

The Role of Norepinephrine in Arousal and Attention:

A human and rat study

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‘Know when you’re finished, and when you are, put your pencil or your paintbrush down.

All the rest is only life’

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-adapted from Duma Key

Stephan King

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ABSTRACT

Background

The locus coeruleus-norepinephrine (LC-NE) system is known to play an integral role in attention and arousal. The LC fires both tonically and phasically. We propose that tonic firing of the LC-NE system needs to be maintained in a critical range for phasic activity of the LC-NE system to enhance the signal-to-noise ratio in stimulus-related neural networks. In addition terminal release of NE from LC neurons responds synergistically with glutamate release from glutamatergic neurons, thereby potentiating its signal. The Yerkes and Dodson (1908) theory of arousal and performance can be represented by an inverted-U shaped curve which serves to relate the proposed functioning of the LC-NE system role (level of arousal) to attentional performance. In the present study we performed both non-invasive human studies (Part A) and invasive rat studies (Part B) to test this hypothesis and elaborate its parameters.

Part A

The first aim of the human study was to determine whether perceived mental effort during performance of an attentional task was reflected by physiological arousal. The second aim was to determine whether healthy individuals with high levels of behavioral impulsivity were physiologically hypo-aroused. The third aim was to determine whether healthy individuals with high levels of anxiety were physiologically hyper-aroused, and whether smoking (nicotine) was used as a form of self-medication. The fourth aim was to determine whether healthy individuals who had experienced childhood traumatic events showed physiological correlates during attentional task performance that were related to arousal and the LC-NE system activity.

Healthy participants were recruited voluntarily, they completed three attentional tasks designed to test different aspect of attention and impulsivity: (1) a continuous performance task, (2) a Go/No-Go task, and (3) a cued target detection task. During completion of the attentional tasks, the participants' arousal levels were continuously recorded: (1) electroencephalogram (EEG), (2) electrocardiogram (ECG), and (3) skin conductance responses. Prior ($\pm$ 5min) to commencement of the attentional tasks participants supplied a saliva sample for cortisol measurement and a second sample was collected immediately after the attentional tasks. Participants also gave blood for future genetic analysis; these samples were obtained on a separate day to that of the attentional tasks. Participants also completed a battery of neuropsychological questionnaires/inventories with respect to their day to day behaviors that were used to relate perceived mental effort, impulsivity, anxiety, and childhood trauma to the physiological measures recorded.

Our results indicate healthy participants' perceived mental effort was related to aspects of physiological arousal, left parietal relative beta power was found to be associated with perceived mental effort during each of the three attentional tasks. In addition each of the tasks, due to their different parameters showed associations with several other relative cortical frequency bands. Only the Go/No-Go task showed associations with peripheral physiological arousal.

We suggested that mental effort in a 'healthy' cohort of participants could be used as a measure of tonic arousal afforded by the LC-NE system, we found several cortical associations. This finding needs to be extended to see if reported mental effort may serve to measure functional LC-NE systems and highlight dysfunction in the LC-NE systems tonic activities in human disorders of 'hypoarousal' such as individuals with ADHD and of 'hyperarousal' such as individuals with anxiety related disorders.

We proposed that organisms that were ‘hypoaroused’ present with decreased tonic activity. Individuals with ADHD have been reported to make increased errors and have short response times. Impulsivity was effectively shown in the present study in the Go/No-Go task response times and self-reported impulsive behavior. Impulsivity within our cohort was measured as short response times in the Go/No-Go task which was reflected by increased relative beta power over both frontal and parietal lobes. These associations dissipated with the progression of the task suggesting that healthy participants with impulsive behaviors were able to improve cortical arousal levels. This finding needs to be extended to include human disorders of ‘hypoarousal’ such as ADHD to ascertain whether ‘hypoaroused’ individuals are unable to reduce these associations as the present ‘healthy’ cohort of participants were able to do, this would further support the present hypothesis.

We proposed that organisms that were ‘hyperaroused’ would present with increased tonic activity. Individuals with anxiety related disorders are reported to be distracted and attempt to passively avoid environmental cues. Anxiety levels were reflected in the present cohort of ‘healthy’ participants’ cortical activities related to the LC-NE system activity tonically by relative frequency power and phasically by the short latency in P300. This finding needs to be extended to ascertain whether ‘hyperaroused’ individuals with disorders such as anxiety related disorders and depression show a similar pattern of activation of tonic and phasic firing of the LC that would further support the present hypothesis.

Interestingly nicotine use showed a similar association, suggesting that nicotine users improved their functional information processing shown by their reduced P300 latency.

Experiences of childhood trauma led to several cortical associations with physical neglect, abuse and emotional abuse, there were no associations with emotional neglect and sexual abuse. For emotional abuse relative right frontal alpha power was negatively associated

across all three attentional tasks. The majority of the associations were found during the Go/No-Go task. Childhood trauma reflected increased recruitment of mental resources as seen by reduced alpha activity, how this is related to the functioning of the LC-NE system needs further interrogation.

Part B

The first aim was to determine whether the relationship (or synergism) between LC-NE terminals and glutamate in hippocampal slices was different in a strain of rat that shows 'hypoarousal' and a rat strain that shows 'hyperarousal'. The second aim was to determine which glutamate receptors played a role in LC-NE terminal release in these two rat strains. The third aim was to determine whether LC-NE terminals responded differentially in several rat strains. The fourth aim was to determine whether the differential release of NE from LC-NE terminals extended to other brain areas that are known to receive NE input from the LC. The fifth aim was to determine whether a change in maternal environment to that of 'hypoaroused' or 'hyperaroused' dam would lead to changes in behaviors related to arousal and whether the synergistic interaction between glutamate and LC-NE terminals was affected by the different rearing conditions. The sixth aim was to determine whether maternal separation affected glutamate stimulated release of NE in hippocampal and prefrontal cortical brain slices. The seventh aim was to determine whether an acute nicotine dose reduced anxiety-like and depressive-like behaviors in several rat strains and to relate it to the effects of acetylcholine on the LC-NE system.

Several strains of rat were used for the animal studies including the spontaneously hypertensive rat (SHR), the Wistar-Kyoto rat (WKY), Sprague-Dawley (SD), Long-Evans hooded-rat, and Wistar rat. The open-field and the elevated-plus maze were used to assess

exploratory behaviors and anxiety-like behavior between P29 and P33. Glutamate-stimulated release of NE from LC-NE terminals was achieved by a superfusion technique.

The 'hyporaroused' rats (SHR) showed exaggerated release of NE from LC-NE hippocampal terminals when stimulated with glutamate as compared to the 'hyperaroused' rats (WKY). Glutamate ionotropic receptor antagonism yielded differential release of NE from LC-NE hippocampal terminals when stimulated with glutamate. WKY rats depended more heavily on AMPA receptor activation for the release of NE, while SHR released greater amounts of NE when the NMDA receptors were antagonized. The exaggeration of NE release in the 'hyporaroused' rats and decrease of NE release in the 'hyperaroused' rats was explained by assessing glutamate stimulated release of NE in hippocampal slices in several strains of rat. Change in maternal environment through cross-fostering of several rat strains showed enhanced anxiety-like behaviors in the 'hyperaroused' rat model when fostered by the 'hyporaroused' dams, while fostering of the 'hyperaroused' rat model onto a reference strain showed reduction in anxiety-like behaviors. When the reference strain was fostered onto the 'hyperaroused' rat strain their anxiety-like behaviors increased. Glutamate stimulated release of NE from the hippocampus and prefrontal cortices were not associated with the changes in behavior. Maternal separation of Sprague-Dawley rats did not affect glutamate stimulated release of NE release from LC-NE terminals of the hippocampus and prefrontal cortex. Acute nicotine led to increased activity in the references strain, however did not affect the behavior of the 'hyporaroused' and 'hyperaroused' rat strains. Acute nicotine did not lead to a change in glutamate stimulated release of NE from hippocampal or prefrontal cortical brain slices.

The exaggerated release of NE from the 'hyporaroused' rat model suggested that there may be reduced synergistic action between the NEergic and glutamatergic terminals. We suggest that this may be a result of increased GABAergic inhibition, resulting in reduced levels of NE at

LC-NE terminals. This suggestion supports the present hypothesis as reduced tonic activity leads to increased terminal release of NE from LC-NE terminals. The reduced response of NE release from the 'hyperaroused' rat model LC-NE terminals suggests a down regulation of glutamate receptors that may result from the increased tonic firing of the LC thereby reducing the synergistic activation between the NEergic and glutamatergic terminals. This suggestion supports the present hypothesis as increased tonic activity would enhance the release of NE, without clearance of NE as the present hypothesis suggests there may be down-regulation of AMPA receptors in vivo. We suggest that the 'hyperaroused' rat model is susceptible to environmental manipulations, while the 'hypoaroused' rat model was resilient to environmental manipulations. These findings led us to suggest that organisms that are 'hypoaroused' may be more resilient to external environmental inputs as a result of increased GABAergic function as changes in glutamate-stimulated release was not affected. This suggestion is complimented by the finding that glutamate-stimulated release of NE was not altered in the maternally separated Sprague-Dawley rats. Interestingly acute nicotine decreased anxiety-like behavior in Sprague-Dawley rats, however no change was seen in either the 'hypoaroused' or the 'hyperaroused' rat models employed.

Conclusions

Cortical arousal as seen by relative frequencies from EEG recordings does serve to assess tonic activity of the LC-NE system. P300 amplitude and latency provides a measure of the phasic activity of the LC-NE system. The relationship between noradrenergic and glutamatergic neurons at terminal loci of the LC-NE system serve to highlight the dysfunction of the LC-NE system in 'hypoaroused' and 'hyperaroused' organisms. Nicotine use does serve as a form of self-medication in the reduction of anxiety. Childhood trauma and environmental manipulations affect arousal regulation.

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LIST OF ABBREVIATIONS

ADHD	Attention deficit-hyperactivity disorder
CRF	Corticotrophin releasing factor
ECG	Electrocardiogram
EEG	Electroencephalogram
ERP	Event-related potential
HPA-axis	Hypothalamic pituitary axis
LC	Locus coeruleus
LC-NE	Locus coeruleus-norepinephrine
NE	Norepinephrine
SHR	Spontaneously hypertensive rat
SD	Sprague Dawley rat
WKY	Wistar-Kyoto rat

1. INTRODUCTION

Norepinephrine (NE) functions as both a neurotransmitter and as a hormone (Ganong, 2001). As a neurotransmitter NE is synthesized and released by dendrites and axon terminals of neurons in the medulla and pons. The NE nuclei can be broadly distinguished by their major fibre bundle pathways: (1) the dorsal noradrenergic bundle which originates from the locus coeruleus (LC) a pontine nucleus and ascends through the medial tegmentum and (2) the ventral noradrenergic bundle which ascends through the ventral tegmentum, originating from several nuclei including two pontine nuclei, the dorsal motor nuclei of the vagi and the nucleus tractus solitarius, and two medullary nuclei of the dorsal tegmentum and lateral tegmentum (Felten and Sladek, 1982, 1983). Both the dorsal and ventral noradrenergic bundles send direct projections to the spinal cord (Theiss and Heckman, 2005). The LC is the major nucleus of the brain which produces NE in the rat, monkey and human (Foote, 1997; Swanson, 1976). The LC projects to several brain regions, and is the sole source of NE to the cerebral cortex, hippocampus and cerebellum (Foote et al., 1983; Jentsch, 2005; Aston-Jones et al., 1999; Southwick et al., 1999; Bunsey and Strupp, 1995). As a hormone, NE is synthesized and released by sympathetic nerve fibres and the adrenal medulla (Ganong, 2001).

The synthesis of NE occurs through a cascade of enzymatic reactions; phenylalanine is hydroxylated to form tyrosine, which is hydroxylated to L-dopa. L-dopa is then decarboxylated to form dopamine. Dopamine is hydroxylated by the enzyme dopamine- β -hydroxylase to form NE (Ganong, 2001; Kandel et al., 2001). Released NE binds to any of several NE metabotropic receptors, the principal receptors being α_1 , α_2 , β_1 , β_2 and β_3 (Ganong, 2001). The α_1 receptors are

further divided into three receptor subtypes α_{1A} , α_{1B} , and α_{1D} . The α_1 subfamily of noradrenergic receptors increases intracellular calcium through G-protein-mediated activation of phospholipase C, leading to the production of 1,4,5-triphosphoinositide and release of Ca^{+2} from internal stores, in addition activation of the α_1 subfamily can lead to direct activation of voltage-gated Ca^{+2} channels which can lead to depolarization of the nerve terminal (Pralong et al., 2002). The α_2 receptors are further divided into three receptor subtypes α_{2A} , α_{2B} , and α_{2C} . The α_2 subfamily of noradrenergic receptors generally inhibit adenylate cyclase through G-protein activation, and can lead to cell hyperpolarization and inhibition of norepinephrine (and glutamate) release, and are classed as autoreceptors in many instances (Pralong et al., 2002). The β subfamily of noradrenergic receptors when activated lead to the activation of adenylate cyclase mediated by G-proteins. β -Receptors also lead to K^+ channel inhibition or presynaptic Ca^{+2} channel activation, these activities lead to the depolarization of the nerve terminal (Pralong et al., 2002). Besides activation of NE receptors, NE can be taken up into presynaptic terminals by NE reuptake transporters, and or by surrounding glia (Iversen, 2006). Catabolism of NE occurs in nerve terminals or glia. Norepinephrine is oxidized by monoamine oxidase to produce 3,4-dihydroxymandelic aldehyde. 3,4-Dihydroxymandelic aldehyde then undergoes methylation by catechol-O-methyltransferase to 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid) or to 3-methoxy-4-hydroxyphenylglycol (MHPG) (Ganong, 2001; Kandel et al., 2001). Systemic NE has a half-life of about two minutes in peripheral circulation, and is then methylated and oxidized by catechol-O-methyltransferase and monoamine oxidase to produce 3-methoxy-4-hydroxymandelic acid (Ganong, 2001).

1.1 Arousal Systems

Arousal systems of the central nervous system arise from several nuclei of the reticular activating system (Moruzzi and Magoun, 1949). These systems are classified by their pathways and their specific neurotransmitters which include the: (1) LC-noradrenergic system (LC-NE), (2) magnocellular basal forebrain/pedunculopontine cholinergic system, (3) substantia nigra/ventral tegmental area dopaminergic system, (4) dorsal raphe serotonergic system, and (5) tuberomammillary hypothalamic histaminergic system (Marrocco et al., 1994). “Arousal is generally a dimension of activity or readiness for activity based on the level of sensory excitability, glandular and hormonal levels and muscular readiness. Or in neurophysiological terms seen as a heightened state of cortical functioning, cortical structures are ‘aroused’ by stimulation from sensory receptors mediated through lower brain structures. Additionally due to the breadth of usage and the generality of meaning, the term arousal is typically used with qualifiers e.g., cortical arousal or sexual arousal” (Reber, 1995). An individual requires a specific level of arousal to perform well within an environment, if the individual is hyperaroused or hypoaroused by environmental stimuli the individual will perform poorly (Yerkes and Dodson, 1908, figure 1.1).

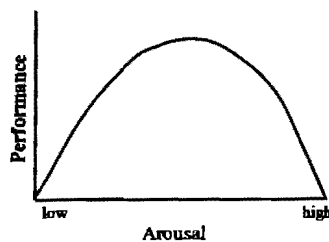


Figure 1.1 Derived from the seminal paper by Yerkes and Dodson (1908), showing the relationship between arousal levels and performance, an example of this would be attentional performance. If the organism is hypoaroused optimal performance is not attainable and if the organism is hyperaroused optimal performance is not attainable.

1.1.1 LC-NE system and cortical arousal

Recording of neural activity from the scalp is achieved by electroencephalography (EEG) and is used to measure cortical arousal (Moruzzi and Magoun, 1949; Timo-Iaria et al., 1970). Broadly cortical arousal can be defined by a reduction in voltage or amplitude of the EEG trace and increased activity within the higher band frequencies (Marrocco and Field, 2003; McCormick and Bal, 1997), this relationship is evident during all states of arousal. Changes in EEG traces including voltage or amplitude are related to the changes in brain wave frequency (Martin, 1998). Fourier transform is a mathematical procedure which decomposes the EEG trace recording into respective sine waves defined by their frequency and phase, giving the power (mV^2) during a selected epoch for each frequency band (Luck, 2005; Martin, 1998). Band frequencies in EEG include: delta (δ), theta (θ), alpha (α), beta (β), and gamma (γ). Delta (1-3 Hz) is associated with deep sleep, i.e. delta wave sleep (Reber, 1995; Guyton and Hall, 2000). Theta (4-7 Hz) activity is associated with temporal and parietal cortical activity (Guyton and Hall, 2000; Ganong, 2001). Alpha activity (7-13 Hz) is a hallmark frequency band that shows pronounced activity during restful wakefulness, especially during closed eyes (Barry et al., 2007, Klimesch, 1999). Beta activity (13-30 Hz) is associated with frontal and parietal cortical activity during mental performance (Reber, 1995; Guyton and Hall, 2000). Gamma activity (30-80 Hz) is associated with high levels of cortical activity (Ganong, 2001).

Electroencephalogram activity recorded during the performance of a cognitive task can additionally be shown as an event-related potential (ERP) (Martin, 1995; Gazzaniga, 2000; Luck, 2005). Event-related potentials are stimulus-locked epochs in the EEG trace (Gazzaniga, 2000).

The epochs extracted permit the overlay or average of cortical activities related to the stimulus presented. The overlay or average of the cortical activities reduces the EEG trace background noise. In doing this a pattern or EEG activity in response to a stimulus presented can be made evident (Luck, 2005). Event-related potentials are interpreted by dividing them into several temporal components (Luck, 2005). The components are defined by their deflection within the ERP, being either positive or negative and having a representative peak (Luck, 2005). For each component several values can be extracted and interpreted including peak amplitude (μV) and latency to peak amplitude (msec) (Luck, 2005). The components of the ERP vary and are dependent on task and modality of stimulus, be it auditory or visual (Luck, 2005). These differences in ERP between modalities of stimuli are suggested to result from the level of neural processing and neural engagement during stimulus presentations (Gazzaniga, 2000). A component of the ERP the P300 originally defined by its occurrence approximately 300 msec post stimulus presentation, during an oddball paradigm (Donchin and Coles, 1988; Polich and Kok, 1995), is currently suggested to be a “cognitive neuroelectric phenomenon” as it is generated during completion of cognitive tasks which require the individual to pay attention, process information and leads to a response from the individual (Polich and Kok, 1995). Anatomical localization of the P300 was originally suggested to originate from the hippocampal formation, as reported by depth electrode recordings. However, destruction of the hippocampal formation was shown to elicit minimal change to the P300 (Polich and Squire, 1993). Recent studies suggest that the P300 is generated from the juncture point of the temporal and parietal cortices only (Verleger et al., 1994; Yamaguchi and Knight, 1991, 1992). P300 during attentional tasks has been shown to be maximal over the parietal cortices (Polich and Kok, 1995). Why the

P300 is generated is presently unknown (Luck, 2005; Polich and Kok, 1995). It is however suggested that the P300 is a representation of the neural processing required for cortical updating (Donchin and Coles, 1988). The 'orienting response' suggests that an individual creates a cortical representation of trials within a task, as the task endures and the individual completes more trials, the cortical representation of the trials are constantly updated (Sokolov, 1960, 1963). The extent of this cortical updating or 'orienting response' is dependent on the individual's state of arousal (Polich and Kok, 1995; Hockey, 1983; Hockey et al., 1986) and the value placed on the information being processed (Sutton and Runkin, 1984). The state of arousal and the effects on P300 generation are suggested to occur (1) tonically (minutes to hours) or (2) phasically (msec to seconds). Tonic changes in arousal include the steady or general state of arousal and can include general changes in EEG activity, heart rate, and endocrine responses (Polich and Kok, 1995). Phasic changes in arousal include the individual's reaction to specific stimuli presentations and can include ERPs, skin conductance responses, and 'orienting responses' (Polich and Kok, 1995). It is important to note that tonic changes affect phasic changes and vice-versa, and cannot be interpreted in isolation (Barry and Sokolov, 1993; Barry et al, 2004). Several additional factors have been shown to change the P300 latency and amplitude and serve to compliment the cortical updating theory. (1) Habituation to task and repetition of task, as shown in an oddball task that led to decrements in P300 amplitude sequentially for each of the ten times the task was repeated (Ravden and Polich, 1998). (2) Task difficulty as shown in a series of mathematical tasks that gradually increased their level of difficulty, the P300 amplitudes obtained formed a U-shaped trend from 'extremely easy' to 'extremely difficult' (Ullsperger et al., 1987). (3) Mental fatigue was shown during an oddball task to decrease P300

amplitude at the end of a three hour testing session, one hour after the three hour testing session the P300 amplitude recovered when the task was re-administered (Murata et al, 2005).

1.1.2 The LC-NE system enhances the signal-to-noise ratio

The LC-NE system projects to the cortex directly and indirectly via relay thalamic nuclei, releasing NE from their terminals (Marrocco et al., 1994). Single cell recordings of the somatosensory cortical area related to foot-tap, were found to lead to either excitation or inhibition of the cells being recorded. With application of NE to the somatosensory cortical area the cells activity was enhanced, either increasing their excitation or increasing their inhibition (Waterhouse and Woodward, 1980). Electrical stimulation of the LC in halothane-anesthetized rats has been shown to increase cortical measures of arousal, and with recovery of LC to its resting state activity reflected decreased measures of arousal (Berridge and Foote, 1994). Stimulation of the LC with cholinergic agonist (bethanechol) has been shown to increase the extracellular levels of NE in the cortex (Berridge and Abercrombie, 1999). This enhanced activity recorded in the cortex was attenuated by intracerebroventricular application of a β antagonist (propranolol) (Berridge and Foote, 1994).

The suprachiasmatic nucleus is known to regulate diurnal rhythms (Ganong, 2001) and has been shown to indirectly innervate the LC via the dorsomedial hypothalamus (Aston-Jones et al., 2001). During slow wave sleep and paradoxical sleep the LC showed minimal or no activity, and was reflected by low frequency band activity and high amplitude in the cortex (Aston-Jones and Bloom, 1981a). However prior to wakefulness and during wakefulness the LC's activity increased, and was reflected by high frequency band activity and low amplitude in the cortex

(Aston-Jones and Bloom, 1981a). Lesions of the indirect pathway between the suprachiasmatic nucleus and the LC were shown to alter the LC's diurnal rhythm (Aston-Jones et al., 2001). In the anesthetized rat stimulation of the LC, with a cholinergic agonist (bethanechol) led to increased cortical arousal, with a delay that varied from five to thirty seconds (Berridge and Foote, 1991). Intravenous infusion of an α_2 -agonist (clonidine) or β -antagonist (propranolol) attenuated the increase in cortical arousal seen with cholinergic agonist application (bethanechol) (Berridge and Foote, 1991). Additionally in the lightly anesthetized rat stimulation of the LC with a cholinergic agonist (bethanechol) led to increased cortical arousal, this arousal was attenuated with infusion of a β -receptor antagonist (timolol) into the medial septal area (Berridge and Foote, 1994). In lightly anesthetized rats electrical stimulation of the LC was not attenuated with unilateral infusion of β -receptor antagonist (timolol) into the medial septal area, however bilateral infusion led to decreased cortical arousal (Berridge and Foote, 1994). Conversely infusion of a β -receptor agonist (isoproterenol) into the medial septal area, increased periods of wakefulness, as was characterized by increased cortical arousal and decreased periods of REM sleep (Berridge and Foote, 1996). However in the awake animal, infusion of a β -receptor antagonist (timolol) into the medial septal area elicited no change in cortical arousal levels during the period that the LC was stimulated (Berridge and Foote, 1994). The release of NE when the LC is stimulated is seen to enhance the 'signal-to-noise' ratio in cortical structures (Berridge and Foote, 1994; Waterhouse and Woodward, 1980; Moxon et al., 2007).

These studies suggested that stimulation of the LC during different arousal states led to enhanced levels of cortical arousal, which in turn led to enhancement of sensory neuronal input and suppression of background ‘non-informative’ neural activity, thereby enhancing the ‘signal-to-noise ratio’ (Berridge and Foote, 1994; Waterhouse and Woodward, 1980; Moxon et al., 2007). Together activation of the LC and its terminal release of NE in the cortex enhanced relevant signals either from external stimuli or internal stimuli to facilitate optimal behavioural output. Incorporation of this suggestion within the Yerkes and Dodson (1908) theory for the relationship between performance and arousal, suggests that optimal performance requires certain levels of LC activity and NE release (figure 1.2).

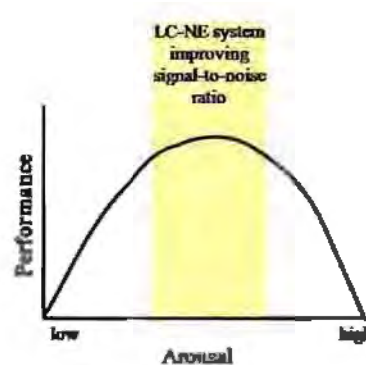


Figure 1.2 Yerkes-Dodson theory (1908) showing the relationship between performance and level of arousal, with the suggested window in which the LC-NE system enhances the signal-to-noise ratio to facilitate optimal performance.

1.1.3 LC-NE system's balance between modes of firing

The LC-NE system has been found to fire in two modes, tonically and phasically (Aston-Jones and Bloom, 1981a,b). During awake states tonic activity of the LC persists, fluctuating between 1 and 3 Hz in the rat and monkey (Aston-Jones and Bloom, 1981a,b; Foote et al., 1980). When vegetative behaviours were interrupted or the animal orientated toward a different behaviour the

LC's firing rate changed to bursts of activity, phasic activity (Aston-Jones and Bloom, 1981a,b). Additionally several studies have demonstrated phasic firing of the LC during novel or unexpected sensory stimulation (Bouret and Sara, 2005; Rajkowski et al., 1994; Sara et al., 1995; Aston-Jones et al., 1991a,b; Grant, 1988). In monkeys the intensity of phasic LC firing was found to be dependent on the type of stimulus, presentation of coloured fruit juice (coloured fruit juice was used as a reward during testing sessions) led to intense phasic burst activity (7-15 Hz). When animals were presented with a novel tone, phasic burst activity occurred but with a lower intensity (2-6 Hz) (Foote et al., 1980). Electrical induction of phasic activity of the rat LC (3.6 pulses, 0.2-0.5 msec duration, 100 Hz) led to brief periods of LC silence, no activity (Waterhouse et al., 1998). This period of silence was dependent on the intensity of the electrical stimulation the LC received (Waterhouse et al., 1998). Chemical manipulations of the LC failed to affect its phasic activity, at least 85% of the LC needed to be lesioned before cortical levels of NE were affected (Waterhouse et al., 1998). In addition dorsal noradrenergic bundle lesion's greater than 85% led to disruptions in waking and reuptake of NE within the prefrontal cortex, however these measures returned to normal levels of function within 25 days (Lidbrink, 1974). Stimulation of the LC and its resultant terminal release of NE was suggested to occur in a linear fashion, however phasic burst activity of the LC was found to lead to greater release of NE cortically than with tonic firing (Florin-Lechner et al., 1996).

Phasic activity of the LC was attenuated by infusion of an α_1 -receptor antagonist (prazosin) or when in combination with a β -receptor antagonist (timolol) which led to decreased cortical arousal (Berridge and Espana, 2000). Additionally infusion of an α_2 -agonist (clonidine) into the

LC decreased cortical arousal an effect that was attenuated by infusion of an α_2 -antagonist (idazoxan) (Berridge et al., 1993; Kawahara et al., 1999). However infusion of an α_2 -antagonist (idazoxan) alone was found to elicit no change in levels of cortical NE (Kawahara et al., 1999). Tonic activity of the LC was reduced by chronic systemic treatment with a NE-reuptake inhibitor (imipramine) which increased cortical NE (Linnér et al., 1999; Swanson et al., 2006). With acute infusions of NE-reuptake inhibitors (atomoxetine, methylphenidate) or α_2 -receptor antagonist (idazoxan) prefrontal levels of NE have been shown to increase (Bymaster et al., 2002; Swanson et al., 2006).

Phasic activity of the LC occurred during an operant conditioning paradigm in monkeys (Aston-Jones et al., 1997). On presentation of target stimuli the LC fired phasically, the animal was then rewarded for correct response with drops of juice (Aston-Jones et al., 1997). When the task was reversed the LC fired phasically to the new condition and did not fire to the previous condition's target stimuli, the phasic firing of the LC occurred before the animal performed the correct behaviours for reversal of the task, the phasic activity additionally attenuated as the animal obtained the new behavioural conditions of the operant conditioning paradigm (Aston-Jones et al., 1997). Tonic activity during a visual discrimination task in monkeys showed that there was a difference in tonic firing between 'poor performance' and 'good performance' which led them to suggest that the tonic activity of the LC needed to be held in a certain range for optimal behavioural performance (Usher et al., 1999). During the good bouts of performance and after phasic activity of the LC, the LC was shown to have a brief period of silence, no activity (Usher et al., 1999). Additionally the period between the presentation of task stimulus and the phasic

activity of the LC was associated with arousal level and attentional performance, suggesting that during low levels of arousal and attentional performance LC phasic activity is delayed (Aston-Jones et al., 1994) or mismatched. Interestingly lesions of the LC have been shown to decrease the amplitude of the P300-like ERP component in monkey EEG during the performance of an oddball paradigm (Pineda et al., 1989).

These studies indicate that the LC fires in two manners that affect behaviour differently. Low levels of tonic firing are related to decreased levels of behavioural arousal and poor performance, and high levels of tonic firing are related to increased behavioural arousal and decreased performance. Suggesting that for optimal performance a level of tonic LC activity is required. Additionally during very low levels of tonic LC activity a paradoxical effect occurs which enhances extracellular NE in the prefrontal cortex, suggesting, that during very low and very high levels of tonic LC firing, NE levels within the cortex are elevated. Incorporation of these suggestions within the Yerkes and Dodson (1908) theory for the relationship between performance and arousal, suggests that optimal performance requires a certain level of LC tonic activity, which permits phasic firing to enhance the 'signal-to-noise ratio' to facilitate optimal performance (figure 1.2).

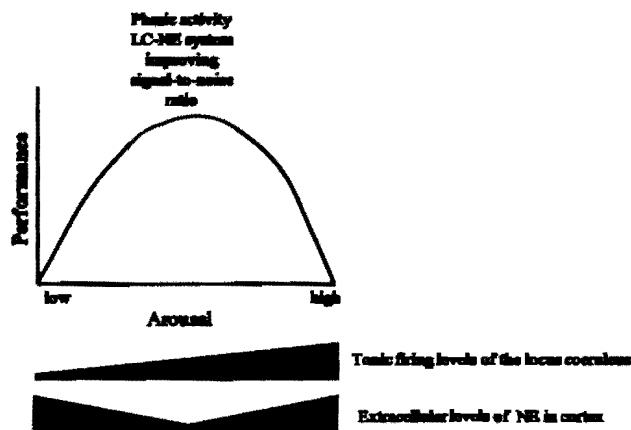


Figure 1.2 Yerkes-Dodson theory (1908) showing the relationship between performance and level of arousal. Tonic and phasic activities of the LC are required during different levels of arousal. Tonic activity of the LC needs to be within a range of activity to allow the phasic firing of the LC and its facilitatory enhancement of the signal-to-noise ratio required for optimal performance.

1.1.4 LC-NE terminal release and regulation

Glutamatergic-thalamocortical projections and their release of glutamate was shown to increase the release of NE from the LC-NE terminals (Marek and Aghajanian, 1999; Marek and Zhang, 2008). Glutamate receptors are broadly defined as non-NMDA, NMDA and metabotropic glutamate receptors. Non-NMDA receptors include AMPA and kainate receptors. When these receptors are activated they elicit fast EPSPs through rapid influx of Na^+ ions (Takahashi, 2005; Kandel et al., 2001). AMPA receptors are fast adapting in the sense that they are capable of rapid desensitization or internalization (Attwell and Gibb, 2005; Rusakov and Kullman, 1998) and are able to translocate to the surface of the postsynaptic terminal (Ashworth-Preece et al., 1999). Desensitization and internalization reduces the excitability of the postsynaptic terminal and translocation to the membrane surface permits reinforcement of the pathway(s) they are involved in (Attwell and Gibb, 2005; Derkach et al., 2007). The NMDA receptors require coincidence for

their activation, requiring depolarization of the nerve terminal prior to the opening of their ion channels (Paoletti and Neyton, 2007). NMDA receptors when activated allow large influx of Ca^{+2} and Na^{+} into the synaptic terminal, their activities are temporally longer than the non-NMDA receptors (Paoletti and Neyton, 2007; Derkach et al., 2007). Calcium entry leads to a cascade of events including internalization and translocation of non-NMDA receptors in the depolarized terminal membrane (Lee et al., 2003). Additionally NMDA receptor subunits are known to down-regulate during particular stages of development (Takahashi, 2005). This down-regulation of NMDA receptor subunits has been related to decreased synaptic plasticity (Takahashi, 2005). These findings are suggested to accentuate the temporal relationship between the ionotropic glutamate receptors that leads to either strengthening of synaptic transmission or a decrease in synaptic transmission. Infusion of a non-NMDA receptor agonist (kainate) into the LC was shown to increase the levels of extracellular NE in the rat cortex (Kawahara et al., 1999; Lehmann, 1992; Kodama and Koyoma, 2006). Antagonism of NMDA receptors had no effect on extracellular levels of NE in the medial prefrontal cortex (Kawahara et al., 1999). Metabotropic glutamate receptors (mGluR1-8) are found in several brain areas including the hippocampus, neocortex and the cerebellum (Ozawa et al., 1997). Postsynaptic metabotropic glutamate receptor activation leads to slow depolarization and increased firing rates (Ozawa et al., 1997) and presynaptic mGluR activation leads to either excitation or inhibition of synaptic transmission (Nakanishi, 1994). Extracellular glutamate is primarily taken-up by glia and the presynaptic terminals (Herrman and Jahr, 2007; Torres and Amara, 2007). Glutamate released in forebrain structures has been related to stress, as seen by tail-pinch (Bagdley and Moghaddam, 1997). The release of glutamate was shown to attenuate with repetition of the stressor (Bagdley

and Moghaddam, 1997). Intraperitoneal injection of a GABA_A receptor agonist (diazepam) prevented this attenuation of glutamate release to repeated tail-pinch (Bagley and Moghaddam, 1997). Norepinephrine has additionally been shown to lead to the release of glutamate within the cortex, through the activation of α_1 -receptors (Marek and Aghajanian, 1999). These findings suggest a synergistic activation and release between glutamatergic and noradrenergic nerve terminals.

Cortical neurons project to the LC and serve as a major afferent, specifically glutamatergic neurons (Arnsten and Goldman-Rakic, 1984; Jodo et al., 1998; Jodo and Aston-Jones, 1997). This feedback has been shown to maintain the tonic activity of the LC in the resting state (Jodo and Aston-Jones, 1997a, b). In addition the LC has axon collaterals that innervate its vast dendritic field, supplying efferent feedback to the LC (Aghajanian et al., 1997; Harley, 2007; Swanson, 1976). Decreased synthesis of NE in the LC through application of α -methyl-p-tyrosine or administration of a NE receptor antagonist (chlorpromazine hydrochloride) decreased the efferent feedback to the LC which decreased the self-reinforcement of its resting state tonic activity (Ritter and Stein, 1973). Two major afferents to the LC located in the rostral medulla are the paragigantocellularis nucleus and the prepositus hypoglossi nucleus (Ennis and Aston-Jones, 1987; Ennis and Aston-Jones, 1989; Singewald and Philippu, 1998). The paragigantocellularis nucleus receives input from a variety of nuclei involved in somatosensory, auditory and autonomic function (van Bockstaele et al., 1993). Paragigantocellularis nucleus afferents to the LC are excitatory and their activity is mediated by non-NMDA receptors (Ennis et al., 1992). The prepositus hypoglossi nucleus has been shown to relay visual shifts in attention

processing, the LC is suggested to integrate the visual information for adjustments during behaviour (Ennis and Aston-Jones, 1989), and this integration of visual information is achieved through inhibition of the LC by activation of GABA receptors (Aston-Jones et al., 1991a,b; van Bockstaele et al., 2001). Earlier studies initially suggested that the nucleus tractus solitarius was a major afferent to the LC, however this was not supported by antidromic stimulation studies of the LC that further emphasized the afferent connections of the prepositus hypoglossi nucleus with the LC (Ennis and Aston-Jones, 1989; van Bockstaele et al., 2001).

The LC receives corticotrophin releasing factor (CRF) from several brain areas including the central amygdala, the bed nucleus of the stria terminalis and Barrington's nucleus (Valentino et al., 1996; Lechner and Valentino, 1999). The CRF receptors of the LC have been found to co-localize with glutamate receptors and are found on the vast dendritic fields of the LC (Valentino et al., 2001). The activation of the LC by CRF leads to increased release of NE at terminals and is suggested to prime the LC to environmental stressors that require change in the behaviour of the organism (Valentino et al., 1983; Valentino et al., 1989; van Bockstaele et al., 1996; Koob et al., 1994; Abercrombie and Jacobs, 1987). The increased release of NE from LC terminals has been shown to act on the β -receptors in the basolateral amygdala (Ferry et al., 1999). During blood volume loss initially both the LC-NE system and sympathetic noradrenergic fibres fire, with continuous decrement in blood volume the sympathetic noradrenergic fibres are inhibited and the LC activity persists (Elam et al., 1986). Electrophysiological recordings of the LC have shown that activation of LC neurons evoked due to the fall of blood pressure in response to nitroprusside was inhibited with intracerebroventricular infusion of a CRF antagonist (α -hCRF),

thus the fall of blood pressure elicited by nitroprusside activated LC neurons by release of CRF (Curtis et al., 1993). Peripheral sensory stimulation led to firing of the LC and sympathetic nerve fibers, firing of the LC-NE system led to increased release of NE at terminal sites and release of NE from the sympathetic nerve fibers (Elam et al., 1986; Theiss and Heckman, 2005). The Barrington's nucleus release of CRF onto the LC has been associated with stimulation of defecation (Mönnikes et al., 1994), inhibition of gastric secretion (Selden et al., 1990), and stimulation of micturition (Elam et al., 1986; Page et al., 1992). Interestingly LC activity is also required for the pre-ovulatory surge of luteinizing hormone (Anselmo-Franci et al., 1997; Szawka et al., 2006; Wright and Jennes, 1993; Morgane and Jacobs, 1979; Leger and Descarries, 1978; McRae-Deguerce and Milon, 1982; Dotti and Talesnik, 1984, Morello and Taleisnik, 1987).

The hippocampus is innervated by noradrenergic fibres arising from the LC (Pasquier and Reinoso-Suarez, 1978; Foote et al., 1983; Jentsch, 2005; Aston-Jones et al. 1999; Southwick et al., 1999; Bunsey and Strupp, 1995). Excitation of the LC leads to synchronization of hippocampal EEG, enhancing theta activity (Waterhouse et al., 1988; Berridge et al., 1993; Berridge and Wifler, 2000; Brown et al., 2005), this synchronization can be attenuated by bilateral LC pretreatment with a β -antagonist (propranolol) (Waterhouse et al., 1988; Berridge and Foote, 1994) or infusion of a β -antagonist (timolol) at the level of the medial septal area in the anesthetized animal (Berridge and Wifler, 2000). In addition, direct bilateral LC infusion of an α_2 -agonist (clonidine) inhibited LC firing and changed hippocampal EEG frequency from its normal prominent theta activity to a range of mixed frequencies (Berridge et al., 1993). Infusion

of NE has been shown to promote long-term potentiation through the activation of β -receptors and the effects of NE were only shown at the local site of the infusion (Harley et al., 1996). In vitro studies confirmed this enhancement by NE (Heginbotham and Dunwiddie, 1991; Stanton and Harvey, 1987). The hippocampus has been implicated in a number of behaviours including novelty detection (Lee et al., 2005; Wu and Sutherland, 2006), spatial memory (Wright et al., 2004), and exploratory behaviour (Wright et al., 2004). Electrical stimulation of the LC or application of glutamate when paired with stimulation of the perforant pathway was shown to cause long-term potentiation within the dentate gyrus (Harley et al., 1989). With intraperitoneal injection of a β -receptor antagonist (propranolol) the pairing of the LC with stimulation of the perforant path led to long-term potentiation in the dentate gyrus only if paired with electrical stimulation of the LC and did not occur with application of glutamate to the LC (Harley et al., 1989; Harley et al., 1985). In a low Mg^{+2} cortical slice preparation NMDA receptors are spontaneously active. With application of an α_1 -receptor agonist to the entorhinal cortex spontaneous NMDA receptor activity was inhibited, and application of a β -receptor agonist enhanced the spontaneous NMDA receptor activities in the dentate gyrus (Stanton et al., 1987). Release of NE at hippocampal terminal sites was facilitated by NMDA receptor activation (Andrés et al., 1993) and glutamate release correlated with β -receptor function of the hippocampus (Harley et al., 1996; Harley, 2007). In freely moving rats infusion of a β -receptor agonist does not facilitate long-term potentiation in the dentate gyrus (Straube and Frey, 2003). Synaptic concentration of NE in the dentate gyrus was required to be thirty times that of basal levels, as three to six times basal level of NE did not elicit potentiation (Harley et al., 1996; Harley, 2007), and higher levels did not facilitate long-term potentiation (Almaguer-Melian et

al., 2005). These findings further accentuate the synergistic activation between glutamatergic and noradrenergic nerve terminals.

Within the CA1 region of the hippocampus, increased extracellular Ca^{+2} has been shown to lead to the release of glutamate from glial cells, and has additionally been shown to cause synchronous firing of the CA1 neurons (Carmignoto and Fellin, 2006). With antagonism of NMDA receptors within the hippocampus, release of NE is significantly decreased. A similar effect was observed with application of a Na^{+} channel antagonist (Andres et al., 1993). β -receptor activation was required for the phosphorylation of Ser845 and Ser831 that are required for the delivery of GluR1 to the synapse. GluR1, a subunit of glutamate receptors, enhances synaptic long-term potentiation. This was directly confirmed by enhancement of memory formation and contextual fear conditioning following β -receptor activation (Hu et al., 2007). Phosphorylation of GluR1 was required for the retention of spatial memory (Lee et al., 2003). In addition, application of NE or electrical stimulation of the LC was shown to provide the required depolarization to meet threshold in neurons that were being held at a subthreshold level in a brain slice preparation (Waterhouse et al., 1988). These studies suggest that LC-NE needs to meet several criteria to function as a neuromodulator and enhance the signal-to-noise ratio to facilitate optimal performance.

1.2 Attention

‘Attention is a general term referring to the selective aspects of perception that function so that at any instant an organism focuses on certain features (salience) of the environment to the exclusion

of other features. Attention may be conscious in that some stimulus elements are actively selected out of the total input, although, by and large, we are not explicitly aware of the factors which cause us to perceive only some small part of the total stimulus array' (Reber, 1995). Attention has been decomposed into brain networks' activated during attentional task performance and include: (1) alerting, (2) orienting, and (3) executive control (Posner et al., 2006). (1) Attentional alerting being the acquisition and maintenance of the alert state required to perform the task. (2) Attentional orienting toward the sensory stimuli that require attention. (3) Attentional executive control which facilitates the resolution of conflict between performance and affect (Posner et al., 2006; Coull, 1998). Posner and Petersen (1990) proposed two attentional networks: (1) anterior attentional network and the (2) posterior attentional network. The (1) anterior attentional network has been suggested to involve the detection of sensory targets and is strongly reliant on the anterior cingulate cortex. The (2) posterior attentional network has been suggested to involve sensory attentional orienting and is reliant on the functioning of the posterior parietal cortex, superior colliculus and thalamic pulvinar. Both of these attentional networks require interaction with arousal systems and with each other to facilitate attentional performance (Posner and Petersen, 1990). A series of positron emission tomography studies during the completion of a rapid visual information processing attentional task revealed that administration of an α_2 -agonist (clonidine, 1.5 $\mu\text{g/kg}$) decreased regional cerebral blood flow (used as a marker of neuronal activity) in the medial occipital cortex, inferior parietal cortex, medial frontal cortex, and inferior frontal gyrus (Coull et al., 1999). The same α_2 -agonist increased the neural connectivity that usually occurred during activation of the attentional networks, between the LC and parietal cortex, and between the parietal cortex,

thalamus, and frontal cortex (Coull et al., 1999). Interestingly during rest, functional neural connectivity was decreased (Coull et al., 1996). This was reinforced by earlier studies where an α_2 -agonist (clonidine 1-4 $\mu\text{g/kg}$) caused participants to become very drowsy and just prior to the commencement of their cognitive task the participants would bring themselves to near fully conscious states (Hall et al., 2001, 2000). These studies accentuate the LC-NE system and its role in the regulation of attention and arousal, through enhancing the connectivity as seen by pathway tracing in positron emission tomography between the LC-NE system and several brain regions required for attention.

Animals with dorsal noradrenergic bundle lesions greater than 84% have been shown to have decreased attentional performance while completing a five choice serial reaction time task, including increased errors and omission when stimuli were presented with increased speed and less predictability (Carli et al., 1983). In a task that serves to measure attention and impulsivity, a delayed alternation performance task in a T-maze, infusion of an α_1 -receptor agonist (phenylephrine) into the prefrontal cortex decreased attentional performance (Arnsten et al., 1999). This decreased attentional performance with the infusion of an α_1 -receptor agonist was reversed by the administration of an α_1 -receptor antagonist (urapidil) (Arnsten et al., 1999). In an attentional-shifting paradigm, which included odor and texture discrimination, administration of an α_2 -antagonist (atipamezole) reduced the number of trials required to learn the reversal of the task. Administration of an α_2 -agonist had no effect (Lapiz and Morilak, 2006), while pretreatment with an α_2 -antagonist (atipamezole) and administration of an α_1 - antagonist (benoxathian) decreased the ability to shift or acquire the reversal of the task (Lapiz and Morilak,

2006). Administration of non-sedative doses of α_2 -agonist (clonidine) or a β -antagonist (propranolol) prevents attention to novel objects in a novel-hole-board paradigm (Sara et al., 1995). The studies accentuated the importance in maintenance of the LC-NE system's integrity relies on differential activation at the level of the LC and the LC-NE nerve terminals this adds additional complexity to the LC-NE system and its role in attention and arousal.

1.3 Hypoarousal and the LC-NE system in attentional performance

The inability to withhold a response is seen as a measure of impulsivity (Aron and Poldrack, 2005). 'Healthy' participants who are not able to withhold responses show higher error rates and shorter response times during attentional performance (Kirkeby and Robinson, 2005; Svebak et al., 1987; Dimoska and Johnstone, 2007), and have characteristics of absentmindedness (Roche et al., 2005), do not take the task seriously (Svebak et al., 1987), and use a more 'reflexive strategy' in responding (Kirkeby and Robinson, 2005; Ruchow et al., 2008). It has been suggested that healthy impulsive individuals fail in-part during updating of stimulus presentation, therefore show deficits in the 'orienting response' (Donchin and Coles, 1988). During a task of response inhibition 'healthy' individuals who performed poorly showed decreased P300 amplitudes (Svebak et al., 1987; Ruchow et al., 2008). A later study showed that the amplitude of the P300 component increased when the participants made an error (Roche et al., 2005). 'Healthy' participants who took the task seriously, showed greater accuracy and lower errors, and greater amplitudes in the P300 component of the ERPs. The administration of a NE reuptake inhibitor (atomoxetine) improved response inhibition in healthy adults (Chamberlain et al., 2006;

Chamberlain et al., 2007). Impulsiveness in 'healthy' individuals has been shown to occur rather broadly and suggests controlling for impulsivity within the 'healthy' controls during studies of impulsivity.

Attention-deficit hyperactivity disorder (ADHD) is presently categorised in the DSMV-IV into three subtypes: (1) predominantly hyperactive-impulsive subtype that includes characteristics of increased motor behaviour, short attention-span, distractibility, impulsiveness, and lack of self-control. (2) Predominantly inattentive subtype that includes characteristics of, difficulty in sustaining attention, inattention to detail and difficulty completing tasks. (3) Combined type that includes characteristics of both subtypes (1 and 2, Sue et al., 2000). An alternative subtyping has recently been suggested to characterize ADHD, with the aim of being able to type individuals with ADHD into three physiologically measurable subtypes. (1) Hypoarousal ADHD characterized by increased theta activity in frontal regions more so than posterior, decreased beta activity with normal alpha activity. (2) Maturation lag ADHD characterized by increased frontal and decreased posterior total power from the filtered EEG trace, and increased delta activity across the scalp. (3) Hyperarousal ADHD characterized by increased beta activity (Clarke et al., 2001, 2002, 2007).

Childhood ADHD has been found to persist into adulthood, 36.3% of 3197 participants showed persistence of ADHD in a study conducted in the USA (Kessler et al., 2005). Individuals with ADHD showed increased levels of theta activity across all ages, while relative delta activity decreased with age, suggesting that increased theta activity is related to impulsivity, while the increased delta activity is related to hyperactivity which rarely persists into adulthood

(Bresnahan et al., 1999). These changes in frequency have been shown to differ topographically for different age groups. Adolescents with ADHD showed increased relative theta power in posterior regions when compared to 'healthy' individuals (Hobbs et al., 2007). Children with ADHD showed higher relative theta power in frontal regions and posterior regions than 'healthy' controls (Clarke et al., 2002). Adults with ADHD showed higher global theta activity than 'healthy' controls (Clarke et al., 2008) indicating differences regionally between age groupings for frequency measurements of EEG (Barry et al., 2003). In addition individuals with ADHD showed increased relative beta activity during resting states (Clarke et al., 2007), and during physical and cognitive activity (Ackerman 1994, 1995). When individuals with ADHD completed a cognitive task beta activity was reduced (Mann et al., 1992) and theta activity increased (Hermens et al., 2005). On finding these differences in theta activity and beta activity in individuals with ADHD, the ratio of theta/beta activity was suggested to be a prominent characteristic found within the EEG of individuals with ADHD (Hobbs et al., 2007, Clarke et al., 2002; Monastra et al., 1999, 2001; Bresnahan et al., 1999, Bresnahan and Barry, 2002).

Two ways in which cognitive task performance and cortical arousal have been improved in individuals with ADHD are: (1) low dose stimulant medication (Chamberlain et al., 2007) and (2) biofeedback in which beta activity was improved/increased and has been shown to reduce hyperactive behaviour and impulsive behaviour whilst simultaneously improving attentional abilities, this form of training also leads to decreased response times and increased P300 amplitudes over parietal cortex (Gruzelier et al., 2006). In addition sympathetic activity as measured by skin conductance levels and skin conductance responses were shown to be reduced

and fewer in individuals with ADHD (O'Connell et al., 2004; Beauchaine et al., 2001). However, during the completion of an auditory oddball task skin conductance responses were non-specific and poorly related to performance of task in individuals with ADHD (Hermens et al., 2005). An interesting aspect of individuals with ADHD was their reported increase in their perception of social stress, however they did not show this association physiologically as plasma cortisol, a measure of hypothalamic pituitary adrenal axis (HPA-axis) activity, was not increased in individuals with ADHD during completion of a stressful cognitive sorting task (King et al., 1998; Randazzo et al., 2007). These studies support the hypoarousal and hyperarousal models suggested by Clark and his colleagues for ADHD (Clarke et al., 2001, 2002, 2008).

The spontaneously hypertensive rat (SHR) was initially developed for the study of adulthood hypertension (Okamoto and Aoki, 1963), this adulthood hypertension is a persistent characteristic of the strain (152 ± 6 mmHg) in comparison to its 'standard' normotensive control the Wistar-Kyoto (WKY, 102 ± 4 mmHg) (Singewald et al., 2000; Kaehler et al., 2004). In later years the SHR was found to exhibit increased locomotor activities and has been used as an animal model of ADHD (Knardahl and Sagvolden, 1979). SHR show high levels of locomotor activity when compared to several strains of rat in the open-field including: WKY (Sagvolden et al., 1993; Sukhanov et al., 2004), Lewis (Hinojosa et al., 2006) and when young adult SHR (PND 30) show higher activity than young adult Wistar (PND 30) (van den Burgh et al., 2006). It should be noted that increased activity within an arena such as the open-field apparatus can also be looked upon as exploratory behaviour, as the animal 'investigates' their environment. When the animal's home-cage was attached to the open-field and the animals were given the choice of

entering the open-field, SHR still spent greater amounts of time ambulating and exploring the open-field than WKY (Wultz et al., 1990; Kaehler et al., 2004). Additionally SHR spent greater amounts of time in the centre area (inner zone) of the open-field arena than WKY, which suggests reduced anxiety (Wultz et al., 1990; Kaehler et al., 2004). When novel objects were placed in the open-field, SHR explored the objects more readily and increased their time spent exploring them on the successive days of testing, in comparison with WKY (Knardahl and Sagvolden, 1979; Rogers et al., 1998). In a novel hole-board experiment SHR showed higher levels of activity in comparison with WKY, Lewis and Fisher 344 (Marti and Armario, 1996). These studies show the persistent hyperactivity of the SHR and its increased exploratory behaviours as compared with several strain of rat.

SHR have been shown to be unable to sustain attention as they show an inability to complete tasks with repeated exposure, and reduced retention of spatial memory shown in a water version of the radial arm maze (Clements and Wainwright, 2006). In the water version of the radial arm maze when an animal found a platform it was removed, this occurred for each of the successfully found platforms, the animal would then be removed from the maze and returned at increasing intervals, SHR made more errors than Sprague-Dawley (SD) and as the weeks progressed SHR performance further deteriorated and they stopped their active search to the ends of the arms as they had initially done to find the platforms (Clements and Wainwright, 2006). In addition when compared with Wistar, SHR rats showed lower levels of acquisition in an eight arm radial maze, and with the introduction of a five second time delay SHR's performance deteriorated and deteriorated further with an hour delay (Nakamura-Palacios et al., 1996). In a reward baited

twelve arm radial maze, SHR's performance excelled in comparison with WKY rats as the SHR varied which arm they entered, while the WKY did not. SHR also excelled in an operant task in which variation of lever press was taken as a measure of performance. However when a single lever press was required, the SHR showed poor performance compared with WKY rats (Mook et al., 1993). This study shows that if a task required high levels of variability in response SHR's performance was better than Wistar and WKY strains. SHR performed poorly in comparison with WKY and Wistar rats in a response inhibition task showing increased impulsivity, when differential reinforcement was delayed (for up to 72 sec), during which time the animals were required to inhibit lever press (van den Bergh et al., 2006). A second study looked at a similar paradigm, finding no difference in acquisition or performance of this task for SHR, WKY and SD rat strains (Ferguson et al., 2007). A second test of response control, a temporal response differentiation task (as used in van den Bergh et al., 2006) found no difference in acquisition and performance between SHR, WKY and SD rats (Ferguson et al., 2007). SHR rats at either P90 or P300 succeeded in the acquisition of an operant food reinforcement task and reversal learning of the task faster than WKY. SHR rats acquired the spatial location of a platform submerged under water within the Morris water maze, more effectively than WKY and Goldblatt renal hypertensive rats (Widy-Tyszkiewicz et al, 1993). However, in acquisition of a spatial food reinforcement task, in a T-maze, SHR and WKY performed equally well/poorly (Łukaszewska and Niewiadomska, 1995).

SHR entered the open arms of an elevated-plus maze, employed as a measure of reduced anxiety, more than Brown Norwegian (Ramos et al., 1997), SD (Ferguson and Gray, 2005),

Wistar Furth (Ramos et al., 1997), Fischer 344 (Ramos et al., 1997), WKY (Ramos et al., 1997; Ferguson and Gray, 2005), and Lewis rats (Hinojosa et al., 2006). Interestingly 'control' (Wistar) animals with low anxiety as shown by open-field and elevated-plus maze behaviours, had reduced levels of corticosterone receptor expression in their hippocampi and showed faster acquisition and memory retention in the water maze than SHR (Herrero et al., 2006). A measure of 'behavioural despair', the forced swim test, is used to test the effectiveness of antidepressant medications (Porsolt et al., 1978). Several studies indicate that SHR have reduced immobility and greater periods of struggling in comparison to Lewis rats (Hinojosa et al., 2006; Lahmame et al., 1997; Armario et al., 1995), WKY (Lahmame et al., 1997; Armario et al., 1995), and Fischer 344 rats (Lahmame et al., 1997; Armario et al., 1995).

These studies serve to confirm the SHR as a behavioural model of ADHD, sharing several characteristics of individuals with ADHD including: hyperactivity, increased exploration, decreased attentional performance, variability in performance, and reduced anxiety. These characteristics slot in with the proposed subtyping of ADHD as a disorder of 'hypoarousal' (Clarke et al., 2001, 2002, 2007). In addition results from the forced swim test suggest that SHR behaviour is resilient to pervasive stressors such as the forced swim test.

The levels of NE within the LC of Wistar rats was shown to be higher than SHR and WKY, shown by HPLC (Felten et al., 1984). Basal levels of NE in the LC and prefrontal cortex was shown to be higher in SHR compared to WKY (de Villiers et al., 1995). No difference was found in basal release of glutamate and GABA in the LC of SHR and WKY rats (Kaehler et al., 2004).

Glutamate infusion into the LC led to increased release of NE in the posterior hypothalamus of SHR, when compared to WKY (Kawasaki et al., 1991). Tail pinch elicited release of GABA and glutamate in the LC, interestingly GABA released from SHR was higher than WKY (Kaehler et al., 2004). With acoustic startle, SHR increased glutamate release in amygdala (50% increase), this increase was not evident for WKY (Singewald et al., 2000). The density of α_2 -receptors was reduced in the cerebral cortex, hypothalamus and medulla oblongata of SHR in comparison with WKY (Olmos et al., 1991). Administration of an α_2 -agonist (guanfacine 0.01-0.1 mg/kg) did not change SHR or WKY performance during a visuospatial divided attention task (Jentsch, 2005). In a second study administration of an α_2 -agonist (guanfacine, 0.3 mg/kg) reduced activity in SHR and marginally improved accuracy in an operant food reward task. In WKY an α_2 -agonist (guanfacine, 0.6 mg/kg) decreased performance in the operant food reward task (Sagvolden, 2006). These studies indicate differences in the basal LC-NE system as manipulations of the LC-NE system led to differential effects behaviourally and neurochemically in the SHR when compared to several strains of rat.

From these studies it is suggested that impulsive 'healthy' individuals, individuals with ADHD and the SHR have dysfunction of the LC-NE system. We suggest overlay of these findings onto the Yerkes and Dodson (1908) relationship between arousal levels and performance (Yerkes and Dodson, 1908). This leads us to suggest that these 'hypoaroused' individuals would appear off centre to the left, as their arousal is insufficient and their performance is poor (figure 1.4).

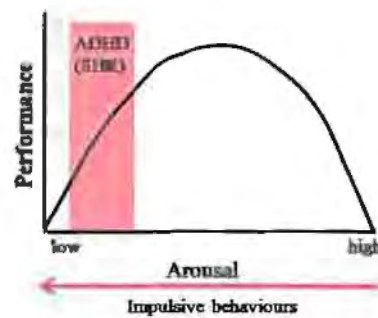


Figure 1.4 The Yerkes & Dodson (1908) relationship between arousal level and performance with the proposed window of 'hypoarousal' that suggests reduced levels of performance as seen in ADHD and SHR due to the LC-NE system dysfunction.

1.4 Hyperarousal and the LC-NE system in attentional performance

Anxiety is 'a vague, unpleasant emotional state with qualities of apprehension, dread, distress and uneasiness. In learning theory, the term is used to represent a secondary (or conditioned) drive which functions to motivate avoidance responding, thus an avoidance response is assumed to be reinforced by a reduction in anxiety' (Rebers). Within 'healthy' participants' high levels of anxiety are associated with poor attentional performance as individuals pay attention to non-informative and aversive stimuli (Avila et al., 2002). In addition EEG recordings of anxious individuals have been found to exhibit decreased theta activity in the frontal midline during cognitive tasks (Ishihara and Yoshii, 1972) and in 'healthy' individuals increased theta activity occurred during personal rumination over parietal and occipital cortices (Andersen et al., 2008). 'Hyperaroused' anxious individuals have also been shown to have increased HPA-axis activity as shown in morning levels of CRF and plasma cortisol (Catalán et al., 1998; van Niekerk et al., 2001).

An anxiety disorder post-traumatic stress disorder is often characterized by the re-experiencing of a traumatic experience, showing characteristic emotional numbing or avoidance of any stimuli related to the traumatic experience, physiologically these individuals present as being 'hyperaroused' (Sue et al., 2000). Post-traumatic stress disorder has been linked to the LC-NE system in several ways including: decreased platelet expression of α_2 -receptors (Southwick et al., 1999), and in culture α_2 -receptors show greater sensitivity (Southwick et al., 1999). Post-mortem studies on war veterans diagnosed with post-traumatic stress disorder revealed decreased LC neuron counts (Bracha et al., 2005). In addition, administration of an α_2 -antagonist (yohimbine) to individuals with post-traumatic stress disorder caused them to recall the traumatic events and/or led to panic attacks (Southwick et al., 1999). A second disorder strongly associated with anxiety is depression. Depression is additionally characterized with feelings of hopelessness, difficulty in decision making, social withdrawal, lowered productivity, psychomotor retardation, sleep disturbances, and several other physiological dysfunctions related to hyperarousal (Sue et al., 2000). The severity of affective symptoms has been associated with a decreased ability to sustain attention (Clarke et al., 2002), and was related to disease progression. Post-mortem studies of individuals who were diagnosed with severe depression were shown to have decreased LC neuron counts (Baumann et al., 1999), disrupted LC ultrastructure (Issidorides et al., 1989), and increased LC α_2 -receptor expression (Ordway et al., 2003). Additionally victims of suicide have been strongly associated with chronic major depression. Post-mortem studies on victims of suicide indicated an association with the LC-NE system finding a 23% decrease in LC neuron count (Arango et al., 1996) and down-regulation of NE reuptake transporters in the LC (Klimek et al., 1997).

The WKY has been used as a 'standard' control for the SHR, and has recently been suggested to model aspects of anxiety-like related behaviours. These behaviours elicited by WKY include, decreased locomotor activity in the open-field in comparison with SHR (Sagvolden et al., 1993; Sukhanov et al., 2004), SD (Pardon et al., 2002), Wistar (Braw et al., 2006; Malkesman et al., 2005), Fischer 344 (Baum et al., 2006) and Lewis rats (Pardon et al., 2002). WKY also spent less time in the open-field than SHR (Sukhanov et al., 2004). With their home cage attached to an open-field, WKY showed decreased exploration when compared with SHR (Wultz et al., 1990). WKY showed less activity in the exploration of a novel hole-board paradigm when compared with SHR, Lewis and Fischer 344 rats (Marti and Armario, 1996). These studies show that WKY have reduced locomotor behaviours and reduced exploration, when compared to several rat strains.

A task in which animals were required to cross an open-field to receive a reward, WKY performed poorly and did not acquire the task when compared to SHR (Rogers et al., 1988). WKY required a longer period of training in the acquisition of an operant food reinforcement task and the reversal thereof than SHR (Widy-Tyszkiewicz et al., 1993). The WKY performed poorly in acquisition of the spatial location of an underwater platform in the Morris water maze when compared with SHR (Widy-Tyszkiewicz et al., 1993). However in an additional study WKY's acquisition of a spatial food reinforcement task in a T-maze was equal to SHR (Łukaszewska and Niewiadomska, 1995). WKY were found to not explore the full length of a water radial arm maze to find an underwater platform, however, with successive exposures the WKY acquisition and performance of task improved (Clements and Wainwright, 2006). WKY

showed decreased entries into the open arms of the elevated-plus maze in comparison to Brown Norwegian (Ramos et al., 1997), Wistar Furth (Ramos et al., 1997), Wistar (Braw et al., 2006), SHR (Ferguson and Gray, 2005) and Fischer 344 rats (Ramos et al., 1997), however, they made a similar number of entries as the Lewis strain (Ramos et al., 1997). Freezing behaviour of WKY was greater than Wistar in the open-field (Braw et al., 2006; Malkesman et al., 2005), and the elevated-plus maze (Braw et al., 2006), and in the conditioned place preference apparatus, the reward being social play (Malkesman et al., 2005). WKY showed greater time immobile and less time struggling in the forced swim test in comparison with SD (Tejani-Butt et al., 2003), Wistar (Tejani-Butt et al., 2003), SHR (Lahmame et al., 1997; Armario et al., 1995; Marti and Armario, 1996), Lewis (Lahmame et al., 1997; Armario et al., 1995; Marti and Armario, 1996) and Fischer 344 rats (Lahmame et al., 1997; Armario et al., 1995; Marti and Armario, 1996). Together these studies suggest that the WKY have increased levels of anxiety and hypoactivity when compared with several strain of rat.

These studies support the suggestion that WKY may serve as a model for anxiety-related disorders. As WKY share several characteristics of individuals with anxiety and with disorders related to anxiety including: increased anxiety, hypoactivity, decreased exploration, decreased attentional performance, and increased immobility. WKY responded to administration of a NE and dopamine reuptake blocker (nomifensine) by increasing their activity and time spent struggling in the forced swim test (Tejani-Butt et al., 2003), while SD and Wistar rats behaviour in the forced swim test was not altered. WKY, SD and Lewis rats showed increased release of adrenocorticoid hormone in response to immobilization stress, however WKY adrenocorticoid

hormone levels persisted for a longer period of time, taking two hours instead of one hour before returning to baseline values (Pardon et al., 2002). Administration of an α_2 -agonist (guanfacine) did not affect WKY performance in a visuospatial divided attention task (Jentsch, 2005), however it decreased performance of WKY in an operant food reward task (guanfacine, 0.6 mg/kg , Sagvolden, 2006). These studies indicate differences in the LC-NE system as manipulations of the LC-NE system led to differential effects behaviourally and neurochemically in the WKY when compared with several strains of rat.

From these studies it is suggested that anxious 'healthy' individuals, individuals with anxiety related disorders and WKY have dysfunction of the LC-NE system. We suggest overlay of these findings onto the Yerkes and Dodson (1908) relationship between arousal levels and performance (Yerkes and Dodson, 1908). This leads us to suggest that these 'hyperaroused' individuals would appear off centre to the right, as their arousal is excessive and their performance is poor (figure 1.5).

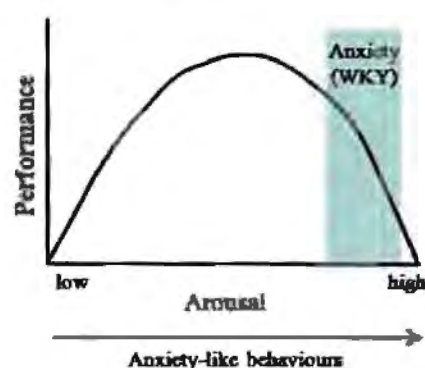


Figure 1.5 Yerkes & Dodson (1908) relationship between arousal level and performance, with the proposed window of 'hyperarousal' suggesting reduced levels of performance as seen in 'anxious' individuals and WKY due to dysfunction of the LC-NE system.

1.5 Nicotine and its effects on the LC-NE system

Attentional deficits have been suggested to lead to the development of nicotine use, as nicotine may serve as a form of self-medication (Newhouse et al., 2004; Rezvani and Levin, 2001), often starting in adolescence (Breslau et al., 2004). Nicotine use in adolescents has been associated with poor family relationships (Breslau et al., 2004). Several psychiatric disorders have been associated with nicotine use including: ADHD, depression, anxiety-related disorders (including obsessive compulsive disorder), schizophrenia, and other substance abuse disorders (Newhouse et al., 2004; Spinella, 2005). Nicotine administration improved performance of 'naïve' individuals on a five choice serial reaction time task as response times were reduced and the number of errors increased (Knott et al., 1999), in addition the variability in response times was reduced (Levin et al., 1996). Administration of nicotine to nicotine-deprived smokers improved their performance by reducing response times and increasing cortical arousal and P300 amplitudes (Knott et al., 1999; 2005). Administration of nicotine to individuals with ADHD has been shown to enhance attentional performance, by reducing their response times and their variability (Levin et al., 1996; Conners et al., 1996). In individuals with obsessive-compulsive disorder administration of nicotine has been shown to reduce anxiety related compulsive behaviors (Lundberg et al., 2004). However an additional study showed that nicotine dependent obsessive-compulsives displayed higher degrees of compulsive behaviors (Spinella, 2005).

Bath application of nicotine to prefrontal cortical slices induced release of both NE and dopamine. This release was achieved through the activation of nicotinic acetylcholine receptors

(Rao et al., 2003). Pre-incubation of brain slices with [³H]NE, followed by exposure to a nicotinic agonist (nicotine or 1,1-dimethyl-4-phenylpiperazine) led to increased release of [³H]NE in several brain regions including the hippocampus and sections of the spinal cord (Lambe et al., 2003). Inhibition of NE release was achieved by application of nicotinic antagonists (mecamylamine, hexamethonium, d-tubocurare and dihydro-β-erythroidine) (Anderson et al., 2000). In addition bath application of nicotine has been shown to increase the excitatory postsynaptic currents in layer V pyramidal neurons, and nicotine was shown to facilitate this release of NE by increasing the release of glutamate (Lambe et al., 2003). Intravenous injection of nicotine (0.35 mg/kg) has been shown to increase cerebral blood volume in the prefrontal cortex, cingulate cortex, amygdala, ventro-caudal hippocampal structures and the anterior portion of the nucleus accumbens (Gozzi et al., 2006). Pretreatment with an injection of a nicotinic antagonist (mecamylamine) cancelled these regional blood volume changes (Gozzi et al., 2006). Subcutaneous injection of nicotine (0.8 mg/kg) has been shown to increase the accumulation of DOPA in NE cell bodies and noradrenergic terminals, which can be used for the synthesis of NE (Mitchell et al., 1990). Intraventricular injection of nicotine (0.065-0.135 mg/kg) has been shown to increase NE release in both the hippocampus and the amygdala in a dose dependent manner (Fu et al., 1998). Direct infusion of nicotine into the LC has been shown to not lead to increased release of NE in the medial prefrontal cortex, and antagonism of nicotinic receptors did not affect the release of NE in the medial prefrontal cortex (Kawahara et al., 1999). Chronic treatment with nicotine has been shown to improve performance on the five choice serial reaction time task (Hahn and Stoleran, 2005). If animals were not able to acquire the five choice serial reaction time task criterion of performance, a nicotinic agonist (nicotine) and

nicotinic $\alpha_4\beta_2$ receptor agonist (SIB 1765F) was able to facilitate the acquisition of the task as number of correct responses increased and response times were reduced, however treatment increased the number of premature responses (Grottick et al., 2000). These studies accentuate the interactions between nicotinic receptor activities and the LC-NE system, and relate this interaction to attentional performance and its possible involvement in regulation of arousal levels through facilitation of the LC-NE system.

In a conditioned place preference task Lewis rats were conditioned to nicotine reward more readily than Fischer 344 rats, suggesting that there is a genetic component to nicotine addiction (Philibin et al., 2005). Chronic nicotine has been shown to accelerate hypertension in adult SHR while having no effect on WKY (Ferrari and Fior-Chadi, 2007). The WKY showed increased expression of nicotinic receptors in frontal cortex and thalamic areas when compared to SHR, however expression was similar to the Wistar strain (Gattu et al., 1997). At the same time chronic nicotine has been shown to increase the expression of nicotinic receptors in WKY for cerebral cortex lamina I, lamina II, prefrontal and cingulate cortex, with no effect in SHR (Hohnadel et al., 2005). With a single pre-treatment of nicotine during adolescence, fear-conditioning was enhanced in adulthood and time spent in the open-field was reduced in Long Evans hooded rat (Smith et al., 2006). Adolescent SD that were pre-treated with nicotine were less sensitive to the effects of acute nicotine treatment in adulthood, as the pre-treated rats did not respond with increased locomotor behaviour as the 'naive' rats did (Faraday et al., 2003). Animals that were chronically treated with nicotine and trained on the five choice serial reaction time task, responded differently to acute dose of nicotine (Grottick et al., 2000). When

chronically treated animals were given a 0.2 mg/kg dose of nicotine subcutaneously the animals decreased their response inhibition and decreased their response latency, and when animals were given a 0.4 mg/kg dose of nicotine subcutaneously the animals increased their number of correct responses (Grottick et al., 2000). These studies suggest the effect of nicotine differs between strains of rat, and needs further elucidation.

Acute doses of nicotine have been shown to increase locomotor activity in adolescent or adult rats (0.1-1.0 mg/kg Long-Evans hooded rat) (Hetzler and Thienpeng, 2004). A specific nicotinic β_4 subunit agonist has been shown to increase locomotor activity, however chronic administration did not show this increase in locomotor activity, additionally the acute dose was not attenuated by administration of nicotinic receptor antagonists (mecamylamine or dihydro- β -erythroidine (Grottick et al., 2001).

The use and effects of nicotine are suggested to decrease attentional deficits and decrease anxiety. How nicotine accomplishes this is presently unclear, however the above studies suggest that nicotine leads to depolarization of glutamatergic terminals or glia. The released glutamate then leads to the release of NE from noradrenergic terminals. Between strains of rat and age of the rats nicotine leads to different effects in the release of NE and behavior. Early use of nicotine in adolescents both human and rat, led to a predisposition to nicotine addiction and the beneficial effects of nicotine use in adulthood suggesting a developmental link. The relationship between nicotine use and the effects on the LC-NE system in arousal and attention is presently poorly understood.

1.6 Nature versus Nurture and the LC-NE system

Environment is known to play a key role in the development of an individual. The question still remains open to what extent. The LC-NE system plays a key role in stimulating the paraventricular nucleus, leading to the release of CRF (Leibowitz et al., 1989). CRF is the critical signal in the HPA-axis mediated 'stress response' and in parallel activates the LC (Valentino and van Bockstaele, 2008; Reyes et al., 2006). In the 'stress response' CRF activates the pituitary gland to release adrenocorticoid hormone (Ganong, 2001). Adrenocorticoid hormone acts on the adrenal cortex to release glucocorticoids. The primary glucocorticoid in humans is cortisol and is analogous to corticosterone in the rats, and is generally considered the main 'stress' hormone.

Childhood trauma has been shown to increase the risk and development of depression, anxiety disorders including post-traumatic stress disorder and substance abuse (de Wilde et al., 1992; Dube et al., 2001; Johnson et al., 2002; Kendler et al., 1995, Kendall-Tackett, 2000). In addition the extent of the trauma has been positively correlated to the development of post-traumatic stress disorder and depression (Suliman et al., 2008). The effect of childhood trauma has been shown to increase HPA-axis activity (Heim and Nemeroff, 2001; Kendall-Tackett, 2000). Childhood sexual abuse is one form of childhood trauma that has been researched quite intensively. Individuals from sexual abusive families have been shown to have a greater risk of development of mental illness in adulthood (Brown and Anderson, 1993), additionally sexual abuse has been associated with attentional problems (Kaplow et al., 2008). Interestingly children

are often misdiagnosed with ADHD in cases of sexual abuse, as their behaviour fulfill criteria of the DSM-IV, presenting with deficits in attention, restlessness and impulsivity, when they have some form of post traumatic stress disorder (Weinstein and Staffelbach, 1998, Weinstein et al., 2000). Resilience to childhood trauma has been found, suggesting that there was an interaction between environment and genetics which may suggest a predisposition to the development of mental disease within a human population (Fincham et al., 2008). These studies additionally accentuate the distinction between ADHD and anxiety-related disorders and their levels of arousal.

Manipulation of the early postnatal period in rats can limit or enhance the HPA-axis environmental adaptive mechanisms as seen in several behaviours (Colorado et al., 2006), particularly manipulations performed in the first two weeks of birth (McCarty and Fields-Okotcha, 1994). This was shown by SHR pups cross-fostered onto WKY dams that resulted in the SHR pups not developing adult hypertension and/or showing reduced mean arterial pressure when compared to SHR pups reared by their biological dams (McCarty and Fields-Okotcha, 1994; McCarty and Lee, 1995), and a reduction in SHR adult hypertension was evidenced in SHR pups reared by SD dams (Cierpial and McCarty, 1991). In addition early handling of pups has been shown to decrease CRF response to stressors (Fenoglio et al., 2006; Zhang et al., 2006). The CRF systems are suggested to be a critical target for the effects of variation in maternal care (Francis and Meaney, 1999). Dams which exhibit high levels of maternal licking/grooming and arched back nursing (in which dam stands over her pups and arches her back to facilitate suckling) was associated with reduced CRF mRNA and enhanced glucocorticoid negative feedback leading to a reduction in the stress response (Champagne and Meaney, 2001). In

addition reduced expression of CRF mRNA and low maternal licking/grooming led to increased levels of adrenocorticoid hormone, increased levels of corticosterone, increased CRF expression in the LC and reduced exploration of the open-field (Champagne et al., 2003). In studies of maternal separation, pups maternally separated showed increased impulsiveness and hyperactivity within a novel environment in adulthood (Colorado et al., 2006). Maternal separation has been shown to increase CRF expression in the vicinity of the LC and raphe nuclei and increase α_2 -receptor binding (Caldji et al., 1998). In addition the effects of manipulating the maternal environments have been shown to be counteracted by early handling of pups by researchers (Colorado et al., 2006) or enriching pups home cage environments, by placement of novel objects or tunnel systems in their cages (Francis et al., 2002). Pups that were handled during the period of maternal rearing have been shown to decrease their startle responsivity and increase their exploration of a novel open-field (Caldji et al., 2000). Interestingly by depriving SHR pups of environmental stimuli by social isolation and/or dark adaptation their developmental hypertension was retarded (Hallback, 1975). Additionally weaning rat pups post the normal suckling period (PND 21) increased exploratory behaviours and decreased anxious behaviours (Ferdman et al., 2007).

Childhood, or early, manipulations of the environment lead to several changes in the development of an appropriately responding HPA-axis. Importantly these changes occur differently in different strains of rat and within the human population. The interactions between the HPA-axis and the LC-NE system during early development and adulthood is an interestingly field of study, that needs further study to reverse the effects of early life trauma.

HYPOTHESIS

We propose the following hypothesis with respect to the functioning of the LC-NE system:

Individuals with ADHD and SHR at rest have low tonic firing of the LC ($< 1-3$ Hz). This leads to increased release of NE at the LC-NE terminals, as the terminals are not maintained appropriately. Feedback to the LC through cortical glutamatergic neurons (Arnsten and Goldman-Rakic, 1984; Jodo et al., 1998; Jodo and Aston-Jones, 1997) and axon collaterals of the LC (Aghajanian et al., 1977; Harley, 2007; Swanson, 1976) that would normally maintain LC tonic activity is lacking. These factor together and result in decreased levels of NE at the LC-NE terminals. We suggest that this results in individuals with ADHD and SHR to be ‘hypoaroused’. Physiologically these organisms are ‘hypoaroused’. Behaviourally this ‘hypoarousal’ is evidenced in individuals with ADHD through their hyperactivity and distractibility. And behaviourally this ‘hypoarousal’ is evidenced in SHR through their hyper-locomotor activities and increased exploratory behaviours (figure 1.6).

When individuals with ADHD or SHR are required to perform an attentional task their LC fires phasically, however the basal levels of NE are reduced. This leads to a reduction of the facilitatory effect afforded in ‘healthy’ individuals or ‘control’ animals by NE enhancement of the signal-to-noise ratio. Behaviourally seen in individuals with ADHD and SHR by their poor attentional performance (including latency and accuracy), and is accentuated by their impulsivity (figure 1.6). Treatment of the noradrenergic dysfunction in ADHD supports this suggestion as NE concentration within the synaptic cleft is enhanced by: (1) low dose stimulant medication

that serve to increase synaptic levels of NE and dopamine (Chamberlain et al., 2007), (2) selective postsynaptic agonists of α_2 -autoreceptors (Berridge and Waterhouse, 2003), and (3) selective NE reuptake inhibitors (Berridge and Waterhouse, 2003).

Similarly, low levels of tonic LC firing may reduce activation of the paraventricular nucleus and release of CRF, which in turn activates the LC and elicits the 'stress response' (Leibowitz et al., 1989; Valentino and van Bockstaele, 2008; Reyes et al., 2006). We suggest that the reduced self-reinforcing activity of the LC results in attenuation of anxiety-like behaviours seen in individuals with ADHD and the SHR. Behaviourally evidenced in individuals with ADHD through their lack of self control. And behaviourally evidenced in SHR by their reduced anxiety-like behaviours and their resilience in stressor tests, in which they perform well.

Individuals with anxiety-related disorders and WKY at rest have high tonic firing levels of the LC ($> 1-3$ Hz). This leads to increased release of NE at the LC-NE nerve terminals, as the terminals are not regulated appropriately. Feedback to the LC through cortical glutamatergic neurons and axon collaterals of the LC exert excessive reinforcement of the LC tonic activity. These factor together and result in increased levels of NE at the LC-NE terminals. We suggest that this results in individuals with anxiety-related disorders and WKY to be 'hyperaroused'. Physiologically these organisms are 'hyperaroused'. Behaviourally this 'hyperarousal' is evidenced in individuals with anxiety-related disorders through their avoidance behaviours and mental preoccupation. And behaviourally this 'hyperarousal' is evidenced in WKY through their hypo-activity and avoidance behaviours (figure 1.6).

When individuals with anxiety-related disorders and WKY are required to perform an attentional task their LC fires phasically, however their basal levels of NE are high. This leads to a reduction in the facilitatory effect afforded by NE enhancement of the signal-to-noise ratio. Behaviourally seen in individuals with anxiety-related disorders and WKY with their poor attentional performance (including latency and accuracy) this is accentuated by their avoidance behaviours (figure 1.6). Treatment of the noradrenergic dysfunction in anxiety and depression supports this suggestion as NE concentration within the synaptic cleft is reduced by: (1) reduction of vesicle concentrations of NE through reuptake transporter blockade (Nemeroff, 2007), (2) reduced synaptic concentrations of glutamate, that serve to reduce glutamate potentiation of NE function by elevating NE concentration within the synaptic cleft (Palucha and Pilc, 2007).

In addition high levels of tonic LC firing would increase the activation of the paraventricular nucleus and release of CRF, which activates the LC and elicits the 'stress response'. We suggest that the increased self-reinforcement of the LC results in the enhancement and prolongation of the 'stress response' which results in heightened anxiety-like behaviours seen in individuals with anxiety-related disorders and WKY. This is behaviourally evidenced in individuals with anxiety-related disorders through their increased avoidance behaviours and in WKY through their enhanced anxiety-like behaviours and in stressor tests where these animals are susceptible to stressors and their performance is poor.

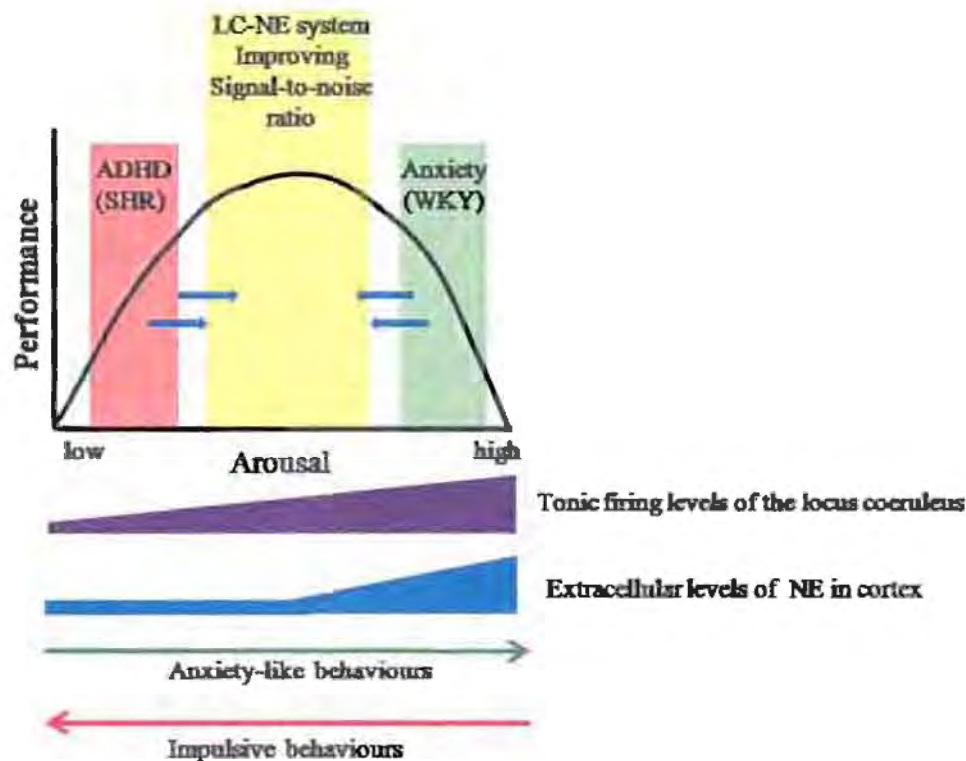


Figure 1.6 Yerkes & Dodson (1908) relationship between arousal level and performance, with overlay of several suggestions to the LC-NE system's role in arousal and attentional performance. Tonic firing of the LC-NE system needs to be held within a range that facilitates phasic burst activity, thereby enhancing the signal-to-noise ratio. 'Hypoaroused' individuals and SHR present with poor attention and decreased cortical and peripheral arousal, and display impulsiveness. 'Hyperaroused' individuals and WKY present with poor attention and increased cortical and peripheral arousal, and display anxiety-like behaviours. We suggest that 'hypoaroused' individuals and SHR require enhancement of their arousal levels in order to perform optimally during attentional tasks and we suggest that 'hyperaroused' individuals and WKY require a reduction in their arousal levels in order to perform optimally during attentional tasks.

A vast number of studies have been carried out to determine the role of the LC-NE system in arousal and attention yielding many amazing functional roles, as reviewed. For the current study we aim to merge both human and rat findings in a series of questions pertaining to the LC-NE system and its role in arousal and attention. These will be performed in an attempt to create a holistic view of the individual and LC-NE system function using the Yerkes and Dodson (1908) relationship between arousal levels and performance as a blueprint.

AIMS AND OBJECTIVES

PART A

- Individuals that are ‘hypoaroused’ or ‘hyperaroused’ express that they require increased recruitment of mental resources, here we examine the relationship between perceived mental effort reported in ‘healthy’ participants during the completion of three attentional tasks while continuous physiological recordings are taken. Hypothesizing that perceived mental effort during attention is associated with physiological parameters of arousal and with the different forms of attention. The reported perceived mental effort was captured with the use of a visual analogue scale. The attentional tasks include: a continuous performance task, a Go/No-Go task, and a cued target detection task. The physiological parameters included: skin conductance responses and durations, heart rate, heart rate variability, and relative EEG frequency power (θ , α , β , θ/β) of frontal and parietal electrodes.
- Individuals that are ‘hypoaroused’ such as seen in majority of individuals with ADHD high levels of impulsivity have been reported (Bresnahan et al., 1999; Mann et al., 1992), here we examine the relationship between reported behavioral impulsivity in ‘healthy’ participants to response times and errors made during three attentional tasks. We then examine whether response times during an attentional task that required response inhibition were related to physiological parameters of arousal measured. Hypothesizing that shortened response times accurately reflect impulsivity in a ‘healthy’ cohort of participants’ and that this is reflected by decreased physiological arousal and may be related to the reduced tonic firing of the LC-NE system seen in disorders of

‘hypoarousal’. Reported behavioral impulsivity was reported with the use of Dickman’s impulsivity inventory. The three attentional tasks include: a continuous performance task, a Go/No-Go task and a cued target detection task. The attentional task that required response inhibition was the Go/No-Go task. The physiological parameters included: skin conductance responses and durations, relative EEG frequency power (θ , α , β , θ/β) of frontal and parietal electrodes, P300 amplitude and latencies from parietal electrodes.

- Individuals that are ‘hyperaroused’ such as in the anxiety disorders report high levels of anxiety (Sue et al., 2000) here we examine the relationship between reported behavioral anxieties in ‘healthy’ participants to physiological parameters of arousal during a sustained attention task were related to physiological parameters of arousal. Hypothesizing that increased behavioural anxiety accurately reflects “hyperarousal” and decreased information processing due to increased tonic activity of the LC-NE system in a ‘healthy’ cohort of participants’. In addition we hypothesize that nicotine use in anxious ‘healthy’ participants is a form of self-medication to improve information processing by impacting on the LC-NE systems tonic firing rate. Reported behavioral anxiety was collected with the use of Spielberger’s trait anxiety inventory and Kessler’s distress scale. The attentional task was a continuous performance task. The physiological parameters included: skin conductance responses and duration, heart rate and heart rate variability, relative EEG frequency power (θ , α , β , θ/β) of frontal and parietal electrodes, P300 amplitude and latencies from parietal electrodes.
- Individuals that have experienced traumatic childhoods have been shown to have deficits in attentional performance and at times being diagnosed with ADHD (hypoarousal) or

even with post traumatic stress disorder (hyperarousal) (Weinstein and Staffelbach, 1998, Weinstein et al., 2000). Here we examine the relationship between childhood trauma and physiological parameters of arousal during the completion of three attentional tasks. Hypothesizing that 'healthy' participants' that experienced traumatic events during their childhood express changes in their physiological arousal and these may be evident during the completion of attentional tasks. Reported childhood traumatic experiences were collected with the use of the Childhood trauma questionnaire. The attentional tasks included: a continuous performance task, a Go/No-Go task, and a cued target detection task. The physiological parameters included: skin conductance responses and durations, heart rate, heart rate variability, and relative EEG frequency power (θ , α , β , θ/β) of frontal and parietal electrodes.

PART B

- Prefrontal cortical slices of SHR have previously been shown to release greater amounts of [^3H]NE when their terminals were stimulated with glutamate, when compared with WKY (normotensive control, Russell and Wiggins, 2000), here we examine whether this difference extends to the hippocampus. We also examine whether this it is concentration dependent and whether release of [^3H]NE is effected by repeated stimulations with glutamate. Hypothesizing that there is a dysregulation between glutamatergic and noradrenergic terminals that leads to a differential release of [^3H]NE when stimulated with glutamate. This was accomplished by incubation of hippocampal brain slices with radioactively labeled [^3H]NE. These hippocampal brain slices were stimulated with different glutamate concentrations used 10mM, 1mM, 100 μM , and 10 μM . The

hippocampal brain slices were exposed three times to their particular glutamate concentration.

- Due to the present controversy related to WKY being a 'normotensive' control of SHR and the possibility of WKY as a model of anxiety-like behaviors (Pardon et al., 2002) we examined the effect of glutamate stimulated release of [^{3} H]NE from several strain of rat. Hypothesizing that SHR and WKY release differential amounts of [^{3} H]NE when compared to different strain of rat. This was accomplished by incubation of hippocampal brain slices with radioactively labeled [^{3} H]NE. The rat strains used included SHR, WKY, SD, Wistar, and Long-Evans hooded rat. Using a glutamate concentration of 1mM to stimulate the release of the loaded [^{3} H]NE.
- SHR have previously been suggested to show dysfunction in glutamate receptor function (Kaehler et al., 2004) here we examine differences between SHR and WKY hippocampal brain slices with antagonism of ionotropic glutamate receptors. We also examine whether release of [^{3} H]NE is effected by repeated stimulations with glutamate. Hypothesizing that there are differential effects in glutamate stimulated release with antagonism of ionotropic glutamatergic receptors. This was accomplished by incubation of hippocampal brain slices with radioactively labeled [^{3} H]NE and incubation of hippocampal brain slices with AMPA receptor antagonist (CNQX) and with NMDA receptor antagonist (MK801). Using a glutamate concentration of 1mM to stimulate the release of the loaded [^{3} H]NE.

- The LC-NE system is the sole innervation of noradrenergic nerve terminals to several brain areas including the prefrontal cortex, hippocampus and the cerebellum (Foote et al., 1983; Jentsch, 2005; Bunsey and Strupp, 1995). Here we examine whether the differential release between SHR and WKY extends to an additional brain area, the cerebellum. Hypothesizing that all LC-NE terminals lead to similar patterns of release in each strain of rat regardless of brain area. This was accomplished by incubation of lateral cerebellar slices and cerebellar vermis brain slices of SHR and WKY with radioactively labeled [^3H]NE. Using a glutamate concentration of 1mM to stimulate the release of the loaded [^3H]NE.
- Environmental manipulation through cross-fostering of SHR onto WKY 'normotensive' controls has been shown to prevent hypertension in adulthood (Cierpial and McCarty, 1991) and may express changes in the development of the LC-NE system. Here we examine whether cross-fostering of SHR and WKY effect pups behavior and/or the neurochemistry of noradrenergic terminals. This was accomplished by cross-fostering of litters of pups borne to SHR, WKY and SD dams on postnatal day 2, to determine the behavioral effects in the open-field and elevated-plus maze and glutamate stimulated release of [^3H]NE from prefrontal cortical and hippocampal brain slices in adolescents (P29-P35). Using a glutamate concentration of 250 μM to stimulate the release of the loaded [^3H]NE.
- Environmental manipulation through maternal separation of SD has been shown to lead to several neuro-endocrine changes (Caldji et al., 1998), here we examine whether mild maternal separation is sufficient to change the synergistic activities between glutamate

and LC-NE nerve terminals. This was accomplished by separating rat pups from their dams between postnatal day 2 through to postnatal day 14 for 3 hrs. SD adolescent pups prefrontal cortex and hippocampal brain slices were incubated with radioactively labeled [^3H]NE, using a glutamate concentration of 1mM to stimulate the release of the loaded [^3H]NE.

- Nicotine has recently been suggested as a form of self-medication and serves to reduce anxiety-related behaviors and improve attentional performance (Newhouse et al., 2004; Rezvani and Levin, 2001), here we explore the effects of an acute dose of nicotine via voluntary oral administration in SHR and WKY, and previous studies have shown robust changes in behavior of SD rats (Grottick et al., 2000) therefore we included SD as a reference strain. Here we examine the acute effects of nicotine effect adolescent pups behavior and/or the neurochemistry of noradrenergic terminals. Behaviour was examined in the open-field and the elevated-plus maze and glutamate stimulated release of [^3H]NE from prefrontal cortical and hippocampal brain slices in adolescents. Using a glutamate concentration of 250 μM to stimulate the release of the loaded [^3H]NE.

PART A

A1. Introduction

Norepinephrine has been implicated in the control of arousal and attention (Jentsch, 2005; Oades et al., 2005; Beane and Marrocco, 2004). Arousal and attentional networks receive neural innervations from the LC-NE system (Aston-Jones et al., 1999; Southwick et al., 1999; Bunsey and Strupp, 1995). In order to maintain attention, an individual is required to discard irrelevant information thereby selecting only that which is relevant. This requires specific levels of arousal (Sarter et al., 2001) and involves several neurotransmitter systems (Berger and Posner, 2000). Deficits in attention have been reported to affect more than 6% of the human population (Beane and Marrocco, 2004). In addition deficits or dysfunction in attention has been evidenced in several psychiatric disorders including: ADHD (Clark et al., 2002, Harmer et al., 2002; El-Badri et al., 2001) and several anxiety related disorders (Avila et al., 2002; Nelson et al, 1993; Sue et al., 2000). Phasic firing of the LC increases cortical activity, as the release of NE increases the ‘signal’ (salient information for maintenance of attention) and decreases the ‘noise’ (background activities not requiring attention) by release of NE in thalamocortical projections (Berridge and Foote, 1994; Waterhouse and Woodward, 1980). The relationship between attentional processes of the brain and peripheral responses are beginning to be re-investigated, changing from the dualistic body/mind perspective to an integrated body-mind approach (Damasio, 2000). Several studies have found associations between task performance and activity of the autonomic nervous system (Polich and Kok, 1995). However, many of these studies incorporated tasks that elicited

some form of heightened stress, suggesting that their results are more closely related with the interaction of the HPA-axis and its impact on attention and arousal. Therefore in the present study non-stressful paradigms were sought to measure the relationship between LC-NE system activation, and its regulation of arousal and attention.

The current study was performed by testing ‘healthy’ participants with a battery of attentional tasks to assess their performance while measures of physiological arousal were recorded. On the day of testing, participant characteristics were gathered by: completion of several subjective questionnaires, collection and archiving of blood samples for future genetic studies depending on the outcomes of the present study, and salivary cortisol measures to assess the activity of the HPA-axis on the day of testing. The cohort that participated in the present study received no material benefit and were informed that the current study was only for research purposes. During the different stages of the testing session, measures of physiological arousal were continuously recorded and included: phasic skin conductance responses; electrocardiogram (ECG); and EEG. Two resting states were recorded and three attentional tasks were performed by the participants.

The three attentional tasks assessed covert reflexive attention and/or sustained attention. The tasks were a continuous performance task, a Go/No-Go task and a cued target detection task. The tasks and their instructions were presented visually on a computer screen and the instructions to each of the tasks were presented prior to the onset of the specific task. Both the continuous performance task and the Go/No-Go task used central stimulus presentation, cues and stimuli were consonants in the English alphabet. The continuous performance task and the

Go/No-Go task required endogenous orienting and served to reinforce the participant's placement of their attention. The cued target detection task required the participant to maintain central fixation on a circle, which was continuously displayed, while cues and stimuli occurred in the left or right peripheral field causing the participants to respond with peripheral processing of the cues and stimuli. The cued target detection task involved both endogenous and exogenous orienting (Hahn et al., 2006). Exogenous orienting involved peripherally-located cues or stimuli that drew participant's attention due to their salience (Kincade et al., 2005). Both sustained and reflexive attention have been found to activate the frontal and parietal cortices when they involve a central fixation point (Kincade et al., 2005; Coull et al., 1996). Additional conditions within the Go/No-Go task allow for assessment of: (1) response inhibition, in which participants are required to inhibit their responses and (2) delayed presentation of stimuli, in which participants are required to wait for a longer period for the presentation of the stimuli.

Mental effort is utilized when a person completes a task voluntarily and requires mental resources to complete the task (Reber, 1995). Mental effort has been correlated to physiological measures within cognitive tasks. These effects of increased mental effort include: increased perceived task difficulty (Smit et al., 2004a,b); increased cortisol (Peters et al., 1998); increased heart rate (Peters et al., 1998); increased relative theta activity (Smit et al., 2005); and reduced alpha activity (Fink et al., 2005; Keil et al., 2006). These studies have either compared resting states to physiological changes during task completion or compared physiological differences during tasks which required higher mental abilities than are asked by the simple attentional tasks employed in the current study. The aims of the present question were to ascertain (1) whether

reported mental effort changed during the three attentional tasks, (2) if there were changes in reported mental effort, whether these changes were accompanied by corresponding changes in physiological arousal levels, (3) whether these changes were related to the attentional task, (4) and whether these physiological changes in arousal during the completion of attentional tasks could be related to the functioning of the LC-NE system.

Hypoarousal is seen as under arousal both in the peripheral and central nervous system and is strongly suggested to be a factor in attentional disorders, such as ADHD (Clarke et al., 2001, 2002, 2007). Individuals with ADHD display several deficits in attentional performance, including the inability to sustain attention and inhibit responses. The inability to withhold a response has been strongly related to behavioural impulsiveness (Lubar, 1991; Mann et al., 1992). 'Healthy' individuals who present with high levels of impulsiveness display deficits in attentional performance as they respond reflexively, displayed by increased errors and shorter response times (Svebek et al., 1987; Aron and Poldrack, 2005; Kirkeby and Robinson, 2005; Dimoska and Johnstone, 2007). The aims of the present question were to determine whether any of the three attentional tasks teased out impulsivity within a 'healthy' population. If such a task was found, apparent physiological associations would be searched for and the relationship between impulsivity within a 'healthy' population and the functioning of the LC-NE system would be determined. The hypothesis that 'hypoarousal' occurs in impulsive individuals would be tested.

A2. Methodology

A2.1 Participants

This study was approved by the Human Ethics Committee of the University of Cape Town. The study was conducted in accordance with the Declaration of Helsinki (2000). Participants were required to be of good health, have matriculated, English was required to be their 1st language, and they needed to be between the ages of 21 and 45 yrs. In addition participants were required to not have any current form or history of brain trauma, organic brain disease, epilepsy or diagnosis of any psychiatric or psychological disorder(s).

A2.2 Recruitment of participants

Flyers and emails were sent out to students and staff members of the University of Cape Town (appendix A1). Participants were recruited in group sessions on the premises of the university. Details of the study were explained to each of the prospective participants during the recruitment process. Participation in the study held no incentive and was voluntary. Exclusion criteria were checked in a second form by completion of a subjective questionnaire. Each of the participants received an information sheet with an explanation of the testing procedure and the measures that would be recorded (appendix A2). If the participant understood and was prepared to participate in the research study, a consent form was signed by both the participant and researcher prior to commencement of any of the experimental procedures (appendix A3). The participants were

Hyperarousal is seen as an over arousal of both the peripheral and the central nervous systems and has been strongly associated with anxiety related disorders (Sue et al., 2000). Within 'healthy' individuals increased anxiety results in poor sustained attention as the individual is distracted with endogenous or exogenous stimuli (Avila et al., 2002; Avila et al., 2002). The aims of the present question were to ascertain whether subjective anxiety levels within a 'healthy' population had associations with physiological arousal and whether these associations differed between the three attentional tasks, and how these can be related to the functioning of the LC-NE system. In addition do 'hyperaroused' individuals resort to self-medication in the form of nicotine use in an attempt to correct their LC-NE systems activity, what physiological associations are there if any.

Childhood trauma has been shown to increase the development of anxiety related disorders (de Wilde et al., 1992; Dube et al., 2001; Johnson et al., 2002; Kendler et al., 1995; Kendall-Tackett, 2000). The aims of the present question were to ascertain the effects of childhood trauma within a 'healthy' population, with respect to physiological arousal during the performance of three attentional tasks. In addition if there are changes in arousal during attentional performance can these be related to the functioning of the LC-NE system.

informed that they could resign from the study without any need for explanation and without prejudice. They were also made aware that their removal from the study would not affect their current or future health care. Strict confidentiality was maintained with respect to all aspects of their participation. To ensure confidentiality all data collected from participants was assigned a number, selected by the relevant participant on the day of enrolment into the research study.

The consent form included the risk of bruising and/or bleeding associated with venepuncture, which was necessary for blood withdrawal. Participants were informed that the risk in blood letting of this volume is minor and were informed that the blood samples would only be used for research purposes. They were also informed that any research conducted on their blood would be performed solely by the University of Cape Town. Two 5 ml samples of blood were then drawn.

The participants were made aware that the psychophysiological measures were non-intrusive, painless and harmless. The only discomfort experienced would be (1) the cleaning of skin areas where electrodes would be placed for the recordings, (2) the cleaning of scalp areas under the electrode cap for ten recording sites and the injection of gel into the hollow of the EEG electrodes to enhance conductance of brain wave activity. The participants were informed that there would be no report back or follow-up with participation in the study and that there were no benefits for participation in the study. The participants were also assured of their anonymity and that the results of the study would be based on their participant number only.

A2.3 The experience of the participant during the present study

As participants entered the group recruitment sessions, they were offered further clarification regarding the study if they so wished. Once participants were satisfied, a consent form was filled in and signed by both the participant and researcher. Each participant chose a number from a sheet of paper with numbers between 1 and 100. The number chosen was carried by the participant through the entire study to ensure anonymity. Blood samples (two 5ml samples) were drawn by either a qualified doctor or a qualified phlebotomist. The psychophysiological testing session for each participant was scheduled on the day of blood drawing or else email was used to set a time at their convenience. Participants did not have their physiological recordings done on the same day of blood drawing. Participants were required to choose from specific (1 hr) slots for the psychophysiological testing. At the recruitment session each participant was handed their 1st batch of questionnaires (section A2.4, appendix A4), which was to be completed and handed to the researcher on the day of psychophysiological testing. Each participant was asked to refrain from caffeine (coffee, tea, and energy drinks), cigarettes, alcohol, or non-prescription drugs for 18 hrs prior to their testing session. If they felt unwell or were recovering from an illness they were asked to reschedule their psychophysiological testing appointment in order to prevent their exclusion from the study. This was due to the possibility that their immune response could affect the psychophysiological recording parameters.

On the day of psychophysiological testing participants were asked whether they had abstained from the restricted substances and whether their health was good. The researcher explained that it was possible to reschedule and that the testing could not be repeated due to the style of the experimental set-up. If this was perceived to be clearly understood by the participant, the testing session proceeded. The participant was asked for the completed batch of questionnaires, which also carried the participant's number (thereby maintaining participant's anonymity). The participant was asked to take a seat in front of the computer screen, which would run the attentional tasks. Participants sat with a relaxed posture approximately 50 cm away from the computer screen. The participant was then asked to place a cotton wool saliva collecting device (Salimetrics, Salivettes®) under their tongue obliquely or diagonally due to size and to keep it there without sucking or biting on it for at least two minutes. During this time the researcher started to wipe electrode placement areas with alcohol wipes. The electrode placement areas were: the ventral surface of the ankles; the anterior surface of the wrists; the left clavicle for the electrocardiogram (ECG); and the forehead in preparation for the prefrontal electrodes of the EEG. All of these areas were cleaned with an alcohol swab. Once the areas had dried the ECG electrode pads were placed in their respective positions and the electrodes were attached. The saliva saturated Salivette® was removed by the participant with a latex glove (a non-latex glove was used if the participant presented with a latex allergy) and deposited into the collection vial. The participant was then informed of the procedure and the function of each electrode. During this explanation the researcher continued to place the equipment in preparation for the testing battery. The electroencephalogram cap was positioned and secured under the chin and an attempt was made to minimize the discomfort to each of the participants. The areas under the electrodes

of interest were cleared of hair by light abrasion with an orange stick placed through the openings in the EEG cap. The cavity of the electrode was then filled with EEG electrode gel.



Figure A2.1 Research participant with electrocardiogram and electroencephalogram cap in place, sitting in front of the computer screen which ran the attentional tasks (consent from participant for insertion of photograph was obtained).

The final electrodes placed on the participant were for the skin conductance response.

The two transducers were placed on digits two and three of the non-dominant hand as the participant was required to use the dominant hand or the hand that usually operated the mouse to respond to attentional tasks by clicking the mouse (refer to section A2.7.2). The following ten to twenty minutes was taken to ensure conductivity and signal strength. After confirmation of the correct functioning of the apparatus, the testing session was started. The participant was told that baseline recordings were to be followed by three simple attentional tasks. The participant was asked to relax and if any noises were heard, to feel that they were expected and accept them as though they were normal. Examples of noises that might occur were given. The lights in the room were turned off to prevent visual distractions from the computerized attentional tasks. The participants were also advised that if they did not understand what a task was asking of them,

that they should ask the researcher while the instructions for the task were on the computer screen. It was suggested that, even if they understood it they may check with the researcher as the testing could not be carried out a second time. Once the participants had completed the testing battery the lights were switched back on and the electrodes were removed by the researcher. The participants then had a second saliva sample taken in the same manner as the first sample and were asked to complete the 2nd batch of questionnaires (section A2.4, appendix A4). The saliva samples were taken within the allotted time (1 hr) of the psychophysiological testing session. The researcher then offered a beverage to the participant who was thanked for his/her participation in the study.

A2.4 Questionnaires

Questionnaires were included in this study to broaden and enrich the data and to ascertain whether subjective responses to questionnaires correlated with performance in the cognitive attentional tasks and physiological recordings. The choice of questionnaires depended on their applicability and the information that they would supply to predict the psychophysiological data. The present study focused on arousal and attention and therefore many of the chosen questionnaires pertained to aspects that may influence either of these two factors. Standardized questionnaires were used and have largely been accepted by the research community and most can be found in peer reviewed publications when assessing 'healthy' individuals. The questionnaires were given in two batches to the participants, the 1st batch on the day of blood

withdrawal and the 2nd batch was given immediately after the recording session. Both questionnaires were returned completed on the day of testing.

The 1st batch of questionnaires comprised: Cloninger's tridimensional personality subscales of harm avoidance and novelty seeking behaviours; Dickmans impulsivity questionnaire including subscales of functional and dysfunctional impulsivity; Edinburgh handedness questionnaire; sensitivity to punishment and reward questionnaire; and the abbreviated Eysenck personality questionnaire. The 2nd batch of questionnaires comprised: Spielberger's trait anxiety inventory with subscales of anxiety present and anxiety absent; drug-use frequency questionnaire (adapted to include South African street drugs); Kessler psychological distress scale; subjective questionnaire (including age, gender, years of post high-school education (post-matriculation), allergies, headaches, and history of mental illness in family); childhood trauma questionnaire short form; and subjective mental effort visual analogue scales for each of the three cognitive tasks completed.

A2.5 Blood samples

Blood samples were drawn by a qualified doctor or phlebotomist. Two 5 ml blood samples (collected in glass/plastic EDTA vacutainer tubes bearing the participant number) were drawn from each participant. The EDTA and blood were gently mixed by the researcher by inverting the vacutainer tube four times. The samples were then stored at 7 degrees Celsius. Within one week of blood collection the 'buffy' layer, containing the white blood cells, of the samples was

removed for future genetic analysis. The 'buffy' layer was removed by Pasteur pipette and transferred into plastic 2 ml Eppendorf tubes. The Eppendorf tubes were centrifuged for 10 min at 3000 rpm so distinction in the layers was clearly visible: plasma, white blood cells and blood. The 'buffys' were then archived at -80°C , in the Department of Human Biology at the University of Cape Town. This archive of blood will be used by our laboratory in future studies related to the hypotheses generated from the results of the present study.

A2.6 Salivary Cortisol

Participants were asked not to eat or drink any fluids except water two hours prior to the onset of his/her psychophysiological testing session. This served as a precautionary measure in order to prevent the collection of non-salivary particulates that may have influenced the saliva sample for the cortisol enzyme immunoassay. All saliva samples were collected between 09h30 and 14h00 in order to limit the effects of the diurnal rhythm of cortisol release.

Collection of saliva samples was made possible by the use of non-citric acid Sarstedt Salivettes® (figure A2.2). Salivette's include a sterile cotton wool roll of approximately 0.6 cm diameter and 3 cm length. The participant was asked to place the cotton wool roll under his/her tongue squarely or obliquely with the use of a clean laboratory glove, and was further requested to not bite or chew the cotton wool roll. They were asked to keep the Salivette® under their tongue until it was saturated and pliable or as long as they were able. The Salivette® was removed if visible discomfort was noted by the researcher. The participant was then asked to remove the cotton wool roll and to replace it in the shaft of the Salivette® tube using a clean laboratory

glove. The researcher then capped the Salivette® tube with the saliva sample and placed it on ice until completion of the testing session. On completion of the testing session the researcher placed the collected samples into a -80°C freezer. The enzyme immuno-assay for salivary cortisol was performed in duplicate after all of the participants' samples had been collected.

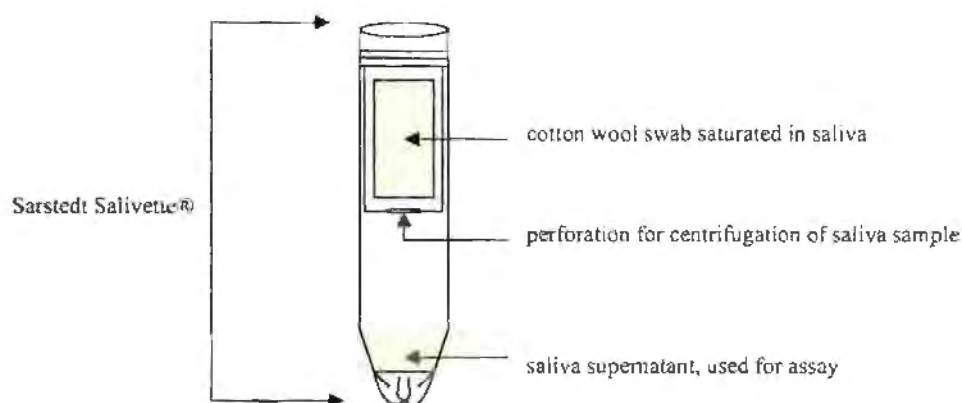


Figure A2.2 Diagram of a Salivette® used for the collection of salivary cortisol

The frozen Salivettes® with saliva samples were thawed at room temperature. Once completely thawed the samples were centrifuged for 15 min at 3000 rpm. Freezing of the samples allowed precipitation of mucins. After the removal of the inner tube holding the cotton wool swab, the clear sample was pipetted into a 2 ml Eppendorf tube and placed on ice. Placement on ice was a precautionary measure due to the number of samples that required thawing on any single day. Salimetrics LLC expanded range high sensitivity salivary cortisol enzyme immunoassay kit was used to determine cortisol concentrations. Assay procedure was carried out as recommended by the insert of the assay kits, Salimetrics Catalog No. 1-3002/1-3012, 96-Well Kit (lower detection limit 0.003µg/dL else 0.083nmol/L). All samples were analyzed in duplicate and all sample pHs were in the range of accuracy for the assay kit used. Layout and controls were in the format

recommended by Salimetrics and included high and low cortisol control solutions which were assayed with the saliva samples. Optical density was measured on a standard plate reader at 450nm. Values were converted in the manner suggested in Salimetrics Catalog No. 1-3002/1-3012, 96-Well Kit.

A2.7 Cognitive attentional tasks

Three attentional tasks were chosen for the testing battery. The three tasks involved only visual presentation; no auditory stimuli were used during the administration and completion of the three attentional tasks. The first task was a modified continuous performance task in which the participant received two cues that were followed by the stimulus. The second task was a Go/No-Go task, which included different interstimulus intervals and response inhibition. The two different conditions occurred in distinct periods of the task. The third task was a cued target detection task in which central fixation was required, cues and stimuli occurred peripherally to the central fixation point.

A2.7.1 Programming

The three attentional tasks were programmed in E-prime Version 1.1 of Psychology Software Tools, Inc. E-prime E-Studio was run on a personal computer running windows 98. The programmed testing session incorporated the instructions in a simplified manner and the layout of the testing session was identical for all participants in the present study. The format and order of the cues and stimuli were also identical. The testing session included baseline recordings of two min duration, starting with eyes open, followed by eyes closed. Termination of the baseline

recording of eyes closed state was achieved by the researcher telling the participant to open their eyes, as all output from the personal computer was visual only. Prior to the onset of the session, participants were informed that the researcher would inform them of the termination of the resting eyes closed session. Instructions regarding the first task were followed by mouse key deflection. All responses by the participants were via mouse key deflection and were recorded via digital input onto the laptop recording the physiological. On completion of the first task the participant was prompted by instructions to continue to the second task. Similarly the participant was prompted by instructions on completion of the second task to continue to the third task. The computerized testing session was continuous. Randomization of the cues and stimuli within a task or task condition was achieved prior to programming such that all participants followed the identical format and order of tasks and task conditions. Participant responses were recorded once only. Thus if an error occurred during the testing session, the participant was excluded. This only occurred once, where the PC froze at the onset of the cued target detection task.

A2.7.2 Acquisition of cognitive task data

The E-prime program was embedded with digital tagging, the E-prime program sent analogue outputs from the printer port to the Biopac MP system via a ribbon cable (DB25M/F, supplied by Biopac Inc.). Input from the participants in the form of mouse clicking was embedded into the E-prime program, which relayed data via the ribbon cable to the laptop collecting the physiological data. Output from the E-prime studio program running the testing battery was embedded with digital signatures for the presentation of the different tasks, their conditions, cues and stimuli. These were all relayed via the ribbon cable to the laptop collecting the physiological data (figure

A2.3). The ribbon cable from the printer port terminated in an isolated digital interface (STP100C Biopac Inc.), which in turn was attached to a universal interface module (UIM100C, Biopac Inc.). Both the STP100C and UIM100C were attached to the Biopac workstation (MP150 system, Biopac Inc.). The analogue input was translated to a 5 volt TTL digital input by setting channels in Acqknowledge acquisition program (Biopac Inc.). The sampling frequency of the software (Biopac Systems Incorporated, Acqknowledge 3.8.1) was set at 500 Hz with the units of recording set as volts. Scaling parameters on software CAL1 input value was set at 10 with scale value 10, CAL2 input value was set at -10 with scale value set at -10 (as recommended by Acqknowledge software manufacturers).

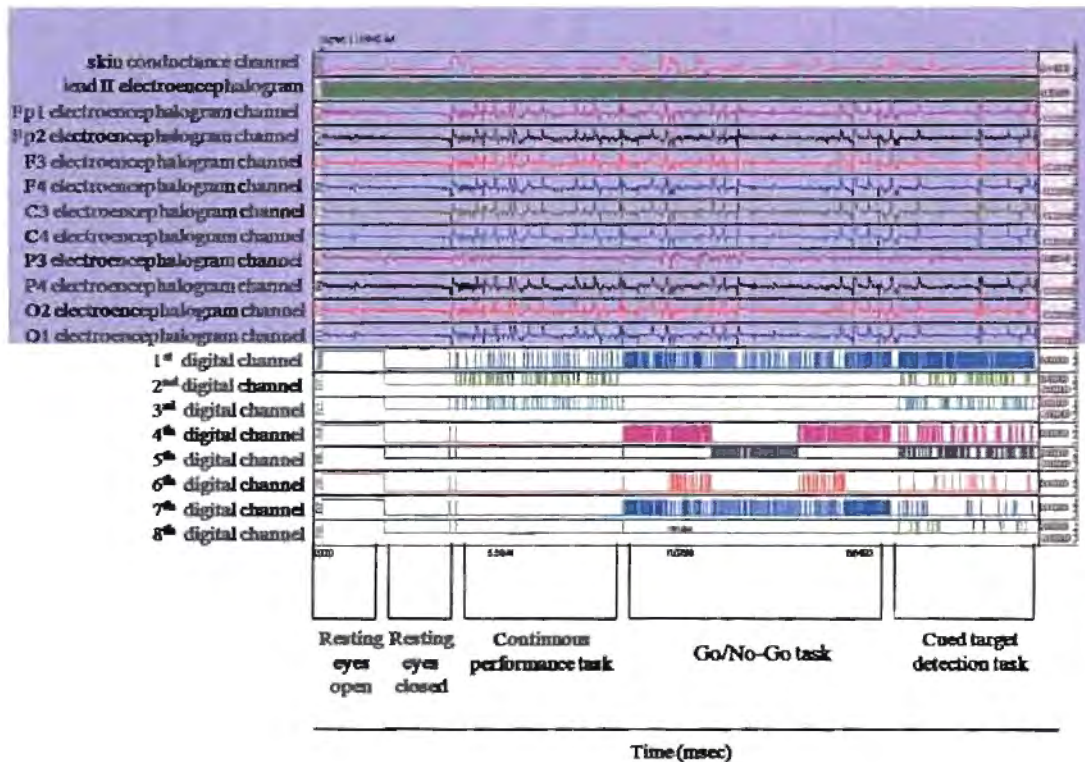


Figure A2.3 Full data set obtained from the Acqknowledge software, indicating the 8 digital channels used as digital channels for the different testing stages.

A2.7.3 Continuous performance task

The continuous performance task measured the ability of a participant to sustain attention and involves cues, stimuli and non-targets. The continuous performance task involved the presentation of two central cues; two successive presentations of the letter S in the centre of the computer screen. The two S's were displayed for 500 msec with an inter-stimulus interval of 100 msec. The onset and offset of these cueing stimuli were digitally captured on the Acqknowledge software on the 3rd digital channel of the Acqknowledge 3.8.1 digital input interface as programmed into Eprime. The stimulus (target) was presented 100 msec after the 2nd cue was presented for 500 msec in the centre of the computer screen. If the subject's response latency was shorter than 500 msec the stimulus was offset (ceased to be presented). The stimulus was captured on the 2nd digital channel of the Acqknowledge 3.8.1 digital input interface as programmed into Eprime. Responses were captured by mouse clicking on the 1st digital channel of the Acqknowledge 3.8.1 digital input interface as programmed into Eprime. Participants received 64 such trial procedures programmed in Eprime for the continuous performance task. Non-target stimuli were presented for 500 msec in the centre of the computer screen with an interval of 100 msec. The non-targets were letters of the alphabet which were not vowels (figure A2.4).

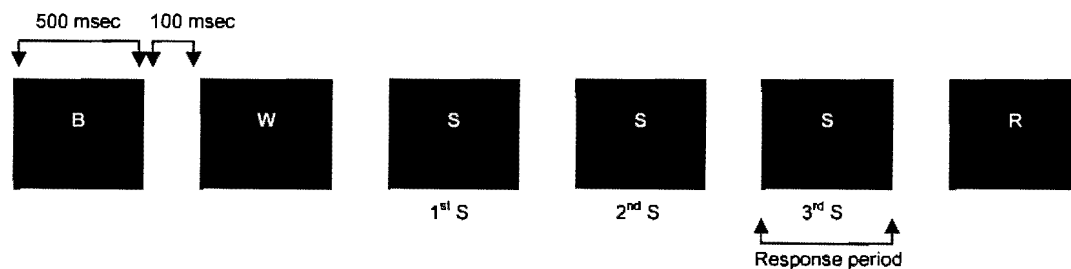


Figure A2.4 Diagrammatic presentation of the continuous performance tasks non-targets, cueing stimuli and target stimuli with intervals and duration of presentation indicated.

Extraction of the response times for the continuous performance task was achieved by selection of the time period in which the task was performed. This was followed by the selection of the digital channel for the stimulus presentation and extraction of the positive peak deflections. These were created by the digital tagging programmed into Eprime which ran the testing battery. The peaks were extracted by an automated peak detection application in Acqknowledge 3.8.1. The digital channel for the mouse clicking was selected and extraction of the positive peak deflections created was performed by the digital tagging programmed into Eprime. The positive peak deflections were further extracted with the automated peak detection application in Acqknowledge 3.8.1. The peak data were set to time in msec. The two extracted data files had a column of time and a column of values to depict the presentation of the stimulus (3rd digital channel) or response (1st digital channel). The response times were calculated by subtraction of stimulus from response presentation. Average response times were calculated for each of the participants. The continuous performance task had 64 trials (figure A2.5).

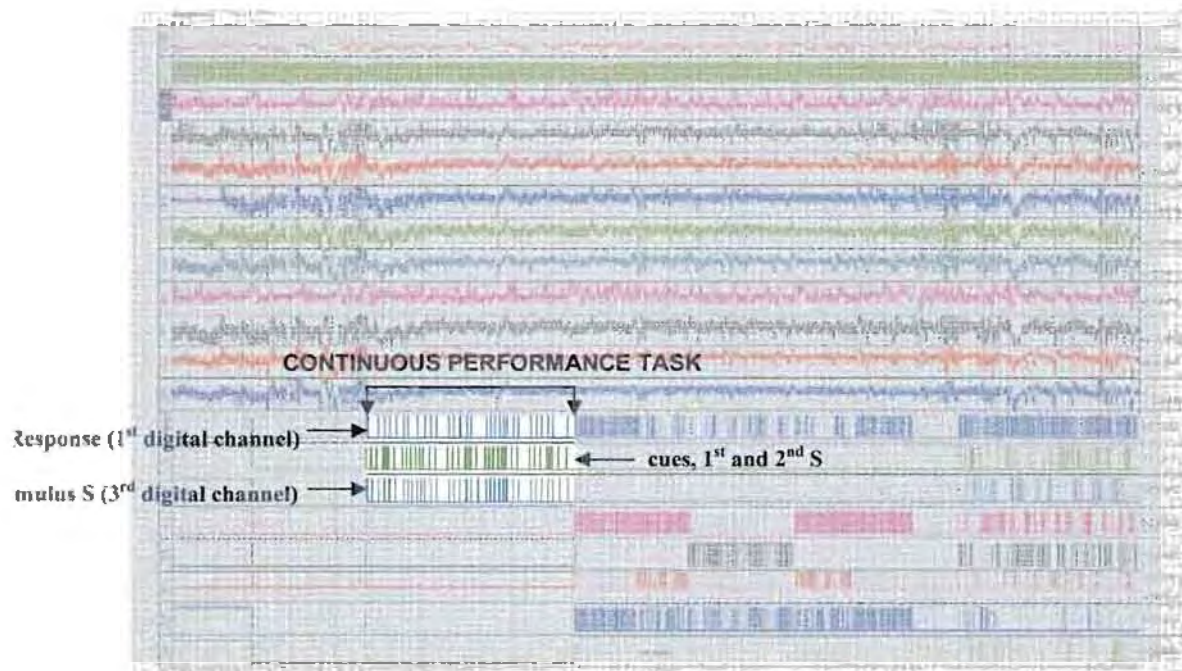


Figure A2.5 Full data set obtained from the Acqknowledge software, demarcating the epoch of the continuous performance task and the two digital channels (1st and 3rd) used to determine response times. The cueing stimuli were captured on the 2nd digital channel.

A2.7.4 Go/No-Go task

The Go/No-Go task involved sustained attention in addition to conditions that required response inhibition and delayed response prior to stimulus presentation. The visual Go/No-Go task used in the present study required the participant to respond by mouse key deflection to all consonants with the exception of the letter 'V' No vowels were used. The 1st condition of the Go/No-Go task was a go condition, in which all stimuli required responses by the participant as no Vs' were presented (figure A2.6).

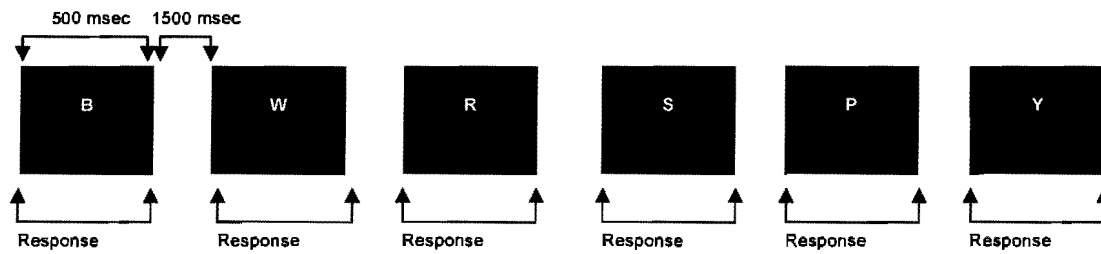


Figure A2.6 Go condition with 1500 msec interval. All consonants required response by participant

The 2nd condition of the Go/No-Go task was a no-go condition, in which participants needed to respond to the consonants that were non-Vs and inhibit their response on the presentation of Vs (figure A2.7).

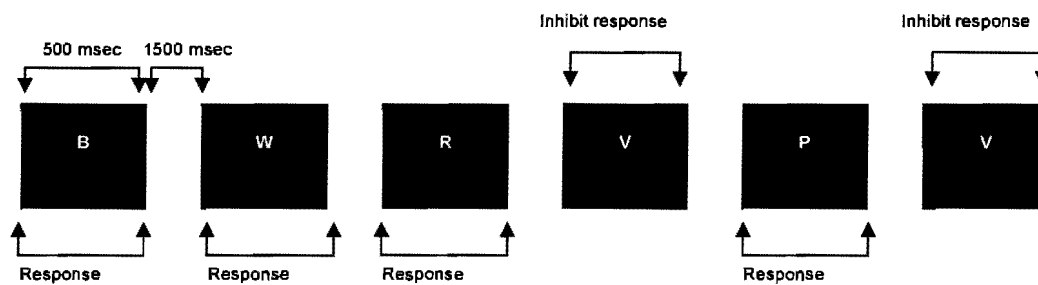


Figure A2.7 No-Go condition with 1500 msec interval. All consonants required response by participant

The 3rd condition of the Go/No-Go task was a go condition with a longer interstimulus interval of 3 500 ms (figure A2.8) unlike the 1st and 2nd condition, which had interstimulus intervals of 1 500 msec.

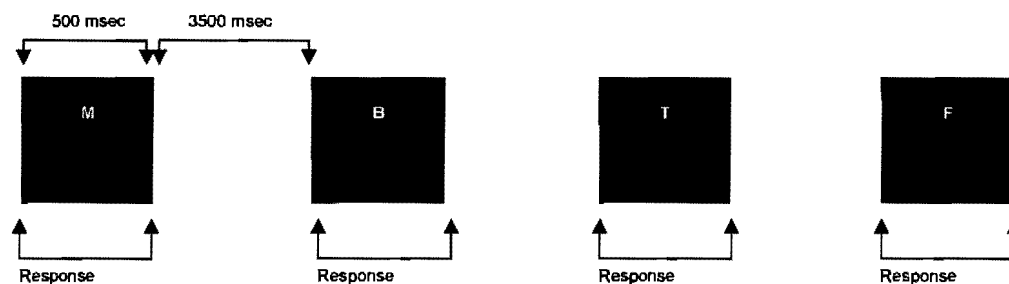


Figure A2.8 No-Go condition with 3500msec interval. All consonants required response by participant

The 1st condition comprised 40 stimuli; the 2nd condition comprised 20 stimuli and 20 Vs, which required response inhibition (50% target, 50% non-target split); and the 3rd condition comprised 20 stimuli. These three conditions were then reversed in order and hence formed a mirror image of the first three conditions. Stimuli, non-targets and inter-stimulus intervals were captured on different digital channels, the onset and offset of stimulus presentation was also recorded. Responses by participants were captured on the 1st digital channel as in the continuous performance task (figures A2.6 ,7 ,8).

Extraction of the response times and errors were carried out in the following manner: the area of the recorded data during which the Go/No-Go task was performed was selected. Three channels (1st for participant responses, 7th for stimuli and 6thfor Vs) were selected individually and peaks were found with peak detection application within Acqknowledge 3.8.2 software. (figure A2.9).

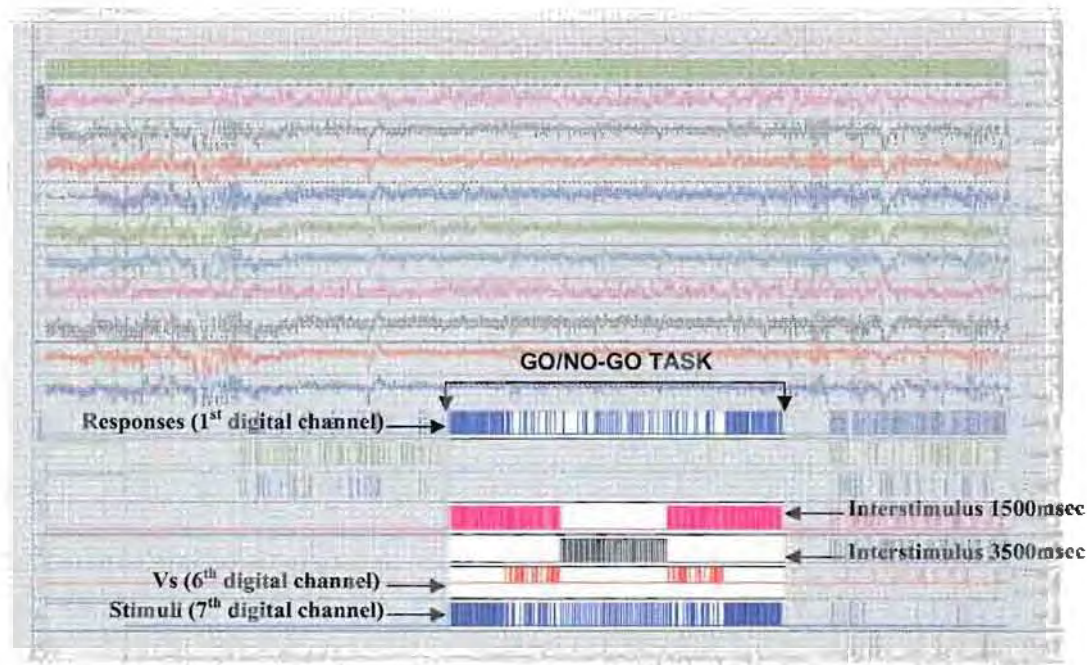


Figure A2.9 Full data set obtained from the Acqknowledge software, demarcating the duration of the Go/No-Go task and the four digital channels used to determine response times and errors during the no-go conditions. Recorded data included responses by the participant collected on the 1st digital channel; interstimulus interval of 1 500 msec collected on the 4th digital channel; interstimulus interval of 3 500 msec collected on the 5th digital channel; presentation and duration of Vs collected on the 6th digital channel; and targets or non-Vs collected on the 7th digital channel.

The peak data was set for time in msec. The times at which responses occurred were subtracted from the stimulus onset times to determine response time for each of the conditions. If the participant responded within 500 msec to a V, an error was recorded.

A2.7.5 Cued target detection task

The cued target detection served to measure covert reflexive attentional ability while maintaining central fixation (Beane and Marrocco, 2004). The cued target detection task required participants to maintain fixation on a central cue: a white circle in the centre of the computer screen. On either side of the central fixation cue was an outline of a rectangle that was grey in colour. The central fixation point and the grey rectangle outlines were present throughout the cognitive task.

The participant was required to respond to the presentation of a square within either of the rectangles. For this task there were four conditions: congruent cue and stimulus presentation; incongruent cue and stimulus presentation; double cue and stimulus presentation; and no cue and stimulus presentation. For the congruent stimulus presentation either the left or the right rectangles brightened i.e. became white, followed by the appearance of the cue in the rectangle that brightened (figure A2.10). Onset and offset of congruent cues were sent to the 5th digital channel (figure A2.14). Incongruent cue and stimulus presentation occurred when the left rectangle brightened and the stimulus occurred in the right rectangle and vice versa (figure A2.11). Onset and offset of incongruent cue was sent to the 6th digital channel (figure A2.14). For the double cue presentation both the left and the right rectangles brightened and the stimulus occurred in either the left or the right rectangle (figure A2.12). Onset and offset of the double cue was captured in the 7th digital channel (figure A2.14). For the no-cue stimulus presentation neither of the rectangles brightened and the square appeared in either the left or the right rectangle (figure A2.13). Onset and offset of no cues were captured in the 8th digital channel (figure A2.14). Cues were presented for 500 msec and the stimulus was presented for 500 msec. The inter-stimulus interval was variable throughout the task, with durations of 500, 1000, or 1500 msec. Inter-stimulus durations were digitally captured in the 3rd, 4th, and 5th channels.

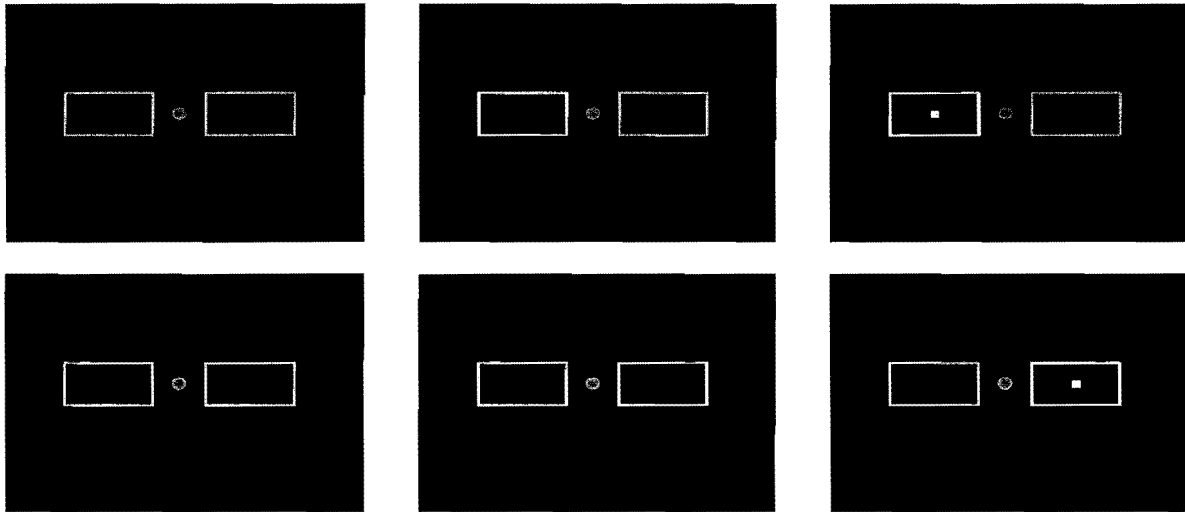


Figure A2.10 Cued target detection task showing the central fixation point and the two peripheral rectangles. Brightening of a rectangle followed by presentation of a square within the brightened rectangle, these reflect congruent trials.

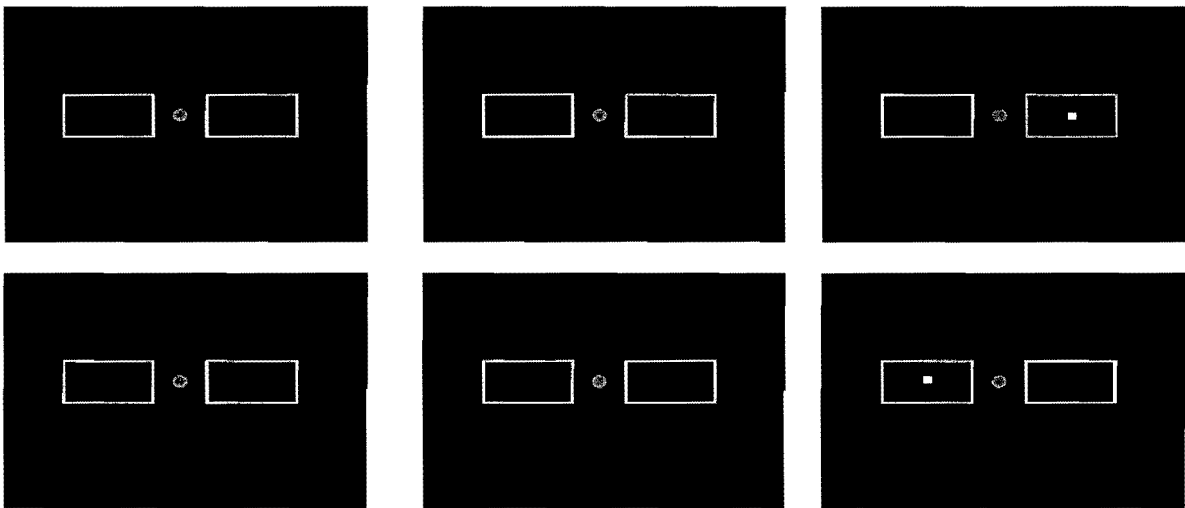


Figure A2.11 Cued target detection task showing the central fixation point and the two peripheral rectangles. Brightening of one of the rectangles followed by presentation of the target in the opposite rectangles served as an incongruent trial.

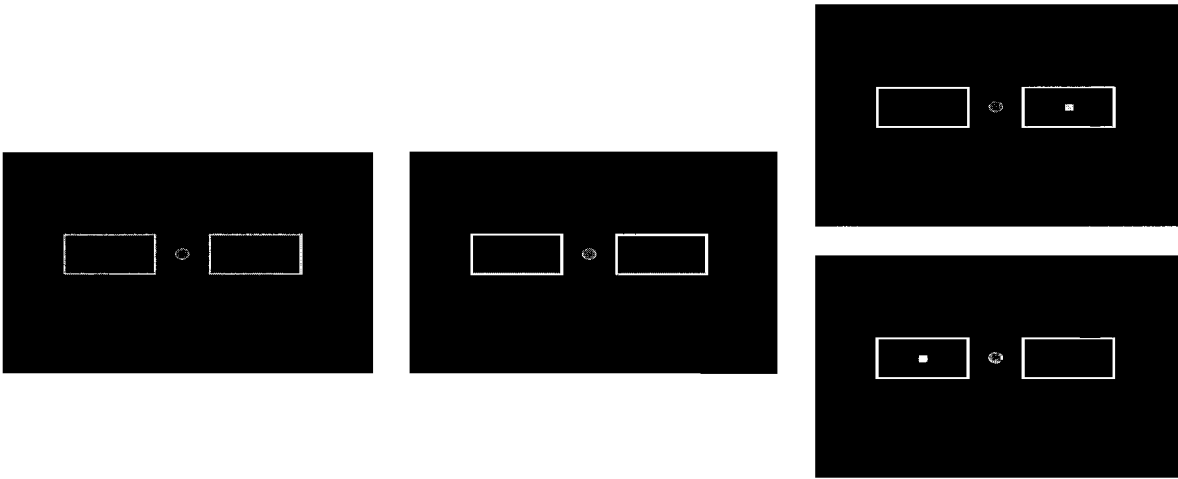


Figure A2.13 Cued target detection task, showing the central fixation point and the two peripheral rectangles. Brightening of both rectangles followed by presentation of the target served as a double cue trial.

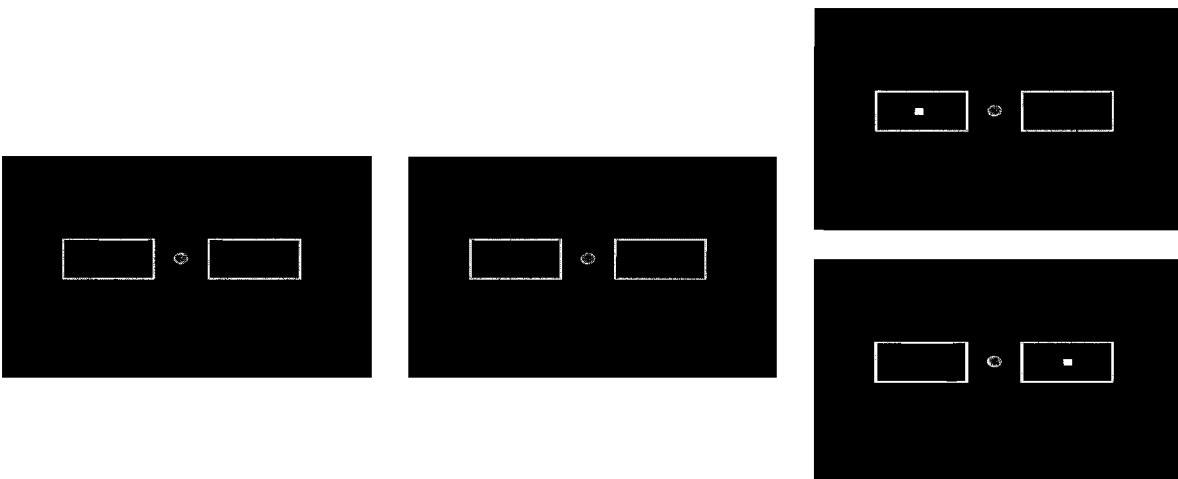


Figure A2.13 Cued target detection task, showing the central fixation point and the two peripheral rectangles. No brightening followed by target presentation served as no-cue trials.

The cued target detection task had 64 stimuli that were congruent; 16 stimuli that were incongruent; 16 stimuli that had double cueing; and 16 stimuli that had no cues. Each condition: congruent, incongruent, double cue and no-cue sent a digital input to the Acqknowledge 3.8.1 software when presented and assigned a different digital signature. These digital channels

allowed easy extraction of response times, as described previously for the continuous performance task and the Go/No-Go task.

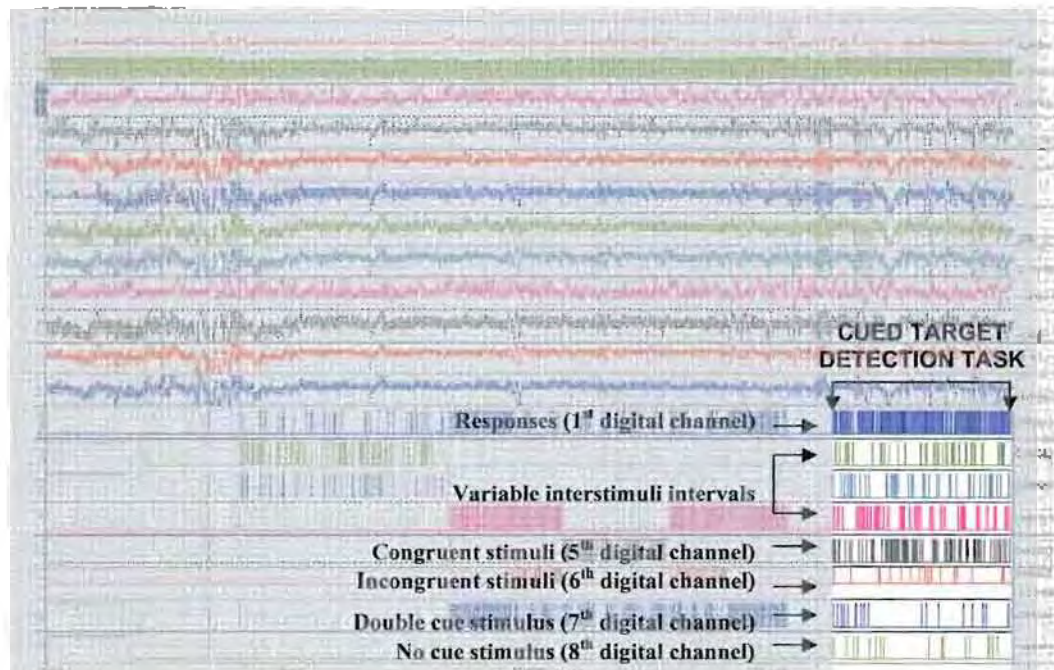


Figure.A2.14 Full data set obtained from the Acqknowledge software, demarcating the duration of the cued target detection task for the eight digital channels used for this task. Responses by the participant were collected on the 1st digital channel, variable inter-stimuli on digital channels 2 through 4, 5th digital channel represents the target for congruent trials, 6th channel represents the target for incongruent trials, 7th channel represents the target for double cue trails, and the 8th channel represents the target for no cue trials.

A2.8 Skin conductance measures

Two transducers were placed on the non-dominant hand or the hand that the participants did not use for mouse key deflection required for completion of the cognitive tasks. The TSD203 transducers were filled with GEL101 (www.biopac.com), composed of a 0.5% isotonic salt solution similar to human physiological salt concentration. The electrodes were strapped with Velcro around the index fingertip and the pointing fingertip. A minimum of 10 minutes passed

prior to commencement of the recording session. Participants' fingertips were not cleaned with alcohol swabs but were rather wiped with toweling. Output from the participants was collected by TSD203 transducer (channel 1 on the MP system, application note 187 from www.biopac.com). The TSD203 transducer terminal ends comprised two Ag-AgCl electrodes housed in polyurethane and Velcro straps for easy positioning on the distal phalanges. The TSD203 transducers provided a small current to the site, allowing for measurement of resistance between the two electrodes. The TSD203 was in turn attached to a MEC100C and into a GSR100C amplifier module (Biopac Systems Incorporated). The Amplifier settings were set for skin conductance response measurements (phasic) and not skin conductance level (tonic). Gain was set at 10 $\mu\text{mho/V}$, the low pass filter was set at 10 Hz and the high pass filter was set at 0.05 Hz (application note 187, www.biopac.com). The sampling frequency of the software (Biopac Systems Incorporated, Acqknowledge 3.8.1) was set at 500 Hz with units of recording μmho the equivalent of $\mu\text{Siemens}$. Scaling parameters on the software CAL1 input value were set at 0 with a scale value set at 0. The CAL2 input value was set at 1 with a scale value set at 10 (as recommended by software manufacturers).

All processing of SCR data was carried out post the recording session with Acqknowledge 3.8.1. Skin conductance responses and their durations were extracted within selected stages of the testing session. . The data was passed through a FIR low pass filter of 1Hz with a hamming window. The number of coefficients was set at 2000, as recommended by Biopac Systems Incorporated (application note 233, www.biopac.com). The data were then analyzed for peaks exceeding a threshold value. Responses greater than 0.05 μmho reflected a spike of activity.

Epochs were extracted from the skin conductance channels during: resting eyes open; resting eyes closed; continuous performance task; Go/No-Go task; and the cued target detection task (figure A2.15). Baseline values of skin conductance were not collected as amplifiers were set for skin conductance responses (a phasic measure) and not skin conductance levels (a tonic measure). Skin conductance responses allow the assessment of what arousal adds to attentive abilities and not just how the performance will vary with any non-specific level of vigilance.

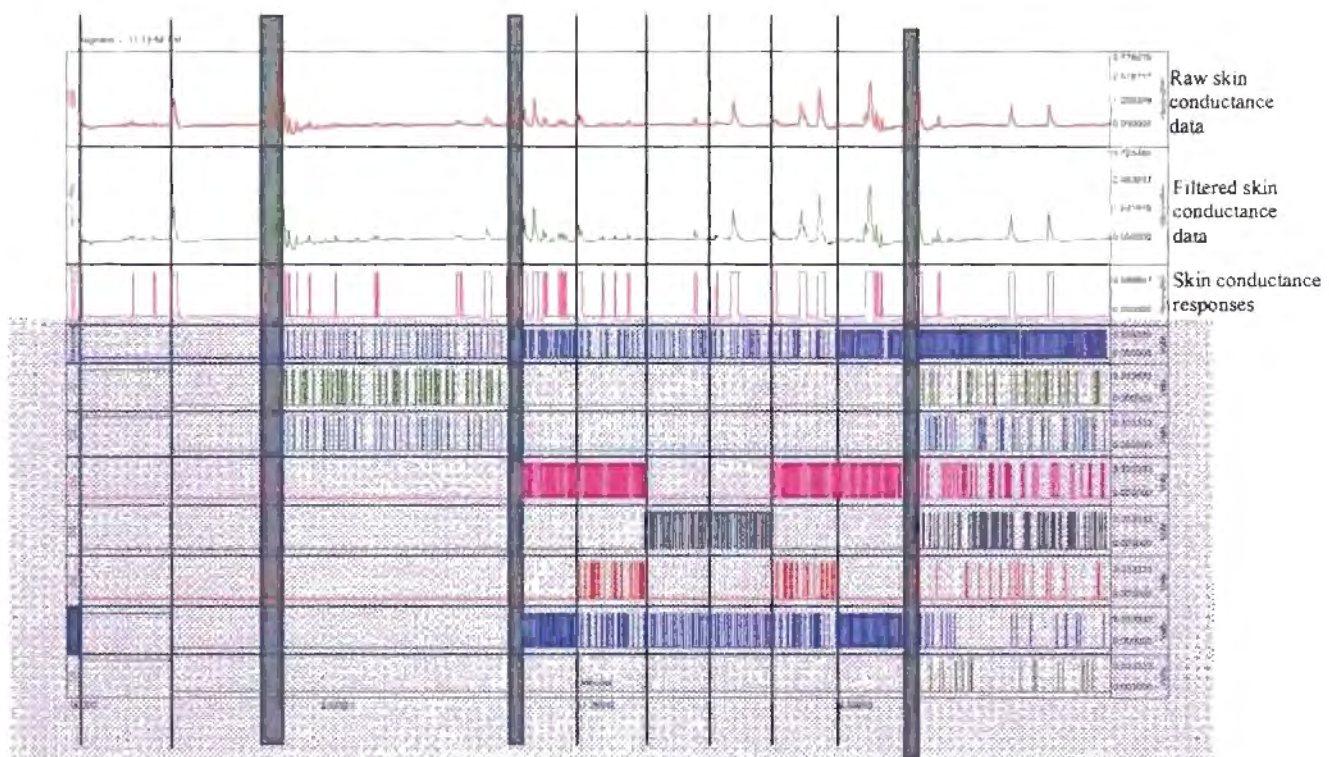


Figure A2.15 Breakdown of skin conductance responses during a recording session. The area between the black lines depicts the period in which skin conductance responses were calculated, gray shaded areas show areas between tasks in which the participant read the instructions for the following task. The 1st channel in this diagram shows the raw skin conductance; the 2nd horizontal channel shows skin conductance response to FIR 1Hz low pass filter and; the 3rd channel shows the number of skin conductance responses and their duration when a threshold of 0.05 μ mho was applied. The remaining channels are the digital input channels programmed into Eprime.

A2.9 Electrocardiogram

Effective placement of the five ECG electrodes was achieved by first: vigorous cleaning of the areas of skin with alcohol swabs, drying these areas, and placing electrode gel pads on the left collarbone (chest lead), left leg and left arm (leads for I) , and right leg and right arm (leads for II). Ground was established with the SCR amplifier. The TSD155C multi-lead ECG cable with built-in Wilson terminal (with five leads) was plugged into the three ECG100C amplifier modules (channels 2, 3, and 4 on the MP system, application note 109 ECG from www.biopac.com). The Biopac Systems Incorporated, MP150 acquisition system was used with three EGC100C amplifier modules. Amplifier settings were as follows: gain set at 1000; mode normal; low pass filter set at 35 Hz; and high pass filter set at 0.5 Hz (application note 233, www.biopac.com). The sampling frequency of the software (Biopac Systems Incorporated, Acqknowledge 3.8.1) was set at 500 Hz with units of recording set at μ volts. The scaling parameters on software CAL1 input value was set at 10 with scale value set at 10, CAL2 input value was set at -10 with scale value set at -10 (as recommended by software manufacturers). The trace was visually checked to confirm good connectivity as seen by the QRS complex.

All processing of ECG data was carried out post recording session. Lead II was used to determine heart rate and heart rate variability (HRV) from the ECG recording. The data was passed through a FIR band pass filter with a hamming window. Low frequency cut-off was set at 0.5 Hz and high frequency cut-off at 35 Hz. The number of coefficients was set at 4000, as recommended by Biopac Systems Incorporated (application note 233, www.biopac.com).

To establish the viability of the data, a tachogram was created with the specialized analysis software on version 3.9 Acqknowledge, run on a Macintosh computer. A tachogram represents the R-R interval from heart beat to heart beat, therefore providing evidence of missed beats or errors during the acquisition of data (figure A2.16). No data was manipulated, participants were removed if their data was erroneous.

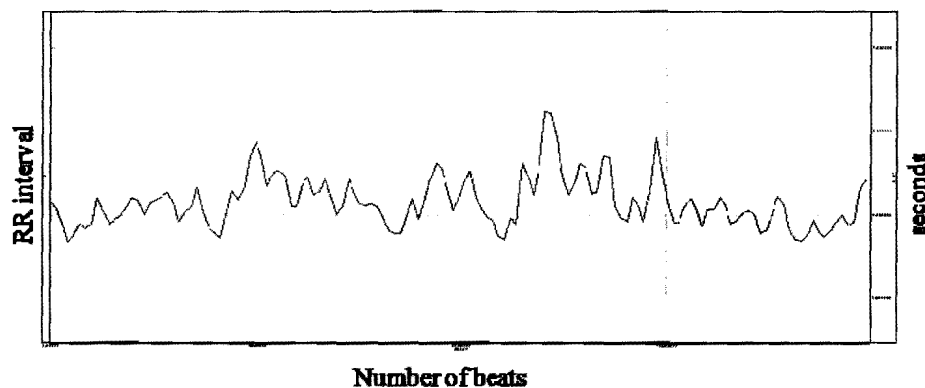


Figure A2.16 Tachogram from the present study obtained in Acqknowledge 3.9

The data underwent a fully automated heart rate variance analysis with the use of heart rate variance analysis software, from the Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland (<http://venda.uku.fi/research/biosignal>). Default parameters set in the software for autoregressive analysis was applied to the tachograms: low frequency 0.04 to 0.15 Hz and high frequency 0.15 to 0.4 Hz. The autoregressive model order was set at 20. HRV measures reported in the present study include: relative low frequency power; relative high frequency power; and the low frequency to high frequency ratio. Heart rate was extracted for both resting eyes open and eyes closed states; during the continuous

performance task; during the Go/No-Go task; and during the cued target detection task. Heart rate variability was obtained for the three cognitive tasks only.

A2.10 Electroencephalogram

Biopac Systems Incorporated, MP150 acquisition system, was used with ten EEG amplifiers, EEG100C (www.biopac.com). Electrodes linking ear lobes were used as the reference in the present study. This was achieved by linking the EEG 100C amplifiers with JUMP 100C jumper cables. In-house constructed jumpers were used initially, as jumpers were not acquired when the initial system was purchased. The use of in-house jumpers reduced the number of recorded participants in respect to EEG measurements, as the in-house jumpers were found to be faulty. Each of the 10 amplifiers was connected via a ribbon cable (1 m long), which ended by fanning into the international 10/20 montage EEG electrode cap (CAP100C).

The EEG100C amplifiers were set on normal mode; with low pass filter on 100 Hz and high pass filter on at 0.1 Hz with a gain of 1000 (application note 233, www.biopac.com). The sampling frequency of software (Biopac Systems Incorporated, Acqknowledge 3.8.1) was set at 500 Hz with the units of recording set as volts. The scaling parameters on the software were set as follows: CAL1 input value was set at 10 with a scale value of 10, and the CAL2 input value was set at -10 with a scale value set at -10 (as recommended by the software manufacturers). Channels of the 10/20 montage recorded were: Fp₁, Fp₂, F₃, F₄, C₃, C₄, P₃, P₄, O₁, and O₂ respectively (figure A2.17).

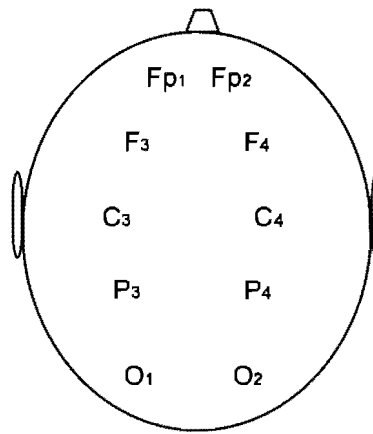


Figure A2.17 Diagrammatic representation of the position of electrodes for the bilateral recording of scalp activity over the prefrontal, frontal, cingulate, parietal and occipital cortices during the testing session, which included three attentional tasks.

Each of the electrodes of the cap had a central opening. An orange stick was used in a circular motion to clear the area of hair. Then each of the ten electrodes was filled with electrode gel (GEL 100C, www.biopac.com). Care was taken to ensure that no air pockets were in the gel thereby ensuring a direct conduction pathway from the scalp to the recording electrode.

A2.10.1 EEG filtering

All processing of EEG data was carried out post recording. Each of the EEG channels (channels 3,4,5,6,7,8,9,10,11,12, figure A2.18) was filtered through a FIR band pass filter with a hamming window. Low frequency cut-off was at 0.5 Hz and high frequency cut-off was at 30 Hz. The number of coefficients was set at 4000, as recommended by Biopac Systems Incorporated (application note 233, www.biopac.com).

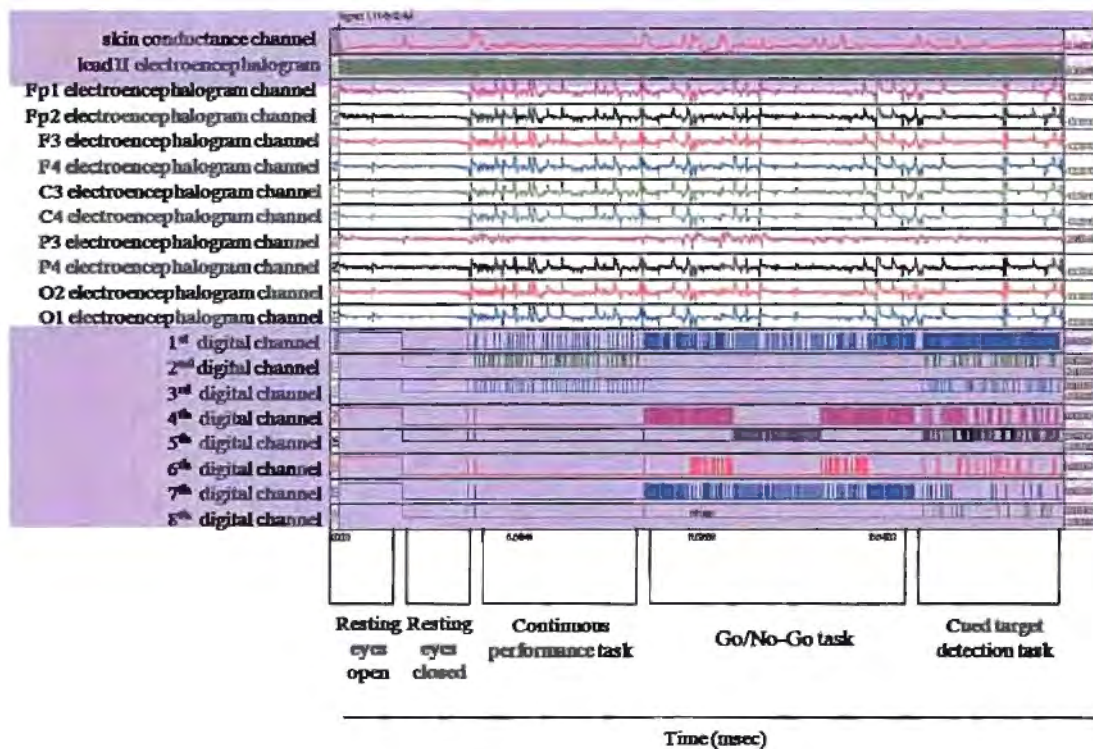


Figure A2.18 Full data set obtained from the Acqknowledge software, demarcating the channels that recorded the EEG, which included the prefrontal cortex (Fp1 and Fp2), frontal cortex (F3 and F4), cingulate cortex (C3 and C4), parietal cortex (P3 and P4), and occipital cortex (O1 and O2).

A2.10.1 EEG extraction of frequencies

Fast Fourier transform of the data was carried out on the filtered EEG data in Acqknowledge 3.8.1 for resting eyes open; resting eyes closed; the continuous performance task; Go/No-Go task; cued target detection task; and for the different conditions of the Go/No-Go task. Absolute power values were obtained for theta (θ , 4 -7 Hz), alpha (α , 7-14Hz), and beta (β , 15-30 Hz) frequency bands. For resting eyes open, resting eyes closed, the continuous performance task, the Go/No-Go task, the cued target detection task, and the conditions of the Go/No-Go task; the

extracted absolute power values were converted into relative power values, by division of a band power (θ or α or β) by the sum of the band powers extracted ($\theta + \alpha + \beta$).

A2.10.1 EEG extraction of event-related potentials

Event-related potentials (ERPs) were extracted slightly differently for each of the three tasks, as will be explained. The extraction of ERPs was set with artifact rejection values greater than +100 μ volts and less than – 100 μ volts. This was followed by visual inspection of the averaged ERP for each participant, as the software used for the extraction did not permit the exclusion of a single ERP waveform or checking a single waveform.

A2.10.1.1 Event-related potentials for the continuous performance task

The continuous performance task ERPs were extracted from the digital signature of the stimulus (presentation of the third S, channel 3, figure A2.4). For the continuous performance task, one ERP was extracted per EEG channel for each participant. To visualize the effect of the cueing, ERPs were extracted 1300 msec prior to the stimulus and 900 msec post stimulus. The extracted average ERP was then baseline-corrected to the 100 msec prior to the first cue. To determine the P300 latency and amplitude, the ERP was baseline-corrected for the 100 msec prior to the stimulus. A window between 200 msec and 500 msec was used to determine the amplitude and latency of the P300 component for the parietal electrodes. Amplitude was calculated as the point of maximum height of the P300, and the latency was calculated as the time at which the maximum amplitude occurred.

A2.10.1.2 Event-related potentials for the Go/No-Go task

The Go/No-Go task ERPs were extracted from the digital signature of the stimulus for each of the five conditions and for the presentation of the Vs' for the two no-go conditions. For the Go/No-Go task, seven ERPs were extracted per channel for each participant and for each of the following conditions: 1st go 1 500 msec, 1st no-go, 1st no-go Vs, Go 3 500 msec, 2nd no-go, 2nd no-go Vs, and 2nd go 1 500 msec conditions. The digital signature of the stimuli (7th digital channel) or Vs (8th digital channel) were extracted from 100 msec prior to either the stimulus or Vs presentation, and 900 msec post either the stimulus or Vs presentation. The average ERPs for each channel was then baseline-corrected for the 100 msec prior to stimulus or V's presentation. A window between 250 msec and 500 msec was used to determine the amplitude and latency of the P300 component for the parietal electrodes. Amplitude was the point at which the height of the P300 was maximal and the latency was the time at which this maximal amplitude occurred.

A2.10.1.3 Event-related potentials for the cued target detection task

The CTD task's ERPs were extracted from the digital signature of the stimulus for each of the four trial types. The digital signatures for the cues were embedded in the digital channels for each of the four trials: congruent trials (5th digital channel), incongruent trials (6th digital channel), double cue trials (7th digital channel) and no-cue trials (8th digital channel). Each of the trials was extracted from 100 msec prior to stimulus presentation and 900 msec post stimulus presentation. The average ERPs for each trial type was then baseline-corrected for the 100 msec prior to stimulus presentation. Only the congruent trials' P300 component was extracted as: (1) digital tagging was insufficient to determine left or right cueing; (2) horizontal and vertical eye

movements were not measured; (3) Only 16 trials were presented for the incongruent trials, double-cue trials, and no-cue trials and therefore were deemed insufficient to determine a true representation of the P300 component of the ERP wave form (Luck, 2005). A window between 400 and 700 msec was used to determine the amplitude and latency of the P300 component from the parietal electrodes. Amplitude was the point at which the height of the P300 was maximal and the latency was the time at which this maximal amplitude occurred.

A2.10.3 Electroencephalogram problems

On acquisition of the recording equipment; 'in-house' jumper cables were used to obtain a reference for the EEG, through the linking of electrodes attached to the earlobes, however these jumpers were faulty. This led the researcher to discard EEG data collected when these 'in-house' jumpers were used. Electro-oculograph measurements were not taken. Prefrontal electrodes Fp1 and Fp2 were used as indicators of high EOG activity. Participants were excluded if the extracted ERP averages for either Fp1 or Fp2 appeared abnormal, from ERP analysis and EEG frequency analysis. The software used to extract the ERPs would not allow the exclusion of a single ERP, which would otherwise have allowed manual removal of the faulty ERP waveforms. Problems related to the cued target detection are listed under A2.10.1.3

A2.11 Statistical analysis

The Statistica 8 software package was used for the statistical analyses. Non-parametric statistics were used for all human data as Shapiro-Wilks W test revealed that the data was not normally

distributed. Mann-Whitney U test was performed for comparison of two independent samples. Kruskal-Wallis ANOVA was performed for comparison of several independent samples. Friedman ANOVA was performed for comparison of several dependent variables. Wilcoxon matched pairs test was performed when comparing two dependent variables. Spearman's Rank R was used to determine correlations between variables. When comparisons were made between dependent variables Bonferonni correction was applied, this was achieved by division of the standard p-value 0.05 by the number of dependent variables. An example of this would be three similar physiological measures that were dependent on each other required a significant value of $p < 0.01667$ ($p < 0.05/3$). Figures report mean $\pm$ SEM. Median and quartile box plot graphs can be found in the respective appendices.

A3 Results

A3.1 Participant characteristics

A3.1.1 Participants

Eighty-four adults participated in the present study (27.1 ± 0.6 years): 50 females and 34 males. Participants had an average of 6 years of post-matriculation education (6.31 ± 0.34 , table A3.1.1). This characteristic was due to participant recruitment that was done within the University of Cape Town population. The cohort comprised 73 right-handed individuals, 5 left-handed individuals and 6 ambidextrous individuals, as ascertained from the Edinburgh's handedness questionnaire. The participants did not report any current form or history of brain trauma, organic brain disease, history of epilepsy or diagnosis of any psychiatric or psychological disorder. Additional information including: allergies; headaches and family psychiatric disease, tabulated in A3.1.2. The Drug-use frequency questionnaire devised by O'Farrell et al. (2003) for the American population was adapted to include common street name drugs and drugs used in South Africa. Results are reported in table A3.1.3.

Table A3.1.1 Participant ages and years of post-matriculation education

	female participants		male participants		All participants	
	n = 50		n = 34		n = 84	
	Mean	SEM	Mean	SEM	Mean	SEM
Years of post matriculation education	6.32	0.35	6.29	0.69	6.31	0.34
Age	26.8	0.7	27.6	0.9	27.1	0.6

(median and quartiles appendix A6)

Table A3.1.2 Additional information obtained from cohort

	n = 84
Allergies	
<i>Chronic prescription for medication of allergies</i>	8
<i>Acute prescription medication of allergies</i>	8
<i>Suffer from allergies but are not medicated</i>	19
Headaches	
<i>Suffer from migraine</i>	12
<i>Suffer from cluster headaches</i>	7
<i>Suffer from acute headaches</i>	45
<i>No headaches reported</i>	35
Family member(s) with psychiatric disorders	
<i>Depression</i>	4
<i>Attention-deficit/hyperactivity disorder</i>	3
<i>Schizophrenia</i>	1
<i>Obsessive compulsive disorder</i>	1
<i>Unknown disorder</i>	1

Table A3.1.3 Reported drug-use

Drug use-frequency (last six months prior to testing session)	n = 84
Smokers (nicotine)	
<i>Regular smokers (smoke 5 or more cigarettes per day)</i>	13
<i>Social smokers (smoke 5-10 cigarettes per week)</i>	7
<i>Non-smokers</i>	64
Alcohol consumption	
<i>Regular drinkers (drink 5-7 days per week)</i>	4
<i>Social drinkers</i>	61
<i>Non-drinkers</i>	19
Caffeine consumption	
<i>Regular drinkers (5-7 days per week)</i>	64
<i>Occasional drinkers</i>	11
<i>No-consumption of caffeine</i>	9
Marijuana use	
<i>Regular smokers</i>	2
<i>Social smokers</i>	4
<i>Have tried marijuana</i>	12

<i>Have had stimulants</i>	4
<i>Have had narcotics</i>	1
<i>Have had cocaine</i>	1
<i>Have had hallucinogenics</i>	1

Mann-Whitney U test revealed significant differences between male and female participants for several of the questionnaires. Male participants reported higher levels of functional impulsivity as seen on the subscale of Dickman's impulsivity scale, and higher reported levels of childhood physical abuse as measured on a subscale of the childhood trauma questionnaire (CTQ-SF). Female participants reported significantly higher levels for both subscales of Spielberger's trait anxiety (trait anxiety present and trait anxiety absent). Female participants reported significantly higher sensitivity to punishment a subscale of the sensitivity to punishment and sensitivity to reward questionnaire (table A3.1.4, appendix A6).

Table A3.1.4 Reported subjective questionnaire scores

	female participants			male participants			All participants		
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
<i>Dickman's impulsivity scale</i>									
<i>ⁿFunctional impulsivity</i>	5.46	0.22	50	6.27	0.29	33	5.78	0.18	83
<i>Dysfunctional impulsivity</i>	4.66	0.25	50	4.88	0.25	33	4.75	0.18	83
<i>Sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ)</i>									
<i>Sensitivity to reward</i>	9.28	0.57	50	10.52	0.7	33	9.77	0.44	83
<i>*Sensitivity to punishment</i>	11.34	0.74	50	8.76	0.96	33	10.31	0.6	83
<i>Abbreviated Eysenck personality questionnaire (EPQR-A)</i>									
<i>Extraversion</i>	3.06	0.17	50	3.15	0.21	34	3.1	0.13	84
<i>Lie Scale</i>	2.96	0.21	50	3.56	0.24	34	3.2	0.16	84
<i>Psychoticism</i>	2.98	0.11	50	3	0.16	34	2.99	0.09	84
<i>Neuroticism</i>	2.8	0.25	50	2.18	0.29	34	2.55	0.19	84
<i>Kessler distress scale (K10)</i>									
	20.9	0.69	50	21.59	1.8	34	21.18	0.83	84

Cloninger's tridimensional personality questionnaire subscales									
<i>Novelty seeking</i>	18.02	0.78	50	17.65	1.22	34	17.87	0.67	84
<i>Harm avoidance</i>	20.42	1.39	50	16.74	1.82	34	18.93	1.12	84
Spielberger's trait anxiety scale (STAI-T)									
<i>*Anxiety absent</i>	18.82	0.65	50	16.71	0.88	34	17.96	0.54	84
<i>*Anxiety present</i>	20.22	0.7	50	17.97	0.81	34	19.31	0.54	84
Childhood trauma questionnaire short form (CTQ-SF)									
<i>#Physical abuse</i>	5.66	0.19	50	6.5	0.28	34	6	0.16	84
<i>Physical neglect</i>	5.8	0.19	50	6.38	0.32	34	6.04	0.18	84
<i>Emotional abuse</i>	7.14	0.42	50	7.62	0.53	34	7.33	0.33	84
<i>Emotional neglect</i>	7.88	0.47	50	8.24	0.6	34	8.02	0.37	84
<i>Sexual abuse</i>	5.28	0.12	50	5.18	0.12	34	5.24	0.09	84
<i>m/denial</i>	0.78	0.16	50	0.68	0.17	34	0.74	0.12	84
Visual analogue scale (VAS) for reported mental effort									
<i>Continuous performance task (cm)</i>	3.17	0.13	49	2.36	0.28	34	2.78	0.22	83
<i>Go/No-Go task (cm)</i>	5.33	0.35	49	4.91	0.50	34	5.15	0.30	83
<i>Cued target detection task (cm)</i>	2.82	0.32	48	2.69	0.37	34	2.77	0.23	82

*Mann-Whitney U test by gender, $p < 0.05$, * females significantly higher than males, # males significantly higher scores than females*

For the subjective questionnaires one participant did not complete Dickman's impulsivity scale and the sensitivity to punishment and sensitivity to reward, one participant did not mark off on the visual analogue scale their perceived level of mental effort and one participant did not complete the cued target detection task and therefore was unable to report on their perceived mental effort for this task. When any of the analyses were performed, if a data point was missing, the participant was removed from the analysis that followed.

A3.1.2 Blood samples

Eighty-four participants' blood samples were stored at -80°C . For each participant two blood buffy layer samples and two plasma samples were stored in 2 ml eppendorff vials. Each of the

two samples was derived from one of the two blood samples collected. The future analysis of these samples is dependent upon the outcomes of the present study.

A3.1.3 Changes in Salivary Cortisol

Kruskal-Wallis ANOVA revealed no significant differences for salivary cortisol prior to or post testing. In addition no significant differences were found for gender. There were no significant differences noted for use of the contraceptive pill (table.A3.1.4, appendix A6).

Table A3.1.4 Participants' salivary cortisol ($\mu\text{g/dL}$) measures prior to and post cognitive testing

	n	Cortisol prior to cognitive testing		Cortisol post cognitive testing	
		Mean	SEM	Mean	SEM
<i>Male participants</i>	34	0.170	0.025	0.200	0.029
<i>Females not taking contraceptive pill</i>	30	0.166	0.016	0.166	0.029
<i>Females taking contraceptive pill</i>	17	0.214	0.035	0.182	0.026
<i>All participants</i>	81	0.177	0.014	0.184	0.017

Mann-Whitney U test grouping gender, $p < 0.05$, no difference found, units $\mu\text{g/dL}$

Three participants were excluded from the cortisol analysis. One participant had exceptionally high cortisol measures (10x higher mean value) and two participants yielded insufficient saliva for the post cognitive testing sample.

A3.1.4 Skin conductance responses and their durations for the stages of the testing session

Friedman ANOVA revealed significant differences for skin conductance duration. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.01$) revealed that the duration of responses during resting eyes open were significantly shorter in comparison to all other stages of the testing session. During resting eyes closed the duration of responses were longer than during the continuous performance task. The duration of responses during the continuous performance task were shorter than responses in the Go/No-Go task and the cued target detection task. The number of skin conductance responses for the different stages of the testing session were not compared. This was due to the different stages being of dissimilar duration (Figure A3.1.1, appendix A6).

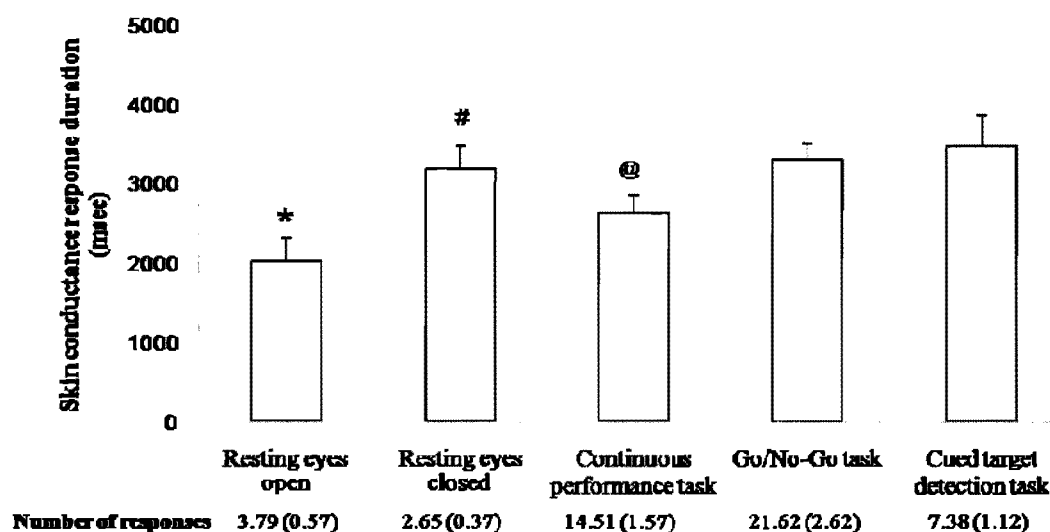


Figure A3.1.1 Skin conductance durations and number of responses. *Duration of response during the brief period of resting eyes open was shorter than all other stages of the testing battery, #during the resting eyes closed state, the duration of skin conductance responses were longer than during the continuous performance task, @ the duration of response during the continuous performance task was shorter than for the Go/No-Go task and the cued target detection task ($p < 0.01$, $n = 84$, mean $\pm$ SEM, median and quartiles appendix A6).

Eighty-four participants' skin conductance responses and durations were recorded. Five participants were 'non'-responders, meaning that they did not reach the lower threshold ($0.05 \mu\text{mho}$, Dawson et al., 2001) set in the current analysis protocol for skin conductance response.

Removal of the ‘non’-responders at any point of the following analyses did not affect the results and therefore were included in the analyses.

A3.1.5 Electrocardiogram heart rate for stages of the testing session

Friedman ANOVA revealed significant differences in heart rate during the different stages of the testing session. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.01$) revealed slower heart rate during resting eyes open and resting eyes closed in comparison to heart rate during the three cognitive tasks. Heart rate was higher during the Go/No-Go task than during the cued target detection task (figure A3.14, appendix A9).

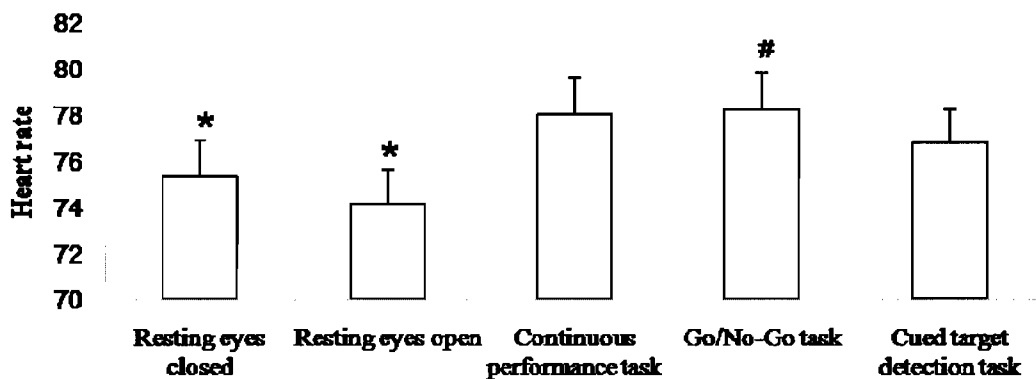


Figure A3.14 Heart rate during the cognitive testing battery, *participants' heart rates were lower during rest, heart rate during the #Go/No-Go task was higher than during the cued target detection task ($p < 0.01$, $n = 76$, mean $\pm$ SEM (median and quartiles appendix A6)).

Seventy-six participants were included for heart rate variance analysis, eight participants were excluded due to errors seen in tachograms.

A3.1.6 Electroencephalogram frequencies during resting eyes open and resting eyes closed

The first four minutes of the testing session comprised two resting states: eyes open and eyes closed. EEG frequency bands for theta, alpha and beta were extracted. Absolute values were comparable, as epochs during the two resting states were similar. Electrodes used for the frequency extraction were frontal (F3 and F4) and parietal (P3 and P4). Wilcoxon matched pairs test with Bonferroni correction ($p < 0.025$) revealed differences between eyes open and eyes closed for absolute (figure A3.1.3) and relative frequency powers (figure A3.1.4). Only one exception was found as absolute theta did not differ for F4 between resting eyes open and resting eyes closed (appendix A6).

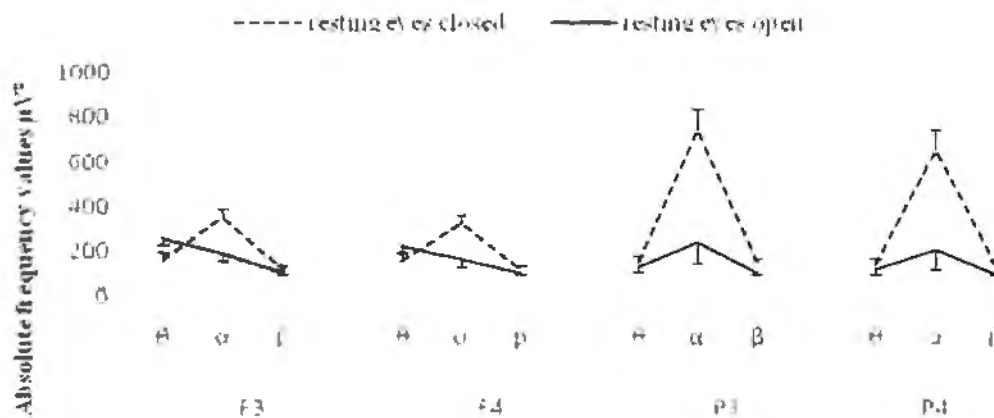


Figure A3.1.3 Absolute frequency power during two resting states prior to the cognitive testing; resting eyes open followed by resting eyes closed. All absolute frequency measurements differed between eyes open and eyes closed, except F4 absolute theta ($p < 0.025$, $n = 47$, mean $\pm$ SEM, median and quartiles appendix A6).

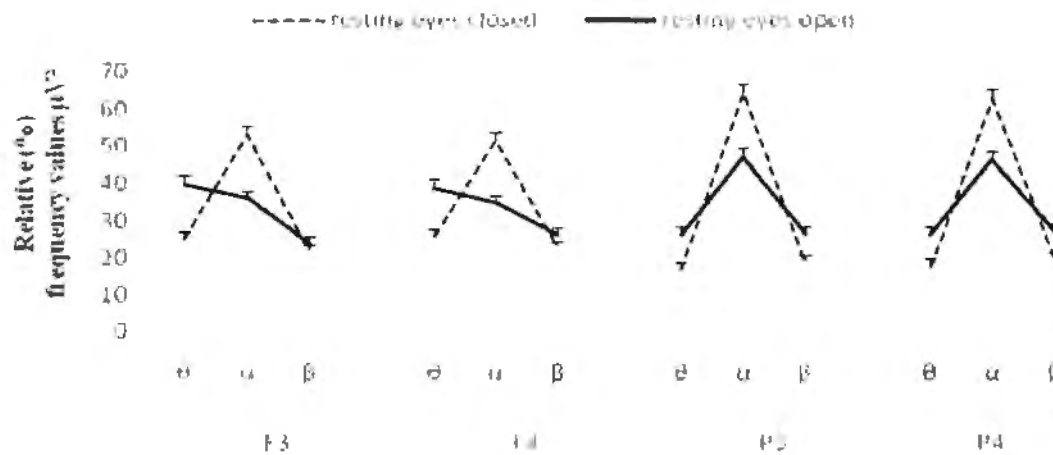


Figure A3.1.4 Relative frequency power during two resting states prior to the cognitive testing; resting eyes open followed by resting eyes closed. All relative frequency measurements differed from eyes open to eyes closed ($p < 0.025$, $n = 47$, mean $\pm$ SEM, median and quartiles appendix A6).

A3.2 Differences between and within the three cognitive tasks

A3.2.1 Response times

Eighty three participants were used in the following analyses, as one participant did not complete the cued target detection task due to software error. This was the only time that this malfunction occurred and does not comprise any of the data collected.

A3.2.1.1 Cognitive tasks response times

For each of the three tasks, response times were summed, and divided by the number of responses to obtain the average response times. Friedman's ANOVA revealed significant differences in response times. Wilcoxon's matched pairs test with Bonferroni correction ($p <$

0.01667) revealed shorter response times for the continuous performance task and Go/No-Go task when compared with the cued target detection task (figure A3.2.1, appendix A7).

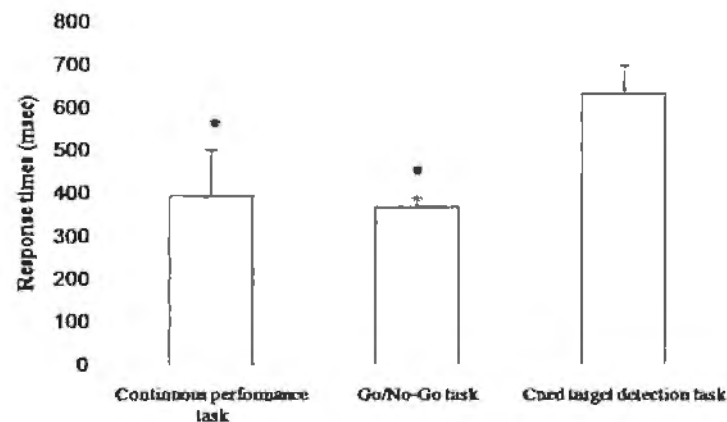


Figure A3.2.1 Average response times for the three cognitive tasks, the *continuous performance task and Go/No-Go task response times were shorter than for the cued target detection task ($p < 0.01667$, $n = 83$, mean $\pm$ SEM (median and quartiles appendix A7)).

Spearman's rank order correlation with Bonferroni correction ($p < 0.01667$) yielded positive correlations between all cognitive tasks for their average response times (table A3.2.1, $p < 0.01$, appendix A7).

Table A3.2.1 Attentional tasks response times relationships

			Spearman	p-level
cued target detection task	continuous performance task	*	0.418538	0.000082
cued target detection task	go/no-go task	*	0.450338	0.000019
continuous performance task	go/no-go task	*	0.398262	0.000192

*Spearman's rank order correlations. Bonferroni correction $p < 0.01667$ $n = 83$. *all conditions significantly correlated*

A3.2.1.2 Response times for the different conditions of the Go/No-Go task

The Go/No-Go task had five conditions, of which two involved stimuli, that required the participants to inhibit their response, the 1st and 2nd no-go conditions. For one of the conditions there was a period of delay prior to the presentation of the stimulus, the Go 3 500 msec condition. Friedman ANOVA revealed significant differences in response times. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.01$) revealed that the 1st go 1 500 msec condition response times were shorter than all other conditions, and that the 2nd go 1 500 msec condition response times were shorter than the remaining conditions' response times (figure A3.2.2, appendix A7).

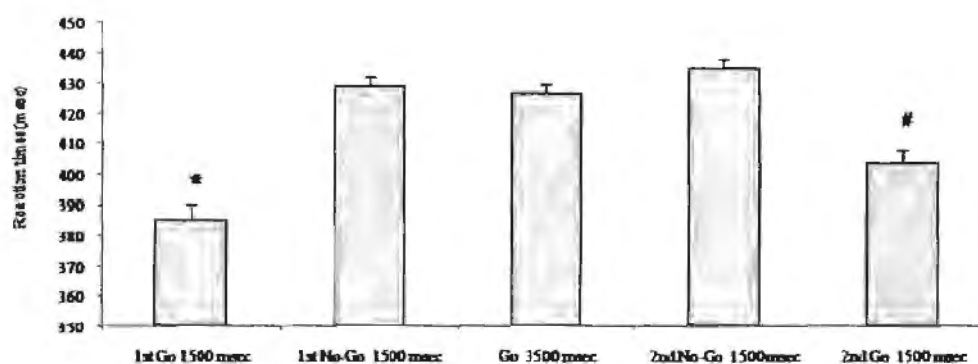


Figure A3.2.2 Response times for the five conditions of the Go/No-Go task, *1st go condition with interstimulus interval of 1 500 msec response times were shorter than all other conditions, #2nd go condition with interstimulus interval of 1 500 msec responses times were shorter for both of the no-go conditions and the 3 500 msec go condition ($p < 0.01$, $n = 83$, mean $\pm$ SEM, median and quartiles appendix A7).

Spearman's rank order correlation with Bonferroni correction ($p < 0.01$) yielded positive correlations between all Go/No-Go task conditions response times (table A3.2.2, appendix A7). Errors made during the two no-go conditions did not differ (figure A3.2.3, appendix A7).

Table A3.2.2. Go/No-Go task conditions response times relationships

				Spearman	p-level
1st go 1 500 msec condition	&	1st no-go condition	*	0.335	0.0020
1st go 1 500 msec condition	&	go 3 500 msec condition	*	0.403	0.0002
1st go 1 500 msec condition	&	2nd no-go condition	*	0.366	0.0007
1st go 1 500 msec condition	&	2nd go 1 500 msec condition	*	0.511	0.0000
1st no-go condition	&	go 3 500 msec condition	*	0.428	0.0001
1st no-go condition	&	2nd no-go condition	*	0.464	0.0000
1st no-go condition	&	2nd go 1 500 msec condition	*	0.559	0.0000
go 3 500 msec condition	&	2nd no-go condition	*	0.475	0.0000
go 3 500 msec condition	&	2nd go 1 500 msec condition	*	0.585	0.0000
2nd no-go condition	&	2nd go 1 500 msec condition	*	0.472	0.0000

*Spearman's rank order correlations, Bonferroni correction $p < 0.01$, $n = 83$, *all conditions significantly correlated (median and quartiles appendix A7)*

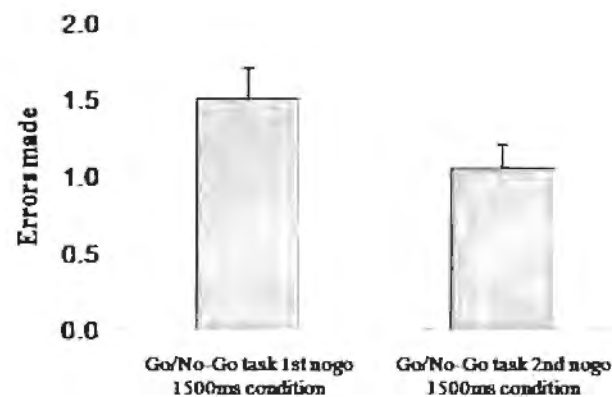


Figure A3.2.3 Number of errors made during the no-go conditions of the Go/No-Go task. No differences between the 1st and 2nd no-go conditions were found ($n = 83$, mean $\pm$ SEM, median and quartiles appendix A7).

A3.2.1.3 Response times for the different conditions of the cued target detection task

The cued target detection task had four different trial conditions, which were interlaced and not serially distinguished. These four trial conditions vary by presentation of different cues. Friedman ANOVA revealed significant differences in response times. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.0125$) revealed that for congruent trials, response times

were shorter than all other trial conditions. For the no-cue trials, response times were longer than all other trial conditions (figure A3.2.4, appendix A7).

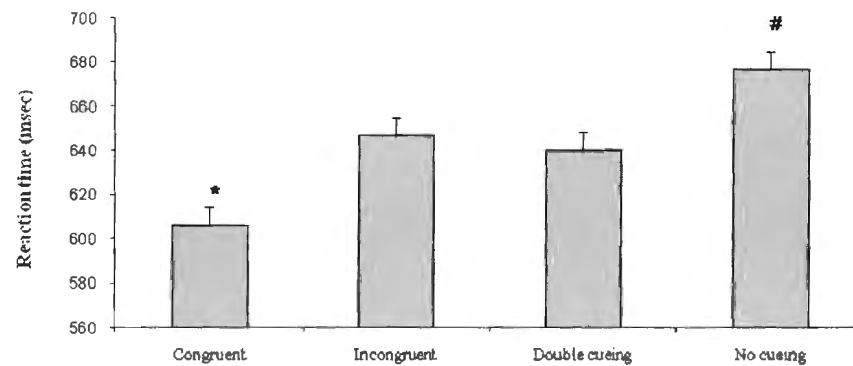


Figure A3.1.8 Response times for the four different trial conditions of the cued target detection task, *congruent trials had shorter response times than all other trial conditions. The absence of a cueing stimulus, in the # no cue trials, resulted in longer response times than all other trial types ($p < 0.0125$, $n = 83$, mean $\pm$ SEM, median and quartiles appendix A7).

Spearman's rank order correlation yielded positive correlations between response times for the different trial conditions of the cued target detection task (table A3.2.3, appendix A7).

Table A3.1.7. Cued target detection task response times relationships

Response times for the cued target detection tasks			Spearman	p-level
congruent	&	incongruent	* 0.879371	$p < 0.0001$
congruent	&	double cue	* 0.915558	$p < 0.0001$
congruent	&	no cue	* 0.799421	$p < 0.0001$
incongruent	&	double cue	* 0.871038	$p < 0.0001$
incongruent	&	no cue	* 0.824881	$p < 0.0001$
double cue	&	no cue	* 0.866777	$p < 0.0001$

*Spearman's rank order correlations, Bonferroni correction $p < 0.0125$, $n = 83$, * all conditions significantly correlated (median and quartiles appendix A7)*

A3.2.2 Skin conductance responses and durations

Eighty-four participants' skin conductance responses and durations are reported. Five participants were 'non'-responders, meaning that they did not reach the lower threshold (0.05 μ mho, Dawson et al., 2001) set in the current analysis protocol for skin conductance response.

A3.2.2.1 Skin conductance responses and durations for the three cognitive tasks

See results section A3.1.5

A3.2.2.2 Skin conductance responses and durations for the Go/No-Go task conditions

Friedman ANOVA carried out on the conditions of the Go/No-Go task revealed significant differences for the values and duration of skin conductance responses. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.0083$) revealed skin conductance durations were longer during the 1st go 1 500 msec condition than for the 1st and 2nd go 3 500 msec conditions. The 1st no-go condition durations were longer than all other conditions except the 2nd no-go condition. The number of skin conductance responses was higher for the 1st no-go condition than both of the go 3 500 msec conditions (figure A3.2.5, appendix A7).

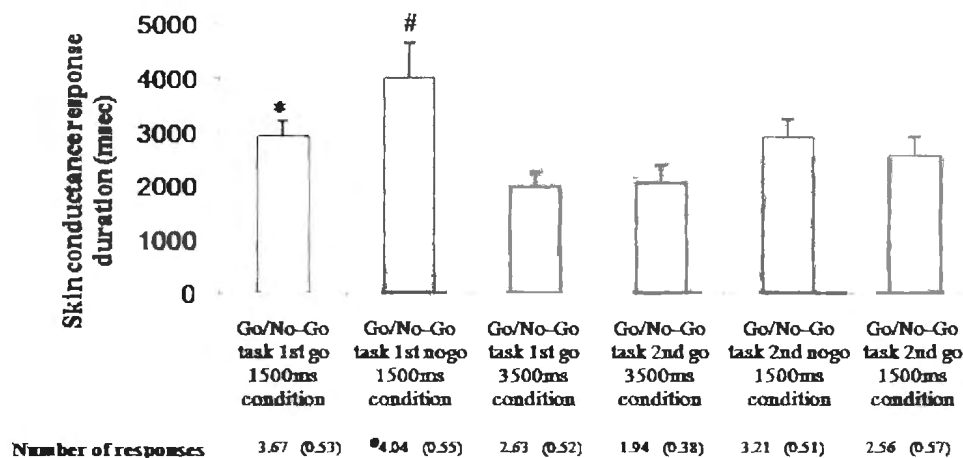
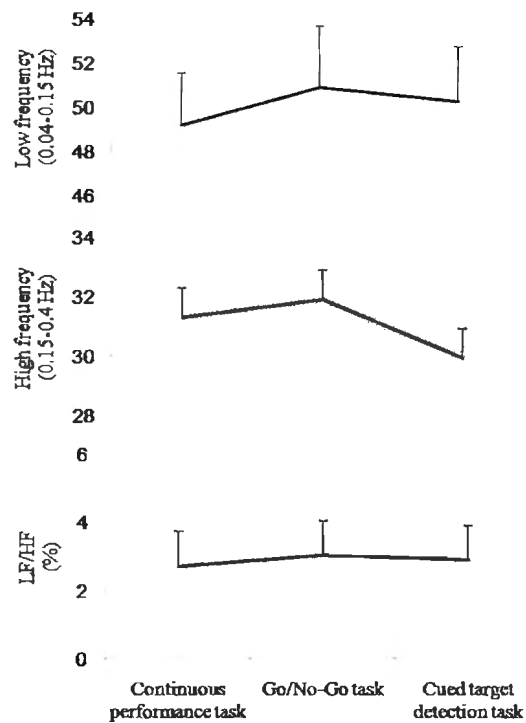


Figure A3.2.5 Skin conductance duration of responses and number of skin conductance responses during the different conditions of the Go/No-Go task. *1st go 1500 msec duration of responses was longer than both of the go 3500 msec conditions. # 1st no-go condition duration of skin conductance responses was longer than all others except the 2nd no-go condition. @ 1st no-go condition number of responses were greater than both of the go 3500 msec conditions ($p < 0.0083$, $n = 83$, mean $\pm$ SEM (median and quartiles appendix A7)).

Friedman ANOVA for heart rate variance parameters: low frequency (0.04 - 0.15 Hz), high frequency (0.15 - 0.4 Hz) and LF/HF ratio, did not yield any significant differences between the three cognitive tasks (figure A3.2.6, appendix A7).



A3.2.6 Heart rate variance parameters for the three cognitive attentional tasks; no differences were found ($n = 76$, mean $\pm$ SEM (median and quartiles appendix A7))

Seventy-six participants were included for heart rate variance analysis, as eight participants were excluded due to errors seen in tachograms. Error in a tachogram is indicative of beats skipped or beats dropped through technical dysfunction.

A3.2.4 Electroencephalogram measures

A3.2.4.1 Relative EEG frequency

A3.2.4.1.1 Relative EEG frequency differences between the three cognitive tasks

Friedman ANOVA revealed differences for relative beta power for frontal electrodes (F3 and F4) and parietal electrodes (P3 and P4). Differences were also found in relative alpha power for parietal electrodes (P3 and P4), and beta to theta ratio for frontal electrodes (F3 and F4). Wilcoxon's matched pairs test with Bonferroni correction ($p < 0.01667$) revealed that frontal electrodes (F3 and F4) relative beta activity were significantly higher for the cued target detection task than for both the continuous performance task and the Go/No-Go task. In addition relative beta power for the continuous performance task was higher than during the Go/No-Go task for frontal electrodes. Relative alpha power for parietal electrodes (P3 and P4) was higher for the Go/No-Go task when compared to the continuous performance task and the cued target detection task. (figure A3.2.7, appendix A7). Note on relative theta being different, however showing high frontal. Theta to beta ratio was higher for the Go/No-Go and the continuous performance task than for the cued target detection task (figure A3.2.8, appendix A7).

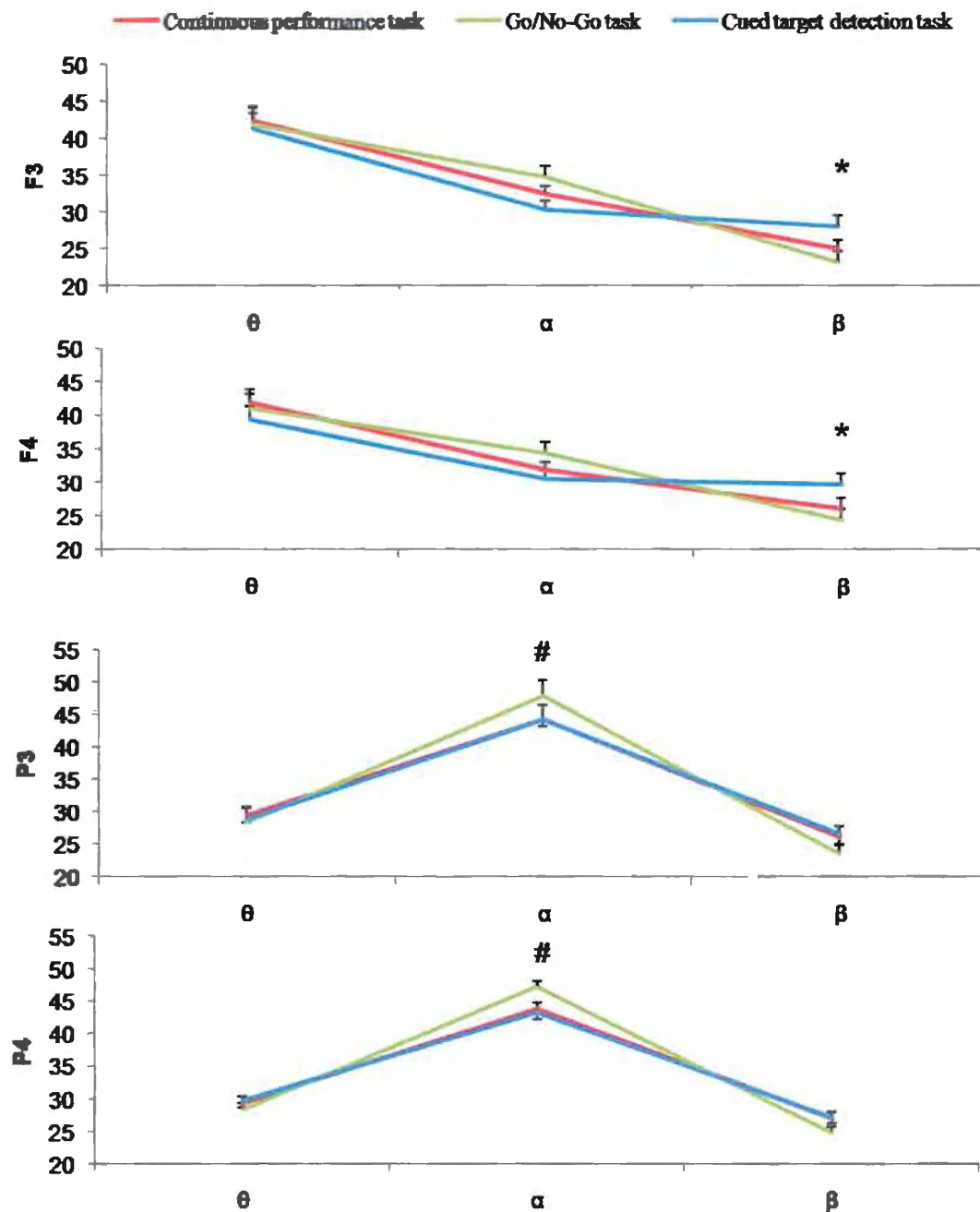


Figure A3.2.7 Frontal and parietal electrodes' relative frequency power for the three cognitive tasks. Frontal electrodes (F3 and F4) *relative beta power for cued target detection task higher than continuous performance task and Go/No-Go task, and the continuous performance task relative beta power was higher than for the Go/No-Go task. Parietal electrodes (P3 and P4) #relative alpha power for Go/No-Go task higher than continuous performance task and cued target detection task ($p < 0.01667$, $n = 46$, mean $\pm$ SEM, median and quartiles in appendix A7).

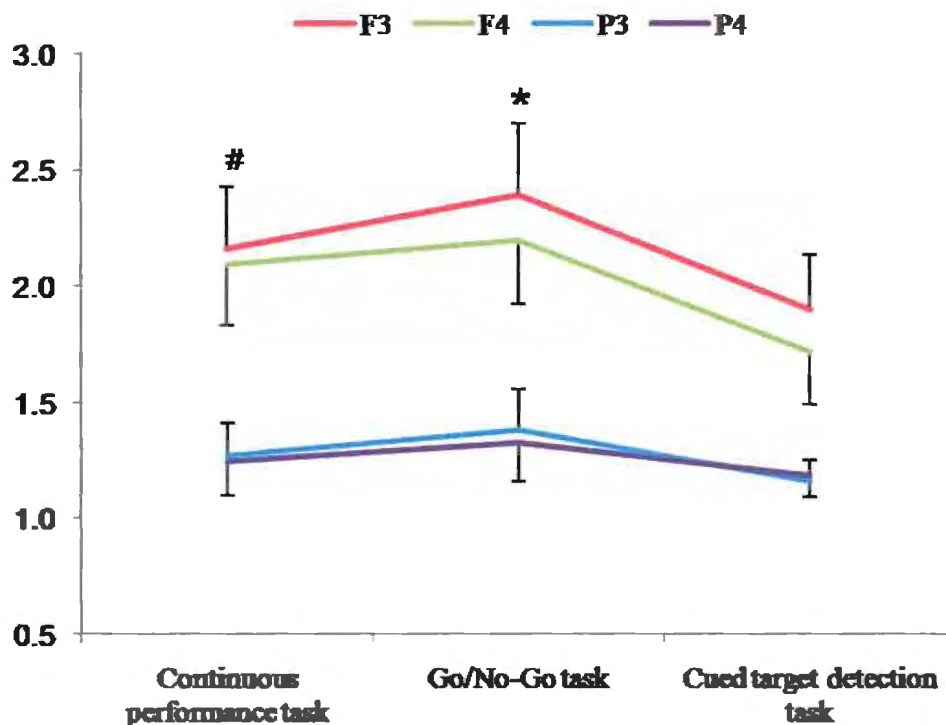


Figure A3.2.8 Relative theta to beta ratios for the three attentional cognitive tasks. *F3 theta/beta ratio for cued target detection was lower than for the continuous performance task and Go/No-Go task. Frontal electrodes (F3 and F4) *relative theta/beta ratio for the Go/No-Go task was higher than continuous performance task and cued target detection task, # the continuous performance task theta/beta ratio was higher than cued target detection task ($p < 0.01667$, $n = 46$, mean $\pm$ SEM (median and quartiles appendix A7)).

A3.2.4.1.2 Relative EEG frequency differences between conditions of the Go/No-Go task

Friedman ANOVA carried out on the relative frequencies of the Go/No-Go task conditions revealed significant differences for relative frequency powers for frontal (F3 and F4) and parietal (P3 and P4) electrodes. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.01$) revealed that F3 relative theta power for the go 3 500 msec was higher than the 1st go 1 500 msec and 1st no-go conditions (table A3.2.4, appendix A7). F3 relative alpha power for the 1st go 1 500 msec was significantly lower than the go 3 500 msec, 2nd no-go and the 2nd go 1 500 msec

conditions. F3 relative alpha power for the 1st no-go was significantly lower than the go 3 500 msec condition. F4 relative theta power was lower during the 1st go 1 500 msec and the 1st no-go condition was lower than the go 3 500 msec condition. F4 relative alpha power during the 1st go 1 500 msec and 1st no-go were lower than during the go 3 500 msec condition. F4 relative beta power for the 1st go 1 500 msec was higher than for the 2nd no-go and 2nd go 1 500 msec conditions.

P3 relative theta power was higher for the 1st go 1 500 msec and the 1st no-go condition than for go 3 500 msec condition. P3 relative alpha power was higher for the go 3 500 msec and the 2nd no-go condition than the 1st go 1 500 msec and 1st no-go conditions. P3 relative alpha power for the go 3 500 msec was higher than the 2nd no-go and 2nd go 1 500 msec conditions. P3 relative beta power was higher for the 1st go 1 500 msec than all other conditions. P4 relative theta power for the 1st go 1 500 msec and 1st no-go was higher than go 3 500 msec condition. P4 relative alpha power for the 1st go 1 500 msec and 1st no-go was lower than go 3 500 msec, 2nd no-go condition, and the 2nd go 1 500 msec conditions. P4 relative alpha power for the go 3 500 msec condition was higher than the 2nd no-go condition. P4 relative beta power for the 1st no-go condition was higher than go 3 500 msec condition (table A3.2.4, appendix 7). Relative theta to beta ratios revealed no differences (table A3.2.5, appendix 7).

Table A3.2.4 Relative frequencies for the conditions of the Go/No-Go task

Relative frequency power		Go/No-Go task 1st go 1500ms condition		Go/No-Go task 1st no-go 1500ms condition		Go/No-Go task 1st go 3500ms condition		Go/No-Go task 2nd no-go 1500ms condition		Go/No-Go task 2nd go 1500ms condition		
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
F3	θ	43.02	2.01	43.31	2.04	*	38.92	2.07	41.19	2.16	41.38	2.22
	α	@ 31.05	1.12	32.79	1.32	*	36.62	1.55	# 33.71	1.33	34.73	1.70
	β	25.93	1.40	23.91	1.33		24.46	1.47	25.10	1.60	23.90	1.39
F4	θ	40.61	2.07	41.90	2.12	*	38.23	2.09	40.50	2.09	40.94	2.35
	α	31.74	1.35	32.56	1.44	*	36.21	1.61	# 33.63	1.43	34.54	1.72
	β	@ 27.65	1.67	25.55	1.69		25.56	1.56	25.87	1.71	24.51	1.53
P3	θ	28.47	1.84	29.87	2.12	*	24.60	1.86	27.20	1.89	28.21	2.40
	α	§ 42.47	2.07	43.83	2.06	*	50.50	2.44	# 46.77	2.18	† 45.89	2.67
	β	‡ 29.06	1.39	26.30	1.39		24.89	1.45	26.03	1.49	25.89	1.65
P4	θ	28.32	1.87	29.19	1.98	*	25.19	1.97	27.40	1.87	27.45	2.43
	α	@ 42.26	2.05	" 42.70	1.98	*	49.19	2.43	# 45.64	2.11	46.21	2.70
	β	‡ 29.42	1.45	28.11	1.48	*	25.63	1.47	26.96	1.51	26.34	1.72

Wilcoxon matched pairs test, Bonferroni correction, p -value < 0.01, $n = 48$, *go 3500ms condition different from 1st go 1500ms condition and 1st no-go condition, # 2nd no-go condition different from go 3500ms condition, @ 1st go 1500ms condition different from 2nd no-go condition and 2nd go condition, † 2nd go condition different from go 3500ms condition, § 1st go 1500ms condition different from 2nd no-go condition, ‡ 1st no-go condition different from 2nd no-go condition and 2nd go 1500ms condition, ‡ 1st go 1500ms condition different from all (medians and quartiles appendix A7)

Table A3.2.5 Relative theta/beta ratios for the different conditions of the Go/No-Go task

Relative theta/beta ratio		Go/No-Go task 1st go 1500ms condition		Go/No-Go task 1st nogo 1500ms condition		Go/No-Go task go 3500ms condition		Go/No-Go task 2nd nogo 1500ms condition		Go/No-Go task 2nd go 1500ms condition	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
F3		2.30	0.32	2.52	0.34	2.19	0.29	2.42	0.36	2.80	0.69
F4		2.09	0.33	2.55	0.40	2.09	0.28	2.23	0.30	2.94	0.83
P3		1.13	0.13	1.35	0.21	1.21	0.20	1.28	0.18	1.77	0.67
P4		1.20	0.19	1.55	0.26	1.22	0.20	1.33	0.19	1.79	0.64

Wilcoxon matched pairs test, Bonferroni correction, p -value < 0.01, $n = 48$, no significant differences were found (medians and quartiles appendix A7)

A3.2.4.2 Event-related potentials

Event-related potentials were extracted for all three cognitive tasks and for the different conditions of the Go/No-Go tasks. Additionally the P300 component amplitude and latency were extracted for the parietal electrodes.

A3.2.4.2.1 Event-related potentials for the continuous performance task

Two cues were presented followed by the stimulus for the continuous performance task. Event related potentials were extracted from all ten electrodes to visualize the cueing process. These ERPs were baseline corrected for the 100 msec prior to the 1st cueing stimulus (figure A3.2.9).

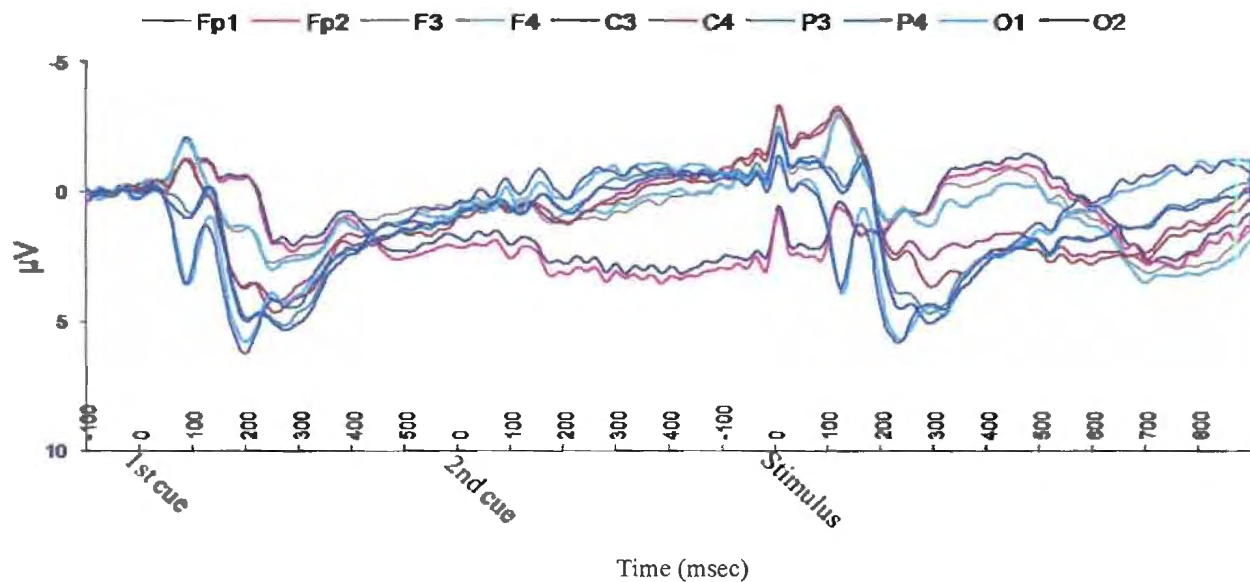


Figure A3.2.9 Continuous performance task ERPs with two cues and stimulus, for the ten scalp electrodes (n = 51, mean).

P300 amplitudes and latencies were extracted from the ERPs relative to stimulus presentation and baseline corrected to 100 msec prior to stimulus presentation (table A3.2.6, figure A3.2.10).

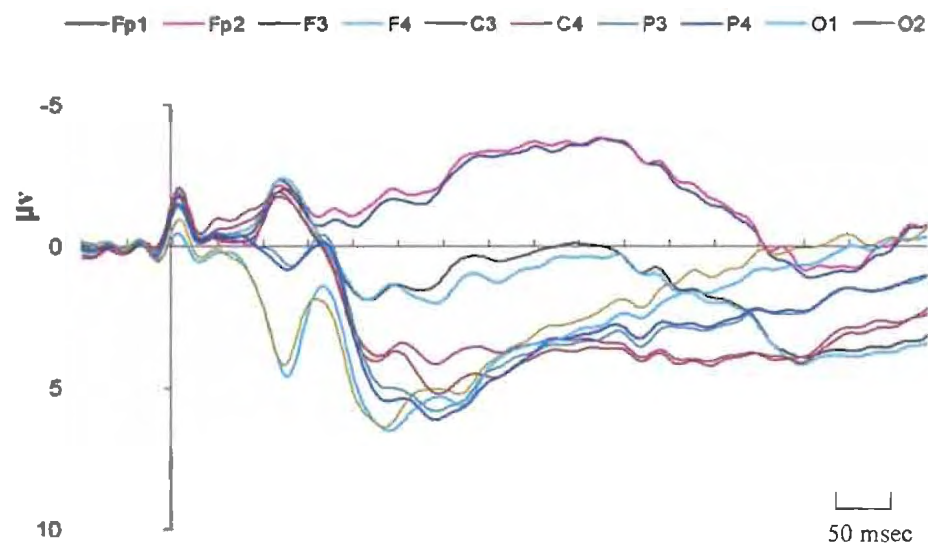


Figure A3.2.10 Continuous performance task ERPs relative to the stimulus and baseline corrected, for the ten scalp electrode (n = 51, mean).

Table A3.2.6 P300 amplitudes and latencies for the continuous performance task ERPs

	Amplitude P300 (µV)		Latency P300 (msec)	
	Mean	SEM	Mean	SEM
P3	7.15	0.50	307.22	10.07
P4	7.26	0.52	297.92	8.19
<i>n = 51 (mean ± SEM)</i>				

A3.2.4.2.2 Event-related potentials Go/No-Go task

Event related potentials were extracted from all ten electrodes. P300 amplitudes and latencies were extracted from the ERPs relative to stimulus presentation and baseline corrected to 100 msec prior to stimulus presentation (figure A3.2.11).

The P300 amplitude and latencies were extracted from the ERP waveforms for the parietal electrodes. Friedman ANOVA carried out on parietal P300 amplitudes and latencies found for the Go/No-Go task conditions revealed significant differences for P300 amplitudes and latencies for parietal ERP waveforms. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.00714$) revealed that P300 amplitudes were higher in 1st and 2nd no-go condition trials for stimuli that required inhibition of response than 1st and 2nd go 1500 msec conditions and the go 3500 msec condition. The P300 amplitude of the 1st go 1500 msec condition was smaller than the 2nd no-go conditions. For the P4 P300 amplitude the 1st no-go condition trials P300 amplitudes were smaller than the no-go condition trials P300 amplitudes which required inhibition of response. Wilcoxon matched pairs test with Bonferroni correction ($p < 0.00714$) revealed that parietal P300 latencies were greater for the 1st and 2nd no-go trials that required inhibition of response than the 1st and 2nd go 1500 msec condition, and the 3500 msec condition. The 1st go 1500 msec condition P300 latency was earlier than for the 1st no-go condition P300 latency. In addition the P3 parietal electrode P300 latency for the 2nd go 1500 msec condition was earlier than the 1st no-go condition P300 latency (table A3.2.6, appendix A9).

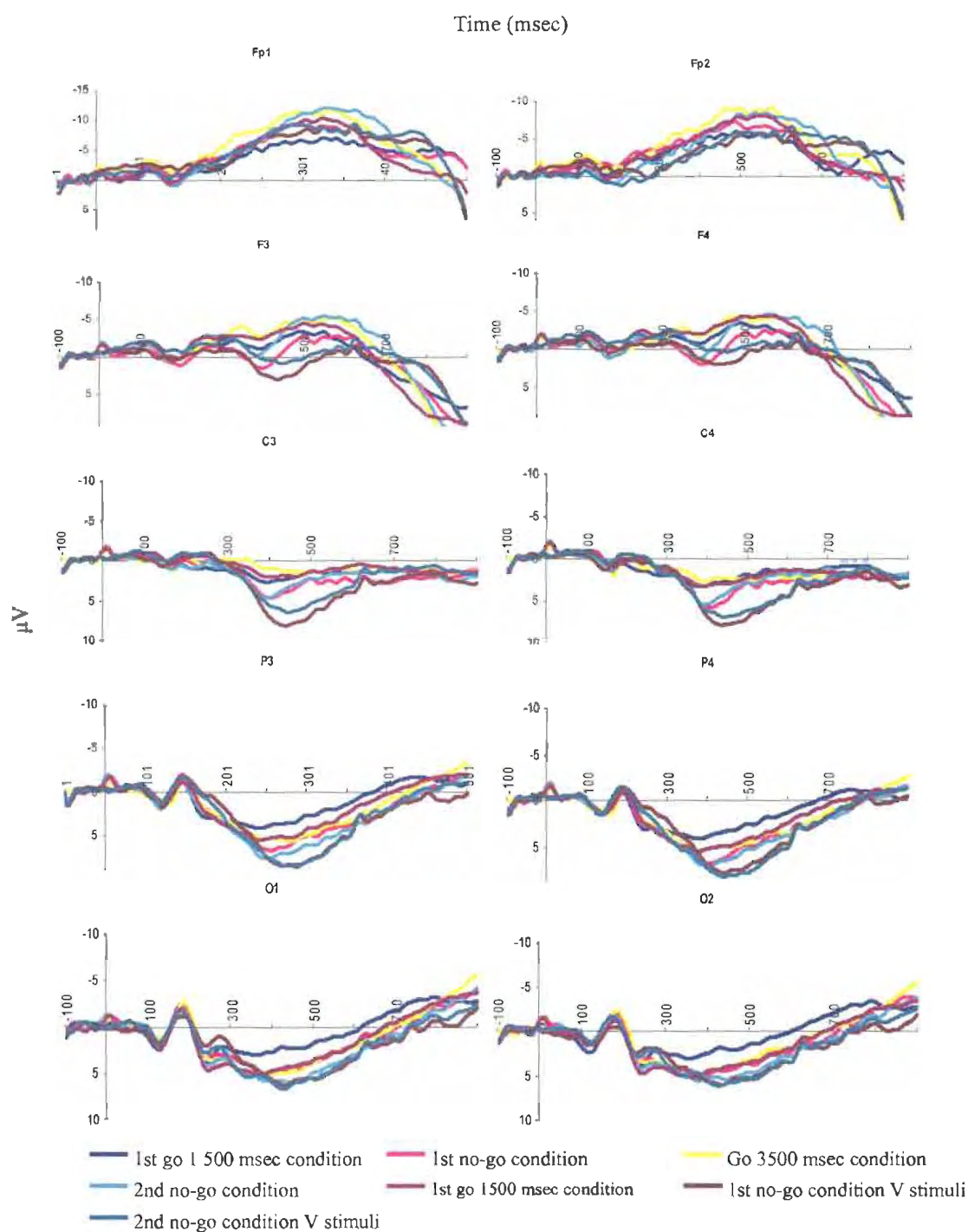


Figure A3.2.11 ERPs in the Go/No-Go task conditions: 1st and 2nd go 1 500 msec condition, Go 3 500 msec condition, 1st and 2nd no-go condition and the 1st and 2nd no-go condition trials that required inhibition of response, for the ten scalp electrodes (n = 48, mean).

A3.2.4.2.3 ERPs in the cued target detection task

ERPs were extracted from all ten electrodes. P300 amplitudes and latencies were extracted from the ERPs relative to stimulus presentation and baseline corrected to 100 msec prior to stimulus presentation (figure A3.2.12).

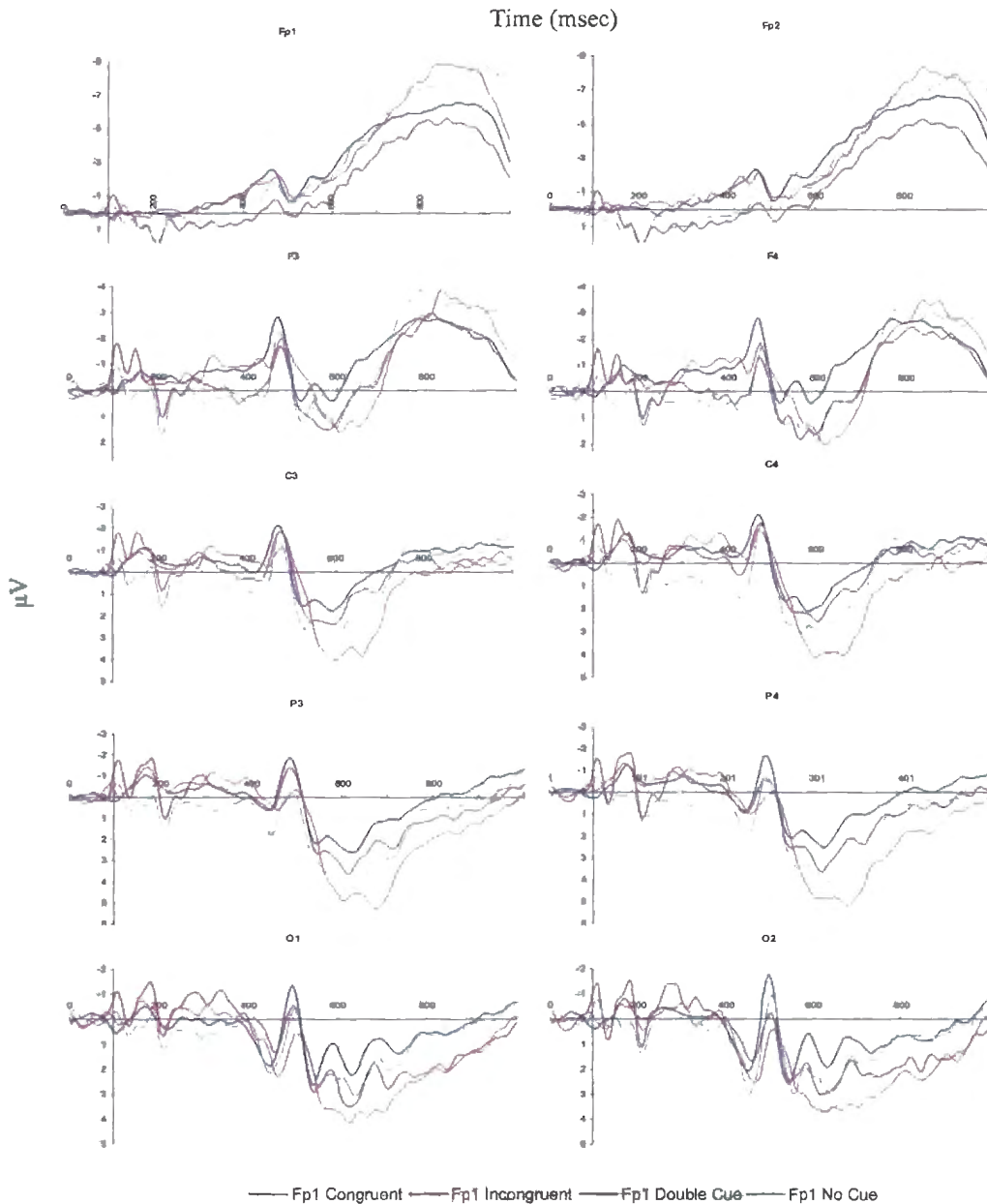


Figure A3.2.12 Cued target detection task ERPs for different trial types: congruent trials, incongruent trials, double cue trials, and no-cue trials, for the ten scalp electrodes ($n = 51$, mean).

Only the congruent trials P300 amplitudes and latencies are reported (table A3.2.7, appendix A9).

Table A3.2.7 Amplitudes and latencies of the parietal P300 for the cued target detection task congruent trials

	Amplitude P300 (μ V)		Latency P300 (msec)	
	Mean	SEM	Mean	SEM
P3	4.17	0.37	536.8	9.03
P4	4.03	0.35	523.1	7.42

n = 51 (*mean* $\pm$ *SEM*)

A3.3 Reported mental effort during the three attentional tasks and their physiological correlates

Wilcoxon matched pairs test with Bonferroni correction for mental effort during the completion of the three cognitive tasks revealed increased mental effort during the Go/No-Go task (table A3.3.1, appendix A8).

Table A3.3.1 Reported mental effort differences for the three cognitive tasks

	Continuous performance task			Go/No-Go task			Cued target detection task	
	Mean	SEM		Mean	SEM		Mean	SEM
Mental effort (visual analogue, cm)	2.85	0.32	*	4.91	0.37		3.60	0.34

*Wilcoxon matched pairs test, Bonferroni correction, significance p -value < 0.01667, $n = 46$, * different to all tasks*

Spearman's rank order correlation analysis was performed to determine relationships between required mental effort and physiological measures during the completion of each of the cognitive tasks. Without Bonferroni correction ($p < 0.05$), mental effort during the Go/No-Go task yielded relationships for heart rate variance parameters; high frequency and low frequency to high frequency ratio. Relative theta frequency power of F3 was negatively correlated with mental effort of the Go/No-Go task and the cued target detection task. Relative beta frequency power of F3 was positively correlated with the perceived mental effort during the cued target detection task. Relative theta power of F4 was negatively correlated with the perceived mental effort during the cued target detection task. Relative beta power of F4 was positively correlated with the mental effort required during the cued target detection task. Relative beta power of the P3 was positively correlated with the perceived mental effort during all three of the attentional tasks. Relative beta power of the P4 was positively correlated with the mental effort required for the

Go/No-Go task. The relative theta to beta ratio for F3 and F4 were negatively correlated with the perceived mental effort during the cued target detection task and relative theta with beta ratio for the P3 was negatively correlated with the perceived mental effort during the Go/No-Go task and during the cued target detection task (table A3.3.2, appendix A8). With Bonferroni correction ($p < 0.01667$) relative theta frequency power of F3 was negatively correlated with the perceived mental effort during the Go/No-Go task and during the cued target detection task. Relative beta frequency power of F3 was positively correlated with the perceived mental effort during the cued target detection task. Relative beta power of the P3 was positively correlated with the perceived mental effort during the Go/No-Go task and during the cued target detection task. Relative beta power of the P4 was positively correlated with the mental effort during the Go/No-Go task. The relative theta to beta ratio of for F3 and F4 were negatively correlated with the perceived mental effort during the cued target detection task and relative theta to beta ratio for the P3 was negatively correlated with the perceived mental effort during the cued target detection task.

Table A3.3.2 Perceived mental effort during three attentional tasks and their physiological correlates

Correlates to Mental Effort	Continuous performance task		Go/No-Go task		Cued target detection task	
	Spearman n	p-level	Spearman	p-level	Spearman n	p-level
Skin conductance response	-0.21	0.170	-0.02	0.907	0.01	0.946
Skin conductance response duration (ms)	-0.13	0.390	0.00	0.976	-0.12	0.436
Heart Rate	-0.08	0.608	0.11	0.454	0.01	0.972
Heart rate variance low frequency	0.12	0.447	0.26	0.085	0.17	0.248
Heart rate variance high frequency	0.03	0.819	* -0.32	0.030	-0.24	0.101
Heart rate variance LF/HF	0.07	0.647	* 0.34	0.022	0.25	0.090
Frontal electrode F3 relative theta power	-0.09	0.533	* -0.37	0.011	* -0.33	0.024
Frontal electrode F3 relative alpha power	-0.08	0.588	0.21	0.160	0.11	0.470
Frontal electrode F3 relative beta power	0.08	0.590	0.24	0.115	** 0.39	0.008
Frontal electrode F4 relative theta power	0.11	0.454	-0.21	0.166	* -0.34	0.021
Frontal electrode F4 relative alpha power	-0.10	0.523	0.10	0.522	-0.02	0.912
Frontal electrode F4 relative beta power	-0.06	0.696	0.25	0.092	** 0.48	0.001
Parietal electrode P3 relative theta power	0.04	0.795	-0.16	0.275	-0.18	0.232
Parietal electrode P3 relative alpha power	-0.27	0.065	0.01	0.949	-0.03	0.819
Parietal electrode P3 relative beta power	* 0.29	0.047	* 0.38	0.009	** 0.40	0.006
Parietal electrode P4 relative theta power	0.01	0.936	-0.17	0.258	-0.23	0.126
Parietal electrode P4 relative alpha power	-0.22	0.143	-0.01	0.944	0.13	0.399
Parietal electrode P4 relative beta power	0.18	0.221	* 0.37	0.010	0.21	0.157
Frontal electrode F3 relative theta/beta ratio	-0.09	0.536	-0.27	0.071	** -0.39	0.007
Frontal electrode F4 relative theta/beta ratio	0.10	0.502	-0.21	0.166	** -0.44	0.002
Parietal electrode P3 relative theta/beta ratio	-0.16	0.288	* -0.30	0.046	** -0.40	0.006
Parietal electrode P4 relative theta/beta ratio	-0.11	0.451	-0.27	0.064	-0.27	0.069

*Spearman's rank order correlations between perceived mental effort and physiological parameters measured during the three attentional tasks: the continuous performance task, the Go/No-Go task and the cued target detection task, n = 46, * significant correlates (p < 0.05) with perceived mental effort during the performance of the three attentional tasks, with Bonferroni correction (p < 0.01667) **significant correlates with required mental effort during the performance of the three attentional tasks*

A3.4 Impulsivity and its relationship with response times, errors and physiological parameters during the three attentional tasks

A3.4.1 Three cognitive tasks and scales of impulsivity

Spearman's rank order correlations were performed to determine which of the three tasks were correlated with Dickman's scale of impulsivity, which was divided into two subscales of (1) functional impulsivity and (2) dysfunctional impulsivity. Significant correlations with dysfunctional impulsivity for several of the Go/No-Go task conditions: the 1st go 1500 msec condition, the 1st go 3500 msec condition, and the 1st no-go condition (table A3.4.1, appendix A9).

Table A3.4.1 Dickman's subscales of functional and dysfunctional impulsivity and their relationships to response times during the three cognitive tasks and their conditions/trials.

	<i>Correlates with Dickman's scale of impulsivity</i>			
	Functional impulsivity		Dysfunctional impulsivity	
	Spearman	<i>p-level</i>	Spearman	<i>p-level</i>
Cued target detection congruent response times	0.11	0.329	-0.01	0.900
Cued target detection incongruent response times	0.17	0.142	-0.01	0.932
Cued target detection double cue response times	0.11	0.332	-0.03	0.825
Cued target detection double cue response times	0.08	0.516	-0.04	0.709
Continuous performance response times	0.05	0.695	-0.17	0.147
Go/No-Go task 1st go 1500ms condition response times	0.10	0.385	* -0.30	0.010
Go/No-Go task 1st no-go 1500ms condition response times	-0.09	0.419	0.10	0.385
Go/No-Go task 1st go 3500ms condition response times	-0.02	0.868	* -0.25	0.031
Go/No-Go task 2nd go 3500ms condition response times	0.03	0.787	-0.05	0.694
Go/No-Go task 2nd no-go 1500ms condition response times	0.09	0.422	0.14	0.229
Go/No-Go task 2nd go 1500ms condition response times	0.13	0.280	-0.08	0.491
Go/No-Go task 1st no-go condition errors	0.00	0.977	* 0.32	0.004
Go/No-Go task 2nd no-go condition errors	-0.08	0.470	0.08	0.499

*Spearman's rank order correlation, p-value < 0.05, n = 48, * significant correlations with Dickman's scale of dysfunctional impulsivity*

A3.4.2 Three cognitive tasks and errors in the Go/No-Go task

Spearman's rank order correlation analysis was performed to determine whether reaction times during the three attentional tasks and their different conditions/trials were related to the number of errors made during the no-go conditions of the Go/No-Go task. Significant correlations were found. The response times of the continuous performance task were negatively correlated with the number of errors made during the no-go conditions. The response times for the congruent trials during the cued target detection task were negatively correlated with the errors made during the 1st no-go condition. The response times for all of the Go/No-Go task conditions were negatively correlated with the number of errors made during the no-go conditions of the Go/No-Go task, except for the response times during the 2nd no-go condition (table A3.4.2, appendix A9).

Table A3.4.2 Response time correlations with errors made during the no-go conditions of the Go/No-Go task

	<i>Go/No-Go task Errors</i>			
	1st no-go condition		2nd no-go condition	
	Spearman	<i>p-level</i>	Spearman	<i>p-level</i>
Cued target detection congruent response times	* -0.26	0.024	-0.07	0.545
Cued target detection incongruent response times	-0.21	0.069	-0.09	0.439
Cued target detection double cue response times	-0.17	0.146	-0.03	0.800
Cued target detection double cue response times	-0.18	0.125	-0.04	0.763
Continuous performance response times	* -0.38	0.001	* -0.30	0.009
Go/No-Go task 1st go 1500ms condition response times	* -0.45	0.000	* -0.30	0.008
Go/No-Go task 1st no-go 1500ms condition response times	-0.25	0.032	* -0.33	0.003
Go/No-Go task 1st go 3500ms condition response times	* -0.34	0.003	* -0.36	0.001
Go/No-Go task 2nd go 3500ms condition response times	-0.24	0.036	* -0.47	0.000
Go/No-Go task 2nd no-go 1500ms condition response times	-0.23	0.049	-0.19	0.108
Go/No-Go task 2nd go 1500ms condition response times	-0.28	0.014	-0.25	0.032

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.025, n = 48, * significant correlations with the 1st or 2nd no-go conditions errors made during the Go/No-Go task*

A3.4.3 Go/No-Go task response time correlates with physiological parameters

Spearman's rank order correlation analysis was performed to determine whether response times during the various conditions of the Go/No-Go task were related to physiological parameters. Without Bonferroni correction ($p < 0.05$), for the response times during the 1st Go 1 500 msec condition was correlated with: (1) F3 relative theta power positively, (2) F3 relative alpha power negatively, and (3) F3 theta/beta ratio positively. For the response times during the 1st no-go condition was correlated with: (1) P4 relative alpha power negatively and (2) P4 relative beta power positively. For the response times during the 1st go 3 500 msec condition were correlated with: (1) F3 relative beta power positively, (2) P3 relative beta power positively, and (3) P4 relative beta power positively. For the number of errors made during the 1st no-go condition was negatively correlated with P4 P300 amplitude. With Bonferroni correction ($p < 0.0083$) response times during the 1st Go 1 500 msec condition were correlated with: (1) F3 relative theta power positively, and (2) F3 relative alpha power negatively.

Table A3.4.3 Go/No-Go task conditions response times and errors made correlations with physiological parameters

	Go/No-Go task								
	Go/No-Go task 1st go 1500ms response times	Go/No-Go task nogo 1500ms response times	Go/No-Go task 1st go 3500ms response times	Go/No-Go task 2nd go 3500ms response times	2nd nogo 1500ms response times	Go/No-Go task 2nd go 1500ms response times	Go/No-Go task 1st nogo 1500ms errors	Go/No-Go task 2nd nogo 1500ms errors	
	p-level	p-level	p-level	p-level	p-level	p-level	p-level	p-level	
Skin conductance responses	-0.01 0.118	-0.08 0.573	0.12 0.426	0.04 0.771	0.15 0.312	0.02 0.919	0.13 0.370	0.05 0.712	
Skin conductance durations (ms)	-0.25 0.150	0.07 0.640	0.15 0.310	0.02 0.901	0.00 0.995	-0.11 0.464	0.00 0.976	0.00 1.000	
Relative frequency power									
Frontal electrode F3 theta	** 0.47 0.001	0.01 0.961	0.23 0.117	0.15 0.307	0.10 0.518	0.21 0.159	0.26 0.076	0.08 0.569	
Frontal electrode F3 alpha	** -0.47 0.001	-0.16 0.263	0.02 0.915	0.07 0.614	-0.17 0.242	-0.22 0.125	0.11 0.447	0.02 0.896	
Frontal electrode F3 beta	-0.26 0.069	0.18 0.228*	0.34 0.017	0.18 0.214	0.19 0.197	-0.11 0.467	0.21 0.161	0.08 0.581	
Frontal electrode F4 theta	0.26 0.071	0.11 0.469	-0.12 0.430	-0.10 0.514	0.01 0.922	0.21 0.143	-0.10 0.510	0.00 0.994	
Frontal electrode F4 alpha	-0.23 0.112	-0.06 0.669	0.12 0.427	0.14 0.326	-0.20 0.165	-0.18 0.222	0.07 0.652	-0.08 0.595	
Frontal electrode F4 beta	-0.24 0.108	0.05 0.761	0.19 0.193	0.08 0.602	0.21 0.148	-0.12 0.398	0.08 0.611	0.10 0.488	
Parietal electrode P3 theta	0.14 0.335	0.03 0.836	0.04 0.801	0.04 0.772	0.02 0.892	0.13 0.379	-0.06 0.698	-0.14 0.340	
Parietal electrode P3 alpha	-0.22 0.125	-0.20 0.178	-0.20 0.170	-0.04 0.799	-0.17 0.251	-0.13 0.366	0.08 0.567	0.14 0.354	
Parietal electrode P3 beta	0.07 0.628	0.19 0.193*	0.29 0.048	0.02 0.890	0.20 0.175	0.12 0.427	0.06 0.700	0.04 0.789	
Parietal electrode P4 theta	0.13 0.388	0.00 0.985	0.08 0.610	0.01 0.922	0.08 0.576	0.09 0.523	0.02 0.914	-0.07 0.652	
Parietal electrode P4 alpha	-0.16 0.272	* -0.29 0.042	-0.24 0.096	-0.06 0.691	-0.18 0.217	-0.13 0.382	0.01 0.951	0.08 0.583	
Parietal electrode P4 beta	-0.04 0.802	* 0.30 0.037*	0.29 0.049	0.05 0.719	0.23 0.123	0.10 0.516	0.08 0.578	0.05 0.757	
Relative theta/beta ratio									
Frontal electrode F3	* 0.37 0.011	-0.11 0.456	-0.28 0.052	-0.14 0.347	-0.13 0.389	0.20 0.177	-0.28 0.057	-0.11 0.454	
Frontal electrode F4	0.24 0.098	0.03 0.817	-0.14 0.355	-0.09 0.532	-0.13 0.394	0.20 0.165	-0.10 0.494	-0.06 0.661	
Parietal electrode P3	0.11 0.448	-0.17 0.234	-0.12 0.400	-0.04 0.767	-0.08 0.568	0.04 0.782	-0.06 0.701	-0.07 0.653	
Parietal electrode P4	0.10 0.505	-0.08 0.575	-0.21 0.142	0.02 0.871	-0.08 0.582	0.05 0.727	-0.09 0.526	-0.13 0.374	
Event-related Potential P3 component									
Parietal electrode P3 amplitude	0.13 0.373	0.15 0.304	-0.04 0.812	-0.04 0.788	-0.23 0.118	-0.07 0.627	-0.11 0.465	0.07 0.644	
Parietal electrode P3 latency	0.10 0.520	-0.09 0.552	0.01 0.920	-0.01 0.951	0.15 0.304	0.03 0.846	-0.12 0.405	0.02 0.902	
Parietal electrode P4 amplitude	0.21 0.152	-0.03 0.862	-0.05 0.741	-0.05 0.736	-0.16 0.277	0.09 0.532*	-0.29 0.050	0.09 0.538	
Parietal electrode P4 latency	0.16 0.275	0.04 0.789	0.00 0.983	0.00 0.999	0.03 0.842	-0.02 0.904	-0.18 0.223	0.13 0.394	

Spearman's rank order correlations between response times and errors with physiological parameters measured during the Go/No-Go task conditions, $n=43$, * significant correlates ($p < 0.05$) with response times and errors made in conditions of the Go/No-Go task, with Bonferroni correction ** significant correlates with response times of the Go/No-Go task

A3.4.4 Dickman's scale of impulsivity and correlates with additional personality questionnaires

Spearman's correlation analysis was performed to determine whether Dickman's scale of impulsivity employed in the present study correlated with other measures of personality traits within the present 'healthy' cohort of participants. The Abbreviated Eysenck personality questionnaire subscale of extraversion correlated positively with reported impulsivity on both subscales of Dickman's impulsivity questionnaire.

Table A3.4.4 Dickman's scale of impulsivity, functional and dysfunctional impulsivity correlates with additional personality measures employed in the present study

Dickman's scale of impulsivity						
	Functional impulsivity		Dysfunctional impulsivity			
	Spearman	p-level	Spearman	p-level		
Sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ)						
Sensitivity to reward	0.23	0.113	0.24		0.100	
Sensitivity to punishment	-0.03	0.841	0.03		0.848	
Abbreviated Eysenck personality questionnaire (EPQR-A)						
Extraversion	*	0.36	0.012	*	0.35	0.017
Lie Scale		0.25	0.085		0.01	0.925
Psychoticism		0.08	0.591		-0.04	0.805
Neuroticism		0.02	0.873		0.06	0.704
Cloningers tridimensional personality questionnaire subscales						
Novelty seeking		0.24	0.105		0.24	0.102
Harm avoidance		-0.15	0.312		-0.02	0.895

*Spearman's rank order correlation, p-value < 0.025, n = 48, * significant correlations with subscales of Dickman's scale of impulsivity*

A3.5 Reported levels of anxiety and their physiological correlates during a sustained attention task

A3.5.1 Scales of anxiety and their physiological correlates during the continuous performance task

Spearman's rank order correlation analysis was performed to determine whether the scales employed to measure anxiety were related. Significant correlations between all three scales of anxiety employed: (1) Kessler distress scale, (2) Spielberger's trait anxiety present, and (3) Spielberger's trait anxiety absent (table A3.5.1, appendix A10).

Table A3.5.1 Relationship of anxiety scales with each other

		Spearman	p-level
Kessler distress scale	& Spielberger's trait anxiety scale - anxiety absent	*0.63	0.0000
Kessler distress scale	& Spielberger's trait anxiety scale - anxiety present	*0.68	0.0000
Spielberger's trait anxiety scale - anxiety absent	& Spielberger's trait anxiety scale - anxiety present	*0.73	0.0000

*Spearman's rank order correlation with Bonferroni correction (p-value < 0.01667), n = 51, * significant correlations for scales of anxiety*

Spearman's rank order correlation analysis was performed to determine whether the scales of anxiety were related to physiological parameters measured during the continuous performance task. Without Bonferroni correction ($p < 0.05$) all three scales of anxiety were correlated with: (1) positively with P3 relative beta power positively, (2) negatively with P3 P300 latency, and (3) negatively with P4 P300 latency. In addition Spielberger's state trait anxiety absent subscale negatively correlated with the number of skin conductance responses. With Bonferroni correction ($p < 0.01667$): Spielberger's state trait anxiety absent subscale correlated: (1) negatively with the number of skin conductance responses, (2) positively with P3 relative beta

power, (3) negatively with P4 P300 latency. Additionally P3 P300 latency negatively correlated with the Kessler distress scale and Spielberger's trait anxiety present subscale (table A3.5.2, appendix A10).

Table A3.5.2 Physiological parameters measured and their correlates with scales of anxiety

	mean	SEM	Kessler distress scale			Spielberger's trait anxiety scale - Trait subscale			
			Spearman	p-level		anxiety absent		anxiety present	
						Spearman	p-level	Spearman	p-level
Response times	377.16	12.21	0.06	0.668		0.15	0.287	0.24	0.083
Skin conductance responses	13.12	1.53	-0.20	0.154	**	-0.34	0.015	-0.15	0.291
Skin conductance durations (ms)	3155	314	0.03	0.857		0.25	0.077	0.06	0.701
Heart rate variance low frequency	49.75	3.12	0.11	0.446		0.02	0.888	0.08	0.574
Heart rate variance high frequency	31.36	2.88	-0.21	0.142		-0.19	0.171	-0.13	0.349
Heart rate variance LF/HF	4.05	1.40	0.21	0.130		0.19	0.173	0.17	0.242
Relative frequency power									
Frontal electrode F3 theta	43.76	1.90	-0.04	0.799		-0.11	0.456	0.01	0.939
Frontal electrode F3 alpha	31.99	1.04	-0.17	0.224		-0.01	0.934	-0.13	0.368
Frontal electrode F3 beta	24.25	1.25	0.13	0.379		0.11	0.443	0.05	0.714
Frontal electrode F4 theta	43.23	1.97	0.09	0.532		-0.01	0.932	0.13	0.357
Frontal electrode F4 alpha	31.53	1.14	-0.18	0.208		0.00	0.995	-0.08	0.585
Frontal electrode F4 beta	25.24	1.58	0.03	0.819		0.08	0.591	-0.06	0.689
Parietal electrode P3 theta	31.29	1.91	-0.01	0.951		0.12	0.419	0.08	0.556
Parietal electrode P3 alpha	43.36	2.03	-0.15	0.301		-0.23	0.099	-0.21	0.141
Parietal electrode P3 beta	25.35	1.20	* 0.32	0.024	**	0.34	0.015	* 0.30	0.035
Parietal electrode P4 theta	30.98	1.87	-0.03	0.855		0.04	0.765	0.04	0.777
Parietal electrode P4 alpha	43.03	1.94	-0.14	0.322		-0.04	0.754	-0.11	0.440
Parietal electrode P4 beta	25.99	1.25	0.26	0.062		0.19	0.186	0.25	0.073
Relative theta/beta ratio									
Frontal electrode F3	2.47	0.30	-0.10	0.489		-0.11	0.434	-0.03	0.817
Frontal electrode F4	2.38	0.28	-0.01	0.922		-0.10	0.490	0.05	0.712
Parietal electrode P3	1.48	0.18	-0.19	0.175		-0.05	0.715	-0.07	0.629
Parietal electrode P4	1.45	0.18	-0.18	0.208		-0.06	0.663	-0.08	0.582
Event-related Potential P3 component									
Parietal electrode P3 amplitude	7.26	0.52	0.17	0.236		0.22	0.116	0.12	0.389
Parietal electrode P3 latency	297.92	8.19	** -0.35	0.011	*	-0.31	0.029	** -0.34	0.015
Parietal electrode P4 amplitude	7.15	0.50	0.10	0.470		0.16	0.277	0.04	0.772
Parietal electrode P4 latency	307.22	10.07	* -0.28	0.045	**	-0.34	0.015	* -0.31	0.028

*Spearman's rank order correlations between scales of anxiety with physiological parameters measured during the continuous performance task, n = 51, * significant correlates (p < 0.05) with scales of anxiety, with Bonferroni correction (p < 0.01667) ** significant correlations with scales of anxiety*

A3.5.2 Nicotine use and correlates with physiological parameters

Nicotine usage was ranked into three groups; participants who have never smoked as 0, participants who are social smokers as 5, and regular smokers as 10. Spearman's rank order correlation was performed to determine whether relationships are present between nicotine use and physiological parameters during the continuous performance task. Nicotine use was correlated negatively to P4 P300 latency during the continuous performance task (table A3.5.4, appendix A10).

Table A3.5.4 Nicotine use correlates with physiological parameters during the continuous performance task

	Nicotine use	
	Spearman	p-level
Kessler distress scale	0.12	0.40
Spielberger's trait anxiety scale - anxiety absent	0.11	0.44
Spielberger's trait anxiety scale - anxiety present	0.22	0.13
Response times	0.11	0.44
Skin conductance responses	-0.08	0.57
Skin conductance durations (ms)	-0.15	0.28
Heart rate variance low frequency	0.14	0.33
Heart rate variance high frequency	-0.15	0.31
Heart rate variance LF/HF	0.10	0.50
Relative frequency power		
Frontal electrode F3 theta	0.19	0.19
Frontal electrode F3 alpha	-0.18	0.20
Frontal electrode F3 beta	-0.11	0.44
Frontal electrode F4 theta	0.13	0.37
Frontal electrode F4 alpha	-0.05	0.71
Frontal electrode F4 beta	-0.14	0.34
Parietal electrode P3 theta	0.04	0.79
Parietal electrode P3 alpha	-0.10	0.50
Parietal electrode P3 beta	0.16	0.27
Parietal electrode P4 theta	0.03	0.82
Parietal electrode P4 alpha	-0.21	0.15
Parietal electrode P4 beta	0.24	0.09
Relative theta/beta ratio		
Frontal electrode F3	0.13	0.35
Frontal electrode F4	0.15	0.31
Parietal electrode P3	-0.09	0.54
Parietal electrode P4	-0.15	0.29
Event-related Potential P3 component		
Parietal electrode P3 amplitude	0.05	0.73
Parietal electrode P3 latency	-0.09	0.53
Parietal electrode P4 amplitude	0.08	0.59
Parietal electrode P4 latency	* -0.30	0.04

*Spearman's rank order correlation, p-value < 0.05, n = 51, * significant correlations with measures of nicotine use*

3.5.3 Scales of anxiety and correlates with additional personality questionnaires

Spearman's correlation analysis was performed to determine whether the scales of anxiety employed in the present study were correlated with additional personality measures within the 'healthy' cohort of participants. Sensitivity to punishment and the abbreviated Eysenck's personality questionnaire subscale of neuroticism were positively correlated with all three of the anxiety scales employed in the present study. Additionally Spielberger's state trait anxiety absent subscale was positively correlated with Eysenck's personality questionnaire subscale of psychoticism (table A3.5.3, appendix A10).

Table A3.5.3 Scales of anxiety: the Kessler distress scale, Spielberger's state trait anxiety subscales with anxiety absent and anxiety present correlates with additional personality measures employed in the present study

	Kessler distress scale				STAI anxiety absent			STAI anxiety present	
	Spearman	p-level		Spearman	p-level		Spearman	p-level	
Sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ)									
Sensitivity to reward		0.17	0.244		0.20	0.170		0.28	0.049
Sensitivity to punishment	*	0.39	0.005	*	0.48	0.000	*	0.55	0.000
Abbreviated Eysenck personality questionnaire (EPQR-A)									
Extraversion		0.06	0.680		-0.07	0.621		0.10	0.464
Lie Scale		-0.06	0.700		0.02	0.898		0.07	0.642
Psychoticism		0.21	0.146	*	0.40	0.003		0.27	0.054
Neuroticism	*	0.65	0.000	*	0.55	0.000	*	0.69	0.000
Cloningers tridimensional personality questionnaire subscales									
Novelty seeking		0.04	0.757		0.04	0.775		0.01	0.947
Harm avoidance		0.06	0.678		0.15	0.286		0.24	0.085

*Spearman's rank order correlation, p-value < 0.05, n = 51, * significant correlations with scales of anxiety*

A3.6 Different forms of childhood trauma physiological correlates during three attentional tasks

Spearman's rank order correlation was performed to determine whether there are associations between the subscales of the childhood trauma questionnaire (short form) with physiological parameters measured during three attentional tasks, with Bonferroni correction ($p < 0.01667$, appendix A11). Physical abuse was negatively correlated with F3 and F4 relative alpha power during the Go/No-Go task (table A3.6.1, appendix A11).

Table A3.6.1 Childhood trauma subscale of physical abuse correlates with physiological parameters measured during the three attentional tasks

	Continuous performance task		Go/No-Go task		Cued target detection task	
	Spearman	p-level	Spearman	p-level	Spearman	p-level
Physical abuse						
Skin conductance response	0.15	0.305	0.11	0.487	0.05	0.728
Skin conductance response duration (ms)	-0.16	0.273	-0.15	0.329	0.05	0.733
Heart Rate	0.08	0.605	-0.01	0.964	0.02	0.883
Heart rate variance low frequency	0.14	0.351	-0.01	0.958	-0.05	0.719
Heart rate variance high frequency	-0.30	0.044	-0.07	0.642	-0.10	0.502
Heart rate variance LF/HF	0.21	0.152	0.09	0.558	0.03	0.821
Frontal electrode F3 relative theta power	0.27	0.070	0.30	0.044	0.31	0.034
Frontal electrode F3 relative alpha power	-0.25	0.096 *	-0.44	0.002	-0.22	0.141
Frontal electrode F3 relative beta power	-0.11	0.463	-0.07	0.653	-0.14	0.358
Frontal electrode F4 relative theta power	0.19	0.197	0.16	0.276	0.11	0.459
Frontal electrode F4 relative alpha power	-0.24	0.113 *	-0.38	0.009	-0.17	0.269
Frontal electrode F4 relative beta power	-0.12	0.439	-0.02	0.900	0.00	0.979
Parietal electrode P3 relative theta power	0.15	0.320	0.19	0.213	0.08	0.596
Parietal electrode P3 relative alpha power	-0.04	0.780	-0.14	0.369	-0.02	0.912
Parietal electrode P3 relative beta power	-0.04	0.785	0.03	0.862	0.02	0.882
Parietal electrode P4 relative theta power	0.26	0.076	0.31	0.037	0.20	0.177
Parietal electrode P4 relative alpha power	-0.25	0.093	-0.33	0.026	-0.22	0.149
Parietal electrode P4 relative beta power	0.15	0.305	0.11	0.465	0.18	0.240
Frontal electrode F3 relative theta/beta ratio	0.15	0.321	0.15	0.307	0.25	0.100
Frontal electrode F4 relative theta/beta ratio	0.13	0.406	0.08	0.613	0.06	0.700
Parietal electrode P3 relative theta/beta ratio	0.17	0.267	0.11	0.464	0.05	0.722
Parietal electrode P4 relative theta/beta ratio	0.10	0.523	0.09	0.532	0.02	0.877

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.01667, n = 46, *significant correlation with physical abuse*

For the childhood trauma subscale of physical neglect, F3 relative theta power and relative theta/beta ratio were positively correlated during the Go/No-Go task, P3 relative theta power was positively correlated and alpha power was negatively correlated during the Go/No-Go task (table A3.6.2, appendix A11).

Table A3.6.2 Childhood trauma subscale of physical neglect correlates with physiological parameters measured during the three attentional tasks

	Continuous performance task		Go/No-Go task		Cued target detection task		
	Spearman	p-level	Spearman	p-level	Spearman	p-level	
Physical neglect							
Skin conductance response	-0.21	0.170	-0.28	0.063	-0.33	0.027	
Skin conductance response duration (ms)	0.17	0.252	-0.08	0.601	-0.20	0.173	
Heart Rate	-0.04	0.784	-0.13	0.379	-0.14	0.362	
Heart rate variance low frequency	-0.32	0.032	-0.15	0.336	-0.09	0.569	
Heart rate variance high frequency	0.08	0.594	0.05	0.753	0.01	0.921	
Heart rate variance LF/HF	-0.15	0.314	-0.12	0.430	-0.02	0.903	
Frontal electrode F3 relative theta power	0.31	0.038	*	0.37	0.011	0.21	0.163
Frontal electrode F3 relative alpha power	-0.25	0.090		-0.27	0.071	-0.09	0.558
Frontal electrode F3 relative beta power	-0.26	0.077		-0.32	0.032	-0.17	0.262
Frontal electrode F4 relative theta power	0.23	0.123		0.32	0.028	0.13	0.396
Frontal electrode F4 relative alpha power	-0.32	0.033		-0.34	0.020	-0.30	0.042
Frontal electrode F4 relative beta power	-0.12	0.430		-0.18	0.230	0.02	0.889
Parietal electrode P3 relative theta power	0.24	0.101	*	0.41	0.004	0.27	0.072
Parietal electrode P3 relative alpha power	-0.31	0.033	*	-0.38	0.009	-0.28	0.060
Parietal electrode P3 relative beta power	0.11	0.462		0.13	0.389	0.15	0.329
Parietal electrode P4 relative theta power	0.29	0.049		0.36	0.014	0.34	0.019
Parietal electrode P4 relative alpha power	-0.20	0.189		-0.27	0.067	-0.25	0.094
Parietal electrode P4 relative beta power	-0.05	0.742		0.01	0.939	-0.02	0.917
Frontal electrode F3 relative theta/beta ratio	0.30	0.041	*	0.42	0.004	0.25	0.098
Frontal electrode F4 relative theta/beta ratio	0.19	0.209		0.33	0.023	0.11	0.457
Parietal electrode P3 relative theta/beta ratio	0.17	0.273		0.31	0.039	0.14	0.348
Parietal electrode P4 relative theta/beta ratio	0.27	0.068		0.36	0.015	0.32	0.030

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.01667, n = 46, *significant correlation with physical neglect*

For the childhood trauma subscale of emotional abuse, F4 relative alpha power was negatively correlated during all three of the attentional tasks. F3, P3 and P4 relative theta power was positively correlated and relative alpha power was negatively correlated during the Go/No-Go task. F3 relative theta power was positively correlated during the cued target detection task (table A3.6.3, appendix A11).

Table A3.6.3 Childhood trauma subscale of emotional abuse correlates with physiological parameters measured during the three attentional tasks

	Continuous performance task			Go/No-Go task			Cued target detection task	
	Spearman	p-level		Spearman	p-level		Spearman	p-level
Emotional abuse								
Skin conductance response	-0.05	0.754		-0.05	0.753		-0.07	0.641
Skin conductance response duration (ms)	-0.07	0.635		-0.20	0.194		-0.25	0.089
Heart Rate	0.09	0.553		-0.01	0.951		-0.01	0.960
Heart rate variance low frequency	0.04	0.772		0.17	0.269		-0.05	0.762
Heart rate variance high frequency	-0.33	0.027		-0.24	0.113		-0.26	0.081
Heart rate variance LF/HF	0.18	0.226		0.22	0.138		0.15	0.319
Frontal electrode F3 relative theta power	0.34	0.020	*	0.39	0.007	*	0.43	0.003
Frontal electrode F3 relative alpha power	-0.23	0.118	*	-0.38	0.009		-0.29	0.053
Frontal electrode F3 relative beta power	-0.29	0.047		-0.30	0.044		-0.35	0.017
Frontal electrode F4 relative theta power	0.33	0.027	*	0.35	0.016		0.33	0.025
Frontal electrode F4 relative alpha power	* -0.42	0.004	*	-0.56	0.000	*	-0.42	0.004
Frontal electrode F4 relative beta power	-0.21	0.152		-0.09	0.531		-0.16	0.289
Parietal electrode P3 relative theta power	0.29	0.052	*	0.39	0.008		0.17	0.260
Parietal electrode P3 relative alpha power	-0.26	0.081	*	-0.37	0.011		-0.14	0.359
Parietal electrode P3 relative beta power	-0.05	0.751		0.09	0.567		0.09	0.545
Parietal electrode P4 relative theta power	0.25	0.092	*	0.41	0.005		0.16	0.285
Parietal electrode P4 relative alpha power	-0.29	0.055	*	-0.38	0.008		-0.22	0.140
Parietal electrode P4 relative beta power	0.06	0.670		0.08	0.587		0.18	0.233
Frontal electrode F3 relative theta/beta ratio	0.26	0.078		0.33	0.025		0.35	0.017
Frontal electrode F4 relative theta/beta ratio	0.23	0.122		0.17	0.273		0.19	0.211
Parietal electrode P3 relative theta/beta ratio	0.21	0.153		0.17	0.255		-0.01	0.972
Parietal electrode P4 relative theta/beta ratio	0.09	0.545		0.14	0.352		-0.05	0.723

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.01667, n = 46, *significant correlation with emotional abuse*

No correlates were found for the childhood trauma questionnaire subscale of emotional neglect with physiological parameters measured during the three attentional tasks (table A3.6.3, Appendix A11).

Table A3.6.3 Childhood trauma subscale of emotional neglect correlates with physiological parameters measured during the three attentional tasks

	Continuous performance task		Go/No-Go task		Cued target detection task	
Emotional neglect	Spearman	p-level	Spearman	p-level	Spearman	p-level
Skin conductance response	-0.28	0.055	-0.35	0.016	-0.31	0.034
Skin conductance response duration (ms)	0.07	0.634	0.00	0.998	-0.19	0.208
Heart Rate	0.04	0.774	0.02	0.894	-0.05	0.759
Heart rate variance low frequency	0.02	0.871	0.24	0.105	-0.06	0.709
Heart rate variance high frequency	-0.17	0.269	-0.22	0.143	-0.15	0.304
Heart rate variance LF/HF	0.12	0.434	0.23	0.124	0.08	0.614
Frontal electrode F3 relative theta power	0.02	0.905	0.14	0.362	0.14	0.341
Frontal electrode F3 relative alpha power	-0.08	0.575	-0.19	0.216	-0.23	0.125
Frontal electrode F3 relative beta power	0.04	0.775	-0.07	0.661	-0.09	0.568
Frontal electrode F4 relative theta power	0.00	0.994	0.15	0.325	0.07	0.655
Frontal electrode F4 relative alpha power	-0.14	0.347	-0.35	0.018	-0.29	0.054
Frontal electrode F4 relative beta power	0.14	0.357	0.14	0.369	0.09	0.532
Parietal electrode P3 relative theta power	0.13	0.375	0.33	0.024	0.12	0.425
Parietal electrode P3 relative alpha power	-0.23	0.119	-0.27	0.069	-0.16	0.286
Parietal electrode P3 relative beta power	0.04	0.811	0.05	0.758	0.08	0.599
Parietal electrode P4 relative theta power	0.27	0.070	0.39	0.008	0.23	0.121
Parietal electrode P4 relative alpha power	-0.27	0.066	-0.29	0.049	-0.25	0.095
Parietal electrode P4 relative beta power	-0.06	0.715	0.01	0.966	-0.01	0.973
Frontal electrode F3 relative theta/beta ratio	-0.01	0.958	0.13	0.385	0.12	0.416
Frontal electrode F4 relative theta/beta ratio	-0.05	0.753	0.02	0.880	0.01	0.956
Parietal electrode P3 relative theta/beta ratio	0.06	0.695	0.26	0.086	0.03	0.847
Parietal electrode P4 relative theta/beta ratio	0.22	0.146	0.32	0.031	0.17	0.255

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.01667, n = 46, *significant correlation with emotional neglect*

No correlates were found for the childhood trauma questionnaire subscale of sexual abuse with physiological parameters measured during the three attentional tasks (table A3.6.3, appendix A11).

Table A3.6.3 Childhood trauma subscale of sexual abuse correlates with physiological parameters measured during the three attentional tasks

	Continuous performance task		Go/No-Go task		Cued target detection task	
	Spearman	p-level	Spearman	p-level	Spearman	p-level
Sexual abuse	man		n		n	
Skin conductance response	0.08	0.618	-0.02	0.906	-0.05	0.753
Skin conductance response duration (ms)	-0.10	0.493	-0.09	0.553	-0.08	0.604
Heart Rate	0.04	0.785	-0.03	0.844	-0.02	0.909
Heart rate variance low frequency	0.01	0.966	-0.16	0.277	-0.22	0.150
Heart rate variance high frequency	-0.15	0.314	0.03	0.837	0.09	0.540
Heart rate variance LF/HF	0.10	0.522	-0.13	0.373	-0.14	0.353
Frontal electrode F3 relative theta power	0.07	0.644	0.06	0.689	0.13	0.381
Frontal electrode F3 relative alpha power	-0.17	0.272	-0.09	0.540	-0.21	0.164
Frontal electrode F3 relative beta power	0.04	0.797	-0.05	0.720	0.02	0.884
Frontal electrode F4 relative theta power	0.10	0.529	0.07	0.662	0.13	0.379
Frontal electrode F4 relative alpha power	-0.11	0.451	-0.07	0.659	-0.15	0.335
Frontal electrode F4 relative beta power	-0.03	0.859	-0.06	0.710	0.04	0.807
Parietal electrode P3 relative theta power	0.13	0.373	0.09	0.556	0.10	0.503
Parietal electrode P3 relative alpha power	-0.20	0.179	-0.15	0.323	-0.23	0.129
Parietal electrode P3 relative beta power	0.08	0.600	0.13	0.391	0.22	0.151
Parietal electrode P4 relative theta power	0.16	0.274	0.12	0.433	0.16	0.280
Parietal electrode P4 relative alpha power	-0.28	0.059	-0.25	0.090	-0.30	0.042
Parietal electrode P4 relative beta power	0.08	0.607	0.09	0.554	0.19	0.216
Frontal electrode F3 relative theta/beta ratio	0.01	0.961	0.04	0.774	0.09	0.543
Frontal electrode F4 relative theta/beta ratio	0.05	0.743	0.09	0.552	0.09	0.559
Parietal electrode P3 relative theta/beta ratio	0.05	0.743	0.02	0.907	-0.05	0.731
Parietal electrode P4 relative theta/beta ratio	0.03	0.856	0.01	0.937	0.00	0.975

*Spearman's rank order correlation, with Bonferroni correction, p-value < 0.01667, n = 46, *significant correlation with sexual abuse*

A4. Discussion

A4.1 Characterization of the present cohort and physiological resting state activities

Male participants (6.27) reported higher levels of functional impulsivity on the Dickman's scale of impulsivity when compared with female participants (5.46). Dickman's scale of functional impulsivity was developed to measure levels of adventurousness, enthusiasm that led to positive outcomes for the individual (Dickman, 1990). No differences were found for reported levels of dysfunctional impulsivity, Dickman's scale of dysfunctional impulsivity was developed to measure levels of disorderliness and the inability to see hard facts leading to negative outcomes for the individual (Dickman, 1990). The present cohort's reported dysfunctional impulsivity of 4.75 and functional impulsivity of 5.78 compared well with a study conducted within a British public cohort of mixed gender as they reported 5.61 for dysfunctional impulsivity and 5.18 for functional impulsivity (Brunas-Wagstaff et al., 1994). The present cohort comprised primarily individuals with an average of 6.32 years post matriculation education, as a result of our recruitment process. The present findings suggest that the present cohort may have been more adventurous, enthusiastic and these forms of impulsivity led to positive outcomes for the individuals. In addition the present cohort showed lower levels of disorderliness and were able to see hard facts that would prevent negative outcomes during behaviour. These traits would support the achievements required during post matriculation education. We suggest the gender differences found within the present cohort were related to gender roles.

The Female participants (11.34) within the present study reported higher levels of sensitivity to punishment on the sensitivity to punishment and sensitivity to reward questionnaire when compared with male participants (8.76). The sensitivity to punishment scale was developed to assess the behavioural inhibition system (Gray, 1982,1987; Gray and McNaughton, 2000), activation of this system leads individuals to respond within fearful and novel environments with passive avoidance behaviours (Torrubia et al., 2001). A previous study conducted at an Italian university, using a university population found no gender (female = 11.98, male = 11.65) differences within the sensitivity to punishment subscale (Torrubia et al., 2001). The results suggest that the male participants in the present cohort did not activate their behavioural inhibition systems when exposed to fearful or novel environments, and showed reduced passivity. We did not find gender differences within the sensitivity to reward subscale (Torrubia et al., 2001). The sensitivity to reward scale was developed to assess the behavioural activation system, activation of this system leads individuals to respond actively and occurs during situations that are non-threatening and hold reward for the individual (Gray, 1982,1987; Gray and McNaughton, 2000). No differences were found for gender for any of the subscales of the abbreviated Eysenck personality questionnaire. Previous studies have shown that female participants scored higher on the neuroticism scale than male participants (Forrest et al., 2000), a measure of expressed anxiety and emotional over-responsiveness (Barrett et al., 1998). In addition male participants had a tendency to score higher on the psychoticism scale (Forrest et al., 2000; Shelvin et al., 2002), a measure associated with anti-social behaviours such as risk taking, sensation seeking, alcohol and drug use (Barrett et al., 1998). No differences were found for gender with the Kessler distress scale. The Kessler distress scale was developed in Australia

to assess the level of stress, and was associated positively with anxiety and affective disorders (www.NTC.AU). The present cohort score (21.18) for the Kessler distress scale indicated that our participants were mildly anxious. Female participants scored higher for Spielberger's trait anxiety subscales, anxiety absent ($18.82 > 16.71$) and anxiety present ($20.22 > 17.97$) than male participants. Spielberger's trait anxiety scale was developed to measure how an individual reacts within an environment that can be perceived as stressful to some and non-stressful to others, and the frequency and intensity of these stressful experiences during past events (Spielberger et al., 1970, 1980, Spielberger, 1985). The anxiety absent subscale measures the individual's 'state of well-being' and serves to measure anxiety when anxiety levels are low (Spielberger et al., 1970, 1980, Spielberger, 1985), while the anxiety present subscale measures the individuals anxiety levels when they are high (Spielberger et al., 1970, 1980, Spielberger, 1985). Our present cohort reported results (all participants = 37, female participants = 39, and male participants = 34) for the Spielberger trait anxiety scale were equivalent to those previously reported (male college students = 38.30, adult working males = 34.89, female college students = 40.40, and working adult females = 34.79). Male participants (6.50) reported higher levels of physical abuse in the childhood trauma questionnaire, when compared with the female participants (5.66). Physical abuse, measured by the childhood trauma questionnaire assessed the assaults made upon a child by an adult or older individual that posed a risk or resulted in injury to the child (Bernstein et al., 2003). Normative data for the childhood trauma questionnaire was collected from a general American cohort ($n = 1007$), male participants between the age of 18 and 24 reported 6.91 on the physical abuse subscale and males between the age of 25 and 44 reported 6.78 on the physical abuse subscale. This suggests that the reported physical abuse in the present

cohort was similar to previously reported data. No differences in gender were found for reported mental effort during the completion of the three attentional tasks. The visual analogue scale allowed each participant to estimate visually how much mental effort they felt was required during the completion of each of the attentional tasks (Azouvi et al., 2004).

The scores obtained from the several questionnaires completed by the participants suggests that the present cohort falls within a normal range of data previously reported (Brunas-Wagstaff et al., 1994; www.NTC.AU ;Bernstein et al., 2003; Spielberger et al., 1970,1980, Spielberger, 1985). In addition differences found between females and males suggest gender differences in a South African cohort, not previously reported. We did not however include a questionnaire to assay attention or distractibility that would have assisted in assessing one of the core constructs in the present study.

No differences were found between salivary cortisol levels taken prior to (0.18 µg/dL) or post the cognitive (0.20 µg/dL) testing session. This suggests that the testing session did not activate the participants' HPA-axis. The reported results and their values were similar to those of a study that assessed cortisol responses in normal participants prior to and post a public speaking task (Furlan et al., 2001 (0.2 µg/dL)). Other reported data for salivary cortisol showed higher baseline levels for cortisol than the present cohort did (Gozansky et al., 2005 (0.8 µg/dL)), Skosnik et al., 2000 (1.18 µg/dL)). Increased salivary cortisol was shown with physical exercise (Gozansky et al., 2005) and after the completion of a social stress test (Lakschewitz et al., 2008). A study has in addition reported that exposure to a psychological stressor prior to cognitive testing did not

increase salivary cortisol levels prior to and post cognitive testing (Skosnik et al., 2000). No gender differences were found in the present cohort, and no differences were found between females on contraception and females not on contraception, as previously reported (Hammerfield et al., 2006).

In finding no differences for salivary cortisol measured we concluded that the cognitive testing session was not perceived as stressful and did not activate the HPA-axis, since the latter would have led to increased levels of salivary cortisol (Skosnik et al., 2000).

At rest the skin conductance responses were greater during the eyes closed condition than with eyes open, and the duration of the skin conductance responses were longer during eyes open condition than with eyes closed. These responses during the first four minutes of the testing session accentuated the effects of the novel testing environment and suggest that these differences were related to non-specific tonic orienting responses (Boucsein 1992; Rushby and Barry, 2007).

Heart rate was lower during resting state conditions than during the three attentional tasks. Increased heart rate was shown to be associated with the individuals' ability to adapt physiologically within the environment, by increasing their arousal when the environmental demands required it (Thayer and Lane, 2000). This suggests that the participants were relaxed at the onset of the testing session as the attentional tasks increased heart rate.

During the two resting conditions, when the participants' eyes were closed absolute and relative alpha power increased over frontal and parietal electrode sites. This is a well known occurrence during rest and is emphasized with closure of eyes (Barry et al., 2007). In addition absolute beta frequency was higher when the participants' eyes were closed, and absolute theta was lower when the participants' eyes were closed, these findings have been suggested to be related to the large changes in alpha activity (Barry et al., 2007). These changes in electrical cortical activity have been suggested to occur due to an uncoupling of thalamo-cortical interactions required during visual information processing (Klimesch et al., 2001; Barry et al., 2007). The reported physiological measures during rest support previously (Klimesch et al., 2001; Barry et al., 2007). These findings served to characterize the present cohort and compared resting state activities, and confer with previously reported literature.

A4.2 Performance and Physiological activities during and within the three attentional tasks

Average response times for the continuous performance task (386 msec), Go/No-Go task (360 msec), and the cued target detection task (626 msec) were similar to reported literature ((Kirmizi-Alsan et al., 2006 (continuous performance task response time = 349 msec, Go/No-Go task response times = 362 msec), Oberlin et al., 2005 (cued target detection task between 440 and 700 msec, due to the different trial types)). The cued target detection response times were longer than both the continuous performance task and the Go/No-Go task. At first glance this may seem counterintuitive, as the cued target detection task is classified as a reflexive attentional task. Reflexive attention is said to recruit primal and instinctual responses with shorter response times than sustained attentional tasks such as the continuous performance task (Beane and Marrocco, 2004). This suggests that the sensory modality of task presentation may have been where this rift between these attentional tasks response times may lie (Beane and Marrocco, 2004). We suggest that the cued target detection response times were longer and were caused by: (1) sensory modality of task presentation, as auditory processing is faster than visual processing which requires additional processing, even with salient information (Shih and Sperling, 2002). (2) The cued target detection task not only involved reflexive attention but also incorporated sustained attention, as individuals were required to maintain central fixation throughout the task and stimuli were peripherally processed (Corbetta et al., 1998). (3) The cued target detection task used in the present study had three different interstimulus intervals (500 msec or 1000 msec or 1500 msec) this may have caused delay in processing as predictability of stimulus presentation was reduced. The above suggests that the extended response times of the cued target detection

task were caused by the maintenance of sustained attention while processing salient reflexive cues, together with the limited predictability of the arrival of the stimuli that required a response.

The continuous performance task resulted in shorter durations in skin conductance responses when compared to the Go/No-Go task and the cued target detection task. Skin conductance response durations have not previously been reported for the different attentional tasks. Skin conductance is commonly used to index the autonomic nervous systems activity, and is controlled solely by the sympathetic nervous system (Boucsein, 1992). Skin conductance responses have been related to phasic changes in arousal during orienting toward a stimulus if novel or salient (Sokolov, 1963; Polich and Kok, 1995). The continuous performance task had a single trial condition unlike the Go/No-Go task and the cued target detection task. The continuous performance task trials involved the presentation of two cues followed by the stimulus that the participants were required to respond to and presented no conflict in cortical updating (Donchin and Coles, 1988). The Go/No-Go task had three different types of trial conditions and did not have cues. In addition two of the conditions required response inhibition that could have created conflict in the orienting response (Sokolov, 1960, 1963). The cued target detection task had four different types of trials defined by their different cueing systems in addition one of the trial types presented a cue in the opposite location to the stimulus presented that could have created conflict in the orienting response (Sokolov, 1960, 1963). Conflict within the Go/No-Go task and the cued target detection task could have increased the duration of the skin conductance response by activation of the orienting response (Donchin and Coles, 1988). To confirm whether these increases in skin conductance response duration are related to these

‘conflicts’ during the Go/No-Go task and the cued target detection task, we would suggest the incorporation conflicting cues, or the display of distractors during the cueing process of the continuous performance task. In addition to reducing the skin conductance response durations during the Go/No-Go task, removal of the no-go trials would change the task to one of sustained attention only. To reduce the skin conductance response durations made during the cued target detection task, removal of the incongruent trials would reduce the conflict in trials presented. If the duration of the skin conductance responses persisted after the removal of the incongruent trials, the no-cue trials and double-cue trials would be removed, as both of these did not supply positive cues as was during the continuous performance task.

Heart rate during the Go/No-Go task was higher than during the cued target detection task. Heart rate differences during different cognitive tasks have been shown to occur with increased task difficulty (Ray and Cole, 1985; De Pascalis and Ray, 1997). This suggests that the present cohort found the Go/No-Go task more difficult than the cued target detection task. This difference may also have been caused by the format of the Go/No-Go task as response inhibition was required and errors could have been made during the no-go conditions. We suggest that the participants’ increased heart rate during the Go/No-Go task may have been caused by (1) difficulty of task or (2) the inclusion of response inhibition trials. There were no differences found between the attentional tasks for measures of heart rate variance. Difficulty of task has previously been found not to affect measures of heart rate variability (Capa et al., 2008). This further promoted the suggestion that the Go/No-Go task leads to increased heart rate caused by the difficulty of the task that in addition suggests increased tonic arousal (Polich and Kok, 1995).

Relative alpha power during the Go/No-Go task was higher over the parietal electrode sites, than during the continuous performance task and the cued target detection task. Decreased alpha activity was associated with increased performance (Ray and Cole, 1985; Klimesch, 1999), and has in addition been related to increased task difficulty or increased mental load caused by parameters of the task being completed (Fink et al., 2005). In the present battery of attentional tasks this would suggest that the reflexive cued target detection task and the continuous performance task were more difficult than the Go/No-Go task. However this decreased alpha power only occurred over the parietal cortices and this may have been of relevance. The parietal cortices have been shown to be intimately involved in orienting within the visual field and posterior attentional network activity (Posner, 1987). Anticipatory waiting during attentional tasks was shown to increase alpha activity over parietal cortices (Bastiaansen et al., 2002). This suggests that alpha power during the Go/No-Go task was higher than the continuous performance task and the cued target detection task, because although the individuals were anticipating the stimulus, they had no warning (cue) of its arrival. Relative beta activity during the cued target detection task was higher over frontal cortical electrodes than during the continuous performance task and the Go/No-Go task. Increased beta activity over the frontal cortex has been associated with deficits in sustained attentional performance in individuals with ADHD (Clarke et al., 2007, 2003). Our results suggest that there is decreased attention related activity over the frontal electrodes during the cued target detection task because it required the activation of the posterior attentional network more than the anterior attentional network (Posner and Petersen, 1990). In addition beta activity was lower during the Go/No-Go task than during

the continuous performance task. This suggests that the Go/No-Go task required greater activation of the anterior attentional network.

The cued target detection task was a reflexive attentional task known to activate the posterior attentional network depending heavily on parietal activity, while the continuous performance task and the Go/No-Go task were voluntary attentional tasks and are known to activate the anterior attentional network depending heavily on the frontal activity (Beane and Marrocco, 2004; Posner and Pertersen, 1990). The cued target detection task showed enhanced engagement of the parietal cortices as shown by decreased alpha activity, and did not engage the frontal cortices as actively as the Go/No-Go task. The Go/No-Go task showed reduced engagement or activation of the parietal cortices as shown by increased alpha power and engaged the frontal cortices more actively as shown by decreased beta power. These support the activation of the posterior attentional network during reflexive, involuntary attentional tasks, and the activation of the anterior network during voluntary attentional tasks. During the Go/No-Go task theta/beta ratios were higher over the frontal areas than during the continuous performance task and the cued target detection task. Theta/beta ratios are considered to serve as an accurate measure of increased arousal and attention (Barry et al., 2004). This suggests that during the Go/No-Go task arousal levels were increased for the performance of the attentional task, this was likely to have been caused by the requirements of the task, (1) response inhibition and (2) delay in stimulus presentation during two of the tasks conditions.

In summary EEG alpha activity over the parietal cortices indicated increased attention during a task that incorporates reflexive attention and was related to cueing stimuli. Beta activity over the frontal cortices indicates reduced activity during reflexive attention tasks. In addition, the theta/beta ratio over the frontal cortices indicated increased arousal and attention employed during a sustained attention task that requires response inhibition and delay of stimulus presentation.

A4.2.1 Performance and physiological activities within the Go/No-Go task

For the Go/No-Go task the first and last conditions (1st Go 1 500 msec and 2nd Go 1 500 msec) were found to have shorter response times than all of the other conditions. During the 1st Go 1 500 msec condition and the 2nd Go 1 500 msec condition, participants were presented with stimuli only. The no-go conditions' stimuli were presented with the same interstimulus interval as the Go 1 500 msec conditions, however the participants were required to inhibit their responses for 50% of the trials, when V's were presented. Longer response times during the no-go conditions suggest that the participants increased attention and processed the visual information more carefully to prevent responding to stimuli that required response inhibition. During the go 3 500 msec condition, stimuli were present with a longer interstimulus interval than the go and no-go conditions (3 500 msec instead of 1 500 msec). Increased interstimulus interval and their related response times that are longer, has not yet been fully elucidated (Beane and Marrocco, 2004). Finding longer response times during the go 3 500 msec condition than the two go 1 500 msec suggests that delay in stimulus presentation was sufficient to lead to longer

response times and was not a result of the Go/No-Go task format. The 1st go 1 500 msec condition had shorter response times than the 2nd go 1 500 msec condition. Reflexive responding in ‘healthy’ individuals during tasks of sustained attention has been suggested to reflect impulsivity (Kirkeby and Robinson, 2005; Ruchow et al., 2008). The response times suggest that initially the participants responded more reflexively/impulsively and as the task progressed they changed their manner of responding to one of more caution, in an attempt to improve performance. Interestingly the number of errors made did not decrease in the 2nd no-go condition when compared with the 1st no-go condition. Therefore their caution did not improve their performance with respect to inhibiting their responses.

For the Go/No-Go task skin conductance durations were greater for the 1st no-go condition in comparison with all other conditions of the Go/No-Go task except for the 2nd no-go condition, this could be related to the conflict in presentation of the no-go stimuli, activating the autonomic nervous system and prolonging the phasic orienting responses (Sokolov, 1963; Polich and Kok, 1995). In addition errors made during the no-go conditions may enhance the autonomic nervous activity leading to the prolonged phasic arousal responses. What was interesting was finding that the 1st go 1 500 msec condition skin conductance durations were greater than for the go 3 500 msec conditions, which supports the suggestion made earlier that there may have been a carry over effect caused by the shorter interstimulus intervals. However we suggest that the increased duration of skin conductance responses during the 1st go 1 500 msec condition was caused by the change in format of task from one of cueing to one of no cueing. In hand with this the task was novel and participants needed to orient toward the new conditions of the task. This suggests

duration of skin conductance responses shortened as participants became habituated to the tasks requirements, reducing the phasic orienting response (Barry and Sokolov, 1993; Critchley et al., 2000; Polich and Kok, 1995, Alexander et al., 2008).

EEG activity during the Go/No-Go task revealed several interesting results. During the 1st go 1 500 msec condition and the 1st no-go condition relative alpha activity for frontal and parietal cortices showed decreased alpha activity compared to the go 3 500 msec condition. Increased alpha activity was related to increased cortical arousal and increased attention (Ray and Cole, 1985; Klimesch, 1999). During the 1st go 1 500 msec condition stimuli were presented without cueing as occurred in the previous task, the continuous performance task. During the 1st no-go condition 50% of the stimuli presented required inhibition of response. Novel conditions within a task have been shown to increase phasic firing of the LC, as shown when an operant conditioning paradigm in monkeys was reversed (Aston-Jones et al., 1997). We suggest that this was related to the initial alerting and orienting toward the new task requirements and was accomplished through activation of the LC-NE system (Bouret and Sara, 2005; Rajkowski et al., 1994; Sara et al., 1995; Aston-Jones et al., 1991; Grant, 1988).

During the 1st go 1 500 msec condition and the 1st no-go condition relative theta activity for frontal and parietal cortices showed greater activity than the go 3 500 msec condition. Increased theta activity was evident in individuals with ADHD and was related to impulsiveness (Bresnahan et al., 1999; Hobbs et al., 2007; Clarke et al., 2002). During the go 3 500 msec condition, during which the stimulus presentation was delayed, there was decreased theta activity

over the frontal and parietal cortices in comparison with the 1st go 1 500 msec and 1st no-go conditions. Increased theta activity has been associated with individuals with ADHD (Clarke et al., 2008; Hermens et al., 2005). This suggests that for delayed stimulus presentation attentional maintenance was achieved through decreased theta activity, and during the initial stages of the Go/No-Go task the participants did not show this maintenance of attention. Our present findings suggest that during the first two conditions of the Go/No-Go task the participants showed increased cortical arousal, increased their attention as seen by the reduced alpha activity and In addition showed impulsive cortical activities as seen by the increased theta activity.

Interestingly during the go 3 500 msec condition relative alpha was lower than all other conditions of the Go/No-Go task over the parietal electrodes. An attentional task was employed to assess the changes in alpha activity during the temporal changes in the interstimulus interval (600 msec to 1 400 msec), finding that lengthening of the interstimulus interval decreased alpha activity over parietal and frontal cortices (Babiloni et al., 2004). This suggests that with increased interstimulus interval cortical arousal over the parietal cortices was increased and can be related to the increased anticipation (Bastiaansen et al., 2002).

A similar relationship was apparent for relative beta activity at the start of the task as relative parietal beta activity was increased during the 1st condition of the Go/No-Go task in comparison with all other conditions. Increased beta activity during attentional tasks has been related to reflexive responding and deficits in attentional performance, due to hyperarousal (Clarke et al.,

2001, 2002, 2007). This suggests that on initiation of the Go/No-Go task participants responded reflexively or impulsively as seen by increased parietal beta activity.

In summary on the initiation of the Go/No-Go task participants showed cortical impulsivity, increased their cortical arousal and responded reflexively as they were 'hyporoused' as was shown by heightened parietal activities. These activities attenuated through the progression of the task.

The parietal ERP P300s were extracted for the different Go/No-Go task conditions. The P300s of the 1st go 1 500 msec condition and the 2nd go 1 500 msec condition had shorter latencies and smaller amplitudes than when the targets which required inhibition were presented, and was apparent for both of the no-go conditions. These results confirm those previously reported in an auditory Go/No-Go task (Broyd et al., 2005). Delay in latency of P300 peak amplitudes has been shown to result from (1) task difficulty (Fallgatter and Strik, 1999), or (2) cortical conflict due to cessation of perseverance of response (Kirmizi-Alsan et al., 2006). These both suggest that the conditions that required response inhibition were difficult and conflict occurred when preventing a response to them. Increased P300 amplitude has been shown to result from several mechanisms: (1) novelty (Bruin and Wijers, 2002; Low and Miller, 1999), (2) inhibition of response (Bruin et al., 2001; Bekker et al., 2004), (3) increased attention (Picton, 1992), and (4) increased cortical processing required to update the cortical representation of the stimulus (Donchin and Coles, 1988). The above mentioned mechanisms that have been found to increase

the amplitude of the P300s for the stimuli that required inhibition of response may all hold true for the present findings.

A4.2.2 Performance within the cued target detection task

Congruent trials of the cued target detection task had shorter response times than the incongruent trials, double cue trials and the no-cue trials. In addition the no-cue trials had longer response times than the incongruent and no-cue trials. These results mirror several other studies previously reported (Perchet et al., 2001; Pardo et al., 2005). Shorter response times during congruent trials when compared with incongruent trials has been called the ‘validity effect’ and is suggested to reflect benefits of cueing in the spatial location where the stimulus would occur and the reduced attention required during congruent trials (Marrocco and Beane, 2004). Longer response times during the no-cue trials when compared to trials with cues, even if not in line with the spatial presentation, served to indicate the pre-potency in presenting a cue related to temporal presentation of the stimulus that would occur. The present results suggest valid cueing is beneficial during attentional performance and in-valid cueing aided responses more than no cue at all.

A4.3 Mental effort as a measure of arousal during three attentional tasks

Mental effort is applied when an individual completes a task voluntarily and when an individual uses mental resources to complete a task (Reber, 1995). Participants were asked to report their perceived levels of mental effort during each of the attentional tasks. This was made possible by completion of a visual analogue scale for each of the three attentional tasks (Azouvi et al., 2004, appendix A4). In the present study participants reported that they perceived higher levels of mental effort for the completion of the Go/No-Go task (4.9 cm) in comparison with the continuous performance task (2.8 cm) and the cued target detection task (3.0 cm).

The Go/No-Go task was the second attentional task that was performed, which suggests that novelty to the testing environment did not play a role in the increased perceived mental effort reported for the Go/No-Go task. In addition it was not the last attentional task performed, and this suggests that the increased perceived mental effort reported for the Go/No-Go task was not related to depletion of mental resources (Kahneman et al., 1973).

What did make the Go/No-Go task unique were its different conditions. There were two conditions within the Go/No-Go task that required the participants to inhibit their responses to 50% of stimuli presented, and a condition that required the participants to delay their response, as stimuli were presented with longer interstimulus intervals. An additional aspect of the Go/No-Go task was that its stimuli were not cued as in the continuous performance task and for the majority of the cued target detection trials.

Physiologically the during the Go/No-Go task participants reported (1) increased heart rate (78 bpm) when compared to the cued target detection task. (2) Skin conductance response durations were longer for the Go/No-Go task (3 373 msec) than during the continuous performance task (2 941 msec). (3) Left frontal beta activity was lower for the Go/No-Go task (23% relative power) than the continuous performance task (25% relative power) and the cued target detection task (28% relative power). (4) Right frontal beta activity was lower for the Go/No-Go task (24% relative power) than the continuous performance task (26% relative power) and the cued target detection task (30% relative power). (5) Left parietal alpha activity was higher for the Go/No-Go task (48% relative power) than the continuous performance task (44% relative power) and the cued target detection task (44% relative power). (6) Right parietal alpha activity was higher for the Go/No-Go task (47% relative power) than the continuous performance task (44% relative power) and the cued target detection task (30% relative power). (7) Left parietal beta activity beta activity was lower for the Go/No-Go task (24% relative power) than the continuous performance task (26% relative power) and the cued target detection task (27% relative power). (8) Right parietal beta activity was lower for the Go/No-Go task (25% relative power) than the continuous performance task (27% relative power) and the cued target detection task (27% relative power). These physiological parameters suggest that increased mental resources were used during the Go/No-Go task. The increased use of mental resources during the Go/No-Go task include increased activation of the anterior attentional network as shown by disengagement of the parietal cortices and increased activation of the frontal cortices, peripheral shown by increased duration of skin conductance response durations and increased heart rate. (section A4.2.1, appendix A7). These physiological parameters suggest that there was increase in tonic

arousal levels (Polich and Kok, 1995) and suggests that perceived mental effort is a suitable measure of physiological arousal changes during different attentional tasks.

Several physiological associations were found with regard to perceived mental effort: (1) left frontal relative theta power was negatively correlated and (2) left and right parietal relative beta power was positively correlated ($p < 0.01667$). Left frontal lateralized activity has been associated with activation of the behavioural activation system as earlier described (Sutton and Davidson, 1997). In addition during cognitive tasks individuals with ADHD show increased theta activity in EEG and has been associated with impulsivity (Bresnahan et al., 1999; Mann et al., 1992). This suggests that the present cohort improved their arousal and attentional performance actively during the Go/No-Go task. Parietal cortical activity has been related to the posterior attentional network (Posner and Petersen, 1990). The Go/No-Go task was shown to increase frontal cortical activities during its completion when compared with the continuous performance task and the cued target detection task. We suggest that with increased perceived mental effort there was disengagement of the posterior attentional network, to allow for greater anterior attentional network activity. Together these associations suggest the present cohort of 'healthy' participants were actively coping with the challenge of the task.

Mental effort reported for the cued target detection task was found to positively correlate with frontal and left parietal beta power). In addition the cued target detection task was found to have greater relative beta power for the frontal and left parietal cortices when compared to the Go/No-Go task and the continuous performance task (section A4.2.1, appendix A7). The cued target

detection task was a task that primarily required reflexive attention, when compared with the Go/No-Go and the continuous performance task that both required sustained attention. Reflexive attention has been shown to activate the posterior attentional network (Posner and Petersen, 1990). Activation of the posterior attentional network suggests that the anterior attentional network was disengaged and when an individual's perceived mental effort increased during the cued target detection, as shown by increased relative beta activity. The theta/beta ratio has been used in several studies as a marker for individuals with ADHD during sustained attentional task performance (Hobbs et al., 2007, Clarke et al., 2002; Monastra et al., 1999, 2001; Bresnahan et al., 1999, Bresnahan and Barry, 2002). Individuals with ADHD have additionally been suggested to respond reflexively and this has been suggested to reflect their impulsivity during sustained attentional tasks (Sue et al., 2000). Here we suggest that a decreased theta/beta ratio was required for performance of the reflexive attentional task, when compared to tasks that required sustained attention. When individuals perceived increases in their mental effort during a reflexive attentional task, their beta activity increased and the theta/beta ratio decreased, these support disengagement of the frontal attentional networks during tasks of reflexive attention. In addition supports the idea the individuals with ADHD respond reflexively when completing sustained attentional tasks (Kirkeby and Robinson, 2005; Ruchow et al., 2008).

The present findings suggest that reflexive attention and sustained attention differentially activate the anterior and posterior attentional networks (Posner and Petersen, 1990). This was evident during the cued target detection task that required disengagement of the anterior attentional network. With increased perceived mental effort as seen during the Go/No-Go task

greater disengagement of the posterior attentional network occurred and activation of the anterior attentional network occurred. In addition suggests that this disengagement is more clearly evident over the left parietal cortex.

It is important to note that cortical measures of activity during different forms of attentional task require different levels of perceived mental effort and follow different patterns of cortical activity. In addition the use of a visual analogue scale of mental effort required to perform reflexive attentional tasks could serve to ascertain the ability of an individual to disengage their anterior attentional network to perform reflexive attentional tasks. This may hold great relevance to mental disorders that have been shown to have deficits in reflexive attention including schizophrenia (Carter et al., 1996), either aiding diagnoses or enabling a better understanding of the neural networks involved.

Incorporating the present findings into our present hypothesis we suggest that perceived mental effort may reflect the intensity of phasic LC activity within a 'healthy' cohort required to facilitate and maintain attentional performance and may reflect the extent to which the LC-NE system enhances the signal-to-noise ratio for different forms of attention.

A4.4 Impulsivity and its role during arousal and attention

Our first aim was to determine whether one of the three tasks that were employed teased out impulsivity within a ‘healthy’ population. Impulsivity within a ‘healthy’ population has been suggested to reflect in short response times and increased errors as the individuals respond reflexively (Kirkeby and Robinson, 2005; Ruchow et al., 2008) or do not take the task seriously (Svebak et al., 1987). Response times during each of the three tasks and their conditions/trials underwent correlation analysis with reported impulsivity in the Dickman’s scale of impulsivity (section A4.1). Dickman’s dysfunctional impulsivity subscale was found to be associated with shorter response times during all three of the attentional tasks: (1) continuous performance task, (2) congruent trials of the cued target detection task, and (3) several of the Go/No-Go task conditions. In addition the number of errors made during the 1st no-go condition were associated with reduced response times for several of the Go/No-Go task conditions. With these results we concluded that the Go/No-Go task is an accurate measure of impulsivity within a ‘healthy’ cohort of participants.

The response times for the 1st Go 1 500 msec condition (385 msec) was reported earlier as having the shortest responses times than all other Go/No-Go task conditions (400 – 430 msec) (section A4.2.2, appendix A7). Concluding that during the 1st Go 1 500 msec condition participants responded reflexively/impulsively and as the participants became familiar with the requirements of the task they changed their form of responding to one of more caution. Reflexive responding in ‘healthy’ individuals during tasks of sustained attention, such as the

Go/No-Go task has been reported to reflect impulsivity (Kirkeby and Robinson, 2005; Ruchow et al., 2008).

Interestingly two physiological associations for the 1st Go 1 500 msec condition response times were found: (1) Left frontal theta activity was positively associated and (2) Left frontal alpha activity was negatively associated with response times.

Taken together these strongly support increased cortical arousal required for improved attentional performance (Klimesch, 1999). A previous report associated increased left frontal activity as seen with magnetic resonance imaging BOLD signal with the behavioural activation system (Sutton and Davidon, 1997). The behavioural activation system is a branch of Gray's behavioural approach system, and was developed to assess behaviour leading the individual to actively cope within an environment that they perceive as non-threatening or rewarding (Gray, 1982,1987; Gray and McNaughton, 2000). The behavioural activation system has additionally been shown to increase arousal and attention (Hewig et al., 2006; Torrubia et al., 2001). The present findings suggest that the present cohort of 'healthy' participants took initiative to improve their performance during the 1st Go 1 500 msec condition which resulted in increased cortical arousal and can be seen as an active coping strategy employed due to the novelty of the new task.

In addition Dickman's subscales of impulsivity were positively associated with Eysenck's extraversion personality questionnaire subscale. The extraversion subscale was developed to

measure “impulsiveness” and “sociability” (Barrett et al., 1998). Eysenck’s personality questionnaire subscale of extraversion has been positively associated with the behavioural activation system questionnaire (Elliot and Thrash, 2002). This relationship reinforces our present findings and discussion in relation to the Go/No-Go task and its 1st Go 1 500 msec as measuring impulsivity within a ‘healthy’ population.

Individuals that responded more reflexively/impulsively had shorter response times during the initial stages of the Go/No-Go task specifically the 1st Go 1 500 msec condition, decreased left frontal theta activity and increased alpha activity. We suggest two alternative explanations in respect to the present hypothesis: (1) individuals with low arousal levels, when the LC phasically fired due to the salience and novelty of the task the release of NE did not enhance the signal-to-noise ratio as effectively as in individuals with higher arousal levels within the present cohort of ‘healthy’ participants. Alternatively (2) the intensity of the LC phasic activity was lower as the salience and novelty of the task was not perceived to require increased levels of attention.

A3.5 Anxiety and its role during arousal and attention

Our first aim for the present question was to determine whether the questionnaires employed in the current study accurately measured anxiety within a ‘healthy’ population. The scores of the two scales: (1) Kessler distress scale and (2) Spielberger’s trait anxiety questionnaire and its subscales (a) anxiety absent and (b) anxiety present scores were positively associated. The Kessler distress scale was developed to assess the levels of perceived stress as reflected by anxious thoughts and behaviours ([section A4.1](#)). Spielberger’s trait anxiety scale was developed to measure how an individual reacts within an environment, whether the individual perceived it as stressful or non-stressful (Spielberger et al., 1970, 1980, Spielberger, 1985). Both of these scales’ outputs assess the level of anxiety an individual presents with.

Physiological parameters were associated with the scores of these two scales: (1) Kessler distress scale and (2) Spielberger’s trait anxiety questionnaire and its subscales (a) anxiety absent and (b) anxiety present. All three of the scales/subscales were positively associated with left parietal relative beta activity, and were negatively associated with their P300 latency ([section A3.4](#)).

Individuals who were reported to be ‘hypoaroused’ (individuals with ADHD) showed reduced beta activity during attentional task performance (Mann et al., 1992). Individuals with anxiety disorders presented with physiological ‘hyperarousal’ (Sue et al., 2000). The present findings suggest that left parietal relative beta activity during sustained attentional task performance may

serve as a marker for anxiety within a 'healthy' cohort. However, anxiety scales/subscales were negatively associated with parietal P300 latency. Increased latency of the P300 has previously been reported in individuals with post traumatic stress disorder during the completion of an auditory oddball task, and it was suggested that the increased latency was due to attentional processing deficits (Reza et al., 2006). The present results suggest that 'healthy' individuals with high levels of anxiety had reduced P300 latencies. We suggest that the present participants that presented with increased anxiety were not aroused to an excessive level, in which attentional performance deteriorates. We conclude that the anxious participants in the present study used their increased arousal levels beneficially by increasing beta activity which improved attentional processing, as seen by the decreased latency of the P300.

A peripheral physiological negative association was found between the Spielberger's trait anxiety absent subscale and the number of skin conductance responses. Spielberger's state trait anxiety absent subscale was developed to measure low levels of anxiety and the 'state of well being' of an individual (Spielberger et al., 1970, 1980, Spielberger, 1985, appendix A4). Spielberger's trait anxiety scale has been suggested to serve as an excellent marker for autonomic reactivity as it was found to strongly correlate with skin conductance levels within a cohort of speech phobic college students (Hofmann and Kim, 2006). A negative association for the skin conductance responses with Spielberger's trait anxiety subscale supports the development of this subscale as a measure of the participants 'state of well being' when individuals anxiety levels are low.

Interestingly with more stringent statistical parameters a pattern formed in respect to different anxiety scales/subscales (section A3.4). Spielberger's trait anxiety absent subscale remained associated with left parietal beta activity and negatively associated with parietal P300 latency. Spielberger's trait anxiety absent subscale served to measure 'state of well being' and serves to measure anxiety levels when they are low (Spielberger et al., 1970,1980, Spielberger, 1985). The associations suggest that within a 'healthy' population that presents with low levels of anxiety, left parietal beta activity and right parietal P300 latency serve as a marker of increased arousal during tasks that required sustained attention. Kessler's distress scale and Spielberger's trait anxiety present subscale both measure anxiety that was present in the individual and was negatively associated with left parietal P300 latency. This association suggests that within a 'healthy' population that presents with high levels of anxiety, left parietal P300 latency may serve as a marker of increased arousal during tasks that require sustained attention.

The scales employed to assess the participants' level of anxiety were positively associated with their reported levels of sensitivity to punishment and neuroticism. Sensitivity to punishment was a subscale of the sensitivity to reward and sensitivity to punishment questionnaire (appendix A3).

The sensitivity to punishment scale was developed to measure the activation of the behavioural activation system. Activation of this system is suggested to assess behaviours of an individual within an environment that they perceive as non-threatening or rewarding leading them to actively cope within the environment (Gray, 1982,1987; Gray and McNaughton, 2000). This

association suggests that 'healthy' anxious individuals were more aware of their environments and employed active coping strategies within non-threatening environments. Neuroticism a subscale of the Eysenck personality questionnaire was developed to measure the expression of anxiety and emotional over-responsiveness (Barrett et al., 1998). Neuroticism has been associated with activation of the behavioural inhibition system, suggesting that individuals respond with fear and passivity within novel environments to avoid aversive events (Elliot and Thrash, 2002). In addition activation of the behavioural inhibition system has been previously associated with neuroticism and Spielberger's trait anxiety, resulting in increased cortical activity (Knyazev et al., 2002). This association suggests that 'healthy' anxious individuals were more aware of their environments and employed passive coping strategies within fearful environments. These associations show that the testing session was not perceived as threatening and did not induce fear as the 'healthy' participants that were anxious adapted positively as shown by their decreased P300 latencies. The lateralization found between the associations of the P300 latency suggest that the behavioural activation and inhibition systems show cortical lateralization and can be associated with information processing during sustained attentional tasks.

In addition Spielberger's trait anxiety absent subscale was positively associated with psychoticism. Psychoticism a subscale of the Eysenck personality questionnaire was developed to measure anti-social behaviours such as risk taking, sensation seeking and substance abuse behaviours (Barrett et al., 1998). This suggests that 'healthy' participants that present with low levels of anxiety had additional personality traits that may reduce their anxiety.

In summary, the Kessler distress scale and Spielberger's state trait anxiety present scores were associated with increased left parietal beta activity, decreased parietal P300 latency specifically the left parietal cortex, increased sensitivity to punishment, and increased neuroticism. If we incorporate these associations into the present hypothesis, 'healthy' individuals with increased levels of anxiety had increased cortical activity and improved their attentional processing. We suggest this was accomplished through increased tonic firing of the LC toward an optimal level or arousal so that phasic activity of the LC was able to improve cognitive performance during the attentional tasks. And this may be related to the 'healthy' anxious individual's fear of punishment or negative outcomes if their performance was poor. In addition Spielberger' state trait anxiety absent scores were associated with decreased skin conductance responses, increased left parietal beta activity, decreased parietal P300 latency specifically the right parietal P300, increased sensitivity to punishment, increased neuroticism and increased psychoticism. If we incorporate these associations into the present hypothesis, 'healthy' individuals presenting with anxiety absent on the day of testing had increased cortical activity and improved attentional processing. This suggests that 'healthy' individuals with decreased skin conductance responses and increased reports of exploratory behaviour, both seen as traits of 'hypoarousal' and ADHD. We suggest that this pattern of physiological activity and behaviours may serve the individual in adapting or reducing their anxiety levels.

A4.5.1 Nicotine use and anxiety regulation

Nicotine was found to negatively associate with right parietal P300 latency. Deficits in attention have been suggested to lead to the use of nicotine as a form of self-medication (Newhouse et al., 2004; Rezvani and Levin, 2001), and has been associated with mental disorders that are characterized in part with deficits of attention (Newhouse et al., 2004; Spinella, 2005). The participants in the present study had abstained from nicotine use for a minimum of eighteen hr prior to the testing session. Chronic nicotine users have been shown to increase their attentional performance as seen by reducing their response times and increasing their P300 amplitudes (Knott et al., 1999; 2003), latencies were not reported. The present association found with nicotine was similarly found for the Spielberger's state trait anxiety absent subscale. From this we suggest that nicotine use serves as part of the compensation that 'healthy' individuals employ to reduce their anxiety.

A4.6 Childhood trauma and its effects on arousal and attention

Several associations were found between the subscales of the childhood trauma questionnaire and physiological responses during the three attentional tasks.

Emotional abuse subscale scores were negatively associated with right frontal alpha activity during the three attentional tasks. The emotional abuse subscale was developed to assess verbal assaults on a child's sense of worth or well-being or any form of humiliation or demeaning behaviour directed toward a child by an adult (Bernstein et al., 2003). The three tasks employed in the present study assessed two forms of attention, sustained and reflexive. Increased right frontal activity has been suggested to be associated with activities of the behavioural inhibition system (Hewig et al., 2006). The behavioural inhibition system questionnaire was developed to assess an individual's fear and passivity within novel environments in an attempt to avoid aversive events (Gray, 1982, 1987; Gray and McNaughton, 2000). Decreased alpha activity is seen as a measure of increased attentional performance (Klimesch, 1999). The present findings suggest that decreased right frontal activity as seen by decreased alpha activity was related to an individual's fear of aversive outcomes during performance of the three attentional tasks, and is pervasive during reflexive and sustained attention.

In addition reported emotional abuse was associated with left and right frontal and parietal alpha activity and negatively associated with theta activity during the Go/No-Go task. Physical abuse was negatively associated with frontal alpha activity during the Go/No-Go task. The physical

abuse subscale was developed to assess bodily assaults on a child by an adult or older person that posed a risk or resulted in injury of the child (Bernstein et al., 2003). This suggests that physical abuse may reflect that individuals that have been physically abused perceive the task as more difficult and try to improve their performance. Physical neglect subscale scores were associated with several cortical band activities during the Go/No-Go task as it was positively correlated with relative theta power over the left frontal and parietal cortices, and theta/beta ratio for the left frontal cortex, and was negatively correlated with right parietal alpha activity. The physical neglect subscale was developed to assess failure of caretakers to provide for the child's basic physical needs (Bernstein et al., 2003). These results suggest that the physiological associations found with emotional abuse, physical abuse and physical neglect were related to the requirements of the Go/No-Go task a task that serves to measure impulsivity within a 'healthy' cohort. We suggest that use of a sustained attentional task that requires greater levels of perceived mental effort (section A4.3) may be capable of reflecting the degree of childhood trauma even if subtle. The Go/No-Go task requires response inhibition conditions during which errors can be made, which suggests that the individuals that have been traumatized, develop cortical strategies to avoid negative or aversive outcomes. Avoidance of negative outcomes to the individuals has been strongly related to anxiety-related disorders including posttraumatic stress disorder and depression.

Childhood trauma has been associated with increased development of mental illness (de Wilde et al., 1992; Dube et al., 2001; Johnson et al., 2002; Kendler et al., 1995, Kendall-Tackett, 2000), and has been positively related to the development of anxiety, increased HPA-axis

activity and has been suggested to result in hyperarousal (Suliman et al., 2008; Heim and Nemeroff, 2001; Kendall-Tackett, 2000). To date only one study has been conducted that assessed the effect of childhood trauma on attentional performance, this study specifically investigated childhood sexual abuse, the children showed deficits in attentional performance within a classroom as scored by the child behaviour checklist for children (Kaplow et al., 2008).

The effects of childhood trauma were clearly evident during the three attentional tasks and were primarily associated with decreased alpha activity. In not finding associations with beta activity, we do not suggest that the present ‘cohort’ were anxious or were ‘hyperaroused’. Presently no studies have reported similar results for childhood trauma that were found in the present study, or looked at physiological parameters during a battery of attentional tasks that assessed sustained and reflexive attention in adulthood. It was very interesting finding ‘healthy’ individuals with reported low levels of childhood trauma to reflect changes in their cortical activities during attentional task performance, and suggests that this avenue of research may facilitate in understanding how these cortical strategies are (1) developed, (2) how they can be corrected, (3) what levels of trauma lead to ‘hyperarousal’ and mental illness, and (4) do the above strategies in cortical activity during attentional performance show resilience.

PART B

B1. Introduction

The spontaneously hypertensive rat (SHR) has been used to model attention-deficit hyperactivity disorder (ADHD), as it exhibits several of the behavioural characteristics of the human disorder. Initially bred for their hypertensive properties in adulthood (Okamoto and Aoki, 1963) these rats show marked deficits in attentional performance (Sagvolden, 2000), behavioural impulsivity (Sagvolden et al., 1993; Clements and Wainwright, 2006), increased locomotor activity and exploratory behaviour (Knardahl and Sagvolden, 1979; Ferguson and Gray, 2005) prior to the development of hypertensive properties.

The Wistar-Kyoto rat (WKY) was initially bred as a control for the hypertensive SHR (Okamoto and Aoki, 1963). However in recent years a number of interesting behaviours have been characterized and the WKY has been suggested to model anxiety-like behaviours, these include the development of stress-ulcers (Paré, 1989a,b), 'learned helplessness' (Paré, 1989b, Armario et al., 1995; Lahamame et al., 1997), passive avoidance (Paré, 1993,1996), stress hyper-reactivity (Grauer and Kapon, 1993), reduced-exploration (Paré, 2000), hypoactivity (Drolet et al., 2002; Marti and Armario, 1996).

The following experiments were aimed at elucidating the roles NE plays in both the SHR and WKY strains of rat, suggesting that SHR are 'hypoaroused' and WKY are 'hyperaroused' and that this in part is due to dysfunction of the LC-NE system.

Incubation of healthy brain slices with a radioactively labelled neurotransmitter leads to uptake into axon varicosities. Placement of these brain slices into columns which allow constant buffer inflow and outflow and permits the collection of 'fractions' of eluate (or runoff from the brain slices). Exposure of these slices to either depolarizing ions or excitatory neurotransmitters leads to release of the labelled transmitter. The continual collection of the 'fractions' of eluate permits the calculation of the relative release during the stimulation. This is termed the superfusion technique. Glutamate is the primary excitatory neurotransmitter in the central nervous system. In the following experiments brain slices were incubated with [^3H]NE and glutamate was used to facilitate depolarization and terminal release of [^3H]NE. This technique allows the determination of the functional role of neurotransmitters released from terminals in vitro, without activation of neural networks that may compensate for the chemical manipulation that could occur in vivo.

Glutamate-stimulated release of [^3H]NE from prefrontal cortical slices has previously been reported to be greater in SHR than WKY (Russell and Wiggins, 2000). Glutamate-stimulated release of NE has not previously been investigated in areas of the brain other than the prefrontal cortex. We measured glutamate-stimulated release of NE from LC-NE terminals in hippocampus and cerebellum of SHR and control rat strains (WKY and SD). In addition we determined whether successive stimulations with glutamate yielded similar patterns of release in SHR and WKY hippocampus (Experiment 1).

SHR is a model of ADHD and WKY has been suggested to model anxiety-like disorders, hence we incorporated three additional rat strains to determine whether there was stratification of glutamate-stimulated release between several rat strains (Experiment 2).

Glutamate receptors when activated lead to the release of NE in several brain areas. Activation of ionotropic glutamate receptors was suggested to play a major role in the release of NE (Russell, 2001). We determined the role of the different ionotropic receptors in glutamate-stimulated release of NE in hippocampal slices, by incubation of hippocampal slices with a non-NMDA receptor antagonist (CNQX), a NMDA receptor antagonist (MK801), or a combination of both (CNQX & MK801) (Experiment 3).

The LC is only the source of NE terminals in the prefrontal cortex, hippocampus and cerebellum (Foote et al., 1983; Jentsch, 2005; Aston-Jones et al., 1999; Southwick et al., 1999; Bunsey and Strupp, 1995). The LC-NE system is implicated in the regulation of arousal and attentional performance (Jentsch, 2005; Oades et al., 2005; Beane and Marrocco, 2004), which is suggested to facilitate secondary behaviours such as exploration, vigilance, and behavioural flexibility (Wu and Sutherland 2006; Aston-Jones et al., 1999; Sara et al., 1995; Aston-Jones and Bloom, 1981). Determining whether all terminals of the LC-NE system respond in similar ways to glutamate stimulated release of NE would facilitate in determining whether SHR exhibit a hyper-noradrenergic system when stimulated with glutamate, in comparison to WKY which may be classed as exhibiting a hypo-noradrenergic system when stimulated with glutamate. Lateral cerebellar slices and cerebellar vermis slices were superfused with glutamate to determine whether differences existed between WKY and SHR strains (Experiment 4).

The interplay between genetics and the environment has been subject to intensive investigation in recent years (Holmes et al., 2005). One way in which early environment can be manipulated is by adjusting or changing the maternal environment of pups.

Cross-fostering is a maternal environmental manipulation in which pups are fostered onto dams which were not their biological mothers. Cross-fostering has been routinely used to study the effect of the dam on the behaviour of her pups that she has reared (Cierpial et al., 1989; Cierpial et al., 1990; Cierpial and McCarty, 1991). We determined whether early postnatal (PND2-PND21) environment determined behavioural and neurochemical outcomes in SHR and WKY strains. SHR, WKY and SD rats were bred and cross-fostered. Behavioural effects of these manipulations of maternal rearing were determined by use of the open-field apparatus which is used as a standardized test of exploratory and locomotor behaviour over a brief interval of fifteen min and the elevated plus maze which is regarded as a robust measure of anxiety-like behaviours (five min, Ramos and Morméde, 1997). Glutamate-stimulated release of NE was determined in both hippocampal and prefrontal cortical slices two days post behavioural testing (Experiment 5).

Maternal separation is a maternal environmental manipulation in which pups and dams are separated for periods of time over the course of maternal care (PND0 – PND21), depending on the paradigm of maternal separation employed. We determined the glutamate-stimulated release of NE from prefrontal and hippocampal slices of Sprague-Dawley rats that had undergone mild maternal separation. Mild maternal separation involved the removal of the rat pups from PND 2 until PND 14 for three hr per day (Experiment 6).

Nicotine use has been suggested to serve as a form of self-medication in individuals with attentional deficits and anxiety-like behaviours, which suggests that administration of nicotine to SHR and WKY rats may yield different effects on behaviour. Acute nicotine has been shown to

decrease hypoactivity and increase locomotion in SD (0.1-1.0 mg/kg) (Faraday et al., 2003; Hetzler and Thienpeng, 2004), but has not been investigated in SHR and WKY. Direct application of nicotine and its agonists has shown increased release of NE in several areas of the brain including the prefrontal cortex (Rao et al., 2003) and hippocampus (Anderson et al., 2000; Fu et al., 1998). We determined the effects of an acute oral dose of nicotine (0.6 mg/kg) on behaviour and glutamate-stimulated release of NE in hippocampal and prefrontal cortical slices of SHR, WKY and 'control' SD. In addition we determined the effect of successive glutamate stimulations of hippocampal and prefrontal cortical slices of SHR, WKY and SD rats (Experiment 7).

B2. Methodology

B2.1 Animals

All animal experiments were approved by the Research Animal Ethics Committee of the University of Cape Town. Rats were housed in grid roofed plexiglass cages with sawdust bedding, the rats were moved to a clean cage three times per week. Food and water were provided *ad libitum*, a twelve hr light/dark cycle was maintained (lights on @ 06h00 and lights off @ 18h00), and temperature was maintained between 21 and 23 °C. These conditions were maintained for all experiments.

B2.2 Rearing

On PND 2 litters were culled to eight pups, males taking preference as males were the only sex used for all of the present studies. All rats for these studies were strictly weaned on PND 21, housed either with litter mates or in same strain pairs, depending on the experimental design of the study. Rats that were used for neurochemical studies were killed by decapitation in their fourth week (PND 29 to 35). All behavioural studies were conducted in their fourth week. If both behaviour and neurochemistry were performed on an animal both the behaviour and neurochemistry were performed in the fourth-week, with two days between. The studies were carried out in the fourth week to prevent any interaction with the development of hypertension in the SHR (de Jong et al., 1995). In addition pre-puberty is the period in which SHR has persistently been shown to display hyper-locomotion (Ferguson and Gray, 2005; van den Bergh et al., 2006).

B2.3 Cross-fostering protocol

Three strains of rat were used for the current study; SHR, WKY and SD. Two to three females were placed with a male (harem mating) of the same strain for four days, the duration of a rat's oestrous cycle, thereby increasing the likelihood of successful mating. The females were housed individually when signs of pregnancy were evident, through visual inspection and light palpation of the abdomen. The day of birth (PND 0) was noted. On PND 2, the litters were culled to eight and the rats were cross-fostered. Cross-fostering on PND 2 was to limit the possibility of cannibalism, which can possibly occur as a result of (1) human handling at too early an age, (2) insufficient time allowed for grooming and removal of traces of delivery, (3) heightened maternal sensitivity during the first few PNDs. This method of cross-fostering on PND 2 was found to be highly successful in all strains used. To note pups born from one litter were cross-fostered as a litter, the litters were not mixed during any stage of the cross-fostering study. Control rats remained with their birth mothers; it has been shown that pups that are reciprocally cross-fostered (which is achieved by fostering pups onto a different dam of the same strain) do not alter their behaviours compared to pups that remain with their birth dams (Cierpial et al., 1989). SHR often yield litters smaller than other strains, for this reason we included SHR litters that were greater than five pups or more for the present study. A maximum of two pups were used from a litter of five pups and a maximum of four pups were used from a culled litter of eight pups. These choices in the exclusion and inclusion, regarding the number of pups used per litter were judged to be the most appropriate for the question being asked of the present study and to limit animal wastage. On PND 21 the rats were weaned and paired with a litter mate of the same strain and rearing condition. Four pups were assessed for their behaviour in the open-

field and the elevated-plus maze between PND 29 and 33 (behaviour was recorded by Leander Bindewald). Two days after the behavioural recordings, the four rats were decapitated to determine glutamate-stimulated release of [^3H]NE from prefrontal and hippocampal slices. The superfusion apparatus only allowed the use of four rats and two brain areas (16 columns).

B2.4 Chronic mild maternal separation protocol

SD dams were harem mated; females were housed individually when signs of pregnancy were evident. Day of pups birth (PND 0) was noted. On PND 2 the pups were culled to eight per litter. From PND 2 through to PND 14 the dams were removed from pups for three hrs per day. The separation occurred between 09h00 and 12h00. Cages containing the pups were transferred to a separate room where the temperature was maintained at 31°C to prevent hypothermia. At 12h00 the cages with pups were returned to the communal rat room and maternal dams were returned to their respective pups. Rats belonging to the control group were raised normally. Pups were weaned in litters on PND 21. On PND 30 rats were housed in pairs. On PND 35 rats were decapitated to determine the glutamate-stimulated release of [^3H] norepinephrine from prefrontal and hippocampal slices.

B2.5 Neurochemistry

Rats were brought to a room adjacent to the laboratory one hr prior to decapitation. For all experiments rats were killed in their light cycle, between 09h00 and 12h00. The rats were decapitated by guillotine. Their brains were rapidly removed with the use of skin scissors, rongeres and eased out with a spatula. The brain was submerged in an ice-cold slush of Krebs buffer (NaCl 118 mM, KCl 4.7 mM, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 1.0 mM, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.2 mM, NaHCO_3 23

mM, D-Glucose 11 mM, EDTA 37.6 μ M and $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ 1.3 mM) and aerated with carbogen (95% O_2 /5% CO_2), for fifteen min.

Prefrontal cortical slices were obtained by manual slicing with a McIlwain tissue chopper. The whole brain was positioned with the ventral side down perpendicular to the McIlwain blade and 0.9 mm slices were cut from the anterior apex of the frontal lobes. No slices were taken once the forceps minor was visible. The olfactory bulb was teased away from the prefrontal cortical slices. The prepared prefrontal cortical slices were then sliced into 0.3 mm by 0.3 mm columns with a McIlwain tissue chopper.

Hippocampi were dissected by placing the brain with dorsal side down. The brain stem and mid brain were removed with forceps. The cortical lobes were gently parted at the level of the lateral ventricle, revealing the complete length of the hippocampus. The hippocampus was then eased out with the use of a spatula. The visible membranes and blood capillaries were teased away from the hippocampal tissue. The hippocampus was then chopped into 0.3 mm by 0.3 mm slices with a McIlwain tissue chopper.

If both the prefrontal cortex and the hippocampus were used in a single experiment the prefrontal cortex was removed first followed by the hippocampus.

Cerebelli were eased away from the brainstem and the paraflocculi were removed. The cerebellum was then sliced either side of the paramedian sulcus, thereby dividing the cerebellum into two lateral cerebelli and a vermis. The three sections were unfolded with a ventral medial cut, and laid flat. These sections were then sliced into 0.3mm by 0.3 mm columns with a McIlwain tissue chopper.

The sliced 0.3mm by 0.3mm columns of brain were transferred from the McIlwain chopping plate with the use of a moist brush into 1ml of ice-cold Krebs buffer with ascorbic acid (5.7 mM) to reduce free radical damage and decrease tissue degeneration. The tissues were lightly agitated with the brush to separate the columns from each other. At all times while the brain tissues were in the vials they were gently surface aerated with carbogen. The vials with the brain slices were then placed in a waterbath to reach and maintain 37°C for ten min, brain tissues were maintained at 37°C for the rest of the experiment. Radioactively labeled norepinephrine 2.67 µl (1-[7,8-³H] NE, 37 MBq/ml, 1.0 mCi/ml, Amersham Biosciences UK Limited) was then pipetted into vials with brain tissue slices. Vials with tissue slices were swirled and slices were incubated for fifteen min, to allow for the uptake of [³H]NE by terminal vesicles. The supernatant was removed and fresh Krebs buffer was added. The slices were then transferred by Pasteur pipette into prepared superfusion chambers and perfused with Krebs buffer for one hr. One hr was sufficient to establish settling of tissues in the chambers to maintain a steady baseline release of radioactivity.

To obtain a single glutamate-stimulated fraction, two baseline fractions of five min were collected, on initiation of collection of the third fraction, the inlet tubes of the superfusion columns were transferred to a glutamate-containing Krebs buffer solution. The inlet tubes were kept in the glutamate-containing Krebs buffer solution for one min, and then returned to the Krebs buffer solution for the remaining four min of the fraction. This fraction serves as the glutamate-stimulated fraction. After collection of the glutamate-stimulated fraction an additional five min baseline fraction was collected. After the final fraction was collected an additional fraction was collected with inlet tubes removed from the Krebs buffer solution, leading to a smaller fraction being collected, as this comprised of Krebs buffer solution remaining in the inlet

tubes. The brain slices were removed from the superfusion columns to determine residual radioactivity that was required for the calculation of fractional release. One milliliter of NaOH (1M) was added to the superfused brain slices to release the remaining [^3H]NE.

In total for a single glutamate stimulation experiment five fractions were collected, including two pre-stimulation fractions, one glutamate stimulation fraction, one full post stimulation fraction, one shorter fraction, and a final vial with brain slices.

To obtain three glutamate-stimulation fractions, a second stimulation fraction (S_2) was collected after the first post stimulation fraction and a third stimulation fraction (S_3) was collected after the second post stimulation fraction. In total for three glutamate stimulations ten fractions were collected, including two pre-stimulation fractions, three glutamate stimulation fractions, three post stimulation fractions, one shorter fraction, and a final vial with brain slices.

B2.6 Calculation of glutamate-stimulated release of norepinephrine

Radioactivity in baseline and stimulation fractions as well as the brain slices at the end of the experiment was analyzed using a Packard 1900 CA TRI-CARB liquid scintillation analyzer. To determine the glutamate-stimulated release relative to baseline, release was calculated as a fraction of the total amount of radioactivity present in the slices at the time of release of that five min fraction, followed by subtraction of the baseline from the stimulation fraction, to obtain glutamate-stimulated [^3H]NE release.

B2.7 Behavioural measures

Between PND 28 and 33 the rats were assessed for their behaviours in the open-field and elevated plus maze, depending on the paradigm. On the day of behavioural recording the rats were taken to a room adjacent to the behavioural room at least 1 hr prior to behavioural recording. For all studies recordings of behaviour occurred between 10h00 and 14h00, in the rats' light cycle. The reason for choosing their light cycle was to avoid the natural foraging for food which occurs in their dark cycle in order to discount food seeking behaviours. The illumination of the behavioural room was kept at a lux of 50. All behaviours were recorded with surveillance cameras and analyzed with Ethovision (version 3.1, Noldus Information Technology, Wageningen, Netherlands) software.

The elevated plus maze provides a robust measure of anxiety in which illumination plays a minimal role. Since novelty is a major contributor to behaviour in the open field, this test was performed initially, at least 2 hrs prior to the elevated plus maze. Each apparatus was cleaned with 20% ethanol between each rat recordings.

B2.7.1 Open-field behaviour

Dimensions of the open field: 1.0 m x 1.0 m black wooden box with 0.5 m high walls. The inner zone (0.70 m x 0.70 m) was demarcated with a white line (figure B2.1). A lux of 50 was chosen to allow animals to explore the novel environment without the complication of exposure to bright light. Each rat was individually placed in the open field facing into a corner, parallel to a wall (figure B2.1). The positioning of the rat within the open field in this manner was to limit locomotion resulting from the stress of handling and placement into the novel environment, which may have yielded a false locomotor response. The rats were recorded for fifteen min each.

Parameters analysed in the open-field apparatus; (a) total distance travelled (m), (b) time taken to enter the inner zone, (c) number of entries into the inner zone, and (d) time spent in the inner zone (sec).

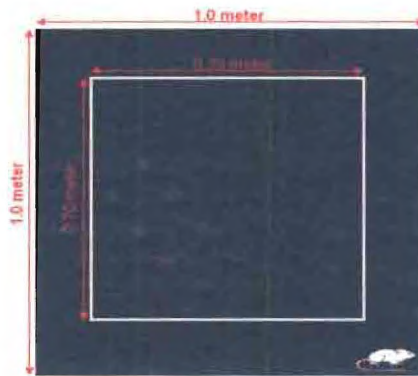


Figure B2.1 The Open-field apparatus is a box with area dimensions of 1 m by 1 m, the wall of the open-field is 0.5 m high, the white line within the box depicts the marking for the inner zone which is 0.7 m by 0.7 m, the positioning of the rat in the diagram indicates the placement of the animals in the open-field apparatus.

B2.7.2 Elevated-plus maze behaviour

The elevated plus maze is a plastic + shaped apparatus, raised above the ground by 0.5 m (figure B2.2). The rats were recorded for five min each in the elevated-plus maze after a two hr period of rest after exploring in the open-field apparatus. Rats were placed in the centre of the elevated-plus maze facing an open arm. Parameters analyzed in the elevated-plus maze; (a) total time in the closed arms (sec), (b) total time in open arms (sec), and the (c) number of entries into the open arms. The lux above the elevated-plus maze was maintained at 50.

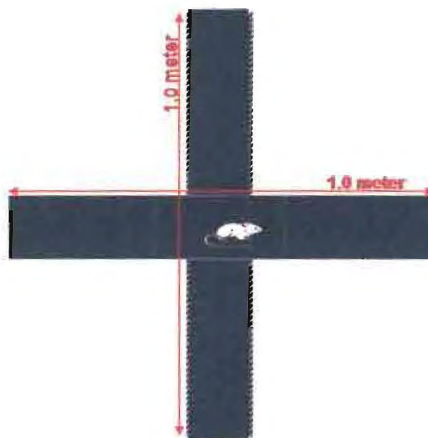


Figure B2.2 The elevated-plus maze, is a plus-shaped object with dimensions of 1 by 1 m, the maze is elevated by 0.5 m, the striped lines in the diagram represent the closed arms, the closed arms have walls of 0.5 m high with open endings, the two other arms have a shallow lip, to prevent animals from falling off with open endings. Placement of the rat into the elevated-plus maze is indicated in the diagram.

B2.8 Nicotine treatment

Pups used in this study were housed individually on PND 28, their cages were placed close together, allowing for oral vocalizations. Separation was unfortunately necessary to allow for oral self-administration of pretreatment and drug within homecage. Oral self-administration of acute nicotine was achieved by giving pups a drop of condensed milk for three consecutive days at 09h00, both treated and control animals received a drop of condensed milk. Pups were weighed on the day of behaviour or neurochemistry to determine the volume of concentrated nicotine solution that needed to be mixed with the condensed milk. The nicotine solution (0.6mg/kg, (-)-nicotine hydrogen tartrate sal, minimum 98% TLC, Sigma-Aldrich) was freshly prepared each day. On the day of behavioural assessment or neurochemistry, 0.6 mg/kg nicotine dose was given to each rat pup one hr prior to manipulation. All animals used in the drug

treatment study were naïve and only received a single acute dose of nicotine. This oral method of self-administration reduced the effects of the stress response to gavage administration or subcutaneous injection.

B2.8 Statistical analysis

The Statistica 8 software package was used for the statistical analyses. Parametric statistics were performed on all neurochemical results, since the Shapiro-Wilks W test revealed that the neurochemical data was normally distributed. Repeated measures ANOVA and one-way ANOVA were applied as appropriate, followed by Tukeys HSD post-hoc test. Non-parametric statistics were used for all behavioural data, since the behavioural data was not normally distributed according to the Shapiro-Wilks W test. When comparing two independent samples Mann-Whitney U test was performed. When comparing several independent samples Kruskal-Wallis ANOVA was performed. Friedman ANOVA was performed when comparing several dependent variables. Wilcoxon matched pairs test was performed when comparing two dependent variables. Results are reported as mean $\pm$ SEM, median and quartiles for the non-parametric statistics can be found in the respective appendices.

B3. Results

B3.1 Concentration-dependent glutamate-stimulated release of [³H]NE from hippocampal slices of SHR and WKY

To determine the optimal glutamate concentration required for the release [³H]NE from rat hippocampal slices, buffer containing varying concentrations of glutamate were superfused onto hippocampal slices of SHR ($n = 6$) and WKY ($n = 6$). This experiment was necessary as glutamate-stimulated release of [³H]NE has not been previously investigated in hippocampal tissue.

One-way ANOVA revealed significant differences between strains at glutamate concentrations of 10^{-2} M and 10^{-3} M ($p < 0.05$, figure B3.1.1a (appendix B1)). Repeated measures ANOVA for each strain revealed that glutamate-stimulated release of [³H]NE at glutamate concentrations of 10^{-2} M and 10^{-3} M was significantly different from all other glutamate concentrations for both SHR (figure B3.1.1b, appendix B1) and WKY (figure B3.1.1c, appendix B1). As a result glutamate was used at a concentration of 10^{-3} M for the second experiment (B3.2).

The results above were achieved by a single stimulation (S_1) of hippocampal slice preparations by exposure to varying concentrations of glutamate. This study was extended by stimulating the tissues for a second (S_2) and third (S_3) time. For each strain independently, repeated measures ANOVA revealed significantly greater release of [³H]NE at S_1 than at S_2 and S_3 , for glutamate concentrations of 10^{-2} M and 10^{-3} M ($p < 0.05$, figure B3.1.2 left column, appendix B1).

Finding similar patterns of [³H]NE release in SHR and WKY for glutamate concentrations of 10^{-2} M and 10^{-3} M, fractional change in release was calculated between the three glutamate

stimulations. This was achieved by dividing the glutamate-stimulated fractional release of [³H]NE by the glutamate-stimulated fraction release of the previous stimulation (S_2/S_1 and S_3/S_2). Repeated measures ANOVA revealed no differences in fractional change between strain or glutamate concentration. Indicating that both strains release of [³H]NE was similar (figure B3.1.2 right column, appendix B1).

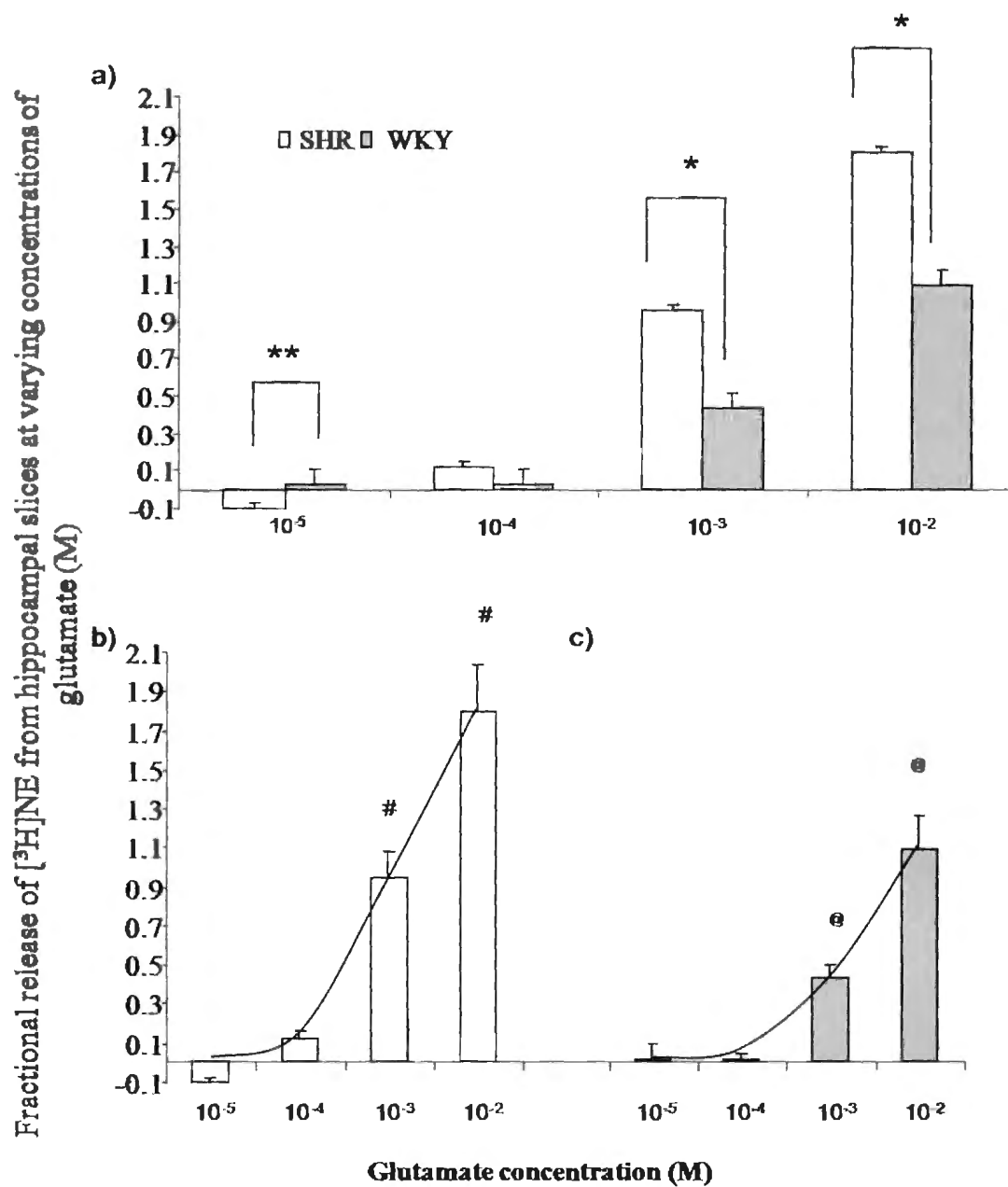


Figure B3.1.1 Concentration-dependent glutamate-stimulated release of $[^3\text{H}]\text{NE}$ from hippocampal slices of (a) SHR ($n=6$) and WKY ($n=6$), *significant differences between SHR and WKY, ** SHR significantly different to WKY. (b) SHR, # significantly different from all other concentrations and (c) WKY, @ significantly different from all other concentrations ($p < 0.05$, mean $\pm$ SEM).

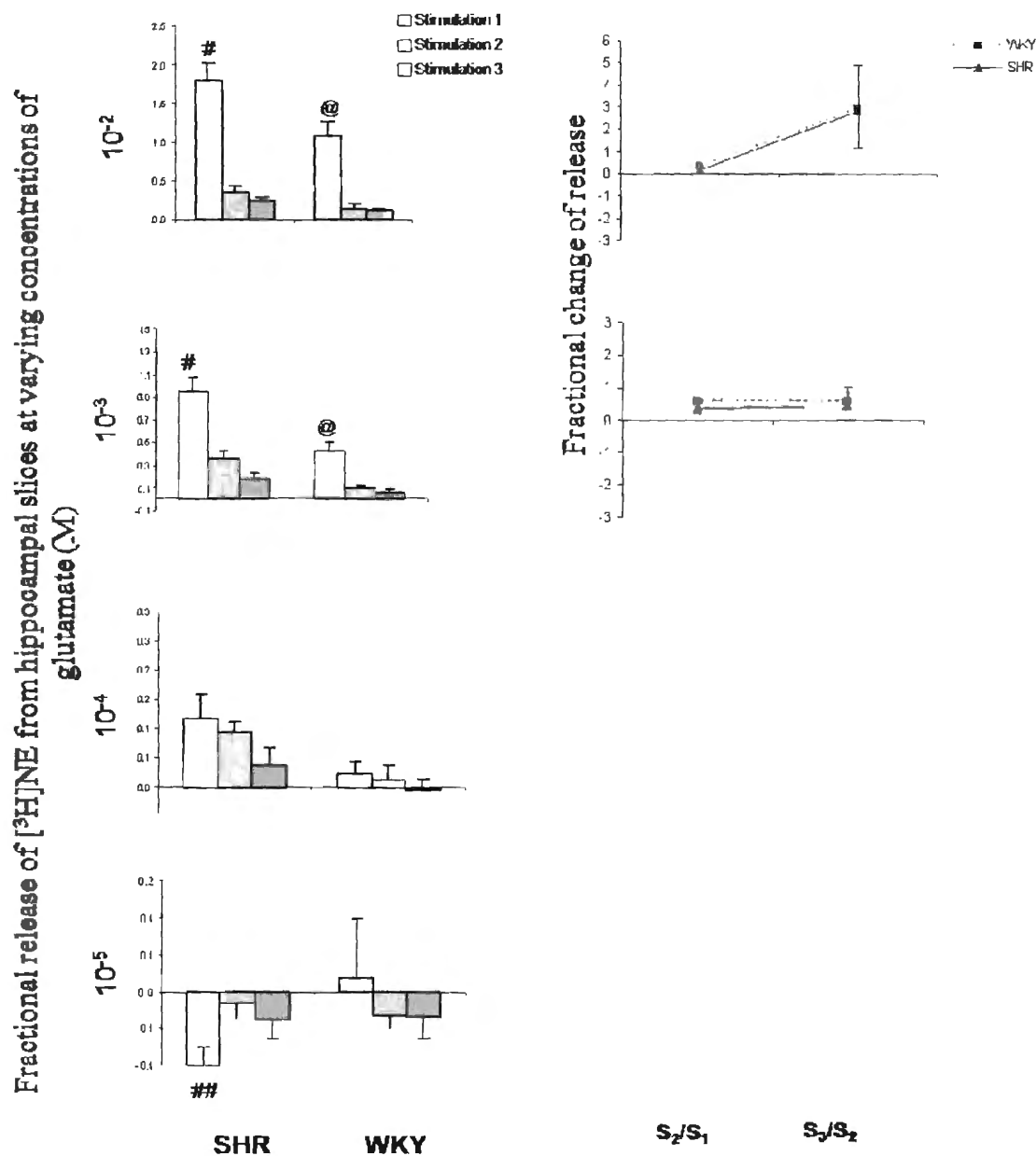


Figure B3.1.2 Concentration-dependent glutamate-stimulated release of $[^3\text{H}]$ NE from hippocampal slices of SHR ($n=6$) and WKY ($n=6$), three stimulations (S1, S2, S3). Left column shows SHR and WKY with repeated exposure to varying concentrations of glutamate. [#] SHR released significantly more $[^3\text{H}]$ NE in their first exposure to glutamate than in their second and third exposure to glutamate at concentrations of 10^{-2}M and 10^{-3}M . [@] WKY released significantly more $[^3\text{H}]$ NE in their first exposure to glutamate than in their second and third exposure to glutamate at concentrations of 10^{-2}M and 10^{-3}M . ^{##} SHR released significantly less $[^3\text{H}]$ NE in their first exposure to glutamate than their third exposure to glutamate at a concentration of 10^{-5}M . Right column, no differences were found for the fractional change (S_2/S_1 and S_3/S_2) in $[^3\text{H}]$ NE release between SHR and WKY ($p < 0.05$, mean $\pm$ SEM).

Due to the controversy with respect to WKY possibly modeling a separate disorder and therefore not serving as a good control for SHR, a study was conducted to determine 10^{-3} M glutamate-stimulated fractional release of [³H]NE in several rats strains, including; SD, Wistar, and Long-Evans hooded rats. Hippocampi were bilaterally removed and independently assessed for glutamate-stimulated release of [³H]NE ($n_{\text{SHR}} = 8$, $n_{\text{WKY}} = 8$, $n_{\text{SD}} = 11$, $n_{\text{Wistar}} = 10$, $n_{\text{Long-Evans hooded rat}} = 11$). Repeated measures ANOVA revealed significant differences for strain. No inter-hemispheric differences were found. Tukeys HSD post-hoc test revealed that SHR released significantly more of [³H]NE than WKY, Sprague-Dawley and Long-Evans hooded rats. WKY released significantly less of [³H]NE than Wistar and Long-Evans hooded rat. SD and Long Evans hooded rat's released significantly less [³H]NE than Wistar rats ($p < 0.05$, figure B3.2.1, appendix B2).

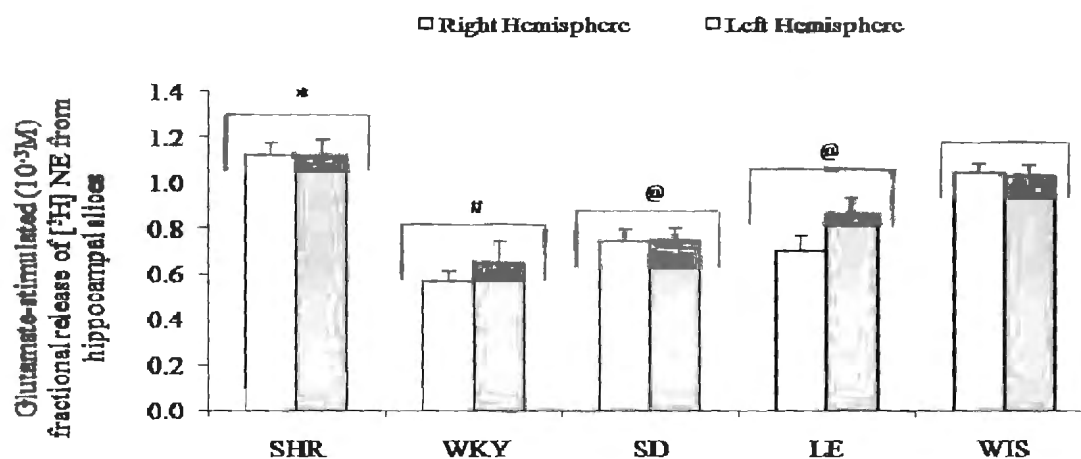


Figure B3.2.1 Differential release of [³H]NE from hippocampal slices of several rat strains, in response to 10^{-3} M glutamate. * SHR ($n_{\text{SHR}} = 8$) released significantly more [³H]NE than WKY ($n_{\text{WKY}} = 8$), SD ($n_{\text{SD}} = 11$) and Long-Evans hooded rats ($n_{\text{LE}} = 11$). @WKY released significantly less [³H]NE than Wistar ($n_{\text{WIS}} = 10$) and Long-Evans hooded rats. @SD and Long-Evans hooded rat released significantly less [³H]NE than Wistar ($p < 0.05$, mean $\pm$ SEM)

B3.3 Glutamate-stimulated release of [³H] NE from hippocampal slices in the presence of ionotropic glutamate receptor antagonists

SHR and WKY rats were used to determine the role of glutamate ionotropic receptors in the release of [³H]NE from noradrenergic terminals in hippocampal slices by exposing tissues to 250 μ M glutamate which was below the K_{off}/K_{on} for glutamate binding of AMPA receptors (Attwell and Gibb, 2005). This was achieved by stimulating the hippocampal slices in the presence of glutamate receptor antagonists; (1) α -amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) antagonist (CNQX at a concentration of 1 μ M, Honore et al., 1988) or (2) N-methyl-D-aspartate (NMDA) antagonist (MK801 at a concentration of 10 μ M, Subramaniam and McGonigle, 1991) or (3) a combination of both AMPA and NMDA antagonists.

One-way ANOVA revealed significant differences between strains for the different antagonists (CNQX alone, MK801 alone, CNQX & MK801, $p < 0.05$). The strains (SHR ($n = 6$) and WKY ($n = 6$)) were found to be significantly different ($p < 0.05$, figure B3.3.1a, appendix B3). Tukeys HSD post-hoc analysis the AMPA antagonist (CNQX and CNQX & MK801) led to significant reductions in the release of [³H]NE from hippocampal slices compared to control ($p < 0.05$, figure B3.3.1b,c, appendix B3). An interesting significant finding for SHR hippocampal slices that were incubated with NMDA receptor agonist only, was that it caused an increase in [³H]NE release ($p < 0.05$, figure B3.3.1b, appendix B3).

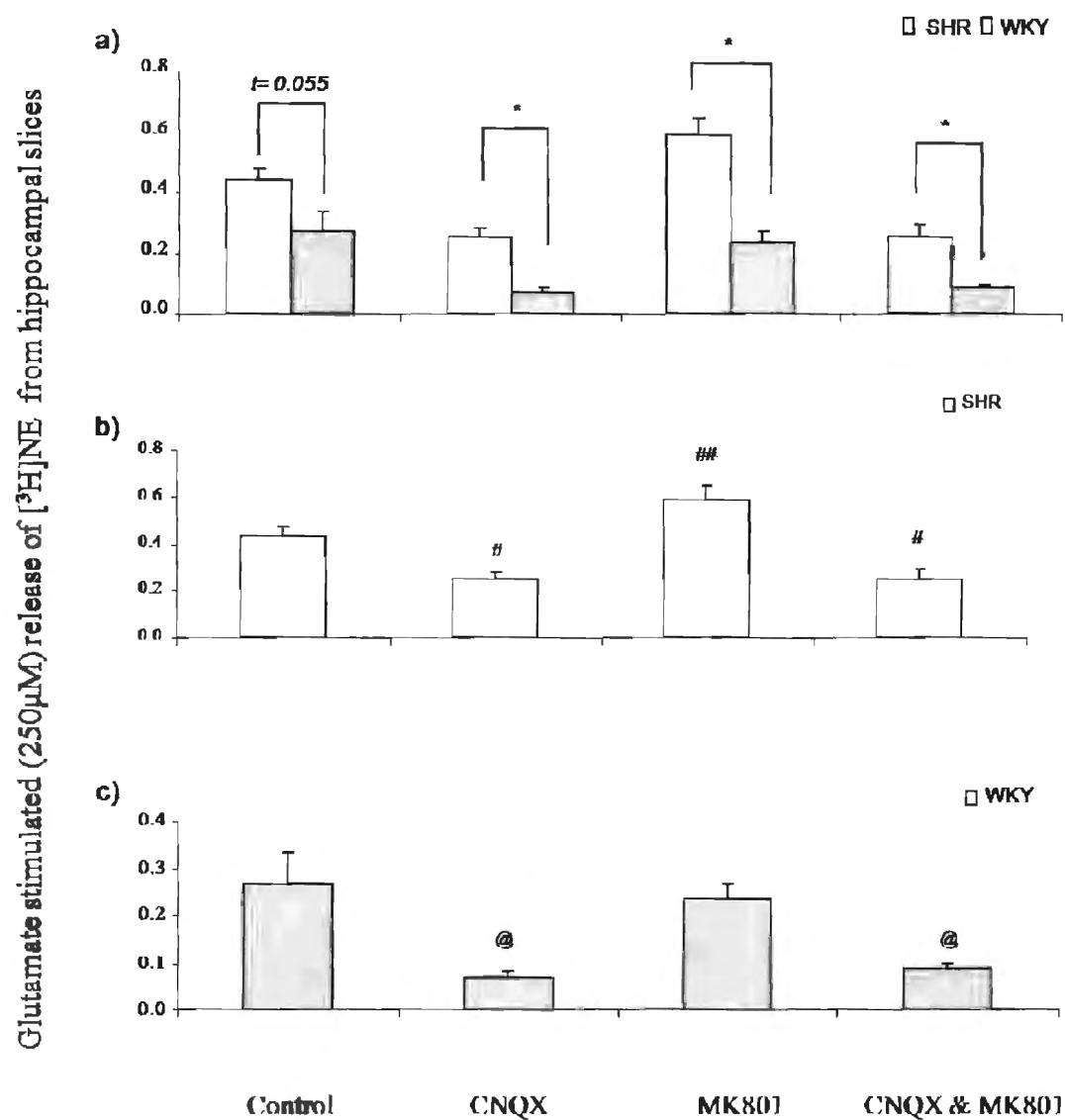


Figure B3.3.1 (a) Ionotropic glutamate receptor antagonist effects on 250 μ M glutamate-stimulated release of [^{3} H]NE from hippocampal slices of SHR ($n=6$) and WKY ($n=6$), *significant differences between SHR and WKY, (b) SHR, # release significantly less than control and slices incubated with NMDA receptor antagonist (MK801), ## significantly greater fractional release of [^{3} H]NE compared to control, (c) WKY, @ release significantly less than control and slices incubated with NMDA receptor antagonist (MK801) ($p < 0.05$, mean $\pm$ SEM).

The results described above were achieved by a single stimulation (S_1) of the hippocampal slice preparation. This study was extended by stimulating the tissues for a second (S_2) and third (S_3) time. Within each strain (SHR and WKY), repeated measures ANOVA revealed significant differences for glutamate-stimulated release of [^{3} H]NE in the presence of antagonists ($p < 0.05$, figure B3.3.2 left column ,appendix B3). Tukeys HSD post-hoc analysis for SHR revealed that S_1 was significantly greater than S_2 and S_3 , except for hippocampal tissue stimulated in the presence of the AMPA receptor antagonist alone which only showed a difference between S_1 and S_2 ($p < 0.05$, figure B3.3.2 SHR column ,appendix B3). Tukeys HSD post-hoc analysis for WKY revealed that S_1 was significantly greater than S_2 and S_3 , except for hippocampal tissue stimulated in the presence of both AMPA and NMDA receptor antagonists which only showed a difference between S_1 and S_3 ($p < 0.05$, figure B3.3.2 WKY column, appendix B3). Again we sought to determine whether there was a similarity in the change of release between strains (SHR and WKY) over the three stimulations. Repeated measures ANOVA revealed no differences within strain or between SHR and WKY in the presence of antagonists (figure B3.3.2 right column, appendix B3).

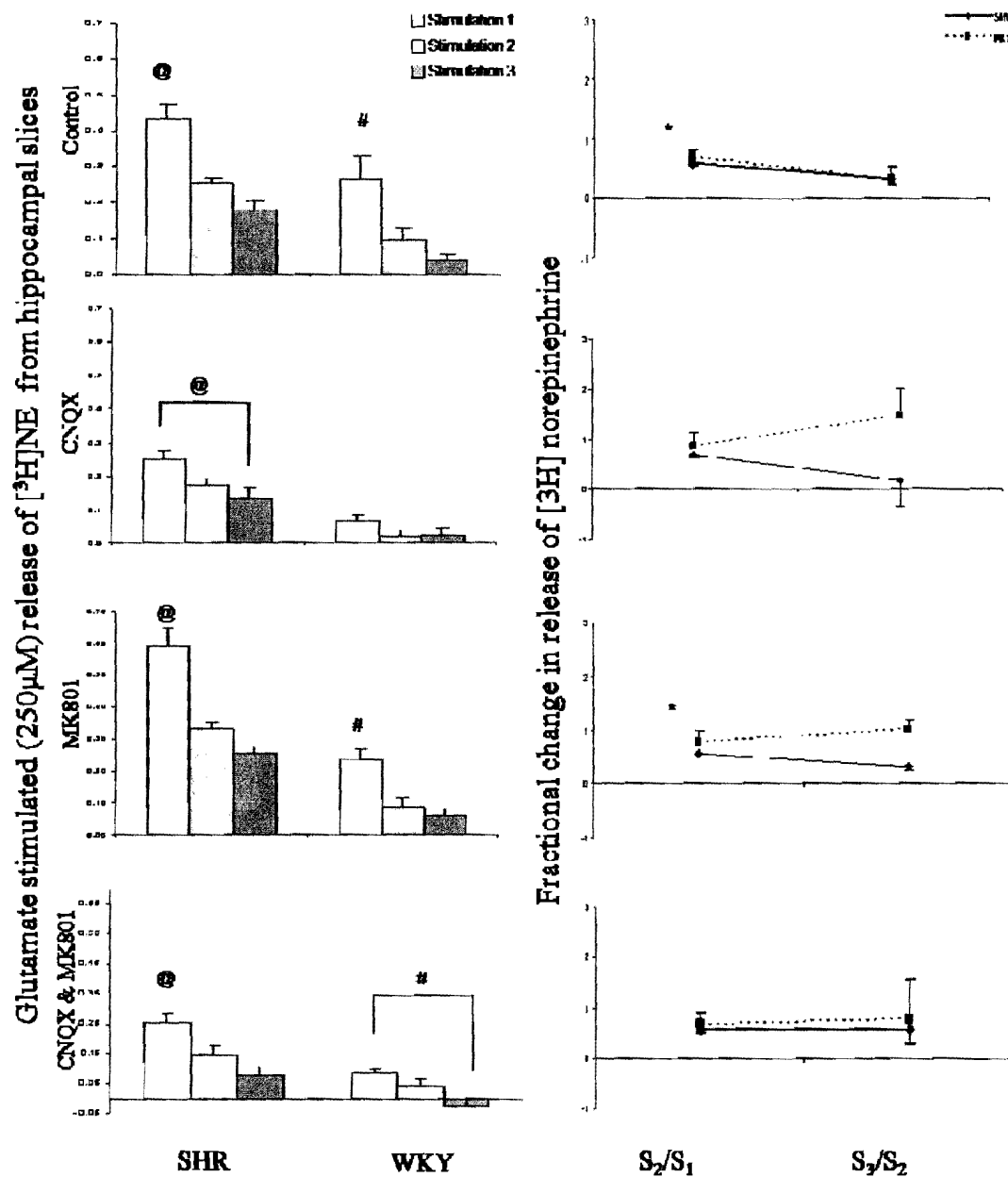


Figure B3.3.2 Ionotropic glutamate receptor antagonist effects on 250 μ M glutamate-stimulated release of [^{3} H]NE from hippocampal slices of SHR ($n = 6$) and WKY ($n = 6$). Three stimulations (S_2 and S_3 were collected, nine and nineteen min after the first stimulation, S_1 , respectively). Left column SHR, @ S_1 significantly greater than S_2 and S_3 , @@ S_1 significantly greater than S_3 , left column WKY, # S_1 significantly greater than S_2 and S_3 , ## S_1 significantly greater than S_3 . Right column no differences in fractional release rate ($p < 0.05$, mean $\pm$ SEM).

B3.4 Glutamate-stimulated release of [³H] norepinephrine from lateral cerebellar slices and vermis cerebellar slices of SHR and WKY

To determine whether glutamate stimulated release of [³H]NE in rat cerebellum, buffer containing 10⁻³M glutamate was superfused onto cerebellar slices of SHR (n = 6) and WKY (n = 6). This experiment was carried out to determine whether global differences in glutamate-stimulated release of [³H]NE was apparent for LC-NE terminals of SHR and WKY. Repeated measures ANOVA revealed a significant effect of strain. SHR released greater amounts of [³H]NE when stimulated with 10⁻³ M glutamate than WKY. No differences were found within strain for glutamate-stimulated fractional release of [³H]NE lateral cerebellar slices or vermis (figure B3.4.1, appendix B4).

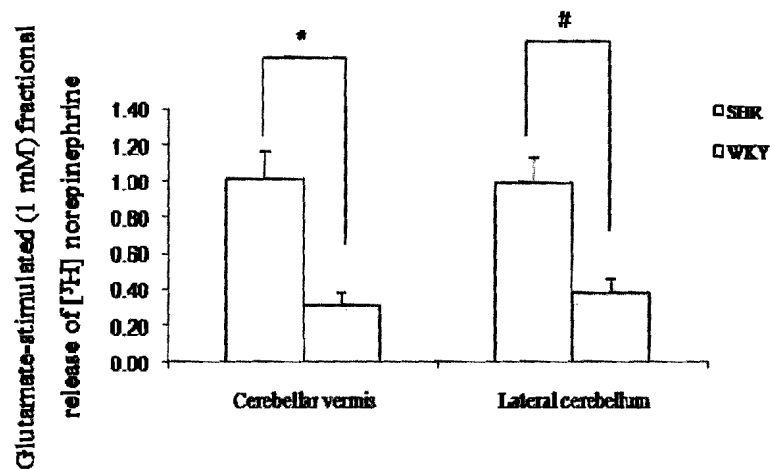


Figure B3.4.1 Differential release of [³H]NE from cerebellar slices of SHR (n = 6) and WKY (n = 6), in response to 10⁻³M glutamate. *SHR released significantly more [³H]NE than WKY for both cerebellar vermis and lateral cerebellar slices (p < 0.05, mean ± SEM)

B3.5 Effects of cross-fostering on behaviour in the open-field and elevated-plus maze and glutamate-stimulated release of [³H]NE from hippocampal and prefrontal cortical slices

Three strains of rat were cross-fostered at PND 2 and reared by different strains of dam, thereby changing their maternal environment. These cross-fostered rats are identified by strain of rearing dam and strain of pup (dam'pup (nSHR'SHR = 12, nWKY'SHR = 14, nSD'SHR = 12, nWKY'WKY = 12, nSHR'WKY = 12, nSD'WKY = 11, nSD'SD = 11, nWKY'SD = 10, nSHR'SD = 15)).

B3.5.1 Behaviour

Kruskal Wallis one-way ANOVA revealed significant effect of maternal environment in behaviour in the open-field behaviours (distance traveled, frequency to the inner-zone, time spent in the inner-zone, latency to enter the inner-zone), and elevated-plus maze (time spent in the closed arms, time spent in the open arms, number of entries to the open-arms) ($p < 0.05$, appendix B5).

B3.5.1.1 Open-field behaviour

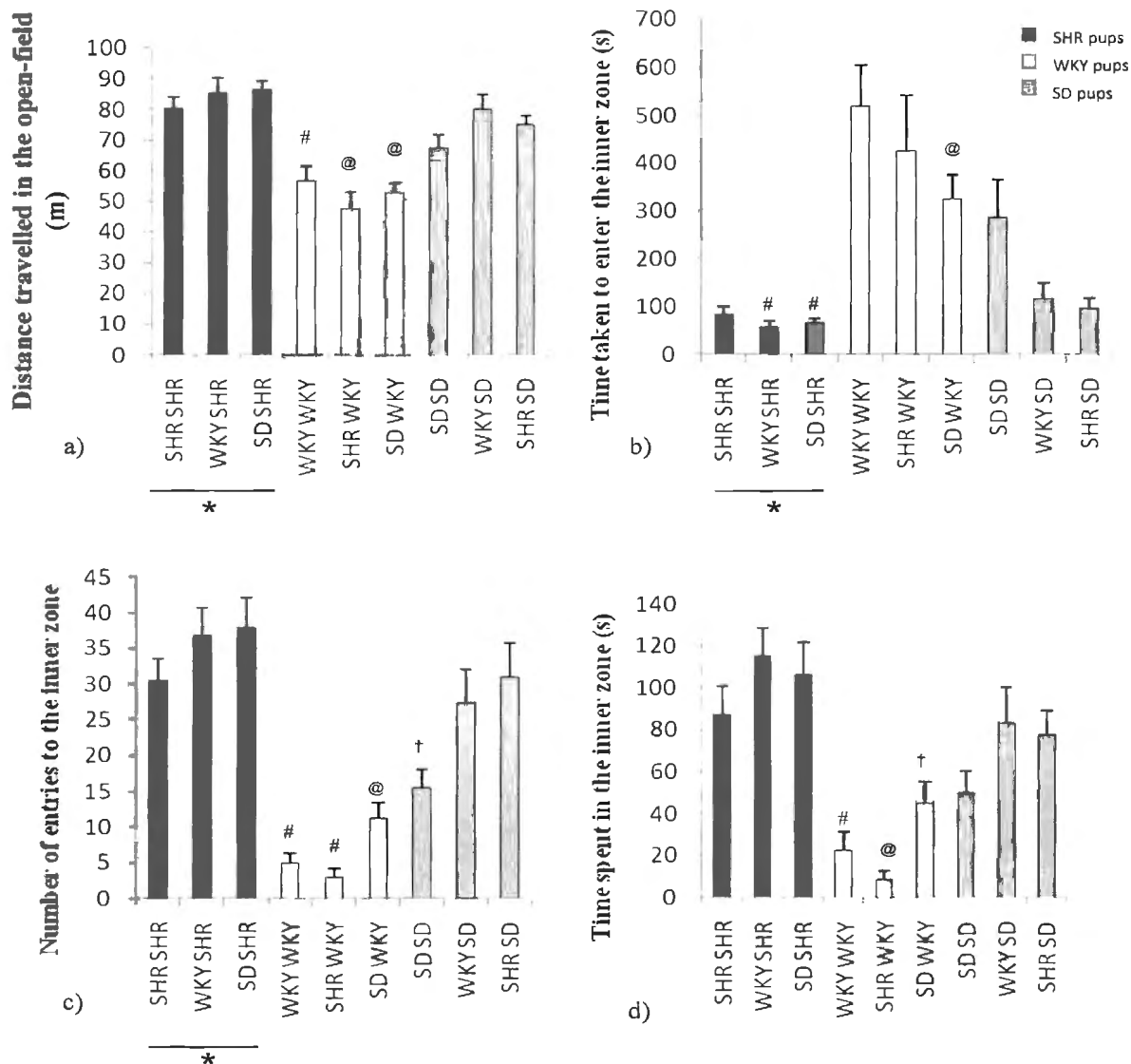
The Mann-Whitney U post hoc test revealed several significant differences between pups in behaviour in the open-field; (a) distance traveled, (b) time taken to enter the inner zone, (c) number of entries to the inner zone, and (d) time spent in the inner zone.

For (a) distance traveled in the open-field: SHR pups traveled more than all WKY pups and SD pups reared by SD dams; WKY pups reared by SHR dams and SD dams traveled less than all SD pups ($p < 0.05$, figure B3.5.1a).

For (b) time taken to enter the inner zone: all SHR pups took less time than all WKY pups and SD pups reared by SD dams to enter the inner zone. SHR pups reared by WKY dams or SD dams took less time to enter the inner zone than SD pups reared by WKY dams or those reared by SHR dams. WKY pups reared by SD dams took less time to enter the inner zone than WKY pups reared by WKY dams or SHR dams ($p < 0.05$, figure B3.5.1b).

For (c) number of entries into the inner zone: all SHR pups entered the inner zone more frequently than all WKY pups and SD pups reared by SD dams. WKY pups reared by WKY dams or SHR dams entered the inner zone less than all SD pups and WKY reared by SD dams. WKY pups reared by SD dams entered the inner zone less than SD pups reared by SHR or WKY dams. SD pups reared by SD dams entered the inner zone less than SD pups reared by SHR dams or WKY dams ($p < 0.05$, figure B3.5.1c).

For (d) time spent in the inner zone: all SHR pups spent more time in the inner zone than all WKY pups and SD pups reared by SD dams. WKY pups reared by WKY dams spent less time in the inner zone than all SD pups and WKY pups reared by SD dams. WKY pups reared by SHR dams spent less time in the inner zone than all SD pups. SD pups reared by SD dams spent less time in the inner zone than SD pups reared by SHR dams ($p < 0.05$, figure B3.5.1d, appendix B5).



B3.5.1 Effects of cross-fostering on behaviours in the open-field apparatus; (a) distance traveled in the open-field: * SHR pups traveled more than all WKY pups, and SD pups reared by SD dams. [#]WKY pups reared by SHR dams and SD dams traveled less than all SD pups. For (b) time taken to enter the inner zone * all SHR pups took less time than all WKY pups and SD pups reared by SD dams to enter the inner zone, [#]SHR pups reared by WKY dams or SD dams took less time to enter the inner zone than SD pups reared by WKY dams or SHR dams. [@]WKY pups reared by SD dams took less time to enter the inner zone than WKY pups reared by WKY dams or SHR dams. For (c) number of entries into the inner zone *all SHR pups entered the inner zone more frequently than all WKY pups and SD pups reared by SD dams. [#]WKY pups reared by WKY dams or SHR dams entered the inner zone less frequently than all SD pups and WKY reared by SD dams, [@]WKY pups reared by SD dams entered the inner zone less frequently than SD pups reared by SHR or WKY dams, [†]SD pups reared by SD dams entered the inner zone less frequently than SD pups reared by SHR dams or WKY dams. For (d) time spent in the inner zone: *all SHR pups spent more time in the inner zone than all WKY pups and SD pups reared by SD dams, [#]WKY pups reared by WKY dams spent less time in the inner zone than all SD pups and WKY pups reared by SD dams, [@]WKY pups reared by SHR dams spent less time in the inner zone than all SD pups, [†]SD pups reared by SD dams spent less time in the inner zone than SD pups reared by SHR dams (p < 0.05, mean ± SEM, median and quartiles appendix B7).

B3.5.1.2 Elevated-plus maze behaviour

Mann-Whitney U post hoc test revealed several differences in behaviour in the elevated-plus maze; (a) time spent in the closed arms, (b) time spent in the open arms, and (c) number of entries into the open arms. For (a) time spent in the closed arms: SHR pups reared by SHR dams spent less time in the closed arms than WKY pups reared by SD and SHR dams and SD pups reared by WKY or SD dams. SHR pups reared by WKY dams spent less time in the closed arms than WKY pups reared by SHR and SD pups reared by WKY or SD dams. SHR pups reared by SD dams spent less time in the closed arms than all WKY pups, SD pups reared by SD dams or WKY dams. SD pups reared by SD or WKY dams spent more time in the closed arms than SD pups reared by SHR dams ($p < 0.05$, figure B3.5.2a).

For (b) time spent in the open arms: SHR pups reared by SHR dams spent more time in the open arms than WKY pups reared by SD dams and SD pups reared by WKY dams and spent less time in the open arms than SHR pups reared by SD dams; SHR pups reared by WKY dams spent more time in the open arms than SD pups reared by SD dams or WKY dams and WKY pups reared by SHR dams or WKY dams. SHR pups reared by SD dams spent more time in the open arms than all WKY pups, than SD pups reared by SD dams or WKY dams. WKY pups reared by SHR dams spent less time in the open arms than SD pups reared by SHR dams ($p < 0.05$, figure B3.5.2b).

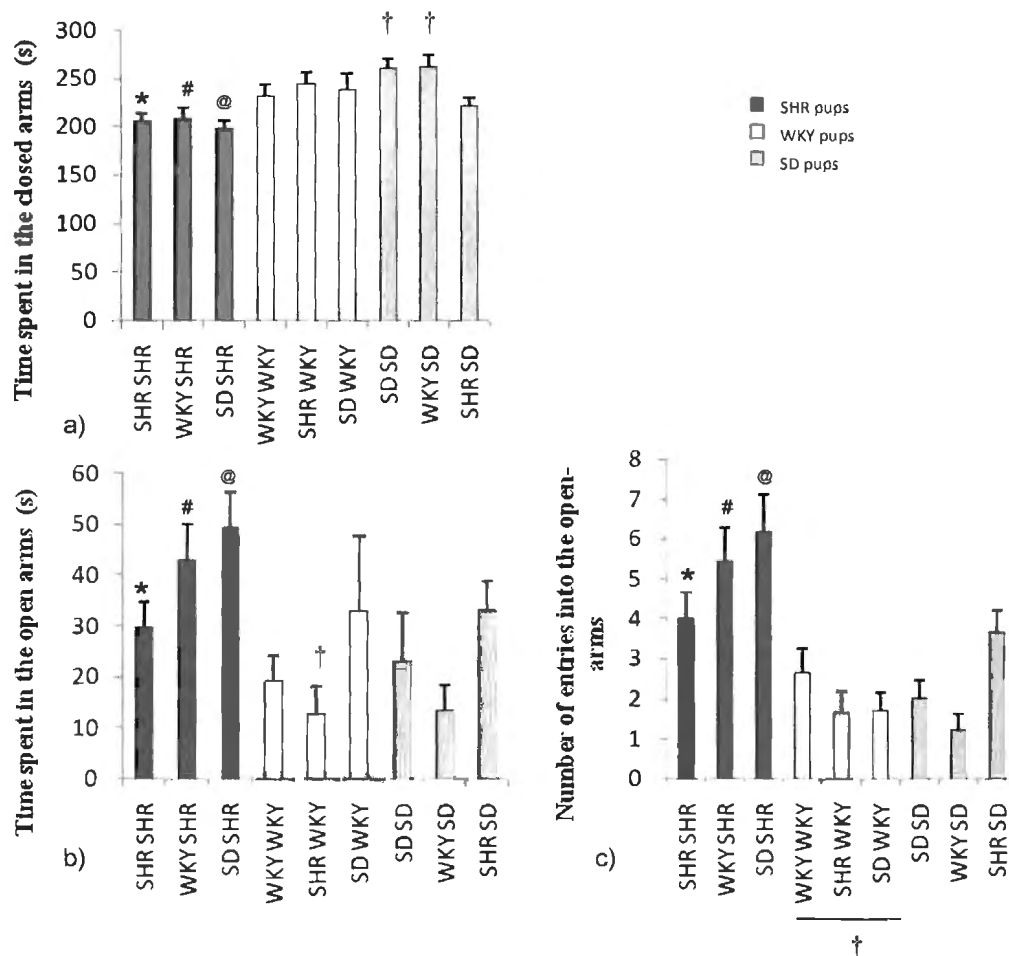


Figure 3.5.2 Effects of cross-fostering on behaviours in the elevated-plus maze; For (a) time spent in the closed arms, *SHR pups reared by SHR dams spent less time in the closed arms than WKY pups reared by SD and SHR dams and SD pups reared by WKY or SD dams, #SHR pups reared by WKY dams spent less time in the closed arms than WKY pups reared by SHR and SD pups reared by WKY or SD dams, @SHR pups reared by SD dams spent less time in the closed arms than all WKY pups, SD pups reared by SD dams or WKY dams, †SD pups reared by SD or WKY dams spent more time in the closed arms than SD pups reared by SHR dams. For (b) time spent in the open arms, *SHR pups reared by SHR dams spent more time in the open arms than WKY pups reared by SD dams and SD pups reared by WKY dams and spent less time in the open arms than SHR pups reared by SD dams, #SHR pups reared by WKY dams spent more time in the open arms than SD pups reared by SD dams or WKY dams and WKY pups reared by SHR dams or WKY dams, @SHR pups reared by SD dams spent more time in the open arms than all WKY pups, than SD pups reared by SD dams or WKY dams, †WKY pups reared by SHR dams spent less time in the open arms than SD pups reared by SHR dams. For (c) number of entries into the open arms, *SHR pups reared by SHR dams entered the open arms more frequently than WKY pups reared by SD dams, #SHR pups reared by WKY dams entered the open arms more frequently than all WKY pups, SD pups reared by SD dams or WKY dams, @SHR pups reared by SD dams entered the open arms more frequently than all SD pups and all WKY pups, †all WKY pups entered the open arms less frequently than SD pups reared by SHR dams ($p < 0.05$, mean $\pm$ SEM, medians and quartiles appendix B7).

For (c) number of entries into the open arms: SHR pups reared by SHR dams entered the open arms more frequently than WKY pups reared by SD dams. SHR pups reared by WKY dams entered the open arms more frequently than all WKY pups, SD pups reared by SD dams or WKY dams. SHR pups reared by SD dams entered the open arms more frequently than all SD pups and all WKY pups. All WKY pups entered the open arms less frequently than SD pups reared by SHR dams ($p < 0.05$, figure B3.5.2c, appendix B5).

B3.5.2 Neurochemistry

One-way ANOVA was carried out on glutamate-stimulated (10^{-3} M) fractional release of [^{3} H] NE revealing significant differences for the prefrontal cortical slices and hippocampal slices ($p < 0.05$, appendix B5).

Tukeys HSD post-hoc test revealed the following significant differences for glutamate-stimulated fractional release of [^{3} H]NE from prefrontal cortical slices: all SHR pups despite rearing condition released greater amounts of [^{3} H]NE than all WKY pups despite rearing condition and SHR pups reared by WKY dams release greater amounts of [^{3} H]NE than SD pups reared by SD dams ($p < 0.05$, figure B3.5.3a, appendix B5).

Tukeys HSD post-hoc test revealed the following significant differences for glutamate-stimulated fractional release of [^{3} H]NE from hippocampal slices: all SHR pups despite rearing condition released greater fractional release of [^{3} H]NE than all WKY pups, all SHR pups despite rearing condition released greater fractional release of [^{3} H]NE than all SD pups except those reared by WKY dams. SD pups reared by WKY dams did not differ significantly from SHR pups reared by WKY dams or SD dams ($p < 0.05$, figure B3.5.3b, appendix B5).

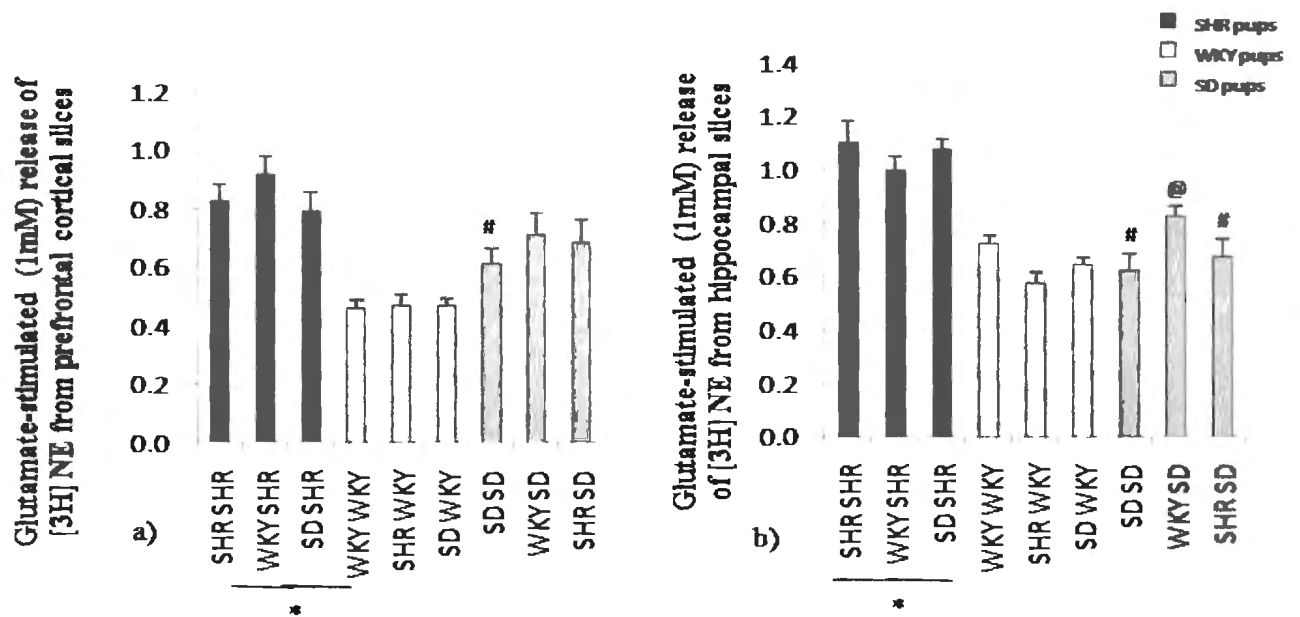


Figure B3.5.1 Differential release of [^3H]NE from (a) prefrontal cortical slices and (b) hippocampal slices of SHR, WKY and SD pups cross-fostered onto different dams. *All SHR pups released significantly greater fractional release of [^3H]NE than all WKY pups despite rearing condition for prefrontal cortical slices and hippocampal slices. (a) [#] SD pups reared by SD dams released significantly less [^3H]NE than SHR pups reared by WKY dams. (b) [#] SD pups reared by SD dams or SHR dams release significantly less [^3H]NE than all SHR pups, [@] SD pups reared by WKY pups released significantly less [^3H]NE than SHR pups reared by SHR dams ($p < 0.05$, mean $\pm$ SEM).

B3.6 Glutamate-stimulated fractional release of [^3H] NE from hippocampal and prefrontal cortical tissue of maternally separated Sprague-Dawley rats

Glutamate-stimulated fractional release of [^3H]NE has not been previously performed on maternally separated rats. An initial study was conducted to determine whether this avenue of research would enrich our understanding of effect the maternal environment on the LC-NE system. One-way ANOVA revealed that maternal separation did not effect glutamate-stimulated release of [^3H]NE from either hippocampal or prefrontal cortical slice preparations of maternally separated rats ($n_{\text{SD}} = 6$) compared to control rats ($n_{\text{SD}} = 6$) (figure B3.6.1 ,appendix B6, Please note that these rats were obtained from Dr Musa Mabandla)

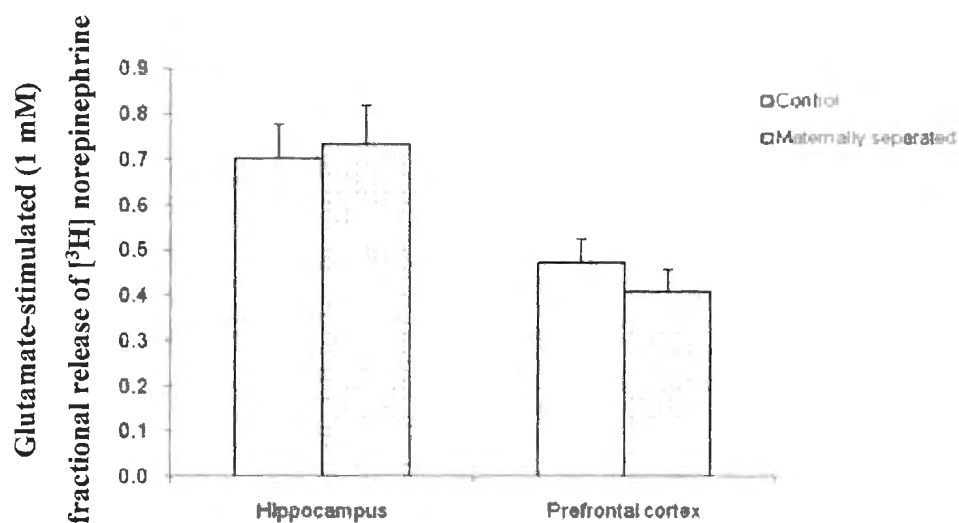


Figure B3.6.1 Glutamate-stimulated release of [^3H]NE from hippocampal and prefrontal cortical slices of SD ($n = 6$) and maternally separated SD rats ($n = 6$). No significant differences were found between control and maternally separated rats (mean $\pm$ SEM).

B3.7 Effects of acute nicotine on behaviour and glutamate-stimulated release of [³H]NE from hippocampal and prefrontal cortical slices in three rat strains

B3.7.1 Behaviour

The effects of an acute dose of nicotine (0.6 mg/kg) on behaviour in the open-field and the elevated-plus maze was determined in three strains of rat ($n_{\text{SHR treated}} = 13$, $n_{\text{WKY treated}} = 10$, $n_{\text{SD treated}} = 11$) in parallel with the controls for each of the three strains ($n_{\text{SHR control}} = 9$, $n_{\text{WKY control}} = 11$, $n_{\text{SD control}} = 13$). Kruskal Wallis ANOVA revealed significant differences for all behavioural parameters for both the open-field and the elevated-plus maze.

B3.7.1.1 Open-field behaviour

Mann-Whitney U post hoc test revealed differences in measurement of the open-field. For (a) distance traveled in the open-field: SHR (control and treated animals) traveled significantly more than WKY (control and treated animals). Control SD animals traveled significantly more than WKY (control and treated animals). Treated SD animals traveled significantly more than WKY (control and treated animals) and SHR (control and treated animals).

For (b) time taken to enter the inner zone; SHR (control and treated animals) took significantly less time to enter the inner zone than WKY (control and treated animals). Control SD animals took significantly less time to enter the inner zone than WKY treated animals. Treated SD animals took significantly less time to enter the inner zone than WKY (control and treated animals).

For (c) number of entries to the inner zone: treated SHR and SD animals entered the inner zone significantly more than WKY (control and treated animals). Control SHR animals entered the inner zone significantly more than WKY (control and treated animals) as well as control SD animals. Treated SHR animals spent significantly more time in the inner zone than treated WKY animals. Treated SD animals spent significantly more time in the inner zone than WKY (control or treated animals).

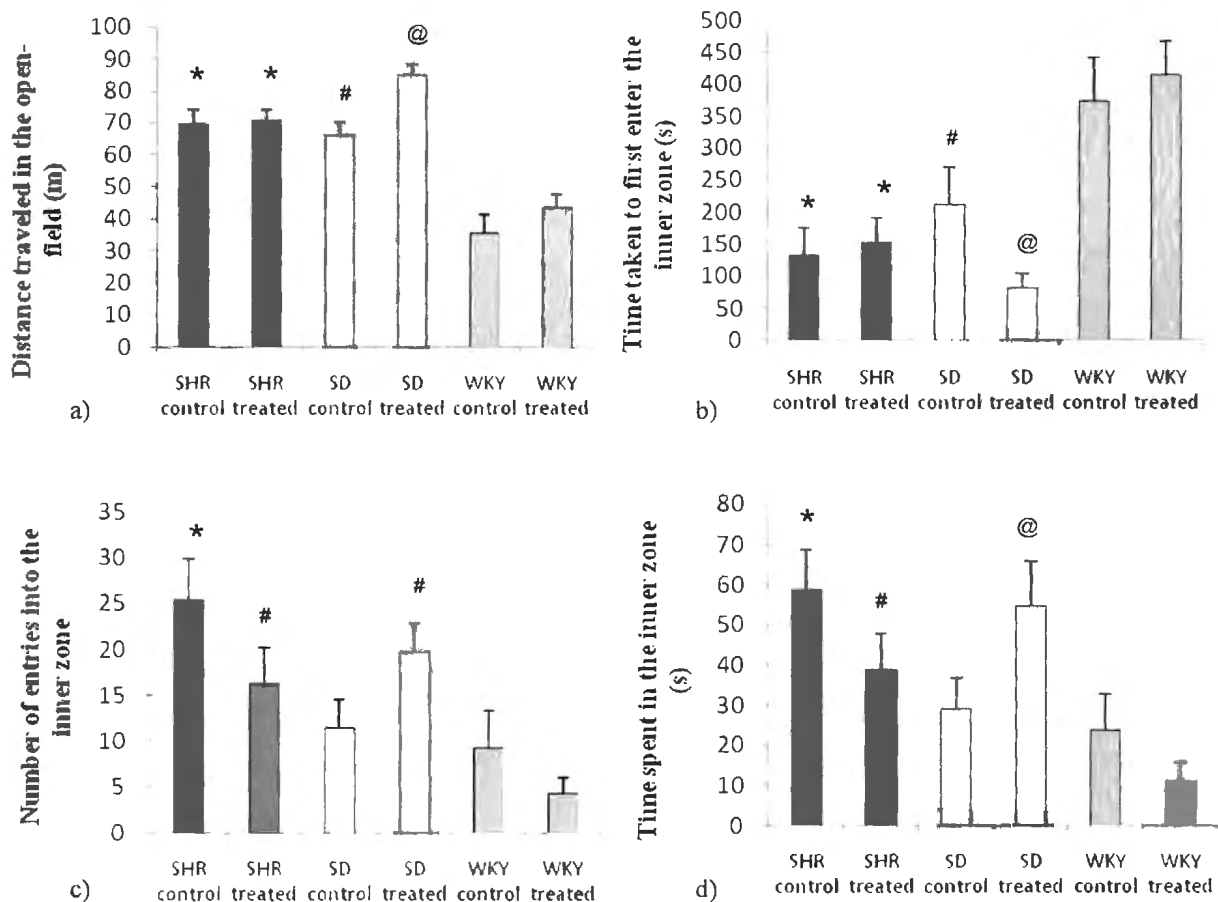
For (d) time spent in the inner zone: control SHR animals spent significantly more time in the inner zone than WKY (control or treated animals) and control SD animals. Treated SHR animals spent significantly more time in the inner zone than treated WKY animals. Treated SD animals spent significantly more time in the inner zone than WKY (control or treated animals) ($p < 0.05$, figure B3.7.1a, b, c, d, appendix B7).

B3.7.1.2 Elevated-plus maze behaviour

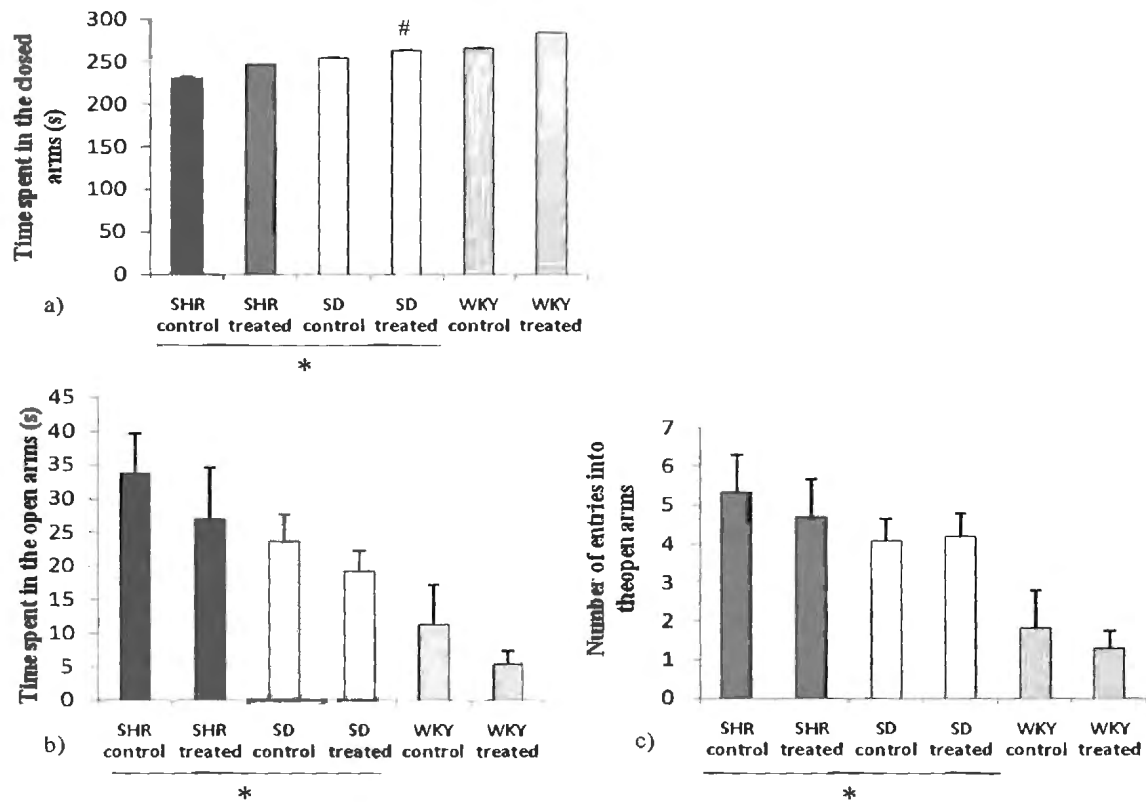
Mann-Whitney U post hoc test revealed several differences in measurement of the elevated-plus maze behaviours. For (a) time spent in the closed arms: control and treated SHR and SD animals spent significantly less time in the closed arms than treated WKY animals. Treated SD animals spent significantly more time in the closed arms than control SHR ($p < 0.05$, figure B3.7.2a).

For (b) time spent in the open arms: control and treated SHR and SD animals spent significantly more time in the open arms than control and treated WKY animals ($p < 0.05$, figure B3.7.2b).

For (c) number of entries into the open arms: control and treated SHR and SD animals entered the open arms more than control and treated WKY animals ($p < 0.05$, figure B3.7.2c, appendix B7).



B3.7.1 Effects of acute nicotine (0.6 mg/kg) on behaviours in the open-field apparatus. For **(a)** distance traveled *control and treated SHR traveled more than control and treated WKY, # control SD animals traveled more than control and treated WKY, @treated SD traveled more than control and treated WKY. For **(b)** time taken to enter the inner zone, *control and treated SHR took less time to enter the inner zone than control and treated WKY, # control SD took less time to enter the inner zone than treated WKY, @treated SD treated took less time to enter the inner zone than control and treated WKY. For **(c)** number of entries to the inner zone, # treated SHR and SD treated entered the inner zone more than control and treated WKY, *control SHR entered the inner zone more than control WKY, treated WKY, and control SD; treated SHR spent more time in the inner zone than treated WKY; treated SD spent more time in the inner zone than control and treated WKY. For **(d)** time spent in the inner zone, *control SHR spent more time in the inner zone than control WKY, treated WKY and SD control, #treated SHR spent more time in the inner zone than treated WKY, @treated SD spent more time in the inner zone than control and treated WKY ($p < 0.05$, mean $\pm$ SEM, median and quartiles appendix B7).



B3.7.2 Effects of acute nicotine (0.6 mg/kg) on behaviours in the elevated-plus maze. For (a) time spent in the closed arms, *control and treated SHR and SD spent less time in the closed arms than treated WKY, [#]treated SD spent more time in the closed arms than control SHR. For (b) time spent in the open arms, * control and treated SHR and SD spent more time in the open arms than control and treated WKY. For (c) number of entries into the open arms, *control and treated SHR and SD entered the open arms more than control and treated WKY ($p < 0.05$, mean $\pm$ SEM, median and quartiles appendix B7).

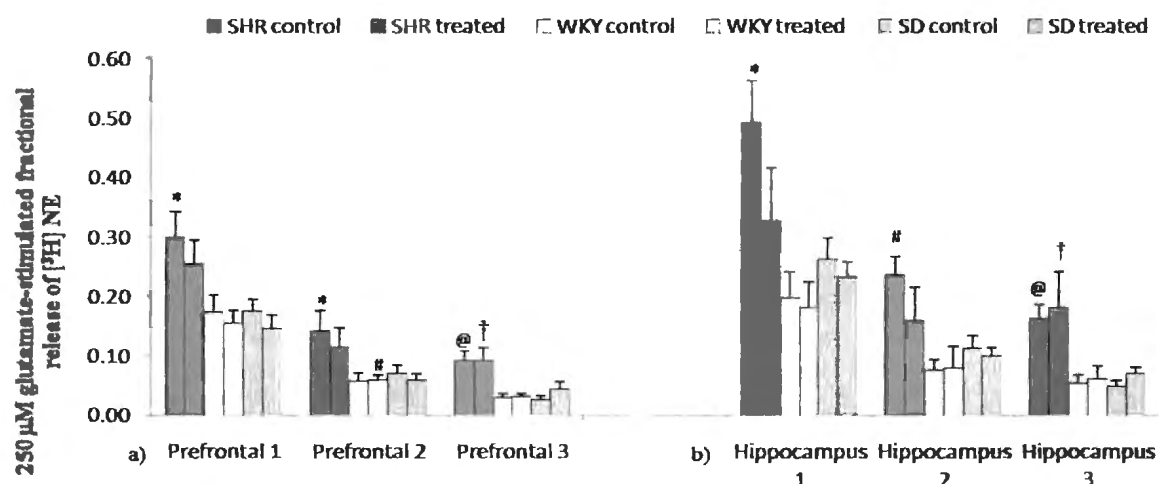
B3.7.2 Neurochemistry

The effects of an acute dose of nicotine (0.6 mg/kg) on glutamate-stimulated fractional release of [³H]NE was determined in three strains of rat ($n_{\text{SHR treated}} = 8$, $n_{\text{WKY treated}} = 11$, $n_{\text{SD treated}} = 10$) in parallel to the controls for each of the three strains ($n_{\text{SHR control}} = 9$, $n_{\text{WKY control}} = 11$, $n_{\text{SD control}} = 13$) were incorporated. Glutamate-stimulated release of [³H]NE was determined for both prefrontal cortical tissue and hippocampal tissue, and underwent three stimulations (S_1 , S_2 , S_3 , described earlier).

One-way ANOVA revealed the following differences for the prefrontal cortex: For the first glutamate-stimulation (S_1) control SHR animals released significantly greater amounts of [³H]NE than treated SD animals. For the second stimulation (S_2) control SHR animals released significantly greater amounts of [³H]NE than treated SD animals and treated WKY animals. For the third stimulation (S_3) control SHR animals released significantly greater amounts of [³H]NE than control and treated WKY animals and control and treated SD animals, and treated SHR animals released significantly greater amounts of [³H]NE than control and treated WKY, and control SD animals ($p < 0.05$, figure B3.7.3a, appendix B7).

One-way ANOVA revealed the following differences for the hippocampus: for the first glutamate-stimulation (S_1) control SHR animals released significantly greater amounts of [³H]NE than control and treated WKY and control and treated SD animals. For the second glutamate-stimulation (S_2) control SHR animals released significantly greater amounts of [³H]NE than control and treated WKY and treated SD animals. For the third glutamate-stimulation (S_3) control SHR animals released significantly greater amounts of [³H]NE than

treated WKY and control SD, and treated SHR animals released significantly greater amounts of [^{3} H]NE than control and treated WKY and control and treated SD animals ($p < 0.05$, figure B3.7.3b ,appendix B7).



B3.7.3 Glutamate-stimulated (250 μ M) release of [^{3} H]NE from prefrontal cortical slices and hippocampal slices, one hr post oral administration of an acute dose of nicotine (0.6 mg/kg). For (a) the prefrontal cortex *control SHR released greater amounts of [^{3} H]NE than treated SD for S_1 and S_2 . # Treated WKY released smaller amounts of [^{3} H]NE than control SHR for S_2 . @ Control SHR released greater amounts of [^{3} H]NE than control and treated WKY and SD for S_3 . † Treated SHR released greater amounts of [^{3} H]NE than control and treated WKY and SD for S_3 . For (b) the hippocampus * control SHR released greater amounts of [^{3} H]NE than control and treated WKY and SD for S_1 . # Treated SHR released greater amounts of [^{3} H]NE than control WKY, treated WKY, and treated SD for S_2 . @ Control SHR released greater amounts of [^{3} H]NE than control WKY, treated and control SD for S_3 . † Treated SHR released greater amounts of [^{3} H]NE than control and treated WKY and SD for S_3 ($p < 0.05$, mean $\pm$ SEM).

Finding similar patterns of [^{3} H]NE release with glutamate stimulation for the three strains of rat: SHR, WKY and SD for the hippocampus and the prefrontal cortex, fractional change in release was calculated between the three glutamate stimulations. This was achieved by dividing the glutamate-stimulated fractional release of [^{3} H]NE by the glutamate-stimulated fraction release of the previous stimulation (S_2/S_1 and S_3/S_2). Repeated measures ANOVA revealed no significant differences in fractional change for strain or treatment (figure B3.7.4, appendix B7).

B4. Discussion

B4.1 Concentration-dependent glutamate-stimulated release of [³H]NE from hippocampal slices of SHR and WKY

SHR released greater amounts of NE from hippocampal slices than WKY at glutamate concentrations of 10^{-2} M and 10^{-3} M. SHR were also found to release greater amounts of NE from prefrontal cortical slices when compared to WKY (Russell and Wiggins, 2000). This finding indicates that glutamate-stimulated release of NE was not restricted to the prefrontal cortex and occurs in an additional brain area that receives its NE innervations from the LC-NE system. These findings suggest that the SHR LC-NE nerve terminals are 'hyper-responsive' to the effects of glutamate, when compared to their 'normotensive' control the WKY.

The differences found in glutamate concentration dependent release of NE may be a result of several potential factors: (1) There may be increased expression of glutamate receptors on the LC-NE terminals of SHR in comparison with WKY, which led to the increased release of NE. Increased expression of AMPA receptor subunits has been observed in the nucleus of the solitary tract of SHR when compared to WKY (Saha et al., 2004). (2) Glutamate receptor subunit composition may differ in SHR compared to WKY, allowing prolonged glutamate receptor phosphorylation and increased release of NE from the nerve terminals (Derkach et al., 2007). (3) NE α_2 -autoreceptor function may be reduced (Russell et al., 2000; Olmos et al., 1991) or desensitized in SHR (Olmos et al., 1991) when compared to WKY. The administration of an α_2 -autoreceptor agonist (guanfacine) has been shown to reduce hyperactivity, impulsivity and improve sustained attention (Sagvolden, 2006). Yet in a second report administration of an α_2 -

autoreceptor agonist (guanfacine) did not improve SHR performance in a divided attentional task (Jentsch, 2005). (4) SHR NE storage vesicles that were released when stimulated with glutamate may hold higher quantities of NE (de Villiers et al., 1995). However no differences were found between SHR and WKY when prefrontal cortical slices were stimulated either electrically or with elevated K^+ (Russell et al., 2000). (5) Increased intracellular Ca^{+2} in SHR may lead to greater externalization of AMPA receptors, when compared to WKY (Horn et al., 1995). With a glutamate concentration of 10^{-4} M differences between SHR and WKY did not persist, suggesting a glutamate concentration dependent effect between these two strains. With a glutamate concentration of 10^{-5} M, SHR showed a negative basal release of NE, suggesting one of two possibilities: (1) low levels of glutamate may activate NMDA receptors leading to the release of GABA, the GABA in turn may activate $GABA_A$ neurons on the noradrenergic terminals, inhibiting release of NE (Vogt and Nicoll, 1999) in SHR. (2) SHR NE reuptake transporters have been shown to work more efficiently in SHR than WKY in the prefrontal cortex and the cerebellum (Myer et al., 1981), this suggests that with low levels of glutamate stimulation and release of NE led to a concentration of NE that stimulates the NE reuptake transporter function, we suggest at low concentrations of glutamate stimulation the release of NE reaches a concentration where the efficiency of NE reuptake is evident when compared to WKY.

These findings were extended by stimulating the hippocampal tissues two additional times (S_2 and S_3), SHR and WKY showed similar decrements in release of NE. These results may be the result of several potential factors: (1) Depletion of available vesicular stores of NE occurs in a similar manner in SHR and WKY. (2) Desensitization of the glutamate receptors occurs at similar rates (Rusakov and Kullman, 1998). (3) Glutamate receptors are internalized at similar

rates in SHR and WKY in response to the one min exposure to glutamate (Sheng and Lee, 2003). An additional factor that may lead to decreased release of NE may be a result of newly synthesized endogenous NE that is taken up into the vesicles, so that when the second and third stimulation occur the packaged NE that is released will be part labeled by radioactively labeled [^3H]NE and part non-labeled NE, therefore the effects and interpretations need to include the possible rundown of labeled transmitter. However calculation of fractional release of radioactivity corrects for this potential error, since the tissue is allowed to equilibrate with the remaining radioactivity labelled NE prior to the 2nd and 3rd stimulation pulses of glutamate.

These results suggest that the LC-NE system operates differently in SHR and WKY the 'normotensive' control for SHR, in response to glutamate stimulation. If we incorporate the present findings in to our present hypothesis: (1) SHR were hyper-responsive to the effects of glutamate at glutamate concentrations 10^{-2} , 10^{-3} and 10^{-4} M and this may be due to decreased synergistic activity that normally occurs between the glutamatergic neurons and the noradrenergic terminals as each have been shown to potentiate each others activity (Marek and Aghajanian, 1999; Harley et al., 1996; Harley, 2007). (2) SHR showed reduced baseline release at glutamate concentration of 10^{-5} M and this may be due to GABA neuron activity or increased activity of NE reuptake transporters.

B3.2 Glutamate-stimulated release of [^3H] NE from hippocampal slices of several rat strains

SHR were found to release greater amounts of NE from hippocampal slices exposed to 10^{-3} M glutamate than WKY and in addition released greater amounts of NE than SD and Long-Evans hooded rat. Suggesting, that SHR was 'hyper-responsive' to glutamate stimulated release of NE, when compared to several 'control' strains of rat. However SHR and Wistar did not differ in response to glutamate stimulated release of NE, which supports the suggestion that Wistar display behavioural characteristics similar to the SHR including impulsiveness, hyperactivity and increased exploratory behaviors (van den Bergh et al., 2006). Albeit that the Wistar is the parent strain to SHR and WKY (Okamoto and Aoki, 1963), the present finding suggests that Wistar should not be used as a behavioural or neurochemical 'control' for SHR or WKY.

WKY were found to release significantly less NE in response to glutamate stimulation of hippocampal slices than SHR, Wistar and Long-Evans hooded rat. Suggesting that WKY are 'hypo-responsive' to the effects of glutamate stimulated release of NE. However WKY glutamate-stimulated release of NE was not different to SD rats. Behaviourally WKY rats have shown high levels of immobility in the forced swim test (Tejani-Butt et al., 2003) and prolonged ACTH activation during acute immobilization stress (Pardon et al., 2002), when compared to SD and Wistar rats. Our present finding and reported studies suggest that WKY is a suitable 'control' for SHR as a model of ADHD when HPA-axis activity is not an aspect of the experiment being performed, and the inclusion of an additional control such as SD would be suitable when HPA-axis activity is being assessed.

If we incorporate the findings into our hypothesis: (1) SHR were ‘hyper-responsive’ to the effects of glutamate when compared to several rat strains, and this may be due to decreased synergistic activity that normally occurs between the glutamatergic neurons and the noradrenergic terminals as each have been shown to potentiate each others activity (Marek and Aghajanian, 1999; Harley et al., 1996; Harley, 2007). (2) WKY was ‘hypo-responsive’ to the effects of glutamate when compared to several rats strains, and this may be due to decreased glutamate receptors or decreased noradrenergic vesicles. Glutamate is known to led to the release of glutamate in several brain structures through activation of the HPA-axis (Bagdley and Moghaddam, 1997) WKY have been shown to respond excessively and for prolonged periods of time to stressors (Pardon et al., 2002). In addition besides WKYs prolonged stress response they have been characterized as ‘hyperaroused’ by their normal behaviours including increased passive avoidance (Paré, 1993,1996) and hypoactivity (Drolet et al., 2002; Marti and Armario, 1996). We suggest that due to the WKYs increased tonic levels of LC activity, a permanent down-regulation of glutamate receptors and decreased vesicular storage of NE occurs in WKY.

B3.3 Glutamate-stimulated release of [³H] NE from hippocampal slices in the presence of ionotropic glutamate receptor antagonists

With antagonism of AMPA receptors in WKY and SHR hippocampal slices, glutamate stimulated release of NE was decreased, WKY showed a greater decrease in release than SHR. This suggests that AMPA receptor activation plays a greater role in WKY than SHR in glutamate stimulated release of NE. These findings were extended by stimulating the hippocampal tissues a second and third time. WKY did not show further decrease in their release of NE. However SHR showed a decrease in release from the first stimulation to the third stimulation, suggesting that an additional glutamate receptor type may be involved in the release of NE.

Antagonism of NMDA receptors had no effect on glutamate stimulated release of NE in WKY rats. However in SHR antagonism of NMDA receptors increased glutamates effect in releasing NE. Previously antagonism of NMDA receptors (MK801) was shown to decrease the release of NE in medulla oblongata slices of SHR and WKY when exposed to glutamate (Tsuda et al., 1994), the medullary NE neurons form part of the ventral noradrenergic bundle, which suggests that this finding may be specific to the LC-NE systems terminals. In addition we suggest that this enhanced release was related to Ca⁺². Influx of Ca⁺² into prefrontal cortical slices of SHR following NMDA receptor activation was found to be higher in WKY than SHR (Lehohla et al., 2004). Intracellular levels of Ca⁺² have been suggested to be elevated in SHR (Horn et al., 1995). In blocking of NMDA receptor influx of Ca⁺² would not have increased to the same extent if NMDA receptors were activated, this suggests that NMDA receptor activation may limit the release of NE.

These findings were extended by stimulating the hippocampal tissues a second and third time. WKY and SHR showed similar decrements in release as in the control hippocampal slices, suggesting the effect seen was primarily through AMPA receptor (1) desensitization (Rusakov and Kullman, 1998) or (2) internalization (Sheng and Lee, 2003).

With antagonism of AMPA and NMDA receptors in WKY and SHR hippocampal slices, glutamate stimulated release of NE was decreased, WKY showed a greater decrease in release than SHR and showed a similar decrement as with AMPA receptor antagonist only. This suggests that AMPA receptor activation in WKY and SHR were the primary receptors that lead to the release of NE when hippocampal tissues were exposed to glutamate.

If we incorporate the findings into our present hypothesis: (1) In SHR the increased release of NE in response to NMDA receptor antagonism, may reflect the limiting effect NMDA receptor activation may have in the LC-NE terminals, which would be able to limit the increased AMPA receptor function that has been suggested in SHR.

B3.4 Glutamate-stimulated release of [³H] norepinephrine from lateral cerebellar slices and cerebellar vermi slices of SHR and WKY

SHR released greater amounts of NE from cerebellar slices than WKY when stimulated with glutamate. This supports our earlier suggestion that the LC-NE nerve terminals are 'hyper-responsive' when exposed to glutamate. Glutamate stimulated release of NE from hippocampal (B2.1) and prefrontal cortical slices (Russell and Wiggins, 2000) were shown to be greater in SHR than WKY. The LC-NE system innervates the hippocampus and prefrontal cortex and is the only source of NE to these brain areas, an additional brain area innervated by the LC-NE system is the cerebellum (Foote et al., 1983; Jentsch, 2005; Aston-Jones et al., 1999; Southwick et al., 1999; Bunsey and Strupp, 1995). In an functional magnetic resonance imaging study, orienting during visual spatial attention showed increased activity of the parietal cortex, frontal frontal cortex as well as the cerebellum (Mao et al., 2007), and the cerebellum has previously been suggested to play a causative role in ADHD (Casey et al., 2007).

If we incorporate the findings in to our present hypothesis: (1) SHRs LC-NE terminals are 'hyper-responsive' in a third area that receives its sole source of NE from the LC, these results support the low tonic activity of the LC and its reduced reinforcement of its tonic activity. These results support our present hypothesis on the function of the LC-NE system and its role in attention and arousal for SHR.

B3.5 Effects of cross-fostering on behaviour in the open-field and elevated-plus maze and glutamate-stimulated release of [³H]NE from hippocampal and prefrontal cortical slices

Open-field behaviours of SHR pups despite their maternal environment showed increased locomotor activity, reduced time taken to enter the inner zone, made a greater number of entries to the inner zone, and increased time spent in the inner zone when compared to the WKY. Suggesting SHR had increased activity and increased levels of exploratory behaviours when compared to WKY within the open-field (Sagvolden et al., 1993; Sukhanov et al., 2004; Wultz et al., 1990; Kaehler et al., 2004). SHR pups differed in several of the open-field parameters when compared to control SD pups as SHR pups showed increased locomotor activity, as they entered the inner zone more frequently and took shorter periods of time to enter the inner zone. SHR activity within the open-field has previously been reported to be higher than SD (Pardon et al., 2002), which supports the present finding. These findings suggest and support SHR as a model of ADHD (Knardahl and Sagvolden, 1979). In addition these 'hyperactive' behaviours elicited by the SHR are resilient to change through maternal environment manipulations, and may be a result of the 'hypoarousal' seen in this model of ADHD.

Elevated-plus maze behaviours of control SHR pups were found to be different to control SD pups and control WKY pups. Control SHR pups spent less time in the closed arms, spent more time in the open arms and entered the open arms more frequently when compared to both control WKY and SD pups. The elevated-plus maze has been used and is reputedly accepted to measure behavioural anxiety (Ramos and Morméde, 1997). Control SHR behaviour within the elevated-

plus maze support the hypothesis that SHR are 'hypoaroused', further supporting the use of SHR as a model of ADHD (Wultz et al., 1990; Kaehler et al., 2004). An interesting finding was the enhancement of these reduced anxiety related behaviours, when the SHR pups were reared by WKY dams and this effect was further enhanced when they were reared by SD dams. Previous studies have shown that cross-fostering of SHR pups onto either WKY or SD dams, reduced mean arterial pressure and reduced their adult hypertension (McCarty and Fields-Okotcha, 1994; McCarty and Lee, 1995; McCarty et al., 1987; Cierpial and McCarty, 1991). It has been suggested that the CRF systems are key to pup and dam interactions and behavioural outcomes (Francis and Meaney, 1999). CRF release has been shown to be controlled by the LC-NE system (Plotsky, 1991). The present findings and reported studies suggest that maternal environment plays an important interactive role with development of anxiety and the development of adult hypertension in SHR, suggesting an interactive role between the LC-NE system and HPA-axis development.

SHR despite their rearing condition showed increased glutamate stimulated release of NE from prefrontal cortical and hippocampal slices, when compared to both WKY and SD rats. Supporting our earlier finding and reported literature of glutamate stimulated release of NE from prefrontal cortical slices (Russell and Wiggins, 2000). Which reinforces the suggestion that the LC-NE system terminals of the SHR are 'hyper-responsive' when stimulated with glutamate.

If we incorporate the findings in to our present hypothesis: (1) SHRs behaviour was related to 'hypoarousal' evidenced by their increased locomotor activities and reduced anxiety, maternal manipulation did not effect these activities if anything accentuated them. We suggest that tonic activities of the LC-NE system may have been affected, leading to a further decrease in the tonic

activity of the LC. These results support our present hypothesis on the function of the LC-NE system and its role in attention and arousal in SHR as a model of ADHD.

Control WKY pups took longer periods of time to enter the inner zone, entered the inner zone less frequently and spent less time in the inner zone of the open-field than control SHR and SD pups. These findings support previously reported studies between SHR and WKY (Wultz et al., 1990; Sukhanov et al., 2004) including decreased activity within the open-field (Sagvolden et al., 1993; Sukhanov et al., 2004). WKY pups when reared by SD or SHR dams showed reduced activity within the open-field than SD pups, however when reared by WKY dams the pups did not show a difference. A previous report found that SD rats showed higher activity in the open-field than WKY rats (Pardon et al., 2002). Interestingly WKY pups reared by SD dams increased their exploratory behaviours in the open-field within similar levels of the control SD pups. The present findings and reported studies suggest that environmental manipulations all be they slight can impact the activity and exploration of WKY strain of rat significantly. Studies have reported that WKY dams supply poor maternal care to their pups when compared to SHR (Cierpial et al., 1990). However an additional report found that cross-fostering of pups lead to changes in the maternal style of the dam toward the biological dam's (Champagne et al., 2003, Champagne and Meaney, 2001). It has been suggested that cross-fostering of hyper-responsive strains can attenuate abnormal behaviours characteristic of the strain (Caldji et al., 2000; Francis et al., 1999; Prakash et al., 2006). We suggest that SD dams may provide a positive effect on the development of WKY pups. One suggestion would be to characterize maternal behaviours and their change with cross-fostering of rat pups to determine whether these behavioural changes are

due to sensory stimulation or changes in levels of maternally related hormones or pheromones that impact the maternal environment for the pups being reared.

Elevated-plus maze behaviors of the WKY pups did not differ significantly from SD pups. When WKY rats were exposed to acute stressors such as the forced swim test and immobilization stress test, they showed reduced struggling and their ACTH levels persisted for longer periods of time than SD rats (Tejani-Butt et al., 2003; Pardon et al., 2002). In addition local infusion of CRF into the LC has been shown to facilitate activity in the forced swim test (Weiss et al., 1994). We suggest the WKY rats require state changes in their arousal to exhibit increased anxiety-like behaviour (Morilak et al., 2005). This increased anxiety-like behaviour has been related to the activation of their HPA-axis (De La Garza II and Mahoney III, 2004), and this was achieved by CRF release through activity of the LC-NE system (Francis and Meaney, 1999).

WKY pups glutamate (250 μ M) stimulated release of NE from hippocampal slices did not differ when compared to SD pups, as previously reported (B3.2, 10^{-3} M glutamate concentration). This study further revealed that this effect was similar in prefrontal cortical slices for these two rat strains, WKY and SD. This reiterates the use of WKY rats as a control for SHR rats (see discussion B3.2), during in vitro studies.

If we incorporate the findings in to our present hypothesis: (1) WKYs behaviour was related to 'hyperarousal' within the open-field. (2) WKYs behaviour within the open-field improved with cross-fostering of WKY pups onto SD dams, we suggest that the maternal environment afforded by SD dam decreases the elevated tonic activity of the LC, how it achieves this is not yet apparent. (3) In addition we suggest that exposure to the elevated-plus maze did not activate the

HPA-axis and the behaviour of SD and WKY was related primarily to the tonic arousal levels rather than the phasic activity of the LC in response to salient sensory stimulation.

Maternal manipulation by cross-fostering SD pups revealed several interesting findings. SD pups cross-fostered onto SHR dams decreased their latency to enter the inner zone and entered the inner zone more frequently in the open-field, spent less time in the closed arms and entered the open arms more frequently in the elevated-plus maze. These findings suggest that SHR dam maternal behaviour toward SD pups increased their exploratory behaviours and possibly attenuated their normal levels of anxiety.

SD pups cross-fostered onto WKY dams decreased their latency to enter the inner zone and entered the inner zone more frequently in the open-field. These findings suggest that WKY dam maternal behaviour toward SD pups increased their exploratory behaviours, but did not affect their anxiety-like behaviours within the elevated-plus maze.

Glutamate stimulated release of NE revealed no significant changes with maternal manipulation by cross-fostering SD pups onto SHR or WKY dams. A suggestion regarding the increased exploratory behaviour could be food seeking behaviour. SHR and WKY dams have been shown to produce poor quality and quantity of milk (Rose and McCarty, 1994). The behavioural studies were conducted in the light cycle, in an attempt to reduce food-seeking behaviours. In addition animals were weaned on P21 and were responsible for intake of the readily available food pellets in their home cage for at least a week. However this point can not be excluded from consideration.

It has previously been suggested that the outcome of early postnatal environmental changes affect different strains to different degrees, suggesting that between strains there is a form of resilience to environmental manipulations due to the genetic make-up of the strain in question (Anisman et al., 1998). In the present study several changes in behavioural parameters were affected by cross-fostering rat pups onto different dams. SHR pups hyperactivity and heightened exploratory behaviours were not effected by different rearing conditions, and a tendency was shown leading to a further reduction in levels of anxiety. WKY pups exploratory behaviour was facilitated by fostering the pups onto SD dams, however their anxiety-like behaviours persisted. We suggest that this goes in par with glutamate-stimulated release of [³H]NE release in hippocampus and prefrontal cortex for both the SHR as a model of ADHD and ‘hypoarousal’ and WKY as a model of anxiety-like behaviour and ‘hyperarousal’. SD pups a ‘control’ strain increased their exploratory behaviours with either SHR or WKY as their fostered dam; we suggest that this was related to food seeking behaviours. SD pups showed decreased anxiety-like behaviours with SHR as fostered dam and increased anxiety-like behaviours with WKY as fostered dam, neurochemically this change was not evidenced with glutamate-stimulated release of NE. However this does not mean that the LC-NE system was not affected.

B3.6 Glutamate-stimulated fractional release of [³H] NE from hippocampal and prefrontal cortical tissue of maternally separated SD rats

Maternally separated SD rats glutamate-stimulated release of NE was not different to 'control' SD rats. Reported behavioural studies have shown that maternal separation increased rat pups stress-responsivity within novel environments and enhanced HPA-axis activities (Colorado et al., 2006).

These results suggest that maternal separation did not change the affects of glutamate stimulation of LC-NE terminals, however does not suggest that the LC-NE system was not affected by the maternal manipulation.

B3.7 Effects of acute nicotine on behaviour and glutamate-stimulated release of [³H]NE from hippocampal and prefrontal cortical slices in three rat strains

Acute nicotine administration (0.6 mg/kg) did not change behaviours in the open-field or elevated plus maze for SHR, WKY or SD rats. A trend was evident in the SD rats to increase their activity and exploration within the open-field but were however not significant. An acute dose of nicotine (0.1 mg/kg or 1.0 mg/kg s.c.) has been shown to increase locomotor activity in SD rats (Faraday et al., 2003; Hetzler and Thienpeng, 2004). Additionally adolescent rats, as were used in the present study have been shown to have a greater response to nicotine (0.5 mg/kg s.c.) (Brielmaier et al., 2007). Oral administration of any form of drug carries a set of risks related to drug absorption. Oral-self administration was followed in the present study to reduce the activation of the HPA-axis 'stress response' which can occur with subcutaneous injection or gavage administration. We suggest that the increased activity seen in the reported acute administration studies was related to the interaction between the 'stress response' and nicotine, and not nicotine alone. Acute nicotine administration (0.6 mg/kg oral) did not affect glutamate-stimulated release of NE in either the prefrontal cortex or hippocampal slices of SHR, WKY or SD rats. In vitro work post oral administration of an acute dose of nicotine has not previously been reported. Direct application of nicotine and analogues thereof to slice preparations of either prefrontal cortex or hippocampal slices has yielded increased release of NE (Rao et al., 2003; Anderson et al., 2000; Fu et al., 1998). The interaction and relationship between the effects of glutamate and nicotine in these brain areas has not been looked at.

From this study it is evident that successive glutamate-stimulated release of [^3H]NE was viable in prefrontal cortical slices which has not previously been reported and confirms two of the current studies experiments, SD rats were not incorporated in the two earlier studies (sections B4.1, B4.3). SHR, WKY and SD rats fractional change in release was similar, suggesting similar (1) internalization and (2) desensitization on noradrenergic nerve terminals to glutamate-stimulated release of [^3H]NE, and (3) production of endogenous NE.

We suggest that the previous reports that showed increased behavioural activity was due to an interaction between nicotine and the stress incurred by handling and injection of the nicotine.

4. CONCLUSIONS

Cortical arousal and changes in arousal were accurately assessed through ‘healthy’ participants’ reports of perceived mental effort during the three attentional tasks. We suggest that perceived mental effort in ‘healthy’ participants is an accurate measure of tonic LC-NE system activity during attention, reflected in relative beta frequency power over the left parietal cortex. Individuals that present with ADHD or anxiety related disorders have been reported to require increased levels of mental effort to complete attentional tasks. Individuals with ADHD require the enhanced mental effort to overcome their ‘hypoarousal’ and individuals with anxiety related disorders require increased levels of mental effort to overcome their distraction, regardless of their poor attentional performance. We suggest that reported perceived mental effort may serve as a physiological measure of tonic LC-NE system activity within a ‘healthy’ cohort of participants. This suggestion could be confirmed by studies of individuals with ADHD and anxiety related disorders. As the present associations found in the ‘healthy’ cohort would not persist.

Impulsivity as seen in reduced cortical arousal was evident in our ‘healthy’ participants however the participants improved their cortical arousal levels to overcome their initial impulsivity. We suggest that ‘hypoaroused’ individuals would be unable to correct their arousal levels due low tonic activity of the LC-NE system. Impulsivity is one of the hallmark characteristics of the SHR the present animal employed as a model of ADHD-like behavior. The SHRs’ behavior within two behavioral measures primarily indicated increased exploratory behaviors (open-field) and reduced levels of anxiety (elevated-plus maze). With maternal manipulation (cross-fostering)

SHRs' behavior within these apparatuses was accentuated, suggesting that their natural 'hypoarousal' was enhanced. The Glutamate stimulated release of NE from SHRs' LC-NE system terminals showed enhanced release when compared to several strains of rat. These results suggest that connectivity and synergistic activities between glutamatergic and noradrenergic terminals is dysfunctional in these 'hypoaroused' animals. We suggest that this is a result of the decreased tonic firing of the LC-NE system and enhanced activity of GABA_A receptors. These factors in turn reduce the signal-to-noise ratio and may account for their poor attentional performance.

Anxiety as seen in increased cortical arousal was evident in our 'healthy' participants by their increased relative beta frequency over the left parietal cortex, similar to that reported for increased mental effort. It is important to note that these individuals did not have anxiety-related disorders and may simply reflect the natural 'healthy' level of tonic LC-NE firing. What is of great interest is the negative association of reported anxiety with P300 latency. This association suggests that the present cohort of 'healthy' participants may rely on their increased tonic LC-NE system firing to increase their information processing abilities or phasic activity of the LC-NE system.

Decreased exploration and passive avoidance in rat has been related to anxiety related human disorders, the WKY rat model has previously been suggested to model anxiety related human disorders. The WKYs' behaviors in the present studies support these suggestions and highlight it as a model of 'hyperarousal'. WKYs' behavior within these apparatuses was accentuated when

reared by alternate strains of dam (cross-fostering), suggesting that their natural ‘hyperarousal’ was enhanced. The WKYs’ LC-NE system terminals showed decreased release of NE when stimulated with glutamate and compared to several strains of rat. These results suggest that connectivity and synergistic activities between glutamatergic and noradrenergic terminals are dysfunctional in these ‘hyperaroused’ animals. We suggest that this is a result of increased tonic activity of the LC-NE system that leads to enhanced concentrations of NE. These factors in turn reduce the signal-to-noise ratio and may account for their poor attentional performance.

The present cohort of ‘healthy’ participants reported low levels of childhood trauma; however several distinct associations were evidenced. Reported literature relates childhood trauma to exacerbation of the HPA-axis development and post traumatic stress disorder. What we find interesting are the differential effects of maternal manipulations (cross-fostering) in the SHR and WKY, suggesting that the outcome may depend more heavily on the resilience of one’s HPA-axis that may lead to the changes in behavior and LC-NE system activity and other additional neurotransmitter systems.

A question then arises as to co-morbidity of individuals presenting with symptoms of ADHD and anxiety. Diagnosis of individuals with ADHD has been one of controversy and remains as such. We support the refinement of ADHD subtypes as is put forth by Clarke and colleagues, who suggest that ADHD is in the majority ‘hypoaroused’ and a minority ‘hyperaroused’ (Clarke et al., 2000, 2001, 2007). In addition we suggest that diagnosis of ADHD has been limited or misused as individuals that are ‘hypoaroused’ and ‘hyperaroused’ show deficits in attentional

performance, and suggest as does Clarke et al (2000, 2001, 2007) that physiological diagnosis may be more suiting and beneficial to the outcomes of these individuals that are labelled under a single umbrella of ADHD. To support this suggestion, studies have shown misdiagnosis of ADHD when the individual should in fact be diagnosed with post traumatic stress disorder (Weinstein and Staffelbach, 1998, Weinstein et al., 2000). We suggest that individuals are either 'hypoaroused' or 'hyperaroused'. If in fact there is co-morbidity in presentation of either of these disorders this may simply be a psychological construct and not the key deficit that the individual presents with that can be treated. Correlation between measures of impulsivity and anxiety were tested in the present cohort of 'healthy' participants, however no associations were found.

Nicotine has in recent years been suggested to serve as a form of self-medication. In the present cohort of 'healthy' participants reduced P300 latency was associated with smoking. In addition we looked at the effects of oral nicotine in rats. Both the human and rat study suggest that anxiety needs to be present in the organism for the positive effects of nicotine to be evident.

The goal of this translational study was to develop our understanding of a system, the LC-NE system and its role in the regulation of arousal and attention and how the present results may facilitate in the treatment of human disorders that are related to 'hypoarousal' and 'hyperarousal'. Anatomically and physiologically the LC-NE system is synonymous in rat and human allowing translational research (reviewed in the introduction). Animal models of human arousal dysfunction such as the SHR and WKY showed distinct differences in behavior and neurochemistry that can be related to the function of the LC-NE system. An additional advantage

of animal models is their reduced complexity compared to the human being. Dysfunction in the synergy found between the glutamatergic and noradrenergic terminals of SHR and WKY support the present treatment regimes for individuals with ADHD and individuals with anxiety related disorders. These results may serve to refine and give better clarity on how to improve the evident dysfunctions in the LC-NE system.

In drawing on similarities between the human LC-NE system function and the rat LC-NE system several parallels were found within the presented studies. These findings support the presented hypothesis of the LC-NE system and its dysfunction in disorders of ‘hypoarousal’ such as ADHD and in disorders of ‘hyperarousal’ such as seen in anxiety related disorders that lead to poor attentional performance. Individuals with ADHD and SHR do not perform optimally when attention is required we posit that this is due to their ‘hypoarousal’ leading to decreased attentional performance behaviourally seen by their shortened response times and increased errors. Vice versa individuals with anxiety related disorder and WKY do not perform optimally when attention is required we posit that this is due to their ‘hyperarousal’ leading to decreased attentional performance, behaviourally seen by their poor response times and increased errors. These present study needs to be extended to incorporate the human disorders of arousal including ADHD and anxiety related disorders.

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APPENDICES

The Role of Norepinephrine in Arousal and Attention:

A human and rat study

Fleur Margaret Howells

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the division of Physiology

Department of Human Biology

Faculty of Health Sciences

UNIVERSITY OF CAPE

January, 2009

Are you interested in participating in a research study?

Title of Study

"A study of electroencephalographic (EEG) activity and autonomic response during the administration of cognitive tasks in human subjects"



Human Ethics Reference: 220/2005

You can participate if you are: Between the age of 21 and 45, have no history of brain trauma and/or suffer from a psychiatric disorder.

Session 1. Blood drawing

A doctor will draw two 5-milliliter blood samples (2 teaspoons in total), to place in storage for later analyses. The genetic analysis will only involve genes related to brain function and only be used for research purposes. You will be given a few questionnaires to fill out. These questionnaires will enrich the recordings of your brain waves. The questions are multiple-choice or yes/no, related to normal daily activities and personal history.

The next date for blood drawing is: **10h00-11h00, 6th March, 2007**
Level 3, Clinical Lab, Sports Science Institute
Boundary Road, Newlands

13h00-14h00, 6th March, 2007
5.03 Neuroscience Lab, Anatomy Building
Medical School, FHS, UCT
Anzio Road, Observatory

If you are unable to make the above date, please contact us, so that we can email you with the next blood drawing date. I'm sure we will still need more volunteers (hwlfle001@uct.ac.za else 021 406 6599) or if you would like more information about the study.

Session 2. Recording (1 hour per person)

The recordings of brain wave activity, heart rate and skin conductance, during the completion of simple computerized attentional tasks, are carried out at the Sports Science Institute. On the day of blood drawing, we will set-up a date and time convenient for you.

Please note for this session, 18 hours prior to the recording session you cannot consume the following: caffeine (coffee, tea, red bull, etc.), cigarettes, alcohol, or non-prescription drugs and do not eat or drink anything except water two hours prior to testing, as this will interfere with the recordings.

A study of brain wave activity and bodily responses during completion of computerized attentional tasks in human subjects

University of Cape Town Human Ethics Committee Reference Number: 220/2005

Participant Information sheet

Attention, is achieved by a specific interplay of neurotransmitters, which has not yet been fully characterized (*Berger & Posner, 2000*). Deficits in attention are found in 6% of the human population (*Beane & Marrocco, 2004*). The reason for conducting this study is to gain better insight and understanding of attentional processes in a normal population.

Hence the study that we would like you to participate in will provide a better understanding of the processes involved when we pay attention, whether a reflex action to an object unconsciously attracting our interest or whether we are deliberately choosing to pay attention to a specific object we can see.

You cannot participate in this study if you are younger than 21 or older than 45, have a history of epilepsy, seizures, brain trauma and you cannot participate if you do not have Grade 12 (Matric). If you want to withdraw from this study you may do so at any time. This study is only for research purposes and not funded by a pharmaceutical company. Your details will be anonymous. This means that all recordings and blood samples will not have your name on them, just a number that will be assigned on the day of the recordings.

The first day, will be a group orientation and blood sampling (5 minutes per person)

A registered nurse/doctor will draw two 5-milliliter blood samples (2 teaspoons in total), to place in storage for later analyses (Please note due to venepuncture, you may have a slight bruise around the puncture area and that the amount of blood drawn will not affect your body at all). The genetic analysis will only involve genes related to brain function and only be used for research purposes. You will be given a few questionnaires, which need to be filled in. These questionnaires will enrich the recordings of your brain waves. The questions are multiple-choice or yes/no, related to normal daily activities and personal history. When answering please attempt to be as honest as possible and know that your name will not in any way be linked to your questionnaire so it will be completely anonymous (please bring the completed questionnaires to the recording session).

The second day we will contact you with times the EEG is available and slot you in, when convenient for you (1 hour)

This session will not take longer than an hour. The recordings of brain wave activity, heart rate and skin response, during the completion of computerized attention tasks (The recordings will not be painful; the electrodes only lie on top of the scalp and won't give you an electric shock). The instructions for the computerized tasks can be read from the computer screen prior to each task. Follow them as best you can. Remember this is not a test, so relax and enjoy. As soon as you have finished these computerized tasks, the researcher will remove all recording instruments. **Please do not consume the following 18 hours prior to the recording session: caffeine (coffee, tea, red bull, etc.), cigarettes, alcohol, or non-prescription drugs and do not eat or drink anything except water two hours prior to testing, thank you.**

Fleur Howells (021 406 6599) else (082 374 3785)
5.02 Neuroscience Lab, 5th Level, Human Biology building
Faculty of Health Sciences, University of Cape Town, Observatory, 7925

A study of electroencephalographic (EEG) activity and autonomic response during administration of cognitive tasks in healthy human subjects

Booklet of Questionnaires to be filled in at home and to be brought back during the recording of your EEG (Electroencephalogram)

Please note when answering, the correct answer is always the first response

To make appointment for EEG please contact, Fleur Howells on (021) 406 6243 or Cell 082 374 3785

Please Note: When you come for the recording of your brain wave activity, to obtain accurate recordings, your hair should be as clean as possible, in the sense of no hair wax, gel, and recently washed.

Thank you

Booklet 1

Questionnaire 1

Cloninger's Subscales of Harm Avoidance and Novelty Seeking

Participant's Number _____

TRUE	FALSE	I often try new things just for the fun or thrills, even if most people think it is a waste of time.
TRUE	FALSE	I usually am confident that everything will go well, even in situations that worry people.
TRUE	FALSE	I often feel tense and worried in unfamiliar situations, even when others feel there is little to worry about.
TRUE	FALSE	I often do things based on how I feel at that moment without thinking about how they were done in the past.
TRUE	FALSE	I am much more reserved and controlled than most people.
TRUE	FALSE	I often have to stop what I am doing because I start worrying about what might go wrong.
TRUE	FALSE	I have less energy and get tired more quickly than most people
TRUE	FALSE	Most of the time I would prefer to do something a little risky (like riding in a automobile over steep hills and sharp turns) rather than having to stay quiet and inactive for a few hours
TRUE	FALSE	I often avoid meeting strangers because I lack confidence with people I don't not know
TRUE	FALSE	I like old "tried and true" ways of doing things much better than trying "new and improved" ways.
TRUE	FALSE	I like to be very organized and set up rules for people whenever I can.
TRUE	FALSE	It is difficult for me to keep the same interests for a long time because my attention often shifts to something else.
TRUE	FALSE	I often spend money until I run out of cash or get into debt from using too much credit.
TRUE	FALSE	I think I will have very good luck in the future.
TRUE	FALSE	I recover more slowly than most people from minor illnesses or stress
TRUE	FALSE	In conversations I am much better as a listener than as a talker.
TRUE	FALSE	I lose my temper more quickly than most people.
TRUE	FALSE	When I have to meet a group of strangers, I am more shy than most people.
TRUE	FALSE	I like to think about things for a long time before I make a decision.
TRUE	FALSE	I often need naps or extra rest periods because I get tired so easily.
TRUE	FALSE	Regardless of any temporary program that I have to overcome, I always think it will turn out well.
TRUE	FALSE	It is hard for me to enjoy spending money on myself, even when I have saved plenty of money.
TRUE	FALSE	I usually stay calm and secure in situations that most people would find physically dangerous.
TRUE	FALSE	I like to stay at home better than to travel or explore new places.
TRUE	FALSE	I like it when people can do whatever they want without strict rules and regulations.
TRUE	FALSE	I would probably stay relaxed and outgoing when meeting a group of strangers, even if I were told they are unfriendly.
TRUE	FALSE	Usually I am more worried than most people that something might go wrong in the future.
TRUE	FALSE	I usually think about all the facts in detail before I make a decision.
TRUE	FALSE	I am usually able to get other people to believe me, even when I know that what I am saying is exaggerated or untrue.
TRUE	FALSE	I need much extra rest, support, or reassurance to recover from a minor illness or stress.
TRUE	FALSE	It is easy for me to organize my thoughts while talking to someone.
TRUE	FALSE	I often react so strongly to unexpected news that I say or do things that I regret.
TRUE	FALSE	I hate to make decisions based only on my first impressions.
TRUE	FALSE	I prefer spending money rather than saving it.
TRUE	FALSE	I can usually do a good job of stretching the truth to tell a funnier story or to play a joke on someone.
TRUE	FALSE	If I am embarrassed or humiliated, I get over it very quickly.
TRUE	FALSE	It is extremely difficult for me to adjust to changes in my usual way of doing things because I get so tense, or worried.
TRUE	FALSE	I usually demand very good practical reasons before I am willing to change my old ways of doing things.
TRUE	FALSE	I nearly always stay relaxed and carefree, even when everyone else is fearful.
TRUE	FALSE	I often follow my instincts, hunches, or intuition without thinking through all the details.
TRUE	FALSE	Other people often think that I am to independent because I won't do what they want.
TRUE	FALSE	I try to do as little work as possible, even then other people expect more of me.
TRUE	FALSE	I am better at saving money than most people.

Questionnaire 1 continued

TRUE	FALSE	Even when most people feel it is not important, I often insist on things being done in a strict and orderly way.
TRUE	FALSE	I feel very confident and sure of myself in almost all social situations.
TRUE	FALSE	I hate to change the way I do things, even if many people tell me there is a new and better way to do it.
TRUE	FALSE	I am more energetic and tire less quickly than most people.
TRUE	FALSE	I like to pay close attention to details in everything I do.
TRUE	FALSE	I often stop what I am doing because I get worried, even when my friends tell me everything will go well.
TRUE	FALSE	Most of the time I would prefer to do something risky (like hang-gliding or parachute jumping), rather than having to stay quiet and inactive for a few hours.
TRUE	FALSE	Because I so often spend too much money on impulse, it is hard for me to save money, even for special plans like a vacation.
TRUE	FALSE	I am not shy with strangers at all.
TRUE	FALSE	I never worry about terrible things that might happen in the future.
TRUE	FALSE	I almost never get so excited that I lose control of myself.
TRUE	FALSE	I prefer to start conversations, rather than waiting for others to talk to me.
TRUE	FALSE	I often have to change my decisions because I had a wrong hunch or mistaken first impression.
TRUE	FALSE	It is fun for me to buy things for myself.
TRUE	FALSE	I recover more quickly than most people from minor illnesses or stress
TRUE	FALSE	I often break rules and regulations when I think I can get away with it.
TRUE	FALSE	I like to make quick decisions so I can get on with what has to be done
TRUE	FALSE	I usually have good luck in whatever I try to do
TRUE	FALSE	I am usually confident that I can easily do things that most people would consider dangerous
TRUE	FALSE	I like to explore new ways to do things
TRUE	FALSE	I enjoy saving money more than spending it on entertainment or thrills
TRUE	FALSE	I usually can stay "on the go" all day without having to push myself
TRUE	FALSE	I nearly always think about all the facts in detail before I make a decision, even when other people demand a quick decision
TRUE	FALSE	I am not very good at talking my way out of trouble when I am caught doing something wrong.
TRUE	FALSE	I think I would stay confident and relaxed when meeting strangers, even if I were told they are angry at me
TRUE	FALSE	People find it easy to come to me for help, sympathy, and warm understanding.
TRUE	FALSE	I have trouble telling a lie, even when it is meant to spare someone else's feelings
TRUE	FALSE	I usually feel tense and worried when I have to do something new and unfamiliar.
TRUE	FALSE	Some people think I am too stingy or tight with my money.
TRUE	FALSE	things often go wrong for me unless I am very careful.
TRUE	FALSE	I usually stay away from social situations where I would have to meet strangers, even if I am assured that they will be friendly
TRUE	FALSE	I usually feel much more confident and energetic than most people, even after minor illness or stress.
TRUE	FALSE	I like to read everything when I asked to sign any papers.
TRUE	FALSE	When nothing new is happening, I usually start looking for something that is thrilling or exciting.

Questionnaire 2

Dickman's Impulsivity Scale (Functional and Dysfunctional)

YES	NO	I don't like to make decisions quickly, even simple decisions, such as choosing what to wear, or what to have for dinner.
YES	NO	I am good at taking advantage of unexpected opportunities, where you have to do something immediately or lose your chance.
YES	NO	Most of the time, I can put my thoughts into words very rapidly.
YES	NO	I am uncomfortable when I have to make up my mind rapidly.
YES	NO	I like to take part in really fast-paced conversations, where you really don't have much time to think before you speak.
YES	NO	I don't like to do things quickly, even when I am doing something that is not very difficult.
YES	NO	I would enjoy working in a job that required me to make a lot of split-second decisions.
YES	NO	I like sports and games in which you have to choose your next move very quickly.
YES	NO	I have often missed out on opportunities because I couldn't make up my mind fast enough.
YES	NO	People have admired me because I can think quickly.
YES	NO	I try to avoid activities where you have to act without much time to think first.
YES	NO	I will often say whatever comes into my head without thinking first.
YES	NO	I enjoy working out problems slowly and carefully.
YES	NO	I frequently make appointments without thinking whether I will be able to keep them.
YES	NO	I frequently buy things without thinking about whether or not I can really afford them.
YES	NO	I often make up my mind without taking the time to consider the situation from all angles.
YES	NO	Often, I don't spend enough time thinking over a situation before I act.
YES	NO	I often get into trouble because I don't think before I act.
YES	NO	Many times the plans I make don't work out because I haven't gone over them carefully enough in advance.
YES	NO	I rarely get involved in projects without first considering the potential problems.
YES	NO	Before making any important decisions, I carefully weigh up the pros and cons.
YES	NO	I am good at careful reasoning.
YES	NO	I often say and do things without considering the consequences.

Questionnaire 3

Edinburgh's

Participant's Number _____

Which Hand?			
Right	Both	Left	
Right	Both	Left	Which hand do you write with?
Right	Both	Left	Which hand do you draw with?
Right	Both	Left	When throwing a ball, which hand do you use?
Right	Both	Left	When using scissors, which hand does the cutting?
Right	Both	Left	When brushing or combing your hair, which hand holds the brush/comb?
Right	Both	Left	When brushing your teeth, which hand holds the toothbrush?
Right	Both	Left	When cutting a loaf of bread, which hand holds the knife?
Right	Both	Left	When eating soup, which hand holds the spoon?
Right	Both	Left	When using a hammer, which hand hammers the nail into the wood?
Right	Both	Left	When using a screwdriver, which hand turns the screwdriver to tighten the screw?
Right	Both	Left	When playing tennis, which hand holds the racket?
Right	Both	Left	When using both a knife and a fork, which hand holds the knife?
Right	Both	Left	When hitting a ball with a cricket bat, which hand is at the top of bat?
Right	Both	Left	When hitting a golf ball with a golf stick, which hand is at the top of the club?
Right	Both	Left	When sweeping with a broom, which hand is at the top of the broom?
Right	Both	Left	When raking leaves up with a rake, which hand is at the top of the rake?
Right	Both	Left	When striking a match, which hand is holding the match?
Right	Both	Left	When opening the match box to get a match, which hand takes the match out?
Right	Both	Left	When dealing cards, which hands deals to the players?
Right	Both	Left	When threading and needle, which hand is used to put the thread through the eye of the needle?

**A study of electroencephalographic (EEG) activity and autonomic response during
administration of cognitive tasks in healthy human subjects**

YES	NO	Do you often refrain from doing something because you are afraid of it being illegal?
YES	NO	Does the good prospect of obtaining money motivate you strongly to do some things?
YES	NO	Do you prefer not to ask for something when you are not sure you will obtain it?
YES	NO	Are you frequently encouraged to act by the possibility of being valued in your work, in your studies, with friends/family?
YES	NO	Are you often afraid of new or unexpected situations?
YES	NO	Do you often meet people that you find physically attractive?
YES	NO	Is it difficult for you to telephone someone you do not know?
YES	NO	Do you like to take some drugs because of the pleasure you get from them?
YES	NO	Do you often renounce your rights when you know you can avoid a quarrel with a person or an organization?
YES	NO	Do you often do things to be praised?
YES	NO	As a child, were you troubled by punishments at home or in school?
YES	NO	Do you like being the center of attention at a party or a social meeting?
YES	NO	In tasks that you are not prepared for, do you attach great importance to the possibility of failure?
YES	NO	Do you spend a lot of your time on obtaining a good image?
YES	NO	Are you easily discouraged in difficult situations?
YES	NO	Do you need people to show their affection for you all the time?
YES	NO	Are you a shy person?
YES	NO	When you are in a group, do you try to make your opinions the most intelligent or the funniest?
YES	NO	Whenever possible, do you avoid demonstrating your skills for fear of being embarrassed?
YES	NO	Do you often take the opportunity to pick up people you find attractive?
YES	NO	When you are with a group, do you have difficulties selecting a good topic to talk about?
YES	NO	As a child, did you do a lot of things to get people's approval?
YES	NO	Is it often difficult for you to fall asleep when you think about things you have done or must do?
YES	NO	Does the possibility of social advancement, move you to action, even if this involves not playing fair?
YES	NO	Do you think a lot before complaining in a restaurant if your meal is not well prepared?
YES	NO	Do you generally give preference to those activities that imply an immediate gain?
YES	NO	Would you be bothered if you had to return to a store when you noticed you were given the wrong change?
YES	NO	Do you often have trouble resisting the temptation of doing forbidden things?
YES	NO	Whenever you can, do you avoid going to unknown places?
YES	NO	Do you like to complete and do everything you can to win?
YES	NO	Are you often worried by things that you said or did?
YES	NO	Is it easy for you to associate tastes and smells to very pleasant events?
YES	NO	Would it be difficult for you to ask you boss for a raise (salary increase)?
YES	NO	Are there a large number of objects or sensations that remind you of pleasant events?
YES	NO	Do you generally try to avoid speaking in public?
YES	NO	When you start to play with a slot machine, is it often difficult for you to stop?
YES	NO	Do you, on a regular basis, think that you could do more things if it was not for your insecurity or fear?
YES	NO	Do you sometimes do things for quick gains?
YES	NO	Comparing yourself to people you know, are you afraid of many things?
YES	NO	Does your attention easily stray from you work in the presence of an attractive stranger?
YES	NO	Do you often find yourself worrying about things to the extent that performance in intellectual abilities is impaired?
YES	NO	Are you interested in money to the point of being able to do risky jobs?
YES	NO	Do you often refrain from doing something you like in order not to be rejected or disapproved of by others?
YES	NO	Do you like to put competitive ingredients to all of your activities?
YES	NO	Generally, do you pay more attention to threats than to pleasant events?
YES	NO	would you like to be a socially powerful person?
YES	NO	Do you often refrain from doing something because of you fear of being embarrassed?
YES	NO	Do you like displaying you physical abilities even though this may involve danger?

Questionnaire 5

Eysenck Personality Questionnaire - Abbreviated

Participant's Number _____

[illegible]

Does your mood often go up and down?
Are you a talkative person?
Would being in debt worry you?
Are you rather lively?
Were you ever greedy by helping yourself to more than your share of anything?
Would you take drugs which may have strange or dangerous effects?
Have you ever blamed someone for doing something you knew was really your fault?
Do you prefer to go your own way rather than act by the rules?
Do you often feel 'fed-up'?
Have you ever taken anything (even a pin or button) that belonged to someone else?
Would you call yourself a nervous person?
Do you think marriage is old-fashioned and should be done away with?
Can you easily get some life into a rather dull party?
Are you a worrier?
Do you tend to keep in the background on social occasions?
Does it worry you if you know there are mistakes in your work?
Have you ever cheated in a game?
Do you suffer from 'nerves'?
Have you ever taken advantage of someone?
Are you mostly quiet when you are with other people?
Do you often feel lonely?
Is it better to follow society's rules than go your own way?
Do other people think of you as being very lively?
Do you always practice what you preach?

Questionnaire 6**K10****Participant's Number** _____

During the last 30 days how often did you.....

Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always
Never	Rarely	Sometimes	Most times	Always

Feel depressed?
Feel so depressed that nothing could cheer you up?
Feel anxious?
Feel nervous?
Feel restless or fidgety?
Feel so restless that you could not sit still?
Feel worthless?
Feel tired out for no good reason?
Feel hopeless?
Feel that everything is an effort?

Questionnaire

Subjective Questionnaire

Participant's Number _____

Do you have an immediate family member with a psychiatric disorder?

If yes which of the following and what relation is this person to you?

Link with pen

YES NO

Mother	Depression
Father	Schizophrenia
Sister	Bipolar Disorder
Sister	Attention-deficit/Hyperactivity disorder
Brother	Obsessive compulsive disorder
Brother	Other

Are you on any forms of prescribed medication? If so what are they:

Do you have a known organic brain disease or any brain trauma?
(such as tumor, stroke, Parkinson's disease)

YES NO

If applicable:

YES NO

Do you or have you suffered from epilepsy or seizures?

Have you lost consciousness for longer than 2 minutes?

YES NO

Have you had one or more of the following If yes mark the applicable

- Meningitis
- Encephalitis (viral)
- Encephalitis (herpes)
- Encephalitis (other or unsure)
- Other disease of the brain
- If yes to other, what?

YES NO

YES NO

YES NO

YES NO

YES NO

YES NO

Do you suffer from allergies?

If yes, do you know what they are?

YES NO

Do you have to take medication for them?

YES NO

If you are female, please circle where in your cycle you are.

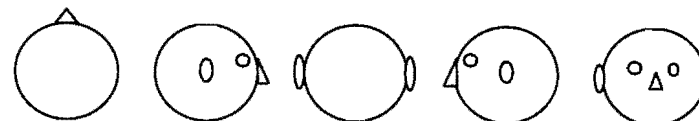
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

Headaches

Do you suffer with headaches? If yes what type of headaches

- Lasting a few hours (acute)
- Lasting a day (cluster) or days
- Migraine (with Nausea)
- Migraine (with Vomiting)
- Migraine (bedridden)

Do they have a specific locality? If yes please best indicate the position



Which best describes you headaches generally?

- Lightning bolts, flashes
- Pounding/ Throbbing
- Dull as if a heavy cloud
- Behind your eyes
- Tension of neck muscles at the back of you head

Questionnaire 8

Spielberger's State-Trait Anxiety Questionnaire (STAI-T) Participant's Number _____

Almost Never	Sometimes	Often	Almost Always	
1	2	3	4	I feel pleasant
1	2	3	4	I feel nervous and restless
1	2	3	4	I feel satisfied with myself
1	2	3	4	I wish I could be as happy as others seem to be
1	2	3	4	I feel like a failure
1	2	3	4	I feel rested
1	2	3	4	I am "calm, cool, and collected"
1	2	3	4	I feel that difficulties are piling up so that I cannot overcome them
1	2	3	4	I worry too much over something that really doesn't matter
1	2	3	4	I am happy
1	2	3	4	I have disturbing thoughts
1	2	3	4	I lack self-confidence
1	2	3	4	I feel secure
1	2	3	4	I make decisions easily
1	2	3	4	I feel inadequate
1	2	3	4	I am content
1	2	3	4	Some unimportant thought runs through my mind and bothers me
1	2	3	4	I take disappointments so keenly that I can't put them out of my mind
1	2	3	4	I am a steady person
1	2	3	4	I get in a state of tension or turmoil as I think over my recent concerns and interests

During your childhood (up until you matriculated from school) did you feel any of the following and to what extent?

[illegible]

I didn't have enough to eat.

I knew there was someone to take care of me and protect me.

People in my family called me things like "stupid", "lazy", or "ugly".

My parents were too drunk or high to take care of me.

There was someone in my family who helped me feel important or special.

I had to wear dirty clothes.

I felt loved.

I thought that my parents wished I had never been born.

I got hit so hard by someone in my family that I had to see a doctor or go to hospital.

There was nothing I wanted to change about my family.

People in my family hit me so hard that it left bruises or marks.

I was punished with a belt, a board, a cord, or some hard object.

People in my family looked out for each other.

People in my family said hurtful or insulting things to me.

I believe that I was physically abused.

I had the perfect childhood.

I go hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor.

I felt that someone in my family hated me.

People in my family felt close to each other.

Someone tried to touch me in a sexual way, or tried to make me touch them.

Someone threatened to hurt me or tell lies about me unless I did something sexual with them.

I had the best family in the world.

Someone tried to make me do sexual things or make me watch sexual things.

Someone molested me.

I believe that I was emotionally abused.

There was someone to take me to the doctor if I needed it.

I believe that I was sexually abused

My family was a source of strength and support.

Visual Analogue Scale (VAS) of Mental fatigue

Azouvi, P., Couillet, J., Leclercq, M., Martin, Y., Asloun, S., and Rousseaux, M. (2004). *Neuropsychologia*. 42:1260-1268.

Please mark between these two ratings ("Very high effort" and "No effort at all"), to indicate how much mental effort was necessary to complete the task. The score is the distance (in centimeters) from the bottom mark of the line.

Task with
three continuous Ss



Task with
Vs as non-targets



Task with
circles in rectangles



Very High Effort

No effort at all

A6
ction A3.1

naires

Gender	years post matriculation education	Dickman's Functional Impulsivity	Dickman's dysfunctional impulsivity	Sensitivity to reward	Sensitivity to punishment	EPQ-R-A Extraversion	EPQ-R-A Lie Scale	EPQ-R-A Psychotism	EPQ-R-A Neuroticism	Kessler distress scale	Cloninger's Novelty seeking subscale	Cloninger's Harm Avoidance subscale	STAI-T anxiety absent	STAI-T anxiety present	CTQ-SF physical abuse	CTQ-SF physical neglect	CTQ-SF Emotional abuse	CTQ-SF emotional neglect	CTQ-SF sexual abuse	CTQ-SF sexual neglect	CPT Q-SF mental fatigue %	G n f
female	8	7	9	13	12	4	1	3	2	35	17	24	23	34	5	5	6	7	5	0	76.47	85
female	4	2	4	9	13	3	5	3	4	26	18	16	20	22	5	5	5	5	5	3	10.50	13
female	8	5	5	4	1	4	5	3	0	17	13	40	18	14	5	6	5	6	5	0	10.08	73
female	10	5	7	13	4	5	2	2	2	26	18	4	14	22	5	5	5	9	5	2	4.20	3
female	6	6	4	7	12	4	3	3	2	16	13	16	11	16	5	5	5	5	5	3	12.61	26
female	6	4	4	6	16	2	2	3	1	17	11	20	21	14	5	7	6	6	5	0	19.33	44
female	10	4	2	7	13	5	0	4	3	21	23	10	13	15	5	8	13	12	5	0	15.13	14
female	6	5	3	10	19	1	4	4	5	20	23	21	27	26	5	5	5	5	7	2	5.88	5
female	4	6	7	10	16	3	2	2	2	19	12	31	20	14	6	5	5	8	5	0	29.41	62
female	5	5	4	13	13	3	5	2	5	15	13	14	14	20	5	5	5	5	5	3	10.92	23
female	6	4	6	15	9	4	0	3	4	25	26	8	22	18	5	5	8	7	5	0	45.38	66
female	4	2	6	9	8	2	4	4	1	21	14	22	16	15	5	9	5	6	5	1	63.03	94
female	8	5	6	8	11	4	3	3	6	26	23	25	23	24	5	6	8	11	5	2	65.55	85
female	9	5	6	6	6	3	3	2	3	19	22	7	11	10	7	5	5	5	5	0	6.72	26
female	0					5	4	2	4	18	0	32	11	20	6	5	7	9	5	1	18.49	10
female	10	7	4	7	4	4	3	3	1	11	14	8	9	13	6	5	8	7	6	0	9.24	9
female	7	6	4	8	8	5	3	3	6	19	26	16	25	24	6	7	6	5	5	3	84.03	85
female	9	5	4	6	9	1	3	2	4	23	10	24	19	17	5	5	5	6	5	0	16.81	3
female	13	6	4	5	5	3	2	2	0	13	21	4	9	12	6	5	5	8	5	0	11.76	27
female	7	6	4	12	10	3	3	4	1	15	23	7	18	19	5	5	5	5	5	2	23.53	76
female	4	3	5	9	17	2	2	3	3	22	16	11	14	18	5	5	5	7	5	0	17.65	44
female	10	6	5	8	20	4	3	3	2	14	9	14	11	15	5	5	5	5	5	2	10.50	20
female	9	6	4	11	11	1	1	4	5	30	23	10	25	18	7	11	5	9	5	0	8.40	4

61	75	female	9	8	4	2	4	3	3	2	2	22	19	6	17	23	8	11	12	16	8	0	44.54	36.97	26.89
62	76	female	8	4	4	2	8	3	6	5	4	23	14	19	17	17	6	5	7	9	5	0	26.05	82.35	11.76
63	77	male	3	4	5	7	4	2	6	2	3	24	15	25	16	20	7	5	7	10	5	0	19.33	59.66	12.61
64	78	male	9	10	4	18	14	4	4	5	4	22	19	16	24	24	8	5	9	10	5	0	20.17	81.51	58.82
65	79	female	8	6	4	11	18	2	2	2	0	19	18	14	18	17	5	7	6	10	5	0	14.29	50.42	42.02
66	80	female	4	4	4	10	17	2	3	2	6	27	20	30	24	28	5	7	7	10	5	0	43.70	42.02	40.34
67	81	female	7	6	4	10	20	2	3	3	2	22	12	24	14	20	5	5	5	6	6	0	9.24	18.49	23.53
68	82	female	4	7	10	20	12	4	3	4	5	29	22	17	25	25	5	6	5	12	5	0	15.13	64.71	10.08
69	83	female	4	6	4	13	7	3	3	3	1	15	21	41	16	19	6	6	5	8	6	2	42.86	64.71	44.54
70	84	female	4	4	4	16	20	3	4	3	3	15	17	17	9	21	6	5	6	5	5	1	35.29	78.99	21.01
71	85	female	7	6	5	14	21	2	3	3	3	27	17	26	23	26	7	5	5	5	5	1	21.01	71.43	21.85
72	86	female	4	7	4	12	19	6	2	3	5	27	24	43	24	26	5	5	7	9	5	0	29.41	50.42	75.63
73	87	female	7	6	8	16	9	4	4	4	2	26	24	14	19	19	9	5	6	6	6	1	37.82	50.42	18.49
74	88	male	3	4	4	13	6	4	4	2	1	15	23	9	12	11	5	8	11	16	5	0	13.45	82.35	47.90
75	90	male	4	5	7	19	8	2	6	4	0	13	27	23	17	18	6	9	16	11	5	0	17.65	36.13	20.17
76	91	male	6	7	5	11	1	4	2	2	0	24	22	4	16	14	5	5	5	7	5	0	62.18	85.71	67.23
77	93	female	10	5	4	4	10	2	3	3	2	28	18	24	20	21	5	5	7	7	5	0	84.03	73.95	25.21
78	94	female	7	4	5	13	7	2	3	3	2	21	19	28	13	16	9	7	5	7	5	0	45.38	83.19	16.81
79	95	male	4	6	4	11	18	3	3	4	4	38	11	23	24	29	5	5	5	5	5	2	37.82	68.07	52.10
80	97	male	1	7	6	17	10	4	4	3	2	23	28	14	17	14	6	6	6	7	5	0	23.53	52.94	13.45
81	98	female	3	4	3	10	9	1	0	4	2	19	15	37	18	19	5	5	6	5	5	0	25.21	42.86	23.53
82	99	male	4	8	4	8	18	3	4	3	5	20	12	22	23	18	10	6	8	9	5	0	9.24	11.76	5.88
83	100	female	8	5	4	8	12	2	2	2	1	19	16	13	19	17	5	5	9	8	5	0	55.46	85.71	21.85
84	101	female	4	8	5	13	9	4	5	3	4	25	21	36	23	29	8	9	13	12	7	0	16.81	36.97	20.17

Descriptive statistics

Breakdown Table of Descriptive Statistics (Questionnaires data.sta)

N=84 (No missing data in dep. var. list)

Gender	Cloninger's Novely seeking subscale Mean s	Cloninger's Novely seeking subscale Mean N	Cloninger's Novely seeking subscale Std.D ev.	Cloninger's Novely seeking subscale Std.E rr.	Cloninger's Harm Avoidance subscale Mean s	Cloninger's Harm Avoidance subscale Mean N	Cloninger's Harm Avoidance subscale Std.D ev.	Cloninger's Harm Avoidance subscale Std.E rr.	STAI-T anxiety absent Mean s	STAI-T anxiety absent N	STAI-T anxiety absent Std.D ev.	STAI-T anxiety absent Std.E rr.	STAI-T anxiety present Mean s	STAI-T anxiety present N	STAI-T anxiety present Std.D ev.	STAI-T anxiety present Std.E rr.
female	18.02	50.00	5.53	0.78	20.42	50.00	9.82	1.39	18.82	50.00	4.62	0.65	20.22	50.00	4.97	0.70
male	17.65	34.00	7.10	1.22	16.74	34.00	10.60	1.82	16.71	34.00	5.16	0.88	17.97	34.00	4.70	0.81
All Grps	17.87	84.00	6.18	0.67	18.93	84.00	10.24	1.12	17.96	84.00	4.93	0.54	19.31	84.00	4.96	0.54

Breakdown Table of Descriptive Statistics (Questionnaires data.sta)

N=84 (No missing data in dep. var. list)

Gender	CTQ-SF physical abuse Mean s	CTQ-SF physical abuse Mean N	CTQ-SF physical abuse Std.D ev.	CTQ-SF physical abuse Std.E rr.	CTQ-SF physical neglect Mean s	CTQ-SF physical neglect Mean N	CTQ-SF physical neglect Std.D ev.	CTQ-SF physical neglect Std.E rr.	CTQ-SF Emotional abuse Mean s	CTQ-SF Emotional abuse Mean N	CTQ-SF Emotional abuse Std.D ev.	CTQ-SF Emotional abuse Std.E rr.	CTQ-SF emotional neglect Mean s	CTQ-SF emotional neglect Mean N	CTQ-SF emotional neglect Std.D ev.	CTQ-SF emotional neglect Std.E rr.	CTQ-SF sexual abuse Mean s	CTQ-SF sexual abuse Mean N	CTQ-SF sexual abuse Std.D ev.	CTQ-SF sexual abuse Std.E rr.	CTQ-SF m/denial Mean s	CTQ-SF m/denial Mean N	CTQ-SF m/denial Std.D ev.	CTQ-SF m/denial Std.E rr.
female	5.66	50.00	1.33	0.19	5.80	50.00	1.36	0.19	7.14	50.00	3.00	0.42	7.88	50.00	3.35	0.47	5.28	50.00	0.83	0.12	0.78	50.00	1.15	0.16
male	6.50	34.00	1.62	0.28	6.38	34.00	1.89	0.32	7.62	34.00	3.08	0.53	8.24	34.00	3.51	0.60	5.18	34.00	0.72	0.12	0.68	34.00	0.98	0.17
All Grps	6.00	84.00	1.51	0.16	6.04	84.00	1.61	0.18	7.33	84.00	3.03	0.33	8.02	84.00	3.40	0.37	5.24	84.00	0.79	0.09	0.74	84.00	1.08	0.12

3	4	female	NO	2.320023	2.611616
4	5	male	NO	0.12584	0.09284773
5	6	female	NO	0.3241199	0.3106799
6	7	female	NO	0.04565187	0.01127313
7	8	female	NO	0.17364	0.15948
8	9	female	NO	0.18156	0.21372
9	10	male	NO	0.08457837	0.09506636
10	11	female	YES	0.3435964	0.3241199
11	12	female	NO	0.22236	0.23892
12	13	male	NO	0.16836	0.04605524
13	14	female	NO	0.1686	0.1242
14	15	male	NO	0.06299734	0.04585356
15	17	male	NO	0.2684399	0.3295469
16	18	female	YES	0.03113007	0.02628946
17	19	female	NO	0.09103252	0.12996
18	20	male	NO	0.1374	0.17556
19	22	female	YES	0.1566881	0.08162505
20	24	male	NO	0.0432745	0.07183339
21	25	male	NO	0.1715821	0.05728196
22	26	female	NO	0.05388207	0.08815279
23	27	female	YES	0.2627569	0.2412212
24	29	female	NO	0.1335438	0.05551401
25	30	male	NO	0.1037922	0.05741794
26	31	female	NO	0.06911347	0.09998439
27	32	male	NO	0.2752357	0.1502475
28	33	male	NO	0.1310114	0.03688269
29	34	female	NO	0.003094091	0.006038633
30	35	male	NO	0.2122384	0.2617506
31	39	female	NO	0.2339755	0.2132448
32	40	female	YES	0.02251161	0.0880168
33	41	female	NO	0.1015196	0.002761394
34	43	male	NO	0.1609147	0.7123805
35	44	male	NO	0.2989855	0.2696001
36	45	female	NO	0.2422275	0.1705757
37	46	male	NO	0.1989886	0.3634807
38	47	female	YES	0.3013724	0.1742545
39	50	male	NO	0.2695422	0.1987859

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77	97	male	NO	0.05131679	0.04334466
78	98	female	YES	0.2054205	0.2483218
79	99	male	NO	0.003436835	0.003381279
80	100	female	NO	0.1136227	0.102186
81	101	female	YES	0.1452157	0.216465

Descriptive statistics

Breakdown Table of Descriptive Statistics (data gender pill)

N=81 (No missing data in dep. var. list)

Gender	Takin g Contr a c e p t i v e	Cortisol prior to cognitive testing Means	Cortisol prior to cognitive testing N	Cortisol prior to cognitive testing Std.Dev.	Cortisol post cognitive testing Means	Cortisol post cognitive testing Std.Dev.
female	NO	0.235315	31	0.396813	0.244619	0.466354
female	YES	0.213502	17	0.143804	0.182176	0.107676
male	NO	0.170845	33	0.146322	0.201766	0.169461
male	YES		0			
All Groups		0.204471	81	0.269444	0.214055	0.309844

Wilcoxon Matched Pairs Test (data gender pill)

Marked tests are significant at $p < .05000$

Pair of Variables

Cortisol prior to cognitive testing & Cortisol post cognitive testing

Valid N	T	Z	p-level
81	1446.000	1.009926	0.312531

Depend.:

Cortisol prior to cognitive testing

Kruskal-Wallis ANOVA by Ranks; Cortisol prior to cognitive testing (data gender pill)

Independent (grouping) variable: Gender

Kruskal-Wallis test: $H(1, N=81) = 1.200665, p = .2732$

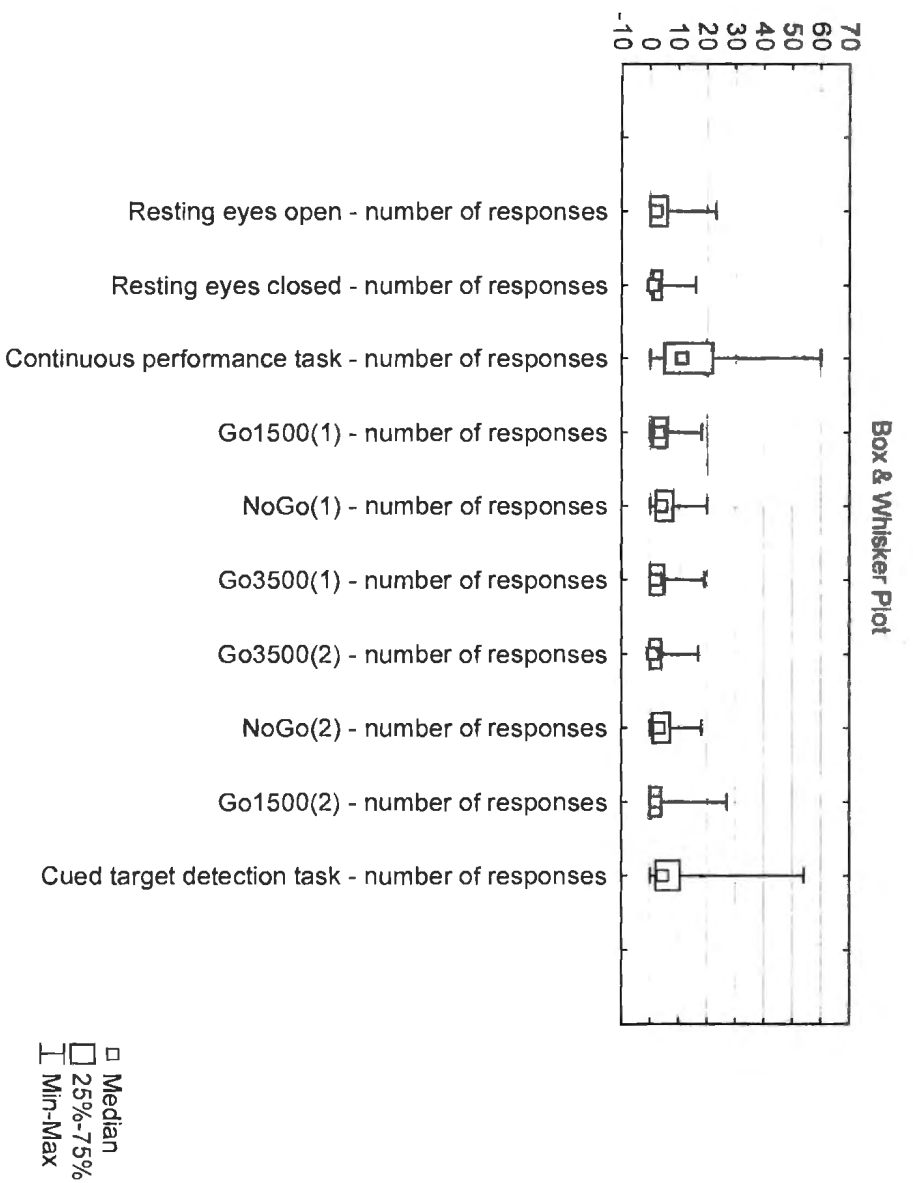
Code	Valid N	Sum of Ranks
female	102	48
male	103	33

22	24	0	0	2	2855	5	1344.8	3	2683.33333	0	0
23	25	2	4073	1	3930	10	3206.2	7	3895.14286	1	6912
24	26	1	2848	1	6990	5	4323.2	8	5585	3	8678.66667
25	27	1	1781	0	0	2	3173	9	5567.44444	0	0
26	29	21	2370	10	2535.8	43	2338.23256	52	2816.90385	0	0
27	30	0	0	1	11076	18	2458.44444	20	2715	17	2863.29412
28	31	0	0	1	4088	2	2899	2	4117	4	2806
29	32	1	4860	1	6804	11	4398.8	25	3948.64	9	5902
30	33	4	1722	3	4348	41	1710.65	74	1465.02703	17	1982.94118
31	34	4	690.5	8	4892.28571	12	3812.33333	11	4860.54545	1	3892
32	35	0	0	0	0	0	0	2	3768	0	0
33	39	0	0	0	0	0	0	0	0	0	0
34	40	23	1241.13043	6	2904.66667	28	2501.21429	37	2140.87806	6	4346.66667
35	41	1	2474	1	4730	1	3502	17	3378.35294	7	3919.14286
36	43	7	3226.28571	8	2738.75	28	3089	63	2888.97835	21	3334
37	44	6	2228.66667	1	4608	22	2638.18182	24	3341.83333	0	0
38	45	0	0	0	0	1	7858	0	0	0	0
39	46	4	3702.5	1	3174	10	5624.6	8	8066.25	3	6908
40	47	0	0	1	9078	1	2218	5	5486.4	3	5980
41	50	0	0	1	8258	6	5550	8	5878.75	3	18340
42	53	4	3949	4	5151.5	13	3644	14	5610.57143	5	23618
43	54	16	1922.66667	14	2040.15385	60	1734.36066	118	1671.80223	41	2315.35
44	55	5	2252.5	1	9170	11	3145.09091	14	4384.71429	3	6929
45	56	3	1451.33333	1	4610	8	3513.5	12	3091.16667	2	4755
46	58	0	0	1	6521	4	1920.75	9	7271.88889	3	6215
47	59	1	2564	1	812	3	2519.33333	6	6450	2	3126
48	60	21	2102.09524	7	2351.14286	51	2165.07692	85	1767.01176	38	2229.36842
49	62	7	2046.57143	4	2836.5	23	2299.30435	30	2466.74167	10	2565.2
50	63	2	527	2	1120	16	1820.375	25	1489.28	5	2108.8
51	64	15	1980.8	11	1958.54545	34	2195.94118	66	2196.01044	19	2132.10526
52	65	9	1712	1	4628	12	2518.33333	15	2697.06667	13	2575.23077
53	66	15	2534.8	16	2538.625	41	2449.17073	67	5323.8209	23	2372.69565
54	67	7	2812.33333	4	2913	29	2007.86207	37	2672.61622	11	3054.72727
55	68	4	3487.5	2	6524	18	2140.22222	33	2841.09091	15	2015.14286
56	69	4	1483.5	2	7299	26	2655	44	2458.04545	11	2707.2
57	70	10	2265.2	6	2367	20	2610.9	19	3201.57895	4	3267.5
58	71	0	0	0	0	0	0	1	628	0	0

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Variable	Descriptive Statistics (Spreadsheet132)			
	Valid N	Mean	Std.Dev.	Standard Error
Resting eyes open - number of responses	84	3.786	5.253	0.5731
Resting eyes open - duration of response	84	2021.781	2622.184	286.1037
Resting eyes closed - number of responses	84	2.655	3.399	0.3709
Resting eyes closed - duration of response	84	3189.968	2601.862	283.8865
Continuous performance task - number of responses	84	14.512	14.387	1.5697
Continuous performance task - duration of response	84	2634.247	2013.792	219.7227
Go/No-Go task - number of responses	84	21.619	23.986	2.6171
Go/No-Go task - duration of responses	84	3305.036	1827.832	199.4328
Cued target detection task - number of responses	84	7.381	10.290	1.1227
Cued target detection - duration of response	84	3468.561	3550.315	387.3711

Skin conductance duration



		Wilcoxon Matched Pairs Test (Spreadsheet132)		
		Marked tests are significant at p < .05000		
Pair of Variables	Valid N	T	Z	p-level
Resting eyes open - duration of response & Resting eyes open - duration of response				
Resting eyes open - duration of response & Resting eyes closed - duration of response	84	459.000	4.247719	0.000022
Resting eyes open - duration of response & Continuous performance task - duration of response	84	750.000	3.164995	0.001551
Resting eyes open - duration of response & Go/No-Go task - duration of responses	84	451.000	5.143262	0.000000
Resting eyes open - duration of response & Cued target detection - duration of response	84	431.000	4.642652	0.000003
Resting eyes closed - duration of response & Resting eyes open - duration of response	84	459.000	4.247719	0.000022
Resting eyes closed - duration of response & Resting eyes closed - duration of response				
Resting eyes closed - duration of response & Continuous performance task - duration of response	84	758.000	2.979515	0.002887
Resting eyes closed - duration of response & Go/No-Go task - duration of responses	84	1395.000	0.158417	0.874129
Resting eyes closed - duration of response & Cued target detection - duration of response	84	1178.000	0.176379	0.859996
Continuous performance task - duration of response & Resting eyes open - duration of response	84	750.000	3.164995	0.001551
Continuous performance task - duration of response & Resting eyes closed - duration of response	84	758.000	2.979515	0.002887
Continuous performance task - duration of response & Continuous performance task - duration of r				
Continuous performance task - duration of response & Go/No-Go task - duration of responses	84	491.000	5.032387	0.000000
Continuous performance task - duration of response & Cued target detection - duration of response	84	727.000	3.427726	0.000609
Go/No-Go task - duration of responses & Resting eyes open - duration of response	84	451.000	5.143262	0.000000
Go/No-Go task - duration of responses & Resting eyes closed - duration of response	84	1395.000	0.158417	0.874129
Go/No-Go task - duration of responses & Continuous performance task - duration of response	84	491.000	5.032387	0.000000
Go/No-Go task - duration of responses & Go/No-Go task - duration of responses				
Go/No-Go task - duration of responses & Cued target detection - duration of response	84	1393.000	0.168978	0.865814
Cued target detection - duration of response & Resting eyes open - duration of response	84	431.000	4.642652	0.000003
Cued target detection - duration of response & Resting eyes closed - duration of response	84	1178.000	0.176379	0.859996
Cued target detection - duration of response & Continuous performance task - duration of response	84	727.000	3.427726	0.000609
Cued target detection - duration of response & Go/No-Go task - duration of responses	84	1393.000	0.168978	0.865814
Cued target detection - duration of response & Cued target detection - duration of response				

Bonferroni correction 0.05/5, significant p-values p < 0.01

Heart rate

Data

Appendix A6

37	50	60.1	58.35	58.03	57.05	57.71
38	53	96.86	84.46	87.29	92.61	92.61
39	54	81.81	78.39	78.55	75.81	75.69
40	55	57.81	59.79	59.76	58.76	55.31
41	56	82.81	82.56	84.84	81.35	77.17
42	58	78.22	74.21	77.17	75.83	72.34
43	59	61.64	60.93	60.7	59.83	59.41
44	60	58.52	62.15	61.01	56.49	55.74
45	62	64.11	65.28	65.48	63.29	63.62
46	63	71.6	71.2	74.16	69.85	70.53
47	64	101.84	102.19	106.35	93.24	91.41
48	65	72.47	65.4	67.37	76.97	75.58
49	66	80.4	81.2	82.09	85.37	79.18
50	67	90.26	84.06	94.07	81.15	74.43
51	68	81.18	84.18	82.37	80.44	75.37
52	69	72.45	72.57	72.96	68.14	64.08
53	70	77.21	70.97	70.21	66.82	68.39
54	71	61.47	62.92	62.4	57.72	58.65
55	72	76.62	77.91	77.7	76.13	74.71
56	73	95.94	99.7	99.87	94.78	94.14
57	75	84.6	78.29	80.12	86.54	84.17
58	77	66.06	66.69	67.32	64.2	65.66
59	78	68.16	66.48	68.28	69.5	67.93
60	79	85.83	83.78	86.56	83.44	80.23
61	80	77.39	75.11	75.97	73.46	73.46
62	81	95.57	86.81	93.74	88.48	91.65
63	83	58.7	59.24	60.06	57.83	57.57
64	84	85.28	84.36	85.71	80.54	81.91
65	85	102.86	101.87	103.98	97.76	98.02
66	86	71.88	66.73	71.51	69.01	67.91
67	87	74.85	72.89	74.3	70.66	68.99
68	88	72.14	69.86	71.16	66.55	64
69	90	78.97	64.34	66.47	64.91	63.81
70	91	64.83	74.68	62.81	61.44	59.84
71	93	78.42	78.02	79.76	74.88	73.45
72	94	74.69	71.99	72.38	68.81	64.83
73	98	83.16	75.51	86.22	76.25	72.49

Appendix A6