayab et al., 2007). The effects of chronic exposure to stress include changes in CRH levels and sub-optimal secretion of cortisol (a profile which, it should be remembered, has been associated with left hippocampal damage; Bremner et al., 1997; Kühn et al., 2013; Mervaala et al., 2000; Stein, 1997; Vythilingam et al., 2002; Winter et al., 2004; Zhang et al., 2011; Seeman et al., 1994; Maras & Baram, 2012; Ivy et al., 2010). Furthermore, chronic exposure to corticosteroids suppresses the secretion of endogenous cortisol, and alters the circadian pattern of cortisol release. This suppression and alteration means that, across the day, in exposed versus non-exposed individuals, (a) average cortisol levels are lower, and (b) night-time cortisol levels tend to be higher.

Hence, Hypothesis 5 stated that individuals with asthma will have higher night-time cortisol levels than healthy controls. This hypothesis was disconfirmed. Although asthmatic

participants tended to have slightly higher levels of night-time cortisol, the between-group difference was not statistically significant, and their cortisol levels were not in the range defined as pathological by normative data (e.g., Clow, 2004; Viardot et al., 2005)

Hypothesis 4(b) also involved analysis of average night-time cortisol levels, stating that there would be an inverse relationship between that variable and hippocampal volume. This prediction was also disconfirmed: Analyses detected no significant association (either across the entire sample or within the asthma group) between average night-time cortisol levels and any of the measures of hippocampal volume.

There are at least two possible explanations for the negative findings with regard to Hypotheses 4(b) and 5, both of which made predictions regarding associations involving night-time cortisol levels. First, the prediction that, in asthmatics, elevations at the trough of circadian cortisol would be associated with greater hippocampal vulnerability was based on the results of previous studies demonstrating that association (e.g., McAuley et al., 2009; Pruessner et al., 2007; Starkman et al., 1992). Those studies, however, featured samples of significantly hypercortisolic individuals (e.g., patients with Cushing's disease). On average, such patients demonstrate significant night-time cortisol elevations compared to healthy controls, whereas the asthmatic sample in the current study did not. Under circumstances such as these, it is less likely that the current analyses would have detected the association observed in those previous studies.

A second explanation for these negative findings involves the choice of measures. In the current study, I collected three saliva samples, one at each of three different critical phases of sleep. Previous studies that have reported higher cortisol levels among asthmatics relative to healthy control, and inverse associations between cortisol and hippocampal volume in clinical populations, have used 24-hour circulating cortisol measures (e.g., Masharani et al., 2005; Skoner et al., 2010; Starkman et al., 1992; Sutherland et al., 200).

Others have found that the magnitude of cortisol elevation in response to stress (rather than levels of circulating cortisol levels per se) is inversely associated with hippocampal volume (e.g., Sindi et al., 2014).

Finally, some studies reporting a relationship between night-time glucocorticoid levels and hippocampal integrity have examined markers of neurogenesis, such as cell proliferation, rather than volumetric measures (Mirescu et al., 2006). One suggestion for future research, then, is that it should be designed with an eye toward (a) capturing sleep-associated patterns of cortisol secretion more accurately, and (b) taking multiple measures of the integrity of the hippocampal structure. Another suggestion is that future studies should first identify participants who have pathologically-elevated night-time cortisol and then compare their hippocampi to carefully-matched healthy controls.

The remaining hypotheses involved predictions regarding the relationships between memory performance, hippocampal volume, and night-time cortisol levels. Earlier, I referred to the suppression of HPA-axis activity by chronic exposure to stress and to corticosteroids. This suppression induces a cascade of metabolic changes affecting the adequate functioning of various neuronal networks, including hippocampal-to-neocortical pathways responsible for the processing of declarative memories (Bender et al., 1991; Brown et al., 2004; Bruehl et al., 2009; Coluccia et al., 2008; Keenan et al., 1996; Lupien et al., 2007; Newcomer et al., 1994; Schmidt et al., 1999; Wolkowitz et al., 1997). Recall also that this suppression is associated with elevated night-time cortisol.

Hence, Hypothesis 3 stated that, across both groups, night-time cortisol levels would correlate negatively with performance on tasks assessing verbal declarative memory. This hypothesis was partially confirmed. Average night-time cortisol levels were not associated significantly with performance on either the immediate or delayed recall components of the VPA-15 task and of the LM subtest. Cortisol did, however, correlate negatively with

measures of retention of verbal declarative material across the night (i.e., VPA Retention and LM Retention scores).

Hypothesis 2 stated that hippocampal volume would correlate positively with performance on verbal declarative memory tasks in the Asthma group but negatively in the Healthy Control group. That hypothesis was partially-confirmed. Indeed, the analyses detected significant, and moderate-to-strong, *negative* associations between hippocampal volume and performance on the immediate and the delayed recall trials of the VPA-15 task. In other words, a proportionally small hippocampus, both left and right, was associated with better performance on the VPA-15 recall trials administered both pre- and post-sleep in the case of the healthy controls and in the sample as a whole.

These findings are consistent with those reported by Pruessner et al. (2007), who studied a sample of healthy young, male adults. Pruessner and colleagues interpret their pattern of results as suggesting that smaller hippocampal volume confers a cognitive advantage in healthy young adults (i.e., having a smaller hippocampus allows for more efficient information processing). Although that interpretation might hold for healthy young adults (and, on the face of it, the current data cannot argue against that statement: analyses detected a significant negative relationship between hippocampal volume and performance on the VPA-15 task in healthy controls), does it hold in asthmatics?

In Chapter One and in the introduction section of the current chapter, I suggest that the relationship between hippocampal volume and declarative memory in asthmatics, would be similar to that found in the elderly, in patients with Cushing's syndrome, with PTSD and MDD because "…sustained hypercortisolemia or prolonged exposure to glucocorticoids are both associated with episodic memory impairment and with reduced hippocampal volume (Lupien et al., 1998; Sapolsky et al., 1990; Sheline et al., 1996; Starkman et al., 1992; Watanabe et al., 1992a, 1992b)…" (p.37), and "given that asthmatics present with

compromised HPA-axis function and make chronic use of glucocorticoid-based treatment, and that learning and memory difficulties have been reported in children with asthma" (p.12). In other words, I predicted that for asthmatics, smaller hippocampal volume would be associated with poorer performance on declarative memory task.

In support of this prediction, the current data set detected associations between cognitive performance and asthma severity and asthma severity and hippocampal volume, which suggest that unlike in healthy, non-asthmatic adults, a smaller hippocampus does not imply better cognitive function in young adults with asthma. Specifically, there was a significant negative correlation between asthma severity and IQ (i.e., those with more severe asthma scored more poorly on the IQ test); there was a significant negative correlation between asthma severity and hippocampal volume (i.e., those with more severe asthma had smaller hippocampi); and there was a significant positive correlation between hippocampal volume and IQ. Despite these findings, the current data set failed to detect any significant relationship between hippocampal volume and memory performance in the Asthma group. Of interest here, however, is that the magnitude of the relationship between memory performance and hippocampal volume varied markedly depending on the values used to represent hippocampal volume. In the main text, I chose to report results from analyses performed using indexed hippocampal volumes. (I did so for the sake of consistency in my approach to controlling for the co-variance between head-size and hippocampal volume.) The results reported in the main text include several significant associations. In Appendix N, I report results from analyses performed using raw volumes with partialled-out head-size residuals. The results reported in that Appendix are all non-significant. Therefore, it appears that the method of head-size correction, and the use of indices versus absolute values, influences the strength of the relationships observed between hippocampal volume and cognitive performance. Correlational data relating to regions of interest ought therefore to be

treated with caution, and the relationships revealed should be replicated in different experimental settings.

The final hypothesis, Hypothesis 6, built on the previous hypotheses by positing that there would be significant relationships between the three variables of interest. Specifically, Hypothesis 6 stated that hippocampal volume would mediate the relationship between average night-time cortisol level and verbal declarative memory performance. That hypothesis was disconfirmed. Indeed, the state of the data did not allow for a mediational analysis because (a) cortisol and hippocampal volume were not significantly correlated, and (b) although both cortisol and hippocampal volume correlated significantly with declarative memory performance, they did not correlate with the *same* memory measures (e.g., cortisol and VPA-15 and LM retention measures were correlated, whereas left, right and total hippocampal volume were correlated with VPA-15 recall performance).

In this regard, the current data are consistent with previous studies. Most of those studies have failed to establish a triadic relationship between hippocampal volume, cortisol levels, and performance on hippocampal-dependent memory tests. Although many studies have reported significant relationships between hippocampal volume and cortisol levels, and between hippocampal volume and declarative memory performance, a common mechanism positing hippocampal-based processing as a mediator for the action of corticosteroids on declarative memory performance is merely inferred. In fact, studies that have observed significant associations between cortisol and declarative memory performance have been unable to demonstrate that hippocampal volume mediates that relationship (Bruehl et al., 2009; MacLullich et al., 2005; O'Brien et al., 2004; Pruessner et al., 2007; Starkman et al., 1992).

Limitations and Directions for Future Research

Several limitations of the current design suggest caution in interpreting the results. In this section, I describe some of those limitations (i.e., those related to the measures used, between-group differences in sociodemographic characteristics, and sample size), and make suggestions as to how future research studies might learn from the way in which they might have affected the power of this study.

One limitation relates to the measures of memory used. As did many previous cross-sectional studies (e.g., Lupien et al., 1994; Newcomer et al., 1994; 1999; Starkman et al., 1992), I employed standardized declarative memory tasks of the kind typically used in clinical assessment settings. However, such tasks are often insensitive to subtle impairments, and are susceptible to ceiling effects when administered to healthy individuals (Ellenbogen, Hulbert, Stickgold, Dinges, & Lezak, 2004; Thompson-Schill, 2006; Rabin et al., 2009). Furthermore, tests assessing discrete cognitive functions, such as verbal memory, have relatively low power to predict regional brain volumes; in fact, in adults, measures of general cognitive ability correlate better with total brain size as well as with regional volumes (MacLullich et al., 2002). Hence, the use of standardized declarative memory tasks might be one reason why this study, and numerous previous research efforts (e.g., Bruehl et al., 2009; MacLullich et al., 2005; O'Brien et al., 2004; Pruessner et al., 2007; Starkman et al. 1992), have failed to detect a significant triadic relationship between glucocorticoid activity, hippocampal morphometry, and memory processing.

A second, and related, limitation relates to the way in which cortisol was measured in the current research. There is well-documented intra-individual variability in HPA-axis function and, consequently, cortisol secretion (e.g., Almeida, Piazza, & Stawski, 2009; Buchanan et al., 2006; Clow, 2004; Kirschbaum et al., 1996; Smyth et al., 1997). One of the consequences of this variability is that studies relying on a few measurement points (e.g.,

measures taken in the morning and/or evening only, or, as in the current study, thrice over the course of the night) generate estimates of daily secretion patterns that are less accurate and representative than studies that take (a) numerous measures repeatedly over a period of hours, days, or weeks (see, e.g., Keenan et al., 1996; Newcomer et al., 1994, 1999) and (b) measures of the magnitude of cortisol response to an environmental challenge or to awakening (e.g., alcoholism study). Of particular interest here is that studies reporting an association between cortisol levels and hippocampal volume tend to be those using, for example, aggregates of 24-hour cortisol collections (Starkman et al., 1992) or measures of the cortisol awakening response (CAR; Pruessner et al., 2007). In fact, there is empirical evidence that individuals with hippocampal damage may present with a normal diurnal cortisol secretion pattern but with an absence of the normal CAR (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004; Wolf et al., 2005). Hence, studies measuring only circulating levels of cortisol may fail to detect an existing association between hippocampal volume and HPA-axis functioning.

A third limitation relates to the fact that there were significant between-group differences with regard to (a) sex distribution and (b) IQ scores. Regarding the former, 7 men and 2 women comprised the Asthma group, whereas 4 men and 6 women comprised the Healthy Control group. This uneven sex distribution was unavoidable given that the current study used a subset of participants from Study 1 who (a) agreed to participate in the neuroimaging component of the research program, and (b) met the eligibility criteria for the MRI study. Hence, participant attrition from the Study 1 sample to the Study 3 sample was beyond my control.

Although a chi-square test of contingency did not detect a significant between-group difference with regard to sex distribution (perhaps because of the small sample size), the effect size estimate approached .40, which suggests that the magnitude of the difference might have some practical significance. This potential significance is amplified when noting

that male participants had smaller hippocampi-to-head size ratios than females. In mitigation of these facts, however, is the observation that between-group differences for left and total hippocampal volumes endured after sex had been controlled for statistically. Nonetheless, future studies in this field should attempt to ensure an equivalent male-female distribution across groups.

Regarding IQ scores, the analyses detected significantly higher IQ scores in the Asthma group than in the Healthy Control group. Although we cannot entirely exclude the possibility that individuals with asthma have higher IQs than their age-matched counterparts, there is little theoretical basis or empirical evidence to assume the presence of superior cognitive performance among individuals with asthma when compared to peers recruited from the population studied here (i.e., English-speaking university students of various races, between the ages of 18 and 35 years). Aside from Mitchell and Dawson (1973), who found that their 7-year-old asthmatic participants had, on average, significantly higher IQ scores (102 points vs. 95 points) than children of the same age, and Benbow (1986) who found a higher prevalence of asthma among adolescents with extreme precocious intellectual abilities than among the general population, the bulk of the evidence assessing the academic and general cognitive ability of children and adults with asthma suggests that their IQ scores are neither significantly higher nor significantly lower than those of their demographically-matched peers (Daramola, Ayoola, & Ogunbiyi, 2010; Ghaffari, Abbaskhanian, & Jalili, 2014; Gilman, Gardener, & Buka, 2008; Griffith , 1965). Hence, it is likely that the current finding is spurious, and is likely a product, again, of the vicissitudes induced by a relatively small sample size.

Furthermore, there was a significant correlation between sex and IQ in the current sample: On the measure of PIQ, male participants outperformed their female counterparts by approximately 10 points (i.e., 2/3 of a standard deviation if one uses the standardization

metric). This phenomenon of higher PIQ among male participants could be the result of sampling bias. Most participants in this series of studies were recruited from the UCT undergraduate psychology student database. Most of those students are women, meaning that there were relatively few eligible male participants (particularly male asthmatic participants) for the three-study project. Hence, I extended recruitment efforts to include students from other faculties. An online advertisement sent out through the university's intranet drew a disproportionately large response from engineering students with asthma who had an interest in the electrophysiological measurement of sleep. Most of these students were men, and their PIQ score range was 120-135.

A fourth limitation relates to sample size. As should be clear from some of the points made in the previous paragraphs of this section, future research can address many of the limitations of the current study by recruiting larger numbers of participants, and by ensuring that groups are well matched with regard to sociodemographic and IQ characteristics. Furthermore, any study using hippocampal volume as either a predictor or outcome variable requires a fairly large sample if it is to avoid being under-powered. This point is emphasized by van Petten (2004), whose meta-analysis of studies investigating the relationship between hippocampal volume and memory performance in healthy adults revealed that inter-individual differences in hippocampal volume accounted for most of the between-study variance, even after controlling for age and sex. The validity of studies with smaller sample sizes, such as this one, is much more likely to be impacted negatively by these inter-individual differences.

Despite these limitations, the current study provides exploratory data on the correlates of declarative memory processing in a population of young adult asthmatics. It is the first study of its kind to investigate a potential mediatory role of the hippocampus in the relationship between cortisol and memory performance in a sample of asthmatic participants

compared to healthy controls. Further strengths of the study are that it featured participants with asthma exclusively, as opposed to participants with loosely-defined inflammatory or atopic diseases; that those asthmatic individuals had well-defined and homogenous treatment regimens; and that those individuals did not experience the possible confounding effects of co-morbid psychiatric disorders.

Summary and Conclusion

The major finding of this study is that, in the current sample, having asthma and being treated with corticosteroids (i.e., having a lifelong experience of specific and persistent respiratory symptoms and being chronically exposed to moderate-to-high doses of inhaled corticosteroids) was associated with smaller hippocampal volume in otherwise healthy young adults. However, despite having smaller left hippocampi and higher IQs, asthmatics did not perform differently to healthy controls on measures of declarative memory performance. An interesting finding was that, among healthy controls, smaller hippocampal volume was associated with better memory performance, but that, among asthmatics, there was no association between hippocampal volume and memory performance. Hence, it appears that, in healthy young adults, smaller hippocampi might be indicative of more efficient synaptic network performance. The question of why there was no similar association in asthmatics remains an open question. One might speculate that the volume reduction induced by chronic inflammation is moderated by certain factors (e.g., IQ, age, inter-individual variability in the response of the HPA axis to different treatment regimes), and that that moderation modifies the association between volume and cognitive performance. It was beyond the scope of the current design to test such speculation.

Finally, the analyses detected a significant association between cortisol and the percentage of word pairs recalled correctly after a period of sleep. However, in contrast to the

a priori prediction, hippocampal volume did not mediate the relationship between cortisol levels and memory performance. In fact, the analyses did not detect any significant association between cortisol and hippocampal volume. Nevertheless, the impact of corticosteroids on the hippocampus is well-documented in previous studies, and hence the possibility of such an association in the current sample cannot be excluded altogether. It is possible that more sensitive measures of HPA-axis functioning (e.g. cortisol awakening response), or more extreme HPA-axis dysfunction (e.g., as is present in patients with Cushing's syndrome), is required for associations with hippocampal volume to become evident. It is also possible that, as Sheline et al. (1996) suggest, the effects of transient pathological fluctuations of glucocorticoid activity endure long after levels of circulating cortisol have returned to normal.

Although the current data cannot provide definitive comment on whether the action of corticosteroids can account for differences in hippocampal volume between asthmatics and healthy controls, one must consider the possibility of alternative mechanisms. Based on (a) the current observation that asthma severity and hippocampal volume were inversely related, and (b) existing evidence linking cytokine activity to hippocampal functioning, it is possible that inflammatory processes act on hippocampal cells, independently of glucocorticoids, to induce neuronal cell death or to slow down the rate of neuroregeneration in young adults with asthma. This possibility remains to be investigated, either through cross-sectional designs involving the correlation of IL-6 levels with hippocampal volume, or (perhaps more effectively) through longitudinal designs charting the course and the intersection of the pathogenesis of asthma with hippocampal development. Another possible alternative mechanism underlying the association between asthma severity and hippocampal volume is the hypoxia associated with asthma attacks. One way to separate the effects of hypoxia from the effects of glucocorticoids would be to monitor participants for sleep apnea, and to test the

association between oxygen saturation and the number of apneic events, on the one hand, and hippocampal volume, on the other.

CHAPTER FIVE:
GENERAL DISCUSSION

The overarching purpose of the dissertation was to explore the relationship between chronic exposure to inhaled corticosteroids and sleep-dependent memory processes and possible neuroanatomical mechanisms underlying that relationship. I measured these memory processes indirectly through performance on episodic memory tasks on the one hand, and the memory content of dream reports on the other. The main aim behind this investigation was to demonstrate that even inhaled corticosteroids with little systemic absorption can be associated with compromised cognitive performance, largely because of their effects on HPA-axis function.

To accomplish these aims, I measured sleep architecture, night-time cortisol levels, memory performance, and dreaming patterns in individuals with asthma who made regular use of inhaled corticosteroids as the main line of treatment for their asthma (Study 1). I then studied, in healthy young adults, the effects of a single dose of oral corticosteroids on the same outcomes (Study 2). There is relatively little data on the prolonged effects of corticosteroids on cognition, in contrast to existing evidence on their acute effects. The main rationale for conducting Study 2 was to provide a platform for comparison on how the impact of chronic exposure differs from acute exposure. Lastly, I investigated the neuroanatomical basis of the relationship between prolonged corticosteroid treatment and potential impairment in episodic memory processing by measuring hippocampal volume in moderate-to-severe asthmatics versus healthy controls (Study 3).

The aim of this chapter is to integrate the findings from the three distinct studies into complementary components which will serve, I hope, to answer the broad questions posed by the overall investigation. First, I discuss whether or not the proposal that corticosteroids affect HPA-axis function (as measured night-time salivary cortisol levels) was confirmed by

the results of Studies 1 and 2. I address this question first as it pertains to the core mechanism of action on which I based most of my other predictions. Second, I discuss whether exposure to corticosteroids and elevated endogenous cortisol are associated with changes in aspects of sleep organization. Third, I discuss (a) the relationships between night-time glucocorticoid levels and memory performance, (b) the relationships between sleep outcome variables and memory performance, and (c) a possible mediating role for sleep in the relationship between glucocorticoids and memory. Fourth, I discuss the role of dreaming in the relationships between cortisol, sleep, and memory. In this latter section, I attempt to examine whether or not dream content can serve as an indicator of how effectively episodic memories are consolidated during sleep, under normal conditions versus conditions of elevated evening cortisol. Finally, the chapter concludes by discussing how the results of Study 3 might fit with those of Study 1. Recall that Study 3 was designed to test the set of hypotheses that together suggested that individuals most exposed to corticosteroids, chronically (i.e., what I defined as *moderate-to-severe* asthmatics in this dissertation), have smaller hippocampal volumes (due to structural damage incurred as a result of that exposure) than healthy controls. If confirmed, this set of hypotheses would provide a potential mechanism through which chronically corticosteroid-exposed individuals might perform less well on tasks assessing neutral declarative memory.

Is Exposure to Corticosteroids Associated with Elevated Cortisol During Sleep?

The first question of interest in this dissertation was whether corticosteroid exposure is associated with elevated cortisol levels during sleep. To address this question, I compared, in Study 1, night-time cortisol levels in five groups: (1) asthmatics with moderate-to-severe exposure to inhaled corticosteroids, (2) asthmatics with mild exposure to inhaled corticosteroids, (3) asthmatics with no exposure to inhaled corticosteroids, (4) non-asthmatics

with topical exposure (eczema controls), and (5) non-asthmatics who had not been exposed to corticosteroids of any sort (healthy controls). First, I measured their cortisol levels during sleep and conducted between-group comparisons testing with the prediction that the group with the most corticosteroid exposure (i.e., the moderate-to-severe asthma group) would have the most elevated levels. Then, I correlated the level of corticosteroid exposure among asthmatics with their average endogenous cortisol levels to test whether a greater level of exposure to exogenous corticosteroids was associated with higher levels of cortisol during sleep.

In Study 1, all the patient groups (i.e., the three asthma groups and the eczema control group) had higher night-time cortisol levels than healthy controls. However, the observed pattern of data did not confirm the a priori predictions that this effect was specific to inhaled corticosteroid exposure, and that there would be a dose-response relationship: Mean cortisol levels were highest in the eczema control group (i.e., the only patient group with topical exposure to corticosteroids), and mean cortisol levels in the moderate-to-severe asthmatic group (i.e., the group most exposed to corticosteroids) did not differ significantly from those of healthy controls. In fact, among the three asthma groups, mean night-time cortisol levels were most elevated in the untreated asthma group. That group consisted of individuals who had less consistent symptom manifestations than asthmatics in the other two groups, and who used their corticosteroid treatment only when they were symptomatic.

Although none of individual participants' cortisol data were in the hypercortisolic range, many of them were above reported evening norms (Clow et al., 2004). This finding is relevant only to the extent that these slight but significantly raised cortisol levels during sleep have been associated with a higher prevalence of nocturnal asthma symptoms in asthmatics (Sutherland et al., 2003), which characteristically are respiratory events which fragment and

disrupt sleep (Barnes et al., 1980; Haxhiu et al., 2006; Klink et al., 1987; Montplaisir et al., 1982, 1983; Rhind et al., 1985; Shapiro et al., 1986).

Is Exposure to Corticosteroids and Elevated Night-time Cortisol Associated with Disrupted Sleep?

Study 1 participants who had been exposed to corticosteroids (i.e., moderate-to-severe asthmatics, mild asthmatics, and eczema controls) displayed the smallest proportions of REM sleep. This time, the predicted dose-response relationship was confirmed, at least partially: Moderate-to-severe asthmatics had smaller proportions of REM than mild and untreated asthmatics, although eczema controls experienced similar levels of REM suppression as moderate-to-severe asthmatics.

In Study 2, prednisone-treated participants presented with similar patterns of proportionally less REM sleep, but they also presented with SWS disruptions. In addition, prednisone-treated participants experienced highly fragmented sleep and less REM Intensification relative to their placebo-control counterparts.

Overall, the Study 2 data (but not the Study 1 data) suggested that cortisol was (a) negatively associated with percentage REM sleep and REM Intensification, and (b) positively associated with WASO. The greater impact of glucocorticoid activity on sleep in Study 2 than in Study 1 was expected, given the greater potency of the glucocorticoid used in that study (i.e., the moderate oral dose of Study 2 versus the mild-to-moderate inhaled doses of Study 1) and the known vulnerability of REM sleep to synthetic glucocorticoids, and to glucocorticoid receptor activation in general (Antonijevic & Steiger, 2003; Besedovsky et al., 2012; Buckley & Schatzberg, 2005; Bohlhalter et al., 1997; Born et al., 1991, 2008; Friess et al., 1994, 2004; García-Borreguero et al., 2000; Gillin et al., 1974; Schmidt et al., 2000). In terms of a direct relationship between night-time cortisol levels and SWS, correlational

analyses of data from Study 1 and Study 2 suggested that cortisol was significantly negatively associated with SWS Distribution.

SWS Distribution is a measure of the preferential concentration of delta sleep in the first half of the night. In other words, elevated night-time cortisol in both Study 1 and Study 2 participants interfered with the homeostatic process S of sleep. The aim of process S is to plunge the sleeper into the deeper stages of sleep shortly after sleep onset, in reaction to preceding waking activity. If this process is disrupted, then sleep is considered non-recuperative (e.g., Borbély Baumann, Brandeis, Strauch, & Lehmann, 1981; Cohen et al., 2010; Durmer & Dinges, 2005; Dauvilliers & Billiard, 2004; Susmakova, 2004; Tononi & Cirelli, 2006; 2015; Werth et al., 1996).

The fact that patients using moderate-to-high doses of inhaled corticosteroids experienced similar REM sleep disruptions as patients using oral corticosteroids has important clinical implications in terms of the selection and management of treatment. As mentioned in Chapter One (p. 52), research on the optimal dose required for the effective management of asthma indicates that high doses of inhaled corticosteroids are not more effective in treating asthma symptoms and in relieving airway inflammation than are low-to-moderate doses (Guleria & Mohan, 2007; Holt et al., 2001; Powell et al., 2003). Furthermore, exposure to high doses of inhaled corticosteroids is associated with physical side effects, such as loss of bone density favoring the development of osteoporosis, cataracts and glaucoma, and skin thinning and bruising (Dahl, 2006). This relatively negative side effect profile of inhaled corticosteroids (to which one can add, given the findings of this study and others (e.g., Javaheri, Storfer-Isser, Rosen, & Redline, 2008; McEwen & Karatsoreos, 2015; Wulff, Gatti, Wettstein, & Foster), the effects of disrupted sleep on cardiovascular health, glucose and fat metabolism, stress resistance, emotion regulation, and cognitive performance), alongside the negligible main effects of high versus low-to-moderate doses of these drugs,

suggests that clinicians should be wary when prescribing treatment regimens to asthmatic patients, and should not simply follow conventional thought regarding side effect profiles of corticosteroids with high systemic absorption (i.e., oral corticosteroids) versus those with low systemic absorption (i.e., inhaled and topical corticosteroids). The negative effects of moderate-to-high doses of inhaled corticosteroids on sleep bear repeating: There are clear indications, based on results reported here, that there are changes in the organization of sleep among individuals exposed to inhaled corticosteroids. Any change in the organization of SWS, and any reduction in REM sleep, is bound to have widespread effects on everyday functioning, given literature on the relationship between both the proportion and the sequence of these particular sleep stages, in relation to one another, to the rest of sleep architecture, and to the time of night (Ambrosini & Giuditta, 2001; Born et al., 2004; Fischer et al., 2006; Nielsen, 2004; Peigneux et al., 2001; Poldrack et al., 2003, 2004; Rasch et al., 2006; Ribeiro et al., 2004; Stickgold et al., 2000; Walker & Stickgold, 2010).

Limitations of the sleep data. Because the designs of Studies 1 and 2 involved collecting only one night of sleep data per participant, those studies are limited in their ability to define sleep architecture among corticosteroid-exposed individuals, asthmatic or otherwise. The limitation here centers largely on ecological validity. First, the absence of a sleep laboratory adaptation night in either study's design means that the sole night on which PSG data were captured might not be an accurate representation of participants' sleep in the home environment. Adaptation nights preceding the actual data collection night(s) allow participants to become comfortable with the unfamiliar laboratory setting and with sleeping surrounded by laboratory equipment, so that sleep on subsequent data collection nights more closely resembles sleep in the familiar home environment (Agnew, Webb, & Williams, 1966; Spoormaker & Montgomery, 2008). The value of an adaptation night has come under scrutiny in some recent studies, however, with some demonstrating that it might not be

strictly required in every PSG study (Bonnet & Arand, 2006; Curcio, Ferrara, Piergianni, Fratello, & De Gennaro, 2004; Sforza, Chapotot, Pigeau, & Buguet, 2008). Second, capturing sleep data on one night rather than longitudinally means that one cannot measure the co-variation of sleep disruption with seasonal or stress-related variation in asthma symptoms. Overall, then, the optimal designs for Studies 1 and 2 might have involved the longitudinal collection of PSG data for each individual, with waves of collection at set intervals over an extended period of time (e.g., 3 nights (including 1 adaptation night) every 3rd month over the course of 1 year).

However, a longitudinal data collection approach was not possible within the framework of a doctoral dissertation, with limited time, human, technical, and financial resources. These limitations are linked to the current status of sleep research in Africa. This is the first project on our continent in the area of sleep and cognition since the 1980s. The handful of sleep laboratories in our country, with the exception of the University of Witwatersrand's *Dial-a-Bed* sleep laboratory, are restricted to clinical assessments of sleep disorders. A single, private sleep disorder center agreed to accommodate the research for 2 nights a week over the period of 1 year, *pro bono*. Hence, I chose to collect one night of sleep data per participant, and to compare the effects of low, high and no corticosteroid exposure, and acute versus chronic exposure, as opposed to running a significantly smaller study featuring a within-subject design and, for instance, 3 nights of data per participant.

Furthermore, I acknowledge the potential confounds introduced by performing two forced awakenings during the test night. Although these awakenings were brief, they might have altered sleep architecture. Such alterations would have been consistent across all participants, however, given that the awakenings were highly standardized.

Despite these limitations, this set of exploratory data can serve as a foundation for further research on the effects of prolonged corticosteroid use on sleep, and on sleep-dependent cognitive processes, in young adults.

Does Sleep Mediate the Relationship Between Cortisol and Memory?

Chapter One reviewed previous literature describing, and speculating about, how glucocorticoid activity affects memory processes during waking and during sleep, and how healthy sleep supports effective memory processing. Below, I discuss whether changes in the organization of sleep mediate, at least in part, the relationship between glucocorticoid activity and memory consolidation during sleep.

Glucocorticoid activity, sleep, and verbal declarative memory. A priori predictions tested in both Study 1 and Study 2 stated that elevated glucocorticoid activity would be associated with relatively poorer performance on episodic memory tasks. These predictions were disconfirmed. Statistical analyses, in both studies, detected no significant between-group differences in episodic memory task performance. Both chronically and acutely corticosteroid-exposed participants in the current studies performed as well as healthy controls, either before or after sleep.

The current data, then, stand in contrast to widespread evidence in the literature suggesting that heightened glucocorticoid activity (either endogenous or exogenous) is associated with specific disruptions in episodic memory performance (for a review, see Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). The current data also stand in contrast to previous reports suggesting that these effects are particularly evident if glucocorticoid activity is raised during its normal trough (i.e., during early sleep; Masharani et al., 2005; McEwen, 1995). Explanations for the contrast between the current results and those from previously published studies may pertain to (1) the use, in the current research, of

standardized tests in a cohort of young adults drawn from a university population, and (2) chronic exposure of participants to inhaled corticosteroids leading to habituation to the drugs' effects. I elaborate on each of those two potential explanations below.

First, the sample of participants tested here consisted of young adults with a university education, with IQs in the average to above-average range. Previous literature suggests that vulnerability to the negative effects of glucocorticoids on memory varies with age, with the elderly being most at risk (Backhaus et al., 2007; Lupien, 1994; Lupien et al., 1998; MacLullich et al., 2005; McAuley et al., 2009; McEwen, 1999; O'Brien et al., 2004; Sapolsky et al., 1986; Van Cauter et al., 1998, 2000). It is possible that age-related plasticity mechanisms offer protection from the deleterious effects of corticosteroid exposure on memory, or that the effects observed in the elderly are cumulative and only become apparent much later than the third decade of life. Furthermore, the episodic memory tests used in the current study are all drawn from standardized clinical neuropsychological test batteries, and so are designed primarily to detect differences between those with and without organic brain damage. They may therefore be relatively insensitive to subtle differences in performance across groups of highly-educated and, aside from asthma, healthy young adults. The fact that correlational analyses in Study 1 and Study 2 detected no significant associations between night-time glucocorticoid levels and performance on subsequent (i.e., morning recall) episodic memory tasks is further evidence for the lack of effect in the current sample; as mentioned above, such effects are much more likely to be detected in older-adult samples.

Second, it is possible that at moderate-to-high levels of exposure to inhaled corticosteroids, a certain habituation to the effects of those drugs occurs, especially in young, otherwise healthy, adults, as far as memory performance is concerned. For instance, Brown and colleagues (2006) found that after first exposure to prednisone, the performance of participants on a list-learning task declined but that, after a second exposure, performance

started to stabilize, even when practice effects on the task were controlled for by the study design. There is however, no empirical evidence yet longitudinally charting the cumulative effects of lifelong use of inhaled corticosteroids on memory performance in adult asthmatics.

Regarding the relationship between sleep outcome variables and performance on hippocampal-dependent episodic memory tasks, the a priori predictions were, for the most part, disconfirmed. Only REM intensification (Study 1) and sleep efficiency (Study 2) were positively associated with performance on delayed recall tasks. In other words, Study 1 participants who experienced progressively longer REM periods as the night progressed, and Study 2 participants who experienced more total time spent sleeping in the period between night-time lights off and morning awakening, performed better on the morning memory tasks. For the most part, however, it appeared that the quality and organization of sleep was, in both Study 1 and Study 2, unrelated to memory performance.

One way to account for this negative finding is by suggesting that the word pairs and stories encoded by the participants immediately before sleep were less important (e.g., of less adaptive value; Anderson et al., 2000; Nairne et al., 2007; Shohami et al., 2010) than other learning in which they had engaged earlier in the day (e.g., learning related to their university degree requirements). In other words, if the study-related information encoded by participants was not of relevance to them (e.g., if the context of the experiment did not provide them with motivation to perform well on the memory tasks), and if it competed with relatively more important information, then it is possible that, in at least some cases, sleep may have played a part in the active weakening of the study-related information. This account is consistent with both animal and human data suggesting that sleep plays a role in simultaneously eliminating relatively superfluous information while consolidating relatively more important information (Ellenbogen et al., 2006; Lipinska et al., 2014; Liu, Faraguna, Cirelli, Tononi, & Gao, 2010).

Glucocorticoid activity, sleep, and procedural memory. Although data from both Study 1 and Study 2 suggested that neither sleep outcome variables nor glucocorticoid activity bore a significant association to performance on episodic memory tasks, data from those studies related to performance on the procedural memory task presented a different picture. For instance, in Study 2 prednisone-treated participants performed significantly more slowly than placebo controls on the finger tapping task (FTT), both after training (i.e., immediately before sleep, but after the baseline measure) and after sleep. Furthermore, sleep did not benefit FTT performance in corticosteroid-exposed participants, whereas it did in placebo controls. Based on their findings on the effects of sleep on FTT performance, Walker et al. (2003) suggest that while training improves performance within a session, sleep provides an offline phase for further consolidation; hence, participants should perform better on the procedural memory task after a period of sleep. The results of Study 2 suggest that exposure to oral corticosteroids before bedtime neutralizes this memory-enhancing function of sleep.

I investigated the nature of this difference further and found that, on the morning FTT trials in Study 2, both speed and accuracy were inversely associated with REM1 and REM2 glucocorticoid levels. Furthermore, speed of performance was positively associated with SWS percentage and SWS Distribution. In an even stronger piece of data, the results of a mediational analysis confirmed that the organization of SWS (i.e., as measured by the SWS Distribution variable) entirely mediated the relationship between night-time glucocorticoid levels and speed of FTT performance. This result stands in contrast to previous literature suggesting that glucocorticoids (i.e., changing the ratio of MR to GR receptors) have no effect on procedural memory, in contrast to their effects on episodic memory. Instead, the current data suggest that statement should be modified: Heightened glucocorticoid activity

before bedtime is associated with disrupted procedural memory performance after sleep, but only *indirectly*, through its association with disruptions in the organization of sleep.

The consolidation of declarative (e.g., episodic) and non-declarative (e.g., procedural) memories rely on the same principles and cellular mechanisms. However, whereas the consolidation of declarative memories often involves recruiting or altering relatively few synapses to assimilate discrete chunks of new information into pre-established schemata, consolidation of non-declarative memories often involves more extensive synaptic modifications and, therefore, can take longer (Ribeiro et al., 2004; Thomson & Kim, 1996). This difference may explain why sleep deprivation is much more detrimental to the consolidation of non-declarative than declarative memories (Fowler et al., 1973; Karni et al., 1994; Laureys et al., 2002; Maquet et al., 2003; Mednick et al., 2003; Smith, 1995, 2001; Stickgold et al., 2000a; Walker et al., 2002). For instance, in a classic study Jenkins and Dallenbach (1924) showed that sleep deprivation is more likely to impair the retention of newly-learnt strings of nonsense syllables than they are real words. In fact, it appears that declarative memory tasks need to be elaborate and cognitively demanding to be affected by post-acquisition sleep deprivation (Ribeiro et al., 2004). This strand of the literature may therefore help explain why, in Study 2, sleep disruption was associated with performance deficits on the procedural memory task, but not on the episodic memory tasks.

The observed association between SWS Distribution and FTT performance is not surprising, given existing evidence linking a concentration of early-night SWS to optimal performance on tasks assessing procedural memory (Huber et al., 2004; Moroni et al., 2008; Robertson et al., 2004). However, the picture is not as straightforward as this: Both SWS *and* REM sleep are required for the effective consolidation of memory (both declarative and non-declarative). Importantly, SWS and REM sleep are optimally effective only if they act in a temporal order, with the effects of SWS preceding those of REM (Gais et al., 2000; Mednick

et al., 2003; Stickgold et al., 2000). Hence, under conditions where SWS is scarce during early sleep, offline consolidation would have an unstable foundation.

In conclusion, Study 1 and Study 2 were not designed specifically to test competing predictions from different models of sleep-dependent memory consolidation. However, the current data disconfirm predictions derived from the dual-process model, which associates SWS with declarative memory exclusively, and REM sleep with non-declarative memory exclusively. Furthermore, the current data support predictions derived from the sequential hypothesis, which posits that the cycling from NREM to REM sleep, together with the evolution of the cycles from early-night SWS-rich sleep to late-night REM-rich sleep, are central to the effectiveness of offline memory consolidation: Variables measuring the organization of SWS and REM sleep (e.g., SWS Distribution, REM Intensification) were stronger predictors of memory performance than were variables measuring the mere proportional presence of those sleep stages (e.g., SWS percentage, REM percentage).

Dreams, Glucocorticoids, Sleep, and Memory

This section summarizes and discusses those aspects of Studies 1 and 2 that focused on dream-related variables as either outcomes (in their relationships to glucocorticoids and to sleep organization), or as a mediator (in the relationship between glucocorticoids and declarative memory performance). First, I investigated whether exposure to exogenous corticosteroids and relatively elevated levels of night-time glucocorticoid activity were associated with lower levels of dream recall, differing circadian patterns of dreaming, poverty of content, and to qualitative aspects of the dreaming experience. Second, I investigated a possible mediating role for sleep staging and organization in the relationship between cortisol and dream content. Third, I investigated whether dream content mediated the relationship

between night-time glucocorticoid levels and declarative memory performance. The following sub-sections discuss these three aspects of Study 1 and 2 in turn.

Is corticosteroid exposure related to dreaming? In both Study 1 and Study 2, the number of dreams reported by corticosteroid-exposed individuals did not differ significantly from that reported by non-exposed individuals. However, this lack of between-group difference does not justify a blanket statement indicating that there is no significant relationship between corticosteroid exposure and dreaming. Instead, examination of the quality of the dreams (e.g., in terms of general poverty of content, and of their memory content), rather than simply their quantity, suggested that corticosteroid exposure might, in fact, bear a significant relationship to dreaming.

Poverty of content. One notable quality of the dreams of corticosteroid-exposed participants was poverty of content. In other words, these individuals appeared to experience mnestic challenges when attempting to recall their dreams. For instance, in Study 1, mild asthmatics recalled a significantly greater number of white dreams (i.e., reports of impressions of dreaming, without any recollection of actual dream content) than participants in other groups. Very few non-asthmatic participants reported white dreams. Similarly, in Study 2, the dream reports given by the prednisone-treated participants were brief, with limited detail of the dream scenarios.

Further regarding poverty of content in the prednisone-treated participants, many of their dream reports were striking for their lack of descriptors. Participants recalled the gist of stories, they reported impressions of having been thinking about a matter, or they stated that they were worried about, or preoccupied by, a particular matter while struggling to elaborate upon why exactly they were worried, or the precise source of the preoccupation. They did, however, report experiencing an association of strong emotions with these seemingly elusive dreams. This pattern of dreaming resonates with the pattern of the white dreams reported by

asthmatics in Study 1, where, as is typical in white dreaming, the visual content of the dream escaped the dreamer but a persistent, compelling emotional impression of having fully engaged with a dream remained.

Why was there such poverty of content in the dream reports of corticosteroid-exposed participants? One piece of conjecture is that between-group differences in SWS and REM sleep organization in Study 1 and in Study 2, as well as considerable sleep fragmentation in prednisone-treated participants in Study 2, may have accounted for the fragility of the dream plot development and of subsequent recall in corticosteroid-exposed individuals. Many of the dream reports collected from those individuals seem to fit the description of typical NREM dreams (i.e., they were brief and lacked perceptual references while containing frequent references to thinking processes) even though they were collected from REM sleep stages.

Consider Nielsen's (2004) proposition that REM and NREM sleep evolve towards each other along a spectrum of consciousness, with a single dream generator at work, drawing from the electrophysiological processes underlying each stage of sleep to generate different aspects of a dream. Placing the currently observed data within that framework, one might argue that the suppression of REM sleep by glucocorticoids resulted in measured mentation more like that typically encountered during NREM sleep periods, even though measures were taken at points in cycles that were nominally REM sleep. In other words, because REM sleep could not always be achieved, and when it was achieved it was frequently disrupted by periods of wakefulness, the ensuing mentation consequently failed to evolve into the rich, textured, fully-formed perceptual experiences that many would consider the only form of sleep mentation properly termed 'dreams' (Cicogna & Bosinelli, 2001; Foulkes & Schmidt, 1983; Herman et al. 1978; Nielsen 1999).

Regardless of why exactly there was such poverty of content in the dream reports of corticosteroid-exposed participants in the current study, future investigations should examine,

for all participants in their studies, report length and should quantify references to content. Such outcome variables may be more effective than the variables used in the current study (i.e., the products of close-ended yes-no questions and cued recall facilitated by the Dream Inventory) in terms of gauging ease of dream recall.

Memory content of dreams. Another notable quality of the dreams of corticosteroid-exposed participants in Study 2 was a relative paucity of waking continuity themes relative to idiosyncratic themes (ratio = 20: 80) and a strong trend toward suppression of references to waking episodes in dreams (i.e., suppressed episodic memory content, $p = .06$ & Cohen's $d = -1.01$) in individuals acutely exposed to corticosteroids. However, in Study 1 data analyses did not detect a circadian influence on broad thematic dream categories relating to waking continuity, among corticosteroid-exposed or among non-corticosteroid control participants. That is to say, at REM3 participants were as likely to report dreams categorized as *residue of the day* or *laboratory-related* as they were to report dreams categorized as *idiosyncratic*.

This pattern of data is consistent with previous studies investigating the memory content of dreams. Baylor and Cavallero (2001) conducted a meta-analytic review of studies investigating the memory content of dreams. Only three studies met their criteria for inclusion in the review. Taken together, those studies suggested that episodic memory content does not change within the same sleep stage, across time, but that episodic memory content is more likely encountered during NREM than during REM sleep. Nevertheless, reviews of the relative influences of ultradian versus circadian factors suggest that over and above qualitative NREM-REM differences, dreams collected during the first half of sleep are more likely to include references to recent events and references to the laboratory setting in the context of an experiment. In contrast, dream reports collected from later awakenings are more likely to include references to one's distant past and to being more creative and idiosyncratic in nature (e.g., *see* Nielsen, 2004; Schredl, 2003 for reviews).

The negative findings in the current study have two possible explanations: First, in Study 1, the small number of participants in each group did not facilitate the individual analysis of trends in each category of participants. It would have been interesting to investigate whether a circadian effect was present among healthy controls. The data reviewed on circadian versus ultradian influences on dream content and themes is derived mostly from healthy populations. Not much is known of how aberrant HPA-axis functioning (such as in asthmatics) modulates the ultradian – circadian ratio of influence on dreaming patterns. Future studies should extend on the current design by using larger samples.

Second, the priori prediction tested in Study 1 that individuals with asthma would be as likely to experience dreams with waking continuity themes (i.e., dreams high in episodic content, here classed as either *residue of the day* or *laboratory-related* dreams) during Late sleep than they would be during Early sleep, whereas healthy controls would experience such dreams mostly during Early rather than Late sleep was based on the expectation that individuals with asthma would display aberrant night-time cortisol secretion patterns (i.e., unlike healthy controls, they would display relatively elevated cortisol levels in Early sleep, and relatively lower levels in Late sleep) and reversals in SWS and REM sleep organization. The absence of the predicted difference in dreaming patterns between asthmatics and controls might be attributed to the fact that, in asthmatic participants, the pattern of circadian cortisol secretion for asthmatic participants was not reversed (although it was relatively high, compared to healthy controls, at all measurement points). For all groups, average cortisol levels were relatively low at REM1, and increased progressively to REM2 and REM3. The absence of the predicted effect might also be attributed to no real pattern of reversals in SWS and REM sleep organization, in either the patient groups or the healthy control group. Although there were between-group differences in SWS Distribution (e.g., some groups showed a more even distribution of SWS across the two halves of the night), no group

experienced more SWS during Late sleep than during Early sleep (i.e., there was no pattern of reversals). Similarly, there were no reversals in the distribution of REM sleep.

Does sleep organization mediate the relationship between cortisol and dream content? A common aim of Studies 1 and 2 was to analyze whether changes in glucocorticoid activity throughout the night were associated with ways in which cycles of sleep across the night differed in their processing of episodic dream content. (Here, each period of measured REM sleep marked the beginning of the end of a sleep cycle, so that for each participant there were three distinct sleep cycles per night.) My examination of this association was not intended to dispute the existence of ultradian differences in the memory content of dreams; in fact, my studies were not designed to control for ultradian influences (because all of my dream reports were collected at the beginning of REM sleep, any comment on the extent of ultradian influences on dreaming is beyond the scope of this research). Instead, my overarching objective was to investigate indirectly (i.e., by assessing dream content) a link between the evolution of memory processing during sleep (i.e., the way that memories were processed differently as SWS stages became shorter, and REM stages longer, as the night progressed), the organization of sleep (especially regarding the greater concentration of SWS during Early sleep and longer REM sleep during Late sleep) and glucocorticoid activity during the night.

In Study 2, prednisone-treated participants showed the expected reversals in glucocorticoid activity and in sleep organization. Unfortunately, because of the small number of participants who reported dreams at all three awakenings, I could not analyze between-group differences in dream distribution across the three measurement points. Future studies might use similar designs to Study 2, but with larger sample sizes and more intense focus on obtaining good-quality dream reports at all awakenings.

Further with regard to the bivariate relationship between sleep organization and the memory content of dream reports, Study 1 analyses suggested that the intensification of REM sleep across the night, but not total percentage of REM sleep, predicted less episodic content (i.e., fewer references to waking experiences) and greater original content (i.e., more references to events, people, or places that the dreamer describes as unfamiliar to him/herself). A high REM Intensification score is achieved if ratio of early-night REM sleep to Late REM sleep is low. The fact that the data suggest that the episodic content of dreams is predicted by REM Intensification, and not by percentage REM sleep per se, is consistent with the argument that the memory content of dreams is influenced by the natural evolution of sleep cycles, in other words, that there is a circadian influence on the quality of dreams (Antrobus, 1991, 1998; Cartwright, 2004; Cicogna & Bosinelli, 2001; Nielsen, 2000).

If one accepts (as I do, as a fundamental aspect of my study rationale) the assumption that variations in the episodic content of dreams at various stages of the night might be used as a proxy for variations in the different aspects of episodic memory that are processed at different times of the night (i.e., at cycles with varying proportions of NREM to REM sleep), then the data described above suggest that the natural evolution of sleep cycles influences processing of different aspects of episodic memory, in a systemic way, across the night. The assumption described above is supported by analyses (again from Study 1 only, due to the paucity of dream reports in Study 2) indicating that (a) performance on episodic memory tests and (b) the episodic content of dreams were similarly related to night-time glucocorticoid levels and to sleep organization. With particular regard to the dream data, both episodic content and original content were predicted by REM Intensification. The more REM intensified from early to late sleep, the more novel dream content tended to be and the less central waking references tended to be. Furthermore, higher cortisol levels during sleep were associated with lower ratings of episodic content. However, because levels of night-time

cortisol did not predict REM Intensification, a mediational role for REM Intensification in the relationship between cortisol and dream content could not be explored.

Taken together, the findings from Study 1 suggest that the normal circadian intensification of REM sleep, and rising levels of cortisol as the night progresses, may indeed be associated with the memory content of dreams. It is well known, from previously published literature (e.g., Antonijevic et al., 1999; Buckley & Schatzberg, 2005; Payne et al., 2004; Nielsen, 2004; Plihal et al., 1999; Steiger, 2003) that, in healthy individuals, cortisol levels start rising during the second half of sleep, and that dreams encountered during that half typically contain more creative and novel elements. The novelty of the current study is that it described a relationship between these occurrences: In the current sample, there was an inverse association between episodic dream content and night-time cortisol. Similarly, it is well known from previous literature (e.g., Schredl, 2003; Nielsen, 2004) that, in healthy individuals, dreams mirroring waking preoccupations are more often encountered during early REM sleep periods while more elaborate, creative dreams that bear looser connections with waking experiences are encountered during later, longer, early-morning REM sleep periods. The further novelty of the current study is that it described a relationship between those occurrences: In the current sample, there was an inverse association between episodic dream content and REM intensification scores. Although these two novel findings are correlational, proposed mechanisms that might drive the associations are contained within well-developed theories (e.g., theories postulating circadian influences on dream content; Antrobus, 1991, 1998; Cartwright, 2004; Cicogna & Bosinelli, 2001; Nielsen, 2000).

Does dream content mediate the relationship between cortisol and episodic memory? Study 1 data analyses detected a significant, direct, bivariate relationship between the degree of episodic content in dream reports and performance on verbal episodic declarative memory tasks on the following morning. Similarly, the analyses detected

significant, direct, bivariate relationships between night-time cortisol levels and (a) episodic dream content, and (b) delayed (morning) paragraph recall. The fact that both of the latter associations were statistically significant argues for the adequacy of the episodic dream content measure as a proxy measure of sleep-dependent memory consolidation. Nonetheless, a model proposing a mediating influence of dream content on the relationship between cortisol and memory performance could not be tested due to non-linearity of these relationships and the limited statistical power conferred by the relatively small sample size.

The significant relationship between the presence of waking inclusions in dreams and performance on episodic memory tasks is consistent with Wamsley's theory (2011) that the processing of episodic memories in dreams reflects engagement with and consolidation of recent waking events. Within this theoretical framework, this processing assists in the successful retrieval of episodic memory, irrespective of how bits of episodes are woven into a dream scenario. Although the current findings provide support, broadly for this theoretical account, the current design did not test the theory directly. For instance, when measuring episodic dream content I did not collect data regarding specific references to the test material learnt before bedtime. I simply asked dreamers and independent raters to quantify (a) the degree of proximity of the dream scenario to any waking experience, and (b) the salience of waking references within the dream scenario.

As noted above, neither Study 1 nor Study 2 could test the critical mediating model linking dream content, glucocorticoid activity, and memory performance. That model proposes that low glucocorticoid activity during the night supports the normal organization of sleep, which in turn supports (a) the reactivation of waking experiences during early sleep (reflected by several physiological events, including enhanced slow-wave activity, as well as by dreams with a high degree of waking continuity or episodic content), and (b) the assimilation of the new information into existing networks (reflected by several physiological

events, including intensification of REM sleep, as well as by the occurrence of dreams with loosely connected events or original content). The model further proposes that the occurrence of both types of dreams referred to above support the effective consolidation of episodic memory (with such consolidation made manifest by, for instance, better performance on declarative episodic memory tasks after a period of sleep than after a period of waking).

Because the current series of studies could not test that model, they provide an insight into the associations between these phenomena without being able to infer causal links that bind them into a temporally-coherent series of events. However, the groundwork is set for future studies to investigate this causal link by manipulating glucocorticoid activity in the same individuals across multiple nights and by measuring waking inclusions in dreams which pertain to controlled pre-sleep experiences. Ideally, these experiences should be emotionally arousing in order to maximize the possibility of their consolidation during sleep and their subsequent recall in the morning.

Glucocorticoid Activity, Hippocampal Volume, and Episodic Memory

Another aim of the series of studies described in this dissertation was to argue that, if present, declarative memory deficits among asthmatics would be mediated by the damaging effects of chronic corticosteroid exposure on the hippocampus. Study 3 pursued this aim, using a subset of participants from Study 1 (viz., a group of moderate-to-severe asthmatics, and a group of healthy controls). However, the data analyses in that study detected (a) no between-group differences in memory performance, and (b) no significant relationship between corticosteroid exposure scores and hippocampal volume. Hence, I could not test the proposed mediating model.

In the remainder of this chapter, I discuss briefly the implications of two statistically significant findings from Study 3: significant associations between hippocampal size and

performance on declarative memory tasks, and significantly smaller left hippocampal volumes in moderate-to-severe asthmatics compared to healthy controls.

Hippocampal size and memory performance. Within the sample of healthy controls, hippocampal volume (right, left and total) was inversely related to performance on the verbal paired associates task. This finding is consistent with previous reports from studies using young adult samples (e.g., Pruessner et al., 2007; Tupler et al., 2006). Pruessner and colleagues (2007) argue that, in adolescents and young adults, the occurrence of pruning and the ongoing development of more efficient neural networks explain why a smaller hippocampus would be associated with better cognitive performance. From this perspective, an inverse relationship between hippocampal volume and memory performance is expected in typically developing individuals.

Within Study 3's sample of asthmatics, however, there was no such inverse relationship. Furthermore, although asthmatics in this study had smaller left hippocampal volumes than their healthy counterparts, they did not perform better on the declarative memory tests. Together, these findings suggest that, in asthmatics, smaller hippocampal volume is not necessarily associated with more efficient cognitive processing, as it seems to be in healthy individuals. In the Discussion section of Chapter 3, I propose some reasons for why asthmatics may have smaller hippocampal volumes, and why such smaller volumes are not associated with improved cognitive performance.

Smaller left hippocampus in asthmatics. The findings that moderate-to-severe young adult asthmatics present with smaller left hippocampi, and that left hippocampal volume in those asthmatics was negatively and moderately correlated with asthma severity scores (i.e., the more severe the asthma, the smaller the left hippocampus), raise concern, and should spur further investigation. Small hippocampi have been associated with greater vulnerability to stress and stress-related neurodegenerative processes (Conrad, 2008;

Gilbertson et al., 2002; Rooij et al., 2015), and severity of illness in asthmatics has been associated with disruptions in academic productivity in children (Haneesh, Krishnakumar, Sukumaran, & Riyaz, 2013; Szefler, 2016). Although it is possible that the effects of this stress-related vulnerability may only become manifest in later adulthood, it is also possible that, if innate, smaller hippocampal size in asthmatics contributes to the lifelong dysfunctional regulation of the HPA axis often observed in those individuals. Confirming and tracking the cause and the course of this phenomenon in asthma could have important implications for management of the disorder.

Although Study 3 data analyses did not detect (a) dysfunction of the HPA axis among the moderate-to-severe asthmatics (i.e., on average, night-time cortisol levels were not significantly higher in that group than in healthy controls), or (b) a significant association between night-time cortisol levels and hippocampal volume, a relationship between HPA-axis function and the hippocampal structure cannot be excluded. Whereas the current study took single cortisol measures and used gross hippocampal volumetric analyses, future studies investigating the relationship between the hippocampus and cortisol in asthmatics should include measures of the cortisol awakening response and/or 24-hour measures of free-circulating cortisol, as well as corresponding measures of hippocampal metabolic activity to capture the dynamics of the hippocampal-HPA axis negative regulatory feedback loop in a temporally meaningful way.

A final note here is to acknowledge that the effects of glucocorticoid imbalances are not restricted to the hippocampus and hippocampal-dependent memory systems. For instance, the prefrontal cortex, parts of which are involved in processes supporting working memory, contains glucocorticoid receptors (Arnsten, 2009; Arriza et al., 1988; de Kloet, 2004; de Kloet et al., 1998; Elzinga & Roelofs, 2005; Nishi, 2011). Together, the results of Study 1 and Study 2 indicated that chronic and acute corticosteroid exposure were associated with

sub-optimal working memory performance, although significant between-group differences were only present in the acute exposure condition. That is to say, prednisone-treated participants performed significantly more poorly than their placebo-control counterparts on the Digit Span Backward task. This finding is consistent with previous studies reporting that the effects of corticosteroid exposure on cognitive performance extend to working memory (Elzinga & Roelofs, 2005). Furthermore, and overall, the findings reported here extend the literature on the effects of endogenous and exogenous corticosteroid activity on brain structure and function, and on higher-level cognition and mentation.

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APPENDICES

Appendix A:

Asthma, Eczema & Sleep

Would you like to participate in a sleep study? The study investigates how your asthma affects the quality of your sleep.

This study is part of a PhD project and has been approved by the Psychology department's ethics committee and the Faculty of Health Sciences' Research Ethics Committee.

Where? The Vincent Pallotti Sleep Laboratory in Pinelands

When? On 1 night, to be arranged.

What will it involve? You will have to sleep overnight at the sleep lab and perform a few neuropsychological tests.

Who? In order to participate, you must either have **ASTHMA** or **ECZEMA** (NOT BOTH) and be a **NON-SMOKER**. You must be between 18 and 45 years of age.

If you are a woman, you must **NOT** be on the contraceptive pill.

***You will be compensated for your time.**

Please contact Ridwana Timol if you have any query.

e-mail: ridwana.timol@gmail.com

Number: 0734925349

Appendix B:
Participant Information Sheet
(All information provided is treated confidentially)

1.Participant's demographic details

Name:……..
Date of
Birth:…….
Gender (Male or female):…….
First language:……..
Years of schooling (incl. primary, secondary and tertiary education if applicable):….....
Telephone:…….
Email address:……
Postal Address:……..
……
……

2. Medical history

Do you smoke? YES/NO

Do you consume any recreational drug? (**This information is for the purposes of this research simply because the effects of certain psychotropic drugs may interfere with the results**)

……

If yes, how frequently do you use this or these drugs? When was the last time you used the drug in question?

……

How long have you been diagnosed with asthma for?**.....**

Do you suffer from any of the following? Select all the symptoms that apply to you, if any and indicate the frequency at which they each occur:

Symptoms related to asthma	**YES** or **NO**	**When is the symptom most prevalent? (***Day only***,** *night only* **or** *both***)**	**Frequency** (*daily*, *weekly* or *monthly*-**specify**)
Coughing			
Shortness of breath			
Wheezing			
Tight chest			
Sneezing			
Blocked nose			

Other (**specify**)			

Specify the type, dose & frequency of asthma medication that you have been using in the past **6 months** (use **n/a** where a class of medication does not apply to you and **d/k** where you don't know the specific information about a medication that you are using):

Type	**Name**	**Dose**	**Frequency**	**Last used**
Inhaler (e.g. Ventolin)		*Average no. of puffs:*		
Preventer (e.g. fluticasone)		*Circle appropriate dose:* *250 µg*/day *250 to 500 µg*/day *≤ 1000 µg*/day *≥1000 µg/*day		
Oral cortisone				*Date & duration:*
Anti-histamine (e.g. Zyrtec)				
Nasal spray		*Average no. of sprays:*		
Homeopathic				
Other (*e.g. Singulair*)				

Are you suffering from any allergies? YES/NO

If yes, specify which……

What treatment are you using if any?.....

Are you suffering from any form of eczema? YES/NO

If yes, specify which……

What treatment are you using if any?.....

Are you suffering from any other respiratory illness (pneumonia, TB, cold, flu, emphysema etc.)?

……

Are you suffering from any medical condition we should be aware of (heart condition, high blood pressure, diabetes, cancer, epilepsy etc.)?

…….

2.6. Do you suffer from any sleep disorder (E.g. sleep apnea, insomnia, narcolepsy) YES/NO; if YES please state the disorder that applies to you:

……

3.0. If you are a woman:

a) Are you sexually active? YES/NO.
   If yes, are you on the pill or the injection? YES/NO

b) Are you pregnant or planning to become pregnant in the near future? YES/NO

c) Do you have a regular menstrual cycle? YES/NO

d) What was the date of your last period?

DD/MM

4.0. G.P. or specialist's details:

GP's or specialist's name:
.....
Address: .....
Telephone: .....

I, ……., confirm that the information contained on this form are correct.

Appendix C:

The Relative Merits of Different Sampling Methods of Collecting Salivary Cortisol

Findings on the relative efficacy of the sampling methods are mixed. At least one study demonstrates that the cotton-cellulose eyespear may have methodological advantages for cortisol measures over the other two (Strazdins et al., 2005). A few others find support for the superiority of passive collection over collections using cotton material (Fischer, Gyllenhaal, Vecchio, & Engeland, 2011; Granger, Schartz, Booth, Curran, & Zakaria, 1999), and still others find no difference in efficacy between passive drooling and the use of Salivettes (e.g., Gallagher, Leitch, Massey, McAllister-Williams, & Young, 2006). In this study, passive collection was excluded on the account of it presenting with challenges with regards to adequate collection and storage of samples as compared to the other two methods. The use of Salivettes is favored for the ease with which they can be manipulated (Nicholson, 2007).

As part of this study, the efficacy of Salivettes and Sorbettes were validated by the UCT Chemical Pathology Laboratory. Samples were centrifuged for 15 minutes at 1000g and analyzed using the *Roche 170 Modular* automated analyzer. The reference ranges were: 2.05 – 11.9 nmol/l for Salivettes and 1.69 – 11.9 nmol/l for Sorbettes, at 5th-95th percentiles.

The objective of this study was to measure cortisol at different times during sleep, and hence it was important not to have the arousals alter the cortisol levels just before collection. The Sorbettes at first seemed to be most practical as they could be slipped easily into the participant's mouth without disrupting sleep. However, collection of saliva using Sorbettes proved to be ineffective in a previous study from our laboratory (Bonitto-Atwood, 2008): A considerable percentage of the samples collected resulted in insufficient amounts of salivary cortisol for analysis (17% vs. 4.35% for the current studies). Furthermore, there have been reports of encountering lower viscosity with Salivette-collected saliva after centrifugation

(Poll et al., 2007). I therefore had to consider the actual implications of waking the participant during saliva collection on cortisol level versus those of losing a considerable amount of data.

Dettenborn, Rosenloeker, and Kirschbaum (2007) showed that forced micro-awakenings during the night do not lead to a cortisol acrophase that is typical of proper morning awakening. It takes approximately 30 minutes for cortisol to peak after a middle-of-the-night awakening, and even when a cortisol surge is observed at that time, it does not reach levels equivalent to morning cortisol acrophase (Hucklebridge et al., 2000). Moreover, Hucklebridge et al. (2000) found that interrupting sleep to measure middle-of-the-night salivary cortisol did not impact on the subsequent morning cortisol measure. Together, these findings imply that it is possible to capture the pattern of salivary cortisol during the course of the night, as sleep cycles evolve, despite the brief awakenings required to chew on Salivettes.

Based on these considerations, the data described above, and the brevity of my awakenings (approximately 5 minutes), I deemed Salivettes the safer instrument for collection of salivary cortisol in the present study.

Appendix D:

Instructions for Coding of Dream Reports- 4 Step Process

Report coding:

Participant code and dream number- REM1= 1, REM2= 2 and REM3= 3

Example: AS003-1 refers to REM1 awakening dream taken from participant AS003.

Dream status:
No dream- 0, Dream without content- 1, Dream with content- 2

Step 1: Dream or No dream?

Step 2: Clear account or "white dream"? A white dream is where person insists they had been dreaming, can recollect emotions associated with the experience but cannot recall the content.

Dream quality:

Is the account more thought-like or is it obviously visual?

Specific theme & Broad category

Step 3: Thematic coding of dream

1. What is the specific theme of the dream?
2. In what broad category (i.e. **residue of the day- 1** OR **experiment/lab- related dream- 2** OR **idiosyncratic-3**) does it fit?

Episodic content score (scale 1-10)

Step 4: Identifying episodic content (i.e. things that bear familiarity to the dreamer's waking existence: a) person(s), b) place(s), c) situation and d) preoccupation.)

List and rate them by using a scale of 0 to 10, where 1 indicates = very little, 10 = a lot of and 0 indicates = the absence of a particular criterion:

A. A person or people dreamer reports knowing =

B. Places familiar to dreamer =

C. An event dreamer is currently experiencing =

D. An event from dreamer's past =

N.B.: you can only rely on the details provided by the dreamer to rate the above. In some cases, these details may not be available. Please note as "report not permitting". The aim is to identify evidence of continuity within the dream plot- is there one single, linear narrative being expressed? In other words, is the story coherent by waking standards?
Does that coherent story bear a connection with events experienced by the dreamer? That is, does the dream contain episodic elements that retain their original context as identified by the dreamer?

Dream report	Dream status	Theme	Broad category	Episodic rating (scale: 0-10)			
				people	places	current event	past event

Appendix E
Informed Consent to Participate in Research and Authorization for Collection, Use, and Disclosure of Sleep Patterns, Dream Reports, Performance on Memory and Attention tasks and Other Personal Data

You are being asked to take part in a research study. This form provides you with information about the study and seeks your authorization for the collection, use and disclosure of your sleep architecture patterns, dream reports and cognitive performance data, as well as other information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. By participating in this study you will not be penalized or lose any benefits to which you would otherwise be entitled.

1. Name of Participant ("Study Subject")

_____

2. Title of Research Study

"Investigating the relationship between cortisol, sleep architecture and memory consolidation in an attempt to explain the content of dreams."

3. Principal Investigator and Telephone Number(s)

Ridwana Timol, PhD candidate
Department of Psychology, office 4.30
University of Cape Town
Contact number: 0734925349

4. Source of Funding or Other Material Support

URC Emerging Researcher Grant

5. What is the purpose of this research study?

This research aims to investigate the effects of using steroids on i) memory and attention, ii) sleep patterns and iii) dreams

6. What will be done if you take part in this research study?

In this experiment, you will be called in for a sleep study on 1 night.
Before commencing the actual study, you will undergo a screening process whereby the Principal Investigator listed in # 3 of this form or her assistant, will administer a short psychiatric questionnaire, a personality questionnaire and an IQ test to you. These tools are not meant to categorise you in any way and won't be used for any diagnostic purpose. They are merely research instruments that allow us to identify certain patterns of interest.

We will also take a comprehensive medical history from you where we will ask you to provide us with details on your asthma and the treatment you are currently undergoing.

The sleep study will be arranged at least one week in advance, at a time convenient to you. You will retain your routine bedtime and waking time but will be asked to avoid caffeine and sugar in your diet for a few hours before bedtime. You will be required to come to the sleep laboratory based at Vincent Pallotti Private Hospital between 19 30 and 20 00 and will be briefed once more, in detail, on the procedure. You will be hooked to a polysomnograph (PSG) which is an EEG machine designed to monitor your sleep pattern. Electrodes will be placed on your head, chest, near your chin and temples; these are completely safe and present no danger whatsoever to your health. They are designed to transmit physiological indications of the stage of sleep you are experiencing at a given point in time, to a computer monitor. One or two researchers will be surveilling the monitor in an adjoining room. They will be available to you for assistance at any time. There is a panic button at your bedside should you need assistance at any point during the night. He or she will also wake you up at two different points during the night and you will be asked to verbally report any dream you remember having before being awakened, in as much detail as possible. An audio-recorder will be placed in the room to record your dream reports. A small cotton bud- shaped swab will be gently placed in your mouth, for two minutes before you are awakened, in order to measure your natural salivary cortisol. This method is non-intrusive and will not harm or cause you discomfort in any way. The same procedure will be followed when you wake up in the morning.

Lastly, you will be required to do a short series of memory and attention tasks that will last approximately 30 minutes. The experiment typically ends at 08 00 on the following morning.

After the sleep sessions are over, you will be informed in detail about the design of the study and the research questions we hope to address with this study. You will also have the opportunity to ask questions and thus learn more about psychological research. If you have any questions now or at any time during the study, you may contact the Principal Investigator listed in #3 of this form.

This study has been approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town and you should feel free to contact Professor Marc Blockman, chairperson of the committee (021 4066496), if you have any concerns about your rights and welfare as a research participant.

7. If you choose to participate in this study, how long will you be expected to participate in the research?

Screening and interview session: approximately 2 hrs and sleep study: 1 night only.

8. How many people are expected to participate in the research?

75

9. What are the possible discomforts and risks?

Sleeping in an environment other than your own bedroom might feel strange and uncomfortable at first. Great precautions will be taken to ensure your safety and comfort. The

sleep laboratory at Vincent Pallotti is fully equipped with a proper bed, clean bedding, restrooms and a kitchenette. It is situated in a secure building with adequate surveillance and alarm system. Attempts will be made to familiarise you with the PSG and the electrodes used will be padded and lubricated so as to be as non-intrusive as possible. We will have asthma rescue medication and equipment at hand should you have an exacerbation during the night and the medical staff at the hospital will attend to you if need be.

10a. What are the possible benefits to you?

You may or may not personally benefit from participating in this study. Participation in this study may, however, improve your understanding of some factors that affect sleep and dreaming and may influence your management of your health generally.

10b. What are the possible benefits to others?

The information from this study may help improve our understanding of certain cognitive mechanisms underlying the process of dreaming and the impact of certain hormones on sleep and dreaming. This research will provide us with a better understanding of the widespread effects of steroids on cognition and on various aspects of daily functioning and this information can be applied to treatment plans that involve the chronic use of corticosteroids such as in conditions like asthma.

11. If you choose to take part in this research study, will it cost you anything?

Participating in this study will not cost you anything.

12. Will you receive compensation for taking part in this research study?

You will receive financial compensation of the amount of R150 for your participation in the study.

13a. Can you withdraw from this research study?

You are free to withdraw your consent and to stop participating in this research study at any time. If you do withdraw your consent, there will be no penalty.

If you have any questions regarding your rights as a research subject, you may phone the Psychology Department offices at 021-650-3430.

13b. If you withdraw, can information about you still be used and/or collected?

Information already collected may be used.

14. Once personal and performance information is collected, how will it be kept secret (confidential) in order to protect your privacy?

Information collected will be stored in locked filing cabinets or in computers with security passwords. Only certain people have the right to review these research records. These people include the researchers for this study and certain University of Cape Town officials. Your

research records will not be released without your permission unless required by law or a court order.

15. What information about you may be collected, used and shared with others?

This information gathered from you will be demographic information, information on the status and management of your asthma, records of your sleep architecture, dream reports, performance on cognitive tests, and scores on the IQ test, personality questionnaire and psychiatric inventory. If you agree to be in this research study, it is possible that some of the information collected might be copied into a "limited data set" to be used for other research purposes. If so, the limited data set may only include information that does not directly identify you. For example, the limited data set **cannot** include your name, address, telephone number, ID number, or any other photographs, numbers, codes, or so forth that link you to the information in the limited data set.

16. How will the researcher(s) benefit from your being in the study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator and others attached to this research project may benefit if the results of this study are presented at scientific meetings or in scientific journals. This study is being undertaken for the Principal Investigator's doctorate degree.

17. Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; and how the participant's performance and other data will be collected, used, and shared with others:

Signature of Person Obtaining Consent and Authorization Date

_____ _____

You have been informed about this study's purpose, procedures, possible benefits, and risks; and how your performance and other data will be collected, used and shared with others. You have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your performance and other data. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing Date

_____ _____

Please indicate below if you would like to be notified of future research projects conducted by our research group:
_____ (initial) Yes, I would like to be added to your research participation pool and be notified of research projects in which I might participate in the future.

Method of contact:

Phone number: _____
E-mail address: _____
Mailing address: _____
_____
_____

Appendix F

The Department of Psychology, University of Cape Town

MEDICAL INDEMNITY FORM

This Form is intended to assist researchers in screening for potential medical conditions that participants may suffer from and to set up structures to care for participants in the case of a medical emergency during the course of the study. All information will be kept confidential.

PERSONAL DETAILS

Participant's Name: _____ DOB: _____
Address: _____ Postcode: ____
Telephone: (H): _____ (C): _____
Name & Address of Family Doctor: _____
_____ Telephone: _____
Medicare No.: _____ Do you have ambulance cover? Y/N
Private Health Insurance Fund: _____
Member No.: _____

MEDICAL CONDITIONS

Please outline any medical condition which you suffer from and which the researchers may need to be aware of:

_____
_____
_____

Allergies to medication: _____
_____
_____
Tetanus Immunisation: Y/N Date of last immunisation: _____

MEDICATION (Both prescription and non-prescription)

_____

Do you expect yourself to be taking any medication during the course of the experiment? Y/N
If YES, please indicate the following:
Name of medication: _____
Dose & frequency: _____
Reason for medication: _____
Any late changes to the above information should be conveyed in writing to the principal investigator of the current study. Please contact Ridwana Timol on 0734925349.

The team of UCT researchers constituting of Dr. Kevin Thomas, Ridwana Timol, Anthony Hodges and Niel Hoogenhout take full responsibility for the provision of any medical attention required by the participants while the latter are in their care. They will be assisted by the Vincent Palloti Hospital staff and trauma unit in the provision of this care. However, the hospital is in no way responsible for the management of the participants and will not be liable for any costs incurred should any form of medical intervention be required. The team of researchers aforementioned take full responsibility for the safety and wellbeing of the participants during the course of the study.

CONSENT TO MEDICAL ATTENTION

I authorise the researchers in charge to:

a) Arrange for medical attention as may be necessary by a medical practitioner, or

b) Administer such First Aid as the researchers in charge may judge to be reasonable in the event of a medical emergency.

Signature of Participant: _____

Date: _____

I,……., hereby declare that the information provided on this form is accurate.

Signature of Participant:……

Date:……

Appendix G

Table A.1.
Regression Analyses Between Sleep Variables and LM Retention Scores.

Regression	R^2, AdjustedR^2	F	p value
Sleep efficiency predicts LM Retention	.028, .012	1.73	.194
Percentage REM sleep predicts LM Retention	.001, -.015	0.47	.829
REM Intensification predicts LM Retention	.064, .049	4.24	.044*
Percentage SWS predicts LM Retention	.003, -.013	0.20	.654
SWS Distribution predicts LM Retention	.011, -.004	0.73	.396

Note. Degrees of freedom were (1, 63) for the above analyses. Significance level was set at *p< .05.

Appendix H

Table A.2.
The relationship between night-time cortisol and the memory content of dreams.

	Group				
Variable	Mild Asthma (*n*=11)	Moderate-to-severe Asthma (*n*=11)	Untreated Asthma (*n*=14)	Eczema Control (*n*=13)	Healthy Control (*n*= 9)
Episodic content*average cortisol	-.24	-.83**[a]	-.13	-.13	-.02
Episodic content*REM2 cortisol	-.18	-.71**[b]	-.08	-.03	.05

Note. The episodic memory outcome measure refers to the objective average episodic memory content scores derived from independent raters and night-time cortisol refers to average night-time cortisol per participant, aggregated over the 3 measurement points. Significance levels were set at $p < .05$. [a]$p = .002$; [b]$p = .007$.

Appendix I

Participant Information Sheet
(All information provided is treated confidentially)

1.Participant's demographic details

Name:……..
Age:…….
Gender (Male or female):…….
First language:……..
Years of schooling (incl. primary, secondary and tertiary education if applicable):…....
Telephone:…….
Email address:……
Postal Address:……..
……
……

2. Medical history

2.1. Do you smoke? YES/NO

2.2. Do you consume any recreational drug? (**This information is for the purposes of this research simply because the effects of certain psychotropic drugs may interfere with the results**)

……

If yes, how frequently do you use this or these drugs? When was the last time you used the drug in question?

……

2.3. Are you suffering from any respiratory illness (asthma, pneumonia, TB, cold, flu, emphysema etc.)?

……

2.4. Are you suffering from any medical condition we should be aware of (heart condition, high blood pressure, diabetes, cancer, epilepsy etc.)?

……

2.5. Do you suffer from any of the following? Tick all the conditions that apply to you, if any.

Peptic ulcer	
Osteoporosis	
Congestive heart failure	
Diabetes mellitus	
Chronic renal failure and uremia	
Quiescent tuberculosis	
Glaucoma,	
Lactose intolerance	
Hypertension	
Myasthenia Gravis	
Thromboembolic disorders	
Other (specify)	

2.6. Do you suffer from any sleep disorder (E.g. sleep apnea, insomnia, narcolepsy) YES/NO; if YES please state the disorder that applies to you:

……

3.0. <u>If you are a woman:</u>

a) Are you sexually active? YES/NO.
   If yes, are you on the pill or the injection? YES/NO

b) Are you pregnant or planning to become pregnant in the near future? YES/NO

c) Do you have a regular menstrual cycle? YES/NO

d) What was the date of your last period?

DD/MM

<u>4.0. G.P. or specialist's details:</u>

GP's or specialist's name:
.....
Address: .....
Telephone: .....

I, ……., confirm that the information contained on this form are correct.

Signed:..... Name: ..... Date: .....

Appendix J

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: hsrecs.emjedi@uct.ac.za

23 April 2008

REC REF: 166/2007

Ms Ridwana Timol
Psychology
Upper Campus

Dear Ms Timol

PROJECT TITLE: INVESTIGATING THE RELATIONSHIP BETWEEN CORTISOL, SLEEP ARCHITECTURE AND MEMORY CONSOLIDATION, IN AN ATTEMPT TO EXPLAIN THE CONTENT OF DREAMS

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Thank you for responding to our comments over the past year and for revising your study as requested. We note that all research team has signed the final amendment.

Approval is granted for one year till the 30th April 2009.
Please submit a progress report before 30th April 2009 so that the study can be renewed for a further year.

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

hsrecjedi

Please quote the REC. REF in all your correspondence.

Yours sincerely

Valery Henry

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

pp

Appendix K

Informed Consent to Participate in Research and Authorization for Collection, Use, and Disclosure of Sleep Patterns, Dream Reports, Performance on Memory and Attention tasks and Other Personal Data

You are being asked to take part in a research study. This form provides you with information about the study and seeks your authorization for the collection, use and disclosure of your sleep architecture patterns, dream reports and cognitive performance data, as well as other information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. By participating in this study you will not be penalized or lose any benefits to which you would otherwise be entitled.

1. Name of Participant ("Study Subject")

_____

2. Title of Research Study

"Investigating the relationship between cortisol, sleep architecture and memory consolidation in an attempt to explain the content of dreams."

3. Principal Investigator and Telephone Number(s)

Ridwana Timol, PhD candidate
Department of Psychology, office 4.30
University of Cape Town
Contact number: 0734925349

4. Source of Funding or Other Material Support

URC Emerging Researcher Grant

5. What is the purpose of this research study?

This research aims to investigate the effects of using steroids on i) memory and attention, ii) sleep patterns and iii) dreams

6. What will be done if you take part in this research study?

In this experiment, you will be called in for a sleep study for 2 consecutive days.

Before commencing the actual study, you will undergo a screening process whereby the Principal Investigator listed in # 3 of this form or her assistant, will administer a short psychiatric questionnaire, a personality questionnaire and an IQ test to you. These tools are not meant to categorise you in any way and won't be used for any diagnostic purpose. They are merely research instruments that allow us to identify certain patterns of interest

We will also take a full medical history from you.

The sleep study will be arranged at least two weeks in advance, at a time convenient to you. You will retain your routine bedtime and waking time but will be asked to avoid caffeine and sugar in your diet for a few hours before bedtime. You will be required to come to the sleep laboratory based at Vincent Pallotti Private Hospital two hours prior to your usual bedtime and will be briefed once more, in detail, on the procedure. You will be hooked to a polysomnograph (PSG) which is an EEG machine designed to monitor your sleep pattern. Electrodes will be placed on your chest, under your chin and on your temples; these are completely safe and present no danger whatsoever to your health. They are designed to transmit physiological indications of the stage of sleep you are experiencing at a given point in time, to a computer monitor. Two researchers will be surveilling the monitor in an adjoining room. One of them will be available to you for assistance at any time. There is a panic button at your bedside should you need assistance at any point during the night. He or she will also wake you up at two different points during the night and you will be asked to verbally report any dream you remember having before being awakened, in as much detail as possible. An audio-recorder will be placed in the room to record your dream reports. A small cotton bud- shaped swab will be gently placed in your mouth, for two minutes before you are awakened, in order to measure your natural salivary cortisol. This method is non-intrusive and will not harm or cause you discomfort in any way. The same procedure will be followed when you wake up spontaneously in the morning.

Lastly, you will be required to do a short series of memory and attention tasks that will last approximately 30 minutes.

On your second and last sleep session, you will randomly be assigned to either the experimental group or the control group but neither you nor the researcher attending to you will know your status. You will consequently either receive 45 mg of oral cortisol (prednisone) or a placebo (sugar tablet). The dose of cortisol used in this study is considered a safe, low dose. A once-off intake of oral cortisol is not known to have any negative long-term effects. You might feel slightly groggy on awakening the following morning but the drug will wash out of your system within 18 hours of administration. There will be a licensed medical practitioner at your disposal should you have any query about taking cortisol.

After the sleep sessions are over, you will be informed in detail about the design of the study and the research questions we hope to address with this study. You will also have the opportunity to ask questions and thus learn more about psychological research.

If you have any questions now or at any time during the study, you may contact the Principal Investigator listed in #3 of this form.

This study has been approved by the Research Ethics Committee in the Faculty of Health Sciences and you should feel free to contact Professor Marc Blockman, chairperson of the committee (021 4066496), if you have any concerns about your rights and welfare as a research participant.

7. If you choose to participate in this study, how long will you be expected to participate in the research?

Screening and interview session: approximately 2 hrs and sleep study: 2 consecutive nights.

8. How many people are expected to participate in the research?

20

9. What are the possible discomforts and risks?

Sleeping in an environment other than your own bedroom might feel strange and uncomfortable at first. Great precautions will be taken to ensure your safety and comfort. The sleep laboratory at Vincent Pallotti is fully equipped with a proper bed, clean bedding, restrooms and a kitchenette. It is situated in a secure building with adequate surveillance and alarm system. Attempts will be made to familiarise you with the PSG and the electrodes used will be padded and lubricated so as to be as non-intrusive as possible. You might feel slightly groggy on the morning following the intake of cortisol, but the drug will wash out of your system within 18 hours of administration.

10a. What are the possible benefits to you?

You may or may not personally benefit from participating in this study. Participation in this study may, however, improve your understanding of some factors that affect sleep and dreaming and may influence your management of your health generally.

10b. What are the possible benefits to others?

The information from this study may help improve our understanding certain cognitive processes underlying the process of dreaming and the impact of certain hormonal processes on sleep and dreaming. This research will provide us with a better understanding of the widespread effects of steroids on cognition and on various aspects of daily functioning and this information can be applied to treatment plans that involve the chronic use of steroids such as in conditions like asthma.

11. If you choose to take part in this research study, will it cost you anything?

Participating in this study will not cost you anything.

12. Will you receive compensation for taking part in this research study?

You will receive financial compensation of the amount of R150 for your participation in the study.

13a. Can you withdraw from this research study?

You are free to withdraw your consent and to stop participating in this research study at any time. If you do withdraw your consent, there will be no penalty.

If you have any questions regarding your rights as a research subject, you may phone the Psychology Department offices at 021-650-3430.

13b. If you withdraw, can information about you still be used and/or collected?

Information already collected may be used.

14. Once personal and performance information is collected, how will it be kept secret (confidential) in order to protect your privacy?

Information collected will be stored in locked filing cabinets or in computers with security passwords. Only certain people have the right to review these research records. These people include the researchers for this study and certain University of Cape Town officials. Your research records will not be released without your permission unless required by law or a court order.

15. What information about you may be collected, used and shared with others?

This information gathered from you will be demographic information, records of your sleep architecture, dream reports, performance on cognitive tests, and scores on the IQ test, personality questionnaire and psychiatric inventory. If you agree to be in this research study, it is possible that some of the information collected might be copied into a "limited data set" to be used for other research purposes. If so, the limited data set may only include information that does not directly identify you. For example, the limited data set cannot include your name, address, telephone number, ID number, or any other photographs, numbers, codes, or so forth that link you to the information in the limited data set.

16. How will the researcher(s) benefit from your being in the study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator and others attached to this research project may benefit if the results of this study are presented at scientific meetings or in scientific journals. This study is being undertaken for the Principal Investigator's doctorate degree.

17. Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; and how the participant's performance and other data will be collected, used, and shared with others:

Signature of Person Obtaining Consent and Authorization Date

_____ _____

You have been informed about this study's purpose, procedures, possible benefits, and risks; and how your performance and other data will be collected, used and shared with others. You have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your performance and other data. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing Date

_____ _____

Please indicate below if you would like to be notified of future research projects conducted by our research group:
_____ (initial) Yes, I would like to be added to your research participation pool and be notified of research projects in which I might participate in the future.

Method of contact:

Phone number: _____
E-mail address: _____
Mailing address: _____
_____
_____

Appendix L

Table A.3.

Regression analysis testing %SWS as a predictor of performance on the FTT.

Regression	R^2, AdjustedR^2	*F*	*p* value
%SWS predicts FTT Speed retest[1]	.16, .11	3.69	.069
%SWS predicts FTT Speed retest[2]	.14, .10	3.36	.082

Note. Degrees of freedom are represented as (1, 21). [1]Refers to the regression analysis performed on the raw data and [2]refers to analysis performed on the rank-transformed data.

Appendix M

Table A.4.
Asthma severity classification scheme

Class A: Classification of severity based on presentation of symptoms		**Score**
Symptoms related to asthma	Shortness of breath	1 point
	Wheezing	1 point
	Tight chest	1 point
	Coughing	1 point
Frequency	Yearly (1< times a year<12)	1 point
	Monthly (1-3 times a month)	2 points
	Weekly (1-3 times a week)	3 points
	Daily (4-7 days a week)	4 points
Prevalence of symptoms	**Total Score (min: 3; max: 16):**	**∑ [symptom x frequency]**

Class B: Classification based on exposure to corticosteroids		**Score**
Corticosteroid treatment (preventer inhaler & nasal spray doses combined)	≤*250 μg*/day Budesonide or equivalent 125 *μg*/day of Fluticasone	1 point
	250 to 500μg/day Budenoside or 125 to 250 *μg*/day of Fluticasone	2 points
	≤ *1000 μg*/day Budesonide or ≤ *500μg*/day of Fluticasone	3 points
	≥*1000 μg*/day Budenoside or ≥*500μg*/day of Fluticasone	4 points
Frequency	Yearly (1< times a year<12)	1 point
	Monthly (1-3 times a month)	2 points
	Weekly (1-3 times a week)	3 points
	Daily (4-7 days a week)	4 points
Oral corticosteroid	Use within 12 months prior to testing (any dose)	1 point
Exposure to corticosteroids	**Total Score (min: 0; max: 10):**	**∑ dose + frequency + oral corticosteroid intake**

Total Severity Score	**(min: 3; max: 26):**	**∑ symptom prevalence score + exposure to corticosteroids score**

Table A.5.
Asthma severity scores and ICS exposure scores Study 3 asthmatic participants (*N* = 9).

Participant number	Severity Scores	ICS exposure score
AS017	19	6
AS018	17	7
AS019	22	7
AS029	15	6
AS031	15	7
AS043	15	7
AS049	15	7
AS061	18	6
AS064	19	7

Note. Participants retained their identifying numbers from Study 1.

Appendix N

Table A.6.
The relationship between average night-time cortisol and raw hippocampal volume

Variable	Right hippocampal volume	Left hippocampal volume	Total hippocampal volume
Average cortisol	.37(.056)	.17(.238)	.32(.089)

Note. Pearson's *r* correlations were performed using raw volumes.*r* values are presented first, followed by *p* values in parentheses.

Table A.7.
The relationship between asthma severity and (a) hippocampal volume and (b) IQ.

Variable	Right hippocampal volume	Left hippocampal volume	Total hippocampal volume	IQ
Asthma severity score	-.50(.086)	-.63(.035)*	-.54(.065)	-.47(.100)

Note. Spearman Rho's*rs* correlations were performed using indexed volumes. *rs* values are presented first, followed by *p* values in parentheses.Significance values are set at $p < .05$ and are denoted by *.

Table A.8.
The relationship between hippocampal volume (indexed) and IQ-corrected performance on the VPA task.

Variables	Immediate recall VPAI/IQ	Delayed recall VPAI/IQ
Right hippocampal volume	-.40*	-.11
Left hippocampal volume	-.38	-.15
Total hippocampal volume	-.41*	-.13
Immediate recall VPAI/IQ	--	.62**
Delayed recall VPAI/IQ	.62**	--

Note. Spearman Rho's *rs* correlations were performed using indexed volumes. *rs* values are presented first, followed by *p* values in parentheses. Significance values are set at $p < .05$ and are denoted by *. **$p < .001$.

Appendix O

Table A.9.
The relationship between hippocampal volume (corrected using residual method) and performance on the VPA task (*N* = 19).

	Immediate recall VPAI	Delayed recall VPAII	VPA Score	Average Cortisol
Right hippocampal volume	-.13	-.03	.13	.33
Left hippocampal volume	-.04	-.07	-.02	.11
Total hippocampal volume	-.10	-.06	.07	.26
Immediate recall VPAI	--	.69**	-.14	-.14
Delayed recall VPAII	.69**	--	.62**	-.49*
VPA Score	-.14	.62**	--	-.50*

Note. Partial correlations were performed by controlling for the effects of ICV. * denotes a significance value of $p < .05$ and ** $p < .005$.