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Social Trauma and the Mu-Opioid System in Depression

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Declaration

I hereby declare that this submission is my own work, both in concept and execution, and that to the best of my knowledge and belief it contains no material written by another person nor material that has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Susan Malcolm-Smith Date

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Abstract

The overarching thesis under investigation is that the endogenous opioid system plays a key role in depression subsequent to traumatic childhood social experiences. This is suggested by the fact that animal work indicates that mu-opioids robustly mediate separation-distress, and that early social stressors lead to long term dysregulation in key related circuitries and neuroanatomical structures. Moreover, although depression remains poorly characterized, it is clear that early social adversity may act as a risk factor for this disorder. The search for effective pharmacological treatments for depression is ongoing, and preliminary clinical evidence suggests that the synthetic opioid buprenorphine may be effective in treating certain cases of refractory depression. As a first step in a program aimed at investigating the role of opioids in depression, the current study examined the impact of an opioid manipulation on subjective experience of affect, social cognition, and neural activation in two groups of medically and psychiatrically healthy normal young adults (N = 32). The groups differed on one key variable – exposure to early social trauma. In this exploratory study, a double-blind, placebo controlled crossover design was employed. It demonstrated that these groups showed a different pattern of response to a low dose of buprenorphine across all domains measured. In terms of baseline characteristics, the trauma-exposed and control groups differed on a primary-process, appetitive emotion system, with the trauma group showing reduced SEEKING. Buprenorphine reduced the experience of both positive and negative emotion in the trauma (but not control) group. In terms of social cognition, it enhanced a positivity bias in the control (but not the trauma) group. In terms of neural activation, region of interest analysis indicated that the trauma group was more sensitive to the effects of buprenorphine in reducing response to negative social signals in the anterior insula. Region of interest analysis also indicated that the trauma group was more responsive to negative than positive social signals on placebo, and that buprenorphine was able to attenuate this effect. Although preliminary, these findings support the idea that long term opioid dysregulation follows early social traumatic experiences, and that the role of opioids in the etiology and treatment of depression requires further investigation.

Introduction

Psychosocial adversity in childhood plays a critical role in at least some cases of depression (Flinn, Muehlenbein & Ponzi, 2009; Heim & Nemeroff, 1999; Heim et al., 2009). Although there are likely to be complex interacting neurochemical mechanisms underpinning this association, the overarching thesis under investigation here is that the endogenous opioid system plays a key role in depression subsequent to traumatic childhood social bonding experiences.

In brief, the key points which give rise to this broad thesis are the following: Major depressive disorder is a heterogenous phenomenon. Its etiology is unresolved, and its treatment remains problematic. Around 30% of all cases do not respond to current treatment options (Mayberg, 2004; Warden, Rush, Trivedi, Fava & Wisniewski, 2007). Many researchers in this field seem to be increasingly convinced that single factor accounts cannot explain the disorder, and that a broader consideration of interacting neurochemistries and variable presentations is required (Drevets, Price & Furey, 2008; Nemeroff, 2007). There is a body of evidence to suggest that early separation experiences predispose to depression, and that depressive episodes are frequently triggered by experiences of social loss (Bowlby, 1969, 1980; Flinn et al., 2009; Heim & Nemeroff, 1999; Heim, Shugart, Craighead & Nemeroff, 2010; Slavich, O'Donovan, Epel & Kemeny, 2010). Preliminary clinical evidence indicates that mu-opioid therapy is effective in certain cases of refractory depression (Bodkin, Zornberg, Lukas & Cole, 1995; Emrich, Vogt & Herz, 1982). Animal research clearly indicates a strong role for the mu-opioid system in mediating social reward and attenuating social loss in several mammalian and avian species (Herman & Panksepp, 1978, 1981; Nelson & Panksepp, 1998; Panksepp, 1980, 2005a). Neuroimaging research is beginning to confirm that the same is true in humans (Eisenberger, Lieberman & Williams, 2003; Kennedy, Koeppe, Young & Zubieta, 2006; Liberzon et al., 2007; Zubieta et al., 2003). Furthermore, it is clear from the animal literature that early social losses, which provoke strong separation-distress responses, impact permanently on stress-related neurochemistries (Anisman & Matheson, 2005; Braun, Lange, Metzger & Poeggel, 2000; de Kloet, Joels & Holsboer, 2005; Meaney, 2001; Meaney et al., 1996; Poeggel et al., 1999; Ziabreva, Schnabel,

Poeggel & Braun, 2003). There is thus good reason to infer that the opioid system, which is closely involved in mediating stress and emotional responses, may also be dysregulated subsequent to early social traumatic experiences.

Systematic investigation of this overarching thesis is thus warranted. Such an investigation requires research at multiple levels (for example, neurophysiological, neurochemical, epigenetic, and behavioral indicators as well as subjective affect need to be explored in both healthy and depressed individuals), which clearly constitutes an enormous and long-term task. The current project represents a first step in this direction, asking whether exposure to early social trauma can lead to identifiable changes in affect, social cognition and neural reactivity, and whether mu-opioid manipulation can alter these effects. If early social trauma leads to long-term mu-opioid dysregulation, there may well be detectable changes in these domains in adults who have experienced such trauma in childhood. Most critically, a different pattern of response to mu-opioid manipulation should be present in these individuals when compared to appropriate controls who have not experienced early social trauma.

Because this is such a complex arena of investigation, with so many potential variables of influence, the present investigation was initiated in a relatively homogenous set of participants. Although the overaching question ultimately concerns depression, due to the heterogeneity present within this disorder it seemed prudent to begin the examination of mu-opioid system function subsequent to early social trauma in a more easily defined group. Medically and psychiatrically healthy young adults were recruited who differed primarily on one key feature – exposure to early social trauma. This exposure was assessed using the Childhood Trauma Questionnaire - Short Form, and stringent inclusion criteria were employed to constitute the trauma and control groups. A double blind, placebo-controlled crossover design, using a low-dose mu-opioid manipulation, was employed. Baseline characteristics including key personality traits and general experience of affect were assessed. In response to the opioid manipulation, current experience of affect, implicit or preconscious evaluation of social stimuli, and neural activation in response to social-emotional stimuli were examined.

The research described in this dissertation is exploratory, and consequently has several notable limitations. These limitations have to be judged in the context of the

larger research program within which the current research is nested, however. The ultimate aim of this program is to clarify whether a particular type of depression exists – one that follows experience of early social trauma, and that is mediated at a neural level, at least in part, by mu-opioid dysregulation. It is thus important to bear in mind that the work reported here is preliminary, seeking to establish whether one small, but important, set of associations exists. Given the significant challenges present in the field of depression research, both in terms of establishing etiology and in terms of successful intervention, the value of the overarching investigative program is evident. Successful research programs must, however, proceed in small successive steps, and that is where the importance of this report lies.

Literature Review

In order to contextualize this research, the review that follows provides an overview of: 1) Panksepp's model of social attachment and emotion; 2) the neurochemistry of the opioid system; and 3) the empirical neuroscience literature on depression. Each of these topics is associated with a substantial body of knowledge. Of necessity, the overviews provided here are extremely limited, and focus only on the information that seems most relevant to the topic at hand. My aim is to embed this investigation within a theoretical framework that will allow us to understand not only *whether*, but also *how* and *why* mu-opioid medication may ameliorate certain cases of depression.

Conceptual Foundations for this Research

This dissertation is grounded in the model of basic emotion systems developed by Jaak Panksepp (1998). Multiple competing models and taxonomies of human emotion currently exist, with little immediate prospect of resolution on the basis of the available evidence. An adequate critical analysis of the strengths and weaknesses of the competing models would require a thesis in itself. For this reason I will not be reviewing any of the other established models of emotion (such as those developed by, for instance, Cosmides & Tooby, 2000; Darwin, 1872/1965; Davidson, 1995; Ekman, 1992; Heller, 1993; Izard, 1977; Tracy & Robins, 2004).

Panksepp's general theoretical articulation of the core emotion systems of the mammalian brain is a work in progress. Its chief strengths lie in the fact that it seeks to build a comprehensive synthesis in multiple ways. Specifically, it looks to evolutionary principles to account for the development and function of emotion, looking back in phylogenetic terms to find the origins of emotion in more primitive, less cerebrally developed (mainly mammalian) species. Furthermore, it seeks to disentangle core emotion systems in neural and neurochemical detail. This approach makes it unique, and incommensurate with other human-centered, more cognitively focused sets of ideas on emotion. Importantly this model, although based mainly on animal research, takes into account the affective feeling states that accompany behavioral and neurochemical changes in animals and humans – an approach that is of great relevance to psychiatric research. Panksepp (2004) argues that psychiatry requires a theory of emotion that is grounded in evolutionarily conserved neural circuitry and neurochemistry, and which incorporates understanding of the resultant subjective experiences, for the core mechanisms of psychiatric disorders to begin to come into focus.

It is thus well worth investigating whether some of the ideas generated from this work can shed light on the neural and neurochemical puzzles of depression. Within this theoretical approach, the argument is that in order to understand depression, one must understand social pain. The following section on Panksepp's core emotion systems will attempt to illustrate how animal research, and subsequent work in humans, has indicated that mu-opioids are involved in the positive feeling states/rewards associated with social contact, and that physical and social pain are closely related, with both being robustly mediated by this neuropeptide.

Specifically, the section that follows will: 1) contextualize the core emotion systems of interest by detailing Panksepp's broad conceptualization of emotion; 2) provide some detail on the two core emotion systems of greatest relevance here; and 3) present some pertinent research involving humans which confirms some of the ideas generated from the animal work; notably, that physical and social pain are strongly related.

Conceptual Foundations: Panksepp's Core Emotion Systems

Panksepp's research has focused on ancient subcortical emotion systems, conserved across mammalian species. Based on this work, he has constructed a general theoretical conceptualization of core emotions and their role in social affects and functioning (Panksepp, 1998; Panksepp, 2005a).

Defining emotion. Panksepp (1998) conceptualizes core emotions as command systems that developed through the course of evolution – they are "evolutionarily prepared and epigenetically refined states of the brain" (p. 122). These core emotions serve to organize mental, physiological, and behavioral responses to events of universal biological significance. The characteristic affective feeling states associated with the various basic emotions serve to encode value: Emotions are thus seen as intrinsic value systems, generating lived experience of the 'goodness' or 'badness' of situations, events, other animals, and conspecifics. Emotion systems, although unconditioned in themselves, are regarded as being modifiable by experience; indeed one of their main functions is to facilitate associative learning, and thus promote adaptive and successful behavior. There is thus a distinction between primary process affects, which are genetically coded and located sub-neortically, and which generate characteristic unconditioned behavioral patterns, and secondary and tertiary processes. Secondary processes include emotional learning via varieties of conditioning, whereas tertiary affects include higher cortical elaborations of emotion, including reflective awareness (Panksepp, 2010). Panksepp's work centers on what have been variously termed basic, core or, most recently, primary process emotion systems.

In sum, core emotions are conceptualized as innate subjective value systems that are generated at the neural level, and which impact in major ways on both simple instinctive, and complex, more cognitively controlled behavior. Panksepp's perspective is that affective consciousness – that is, the experience of these basic emotion states – preceded the evolution of higher forms of cognitively resolved consciousness.

Brain substrates of emotion. The core emotions are instantiated largely subcortically, but also involve areas of archicortex. The core emotion command systems

possess a remarkable degree of cross-mammalian similarity at the neural level (Panksepp, 1998; 2005a). Both the so-called reptilian brain (including the basal ganglia) and the limbic regions of the brain are similar in proportional size across mammalian species. The basal ganglia encode basic motor plans including those related to fear, anger and sexual behavior. The limbic regions encode more social emotions including maternal care, social bonding, rough-and-tumble play and separation-distress. A key point in this model is that neocortex, although it is influenced by and in turn influences emotion in the brain, is not necessary for the generation, experience, or enactment of core emotions.

Core emotion systems. These include four emotions that are evident soon after birth, viz. SEEKING, FEAR, ANGER, and SEPARATION-DISTRESS.[1] Other important emotion systems including PLAY and CARE become evident later in development. These terms are capitalized in order to prevent confusion with colloquial usages of the same terms, and the multiple associations attached to everyday emotional terminology. The capitals indicate specific reference to the core emotion systems that have been distinguished at a neural level (Panksepp, 2010). Ongoing neural activity in these emotion systems results in the generation of moods, and in the long term, in the development of personality characteristics, such as tendencies to be cheerful, aggressive, fearful or depressed (Panksepp, 1998, 2004).

Clearly, adopting Panksepp's theoretical framework indicates an acceptance of the relevance of animal work to human research. This standpoint is by no means uncontroversial (see, e.g., Blumberg & Sokoloff, 2001), but many arguments in support of such an approach have been made. For instance, Darwin (1872/1965), in his celebrated work on emotion, argued that differences between animals are likely to be differences in degree, rather than differences in kind. Many current researchers regard animal work as relevant, due to clear phylogenetic continuities in mammalian brain anatomy and behavior; with structural and proportional size similarities in key brain regions in all mammals, and a great deal of shared genetic material (Adolphs, 2003;

[1] I choose to use the term SEPARATION-DISTRESS as it relates most clearly to the ideas under investigation here. Panksepp uses this term as well as PANIC/GRIEF or SADNESS at different times to refer to the same system.

Damasio, 1994; Damasio et al., 2000). In light of these cross-mammalian continuities, the idea that animal and human emotion exists on a continuum is acceptable. From this standpoint, a particularly compelling argument is that understanding neural emotion systems may be easier in animal models, where causal relationships can be studied directly, and where more complex, later developing emotions, characteristic perhaps only of humans, are not present (Panksepp, 2003, 2005b).

Hence, the research described here proceeds from the perspective that core emotion systems evolved to guide human behavior in adaptive ways, and that these evolutionarily ancient, bottom-up processes impact in major ways on the nature of subjective lived experience. In depression, this adaptive function seems to go awry, but the emotions continue to exert a disproportionate and negative impact on the individual. To date a single coherent account of the role and dysfunction of emotion in depression is lacking: Low mood, sadness, and anxiety all feature, alongside anhedonia, lack of motivation and psychomotor retardation, and a variety of other symptom clusters, with no satisfactory explanation of why all these features, and so many varied clinical manifestations, may occur. The importance of a focus on emotion in depression research is evident.

I propose that two of the core emotion systems identified by Panksepp are worth considering in this regard: SEEKING and SEPARATION-DISTRESS. In fact, the newest articulations of what is now termed the separation-distress hypothesis of depression allocate a role to both systems in the genesis of depression[2] (Alcaro & Panksepp, in press; Panksepp & Watt, 2011; Zellner, Watt, Solms & Panksepp, in press). SEEKING is mediated by dopamine, and SEPARATION-DISTRESS by the mu-opioid system; two chemistries that are closely interactive. Social loss and social pain are often key features of depression, and are the defining characteristics of SEPARATION-DISTRESS. Anhedonia, the loss of interest or pleasure in a variety of activities, is commonly seen in depression, and can be argued to reflect downregulation of the SEEKING system. I provide a brief overview of these two core emotion systems below.

[2] Because these new articulations of the separation-distress hypothesis were published after the current research had been designed and executed, and therefore did not shape the focus of this study, it seems more appropriate to present detail on them in the Discussion section.

The SEEKING system. The SEEKING system is one of two positive emotion systems that have been the focus of much research across mammalian species – viz., this dopamine modulated SEEKING system, and the opioid modulated system of social and consummatory pleasure (Alcaro, Huber & Panksepp, 2007). Both systems are involved in 'rewarding' affective states, although the precise details of their roles remain controversial. The systems interact closely, with opioid and dopamine levels in key circuits influencing each other in various ways. It is thus difficult to examine the effects of opioids without considering interactions with this system.

The SEEKING system is conceptualized as an expectancy system, energizing all appetitive behavior: it is not specific to any particular drive or need. It stimulates animals to engage with the world in order to meet a multiplicity of needs. Through experience and learning, it also generates expectancy states that enable animals to anticipate reward. The associated affective experiential state is clearly positive, and has been described as "a generalized incentive 'emotional' reward state, that does not reflect the pleasure of sensation, but rather the euphoria of appetitive eagerness" (Alcaro & Panksepp, in press; this paper provides a comprehensive recent review of this system).

Brain substrates of the SEEKING system. The neural circuits of the SEEKING SYSTEM run in the extended lateral hypothalamic corridor, from area A10 in the ventral tegmental area to the ventral striatum or nucleus accumbens, which projects to the prefrontal cortex and other structures. Direct electrical stimulation along this pathway results in the "most energized exploratory and search behaviors an animal is capable of exhibiting" (Panksepp, 1998, p. 155). The ascending mesolimbic dopamine pathways delineate this system fairly well, but other components also exist, most importantly descending glutamatergic pathways.

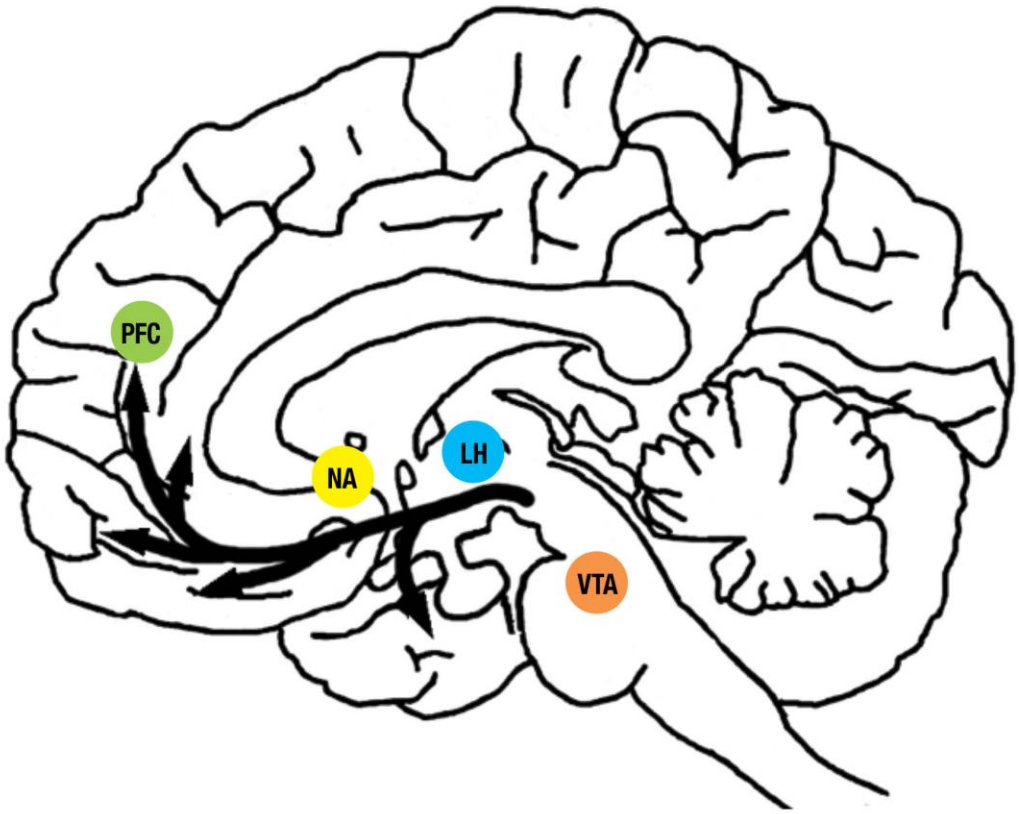

Figure 1:
Brain substrates of the SEEKING system.
Abbreviations: PFC = Prefrontal cortex; NA = Nucleus accumbens;
LH = Lateral hypothalamus; VTA = Ventral tegmental area.

A brief aside is necessary regarding the arguments around this dopamine system and reward, as it clarifies some of the relevant dopamine-opioid interactions. Researchers following the behaviorist tradition conceived of this mesolimbic dopamine system as a *reward* system, largely due to the fact that research animals will self-stimulate here to the point of collapse: they clearly find such stimulation rewarding, and will ignore more basic reinforcers. Those following an affective neuroethological tradition take issue with the *reward system* designation, as the system seems more responsive to the *possibility* than to the *presence* of reward. In fact, dopamine activity is reduced in consummatory behavior, and another system, modulated by opioids, is

activated (Alcaro & Panksepp, in press; Panksepp, 1998). There seems to be, however, a complex interaction between appetitive SEEKING and consummatory behavior. The chemistries interact in several ways, with for example, ventral tegmental area opioids stimulating dopamine activity, while opioids in the striatum inhibit dopamine. It should also be noted that mild opioid stimulation arouses the SEEKING system, while high stimulation reduces the desire for all rewards (Panksepp, 1998). Broadening the conceptualization of this system from a pure learning systems to an affective neuroethological perspective allows a fuller understanding of its key features, for example, the fact that stimulation of the system generates an *unconditioned* and coherent emotional response pattern (foraging or SEEKING), which consists of energized, goal-directed activity (Alcaro et al., 2007). Most critically, the learning system conceptualizations are silent on the affect associated with the activation of this system, and thus cannot contribute to an understanding of its key psychological and psychiatric aspects. Understanding the full emotional picture is integral to understanding what 'reward' or 'reinforcement' actually mean (Panksepp, 2005a).

It is perhaps interesting to note that throughout history, drugs of abuse for humans have consistently been psychostimulants, which act on the ascending mesolimbic dopamine SEEKING pathway, and opiates, which act on the opioid receptors present at the consummatory end of this pathway (Panksepp, 1998). The interaction of dopamine and opioids in SEEKING and reward is fairly clear, and is something that must be borne in mind when investigating opioid involvement in depression. Opioids are also strongly implicated in positive social contact, and in mediating the pain of social loss.

Social bonding and the SEPARATION-DISTRESS system. The brain chemicals involved in social bonding – those that are activated by positive social engagement such as physical contact, grooming, play and sex – include oxytocin, prolactin and the endogenous opioids. Evolution has provided mammals with a neural system characterized by motivation to form affective social bonds, the first exemplar of which is the attachment of the infant to its mother. Evidence for this attachment is apparent across mammalian species: Infants form bonds and demonstrate affiliative

behavior toward their primary caregiver (usually the mother). These bonds are characterized by infants displaying very selective approach and interaction behaviors towards these caregivers, alongside manifest distress if they are separated from the caregiver (Ainsworth, 1967; Bowlby, 1969, 1973, 1980; Harlow, Dodsworth & Harlow, 1965). This system continues to play a critical role in social bonding across the lifespan (Nelson & Panksepp, 1998; Panksepp, 1998).

This system is regarded as responsible for monitoring both social presence and social absence. It has dichotomous associated affective states: positive states associated with social reward, such as contact comfort, and negative states associated with separation-distress or social loss. The neural and neurochemical systems that modulate both affective states developed from more primitive, already extant systems. The system for mediating the positive affects of social contact and bonding may have developed as an extension of existing mechanisms that serve to regulate energy balance, thermoregulation and place preferences, while the system that mediates separation-distress and social loss is an extension of that for physical pain (Panksepp, Nelson & Bekkedal, 1997).

Various chemistries, as mentioned above, appear to play a role in positive affective states associated with social contact, and they may need to act in concert to exert their full impact. It is known that opioids are not invariably involved in social reward, but in many cases they are strongly implicated (Panksepp et al., 1997).

The fact that opioids are involved in certain positive affective experiences related to social bonding has been clearly established. Mu-opioids are experienced as rewarding, as illustrated by the fact that they successfully condition odor- and place-preferences (Nelson & Panksepp, 1998). It is known that physical contact stimulates opioid activation (Nelson & Panksepp, 1998), and this perhaps accounts for the ability of touch to lower heart rate and reduce distress vocalizations (Bermant, 1963; Panksepp, Herman, Vilberg, Bishop & DeEskinazi, 1980b). Social interaction stimulates opioid release – this is particularly true of prosocial activities such as play or grooming (Keverne, Martensz & Tuite, 1989; Panksepp & Bishop, 1981). In animals, proximity of friendly, caring others or positive social interaction can in fact lead to relief of physical pain via opioid release (D'Amato & Pavone, 1993; McMillan, 2000). Gentle touch has been shown to reduce

both physical and emotional pain (Panksepp, Burgdorf, Beinfeld, Kroes & Moskal, 2004; Uvnas-Moberg, 1998).

It is also critical to note that altered levels of opioids can alter social behavior. A key study indicated that mu-opioid receptor knockout mice displayed markedly altered attachment behavior toward their mothers, seeming apparently blind to maternal rewards (Moles, Kieffer & D'Amato, 2004). Other animal research provides important evidence regarding the effects of opioids on social behavior. Work using receptor blockades, antagonists, and agonists indicates that reduced levels of mu-opioids result in increased seeking of social contact, while high levels appear to reduce motivation to seek out such contact (Fabre-Nys, Meller & Keverne, 1982; Herman & Panksepp, 1978; Kalin, Shelton & Lynn, 1995; Keverne et al., 1989; Martel, Nevison, Simpson & Keverne, 1995; Panksepp, Najam & Soares, 1979). These findings are in line with the hypothesis that any agent which activates social reward processes in the brain should reduce pro-social behavior, and vice-versa (Panksepp et al., 1997).

Although the systems and chemistries involved in social reward are not comprehensively delineated, it is clear that opioids play an important role in social bonding and in social reward. The system that mediates social distress or emotional pain is closely related, and seems to have emerged from the existing system for modulating physical pain. This system is more clearly delineated, in part because distress is easier to observe and measure, but also because social distress is more immediately compelling in terms of its clinical implications (Panksepp et al., 1997).

Researchers who have explored the hypothesized relationship between physical and social pain have pointed out that we use words for physical pain to describe social pain (for example *hurt* feelings, or a *broken* heart), and their research indicates that these constructions may well reflect more than the use of metaphor (Eisenberger et al., 2003). Social bonds carry great adaptive value for mammals, so it seems plausible to reason that evolution might have 'wired in' a warning mechanism – similar to pain sensation – to elicit responses that attempt to re-establish vital social contact. Pain is extremely aversive, and thus promotes rapid responses aimed at its alleviation. It may be that the experience of *social* pain, and the associated underlying neural circuitry are uniquely found in mammals (Eisenberger & Lieberman, 2004).

Evidence that the SEPARATION-DISTRESS system is closely related to the pain system is strong. This evidence is highly relevant to the current study for the following reason: We know that mu-opioids are among the most effective painkillers. If social and physical pain are shown to be related, this connection not only supports the contention that opioid intervention should be effective in reducing social pain, it helps to explain *why* this should be so.

All mammals 'cry' when separated from their mothers. This distress is not trivial. It has been shown that many young animals, if isolated, seem to experience such intense pain due to this social loss that other life regulatory systems are impacted – the animals evidence anaclytic depression and often die (Yates, Panksepp, Ikemoto, Nelson & Conner, 1991). Panksepp thus hypothesized that intense social distress could be monitored using distress vocalizations as objective indicators of its presence. Panksepp and colleagues (Herman & Panksepp, 1978, 1981; Nelson & Panksepp, 1998; Panksepp, Herman, Conner, Bishop & Scott, 1978) demonstrated that opioids, which had already been established as powerful analgesics, are remarkably effective at substantially reducing or even eliminating separation-related distress vocalizations, and that these effects are mediated by the mu-opioid system. This was shown to be true for a number of animal species, including guinea-pigs, dogs, and primates (Herman & Panksepp, 1978; Kalin & Shelton, 1989; Panksepp et al., 1978). In fact, it became evident that opioids are even more successful at mediating social distress than physical pain (Panksepp, 1998; Panksepp, Bean, Bishop, Vilberg & Sahley, 1980a; Panksepp, Meeker & Bean, 1980c). These findings have been independently confirmed by other laboratories (e.g. Kalin, Shelton & Barksdale, 1988; Kehoe & Blass, 1986).

Although other neurochemicals also modulate SEPARATION-DISTRESS (e.g. oxytocin and prolactin; notably also chemistries that are involved in social reward), opioids achieve this marked effect at extremely small doses. It is hypothesized that their effectiveness is due to the fact that they cause feelings associated with positive social bonding and social reward (Panksepp et al., 1980a; Panksepp et al., 1980b).

In Panksepp's conceptualization, opioids are particularly important in social bonding and SEPARATION-DISTRESS for the following key reasons: 1) Although the chemistries of reward in general, and of social reward in particular, are not

comprehensively understood (Panksepp et al., 1997), opioids clearly play a critical role in mediating social behavior and social affiliation; 2) opioids have been shown to exert a very strong influence on social distress, even at very low doses, suggesting that they are powerful mediators of social pain; 3) the opioid system is perhaps unique in that its anatomy and function both indicate a clear association between social and physical pain (Panksepp, 2005b). The connection between physical and social pain is central to Panksepp's theoretical construction, where the regulatory mechanisms for social pain are thought to have evolved as an extension of those already in place for regulating physical pain. This link is also central to the model of depression proposed in this dissertation.

Brain substrates of the SEPARATION-DISTRESS system. The system arises in the periaquaductal gray of the midbrain, close to sites where physical pain can be elicited. (Note that the periaquaductal gray is the area at which emotional distress is most easily evoked in both humans and animals). The pathway progresses through the dorsomedial thalamus, to the ventral septal area, the preoptic area and the bed nucleus of the stria terminalis. In higher animals it also continues to the anterior cingulate cortex and some amygdala sites. This path is very similar to the corticotrophin releasing factor (CRF) and opioid systems. Notably, CRF seems to increase distress vocalizations, while opioids reduce them (Panksepp, 1998).

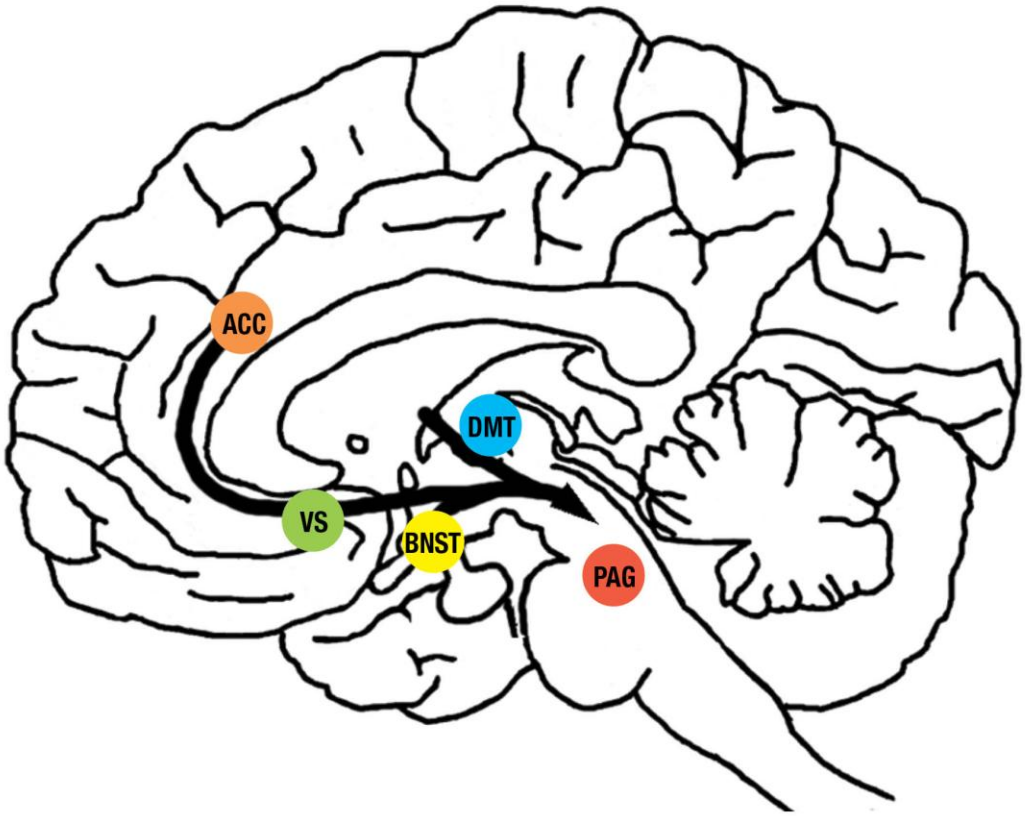

Figure 2:
Brain substrates of the SEPARATION-DISTRESS system.
Abbreviations: ACC, = Anterior cingulate cortex; VS = Ventral septal area;
DMT = Dorsomedial nucleus of the thalamus; BNST = Bed nucleus of the stria terminalis;
PAG = Periaqueductal grey.

It is of the greatest importance to note that when separation-distress is not followed by a successful reunion, the conditions for depression are realized. This has been suggested by both animal research and studies of human children (Bowlby, 1969; Harlow et al., 1965; Panksepp, 1998), where the initial distress vocalization phase (the 'protest' phase) gives way to a depressed, inert state (the 'despair' phase). Alterations in neurochemistries account for this transition: the initial distress phase involves raised corticotrophin releasing factor (CRF) and increased activity in the hypothalamic–pituitary-adrenal (HPA) stress axis. These processes rapidly result in depletion of

norepinephrine, serotonin, and some dopamine reserves in the brain. Depressive behaviors can in fact be artificially induced by creating these exact physiological changes. It is hypothesized that this inert state has benefits in evolutionary terms: the isolated youngster conserves energy, does not wander any further from 'home' seeking the mother, and is less likely to alert predators to its presence and vulnerable state. The depressed state may thus increase the chances of the mother eventually finding the youngster alive (Panksepp, 1998). However, the potential negative psychiatric implications for humans in whom this mechanism has been over-sensitized, putatively following traumatic early social loss, are evident.

There are indeed indications from the animal literature that early social distress can lead to long-term alterations in key structures and circuits. Studies have demonstrated that 24 hours of neonatal maternal separation disinhibits the infant's HPA system, and that this alteration in glucocorticoid feedback mechanisms persists into adulthood (Anisman, Zaharia, Meaney & Merali, 1998; Biagini, Pich, Carani, Marrama & Agnati, 1998; Meaney et al., 1996; Vazquez, Lopez, Van Hoers, Watson & Levine, 2000).

HPA axis changes are not the only sequelae. Notably, in a recent paper on the SEEKING system, Alcaro and Panksepp (in press) reviewed a body of work that demonstrates that chronic stress protocols resulting in behavioral depression, also result in a simultaneous dysregulation of mesolimbic dopamine transmission and receptor function.

Furthermore, an important series of studies on rats (Braun et al., 2000; Poeggel et al., 1999; Ziabreva et al., 2003) indicates that repeated maternal separation and social isolation result in alterations in neuronal development, most notably in the medial prefrontal cortex, including the anterior cingulate cortex and the nucleus accumbens core. The authors call these changes 'functional scars' in emotional brain circuits. Importantly, these authors illustrated that having access to some social contact (viz., the mother's voice) during separation protected against this effect. They also demonstrated that early maternal separation resulted in a reduced number of neurons implicated in inhibitory GABA-ergic function, notably in the anterior cingulate cortex and nucleus accumbens core. They suggest this neuronal reduction may lead to increased excitatory output from

the anterior cingulate cortex, which could in turn result in significantly altered limbic function.

Although the studies reviewed above do not refer specifically to opioid substrates, they do indicate long-term dysregulation in relevant chemistries, as well as structural changes in regions with dense concentrations of mu-opioid receptors. The hypothesis under examination here is that early exposure to significant social trauma results in long-term dysregulation not only of stress-related chemistries, but also of the mu-opioid system which has a significant role in mediating these effects.

To summarize, the animal literature I have reviewed so far indicates that:

- The mu-opioid system is involved in positive affect associated with social contact, and has a role in mediating social behavior.
- A close relationship exists between physical and social pain, with common neural structures involved, and with interchangeable effects in their mediation; what were traditionally thought of as physical pain mediators (mu-opioids) robustly resolve social distress, while social contact seems able to alleviate physical pain.
- Early social stress leads to long-term dysregulation in stress and emotion systems, including the mesolimbic dopamine system (which interacts with the mu-opioid system), and to altered development in regions with high concentrations of mu-opioid receptors. The effect of early social stress has not been investigated directly for the opioid system but it seems reasonable to propose that this system may be dysregulated in the long-term following early social trauma.

Critical Evidence from Human Neuroimaging

Evidence from human neuroimaging research supports the contention derived from the animal literature that physical and social pain are closely related. This link is important to establish because showing that physical and social pain share neural substrates accounts for why mu-opioids should attenuate social pain and should thus be effective in treating depression. The work reviewed below provides evidence that

regions high in opioid receptors are key in both the affective components of physical pain and in the pain of social loss or rejection. It also gives some insight into the way in which social contact might mediate both physical and social pain, through its mediation of stress responses, which according to the literature reviewed above, is likely to involve opioids.

Shared neural substrates for physical and social pain: A number of human neuroimaging studies suggest there may be shared neural substrates for social and physical pain. Functional magnetic resonance imaging (fMRI) studies attempting to elicit feelings of grief have noted activation in pain circuitry; notably in the dorsal anterior cingulate and periaqueductal gray and insula (Gundel, O'Connor, Littrell, Fort & Lane, 2003; O'Connor et al., 2008). Damasio and colleagues (2000) demonstrated, using positron emission tomography (PET), that the experience of sustained sadness correlates with activation in regions known to be implicated in physical pain, including the insula bilaterally, and the anterior cingulate and dorsal pons.

In 2003, Eisenberger and colleagues published the first in a series of studies aimed at directly testing the hypothesis that social and physical pain share common neural substrates in humans. Concurring with Panksepp (1998), they proposed that social pain represents a similar affective experience to physical pain, with the cause being actual or potential separation from significant others or the social group, rather than physical damage.

In this study, Eisenberger et al. (2003) used an fMRI protocol, where participants were initially included in, and then later excluded from, a computer-based social game (tossing a ball between three people). During the game, it appeared that the other two players simply stopped including the participant and only threw to each other. Participants were asked to indicate how distressed they felt during this exclusion. The areas found to be strongly associated with the pain of social rejection were the dorsal anterior cingulate cortex, and a region in the right ventral prefrontal cortex.

The anterior cingulate is known to be involved in the affective component of physical pain (Peyron, Laurent & Garcia-Larrea, 2000; Rainville, Duncan, Price, Carrier & Bushnell, 1997; Tolle et al., 1999). There is also evidence regarding the importance of

the anterior cingulate cortex in social bonding and separation-distress. Research has indicated that the cingulate gyrus must be intact for the generation of distress vocalizations (Lorberbaum et al., 2002): Stimulation of the anterior cingulate cortex results in the production of distress vocalizations, whereas ablation can eliminate distress vocalizations entirely (Kirzinger & Jurgens, 1982; MacLean & Newman, 1988; Robinson, 1967; Smith, 1945). Ablation of the anterior cingulate cortex in primate young has also been shown to lead to reduced affiliative behavior (Hadland, Rushworth, Gaffan & Passingham, 2003; Ward, 1948). The authors thus argued that the anterior cingulate region was implicated in generating the feelings of social pain, and that the right ventral prefrontal region was involved in regulating that distress response.

Eisenberger et al. (2003) thus provided critical evidence of a shared neural substrate for social and physical pain in humans. Ongoing work has confirmed activation of the anterior cingulate region in distress related to social rejection. Additionally, anterior insula regions – also implicated in affective aspects of physical pain – have been shown to be involved in this response (DeWall et al., 2010; Masten et al., 2009; Masten, Telzer, Fuligni, Lieberman & Eisenberger, in press).

DeWall et al. (2010) provide additional evidence of the overlap between physical and social pain systems. They illustrated that a physical pain reliever reduced both social pain and neural activity in regions involved in physical and social pain. Acetaminophen, a common physical pain reliever, whose mechanism of action is unclear, was contrasted with placebo over a 3-week administration period. Administration of acetaminophen led to reduced self-reports of daily social pain, and in an fMRI task, those who had taken the drug for 3 weeks showed reduced activity in regions known to respond to affective components of physical pain – the dorsal anterior cingulate and anterior insula.

Across all studies reviewed thus far, during the affective experience of social loss or social pain, areas known to be implicated in physical pain, notably the periaqueductal gray, and very consistently, the anterior cingulate cortex and anterior insula, are activated.

Subsequent work has provided further evidence supporting the link between physical and social pain, by demonstrating shared sensitivity to both. Because of the strong neural link between physical and social pain, Eisenberger et al. (2006)

hypothesized that there should be a relationship between sensitivity to both types of pain. There was pre-existing evidence in support of this idea: Young children who are in physical pain tend to exhibit increased sensitivity to social pain (Bowlby, 1969), and individuals who suffer from chronic pain tend to have anxious attachment styles, indicating fear of social loss (Ciechanowski, Sullivan, Jensen, Romano & Summers, 2003). Furthermore, those who are extremely sensitive to social rejection report greater distress at seeing others in physical pain (Macdonald & Shaw, 2005). Social support is also known to mediate both physical and social pain (Brown, Sheffield, Leary & Robinson, 2003; Hoogendoorn, van Poppel, Bongers, Koes & Bouter, 2000; Kennell, Klaus, McGrath, Robertson & Hinkley, 1991; King, Reis, Porter & Norsen, 1993; Leary & Springer, 2000; Macdonald & Leary, 2005; Zaza & Baine, 2002), and this review has already covered evidence indicating that mu-opioid medication seems to attenuate both types of pain, at least in non-human animals.

Eisenberger and colleagues (2006) conducted a study following up on some of these ideas. They tested two hypotheses: 1) That baseline sensitivity to physical pain should predict sensitivity to social rejection, and 2) that experiences that lead to increased social distress should increase pain sensitivity. Both hypotheses were supported: Participants who scored higher on baseline sensitivity to pain manifested greater levels of social distress when excluded from social activity, while participants who experienced social rejection in the study rated pain stimuli administered towards the end of the assessment as more aversive. These relationships remained significant after neuroticism was controlled for; the results thus cannot be ascribed simply to high anxiety across conditions (Eisenberger et al., 2006). This work provides further evidence for a strong association between physical and social pain in humans.

Subsequent work has investigated mechanisms linking social support to reduced physiological reactivity to stressors, and suggests that opioids may be implicated. The effects of social support are not trivial: Lack of social support is known to be associated with a markedly increased risk for morbidity and mortality, even compared to well-known risk factors such as smoking, obesity and hypertension. The authors found that, over a period of 10 days, participants who had regular supportive interactions had lower cortisol reactivity to a social stressor (Eisenberger, Taylor, Gable, Hilmert & Lieberman,

2007). Critically, both greater social support and reduced cortisol reactivity were associated with reduced acitivity in regions previously shown to be associated with social disress (dorsal anterior cingulate and right ventral prefrontal regions). The authors point to two possible ways in which social support could reduce physiological reactivity to stressors. The first possibility is that social support changes perception of stressors; these are simply not perceived as threatening and thus the physiological stress response is not elicited. If this were true, they argue you would expect to see reduced activity in neural structures usually involved in response to threats. The second possibility is that social stressors might exert their effect after threat perception but before the full physiological response takes effect – this would mean that those with social support are better able to regulate their responses to stress. If this is the case, one would expect to see increased activation in prefrontal regions known to be associated with regulation.

Eisenberger et al. found, confirming results from previous studies, that increased dorsal anterior cingulate activity correlated with greater self-reports of distress. During social exclusion, greater right-sided activation in the ventral prefrontal region was associated with less self-reported distress. This region is known to be involved in inhibition and affect regulation (Aron, Robbins & Poldrack, 2004; Lieberman et al., 2007; Ochsner et al., 2004). They concluded that this evidence lends support to the second of the two possibilities listed above; that is, that social support exerts its effect in aiding individuals to better regulate their responses to stressors. Furthermore, they suggested that social support acts on the anterior cingulate as follows: The anterior cingulate has one of the highest densities of opioid receptors (Schlaepfer et al., 1998; Vogt, Wiley & Jensen, 1995); Eisenberger and her colleagues (2007) suggested that, over time, consistent social support leads to regular release of opioids, which in turn desensitizes the anterior cingulate and results in an attenuated response to stress.

Recent work provides further support for the idea that social support may desensitize individuals to social stressors (Masten et al., in press). In a prospective study, high school students recorded daily time spent in interaction with friends and 2 years later took part in an fMRI study featuring social exclusion. The authors found that greater time spent with friends 2 years previously related to reduced current activity in areas associated with social pain (dorsal anterior cingulate and anterior insula). They

suggested that these findings indicate that social support can lead to reduced social sensitivity, and thus reduced reactivity to social stressors. Of course these findings also suggest the opposite possibility – that social loss or lack of supportive social interaction may promote sensitivity to social pain, with increased activation in areas associated with the experience of these negative feelings.

To summarize, the human neuroimaging work reviewed here indicates that:

- Physical and social pain share common neural substrates, notably in the anterior cingulate cortex and anterior insula. (The fact that some deeper subcortical regions implicated in animal work have not been identified in human fMRI studies is unsurprising, and by no means excludes the possibility that they form part of the SEPARATION-DISTRESS system in humans. fMRI remains better suited to examining cortical than subcortical activations, with very particular imaging sequences being required to access deeper regions with any degree of accuracy. Moreover, although fMRI's spatial resolution is considered good (in contrast to EEG, for example) commonly used preprocessing techniques like spatial smoothing limit its ability to accurately identify BOLD responses in small regions).
- Further supportive evidence of the link between physical and social pain is demonstrated by shared sensitivities to both types of pain.
- Social support is associated with desensitization to social stressors, and with reduced activity in regions known to be associated with response to physical and social pain (anterior cingulate and anterior insula). It is entirely possible that this effect is opioid-mediated; however, this cannot be concluded from the reviewed research. These findings also raise the possibility that adverse social circumstances and lack of social support could have the opposite impact on sensitivity and neural activity in the face of social stressors.

In the following section of the literature review I present a brief overview of the opioid system, and present some human neuroimaging findings that confirm the role of mu-opioids in dysregulated stress and affective responses.

Conceptual Foundations: The Endogenous Opioid System

Current knowledge of the opioid system. Despite a long history of both use and abuse of plant-derived opiates, the existence of endogenous opioid neuropeptides was only established in the 1970's (Stefano et al., 2000). The question of why opiate sensitive receptors existed in the brain at all led to the search for naturally occurring chemistries that interacted with these receptors, and hence to the ongoing investigation and elucidation of the endogenous opioid system. Delineating the full complexity and function of this system remains an active area of investigation.

Three types of G protein-coupled opioid receptors have been identified – mu, delta, and kappa receptors (Gillan & Kosterlitz, 1982; James & Goldstein, 1984; Martin, Eades, Thompson, Huppler & Gilbert, 1976). Although various other potential receptors and receptor subtypes have been considered, the general consensus seems to be that there is insufficient evidence to accept any of these as opioid receptors proper. It should, however, be noted that Opioid-Receptor-Like 1 (ORL-1) is often included in lists of opioid receptors, and that splice variants of mu-opioid receptors have also been identified (Evans, 2004; Henriksen & Willoch, 2008; Pasternak, 2004).

Three families of endogenous ligands were identified by the end of the 1970's – enkephalins, dynorphins, and endorphins. Each derives from a specific precursor protein and has high binding affinity with particular receptor types. The enkephalins methionine and leucine derive from proenkephalin and show high affinity for delta receptors. The dynorphins A, B and neo-endorphin derive from prodynorphin, and bind with high affinity to kappa receptors. The endorphin, beta-endorphin derives from pro-opimelanacortin, and has high affinity for both mu and delta receptors (Drolet et al., 2001; Henriksen & Willoch, 2008; Stefano et al., 2000). Mu-opioid receptors were soon shown to be critically implicated in analgesia and in other key opioid functions (Evans, 2004). Some early researchers thus wondered why no mu-selective ligand had been

found: beta-endorphin displays equal affinity for both mu and delta receptors. This question led to the discovery in the 1990s of two new peptides that demonstrate a very high and selective affinity for mu-opioid receptors – the endomorphins, EM1 and EM2 (Zadina et al., 1999).

Opioid receptor distribution. The opioid system is represented, with varying densities and profiles of receptors, throughout the mammalian nervous system. It is found not only in the central nervous system, but also in the peripheral and autonomic systems. Moreover, it is implicated in a wide spectrum of functions, from regulating autonomic system function, to modulating physical pain, to involvement in processing reward and social emotions. Clearly, both the complexity of its components and the multiple processes in which it is involved indicate that the opioid system cannot be considered a single functional unit (Drolet et al., 2001; Henriksen & Willoch, 2008). A full understanding of the biological advantages conferred by the multiple opioid peptides has yet to be achieved (Evans, 2004).

The broad research program within which my study is nested centers on affective processes mediated by mu-opioid receptors and mu-agonists, hence this brief overview will focus on this component of this complex system. There is a substantial body of animal research, and a growing body of human research, indicating that the mu-opioid system is distributed in, and serves to modulate the function of, brain regions known to be critically involved in affective processing (Galynker et al., 1996; Liberzon et al., 2007; Sanders, Kieffer & Fanselow, 2005; Zubieta et al., 2003).

Although much animal work has been done to map opioid receptors in general, with some focused on mapping mu-opioid receptors, I have been unable to find a single source that sets out specifically to map the distribution of mu-opioid receptors in the human brain. It is not possible to extrapolate without some caution from animal to human systems, as inter-species variability has been demonstrated (Henriksen & Willoch, 2008). However, given the lack of human mapping work, it is worth looking to animal studies for some guidelines on the distribution patterns of receptors.

Animal research has indicated that opioid receptors are represented throughout the brainstem, limbic system, and cortex, although the density of receptor subtypes varies (Le

Merrer, Becker, Befort & Kieffer, 2009). Most sources focus on mapping mu-opioid receptors in deep and limbic brain regions, and give very little detail on their distribution in cortex. In rats, mu-opioid receptors are synthesized in greatest numbers in the thalamus, striatum, locus coeruleus, and the nucleus of the solitary tract (Mansour, Fox, Akil & Watson, 1995). In non-human primates, very high densities of mu-opioid receptors are found in the periaqueductal gray, medial hypothalamic and limbic regions of the brain, including the amygdala and hippocampus (Snyder, 2004). Subcortically, mu-opioid receptors are found in the nucleus accumbens, caudate-putamen, diagonal band of Broca, globus pallidus, ventral pallidum, bed nucleus of the stria terminalis, and nucleus of the solitary tract. They are also found in the ventral rostral medulla (raphe nucleus and nucleus coeruleus), septum, parabrachial complex, and medial preoptic area (Drolet et al., 2001; Panksepp, 1998; Sanders et al., 2005; Stefano et al., 2000). Taking a broad overview, it seems that animal research indicates that mu-opioid receptors are highly represented in brain regions involved in pain, reward, and affective processing.

Most human research has focused on particular questions or specific regions, rather than on mapping per se. Human post-mortem studies have indicated a high concentration of mu-opioid receptors in the prefrontal cortex, cingulate, temporal cortex, basal ganglia, ventral tegmental area, cerebellum, thalamus, and hypothalamus (Kennedy et al., 2006; Maurer, Cortes, Probst & Palacios, 1983; Pfeiffer, Pasi, Mehraein & Herz, 1982). Functional imaging work using SPECT and PET has indicated that administration of mu-agonists leads to increased cerebral blood flow in prefrontal cortex, anterior cingulate cortex, bilateral amygdala, and thalamus (Adler et al., 1997; Jones et al., 1991; Schlaepfer et al., 1998), while a mu-opioid antagonist resulted in reduced blood flow in the anterior cingulate (van Dyck et al., 1994). High densities of mu-opioid receptors are found in brain regions known to participate in emotion, stress, and reward responses – including the thalamus, striatopallidal circuitry, extended amygdala, insula, anterior cingulate and prefrontal cortex. Mu-opioids are thought to regulate the function of these regions and the neurochemistries associated with them (Greenwald et al., 2007; Zubieta et al., 2003).

A brief note on opioid function. Opioids are implicated in human affective responses (Liberzon et al., 2007; Schlaepfer et al., 1998). In general, it seems that agonists for mu and delta receptors produce effects that are analgesic and rewarding, whereas those that act on kappa receptors produce aversive affects such as dysphoria and psychotomimesis. It may be that mu and delta agonists have these positive effects, at least in part, because both increase dopamine release in the nucleus accumbens, whereas kappa agonists decrease dopamine release in this region (Henriksen & Willoch, 2008; Mansour et al., 1995).

Mu-opioids have been shown to be critically implicated in key opioid effects, including analgesia, euphoria, and reward (Evans, 2004; Mansour et al., 1995). This role has been verified by studies using mu-opioid receptor knockout mice, where morphine-induced analgesia and place-preference conditioning do not occur (Gaveriaux-Ruff & Kieffer, 2002). The role of mu-opioids in human affective function will be discussed in some detail below.

Opioid interactions with other neurochemistries. Although it is important to attempt to tease out the role played by any single neurochemical, it is misleading to think of these chemistries as acting independently of each other. Opioids are no exception, and their interactions with other neurotransmitters and neuropeptides are multiple and complex. Given the focus of this study, the interacting chemistries relevant to stress and depression are highlighted below.

Focusing again on mu-opioid effects, this peptide is known to impact on monoamines: it inhibits norepinephrine, and stimulates release of dopamine and serotonin (Mansour et al., 1995). Norepinephrine inhibition occurs via mu-opioid activity in the locus coeruleus, which inhibits neuronal firing in this region and hence norepinephrine release in the cortex. Stimulation of dopamine release occurs via mu-opioid activity in the nigrostriatal and mesolimbic dopamine systems, through inhibition of inhibitory GABA interneurons, which results in increased release of dopamine. (Note that delta agonists also increase striatal dopamine, while kappa agonists have the opposite effect.) Mu-opioid stimulation of serotonin release occurs in the raphe nucleus. Thus, it is evident that mu-opioids can exert a significant effect on neurochemistries already

implicated in depression (more detail on the role of monoamines is provided in the section on depression). Of particular interest here, however, is their interaction with stress-related chemistries.

Of importance with regard to depression is the impact mu-opioids have on corticosteroid release in relation to stress responses. Mu- and delta-opioids impact upon neuroendocrine and autonomic responses to stress stimuli; specifically, they act to reduce or halt the stress response (Drolet et al., 2001). They thus play a critical role in ongoing adaptation to chronic stress. Unsuccessful regulation of stress responses, in which mu-opioids may well be implicated, is thought to result in both physical and affective disorders (see, e.g., Heim & Nemeroff, 1999; Heim et al., 2000; Heim et al., 2002; Heim et al., 2009; Lupien, McEwen, Gunnar & Heim, 2009; McEwen, 2007; McEwen & Stellar, 1993).

A vital consideration is the possibility of permanent dysregulation in the stress and mesolimbic dopamine systems following early trauma. Chronic stress results in changes in both the mu- and delta-opioid systems (Drolet et al., 2001). Critically important recent research illustrates that long-term mu-opioid mediated changes in mesolimbic dopamine pathways may well occur following social stress. Nikulina, Miczek and Hammer (2005) initially showed that ventral tegmental area mu-opioid receptor stimulation in stressed rats led to increased mesolimbic dopamine levels, which in turn led to increased Fos protein expression in the ventral striatum/nucleus accumbens. Moreover, acute social stress led to rapid upregulation of mu-opioid receptors in the ventral tegmental area and periaqueductal gray. A follow-up study showed that these effects are long-lasting (Nikulina, Arrillaga-Romany, Miczek & Hammer, 2008). Following repeated social stress, mu-opioid receptor messenger RNA in the ventral tegmental area doubled, and remained high for 21 days. Fos changes occurred in the nucleus accumbens, striatum and amygdala, and similarly persisted for 21 days. Furthermore, they unequivocally demonstrated the role of ventral tegmental area mu-opioid receptors in this process by stimulating these cells with a mu-opioid receptor agonist several days after the stress manipulation; this stimulation resulted in increased Fos changes in the nucleus accumbens. They also showed that the induction of these protein changes in the nucleus accumbens is eliminated by administration of a mu-opioid

receptor antagonist. Repeated social stress thus led to long lasting mu-opioid-mediated changes in mesolimbic dopamine function. It seems clear that the mu-opioid system may have a critical role in mediating long-term effects of social stress.

In summary, this brief review focused on mu-opioids has indicated that:

- Mu-opioid receptors are found in high concentrations in brain regions involved in pain and affective processing
- Mu-opioids interact with neurochemistries already known to be implicated in depression; viz. the monoamines.
- Mu-opioids play a role in regulating (attenuating) stress responses; and it is known that unsuccessful regulation of stress responses is implicated in the development of physical and affective disorders.
- There is evidence from animal research that early trauma results in permanent dysregulation of the stress and mesolimbic dopamine systems; and specifically that social stress leads to lasting mu-opioid mediated changes in mesolimbic dopamine function.

Critical Evidence from Human Neuroimaging

Mu-opioid involvement in affective dysregulation. A series of studies by Zubieta and colleagues has made a valuable contribution to knowledge of mu-opioid function in relation to affective processing in humans. Using PET imaging, with the mu-opioid selective radiotracer carfentanil, they have illustrated that mu-opioid activity is associated with the regulation of both affective components of physical pain, and of social pain.

This work has shown that mu-opioid activation mediates the experience of pain, and that this may differ across men and women. Activity in the dorsal anterior cingulate, anterior thalamus and ventral basal ganglia plays a key role in suppressing aversive affective perception of pain (Zubieta et al., 2001). Females are known to show higher pain reactivity, and a study by this group demonstrated that hyperalgesia in females was associated with mu-opioid deactivation in the nucleus accumbens (Zubieta et al., 2002).

Given the increased incidence of depression in women, sex differences of this nature may be implicated in this disorder.

Moreover, in the first study to show dynamic changes in a human neurotransmitter system during a sustained emotional state, this research group examined patterns of activity exhibited during sustained sadness versus a sustained neutral state (Zubieta et al., 2003). In the sadness condition, the authors noted a significant deactivation of mu-opioid transmission in key limbic and striatal regions – the rostral anterior cingulate, ventral pallidum, amygdala, and inferior temporal cortex. Deactivation in the ventral pallidum and amygdala was found to correlate with self-reports of increased negative and reduced positive feeling states.

Subsequently, this research group went on to show that mu-opioid emotion regulation circuitry in women with major depression appears to function abnormally (Kennedy et al., 2006). They again used carfentanil in a PET study, and contrasted mu-opioid receptor binding potential in sustained sadness state (vs. neutral) in women with major depression compared to matched controls. They found that, in the normal controls, sustained sadness was associated with deactivation in the rostral anterior cingulate, amygdala, inferior temporal cortex, ventral striatum/nucleus accumbens, and hypothalamus. The rostral anterior cingulate deactivation furthermore correlated with increased reported negative affect. This constituted a replication of their previous findings in normals, and is consistent with the idea that mu-opioids function to regulate and dampen negative affective responses.

Kennedy et al. (2006) also found that, in contrast to psychiatrically healthy women, in women with major depression, sustained sadness was associated with increased activation in the anterior cingulate, amygdala, inferior temporal cortex, nucleus accumbens and hypothalamus. They also observed a correlation between anterior cingulate activation and plasma cortisol levels in the major depression group, and – the argued that this was another indicator that the mu-opioid system is involved in regulating stress responses. Kennedy et al. further examined this response in those who later did and did not respond to a follow-up SSRI intervention. They found that rostral anterior cingulate activation in these groups was different as follows: Those who later responded to the SSRI evidenced reduced rostral anterior cingulate activation patterns, similar to

those seen in the normal controls, whereas those who did not respond to SSRIs showed increased rostral anterior cingulate activation. Thus we see an abnormal mu-opioid response in key limbic and subcortical regions in the depressed subjects. Furthermore, this abnormal response is associated with raised plasma cortisol levels, and with non-response to a SSRI trial.

Given the role of mu-opioids in mediating stress responses, this research group went on to investigate mu-opioid function after trauma, comparing healthy normals to participants exposed to war (Liberzon et al., 2007). The latter comprised two groups – those with and those without post-traumatic stress disorder (PTSD). In this protocol, PET imaging was done at rest, so the findings indicate differences in baseline mu-opioid function, rather than a response to any challenge or cognitive task. They found distinct between-group differences, with both trauma groups showing reduced binding potential in the extended amygdala, insula, nucleus accumbens, and dorsal prefrontal cortex. The PTSD group had lower binding potential than both non-PTSD groups in the anterior cingulate, while the trauma non-PTSD group had lower binding potential than both other groups in the amygdala and higher binding potential in the orbitofrontal cortex.

The authors interpreted these alterations in baseline mu-opioid function as indicating adaptation: they argued that trauma exposure leads to opioid release, with subsequent downregulation of mu-opioid receptors; hence the reduced binding potential in important and highly interconnected areas (extended amygdala, thalamus and dorsal prefrontal cortex) in both trauma groups. In contrast, the orbitofrontal cortex's primary function appears to be inhibitory: It modulates responsiveness in lower limbic regions, with the anterior cingulate perhaps contributing to this modulation. The non-PTSD trauma group demonstrated greater upregulation in the orbitofrontal cortex, and the authors suggest this indicates that failure to fully upregulate mu-opioid activity in this region may be an important characteristic of PTSD. This failure to upregulate is suggested to have permissive effects on mu-opioid activity in lower regions, which in their previous work was shown to be associated with sustained negative mood in women with depression.

An important suggestion has been made by Liberzon et al. (2007): If exposure to trauma in adults results in changes in baseline mu-opioid function, it is entirely possible

that early trauma may lead to changes in the development of the opioid system. This contention is also made by Panksepp based on his animal work. This possibility is a key factor that the current research program seeks to examine.

In summary, the PET imaging work reviewed above has shown that:

- Mu-opioids regulate physical and social pain in humans. There may be a sex difference in this function, which would be of obvious relevance to depression.
- In healthy normal subjects, reduced mu-opioid activity in limbic and striatal areas is associated with increased experience of sadness.
- This pattern is inverted in women with major depressive disorder, suggesting that mu-opioid function is dysregulated in this disorder.
- Changes in baseline mu-opioid function are evident subsequent to exposure to trauma in adult life.
- Seeing altered patterns of mu-opioid activity in these two disorders (depression and PTSD) supports the contention that this neuropeptide is critical in problematic regulation of stress responses and affective modulation.

I now turn to the literature on depression. It is far beyond the scope of this dissertation to provide a comprehensive review of the enormous body of research done in the field of depression over the past five or six decades. Hence, in the section that follows, I will only address the issues that seem most pertinent to the current research question, with some focus on work around the neurobiology of depression.

Conceptual Foundations: Major Depression

Classification and diagnosis. Depression is defined as a disorder of mood, and is most often diagnosed according to criteria laid out in the Diagnostic and Statistical Manual of the American Psychiatric Association, the current edition of which is the DSM-IV-TR (APA, 2000). The DSM-IV-TR recognizes two forms of unipolar

depression – major depressive disorder and dysthymia. Major depressive disorder is diagnosed when the following criteria are met: Sad mood and/or anhedonia present for at least 2 weeks, in addition to four of the following symptoms: changes in sleep, weight or appetite; psychomotor changes; difficulties with decision making; feelings of worthlessness, excessive guilt, or suicidal ideation. Thus, despite being regarded as a disorder of mood, depression impacts on multiple domains of function. Although single episodes of major depression do occasionally occur, it is generally regarded as a chronic, remitting and relapsing disorder (Mayberg, 2004). Dysthymia is a related mood disorder, characterized by longer chronicity alongside a less severe symptom profile. Dysthymia is diagnosed when sad mood and at least two symptoms of major depression (excluding psychomotor agitation and suicidal agitation) are present for at least 2 years. This review will focus on major depressive disorder.

The DSM-IV-TR delineates a number of subtypes of major depressive disorder, which are termed specifiers of the disorder. The first subtype, melancholic, has the longest history, actually predating the term 'depression' itself. In the modern classification system, melancholic depression's presentation profile features marked anhedonia and endogenous low mood (Rush, 2007). This subtype contrasts with atypical depression, where mood is defined as reactive to external events. In addition, marked sensitivity to rejection, and an inverse pattern of vegetative symptoms (i.e., hypersomnia, increased appetite, and weight gain) are said to characterize atypical depression (APA, 2000).

Four other subypes are recognized by the DSM-IV-TR – two very severe forms (major depressive disorder with psychotic features and catatonic major depression), and two subtypes that have specific onsets (postpartum depression and seasonal affective disorder). A further subtype, anxious depression, is often mentioned in the literature but is not part of DSM nosology. In this classification system, a diagnosis of major depressive disorder is often noted as being accompanied by marked anxiety, or a comorbid anxiety disorder is diagnosed (Drevets et al., 2008).

Epidemiology and impact estimates. Major depressive disorder is one of the most common serious psychiatric disorders, causing great cost to affected individuals,

health services and national productivity (Nemeroff, 2007). By 2020, it is projected to be the second leading cause of disability in the developed world (Giacobbe, Mayberg & Lozano, 2009; Kaplan & Harvey, 2009). The National Comorbidity Study conducted in the United States indicated a 12-month prevalence of 5.3%, and a lifetime prevalence of 13.3%, for major depression. Rates are at least twice as high in women than in men (Kessing, 2007; Mayberg, 2004). Rates in South Africa appear to be slightly lower, with a 12-month prevalence of 4.9%, and lifetime prevalence of 9.7% (Tomlinson, Grimsrud, Stein, Williams & Myer, 2009).

Major depression has been estimated to result in moderate role impairment in 28% of cases, and in severe role impairment in 59% of cases. This impairment leads to an inability to work or to carry out normal daily activities for an average of 35 days per annum for each affected individual (Wang et al., 2003). In the South African epidemiology study, 90% of those with depression reported global role impairment (Tomlinson et al., 2009). Major depressive disorder is associated with increased risk of medical disease, including heart disease, stroke, and diabetes, along with increased risk of suicide (Golden et al., 2004; van Melle et al., 2004; Williams, 2005). Because of its high prevalence rates and marked impact on function, major depression ranks as number two in the developed world when calculating disability-adjusted life years (WHO, 2001).

Heterogeneity of major depression. Depression is thus clearly a major health problem. Yet despite decades of research, and ongoing attempts to provide nosological clarity, it seems that the single point of agreement among researchers and clinicians in this field is that the disorder is poorly characterized, and that, as a result, pharmacological treatment options remain unsatisfactory.

To date, major depressive disorder remains a clinical syndrome; this means that it does not meet criteria to be defined as a disease. The latter requires that diagnosis can be made upon determining the presence of a specific etiology and pathophysiology, and that a specific clinical course for the disease is known. Major depressive disorder, in contrast, is marked by heterogeneity of "presentation, genetics, neurobiology, clinical course and treatment responsiveness" (Rush, 2007, p.4). In the face of this heterogeneity, attempting to define subtypes that have unique etiological, symptomatic, and medication sensitivity

profiles seems a sensible route to take, but the current nosological subtypes appear to have, at best, questionable validity.

At the most basic level, the distinction between major depressive disorder and dysthymia is problematic. Long-term studies indicate that in most individuals, depressive symptoms fluctuate over time, meeting criteria for different diagnoses at different times, and ranging from subclinical states to dysthymia to major depressive disorder. It seems clear that there is no categorical distinction between these nosological entities – they simply represent different points on a continuum of symptom severity (Kessing, 2007; Rush, 2007).

Definitive differentiation of subtypes is similarly lacking. Researchers are not convinced that there is empirical support for the two most commonly diagnosed DSM-IV-TR subtypes (melancholic and atypical depression). Some reviewers argue that various studies have found no reliable differences in clinical presentation or outcome for these subtypes (Kessing, 2007; Nandi, Beard & Galea, 2009). In contrast, other reviewers argue strongly for the existence of the melancholic subtype, based on evidence of a specific symptom profile, and the presence of certain biological markers, notably increased cortisol levels (Rush, 2007).

Despite being the most commonly identified subtype, atypical depression is a problematic category. The presence of one of its key characteristics, reactive mood, has not been supported empirically (Kaplan & Harvey, 2009; Nandi et al., 2009). It seems the reverse vegetative symptoms constitute a more reliable differentiator (Matza, Revicki, Davidson & Stewart, 2003; Seemuller et al., 2008; Thase, 2007). The attempt to define subtypes based on symptom profiles has thus not been particularly successful.

Another problem with the current nosological system is that it views anxiety as belonging to a separate set of disorders. However, around half of all depression cases feature marked anxiety, and this feature is associated with greater severity and increased suicidal ideation (Rush, 2007). The current nosology thus excludes an important symptom of the disorder.

Several reviewers concur that it may be incorrect to conceptualize major depression as a homogenous disorder with a single underlying pathophysiology (Nandi et al., 2009; Nemeroff, 2007; Wijeratne & Sachdev, 2008). Most authors highlight the need

to determine the etiology of depression. Such a step would enable a far more reliable diagnostic system that would include subtyping based on distinct pathophysiological mechanisms (Drevets et al., 2008; Kessing, 2007). However, currently there is little consensus in the literature as to what these mechanisms may be.

Indeterminate etiology of major depression. There is as yet no clarity on the biophysiological mechanisms of depression. The once-promising monoamine hypothesis (Shildkraut, 1965) cannot fully account for the spectrum of the disorder, and some regard it as having impeded progress in the attempt to elucidate the biological underpinnings of depression (Norman, 2006).

The monoamine hypothesis originated in the 1950s, when it became evident that medications that provided relief from depressive symptoms acted on the monoamine neurotransmitter systems, particularly the serotonin or norepinephrine systems, by increasing their synaptic availability. Investigation of these neurotransmitters has remained at the forefront of psychopharmacological research for five decades, with the successful development of selective serotonin reuptake inhibitors (SSRIs) only serving to strengthen this focus (Lee, Jeong, Kwak & Park, 2010; Lopez-Munoz & Alamo, 2009; Mayberg, 2004). Relatively recently, profound problems with this model have become increasingly evident. The idea that dysregulation in these specific systems is of fundamental significance is not tenable for the following reasons: 1) years of research have failed to demonstrate that depression results from any specific monoamine dysfunction or that monoamine depletion can produce depression in normal people, and 2) certain drugs that provide relief from depression do not act in accordance with the hypothesis, acting either to reduce monoamine availability, or acting on other chemistries entirely (Mayberg, 2004; Watt & Panksepp, 2009).

Research into the biological basis of depression has continued to investigate the monoamine neurochemistries, but has also expanded to include investigation of many other chemistries and peptides, as well as the examination of genetics and intracellular mechanisms, and structural and functional correlates of depression. To date, no definite pathogenesis has been defined, and preclinical markers have yet to be established (Drevets et al., 2008; Giacobbe et al., 2009; Lee et al., 2010). In the sections to follow I

will give a very brief indication of the current breadth of investigation in these enormously complex arenas.

Structural and functional neuroanatomical markers. In unipolar depression, no reliable gross structural abnormalities have been established. Postmortem studies have indicated that glial loss occurs in depression, but that this loss appears to take place in various distributed areas; no critical region has been defined (Bowley, Drevets, Ongur & Price, 2002; Drevets et al., 2008; Mayberg, 2009; Ongur, Drevets & Price, 1998). Structural neuroimaging studies have indicated the presence of volumetric changes in various frontal, limbic, paralimbic and cortico-striatal structures. Various regions of the cingulate gyrus (most often, but not always, anterior) have been implicated. Temporal areas including the superior temporal gyrus, temporal poles, hippocampus, parahippocampal gyrus and amygdala have been listed. Key subcortical regions include the ventromedial striatum and thalamic nuclei. Reviewers agree that a great deal of variability is present in this literature, both in terms of the types of changes seen, and in the precise regions implicated (Drevets et al., 2008; Mayberg, 2009; Pizzagalli et al., 2004; Sheline, 2003).

Functional changes (in blood flow and glucose metabolism) have also been noted in many of these, and other, areas (Baxter et al., 1985; Drevets et al., 2008; Drevets et al., 1992; Fitzgerald, Laird, Maller & Daskalakis, 2008; Mayberg, 2003). Several researchers argue that depression is characterized by reduced frontal activity alongside increased limbic and subcortical activity, indicating a failure of emotion regulation (Dannlowski et al., 2009; Drevets et al., 2008; Giacobbe et al., 2009; Johnstone, van Reekum, Urry, Kalin & Davidson, 2007; Mayberg, 2004). Difficulties with this model, however, stem from the fact that the literature is not consistent: although hypoactive frontal regions along with hyperactive limbic and subcortical regions are often shown, many studies provide contradictory evidence – for example, of normal, or hyperactive states in frontal cortex, and reduced activity in the limbic or subcortical regions. The possible reasons for this variability in the literature are multiple and include: 1) functional imaging data is impacted by structural volume loss, but most studies do not correct for this (Drevets et al., 2008); and 2) despite the general consensus that great heterogeneity is

present in depression, much work in the field includes cases with major depression as though they constitute a homogenous group; thus participants are likely to have very different clinical symptom profiles, which may reflect differing underlying mechanisms. This heterogeneity may thus also serve to confuse matters (Mayberg, 2009). As yet, there is no consensus regarding the reliability of these observed structural and functional changes, and thus deriving an account of what they may mean is problematic.

A more robust set of observations indicates that resting state activity is abnormal in depression (Greicius et al., 2007; Grimm et al., 2009; Sheline et al., 2009). These observations have led to the generation of a complex model of depression as a systems-level disorder, which impacts at multiple anatomical and functional levels (Northoff, Wiebking, Feinberg & Panksepp, in press). The abnormal patterns of resting-state activation in depression feature hyperactivity in medial, limbic regions and hypoactivity in lateral cortical regions. This model emphasizes the fact that medial regions are implicated in core emotions and in self-representation, whereas lateral cortical regions deal primarily with processing and reacting to external stimuli. The resting-state imbalance is thought to allow the medial, primitive emotion systems to derail higher cortical emotional and cognitive functions. This model is very promising in that it presents details of nested functional neuroanatomical hierarchies that account for how the systems interact, and how multiple domains of function may be impacted.

Neurochemistries implicated in depression. Changes in multiple neurochemistries have been documented in depression, including the monoamine systems, and the cholinergic, glutamatergic, GABA-ergic, and glucocorticoid chemistries (Drevets et al., 2008; Mayberg, 2004; Mineur & Picciotto, 2010). Many neuropeptides and hormones are currently being investigated, including, for example, galanin, substance p, neuropeptides s and y, argnine vasopressin, melatonin, oxytocin and the opioid system (Alldredge, 2010; Bourin & Prica, 2009; Machado-Vieira, Salvadore, Ibrahim, Diaz-Granados & Zarate, 2009; Parker et al., 2010). The possible role of opioids will be discussed in more detail in a later section. Here I will focus briefly on the more widely investigated substances – the monoamines and stress-related chemistries.

Classic monoaminergic models of depression centered on norepinephrine or serotonin. Although there is a well-established correlation between changes in the norepinephrine system and depression, it has not been possible to show a direct causal role for norepinephrine in depression (see, e.g., Charney, 1998; Delgado & Moreno, 2000). Similarly, although decreased serotonin function is often present in depression, this is not invariable (for a recent review, see Jans, Riedell, Markus & Blokland, 2007). More recent monoaminergic theories of depression propose that all three monoamines are involved, and that dysfunction is present in brain regions modulated by these chemistries (for example, frontal cortex, hippocampus, amygdala, and basal ganglia). In such models, decreased motivation is thought to result from dysregulation in the norepinephrine and dopamine systems, while increased anxiety and irritability may be due to dysregulation in the norepinephrine and serotonin systems (Mayberg, 2004). Although these amines clearly are implicated in depression, precisely what their role is remains unclear (see Watt & Panksepp, 2009, for a detailed review of this literature).

A key problem with regarding norepinephrine or serotonin as primary causative factors in depression is the delay in response to treatment targeting these chemistries: on average a delay of 3-6 weeks is present before therapeutic effects are seen. This delay strongly suggests that these drugs exert indirect effects: that is, antidepressive effects are not due to direct manipulation of the monoamines themselves (Lopez-Munoz & Alamo, 2009). This line of thinking has led to investigations of downstream molecular events, such as impact on second-messenger systems and gene expression, to account for the observed antidepressant effects. Some of this research will be discussed below in the section on genetics.

Recently, investigations focused on the dopamine system have increased. The link between this system and anhedonia seems intuitively obvious, and altered dopaminergic function in the ventral tegmental area and nucleus accumbens has been found in depression (Nestler & Carlezon, 2006). Furthermore, medications that target this system have been found to relieve some symptoms of depression. For example, methylphenidate (Ritalin) has been shown to improve mood and drive (Mayberg, 2004), while the dopamine reuptake inhibitor Bupropion has been found to be effective in treating some cases (Watt & Panksepp, 2009). Reduced dopamine activity is now widely

considered to be a key feature of the amotivational and anhedonic state that is a major characteristic of depression (Lee et al., 2010; Malhi & Berk, 2007; Zellner et al., in press).

One currently influential line of thinking sees depression as a stress-related illness. Not only does a stressful life event often act as the trigger for (at least) the first episode of depression, it seems that changes in corticotrophin releasing factor and glucocorticoid signaling may be a key factor in depression (de Kloet et al., 2005; Holsboer, 2000). Corticotrophin releasing factor (CRF) modulates hypothalamic-pituitary-adrenal axis (HPA) stress responses. Dysregulated CRF function, chronically elevated cortisol levels and altered glucocorticoid signaling are often evident in depression (Burke, Davis, Ottec & Mohra, 2005; Maier & Watkins, 2005; Rush et al., 1996). Immune responses may also be implicated here: some evidence indicates that cytokines impact on glucocorticoid receptors, resulting in ineffective signalling (Pace & Miller, 2009).

The role of chronic stress and the resultant complex physiological responses is clearly important in major depression. For the current investigation, the impact of early social separation-distress or social trauma is of primary concern. As reviewed above, early social trauma in the form of separation-distress strongly arouses the HPA axis, and can cause long-term alterations in the functioning of this system, with permanent dysregulation of CRF. These events may result in a lifelong vulnerability to depressive episodes (Heim & Nemeroff, 1999; Levine, 2001, 2005; Rosenfeld, Suchecki & Levin, 1992; this literature will be discussed in more detail below).

Genetic factors in depression. Research into the role of genetics appears to be yielding similarly complex results. Studies of heritability indicate a strong genetic component in vulnerability to unipolar depression (see Sullivan, Neale & Kendler, 2000, for a review of this literature). Work attempting to identify which particular gene may be implicated has not been successful, however. The initial optimism around a pivotal role for the serotonin transporter (5-HTT) has not been maintained. Early research indicated that the short variant of this gene was strongly associated with neuroticism and with greater susceptibility to depression (Caspi et al., 2003; Kaufman et al., 2004; Ogilvie et

al., 1996). However, later findings have been mixed, with meta-analyses failing to substantiate the association of this gene variant with depression, possibly due to small effect sizes (Canli & Lesch, 2007; Gillespie, Whitfield, Williams, Heath & Martin, 2005; Risch et al., 2009). It seems that although genetic factors are clearly implicated in the risk for developing major depression, the search for a single candidate gene has been unsuccessful (Lee et al., 2010; Shyn & Hamilton, 2010). It appears that multiple genes may be involved, each having only a small effect; neurotrophic, glutamatergic, cholinergic, serotonergic, and intracellular signaling pathways all appear to be implicated (Drevets et al., 2008).

Some genetic research supports a neurogenesis hypothesis. CRF dysregulation and chronically elevated cortisol levels are thought to lead to neuronal atrophy, particularly in the hippocampus (Bremner et al., 2000; Dranovsky & Hen, 2006). The neurogenesis hypothesis states that antidepressant treatments, including monoaminergic drugs and electroconvulsive therapy, achieve their effects by promoting cell growth. Monoaminergic therapy may achieve its effects by causing multiple changes in intracellular messengers (for instance G proteins and cyclic AMP), leading to modified gene expression, which in turn leads to synthesis of certain critical substances, like proenkephalin and brain-derived neurotrophic factor (BDNF; Lopez-Munoz & Alamo, 2009; Martinowich & Lu, 2008). Thus antidepressant therapy, through indirect effects, promotes neurogenesis and the synthesis of neurotrophins (Jacobs, Praag & Gage, 2000; Lee et al., 2010; Norman, 2006; Savitz, Lucki & Drevets, 2009; Valdez, 2009).

Precisely how neurogenesis leads to improvement in depressive symptoms has yet to be established, however. For example, although upregulation of BDNF clearly accompanies improvement, BDNF itself does not directly modulate mood (Groves, 2007; Martinowich, Manji & Lu, 2007). The impact of opioid synthesis has yet to be elucidated.

Inefficacy of current pharmacological treatment for major depression. A wide variety of antidepressant medications, most targeting the monoamine systems, are currently the first choice in treating depression (Norman, 2006). As will be discussed below, the efficacy of these medications remains unsatisfactory.

In the 1950s, tricyclic antidepressants and monoamine oxydase inhibitors were developed, and remained standard treatment for depression until the 1980s. Tricyclic antidepressants act to inhibit the uptake of serotonin or norepinephrine, but also have anticholinergic and antihistamine effects. They thus have a poor side-effect profile, poor tolerability, and notable safety concerns (Roose, 2003). They are no longer considered the first choice for initial treatment, but are broadly considered to be useful for treatment of severe melancholic or treatment resistant depression.[3] Early monoamine oxydase inhibitors also have problematic tolerance and safety concerns, and are now used mainly for treating atypical or treatment resistant depression. Newer reversible monoamine oxydase inhibitors appear to have a better safety profile. Selective serotonin reuptake inhibitors (SSRIs) are currently the first choice in treating depression. They are widely regarded as effective, and have far better tolerability and safety profiles than the early medications (Nemeroff, 2007).

However, many researchers and clinicians remain unsatisfied with the efficacy of available treatment, as it is known that a substantial proportion of cases do not respond to standard interventions. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study was conducted to assess the efficacy of first-choice and standard alternatives in treating major depression. This constituted a 7-year prospective randomized controlled clinical trial, conducted at multiple sites in the United States, including primary health care and psychiatric facilities, with a total of 3671 outpatients enrolled (Warden et al., 2007). The results of this study confirmed that current medical treatment options are not particularly effective.

The STAR*D study found no indication that any particular antidepressant medication was better than any other in treating major depression (Norman, 2006; Rush et al., 2006b). Moreover, contrary to what was commonly believed, the newer SSRIs were not shown to have greater efficacy than older medications (Wijeratne & Sachdev, 2008). It was also found that when initial treatment trials were not successful, conventional alternatives – for example augmenting antidepressant medication, using older generation antidepressants, or using ECT – simply were not as effective as had been

[3] Treatment resistant depression is usually defined when a case does not respond to a minimum of two trials of different antidepressant medications (Nemeroff, 2007).

thought. The standard strategy of sequential trials of medication was shown to yield diminishing results (Giacobbe et al., 2009; Wijeratne & Sachdev, 2008).

Only 28% of the STAR*D sample achieved remission of symptoms following a single trial of SSRIs. Sixty percent of cases required all four sequenced levels of treatment to achieve remission, and cases requiring multiple treatment trials were shown to have higher relapse rates: of those who required the 4th level of treatment, 50% relapsed within 4 months (Little, 2009; Rush et al., 2006a; Warden et al., 2007).

Researchers in the field were alarmed by these findings: "The results of STAR*D are as sobering as they are unequivocal. The results highlight that currently available antidepressant medications and the dominant paradigm of the pathophysiology of depression as a deficiency of monoaminergic transmission, have major limitations" (Giacobbe et al., 2009, p. 45). In sum, standard first choice treatments do not work for most cases, and relapse occurs at alarmingly high rates.

Treatment resistant depression is thus clearly not uncommon. However, attempting to identify which cases are likely to be refractory is also not simple, as treatment resistance is not synonymous with severity, nor is it associated with any particular subtype in the current nosology (Nemeroff, 2007; Wijeratne & Sachdev, 2008).

Invasive techniques may be used in the attempt to obtain relief for patients with treatment resistant depression. Electroconvulsive therapy (ECT), despite its known side-effects, is often used when depression is refractory. Cognitive deficits such as memory loss and reduced attention occur subsequent to ECT, although these are often argued to be temporary. It seems, however, that an inverse relationship exists between treatment efficacy and the severity of the cognitive deficits (Little, 2009). Furthermore, ECT's efficacy is not long-lasting, with relapse appearing to be the rule. A double-blind placebo controlled trial showed that almost all (N = 84) patients relapsed within 6 months of ceasing ECT (Nemeroff, 2007).

Newer invasive treatments have been developed, but their long-term efficacies have yet to be established. For instance, Vagal Nerve Stimulation is approved for use in severe or refractory cases. It involves electrical stimulation of the vagus nerve, requiring surgery and permanent placement of a stimulator, usually under the skin of the chest. Repetitive Transcranial Magnetic Stimulation (rTMS) involves repetitive intense

magnetic stimulation of 1 Tesla being applied to areas of cortex (Little, 2009; Nemeroff, 2007; Wijeratne & Sachdev, 2008). Deep brain stimulation, especially targeting particular regions beneath the anterior cingulate shows promise (Giacobbe et al., 2009; Mayberg, 2009). Obviously, improved medication treatment options would be preferable to these invasive methods, and a great deal of very recent work has focused on investigating alternative neurochemical and hormonal targets. Opioids, however, have not received much attention.

In conclusion, although the move to look beyond the monoamine hypothesis of depression must be regarded as progression, the field is not yet any closer to a definitive understanding of the disorder. It is now confronted with fragmented neurobiological correlates, and the task of attempting to determine whether unipolar depression comprises a set of relatively distinct disorders, or whether it is a systems level disease that impacts variably at multiple levels (Lee et al., 2010; Northoff et al., in press). It is clear that neurochemistries involved in responses to stress play a key role in this complex picture. The contention of the broad research program within which this current study is nested is that opioids are also critically implicated in these responses. Hence the investigation of the effects of buprenorphine, a partial mu-opioid agonist, is required.

Opioids and Depression

The use of opiates in treating mental disorders has a long history, starting with the ancient civilizations of Sumeria, Persia, and Egypt (Stefano et al., 2000). Throughout the nineteenth century, opiates were the first choice in treating melancholia, recommended by well-regarded physicians such as Emil Kraepelin (Carlson & Simpson, 1963; Emrich et al., 1982). Until as late as the 1950s their use was recommended in psychiatric textbooks (Mayer-Gross, Slater & Roth, 1956). Around this time, however, ECT and monoamine therapy replaced opiates as the medical treatments of choice for depression, to some extent simply because they did not have the same addiction risks (Bodkin et al., 1995). Thus despite their apparent efficacy in treating depression, their high addiction liability has, in the past, mitigated against empirical investigation of their therapeutic effects.

It therefore seems well worth investigating the therapeutic potential of new generation synthetic opioids, buprenorphine in particular, for treatment of depression. Buprenorphine has pharmacological properties that make it far safer than traditional opiates. It is a mixed agonist-antagonist, acting as a partial agonist at mu-receptors, and as an antagonist at kappa-receptors. It is an exponentially more powerful analgesic than morphine (Cowan, Lewis & Macfarlane, 1977; Jasinski, Pevnick & Griffith, 1978), and yet is safe even in overdosage. This is due to it being a *partial* mu-agonist: at high doses it acts as an *antagonist* at mu-receptors, thus the respiratory suppression associated with high doses of full mu-agonists does not occur (Galynker et al., 1996).

Buprenorphine has limited addiction liability (Stefano et al., 2000). Rather than the euphoria associated with opiates such as heroin and opium, buprenorphine has been shown to have only modest mood elevating effects in humans, which tend to decline as dose increases. Most subjective effects plateau at a relatively low dose (Ciraulo et al., 2006; Galynker et al., 1996; McAleer et al., 2003; Zacny, Conley & Galinkin, 1997). These ceiling effects on typical opiate effects (euphoria and sedation) increase buprenorphine's safety and reduce its abuse risk (Walsh, Preston, Bigelow & Stitzer, 1995). It is also important to note that in studies using opiate-naïve individuals, subjects show high side effects on low doses, including vomiting and incapacitation. It is argued that these aversive effects should also contribute to reducing addiction liability (Zacny et al., 1997).

Some researchers are not convinced that buprenorphine has a low addiction risk, however. For example, Evans (2004) argued that buprenorphine usage may result in upregulation of mu-opioid receptors, which may lead to increased tolerance of the drug. However, Sittl, Nuitjen and Nautrap (2006) examined escalating doses of buprenorphine and fentanyl used for analgesic purposes in patients with severe pain. Previous clinical reports on use of buprenorphine for pain management had suggested that increasing dose was not required, and this study confirmed that fact. The researchers found that, unlike fentanyl, buprenorphine doses remained stable over time, indicating that patients did not develop tolerance to the medication, and that its analgesic effects continued to be effective at the same dose. These findings argue against concerns regarding high addiction liability.

Buprenorphine and depression. Buprenorphine is widely used as the primary treatment for opiate dependence, and is known to be safe for use in this context (Johnson, Jaffe & Fudala, 1992; Johnson, Strain & Amass, 2003; Ling & Wesson, 2003; Mello & Mendelson, 1980; Resnick et al., 1992). Importantly, studies indicate that in recovering addicts its use is associated with substantial improvements in comorbid depressive symptoms (Kosten, Morgan & Kosten, 1990; Nunes & Levin, 2004). Moreover, preliminary clinical data support the value of buprenorphine in treating certain cases of refractory major depression.

Emrich et al. (1982) conducted the first published study to investigate the efficacy of buprenorphine in treating depression. Their sample consisted of ten psychiatric inpatients with major depression, most of whom had not responded to standard treatments. They used a double-blind, placebo-controlled design, with a low dose of buprenorphine (0.2mg p/day), and demonstrated significant improvement in depressive symptoms over 5-8 days. Another small, placebo-controlled study with non-dependent depressed psychiatric patients ($N = 8$) indicated marked improvement in mood and behavior in 75% of the sample (Mongan & Calloway, 1990). Bodkin et al. (1995) used buprenorphine to treat 10 psychiatric inpatients with refractory depression over a period of 6-8 weeks (with a maximum daily dose of 1.8mg). Three patients could not tolerate the medication and treatment was discontinued. One of the remaining cases deteriorated. However, two cases showed moderate improvement, and four achieved total remission of symptoms. These small studies indicate that for some treatment resistant patients, buprenorphine may be able to provide relief. Systematic investigation of this drug's efficacy is therefore indicated, perhaps particularly in cases of depression following childhood social trauma, as is discussed below.

Childhood Trauma and Depression.

This research program is based on the idea that early social trauma leads to vulnerability to depression at least in part because of mu-opioid dysregulation. A critical study (Nemeroff et al., 2003) indicates that exposure to early childhood social trauma may be a factor in poor response to pharmacological treatment of depression. The investigators examined data from 681 cases with chronic forms of major depression,

assessing the efficacy of psychotherapy, medication or combination therapy. They found that the results differed markedly once the cases were stratified according to whether or not the depressed individuals had experienced early childhood trauma (defined as loss of parents, physical or sexual abuse, or neglect). Those with a history of such trauma were significantly less responsive to antidepressant medication (the medication used was nefadozone, which acts to inhibit reuptake of serotonin, norepinephrine and dopamine). In these cases, targeting the monoamines did not seem to work. Investigation of the efficacy of opioid medication for depression following early social trauma is thus necessary.

There is a well-established literature that clearly shows a strong association between early life difficulties and later depression (see, e.g., Bowlby, 1980; Heim & Nemeroff, 1999; Heim et al., 2009; Kendler, Thornton & Gardner, 2001; Reite, Short, Seiler & Pauley, 1981; Slavich et al., 2010). Space prohibits a full review of this substantial literature, but in brief the key points are as follows: Classic attachment theory predicts that bonding experiences with the primary caregiver in the first 2 years of life set a template for the individual's sense of self, and for all future relationships (Bowlby, 1969). The implications for those with problematic attachment experiences are clear; even though other bonds and social attachment experiences also exert important influences across development (Flinn & Leone, 2006). A great deal of research supports the critical role played by aversive or disrupted early social relational experiences in later psychopathology (see, e.g., Flinn et al., 2009; Heim et al., 2000; Heim et al., 2002; Heim et al., 2010; Zlotnick, Mattia & Zimmerman, 2001; Zlotnick, Warshaw, Shea & Keller, 1997).

Evidence of this association from prospective studies is particulary compelling. For example, in a study examining 676 children with confirmed abuse or neglect prior to the age of 11 years, researchers found a significantly increased risk for depression in adulthood, and earlier onset of depression, in these individuals compared to controls (Widom, DuMont & Czaja, 2007). Research by Flinn and colleagues has illustrated the particular role played by social, as opposed to other severe stressors. They showed that at age 11, children in a Dominican village who had been exposed to equivalently severe stressors showed a different long-term response depending on the type of stressor: Those

who had been exposed to a serious hurricane or political upheaveal in early childhood did not have elevated cortisol levels, whereas cortisol levels were elevated in those who had experienced early family trauma (defined as parental conflict, parental loss, or abuse; Flinn, Nepomnaschy, Muehlenbein & Ponzi, 2011).

It seems evident that severe and chronic early stress is pathological (Anisman et al., 1998; Chrousos, 1998, 2009; Seckl & Meaney, 2004). The impact of early stressors on the HPA axis and stress system has been clearly demonstrated (Champagne, 2010; Flinn et al., 2011; Glover, O'Connor & O'Donnell, 2010; Levine, 2001, 2005), and animal research on this relationship has been reviewed above. In humans, early childhood appears to be a critical developmental period for the HPA system. Early trauma, which includes problematic social attachment experiences, leads to permanent dysregulation of the HPA system, and to elevated levels of CRF and cortisol (Champagne, 2010; Lupien et al., 2009; Mirescu, Peters & Gould, 2004; Nepomnaschy & Flinn, 2009). In the stress literature there is also evidence that the HPA axis is particularly sensitive to social challenges during development (Flinn & Leone, 2006; Flinn et al., 2009; Hennessey, Kaiser & Sachser, 2009; Yim, Quas, Cahill & Haykawa, 2010).

The links between early stress and dysregulated stress responsivity later in life are well-established in the literature, but investigation of the role of the opioid system in these stress responses is lacking. The link between the specific stress of early social trauma and long-term opioid functioning is of central interest here. The broader thesis under investigation is that stressors that disrupt early social bonding (i.e. that activate the separation-distress system, which elicits a strong stress response) may have a particular role in the genesis of depression, and that opioid dysregulation is centrally implicated in this trajectory.

Specific Aims and Rationale

The literature review has indicated that a satisfactory explanatory model for depression has yet to be established. The disorder is heterogenous, manifesting in a variety of clinical presentations, alongside variable responsivity to currently available pharmacotherapeutic options. Of significant concern is the substantial number of patients

who are unable to obtain symptom relief. Investigating the possible therapeutic effects of a new generation opioid with low addiction liability thus seems warranted. My intention is that this investigation should not simply test the efficacy of the medication, but should aim to elucidate both the neurobiological and emotional mechanisms of its actions. As stated at the start of the Literature Review, I would like to determine not only whether, but also how and why this opioid may provide effective treatment for some cases of depression.

For this reason, the current study is grounded in a theory of emotion that views social attachment and social loss as primary emotional states, both of which are mediated by mu-opioids. The pain of social loss across the lifespan is viewed as an extension of the primary separation-distress response evinced by all infant mammals; a distress response as powerful and as critical as that elicited by threats to physical integrity. Support for this model comes from evidence that social and physical pain systems overlap, both in terms of neuroanatomical substrates, and in neurochemical mediation by mu-opioids. The broad thesis under investigation here is that early experiences involving the distress of social loss create a lifelong vulnerability to depression, because they result in long-term neurochemical dysregulation, in which the mu-opioid system is implicated.

In humans, early adverse psychosocial experiences have a clear link to adverse outcomes in adulthood, in which mood and anxiety disorders feature frequently. This trajectory has been mapped out to some extent at a neurobiological level, in terms of alterations in HPA function. Both animal models and research in humans indicate that early stress results in dysregulated CRF function and in dysregulated glucocorticoid signaling. The contention under examination in the broader research project, within which the current study is nested, is that the opioid system is critically involved in this developmental trajectory.

Given the evidence that neurochemistries with which mu-opioids are strongly interactive (notably, stress neurochemistries, and the mesolimbic dopamine system) show long term changes following early social trauma, and that neuroanatomical changes in areas high in mu-opioid receptors are also evident, it seems reasonable to hypothesize that early social trauma will also lead to permanent dysregulation in this system. Previous studies have shown that mu-opioid function may be dysregulated in depression

and in PTSD, but to date, no-one has directly examined the link between early social trauma and mu-opioid dysfunction. This is precisely what the current study set out to do.

Of course, the question of what exactly constitutes early social trauma is difficult to answer definitively. This study used a very broad definition, based on indications in the literature that a range of aversive social experiences can be pathogenic. It seems that various kinds of ill-treatment in childhood, including sexual, physical and emotional abuse, as well as physical and emotional neglect, can result in long term HPA alterations, and in depression (Bowlby, 1969, 1980; Champagne, 2010; Flinn et al., 2009; Heim et al., 2000; Heim et al., 2002; Kendler, Gardner & Prescott, 2002; Mayberg, 2004; McGowan et al., 2009; Slavich et al., 2010; Teicher, Andersen, Polcari, Anderson & Navalta, 2002; Teicher et al., 2003; Teicher, Samson, Polcari & McGreenery, 2006). It is important to note that all these forms of abuse and neglect involve close social relationships, most often within the immediate family. The trauma focus here is social, and concerns disrupted early experiences of attachment and psychosocial relatedness.

According to the overarching thesis, exposure to early social trauma should result in long-term changes to the mu-opioid system. The aim in the present study was to examine the impact of an opioid manipulation in healthy normal individuals. This choice of participants may seem counterintuitive, as the ultimate population of interest is those with depression. However, given the current lack of nosological clarity, the heterogeneity of presentations, and the multitude of neurobiological correlates identified in major depressive disorder, it seemed premature to include cases of depression at this point, as they would most likely cloud the data. If the overarching thesis is true, even psychiatrically healthy individuals who were exposed to early social trauma should show some indications of altered opioid function. Being able to examine this possibility 'cleanly', without the added complexities and possible confounding factors associated with depression, seemed the best option for a first step in this research program.

A further consideration is what exactly is meant by 'opioid dysregulation'. At this point of outset in the research program, it was not entirely clear what form(s) this dysregulation would take. For example, the Kennedy et al. (2006) study showed dysregulated mu-opioid activity in depression, but this took the form of *increased* activity during sadnesss. Given the activity patterns in normals (*reduced* activity during sadness)

one might have expected to see even greater decreases in this population, given their propensity to sad mood. The form of the demonstrated opioid dysregulation was thus unexpected.

The most important goal at this initial stage of the overarching research program was to provide some proof of concept – to demonstrate differences in opioid function between individuals who had and had not been exposed to early social trauma. Thus the predictions made below are heuristic rather than definitive, based on best guesses given what is known about affect and cognitive style in depression. Of greatest interest is whether or not between-group differences in the response to the opioid manipulation can be shown – this is the most critical test the data must pass.

The aim of this study was thus to examine the effects of a once-off low dose opioid manipulation in healthy normal subjects with and without a history of exposure to early social trauma. Information regarding changes in affective experience, social cognition, and neural activation was obtained. The following specific questions were asked, and heuristic predictions were made:

1) Do the groups of healthy normals differ on key baseline characteristics?

Depression: The groups will not differ significantly on depression scores, as only psychiatrically healthy individuals were included.

Hypothesis 1: trauma = control on depression scores

General experience of affect: The groups will differ on general experience of affect. Although only individuals who were not depressed were included, I thought it possible that in general, the group exposed to early social trauma might experience more negative affect, and less positive affect.

Hypothesis 2: trauma > control on negative affect scores
Hypothesis 3: trauma < control on positive affect scores

Personality traits based on core emotion systems: The groups will differ on personality traits related to key core emotion systems as follows:

Hypothesis 4: trauma > control on SADNESS
Hypothesis 5: trauma < control on SEEKING

2) Do the groups differ in their response to opioid manipulation in terms of their subjective experience of affect?

Current experience of affect: The groups will demonstrate the same pattern of affect on placebo as that predicted for general experience of affect; that is, the control group will report greater positive affect, and the trauma group will report greater negative affect. Furthermore, the groups will differ in their response to the opioid buprenorphine, in that the trauma group will respond more strongly to the medication, showing decreased negative affect, and increased positive affect.

Hypothesis 6: On placebo, trauma > control group on negative affect scores
Hypothesis 7: On placebo, trauma < control on positive affect scores
Hypothesis 8: On medication, trauma = control on negative affect scores
Hypothesis 9: On medication, trauma = control group on positive affect scores

3) Do the groups differ in their response to opioid manipulation in terms of social cognition?

Biased judgment of emotional expressions: The groups will differ on placebo, in that a negativity bias will be present in the trauma-exposed participants. These participants will be more likely than controls to judge neutral faces as showing negative emotions (anger, sadness and fear). The opioid buprenorphine will reduce this bias.

Hypothesis 10: On placebo, trauma > control in judging neutral faces as showing negative emotions

Hypothesis 11: On medication, trauma = control in judging neutral faces as showing negative emotions

4) Do the groups differ in their response to opioid manipulation in terms of neural response to social-emotional stimuli?

Hypothesis 12: For all participants, response to negative social signals (faces showing anger and fear) will be reduced on medication compared to placebo.

Hypothesis 13: This effect will be significantly greater for the trauma group than for the control group.

Methods

Research Design

The work reported here constituted a double-blind, placebo-controlled crossover design. It incorporated two groups of healthy individuals – those exposed and those not exposed to early social trauma. None of the participants met diagnostic criteria for any major psychiatric disorder, including depression and anxiety disorders. All participants were assessed both on and off the opioid medication, buprenorphine. The order of administration of buprenorphine/placebo was counterbalanced across participants, as the small sample size did not permit effective randomization. Both the participants and the researchers involved in data collection were blind to medication condition and to trauma status.

Procedure

Recruitment was initiated by placing poster advertisements across the various campuses at the University of Cape Town, and continued for a period of 24 months (09/2008 - 08/2010), with participants at various stages of the research protocol throughout. The stages of the protocol are set out in the following figures: Figure 3 provides an outline of the sequential stages of the protocol and Figure 4 gives details of the full protocol (i.e. of the larger study within which this study is nested). This report focuses on a subset of measures from the full protocol; these are listed in Table 1.

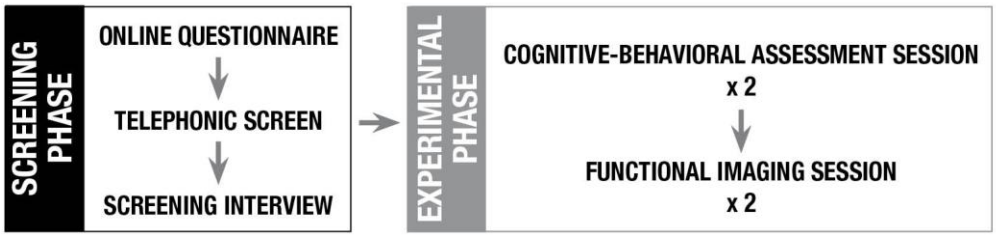

Figure 3:
Sequential stages of protocol.

Initially, potential participants were asked to complete an online screening battery, that included the Childhood Trauma Questionnaire – Short Form (CTQ-SF; Bernstein et al., 2003). Thereafter, individuals who had completed the online screen were contacted telephonically. Those who were excluded at this stage were informed, while those who met preliminary inclusion criteria were asked to provide verbal consent to participate, and further screening information was obtained from them (see Appendix A). At the end of the telephonic screen, those who met inclusion criteria were asked to come in for a full screening interview. At that session, they completed the CTQ-SF for the second time, and the The Mini-International Neuropsychiatric Interview (MINI; Lecrubier et al., 1997; Sheehan et al., 1997) and Beck Depression Inventory-Second Edition (BDI-II ; Beck, Steer & Brown, 1996) were administered.

Again at this stage a number of individuals were excluded from further participation (see Figure 5 for details of inclusion/exclusion of participants). Those who continued first completed two cognitive-behavioral assessment sessions, then two functional imaging sessions. In the cognitive-behavioral sessions, a task assessing subliminal bias in attributing emotion was administered. In the functional imaging sessions, current experience of affect was assessed, and a passive emotion viewing task was administered

Table 1

Screening and Assessment Measures used in the Current Study

Online Screen
Edinburgh Handedness Inventory
List of Threatening Events (LTE)
Childhood Trauma Questionnaire – Short Form (CTQ-SF)
Screening Interview
Childhood Trauma Questionnaire – Short Form (CTQ-SF)
Beck Depression Inventory – 2nd Ed (BDI-II)
Mini-International Neuropsychiatric Interview (MINI)
Positive and Negative Affect Schedule (PANAS - general ratings)
Affective Neuroscience Personality Scales (ANPS)
Cognitive- Behavioral Assessment Sessions
Response Bias Task
Functional Imaging Sessions
Positive and Negative Affect Schedule (PANAS – moment ratings)
Passive Emotion Viewing Task

ONLINE SCREEN

Edinburgh Handedness Inventory
List of Threatening Events
Childhood Trauma Questionnaire – Short Form
Kessler Psychological Distress Scale
Temperament and Character Inventory – Revised; 240 item computerized version

TELEPHONIC SCREEN

See Appendix A

SCREENING INTERVIEW

Childhood Trauma Questionnaire – Short Form
Beck Depression Inventory - II
The Mini-International Neuropsychiatric Interview
Affective Neuroscience Personality Scales
Positive and Negative Affect Schedule
Coping Strategy Indicator
Connor-Davidson Resilience Scale
Downey's Sensitivity to Rejection Scale
Mehrrabian Sensitivity to Rejection Scale
Reading the Mind in the Eyes

COGNITIVE-BEHAVIORAL ASSESMENT SESSIONS

Questionnaires:
Behavioral Inhibition/Behavioral Activation Scale
Visual Analog Mood Scale
Cognitive Failures Questionnaire

Tasks:
Wechsler Abbrieviated Scale of Intelligence (Matrix Reasoning and Block Design subtests)
Prisoner's Dilemma
Object Relocation Task
Response Bias Task
Emotion Recognition Task
CANTAB spatial memory battery
Autobiographical Memory Test

Physiological measures:
Blood drawn for genetic analysis
EEG recording

FUNCTIONAL IMAGING SESSIONS

Questionnaires:
Kessler Psychological Distress Scale-10
Positive and Negative Affect Schedule
Profile of Mood States
Visual Analog Mood Scale
Eysenck Personality Questionnaire Revised - Short Scale

Scanner Tasks:
Passive viewing of emotional expressions (12mins)
Affective Go/No-Go (20 mins)
Computer Generated Arena (15 mins)
Spatial memory task (15 mins)

Physiological measures:
Blood drawn for genetic analysis

Figure 4:
Full protocol

Each of these sessions was separated by a period of 10-14 days. At the start of all experimental sessions, participants received either placebo or buprenorphine tablets. The medication was administered by a nurse according to a counterbalanced schedule set up by the study administrator, thus maintaining the blinding for participants and research assistants involved in data collection. The participants' trauma status was also only known to the study administrator.

Due to variable opioid activity across the menstrual cycle, and the menstrual cycle's impact on closely related reward circuitry, female participants were all assessed and scanned during the luteal phase (Dreher et al., 2007; Kraemer et al., 2006; Taylor, Goubillon, Broad & Robinson, 2007). This was accomplished by having female participants record their cycles (once they had completed all screening phases, and agreed to participate in the experimental sessions), and then scheduling sessions at appropriate points in these cycles. Participants were required to refrain from smoking or consuming caffeine on assessment and imaging days (Wewers, Dhatt, Snively & Tejwani, 1999). Apart from a light meal at least 2 hours prior to assessment, they were also required not to eat or drink before the experimental sessions.

In order to perform cognitive-behavioral assessments and obtain the functional imaging data during the period when the opioid was most active, testing commenced 90 minutes after the medication had been ingested. Time to peak plasma concentration for sublingual buprenorphine is between 1 and 3 hours (Bullingham, McQuay, Porter, Allen & Moore, 1982; McAleer et al., 2003; Mendelson, Upton, Everhart, Jacob & Jones, 1997). Subjective effects can be felt within 30 minutes, peak a little later, and remain high for up to 6 hours (Harris, Mendelson, Lin, Upton & Jones, 2004). The timing was thus designed to ensure that assessment occurred while the opioid was exerting its strongest effect. Including the waiting period, each experimental session was 3 hours long.

The cognitive-behavioral assessment sessions were carried out first in order to ascertain if any participants experienced negative side-effects of the medication (prior to placing them in the scanner). Nausea and vomiting following buprenorphine administration do occur in a small percentage of the population, and in fact five individuals were not able to continue participation for this reason.

Both cognitive-behavioral assessment sessions were conducted at the University of Cape Town Department of Psychiatry. In these sessions, a nurse took blood, and research assistants obtained electro-encephalograph recordings, and administered a number of computer-based tasks and pencil-and-paper questionnaires (see Figure 4 for details of all measures administered in the full protocol).

The functional imaging sessions were conducted at the Cape Universities Brain Imaging Center (CUBIC) at the Stellenbosch University Medical Campus, Tygerberg. Again, blood was drawn, and a number of pencil-and-paper questionnaires and tasks were administered outside the scanner, before the participant completed several tasks within the scanner (see Figure 4 for details).

Sample

Participants were 32 right-handed volunteers, recruited from the University of Cape Town undergraduate student body, and aged 18-25 years. All of these individuals completed two cognitive-behavioral testing and two functional imaging sessions, but only 22 had usable fMRI data.

The criteria for inclusion were extremely rigorous, with the aim of controlling for as many potential confounding variables as possible (see Table 2 for exclusion criteria). The final sample was derived in the following way (see Figure 5 for flowchart): 555 students performed an online preliminary screening assessment. Forty-nine were excluded immediately due to being left-handed. We were unable to contact 54 of the remaining number. Eleven of the remainder were excluded due to having experienced a major stressful life event in the 6 months prior to survey. Furthermore, 38 provided questionable profiles (minimization or denial) on the CTQ-SF and were thus excluded.

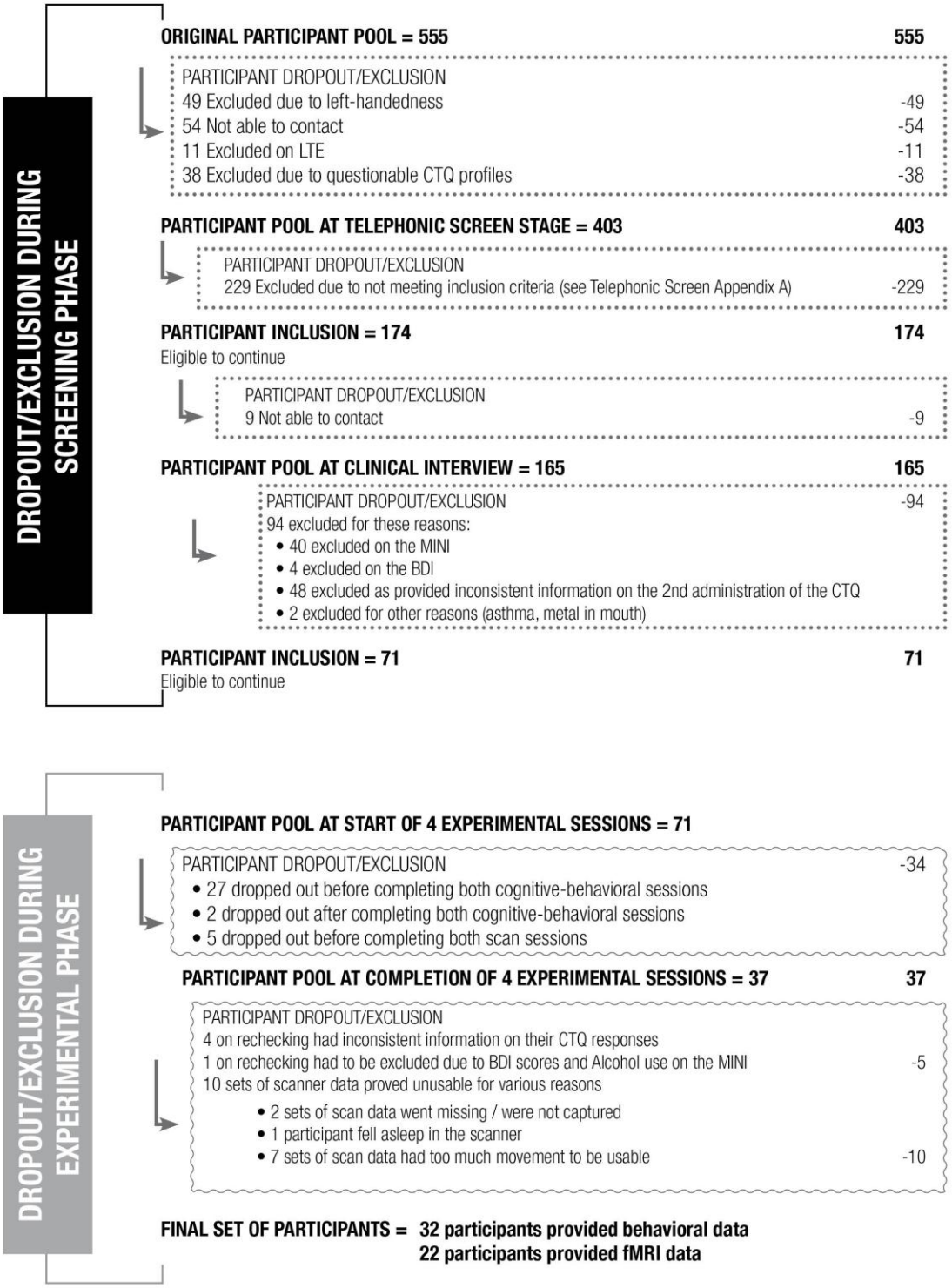

Figure 5:
Flowchart illustrating participant inclusion, exclusion and dropout figures.

Table 2

Exclusion Criteria

Age < 18 or > 25 yrs
Left handedness
Asthma; respiratory or hepatic insufficiency
Any major psychopathology, including affective and psychotic disorders
Psychoactive medication
Previous head injury
Serious medical condition (e.g. renal, cardiac, or neurological illness)
Any neurological condition impacting the central nervous system
Pregnancy or lactation
Any factors that would preclude scanning (e.g. metal pacemaker or prosthesis)
Major stressful life-event in past 6 months
Unsuitable profile on the Childhood Trauma Questionnaire –Short Form

Note: Use of buprenorphine is contra-indicated in anyone with any respiratory or hepatic insufficiency. Its safety during pregnancy and lactation has not been established.

Those meeting preliminary inclusion criteria (N = 403) were contacted telephonically. During that telephone call, a member of the research team obtained further screening information from the potential participant. This information included verbal consent to participate further, and details of any medical condition, medication, or other factors that would preclude participation (see Appendix A for a copy of the telephonic screening questionnaire).

One hundred and seventy-four individuals consented and were eligible to continue participation, but only 165 proved contactable for the next stage. These 165 students then completed a full screening interview, where the CTQ-SF was administered for a second time, and the MINI and BDI-II were administered to check for the presence of psychopathology. Only those who provided consistent CTQ-SF reports across both administrations (i.e. whose online and interview CTQ-SF profiles fit the same clinical interpretations across all subscales), who showed no indication of psychopathology on the MINI and BDI-II, and who met all other inclusion requirements continued as

participants in the study. Ninety-four individuals were excluded. Thus, after the screening phase 71 individuals were eligible to participate in the experimental phase of the study (see Figure 5).

During the experimental phase, there was a high attrition rate: 34 participants dropped out of study before all four sessions were completed. Most dropouts (27) occurred after the first cognitive-behavioral assessment session, two more occurred after the second cognitive-behavioral assessment session, and a further five occurred after the first functional imaging session. Of these 34 participants, only five dropped out due to adverse reactions to the medication (nausea, headaches; four after one cognitive-behavioral session, and one after the first scan). Feedback from the others indicated various reasons for discontinuing. Prohibitive work commitments, scheduling clashes, lengthy research sessions, and distance to the CUBIC facility all appear to have been disincentives to continued participation.

Thirty-seven participants completed all four sessions. However, data from 15 of them had to be excluded from the analysis for the following reasons: on rechecking, four participants had inconsistent profiles on the CTQ-SF; one had to be excluded due to MINI/BDI scores indicating depression; and 10 sets of scanner data proved unusable (see Figure 5 for details).

Participants were compensated for their time at a rate of ZAR100 per session, and were additionally offered a completion bonus (ZAR100) in an attempt to reduce attrition across the multiple assessment sessions. All participants provided written informed consent for both the cognitive-behavioral assessment and functional imaging components of the study (see Appendix B for information and consent sheets). Ethical approval for the study was granted by the University of Cape Town Faculty of Health Sciences' Research Ethics Committee, and by Stellenbosch University's Committee for Clinical Trials. The study was registered with the South African National Clinical Trials Register. We adhered to the principles for research with human subjects outlined in the Declaration of Helsinki (2008).

Establishing trauma status. The CTQ-SF was used to determine trauma group assignment (see Appendix C for guidelines to clinical interpretation of CTQ scores). It

was administered twice, and, as noted above, only participants whose reports yielded the same trauma profile across all subscales at both administrations were included. This double administration of the CTQ-SF was a conservative check aimed at ensuring that participants were reporting reliable information, and that the resulting group allocation was thus valid. Given the central nature of exposure to early social trauma to the research question, and given that I had to rely on retrospective self-report to gauge exposure, it seemed best to err on the side of caution in establishing the two comparison groups.

The CTQ-SF profiles of all eligible participants had to fall into one of two categories: *no/minimal exposure to trauma* or *moderate-severe exposure to trauma.* To be considered part of the control group, participants had to have a maximum of a low-moderate score on a *single* subscale. To be considered part of the trauma group, participants had to have a moderate-severe score on *at least* one subscale. Participants whose profiles fell between these two extremes were excluded. This conservative approach was adopted – rather than using a median split on the total score, for example – in an effort to maximally differentiate the groups on this central criterion.

Participant characteristics. The full sample whose behavioral data were used for analysis consisted of 32 individuals; 14 had been exposed to early social trauma as assessed by the CTQ-SF, and 18 had not. The groups were matched on age and gender, but the race demographic was a little uneven (see Table 3), with a significantly greater number of white participants in the control than in the trauma group.

Table 3

Demographic Characteristics of the Full Sample

	Trauma group (n = 14)	Control group (n = 18)	X^2 / t	p
Gender (Male:Female)	7:7	10:8	0.1	.76
Race (White:Indian:Colored:Black)[a]	1:3:6:4	8:0:4:6	8.9	.03
Age (Mean (SD))	20.36 (1.95)	20.94 (2.55)	0.7	.49

Note: a. In South Africa, due to the legacy of apartheid, census data remains classified in this way as the groups continue to differ in important ways; see Statistics South Africa; http://www.statssa.gov.sa.

Due to the ongoing nature of recruitment and assessment, the study administrator was able to manage over time the matching of participants on key factors, such as trauma status, gender and race, and attempted to ensure that the matching was particularly good for the fMRI sample. The fMRI subset sample thus comprised 22 individuals, 11 in the trauma group and 11 in the control group. These groups were well matched on age and gender. Although the race composition across the groups remained a little more variable, the difference for the subset was not significant (see Table 4).

Table 4

Demographic Characteristics of the fMRI Subset

	Trauma group (*n* = 11)	Control group (*n* = 11)	X^2 / *t*	*p*
Gender (Male:Female)	6:5	6:5	0.0	1.00
Race (White:Indian:Colored:Black)	1:3:3:4	6:0:2:3	6.9	.08
Age (Mean (SD))	20.46 (2.02)	21.36 (2.87)	0.9	.41

CTQ-SF-based allocation to the trauma and control groups was successful: The groups differed significantly on their trauma scores across all subscales. Effect sizes were large (see Tables 5 and 6), for both the full sample and the fMRI subset.

Table 5

CTQ-SF: Group Differences for the Full Sample on Early Exposure to Social Trauma

	Trauma group (*n* = 14)	Control group (*n* = 18)	*t*	*p*	*d*
Emotional Abuse	13.14 (4.99)	6.39 (0.78)	4.46	.001	1.9
	Mod-Severe	*None/Minimal*			
Physical Abuse	9.50 (3.74)	5.61 (0.78)	3.67	.001	1.4
	Mod-Severe	*None/Minimal*			
Sexual Abuse	8.36 (4.09)	5.06 (0.24)	3.04	.005	1.1
	Mod-Severe	*None/Minimal*			
Emotional Neglect	13.14 (5.08)	6.33 (1.71)	4.85	.001	1.8
	Low - Mod	*None/Minimal*			
Physical Neglect	8.21 (3.33)	5.44 (1.04)	3.07	.005	1.1
	Low - Mod	*None/Minimal*			
Total Score	52.36 (13.71)	28.83 (2.18)	5.28	.001	2.4

Note: Means, with standard deviations in brackets, and clinical interpretations are provided for each subscale.

Table 6

CTQ-SF: Group Differences for the fMRI Subset on Early Exposure to Social Trauma

	Trauma group (*n* = 11)	Control group (*n* = 11)	*t*	*p*	*d*
Emotional Abuse	13.36 (5.59)	6.09 (0.70)	3.55	.002	1.8
	Mod-Severe	*None/Minimal*			
Physical Abuse	9.64 (4.15)	5.73 (0.90)	3.25	.004	1.3
	Mod-Severe	*None/Minimal*			
Sexual Abuse	8.55 (4.23)	5 (0.00)	2.60	.017	1.2
	Mod-Severe	*None/Minimal*			
Emotional Neglect	11.91 (4.99)	6.27 (1.95)	3.25	.004	1.5
	Low - Mod	*None/Minimal*			
Physical Neglect	8.09 (3.78)	5.36 (0.67)	2.34	.030	1.0
	Low - Mod	*None/Minimal*			
Total Score	51.55 (15.38)	28.45 (2.16)	4.00	.001	2.1

Note: Means, with standard deviations in brackets, and clinical interpretations are provided for each subscale

Materials

Measures. Figure 4 shows the larger study's full protocol. In this section, I describe only the measures of direct relevance to the work reported in this dissertation. Note also that I provide psychometric detail to substantiate the use of measures central to the research question.

Online screening and assessment instruments. *The Edinburgh Handedness Inventory* (Oldfield, 1971) was developed to provide a brief and simple quantitative assessment of handedness for use in clinical and research contexts. The author explicitly aimed to devise an instrument that would be applicable as universally as possible, to enable comparisons across gender, nationality, culture and socioeconomic status. It was thus ideally suited as a screening instrument in this study. Handedness must always be taken into consideration in functional imaging studies, as a different functional cerebral organization (viz., crossed lateralization or a lesser degree of hemispheric asymmetry) is possible in left-handers. This measure was used to exclude all participants who were not right handed.

The List of Threatening Events (Brugha & Cragg, 1990*)* is a brief screening instrument that was used to exclude potential participants who had experienced a major life-stressor in the six months prior to survey. The research team reasoned that such a recent event might have caused alterations in physiological and neurochemical function that could confound our results. Items include questions about serious illness, financial crises, and loss or death of significant individuals. The measure has good reliability, sensitivity and concurrent validity (Brugha & Cragg, 1990; Humke & Radnitz, 2005).

The Childhood Trauma Questionnaire – Short Form (CTQ-SF; Bernstein et al., 2003) is a widely used retrospective measure that assesses exposure to various kinds of trauma during early childhood. Its popularity is due to the fact that it is almost unique in addressing more than a single type of traumatic experience. It was particularly well suited for use in this study, as the domains it assesses, notably emotional and physical

abuse and neglect, indicate clearly the absence of appropriate nurturing and caretaking in critical early social bonds.

The CTQ-SF is a 28-item self-report scale, with five clinical subscales: it assesses physical, sexual, and emotional abuse, and physical and emotional neglect. It also features a minimization/denial scale that detects under-reporters. Abuse and neglect are identified at three levels – low, moderate and severe. It is also possible to have a 'no exposure' profile. Item responses are scored on a 5-point Likert scale ranging from 'never true' to 'very often true'.

The original CTQ consisted of 70 items. It has excellent test-retest reliability, and demonstrated convergent and discriminant validity. It has good sensitivity and specificity (Bernstein, Ahluvalia, Pogge & Handelsman, 1997; Bernstein et al., 1994). The short form was later developed by the original research group (Bernstein et al., 2003), who aimed not only to provide a short yet reliable and valid instrument, but also to establish measurement invariance. The short form has been validated for use in both clinical and normal populations. The validity checks were performed on a sample of 1978 participants, constituting four groups (three psychiatric, one normal). Analysis indicated measurement invariance is present, with the five-factor structure (corresponding to the five subscales) fitting all four groups. Strong criterion-related validity was also demonstrated. The CTQ-SF has been used effectively across different race and cultural groups (Thombs et al., 2007a; Thombs, Lewis, Bernstein, Medrano & Hatch, 2007b), and was thus suitable for use in this study.

Telephonic screen. This instrument (see Appendix A) served to screen potential participants for any relevant medical condition (e.g., previous head injury, epilepsy), use of medication, or the presence of metal prostheses or objects in the body. The screen also served to exclude any potential female participants who were pregnant or breastfeeding, as the safety of buprenorphine has not been established for these conditions. Participants with asthma, and/or respiratory or hepatic insufficiency were excluded, as use of buprenorphine is contraindicated in these contexts. Anyone previously diagnosed with a psychiatric disorder was also identified and excluded.

Screening interview measures. *The Beck Depression Inventory – 2nd Edition* (BDI-II; Beck et al., 1996) is one of the most widely used measures of depression in research. It features 21 self-report items. The BDI-II has been well validated, and has strong psychometric properties, including very good sensitivity and specificity (Beck, Steer & Gabin, 1988; Subramaniam, Harrell, Huntley & Tracy, 2009). This measure was included to complement the MINI ratings of depression. Participants with a score of 14 or higher – i.e., those who reported the presence of at least mild depressive symptoms – were excluded (see Appendix C for guidelines to clinical interpretation of BDI-II scores).

The Mini-International Neuropsychiatric Interview 5.0 (MINI 5.0; Lecrubier et al., 1997; Sheehan et al., 1998; Sheehan et al., 1997) is a short structured diagnostic interview, and was used to screen participants for DSM Axis I psychopathology, and for antisocial personality disorder. It was developed by psychiatrists and clinical psychologists to provide a brief but accurate diagnostic assessment instrument, useful in both research and clinical settings. In its development the authors aimed to create an instrument that is highly sensitive, specific, and compatible with international diagnostic criteria, including those of the DSM and the International Classification of Diseases (ICD). The instrument was deliberately designed to be conservative with regard to false negatives; this means that it is more likely to falsely identify someone as meeting diagnostic criteria, than to wrongly miss the actual presence of pathology (Sheehan et al., 1998).

The MINI takes approximately 15-20 minutes to administer, and may be administered by laypersons who have received appropriate training. The instrument covers 19 diagnostic categories, including mood and anxiety disorders, suicidality, eating disorders, psychotic disorders, PTSD, alcohol and substance abuse/dependence, and antisocial personality disorder.

The MINI was validated on sample of 636 participants, from specified clinical subgroups. Its performance was compared with that of two longer, well-established structured diagnostic interviews: the Structured Clinical Interview for DSM (First, Spitzer, Gibbon & William, 1997) and the Composite International Diagnostic Interview (Robins et al., 1989) and with that of expert clinical diagnoses. The authors found

predominantly good or very good correspondence between the instruments. Specificity and positive and negative predictive values were also very good. Agreement with expert diagnosis was found in over 85% of cases. The authors concluded that the MINI is a reliable and valid instrument, capable of appropriately detecting the presence of psychopathology as defined by the DSM and ICD (Lecrubier et al., 1997; Sheehan et al., 1998; Sheehan et al., 1997).

The MINI has been widely used since its development, and has been translated into a number of different languages. It has proved useful in European, Arabic, South American and Japanese populations (de Azevedo Marques & Zuardi, 2008; Kadri et al., 2005; Otsubo et al., 2005; Rossi et al., 2004), and this applicability across cultures suggests that it is suitable for use in South Africa.

The Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegen, 1988*)* was developed to provide a brief and easy to administer, yet psychometrically sound, measure of positive and negative affect. This measure is very well-validated and has been used in over 2000 published studies (Thompson, 2007). It has been effectively used across Europe, as well as in Australia, China and the Middle-East, suggesting that it is suitable for use in multiple cultural contexts (Ayyash-Abdo & Alamuddin, 2007; Kiernan, Laurent, Joiner, Catazaro & MacLachlan, 2001; Melvin & Molloy, 2000; Shi, Wang & Li, 2009).

The PANAS can measure both state- and trait-related affect (Tellegen, 1985; Watson & Clark, 1984). Positive and negative affect represent distinct domains of emotional experience; they are orthogonal rather than inter-correlated. High positive affect indicates that an individual feels energized, and positively engaged, whereas low positive affect indicates sadness and a lack of energy. In contrast, high negative affect indicates a range of aversive mood states, including anger, fear and anxiety, whereas low negative affect indicates a state of calm or contentment (Watson et al., 1988). Research has indicated that these two dimensions are stable across cultures (Almagor & Ben-Porath, 1989; Balatsky & Diener, 1993; Melvin & Molloy, 2000).

The developers of this instrument aimed to select mood descriptors that related unequivocally to one or the other affective dimension. They used factor analysis to

identify terms that loaded high only on one factor and had no significant loading on the second factor (loadings of less than .25). The positive affect terms thus derived are *attentive, interested, alert, excited, enthusiastic, inspired, proud, determined, strong*, and *active.* The negative affect terms are *distressed, upset, hostile, irritable, scared, afraid, ashamed, guilty, nervous* and *jittery* (Watson et al., 1988). Participants indicate the extent to which they experience each mood state in a given period of time (e.g. in general, last week, now) on a 5-point Likert scale, ranging from 'very slightly or not at all' to 'very much'.

The original psychometric study (which examined both reliability and validity) indicated good internal consistency for all time period instructions. Cronbach's alpha for positive affect ranged between .86 and .90; for negative affect, it ranged between .84 and .87. The scales were not correlated (*r* ranged from -.1 to -.2; Watson et al., 1988). Independent research has confirmed these statistics (Melvin & Molloy, 2000; Munz & Munz, 1997). As is expected in mood measures, test-retest reliability was found to be higher for the longer time period instructions (e.g. "how much have you felt anxious in the last few weeks?"); in fact, the measure's developers state that the stability coefficients of the general ratings are high enough for these to be treated as trait measurements (Watson et al., 1988). Importantly, even the moment ratings were relatively stable, which the developers argue reflects the fact that even momentary mood states reflect an individual's general experience of affect. The moment measures are, however, capable of reflecting changing moods (Watson et al., 1988). This instrument was thus ideally suited to establish participants' general experience of affect, and to track changing experience of affect on buprenorphine versus placebo.

The Affective Neuroscience Personality Scales (Davis, Panksepp & Normansell, 2003) were constructed to identify relative strengths and weaknesses in the six core emotion systems defined by Panksepp (1998), viz., SEEKING, FEAR, ANGER, SADNESS[4], CARE and PLAY. The developers report that all items were constructed to elicit reports of personal feelings and actual behavior, rather than cognitive social

[4] The term SADNESS is used in this scale. It is a variant of the term SEPARATION-DISTRESS used in the literature review and throughout this dissertation. It refers to the same core emotion system.

judgments. Each core emotion is assessed by 14 items, half of which are reverse scored. Filler items are included as validity checks.

Internal consistency for this measure is good, with Cronbach's alpha for the six scales ranging from .65 - .86, a range commonly found in the field of personality research (see, e.g., Deal, Halverson, Martin, Victor & Baker, 2007; Korner et al., 2008; Tromp & Koot, 2008). Construct validity was assessed by obtaining concurrent measures on a Five Factor Model scale. The 'Big Five' approach to personality has yielded consistent empirical results, and some of the components are thought to be related to underlying physiological systems (Coker, Samuel & Widiger, 2002; Goldberg, 1990; Graziano & Ward, 1992; Jang, Livesley & Vernon, 1996; Morey, Gunderson, Quigley & Lyons, 2000; Paunonen, 2003). SEEKING was found to be significantly associated with the Openness to Experience factor (r = .47, p <.001), while SADNESS was strongly negatively correlated with Emotional Stability (r = -.68, p <.001). Confirmatory factor analysis affirmed the strong inter-relationships between constructs in the two personality measures, with the three negative emotions and emotional stability (inversely) loading onto one factor; agreeableness and CARE loading on a second factor; Extraversion and PLAY loading on a third; and openness to experience and SEEKING loading on a fourth. Inclusion of this scale in the current study enabled detection of possible weaknesses or strengths in the key systems of interest (i.e. SEEKING and SADNESS).

Experimental measures. In the cognitive-behavioral assessment sessions, I used an adapted version of a *Response Bias Task* designed to examine bias in emotion recognition (Arce et al., 2009; Surguladze et al., 2004). Facial expressions convey critical social information, and some evidence suggests that depressed individuals show impaired recognition of emotion, with a bias towards negative emotion (Hale, 1998; Persad & Polivy, 1993; Rubinow & Post, 1992; Surguladze et al., 2004). In these healthy normal participants, I wanted to examine whether any group difference existed in their tendency to perceive others as conveying negative rather than positive emotional information, and if this difference changed on opioid medication.

The specific task I used was developed recently by a research team at the Helmholz Research Institute, Utrecht University (J. van Honk, personal communication,

16 November, 2009). It was delivered via EPrime, and featured eight male and eight female faces sourced from standard sets of photos of affective faces (Ekman & Friesen, 1976; Lundqvist, Flykt & Ohman, 1998). Images were normalized for size and luminance, with a standard grey background. Anger, happiness, sadness, and fear, as well as neutral expressions, were included. Because cognitive processing can over-ride or hide automatic response tendencies or biases, stimulus exposure was extremely brief (92ms), to prevent conscious recognition of the emotion expressed (Hermans, Nijenhuis, van Honk, Huntjens & van der Hart, 2006; Putman, Hermans & van Honk, 2004; Putman, Hermans, Koppeschaar, van Schijndel & van Honk, 2007a).

Each participant saw 96-100 faces, half of which were emotional, and half of which were neutral. The order of presentation was randomized, and differed for each participant and across medication conditions. Participants were asked to guess which emotion they had seen after each stimulus presentation, and were given a forced choice response format with the options ANGER, HAPPINESS, SADNESS and FEAR. The next stimulus was presented once they had indicated their choice, hence each inter-stimulus interval depended on their response time. The question of interest was this: what emotion do participants tend to ascribe to the neutral faces? Following Surguladze et al. (2004), responses were normalized to indicate what percentage of the neutral faces each participant rated as angry, happy, sad or fearful.

For the functional imaging protocol, I used an adapted version of the *Passive Viewing of Emotional Expressions* protocol developed by Sato and colleagues (2004). To date, most functional imaging studies in this field have used static faces, often in conjunction with an active emotion recognition task (see, e.g., Hariri, Tessitore, Mattay, Fera & Weinberger, 2002; Morris et al., 1998; Phillips et al., 2001; Whalen et al., 2001). The ecological validity of this latter paradigm is open to question. In the real world, facial expressions are dynamic, and we are seldom called upon to make conscious, cognitively mediated decisions about which emotion we are seeing. A strong argument can be made that investigation of neural activity in response to these very important social signals should use dynamic stimuli that require no cognitive judgment to be made.

This approach has been adopted by Sato and his colleagues. They developed a task, using static images taken from standard pictures of affect (Ekman & Friesen, 1976), and used computer morphing techniques to create dynamic expressions that transition from neutral to an intense emotional expression. Their work indicates that, 1) the morphing expressions seem natural to participants, and 2) it is not evident to participants that the morphing faces are artificial computer generations (Sato, 2004). They have also demonstrated that, compared to static stimuli, this task elicits greater neural activity in key regions (Sato et al., 2004). This greater neural reactivity in key regions has been confirmed in independent research using a different set of stimuli (Trautmann, Fehr & Herrmann, 2009).

Stimuli for the current protocol were sourced from standard sets of affective faces (Ekman & Friesen, 1976; Lundqvist et al., 1998). Four female and four male faces were used. Images were normalized for size and luminance, with a standard grey background. Starting with static images, computer morphing animation created a dynamic expression that started at neutral and used a series of 50 frames to transition smoothly to maximum intensity of expression (see Figure 6).

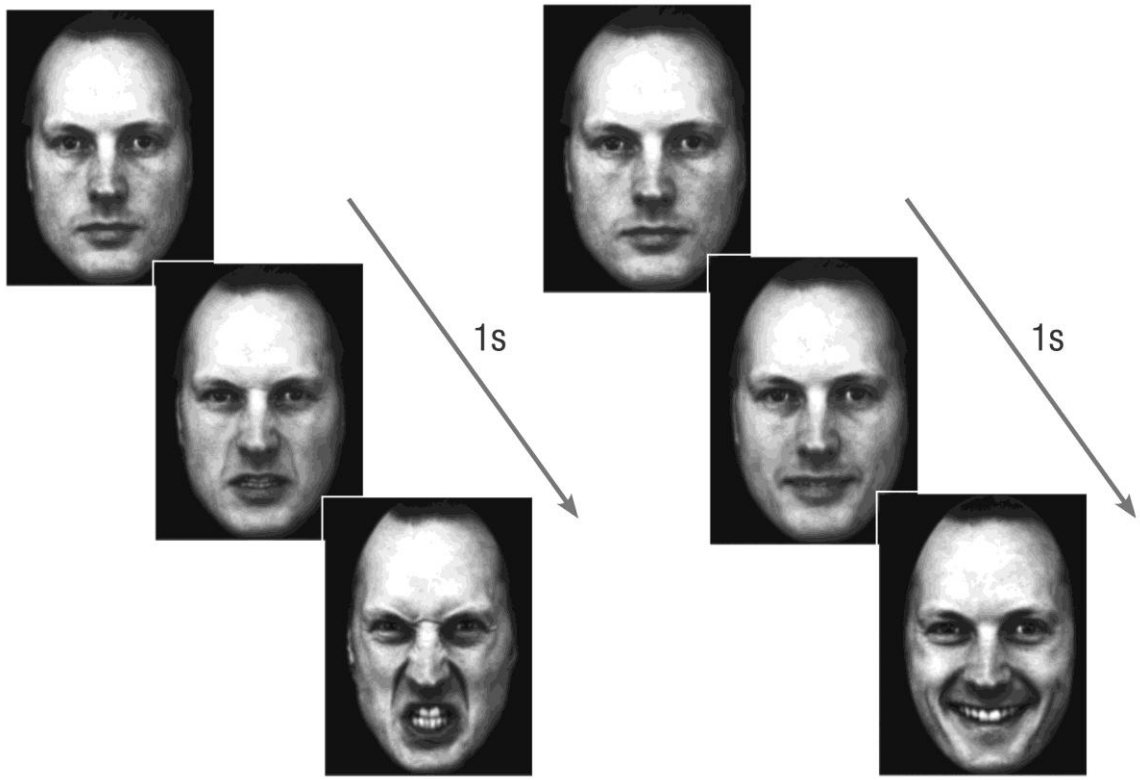

Figure 6:
Passive emotion viewing task: faces morph from neutral to intense expression in about 1 sec.

Only a subset of basic emotions could be included in the investigation, due to time constraints imposed by the larger study. Fear and anger were selected to represent negative emotions, as the processing of these emotional social signals has been extensively investigated, and different styles of processing have been identified for individuals who are avoidant- rather than approach-oriented[5] (d'Alfonso, van Honk, Hermans, Postma & de Haan, 2000; Putman, Hermans & van Honk, 2006; Putman et al., 2007a; Putman, Hermans & van Honk, 2007b; Roelofs, Bakvis, Hermans, van Pelt & van Honk, 2007; van Honk et al., 2000). Happiness was selected as a positive control.

The task was set up as a block design protocol, with epochs of the different emotional expressions alternating with rest periods (see Figure 7). Two blocks of each emotion were included to improve power (Liu, 2004; Savoy, 1999). Anger and happy

[5] This difference in processing was particularly important for the analysis of the affective go/no-go task that formed part of the full protocol, and it was decided to use the same set of emotions for both fMRI protocols.

epochs were presented first, and the fear epochs were presented last. This approach was adopted due to concern that, as fear is a highly salient social stimulus, it might create a long-lasting neural response that would then contaminate data from the subsequent epochs (Davis & Whalen, 2001; Phan, Wager, Taylor & Liberzon, 2002; Putman et al., 2006; Putman et al., 2007a; van Honk, Peper & Schutter, 2005). Because this was the first time this particular scanner protocol was used, I was uncertain of the effects the stimuli would have, and thus decided to err on the side of caution with regard to the fear stimuli.

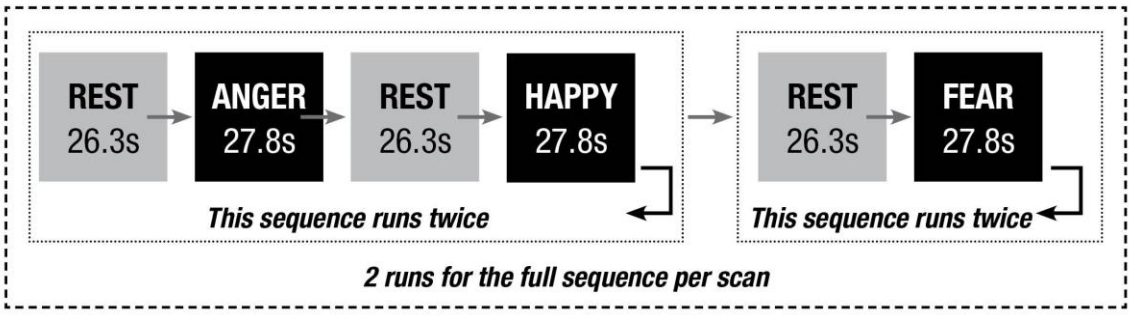

Figure 7:
fMRI block design protocol sequence and timing.

The length of the epochs was varied slightly on a random schedule, to avoid setting any patterned habitual neural response in the participants (Liu, 2004; Savoy, 2005). For the rest periods the average epoch length was 26.3s (*SD* = 0.9, min = 21.4, max = 35.5). For the emotion epochs, the average length was 27.8s (*SD* = 0.8, min = 26.5, max = 31.7).

On average, each run was 350.9s long, and the entire protocol was run twice to improve power. In total both runs took on average 12 minutes. (Note the runs were separated in time by a number of runs of the affective go/no-go task which is part of the total protocol; see Figure 4).

Medication. Buprenorphine in the form of 0.2mg sublingual tablets (brand name: Temgesic) was used. Sugar pills of a similar size and color, also taken sublingually, were used for the placebo condition.

Buprenorphine hydrochloride is a partial mu-opioid receptor agonist and kappa-opioid receptor antagonist. It has a particularly long duration of action, dissociating very slowly from opioid receptors (Correia, Walsh, Bigelow & Strain, 2006; Harris et al., 2004). Time to peak plasma concentration is around one hour, with a half-life of between 20 and 30 hours (Ciraulo et al., 2006; Harris et al., 2004; McAleer et al., 2003). Subjective effects can be felt within 30 minutes, peak a little later, and stay high for up to 6 hours (Harris et al., 2004). It is important to note that significant pain and psychomotor effects persist for up to 240 minutes despite decreasing plasma levels. These effects are due to very slow dissociation of the drug from brain opioid receptors (Escher et al., 2007; Galynker et al., 1996; Zacny et al., 1997).

It must be noted that in the literature, buprenorphine's positive effects seem to be ascribed to its actions on mu-receptors, and this is reasonable given the evidence (reviewed above) that mu-opioids promote positive opioid effects such as euphoria. However, the medication does also act on kappa opioids, blocking their effects. The implications of its kappa opioid effects will be considered in the Discussion.

Buprenorphine is a registered medicine, and is used as an analgesic for moderate to severe pain. The dose used in this study (0.2mg) is the smallest sublingual dosage available in South Africa. When used for pain, 0.2mg-0.6mg sublingual tablets every 6-8 hours is a standard dose protocol (Escher et al., 2007). It is notable that low doses are very effective; in fact, 0.3mg of buprenorphine has been found to have greater subjective effects than 10mg of morphine (Zacny et al., 1997).

Buprenorphine is also commonly used in far larger doses to treat opiate dependence (Boothby & Doering, 2007). In such programs, opiate-dependent individuals are often started on 8mg of buprenorphine daily, with a maximum daily dose of 32mg. These doses are clearly many times greater than those used for analgesic purposes, and high-dose pharmacokinetics are not particularly well characterized (Harris et al., 2004; McAleer et al., 2003).

Common side-effects include drowsiness, nausea, and vomiting. Incidence of these effects seems more pronounced in opiate-naïve individuals, and may increase along with increased dose (McAleer et al., 2003; Zacny et al., 1997). Very few studies of buprenorphine in opiate-naïve individuals have been conducted, but it appears that

tolerability of the medication, even at low doses, may be problematic. Route of administration probably impacts on these effects, but little specific information on sublingual buprenorphine is available. A recent study using low doses (0.002mg/kg intravenous) in opiate-naïve individuals (N = 12) found that all participants experienced drowsiness, 75% felt nauseous, and 42% vomited (Escher et al., 2007). Researchers using 0.3mg intramuscular dose found that 44% of their sample vomited, while 9 of 12 participants were too incapacitated to continue (MacDonald, Gough, Nicoll & Dow, 1989). In one clinical study of effects on depression, very low oral doses (0.15mg - 1.8mg) were used over a 2-week period. Nonetheless, 3 of 10 patients dropped out of this study due to negative experience of these side-effects (Bodkin et al., 1995). In another study using higher doses (2mg-16 mg oral administration) and again conducted with opiate-naïve individuals, 5 of 26 participants withdrew due to aversive effects of the medication (McAleer et al., 2003). The authors found that although standard subjective and physiological effects associated with the drug did not increase with dosage, the acceptability of the drug decreased, dropping from 68% at 4mg to 25% at 16mg. Thus, with increasing dosage side-effects appear to become more problematic, while standard opioid effects appear to plateau (Harris et al., 2004; McAleer et al., 2003).

Due to concerns around negative experience of the medication in healthy normals, and taking into account the sensitivity to low doses seen in opiate-naïve individuals, we decided to use the lowest possible dose in this study. This was, admittedly, a risky decision, as the low dose in combination with a novel fMRI protocol might well have resulted in null findings. However, this course of action was highly recommended by one of our senior co-investigators who has a great deal of experience with opioids in animal work, and given the risks of adverse experience and participant dropout associated with higher doses, we thought this was the safest choice.

Data Analysis

Cognitive-behavioral data analysis. Analyses were conducted using SPSS version 19. Chi-square tests, independent group *t*-tests, and mixed-design ANOVA analyses were used where appropriate.

For analysis of the PANAS ratings of current experience of affect, the factors were group (trauma vs. control); medication (buprenorphine vs. placebo) and emotion (positive vs. negative). I was not interested in the independent main effects for medication or emotion – the aim of this analysis was to ascertain whether or not group differences existed in emotion on and off medication. I thus combined the two repeated-measures (RM) factors into a single factor with the following four levels: medication positive affect, medication negative affect, placebo positive affect and placebo negative affect.

Similarly for analysis of the response bias task data, the factors were group (trauma vs. control); medication (buprenorphine vs. placebo) and emotion (angry, happy, sad, fearful). Again, I was not interested in the independent effects of medication and emotion, but wanted to examine group differences in how judgment of emotion changed on medication. I thus combined the two RM factors into a single factor with 8 levels: medication anger, placebo anger, medication happy, placebo happy, medication sadness, placebo sadness, medication fear, and placebo fear.

Given that the information provided by the independent main effects of these repeated measures in the analyses discussed above was not of interest, reducing the number of factors in the analyses had the added benefit of substantially reducing the number of post-hoc contrasts required, and hence reduced the associated risk of Type 1 error. Fewer RM factors also reduced sphericity demands; where the assumption of sphericity was violated, however, Greenhouse-Geisser corrected values are reported.

Although overall a number of inferential analyses were necessary, the resultant increased risk of Type 1 error had to be weighed against two factors: 1) the exploratory nature of this research, and 2) the small sample size and consequent high risk of Type 2 error. Given these concerns, alpha was not adjusted and remained at the conventional .05 level.

fMRI data acquisition. Data were acquired on a Siemens Allegra Magnetom 3T scanner (Siemens Medical Systems, Erlangen, Germany) using a standard single-channel head coil. For each participant a high-resolution T1- weighted magnetization prepared rapid gradient echo (MPRAGE) image was acquired with the following parameters:

TR/TE/TI = 2300/2.93/1100 ms; FOV = 256 × 240 mm; slices = 160; voxel size = $1.3 \times 1 \times 1$ mm^3; flip angle = 12 degrees; scan time = 6:28.

Gradient echo T2*-weighted echo planar images were acquired using the following parameters: TR/TE: 2000/30 ms; FOV = 220×200 mm^2; resolution = 3.4×3.4 mm^2; slices = 34; slice thickness = 3 mm (1.2 mm gap); flip angle = 90 degrees; 184 volumes. Slices were interleaved, and head movement was minimized with padding.

fMRI preprocessing and data reduction. Data preprocessing and analysis were completed using BrainVoyager QX, version 2.1.2. Preprocessing was standard, and included correction for different slice acquisition times, motion correction, linear trend removal, and high-frequency temporal filtering. The functional images were corrected for different slice acquisition times using cubic-spline interpolation based on TR (2000ms) and the order of slice scanning (ascending, interleaved). Temporal smoothing was done using a high-pass GLM-Fourier filter over two cycles. Motion correction using trilinear interpolation aligned all volumes from each subject to the first volume of his/her functional run. For each participant, translation and rotation parameters were inspected, and where movement exceeded 3mm displacement or 3 degrees rotation, the data were excluded from analysis. Each participant's functional data were co-registered to their high-resolution structural MRI, rotated into the AC-PC plane, and normalized to Talairach space (Talairach & Tournoux, 1988).

fMRI data analysis. Analysis was based on the General Linear Model (GLM), which, in this context, aims to explain changes in the voxel time courses measured by scanning (Friston et al., 1994). Multiple runs of each condition in the block design protocol improved power and increased the ability to distinguish task-related activation from potential low-frequency drifts (Liu, 2004). The block design was set up as a boxcar function, convolved with the standard hemodynamic response function implemented by BrainVoyager (Boynton HRF: delta = 2.5, tau = 1.25). Estimated motion parameters and drift predictors were *z*-transformed and then included as predictors of no interest, to reduce unexplained error variance in the model.

Each participant's data were analyzed at the individual level, and were then entered into a second-level whole brain analysis using a random effects mixed-design ANOVA with two repeated measures [2 (group) x 2 (medication condition) x 3 (emotion)]. Region of interest (ROI) analysis was then conducted to test specific predictions. ROIs were functionally defined, but were independent of the whole brain analysis as orthogonal contrasts were investigated (Friston, Rotshtein, Geng, Sterzer & Henson, 2006; Poldrack et al., 2008). Because BrainVoyager conducts 2-tailed tests by default, ROI *p* values were corrected for the directional hypotheses tested. Anatomical labeling was done using the Duvernoy (1999) atlas, with the assistance of a neuroanatomist.

Results

In the following subsections (with the exception of the fMRI results subsection), I report results for the full sample (N = 32). All the full-sample analyses described below were also conducted for the fMRI subset sample (N = 22), to establish whether the subgroup whose fMRI data were usable for analysis differed from the full sample in any appreciable manner. The results of these analyses are presented in Appendix D. As shown there, the same patterns and significant effects as those found for the full sample were found for the fMRI subset.

Participant Characteristics

BDI-II. The trauma and control groups did not differ significantly on depression scores. Participants' scores all fell within the *None–Minimal* range of depressive symptoms (see Table 7).

Table 7

BDI-II: Group means

Trauma group (n = 11)	Control group (n = 15)	t	p	d
6.00 (4.33)	4.87 (4.72)	0.59	.56	.25

Note: Means, with standard deviations in brackets, are presented.
It must be noted that, unfortunately, BDI-II data were missing for 8 participants[6]: five from the trauma group and three from the control group.

[6] Where data is missing it is because, due to researcher error, not all measures were collected from all participants. When these omissions were noted, all efforts were made to contact the participants and have them complete the outstanding questionnaires/assessments; we were not able to contact some participants, however.

PANAS – general ratings. A 2 (group) x 2 (affect type) mixed-design ANOVA was conducted on the ratings of general experience of positive versus negative affect. Contrary to the *a priori* predictions, there was no interaction between trauma status and type of affect generally experienced, $F(1, 29) = 0.57$, $p = .46$, *partial eta*$^2 = .02$. In fact, participants in both the trauma and control groups reported experiencing significantly more positive than negative affect, $F(1, 29) = 323.97$, $p < .0001$, *partial eta*$^2 = .92$. (See Table 8 for descriptive statistics).

Table 8

PANAS – General Ratings: Group means

	Trauma group (*n* = 18)	Control group (*n* = 13)
Positive affect	34.08 (5.59)	34.56 (4.37)
Negative affect	14.31 (3.92)	13.06 (3.28)

Note: Means, with standard deviations in brackets, are presented.
Data were missing for one trauma group participant.

Affective Neuroscience Personality Scales (ANPS). The *a-priori* prediction was that group differences would be present on the SEEKING and SADNESS subscales, with the control group scoring higher on SEEKING, and the trauma group scoring higher on SADNESS.[7] Because only these specific predictions needed to be tested, a mixed-design ANOVA including the other four emotion systems was considered inappropriate.

A one-tailed *t*-test for independent groups indicated a significant effect for SEEKING, $t(28) = 2.15$, $p = .02$, $d = .77$, with the control group scoring higher, as predicted. However, the prediction that the trauma group would score higher on the SADNESS subscale was not supported, $t(28) = 0.08$, $p = .47$, $d = .03$. (See Table 9 for scores on all the subscales).

[7] The term SADNESS is used in this scale. It is a synonym for the term SEPARATION-DISTRESS used in this dissertation.

Table 9

ANPS Subscales: Group means

ANPS Subscale	Trauma group (*n* = 13)	Control group (*n* = 17)
SEEKING	20.69 (2.66)	22.47 (1.88)
FEAR	16.92 (1.75)	16.82 (2.79)
CARE	20.39 (3.50)	18.59 (4.62)
ANGER	15.39 (4.45)	16.53 (3.12)
PLAY	20.08 (3.97)	21.18 (2.81)
SADNESS	16.85 (3.31)	16.77 (2.33)

Note: Means, with standard deviations in brackets, are presented.
Data were missing for one participant from each group.

Interim summary: Participant characteristics. The recruitment strategy ensured that the trauma and control groups differed significantly and substantially on exposure to early childhood trauma. These groups of otherwise healthy normal participants did not differ on general experience of positive and negative affect, or on signs of depression. The ANPS data indicated a significant difference between the groups on the SEEKING subscale, with the control group scoring significantly higher on this trait. There was no between-group difference on the SADNESS subscale.

Impact of Opioid Manipulation on Subjective Experience of Affect

PANAS – current affect ratings. During both the medication and placebo sessions, participants were asked to rate their current affective state. A 2 (group) x 4 (medication condition-PANAS rating) mixed-design ANOVA was conducted. As with the baseline general affect ratings, both the trauma and control groups reported far more positive than negative affect, $F(2.27, 35.22) = 78.13$, $p < .0001$, *partial eta*$^2 = .74$. However, for the ratings of current affect (moment ratings), a significant interaction between trauma status and medication-PANAS ratings was found, $F(2.27, 35.22) = 5.23$, $p < .006$, *partial eta*$^2 = .16$ (See Table 10 for descriptive statistics, and Figure 8 for a graphical representation of this interaction).

Table 10

PANAS current affect ratings: Group means on and off buprenorphine

	Trauma group (*n* = 13)	Control group (*n* = 16)
Placebo - positive affect	27.39 (9.36)	29.69 (5.67)
Placebo – negative affect	15.00 (4.93)	11.88 (2.80)
Medication – positive affect	23.62 (6.91)	30.56 (6.76)
Medication – negative affect	13.31 (4.29)	12.19 (2.95)

Note: Means, with standard deviations in brackets, are presented.
Data were missing for 1 trauma group and 2 control group participants

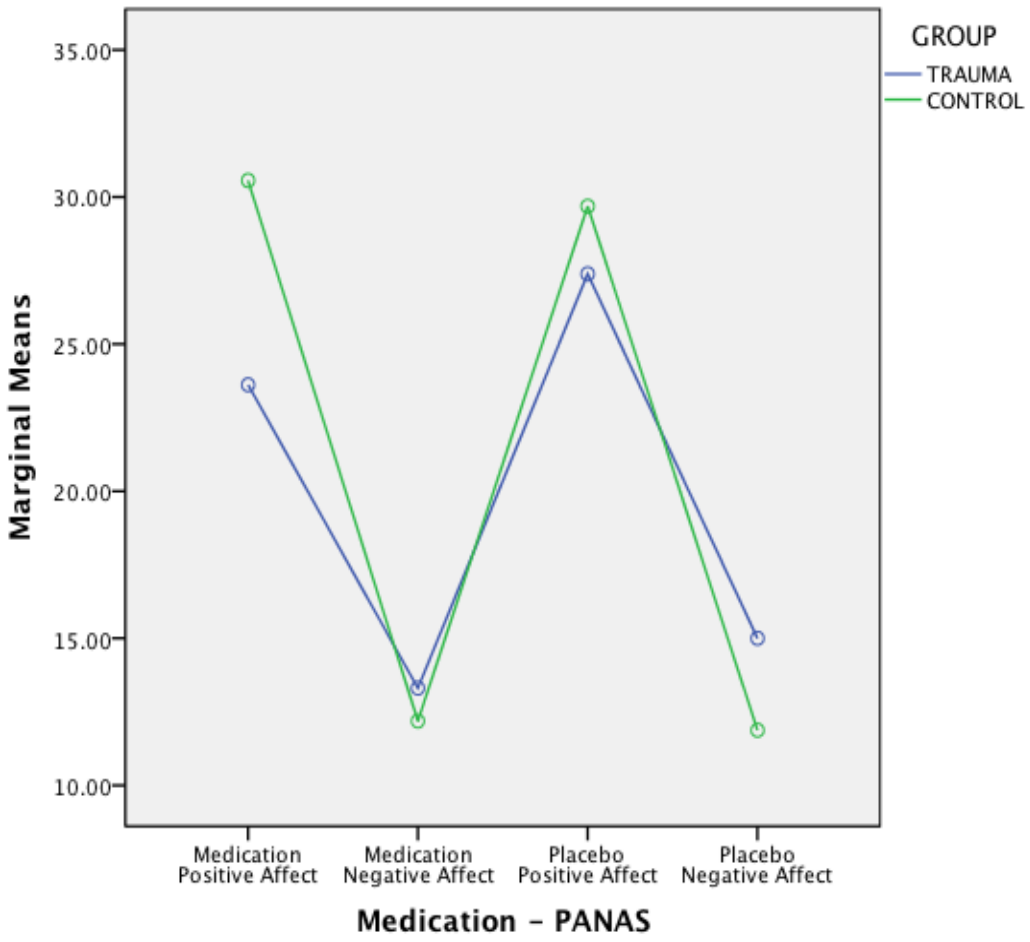

Figure 8:
Significant interaction of trauma status with medication – PANAS ratings of current affect.

Simple effects analysis of the interaction indicated the following: First, there were no significant within-groups effects. That is, within the trauma and control groups, no significant change in either positive or negative affect on medication versus placebo was observed. One interpretation of this pattern of data is that the opioid manipulation was not sufficiently powerful to result in statistically significantly altered experience of emotion within either of the groups.

Second, in terms of positive affect, participants in both the trauma and control groups reported very similar scores on placebo (p = .42). However, on the opioid

medication, the control group reported significantly more positive affect than the trauma group (p = .011).

In terms of negative affect, on placebo the trauma group reported significantly more negative affect than the control group (p =.04). However, the between-group difference was non-significant in the medication condition (p = .41).

Interim summary: Impact of opioid manipulation on subjective experience of affect. In sum, these results indicate that the groups responded differently to opioid manipulation in terms of their subjective experience of affect, albeit not entirely as predicted. The results for negative affect were in the predicted direction. The trauma group reported significantly more negative affect than the control group on placebo, and this difference was reduced (to non-significance) on medication. However, on medication, a change in positive affect was seen only in the control group, who moved from equivalent scores to the trauma group on placebo to significantly higher scores on medication.

Impact of Opioid Manipulation on Social Cognition

Response bias task. A 2 (group) x 8 (medication condition-emotion) mixed-design ANOVA indicated a significant interaction between trauma status and types of errors made for neutral faces on and off medication, $F(2.93, 85.02) = 3.61$, $p < .017$, *partial eta*^{2} = .11. (See Table 11 for descriptive statistics, and Figure 9 for a graphical representation of the interaction).

Table 11

Response Bias: Emotion choices (as % of all neutral faces) on and off buprenorphine

		Trauma group *(n =13)*	Control group *(n =18)*
Placebo	Anger	23.4 (11.7)	25.1 (9.6)
	Happy	9.4 (8.4)	18.0 (17.6)
	Sad	57.8 (16.4)	46.2 (14.8)
	Fear	11.4 (8.3)	9.1 (5.8)
Medication	Anger	20.4 (10.6)	22.4 (10.2)
	Happy	8.5 (5.8)	20.2 (13.3)
	Sad	60.5 (18.6)	45.2 (18.2)
	Fear	10.4 (8.6)	10.1 (7.4)

Note: Means, with standard deviations in brackets, presented.
Data were missing for one trauma group participant.

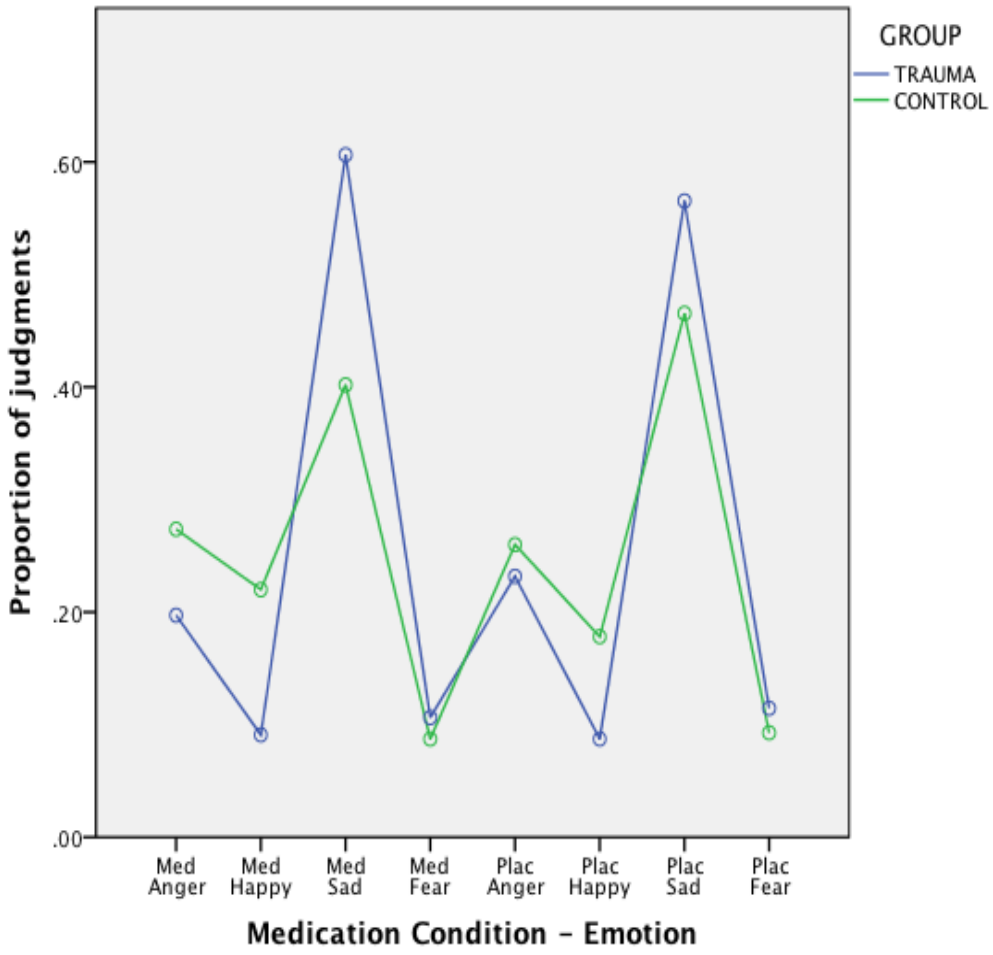

Figure 9:
Interaction of trauma status with medication–emotion judgment.

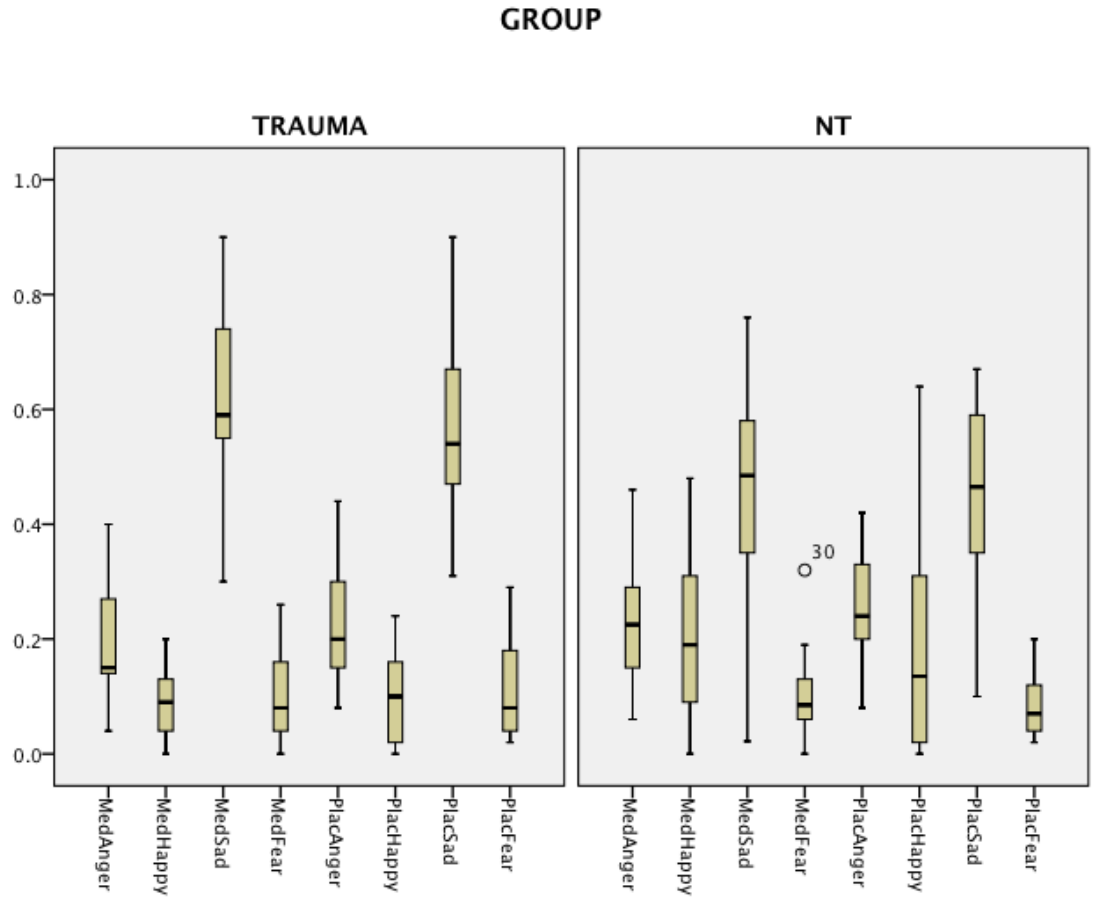

Figure 10:
Boxplot illustrating clear trend for the trauma group to judge neutral faces as sad, while greater variability is present in the control group.

Simple effects analysis of the interaction indicated the following: First, on placebo, the trauma group was significantly more likely than the control group to judge neutral faces as sad ($p = .049$). Second, on medication, two group differences were found: First, the control group was significantly more inclined to judge neutral expressions as showing happiness than was the trauma group ($p < .006$); and second, the trauma group was significantly more inclined to judge neutral expressions as showing sadness than was the control group ($p = .031$). (See Figure 10 for boxplots indicating dispersion of the data).

Interim summary: Impact of opioid manipulation on social cognition. In sum, these results indicate that the groups responded differently to opioid manipulation, but not entirely as predicted. On both placebo and medication, the trauma group was more likely to judge neutral faces as sad. On medication the control group was significantly more likely than the trauma group to judge neutral faces as happy.

Impact of Opioid Manipulation on Neural Activation

The following results are based on data from the fMRI subset sample which comprised 22 participants; 11 had been exposed to early social trauma, and 11 had not. The results for the fMRI subset on all the measures listed above followed the same trends as those for the full sample, and can be found in Appendix D.

fMRI results. The fMRI protocol featured a three-factor crossover design, with one between-group factor (trauma status) and two repeated measures (medication condition and emotion). With this complex design, a multitude of contrasts were possible. However, I had a very specific set of predictions that I wished to test, which enabled me to limit the number of contrasts. First, it was important to establish whether the low dose of buprenorphine resulted in any observable change in brain activation. Specifically, I predicted that relative to placebo, medication would result in reduced activation in response to negative social signals (i.e. faces expressing fear and anger). I predicted that the response to the control condition (faces expressing happiness) would be unchanged from placebo to medication.

Second, I aimed to investigate whether any change due to medication differed across the groups. I predicted that participants who had been exposed to early social trauma would show enhanced response to the opioid manipulation. This would mean that, on medication, the trauma group would show greater reduction in response to negative emotions than the control group.

Whole brain analysis. A 3-way random effects ANOVA with two repeated measures – 2 (group) x 2 (medication condition) x 3 (emotion) – was run, using

percentage transformation and separate subject predictors. Significant effects were indicated (q(FDR) < .05).

With regard to establishing that the dose of buprenorphine resulted in an observable change in brain activation, a significant effect for medication was seen for all three emotions (see Tables 12, 13, and 14), indicating a difference in neural activation on medication relative to placebo for viewing fearful, angry and happy faces.

The prediction of reduced activation in response to negative emotions on medication was not supported in this analysis, however. Although more activations in response to fearful faces indicated a reduced response on medication, almost as many indicated the opposite (see Table 12, negative activations). Greater activation on buprenorphine was generally observed for angry and for happy faces. Activations in response to the three emotions were seen in a variety of regions, including cortical, subcortical, and limbic areas. The largest areas of activation when viewing fearful faces (indicating reduced activation on medication compared to placebo) involved the precuneus and anterior cingulate cortex. The largest areas of activation when viewing angry faces (in this instance indicating increased activation on medication compared to placebo) also involved the precuneus and anterior cingulate cortex; in addition, a large area of activation was evident in the lingual gyrus. The largest area of activation when viewing happy faces (indicating increased activation on medication compared to placebo) involved the left middle frontal gyrus.

With regard to establishing whether any change due to medication differed across the groups, the interaction of group by medication was not significant in this analysis.

Given the unexpected direction of the effects for medication indicated above, and the *a priori* prediction that there would be a between-groups difference in response to the opioid manipulation, I considered it possible that group differences were being obscured in the whole brain analysis. It is very likely that the small sample size (only 11 per group), the huge number of voxels under analysis, and the rigors of the random effects analysis resulted in insufficient power to detect small effects. I therefore conducted region of interest (ROI) analysis to investigate group differences.

Table 12

Whole brain analysis: Fearful faces elicit greater activation on placebo than buprenorphine (peak voxels reported)

		Talairach	Co-ordinates	(mm)			Cluster size
POSITIVE ACTIVATIONS	**Side**	***x***	***y***	***z***	***t***	***p***	**(voxels)**
CORTICAL REGIONS							
Superior frontal gyrus	R	21	23	46	2.80	0.00762	155
Middle frontal gyrus	R	30	14	49	3.75	0.00053	190
Superior temporal gyrus[b,1]	R	60	-4	1	3.19	0.00272	163
Superior temporal gyrus	R	45	-46	22	3.59	0.00086	344
Superior temporal gyrus	R	48	-37	16	3.42	0.00140	115
Middle temporal gyrus	R	39	-61	10	3.42	0.00142	525
Superior frontal gyrus	L	-21	29	43	3.56	0.00093	454
Middle frontal gyrus	L	-39	11	37	3.28	0.00208	361
Frontal - precentral gyrus	L	-42	-4	37	2.82	0.00726	163
Middle temporal gyrus	L	-63	-16	-5	3.45	0.00128	291
Parietal - precuneus	L	-9	-49	40	3.62	0.00079	1835
Parietal - precuneus	L	0	-73	40	2.92	0.00563	253
Middle occipital gyrus	L	-15	-94	7	3.50	0.00110	482
Middle occipital gyrus	L	-42	-64	7	3.14	0.00309	338
LIMBIC REGIONS							
Anterior cingulate[a]	R	3	5	-5	3.14	0.00306	148
Anterior cingulate[a]	L	-6	23	-2	3.05	0.00393	180
Caudate[a]	R	18	-10	28	3.01	0.00446	118
SUBCORTICAL							
Pons[b,2]	L	-6	-25	-26	2.97	0.00497	260
NEGATIVE ACTIVATIONS							
CORTICAL REGIONS							
Inferior frontal gyrus	R	42	26	4	3.71	0.00060	269
Supramarginal gyrus	R	51	-34	37	4.19	0.00014	213
Superior occipital gyrus	R	21	-73	34	2.98	0.00471	494
Occipital - cuneus	R	12	-79	1	2.90	0.00590	227
Frontal - precentral gyrus	L	-57	5	28	3.49	0.00114	373
Parietal - postcentral gyrus	L	-39	-28	34	3.19	0.00268	411
Inferior parietal lobule	L	-54	-28	22	4.02	0.00024	524
LIMBIC REGIONS							
Anterior cingulate[a]	L	-3	23	13	3.60	0.00082	2863
Anterior cingulate[a]	L	-21	38	7	3.17	0.00288	1037
SUBCORTICAL							
Thalamus[a]	R	18	-19	1	3.09	0.00352	139
Pons[a]	R	6	-37	-29	3.70	0.00062	143

Note: Uncorrected *p* values reported.

a: Regions of interest selected for planned contrasts

b: Regions of interest featuring significant group differences on planned contrasts.

1 – 2: The numbers refer to regions in the ROI tables below.

Table 13

Whole brain analysis: Angry faces elicit greater activation on buprenorphine than placebo (peak voxels reported)

		Talairach Co-ordinates (mm)					Cluster size
POSITIVE ACTIVATIONS	**Side**	***x***	***y***	***z***	***t***	***p***	**(voxels)**
CORTICAL REGIONS							
Inferior frontal gyrus	R	48	26	7	3.32	0.00188	162
Orbital frontal gyrus	R	39	35	-5	2.67	0.01070	240
Middle frontal gyrus	R	21	44	13	2.88	0.00622	750
Parietal - precuneus	R	21	-46	34	3.75	0.00054	1589
Parietal - precuneus	R	6	-67	37	3.06	0.00387	119
Occipital - precuneus	R	21	-67	28	3.87	0.00037	1553
Occipital - lingual gyrus	R	12	-52	4	4.03	0.00023	422
Occipital - lingual gyrus	R	12	-82	7	3.71	0.00060	1091
Superior frontal gyrus[b,3]	L	-12	5	46	2.76	0.00862	115
Middle frontal gyrus	L	-24	47	10	2.95	0.00525	118
Middle frontal gyrus	L	-30	26	28	2.70	0.00983	149
Frontal - precentral gyrus	L	-57	2	28	3.18	0.00277	254
Parietal - postcentral gyrus	L	-54	-28	19	3.05	0.00400	120
Inferior temporal gyrus	L	-48	-67	-2	3.45	0.00130	203
Middle temporal gyrus	L	-51	-19	-14	3.59	0.00085	125
Middle temporal gyrus	L	-57	-52	-8	3.23	0.00242	131
Occipital - lingual gyrus	L	-15	-61	1	3.02	0.00427	175
LIMBIC REGIONS							
Insula[b,4]	R	39	14	-2	3.27	0.00216	458
Anterior cingulate[a]	L	-18	35	16	3.24	0.00235	923
Parahippocampal gyrus	L	-27	-40	-5	3.47	0.00120	714
SUBCORTICAL							
Claustrum[a]	R	27	14	13	2.93	0.00550	108
Caudate[a]	R	9	20	7	2.61	0.01237	112
Midbrain[a]	L	0	-10	-8	3.00	0.00449	172
Head of caudate[a]	L	-12	14	-2	2.80	0.00762	148
NEGATIVE ACTIVATIONS							
Superior temporal gyrus[b,5]	R	60	-4	-2	3.09	0.00355	110
Cingulate gyrus[a]	R	9	-10	46	3.17	0.00287	198
Paracentral lobule	L	-12	-22	49	3.80	0.00046	250
Superior occipital gyrus	L	-18	-94	1	3.51	0.00107	705
Pons[a]	L	0	-28	-32	3.11	0.00338	415

Note: Uncorrected *p* values reported.

a: Regions of interest selected for planned contrasts

b: Regions of interest featuring significant group differences on planned contrasts.

3 – 5: The numbers refer to regions in the ROI tables below.

Table 14

Whole brain analysis: Happy faces elicit greater activation on buprenorphine than placebo (peak voxels reported)

		Talairach Co-ordinates (mm)					Cluster size
POSITIVE ACTIVATIONS	**Side**	***x***	***y***	***z***	***t***	***p***	**(voxels)**
CORTICAL REGIONS							
Superior frontal gyrus	R	15	23	49	4.26	0.00011	414
Superior frontal gyrus[b,6]	R	9	2	52	3.57	0.00091	501
Middle frontal gyrus	R	36	20	34	3.66	0.00070	392
Middle frontal gyrus	R	24	-10	49	3.96	0.00029	913
Frontal - paracentral lobule	R	6	-31	46	3.87	0.00038	503
Parietal - postcentral gyrus	R	33	-22	46	4.34	0.00010	329
Parietal - postcentral gyrus	R	51	-25	40	3.74	0.00055	233
Parietal - precuneus	R	18	-79	34	3.44	0.00132	513
Occipital - cuneus	R	12	-52	4	3.57	0.00091	119
Occipital - lingual gyrus	R	9	-76	-2	3.81	0.00045	165
Superior frontal gyrus	L	-12	-22	64	4.99	0.00001	384
Middle frontal gyrus	L	-21	2	61	4.98	0.00001	1910
Middle frontal gyrus	L	-33	20	40	3.07	0.00378	206
Frontal - precentral gyrus	L	-28	-16	67	3.46	0.00124	171
Superior temporal gyrus	L	-39	-52	28	3.55	0.00095	349
Parietal - postcentral gyrus	L	-21	-37	52	4.15	0.00016	210
Parietal lobule	L	-6	-7	55	4.64	0.00003	421
Occipital - precuneus	L	-15	-55	16	3.22	0.00246	261
LIMBIC REGIONS							
Cingulate gyrus[a]	R	9	-16	34	3.92	0.00032	247
Cingulate gyrus[a]	R	15	-1	46	3.45	0.00129	242
Cingulate gyrus[a]	R	6	-1	28	4.07	0.00020	116
Posterior cingulate	R	12	-67	7	3.31	0.00190	166
Insula[b,7]	R	45	-7	22	3.63	0.00077	322
Anterior cingulate[a]	L	-3	23	16	3.10	0.00346	109
Insula[a]	L	-36	-7	10	3.71	0.00061	505
Insula[b,8]	L	-45	-13	19	3.53	0.00101	127
SUBCORTICAL							
Caudate[a]	L	-18	26	1	4.22	0.00013	540
NEGATIVE ACTIVATIONS							
CORTICAL REGIONS							
Superior frontal gyrus	L	-9	59	13	3.47	0.00121	186
Middle temporal gyrus	R	48	-61	10	3.57	0.00092	353

Note: Uncorrected *p* values reported.
a: Regions of interest selected for planned contrasts
b: Regions of interest featuring significant group differences on planned contrasts.
6 – 8: The numbers refer to regions in the ROI tables below.

Region of interest analysis. The key question to be addressed in the functional imaging analysis was whether group differences in the response to the opioid medication could be established, particularly with regard to the negative social signals conveyed by fearful and angry faces. ROI analysis provided greater power for this critical between-groups comparison, as exponentially fewer voxels were included in each analysis. ROIs were functionally defined: Areas of theoretical interest that reached significance in the whole brain analysis were selected as ROIs, within which specific contrasts were investigated. These areas of theoretical interest were, chiefly, anterior limbic and subcortical structures (Alcaro & Panksepp, in press; Braun et al., 2000; Damasio et al., 2000; Eisenberger et al., 2006; Eisenberger et al., 2003; Panksepp, 1998; Panksepp, 2005a; Poeggel et al., 1999; Ziabreva et al., 2003; Zubieta, Dannals & Frost, 1999; Zubieta et al., 2003). Only two posterior cortical regions were included: these were both anterior temporal regions that are high in mu-opioid receptors, and that are often implicated in limbic/emotional function (Maurer et al., 1983; Pfeiffer et al., 1982; Phan et al., 2002). I also included regions falling in ventral and mesial (as opposed to dorsal and lateral) prefrontal areas (see Tables 12, 13 and 14; Kennedy et al., 2006; Liberzon et al., 2007; Northoff et al., in press). These regions are all important in processing social-emotional information or mood states, or are part of the SEPARATION-DISTRESS or SEEKING systems.

The subsequent ROI analyses were independent of the original whole brain analysis, consisting of orthogonal planned contrasts. Random effects general linear model regressions tested the overall contrasts, followed by *t*-tests to examine possible group differences in the specified pattern of response. Once again for the ROI analysis, a number of comparisons were possible. I restricted the analysis to the following three contrasts that seemed particularly important to the research question under examination.

1) Anger + Fear (medication) < Anger + Fear (placebo)

This contrast tested the most critical prediction that compared to placebo, reduced response to negative social signals would be seen on buprenorphine. I predicted that this effect would be significantly greater for the trauma group than for the control group in key regions.

Table 15

Region of interest analysis: Response to negative social signals is reduced on buprenorphine compared to placebo

Region	Cluster size (voxels)	Contrast 1 Means		*t*	*p*
		Trauma group (n = 11)	Control group (n = 11)		
R Insula[4]	458	-0.05	-0.54	2.3	.02
L Insula[8]	505	0.18	-0.32	2.2	.02

Note: The numbers refer back to the regions selected from the whole brain analysis. Uncorrected *p* values reported.

The ROI in the right insula extended over the anterior region of the insula. The ROI in the left insula included activation in the orbital region of the inferior frontal gyrus, which extended to include the short gyri of the anterior insula (triangular part; see Figures 11 and 12).

The contrast means for the between-groups *t*-tests represent average percent signal change as weighted by the specified linear combination. They should be interpreted as follows: a positive group mean indicates that the change in activation is in the direction specified by the linear contrast. In this instance, a positive mean thus indicates that activation in response to negative social signals is less on medication than on placebo. A negative mean indicates the opposite pattern of activation to that specified in the linear contrast. In this instance, a negative mean indicates that activation in response to negative social signals is greater on medication than on placebo.

In both regions where a significant group difference was found, the difference was in the direction predicted (see Table 15). That is to say, the trauma group showed a reduced response to negative social signals on medication in comparison to the control group.

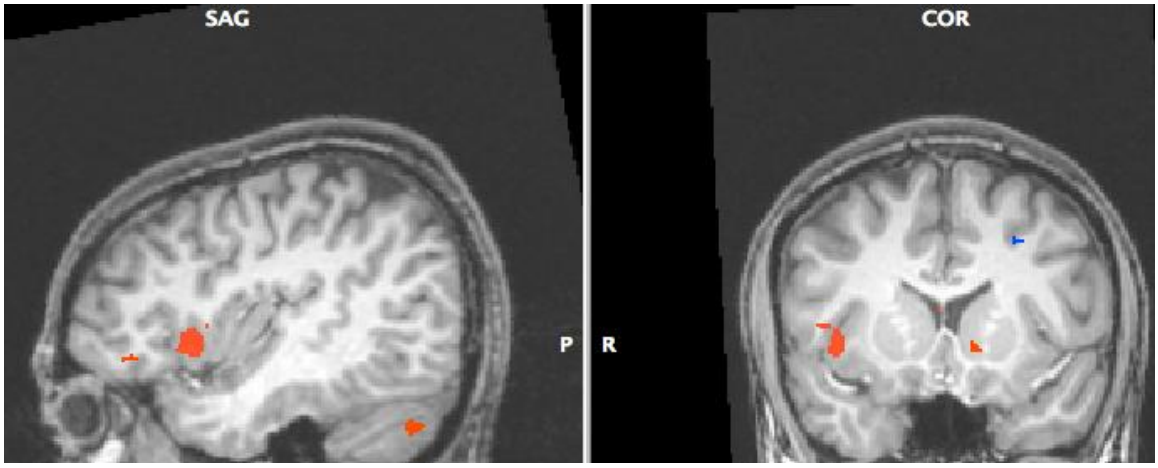

Figure 11:
ROI analysis: Group difference in reduced response to negative social signals on buprenorphine compared to placebo in the right anterior insula (peak voxels 39, 14, -2); t = 2.3, p < .02 (uncorrected); cluster size (voxels) = 458

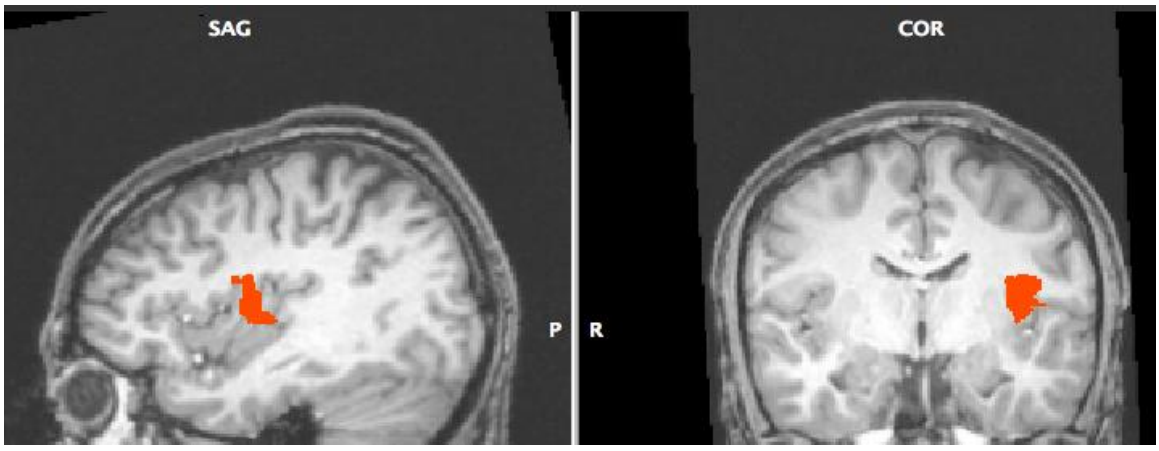

Figure 12:
ROI analysis: Group difference in reduced response to negative social signals on buprenorphine compared to placebo in the left anterior insula (peak voxels -45, -13, 19); t = 2.2, p < .02 (uncorrected); cluster size (voxels) = 505

With regard to the remaining contrasts, I predicted that, on placebo and compared to the control group, the trauma group would show greater reactivity in response to negative than positive social signals; a pattern of response that is, arguably, similar to or indicative of their normal, unmedicated response to such social signals. I also predicted that opioid manipulation would attenuate this effect.

2) Anger + Fear (placebo) > Happy (placebo)

This contrast tested the prediction that on placebo, greater activation would be seen in response to negative compared to positive social signals. I predicted that this effect would be significantly greater for the trauma group than for the control group. Group differences were found in six regions (see Table 16).

3) Anger + Fear (medication) > Happy (medication)

This contrast was conducted only in the six regions where group differences on response to negative social signals on placebo were found, to examine whether this effect was also evident on opioid medication. Group differences in response to negative social signals were not evident on buprenorphine (see Table 16).

Table 16

Region of interest analysis: Greater response to negative than to positive social signals on placebo (Contrast 2) and on medication (Contrast 3).

Region	Cluster size (voxels)	Contrast 2 Means		*t*	*p*	Contrast 3 Means		*t*	*p*
		Trauma (n = 11)	Control (n = 11)			Trauma (n = 11)	Control (n = 11)		
R Superior frontal gyrus[6]	501	0.17	-0.07	2.0	.03	-0.09	0.01	1.1	.15
L Superior frontal gyrus[3]	115	0.07	-0.10	2.4	.01	-0.01	-0.09	1.0	.16
R Superior temporal gyrus[5]	110	0.64	0.10	2.3	.02	-0.07	-0.08	0.1	.49
R Superior temporal gyrus[1]	163	0.59	0.15	2.0	.03	-0.12	-0.01	0.4	.33
R Insula[7]	322	0.17	-0.05	1.9	.04	-0.01	-0.07	0.7	.25
L Pons[2]	260	0.07	0.33	2.5	.01	-0.05,	-0.04	0.2	.40

Note: The numbers refer back to the regions selected from the whole brain analysis. Uncorrected *p* values reported.

Areas in the right and left superior frontal gyrus activated on Contrast 2; the left lateralized region involved the medial region of the superior frontal gyrus. Two rostral regions of the right superior temporal gyrus were implicated. In the first of these regions, the activation incorporated the superior temporal gyrus and superior temporal sulcus, but

did not extend to the middle temporal gyrus. In the second right temporal region, the activation also included superior temporal gyrus and sulcus, and did extend to the middle temporal gyrus. The right insula activation included the inferior border of the inferior frontal gyrus (posterior to orbital region), and extended to the long gyrus of the insula. The region of activation in the left pons encompassed a large area; any number of nuclei could be involved, including the ARAS, raphe nuclei and the trigeminal nerve.

In all five cortical regions where significant group differences were indicated, the trauma group showed the pattern predicted by the contrast – i.e., on placebo they showed greater activation than the control group in response to negative than positive social signals. In the pons, the trauma group again evidenced greater activation in response to negative than positive signals; however in this region this effect was greater in the control group. Group differences were not evident on opioid medication (see Table 16).

The patterns of activation shown by the control group were more variable: In three regions, the control group showed the opposite pattern to that predicted – i.e. on placebo, they showed reduced activation in response to negative compared to positive social signals.

In three regions the control group showed the same pattern as the trauma group (i.e. the pattern predicted by the contrast); in two of these regions the effect was significantly less pronounced in the control group than in the trauma group. In only one region (left pons) where both groups showed the predicted pattern of increased activation in response to negative emotions, was this effect greater in the control than the trauma group.

Interim summary: Impact of opioid manipulation on neural activation. In summary, the fMRI results indicate that the low dose opioid was sufficient to produce observable changes in neural activation relative to placebo. The ROI analysis confirmed that the groups responded differently to opioid manipulation, with reduced response to negative social signals on medication generally being more apparent in the trauma group.

Discussion

This study constitutes the first step in an investigation of the premise that opioid dysregulation following early social trauma plays a key role in the etiology of certain cases of unipolar depression. By examining baseline characteristics, and the impact of opioid manipulation on subjective affective states, social cognition, and neural activation in medically and psychiatrically healthy normal participants who differed only in terms of exposure to early social trauma, I hoped to find indications that opioid function differed across these individuals. Although not all predictions were upheld, I found between-group differences in all measured domains. The most important indication that opioid function may be dysregulated in trauma-exposed, but psychiatrically healthy individuals is the fact that those participants consistently differed from controls in terms of their response to the opioid manipulation.

Participant Characteristics

The sample consisted of psychiatrically healthy young adults. Participants differed primarily on one key variable: exposure to early social trauma. As indicated in the rationale for this study, this construct was very broadly defined, due to clear indications in the literature that various interpersonal social traumas are associated with negative outcomes (see, e.g., Flinn et al., 2011; Heim & Nemeroff, 1999; Heim et al., 2010; Nepomnaschy & Flinn, 2009; Slavich et al., 2010; Widom et al., 2007). Early social trauma was operationalized using the Childhood Trauma Questionnaire – Short Form (CTQ-SF; Bernstein et al., 2003), which measures childhood exposure to physical and emotional neglect, as well as to sexual, physical and emotional abuse.

The trauma and control groups differed significantly on all five CTQ-SF subscales. Estimated effect sizes indicated substantial between-group differences on all subscales. The control group's subscale scores all fell within the *none-minimal* clinical range, whereas the trauma group's scores fell in the *low-moderate* range for physical and emotional neglect, and in the *moderate-severe* range for sexual, physical and emotional abuse. I was thus successful in using recruitment and exclusion criteria to set up

comparison groups with real differences in their exposure to relevant early social traumatic experiences.

The Mini International Neuropsychiatric Interview (MINI; Lecrubier et al., 1997; Sheehan et al., 1997) was used to screen out participants with any psychopathology, including anxiety disorders and depression. The Beck Depression Inventory-Second Edition (BDI-II ; Beck et al., 1996) was used to complement the MINI in screening for depression, and any individual who scored in the *mild* symptom range, or higher, was excluded. The groups were thus equivalent on BDI-II depression scores, scoring in the *none-minimal* symptom range on this measure.

It is true that a number of participants did not complete the BDI-II, and that of these, disproportionately more were from the trauma than from the control group. The data collected showed that, although no significant between-group differences were present, the trauma group scored slightly higher than controls. It is thus possible that this difference might have been more pronounced had all the trauma group participants completed the BDI-II. In other words, it is possible that some of the trauma group participants who did not complete this measure might have been depressed. However, this scenario is unlikely, as I also screened for depression using the MINI, a sensitive measure of psychiatric dysfunction that errs on the side of false positives (Sheehan et al., 1998). The missing data are therefore unlikely to have impacted unduly on participant inclusion, or on between-group differences in depression scores.

That being said, it is not unreasonable to think that even psychiatrically normal individuals who have had difficult childhoods might have a tendency to experience more negative affect than controls. This indeed was hypothesized for negative affect on the Positive and Negative Affect Schedule *(PANAS; Watson et al., 1988)* and for the SADNESS subscale on the Affective Neuroscience Personality Scales (Davis et al., 2003). However, as is made evident below, the trauma and control groups showed no signs of differing on any baseline measures of negative affect.

I used the PANAS-general ratings to obtain a measure of participants' everyday emotional experience. I predicted that the trauma group would experience more negative emotion, and less positive emotion, than the control group. These predictions were not supported. There were no between-group differences on experience of either positive or

negative affect, and both groups reported experiencing significantly more positive than negative affect. Given that our trauma group participants were selected specifically for being psychiatrically healthy (and even more specifically, for not being depressed), this lack of a between-group difference is, perhaps, unsurprising. It is entirely possible that, in terms of the hypothesized mechanisms of depression, this group represents atypical and especially resilient individuals. The implications of this possibility are discussed further below.

The only baseline group difference I did find was on the ANPS – notably, the only measure designed specifically to measure long-term differences in the key core emotion systems that are the focus of interest in this study. As with negative affect on the PANAS, I predicted that the groups would differ on the ANPS SADNESS subscale, with the trauma group scoring higher than the control group. However, this prediction was not confirmed; the groups obtained similar scores on the SADNESS subscale. The predicted group difference on the SEEKING subscale was, however, confirmed; the control group scored significantly higher than the trauma group, and the estimated effect size indicated that a large between-group difference was present. This result indicates that general motivational and positive expectancy states were greater in the control than in the trauma group.

As mentioned above, the groups were both psychiatrically healthy, so once again finding no between-group difference on negative feelings, in this case SADNESS, should perhaps not be surprising. The groups also scored very similarly on the other negative emotion subscales (FEAR and ANGER). The possibility, outlined briefly above, that the trauma group is perhaps atypical and resilient in terms of the hypothesized mechanisms of depression under investigation here, is one that must be considered. The broad thesis is that early separation-distress may lead to dysregulation of opioid function, which then plays a causal role as a mechanism underlying depression; yet the current study's sample was comprised of only non-depressed trauma-exposed individuals. As discussed in the rationale for this research, there was good reason to include only healthy individuals at this point in the research program, but the fact remains that I have assessed an atypical and perhaps very resilient group. These participants were not depressed, they did not experience more negative affect in general, and they specifically did not differ from the

control group on the SADNESS subscale. The fact that the data were able to demonstrate a between-group difference on SEEKING, however, is most encouraging.

These ANPS results can be explained in terms of the most recent articulations of how early separation-distress feeds into depression. As indicated in the literature review, from this perspective depression is conceptualized as resulting from an evolutionarily conserved mechanism that serves to end ongoing activation of the SEPARATION-DISTRESS system. In Bowlby's (1969) terms, separation-distress constitutes a 'protest' phase that can be observed in the vocalizations of young animals and children. This social pain motivates the animal or child to attempt to re-establish vital social contact. However, if this contact is not achieved, a second phase – 'despair' – sets in.

The despair phase is characterized by the shutdown of SEPARATION-DISTRESS behavior and chemistries. As explained in the review above, this shutdown doubtless has benefits in evolutionary terms, as the youngster does not stray too far, avoids metabolic exhaustion, and ceases to announce its vulnerability to potential predators. The shutdown state is characterized by the abandonment of goals, viz., reduced SEEKING. A key point made in the recent papers (Alcaro & Panksepp, in press; Panksepp & Watt, 2011; Watt & Panksepp, 2009) is that, although this shutdown is usually self-limiting, it can become released from normal control mechanisms. In this instance it can become pathologically active in response to even mild stressors, which can result in depression This new articulation of the separation-distress model of depression thus directly links the SEPARATION-DISTRESS and SEEKING systems in the genesis of depression.

This recent work also elucidates the chemical mechanisms that accomplish the switch from distress or protest to despair. The neurochemical underpinnings of this switch are thought to involve, primarily, raised levels of kappa-opioids, particularly in the nucleus accumbens and ventral tegmental area. Kappa-opioids promote dysphoria and reduce pleasure by inhibiting SEEKING – they downregulate mesolimbic dopamine expression and transmission (Alcaro & Panksepp, in press; Zellner et al., in press).

It may thus be that the experience of early social trauma in the healthy participants in the current study has led to long-term downregulation of this system – not at a pathological level, as they have no psychiatric disorder – but at a level sufficient to

be discernible on a trait measure of this SEEKING disposition. Because they are atypical and resilient, elevated negative affect and signs of depression are not evident. However, we do see a reduction in motivation to engage with the world, and in pleasurable expectancy and anticipation states. In a sample of depressed participants, we might expect to see elevated negative affect, along with more profound inhibition of the SEEKING system.

In terms of limitations, it may seem problematic that I chose to begin the examination of a broad thesis proposing that early social trauma plays a role in the etiology of depression by assessing only non-depressed, psychiatrically healthy participants. In terms of this broad thesis, these participants constitute an atypical group. However, I must reiterate that there were sound scientific reasons for beginning the investigation in healthy, normal individuals. Exposure to early social adversity is not synonymous with depression. It is important to investigate opioid function following such exposure in its own right, prior to investigating it in the clinical population. The heterogeneity (of both clinical presentations and neurobiological correlates) that exists within major depressive disorder presents a significant challenge when conducting research with this population. The information regarding the nature of opioid dysregulation subsequent to early social trauma that has now been provided by using a 'clean' population gives a basis from which to build, and will inform future work that does include individuals with depression.

Two ethical concerns were also accounted for, given this choice of participants: 1) Little work using buprenorphine has been done in opiate-naïve participants; it was thus important to be sure of the medication's effects and tolerability at the chosen dose, before administering it to individuals from a clinical population. 2) The study featured a novel fMRI protocol and a very low-dose pharmacological challenge; it was important to ascertain whether these methods could yield any discernible effects, prior to implementing them in a clinical population.

Although in using non-depressed participants I incurred the risk of seeing weaker effects than would be present in depressed participants, it was nonetheless possible that some difference in opioid function would be discernible in this group of trauma-exposed participants. Given that this group was probably atypical and resilient, the fact that a

substantial between-group difference on SEEKING was evident, is a very promising sign that effects of early social trauma on opioid function are indeed present and well worth investigating further.

Another interesting observation is that the groups seem to score differently on the CARE subscale, with the trauma group scoring higher on this dispositional trait. Recent papers suggest that prosocial activities involving the CARE and PLAY systems may act to protect against the effects of early social losses, and may have therapeutic effects in depression, as they result in the release of neurochemistries that promote positive social-affective states (Panksepp, 2010; Panksepp & Watt, 2011; Watt & Panksepp, 2009). In the trauma group, elevated CARE subscale scores thus might reflect a resilience factor. This possibility should certainly be pursued in future work.

Impact of Opioid Manipulation on Subjective Experience of Affect

I used the PANAS-moment ratings (i.e., participants self-ratings of how they felt in the moment) to assess whether the administration of buprenorphine had any observable impact on current experience of affect. I predicted that on placebo, the groups would show the same pattern of affective experience as that predicted for general experience of emotion: that is, the trauma group would experience greater negative affect and less positive affect than the control group. I also predicted that the groups would differ in their response to the opioid manipulation, with the trauma group responding more strongly. I predicted that this stronger response to the opioid manipulation in the trauma group would result in the experience of positive and negative affect being equalized across the groups; that is, on medication the trauma group would not differ from the control group on negative or positive affect. Overall, the results indicated that the trauma group did respond more strongly to the medication, but not in a straightforward way.

There was a significant interaction between trauma status and the combined medication-condition/affect factor. Simple effects analysis of this interaction indicated the following with regard to positive affect: on placebo, the groups reported experiencing similar levels of positive affect. However, on medication, the control group reported experiencing significantly more positive affect than did the trauma group. With regard to

negative affect, on placebo the trauma group reported significantly more negative affect than did the control group. However, on medication, this difference was not evident.

It must be noted that there were no significant within-group effects. In other words, the opioid manipulation did not result in a significant change in the experience of either positive or negative affect in either group. This may well be due to the small sizes of the groups, and resultant low power. (Post-hoc power calculations for this sample size indicate a < 30% chance of detecting small effects; and as indicated below, a small effect may have been present in the trauma group).

Close inspection of the data indicates that the between-group interaction effects were largely due to decreases in the trauma group scores on medication. The observed interaction effect where the control group reported significantly more positive affect on medication is in fact a function of the trauma group's score dropping by almost 4 points (on a scale with a maximum of 50), and the control group going up very slightly (almost 1 point) on medication. The observed interaction effect where the between-group difference in negative affect on placebo disappeared on medication is again due to the trauma group's score dropping (by nearly 2 points), and the control group going up very slightly (nearly 0.5 points).

Thus, the opioid manipulation did not have a straightforward effect of increasing positive affect and reducing negative affect in either group. It seems that buprenorphine reduced the experience of both positive and negative emotion in the trauma group, and had little effect on emotional experience in the controls. Two points are worth considering with regard to these results: first, buprenorphine is an analgesic, so as part of its sedative effect a general dampening of emotional experience is possible (Boothby & Doering, 2007; Greenwald et al., 2007). However, this pattern was *only* evident in the trauma group, so it being the result of a general sedative effect is unlikely. Perhaps the important observation here is that the groups responded *differently* to the opioid manipulation. One might argue that this difference reflects different functioning of the opioid system across the groups, and in fact supports the hypothesis that individuals exposed to early social trauma would be more sensitive to opioid manipulation.

Second, the effects of buprenorphine on mood in opiate-naïve participants are not unequivocal. Buprenorphine is not a full mu-opioid agonist, and so it does not create the

marked euphoria characteristic of those substances; it generally has only modest mood elevating effects (Ciraulo et al., 2006; Stefano et al., 2000). The documented mood improvements in recovering addicts and in small clinical trials using buprenorphine occur following periods of consistent administration, from 5 days to several weeks (Bodkin et al., 1995; Emrich et al., 1982; Kosten et al., 1990; Mongan & Calloway, 1990; Nunes & Levin, 2004). It is thus possible that the ameliorative effects on mood are not evident during a once-off pharmacological challenge, and only occur following repeated administration.

With regard to limitations, the very stringent inclusion/exclusion criteria employed resulted in very small comparison groups, despite prolonged recruitment efforts. Having small groups impacts on results not only in the simple terms of reduced statistical power; it in fact affects the likelihood that the patterns seen in the data are reliable. Those described here thus need to be confirmed in future work. One encouraging sign that these results may reflect reliable data patterns is that the same patterns were found for both the full sample and the fMRI subset: if unusual individuals in the small sample were skewing the data, it would be more likely that data patterns would be unstable across the samples.

Impact of Opioid Manipulation on Social Cognition

Subliminal presentation of faces showing four basic emotions (anger, fear, sadness, and happiness) was used to evaluate bias in assigning one of these four emotions to the presentation of neutral faces. The opioid system is strongly implicated in social functioning. If the idea is correct that exposure to early social trauma leads to opioid dysfunction, which in turn contributes to depression, negativity biases in processing social-emotional information might exist in the trauma group. Negativity biases exist in depression, and are evident, for example, in tendencies to have better recall for negatively valenced material, and to attend more closely to negative stimuli (Beck, 1967, 1987; Matt, Vazquez & Campbell, 1992). They are also evident in processing social and emotional stimuli (Gilboa-Schechtman, Erhard-Weiss & Jecsemien, 2002; Miskowiak et al., 2007; Poulsen, Luu, Crane, Quiring & Tucker, 2009). A specific bias towards interpreting neutral faces as showing negative emotions has been documented in

depression (see, e.g., Bouhuys, Geerts, Mersch & Jenner, 1996; Gur, Erwin, Gur & Zwil, 1992). Thus I predicted that this bias would be present, to some extent, in these psychiatrically healthy trauma-exposed participants.

I predicted that on placebo, a negativity bias would be evident in the trauma group. These participants would thus be more likely than controls to judge neutral faces as showing negative emotions (anger, sadness or fear). I predicted that the opioid manipulation would attenuate this bias, and that therefore, on medication, this tendency would no longer be evident in the trauma group.

Results showed a significant interaction between trauma status and the combined medication condition/emotion factor. Simple effects analysis indicated that on both placebo and medication, the trauma group was more likely than the control group to judge neutral faces as showing sadness. On the opioid medication, the control group was more likely than the trauma group to judge neutral faces as showing happiness.

The predicted negativity bias in the trauma group on placebo was thus only evident for sadness; both groups judged similar proportions of neutral faces as showing anger or fear. Contrary to prediction, buprenorphine administration did not reduce the bias in the trauma group: these participants also judged significantly more neutral faces as sad on medication.

Buprenorphine administration did result in a significant between-group difference in the proportion of neutral faces judged to be showing happiness: the control group made significantly more of these judgments on medication than did the trauma group. This trend was present on placebo (9.4% vs. 18% of neutral faces judged happy by the trauma and control groups respectively), but the difference became statistically significant on medication (8.5% vs. 20.2%).

It should be noted that there were no within-group differences, so there is no direct evidence of the opioid medication producing marked changes in each group's interpretation of the neutral faces. Once again, this is perhaps due to the impact of the small group sizes on statistical power to detect small effects; alternatively, there is the possibility that consistent administration of buprenorphine might be required to produce significant effects.

It should also be noted that the greatest proportion of all judgments for both groups on and off opioid medication featured sadness (58.7% and 60.5% for the trauma group and 46.2% and 45.2% for the control group, respectively). It thus seems that neutral expressions were most easily mistaken for sadness by all participants. A number of earlier studies have reported similar findings (Arce et al., 2009; Gur et al., 2002; Rojahn & Warren, 1997). In those studies, the tendency to attribute sadness at greater rates than other basic emotions to neutral faces was found in both normal and psychiatric populations. This tendency to perceive neutral faces as sad is arguably due to the fact that sad facial expressions are least distinctive when compared to angry, fearful or happy expressions, and hence closest to neutral.

However, the increased tendency to interpret neutral faces as sad was clearly more pronounced in the trauma group (Figure 10 clearly illustrates this point). Difficulties recognizing facial emotions and a bias towards interpreting neutral faces as showing negative emotions have been documented in depression (Bouhuys et al., 1996; Gur et al., 1992), but are also evident in subclinical cases of depression, and in highly neurotic individuals who are not depressed (Arce et al., 2009; Chan, Goodwin & Harmer, 2007; Williams, Watts, MacLeod & Mathews, 1997). It has thus been argued that the negativity bias precedes and is a risk factor for depression (Chan, Harmer, Goodwin & Norbury, 2008). Because interpreting ambiguous facial expressions is thought to play an important role in social and interpersonal relationships, examining the way in which individuals interpret neutral expressions gives insight into how they are likely to respond in complex social situations (Arce et al., 2009; Bouhuys et al., 1996; Gilboa-Schechtman et al., 2002; Gur et al., 1992; Surguladze et al., 2004). As indicated above, negativity biases, including a tendency to interpret ambiguous social situations in a negative light, are thought to represent a vulnerability factor for depression. The trauma group's tendency to interpret ambiguous social signals as negative might thus represent such a vulnerability factor in this group of participants.

On medication, there was an increased bias in the control group towards judging neutral faces as happy. This bias has previously been documented in highly resilient individuals (Arce et al., 2009), and tends to be interpreted as supporting the argument that resilient individuals are able to focus on and generate positive emotions, which helps

them cope successfully with stress (Ong, Bergman, Bisconti & Wallace, 2006; Tugade, Fredrickson & Barrett, 2004; Werner & Smith, 1992). This data thus suggests that opioid manipulation strengthens a positivity bias in interpreting ambiguous social signals in individuals who have not been exposed to early social trauma.

Overall, the data thus support the contention that exposure to early social trauma has a long-term effect on social cognition, even in psychiatrically healthy individuals. Trauma-exposed participants showed a persistent negative bias towards sadness, which was not attenuated by opioid medication. These participants showed no sign of the positive bias exhibited by the controls. A negative bias in interpreting complex social emotional information may have important effects in social interactions, and may represent a vulnerability factor in the trauma group. Importantly, these data again indicated that the groups responded differently to the opioid manipulation, supporting the contention that opioid function is dysregulated in individuals exposed to early social trauma.

In terms of limitations, why did the medication not attenuate the negativity bias towards sadness evident in the trauma group? Perhaps, as argued in the section above in relation to mood, longer periods of consistent administration of buprenorphine might be required to restore the opioid system to relatively normal functioning. Long-term treatment of depression with SSRIs can reduce negativity biases (Curry et al., 2006; Simons, Garfield & Murphy, 1984), and there are indications in the literature that even once-off administration of SSRIs can reduce such biases in processing emotional material (Browning, Reid, Cowen, Goodwin & Harmer, 2007; Miskowiak et al., 2007; Norbury, Mackay, Cowen, Goodwin & Harmer, 2008).

However, although SSRI treatment may reduce the negativity bias, this bias may exist independently of diagnosable depression. It is seen in subclinical cases and in highly neurotic never-depressed individuals; it may remain present even when depressive symptoms remit following treatment (Bouhuys et al., 1996; Mayberg, 2004; Williams et al., 1997). It could be that medications targeting chemistries that primarily mediate affect have limited impact on eradicating negative cognitive biases – these might need to be unlearned in a structured way. Further work will be needed to ascertain whether the negativity bias demonstrated here responds to consistent administration of buprenorphine,

and to what extent this bias acts as a vulnerability factor for increased negative affect or depression in individuals exposed to early social trauma.

Impact of Opioid Manipulation on Neural Activation

The main goal of the fMRI investigation was to establish whether or not the opioid manipulation led to observable between-group differences in neural activation, particularly in response to negative social signals. I used a neuroimaging task that featured dynamic morphing of facial expressions, from neutral to extreme emotion, to investigate this possibility. In the following subsections, I will first discuss this neuroimaging task, then the whole brain analysis, and then the ROI analysis, before moving on to a consideration of limitations of the neuroimaging component of this study.

The passive viewing of emotions protocol. Because the use of passive viewing of dynamic stimuli is fairly unusual, and because the use of a novel fMRI protocol can be problematic, it seems important to justify the selection of this particular protocol. The aim of the task was to expose participants to powerful negative social stimuli in the form of faces that morphed quickly from neutral to extreme fearful or angry expressions (with happiness serving as a positive control).[8] I wanted to access participants' unmediated automatic responses to these signals, and thus no cognitive task was involved. Participants were not required to perform an explicit emotion recognition task; they simply watched the stimuli.

As mentioned briefly in the Methods section, the bulk of functional neuroimaging work investigating the processing of facial emotion has used static images (see, e.g., Costafreda, Brammer, David & Fu, 2008; Habel et al., 2007; Hariri et al., 2002; Morris et al., 1998; Norbury, Mackay, Cowen, Goodwin & Harmer, 2007; Phillips et al., 2001; Whalen et al., 2001). In real life, however, dynamic changes in expression provide vital social information in that they give us immediate indications of changes in others' emotional states.

[8] As indicated in the Methods section, time constraints and considerations from the larger study mandated that only these emotions could be included in the protocol. Given the results regarding sadness in the previous section, it would be most beneficial to include this emotion in this task in future work.

A small body of research shows that using dynamic stimuli to present facial expressions has important consequences. These dynamic stimuli evoke different perceptual, cognitive and emotional processing (Pelphrey, Singerman, Allison & McCarthy, 2003; Puce, Allison, Bentin, Gore & McCarthy, 1998; Wicker, Michel, Henaff & Decety, 1998). They have been shown to 1) improve emotion recognition (de Gelder, Vroomen, Pourtois & Weiskrantz, 1999; Harwood, Hall & Shinkfield, 1999); 2) improve the ability to distinguish actual from posed emotional expressions (Hess & Kleck, 1990); and 3) improve age and familiarity judgments (Berry, 1990; Lander, Christie & Bruce, 1999). Furthermore, a handful of neuroimaging studies have indicated that the use of dynamic facial expressions results in both increased activation in the regions known to activate in response to static faces, and in the activation of a more widespread network that includes these regions (Kilts, Egan, Gideon, Ely & Hoffman, 2003; LaBar, Crupain, Voyvodic & McCarthy, 2003; Sato et al., 2004; Trautmann et al., 2009). Some researchers thus regard the use of dynamic rather than static stimuli in studying responses to facial emotion as a more ecologically valid method, particularly when studying social functioning. As my interest was to see the spontaneous emotional responses elicited by these negative social stimuli, it was important to use the most sensitive protocol.

Passive viewing, rather than a protocol featuring an explicit emotion recognition or other active cognitive task was chosen deliberately in an attempt to exclude conscious cognitive processing. Researchers argue that cognitive demands result in the inhibition of deeper emotion processing structures or systems by the prefrontal cortex (Blair et al., 2007; Ochsner & Gross, 2005; Rosenkranz, Moore & Grace, 2003), and a number of functional imaging studies support this contention (Hariri, Mattay, Tessitore, Fera & Weinberger, 2003; Ochsner, Bunge, Gross & Gabrieli, 2002; Pezawas et al., 2005). In fact, a meta-analysis of 385 neuroimaging studies indicated that increased amygdala activation was evident for passive, compared to active, viewing tasks (Costafreda et al., 2008). The passive viewing of emotions thus seemed a better choice in terms of its ability to strongly elicit emotional processing, and also because I wanted to examine emotional, rather than cognitively mediated, responses to negative social stimuli.

Whole brain analysis. Given the very low dose of buprenorphine used, it was possible that the opioid manipulation would not be strong enough to produce any detectable differences in neural activation. The whole brain analysis was able to show, however, that the medication had a significant effect across all three emotions included in the protocol. Although the general response of all participants to the opioid manipulation was not the focus of this investigation, it was encouraging to note that several regions flagged as important in the literature review demonstrated differences in activation on and off medication. These included various regions in the prefrontal cortex, anterior cingulate, basal ganglia, midbrain and pons – areas implicated in the SEPARATION-DISTRESS and SEEKING systems.

Given that buprenorphine is an analgesic with mild mood-elevating properties, I predicted that on medication compared to placebo, both groups would show reduced neural activation in response to negative social signals (fearful and angry faces). Because the hypothesis was that exposure to early social trauma has a long-term effect on opioid circuitry and opioid function, I predicted that the trauma group would show a different pattern of response to the opioid manipulation when compared to the control group: specifically, that significantly greater reduction in neural response to negative social signals would be evident in the trauma group compared to controls.

When examining the impact of buprenorphine without taking trauma status into consideration, the prediction that medication would reduce response to negative social signals was not supported. In response to viewing fearful faces on medication, more regions showed reduced than increased activation. This does fit the prediction. However, increased activation was also fairly common in response to fearful faces (as illustrated in Table 12, which shows 18 areas of reduced activation, and 11 areas of increased activation on medication). In response to both angry and happy faces, predominantly increased activation was seen on medication.

What could account for this unexpected direction of the effect for medication? It seems that, overall, participants in the two groups responded similarly to anger and happiness on medication, but showed a different pattern of response to fearful faces. Different processing styles for fear and anger stimuli are known to exist: a well-established body of research indicates that socially dominant, approach-oriented

individuals respond similarly to angry and happy faces, whereas those who are more avoidant are likely to react strongly to fear stimuli, but to avoid anger stimuli. This literature uses a model that conceptualizes emotions and their associated behavioral tendencies in terms of a dichotomy: they are all related to either approach or withdrawal tendencies. Research seems to support the contention that many individuals develop personality traits that are either predominantly approach-oriented, motivated by reward-seeking, or avoidant, motivated by the desire to escape punishment (Allen, Coan & Nazarian, 2004; Allen & Kline, 2004; Davidson, 1995; Pizzagalli, Sherwood, Henriques & Davidson, 2005). Critically, this work indicates that those who are more approach-oriented and reward-sensitive will respond differently to anger stimuli than those who are more avoidant-oriented and punishment sensitive. Specifically, approach-oriented individuals attend to and hence respond strongly to anger, whereas avoidant individuals attend to and respond strongly to fear, while avoiding attending to and processing anger stimuli. The idea is that approach-oriented, dominant individuals see anger as a challenge, with reward following if the challenge is successfully handled; in contrast, avoidant, submissive individuals experience anger as punishing, while fear is the most salient social signal (Hermans, Ramsey & van Honk, 2008; Putman et al., 2004; Putman et al., 2007a; van Honk et al., 2002; van Honk & Schutter, 2006; van Honk et al., 2004; van Honk et al., 2000).

It is thus possible that buprenorphine increased the tendency in all participants to respond in approach-related ways; therefore they showed greater activation in response to angry and happy faces on medication. It is, of course, also possible that personality differences of this nature (participants with greater approach or avoidant tendencies) were present in this sample, which could result in very different patterns of neural response in different individuals, thus confounding the analysis. Within-group personality differences of this kind might account for the variable pattern of response to fearful faces, where there were both increases and decreases of activation on medication, in some instances in areas of the same structure (e.g. anterior cingulate and pons); however this line of reasoning is only speculative. What must be considered is the fact that, given that the trauma group probably comprised an atypical set of resilient individuals, it is unlikely that all these participants were avoidant, or that all the participants in the control group

were approach-oriented. This factor could thus constitute an important confounding variable in the between-groups fMRI analysis discussed below. It would be highly advisable to include examination of approach/avoidance tendencies in future work.[9]

Turning to the between-groups comparison, which takes trauma status into account in the examination of the impact of buprenorphine on neural activation, I found no significant interaction between group and medication condition in the whole brain analysis. Thus, the prediction that the groups would respond differently to opioid medication was not supported, and post-hoc investigation of possible different responses to the different emotions (especially the combined negative emotions) could not be conducted.

A primary concern regarding this result was that the rigors of the random effects method in the whole brain analysis, especially given the very small group size (n = 11 per group), resulted in group differences being undetectable. Given the unexpected direction of the effect for medication discussed above, and the power problem in the whole-brain analysis, group differences that consisted of small effects might well have gone undetected. Hence, I turned to the more powerful ROI analysis, and examined very specific planned contrasts.

Region of interest analysis. The ROIs included in the analysis were functionally defined: areas that showed significant activations in the whole brain analysis were used for the ROI analysis. I restricted this exploratory analysis to regions that the literature review had indicated were likely to be of importance: anterior subcortical, anterior limbic and paralimbic, anterior temporal, and prefrontal regions (the latter consisting of ventral and medial areas, rather than dorsal and lateral areas). These are all areas involved in emotion, and more specifically many are implicated in the SEPARATION-DISTRESS and SEEKING systems (Panksepp, 1998; 2005a). In the ROI analysis, I carried out three planned contrasts.

[9] Individual differences in these personality traits are tapped by the BIS/BAS scale and differences in baseline lateralized prefrontal activity as measured by EEG. These measures are not part of the study reported here – they do, however, form an integral part of another PhD being written up on other parts of the dataset from the full protocol. It is for this reason that this analysis is not included in this thesis, despite its obvious relevance.

The first contrast examined the most critical prediction: that opioid manipulation would reduce the trauma group's response to negative social signals significantly more than it would that of the control group. This prediction was confirmed in two anterior regions of the insula. The left-sided region of activation included the short gyri of the anterior insula, and extended to the orbital region of the adjacent inferior frontal gyrus. The right-sided region of activation extended over the anterior region of the insula. In these regions, the opioid manipulation (in comparison to placebo) resulted in the trauma group showing significantly less reactivity to negative social signals than the control group.

It should be noted that the region of activation in the left anterior insula extended to include part of the adjacent inferior frontal gyrus (orbitofrontal region). Previously, opposing patterns of mu-opioid activity in the insula and orbitofrontal cortex have been found (Kennedy et al., 2006; these were interpreted as the insula and orbitofrontal region exerting different influences). In this instance, however, the activation represents a single cluster. A number of authors have noted that spillover of anterior insula activation into this region is in fact common. For example, Ochsner et al. (2006) indicated that the orbitofrontal cortex adjacent to the anterior insula activated in concert with the latter structure during the experience of the affective component of physical pain. In a review of insula function, Craig (2003) stated that insula activation during the experience of emotion often extends to the neighbouring inferior frontal gyrus, and terms this region the fronto-insula junction. It is therefore not unusual to see joint orbitofrontal and anterior insula activation representing a single response or function.

How does this group difference in response to negative social signals in these anterior insular regions fit with (a) reasoning around the overlap between physical and social pain, and (b) the role of the mu-opioid system in these affective states?

The insula is best known for its role in interoception: the representation of the visceral, physiological state of the entire body, which is primarily carried out in the posterior regions (Craig, 2003). The fact that the insula is also implicated in emotion is clear, with work around its role in disgust being best known (see, e.g., Jabbi, Bastiaansen & Keysers, 2008; Phillips et al., 1997; Rozin & Fallon, 1987; Rozin, Haidt & McCauley, 1993). However, the insula does appear to participate in emotion more broadly. Various

studies have indicated that the insula, particularly anterior regions, is activated during the experience of emotions, especially sadness (Damasio et al., 2000; George et al., 1995; Reiman et al., 1997). If we consider Damasio's (1999) conception of emotions as readouts of the body's current state in the world, the insula's involvement in emotion is certainly coherent with its interoceptive functions.

Both areas of significant activation in this analysis fell in the anterior insula. This region has extensive connections to areas of the brain known to be involved in emotion (Craig, 2003, 2009). The right anterior insula seems to have a very particular role. In his reviews of insula function, Craig states that the right anterior insula re-represents interoceptive activity from posterior insula regions, and that this re-representation correlates with subjective awareness of feelings. Insula regions are thus associated with conscious awareness of affective states (see also Damasio, 1999; Damasio et al., 2000).

Furthermore, the insula has a specific role in pain. As part of its interoceptive function, it represents both the sensory and affective components of physical pain (Brooks, Nurmikko, Bimson, Singh & Roberts, 2002; Petrovic, Kalso, Petersson & Ingvar, 2002; Peyron et al., 2000; Price, 2000). It forms part of a descending pain modulation system (incorporating the prefrontal cortex, anterior cingulate cortex, insula, amygdala, hypothalamus, and various brainstem regions including the periaqueductal grey) that can also enhance bottom-up pain signalling, and that appears to have a role in the emotional modulation of pain (Wiech & Tracey, 2009).

Given the evidence of the overlap between brain systems for physical and social pain reviewed earlier, it is unsurprising that the insula plays a role in representing social pain. The insula is involved in empathy for both physical pain (Gu & Han, 2007; Jackson, Brunet, Meltzoff & Decety, 2006; Singer et al., 2004) and empathy for social pain (Masten, Morelli & Eisenberger, 2011). The latter study in fact found anterior insula activation during empathy for social rejection. Moreover, studies investigating the pain of loss have shown that the insula is activated during the experience of grief (Gundel et al., 2003; O'Connor et al., 2008). A recent meta-analysis of functional imaging work on emotions confirmed that the insula is particularly active during the experience of negative emotions (Wager, Phan, Liberzon & Taylor, 2003), and that, as indicated above, it is particularly involved in sadness. Recent work indicates that the insula, and perhaps

particularly the right anterior insula, is activated during the experience of social rejection, and that this activation correlates with increased self-reported distress (Masten et al., 2009; Way, Taylor & Eisenberger, 2009). It thus seems that the insula may be an important part of the human sadness, or SEPARATION-DISTRESS system.

Moreover, there is evidence of mu-opioid involvement in insula regions during the experience of negative emotions. Studies have found an association between mu-opioid activity in both the left (Zubieta et al., 2003) and right (Kennedy et al., 2006) insula and heightened feelings of sadness. These studies have demonstrated that in healthy controls, mu-opioid activity decreases during feelings of sadness, but that in depressed individuals, opioid function appears to be dysregulated. Critically, Kennedy et al. (2006) found that in participants with major depressive disorder, increased mu-opioid activity was evident in the right anterior insula during sadness. Other work has indicated that heightened activity in the anterior insula is greater in individuals who are prone to anxiety when processing emotionally aversive stimuli (Paulus, Feinstein, Castillo, Simmons & Stein, 2005; Simmons, Matthews, Stein & Paulus, 2004; Stein, Simmons, Feinstein & Paulus, 2007).

Another important line of evidence comes from a study that examined baseline mu-opioid activity in groups with exposure to war trauma and in healthy controls (Liberzon et al., 2007). The authors found that all those exposed to trauma had reduced baseline mu-opioid binding potential in the insula bilaterally. The authors suggested that trauma exposure leads to functional changes in the mu-opioid system, and that these changes consist of downregulation of activity in the limbic forebrain and associated cortical areas, including the insula. Given these findings, they also raised the possibility that early trauma may alter the development of the endogenous opioid system. In all the papers investigating the mu-opioid system cited above, the authors regard mu-opioid activity as responsive to negative emotional states; its purpose being the regulation or inhibition of these negative emotions. This function appears to be dysregulated in major depression and in individuals exposed to trauma.

In sum, it seems that the insula is involved in representing and regulating both physical and social pain. We know that the insula is strongly activated during negative emotions, and there are indications that this responsivity is dysregulated (heightened) in

individuals with negative mood states, such as major depression and anxiety. Furthermore, individuals exposed to trauma show reduced baseline mu-opioid activity in this region.

In the current study, buprenorphine administration led to reduced responsivity to negative social signals in trauma-exposed individuals compared to controls. I suggest it is possible that, due to their exposure to early social trauma, mu-opioid function was downregulated in these regions in the trauma group. Given what the literature reviewed above indicates, the observed effect could have come about as follows: exposure to negative social signals could have resulted in activation here, and due to mu-opioid downregulation, trauma-exposed participants could have been less able to adequately self-regulate or inhibit this negative emotional response via mu-opioid release. Buprenorphine administration could thus have had a disproportionate effect on individuals in this group, in reducing insula reactivity, and hence reducing the negative affective state.

Two subsequent contrasts served to explore further the impact of the opioid manipulation on the groups' responses to social signals. First, I hypothesized that the trauma group would be more reactive than the control group to negative than positive social signals. This pattern of response would be another instance of the negativity bias that can be seen in even in subclinical depression and in highly neurotic, never-depressed individuals (Bouhuys et al., 1996; Mayberg, 2004; Williams et al., 1997). I used activation on placebo as a marker for this, reasoning that those responses should approximate normal neural responsivity. Next, I did an indirect contrast aimed at investigating whether or not any such effects seen on placebo were also present on opioid medication. In other words, did the trauma group remain more reactive than controls to negative compared to positive social signals on opioid medication?

The prediction that, on placebo, the trauma group would be more responsive than the control group to negative than positive social signals was confirmed. Significant between-group differences on this contrast were found in five regions: the right and left superior frontal gyrus; two areas of the right superior temporal gyrus; and the right insula.

The superior frontal gyrus activation was not bilateral; different regions were implicated. The activations in the right superior temporal gyrus were both rostral. In the first of these temporal regions, the activation incorporated the superior temporal gyrus and superior temporal sulcus, but did not extend to the middle temporal gyrus. In the second of these regions, the activation again included the superior temporal gyrus and sulcus, and extended to the middle temporal gyrus. It must be noted that these regions seem to be more anterior than the regions of superior temporal gyrus/sulcus that are typically implicated in the processing of facial movement (Haxby, Hoffman & Gobbini, 2000; Sato, 2004; Trautmann et al., 2009). The right insula region of activation extended to include the inferior border of the inferior frontal gyrus (posterior to the orbital region).

As indicated by various meta-analyses, these cortical regions are activated when processing emotional faces (Fusar-Poli et al., 2009; Phan et al., 2002; Sabatinelli et al., 2011; Trautmann et al., 2009; Wager et al., 2003). Emotional face processing is known to activate areas, such as these, that are themselves involved in the experience of emotion (Adolphs, 2002; Davidson & Irwin, 1999; Haxby et al., 2000; Murphy, Nimmo-Smith & Lawrence, 2003). In all these cortical areas, on placebo, the trauma group exhibited a significantly greater response to angry and fearful faces (compared to happy faces) than did the control group.

Subcortically, there was a significant between-group difference in response to these social signals in the left pons; however, here the predicted pattern was reversed. In this area, on placebo, the control group showed greater activation than the trauma group in response to negative compared to positive social signals. It is not entirely clear what this pattern of activation in the pons means. The area covers a large region and could involve a number of pontine nuclei, which serve very different functions (e.g., it might encompass a portion of the reticular activating system, the trigeminal nerve, and the raphe nuclei). In terms of emotion processing, Damasio et al. (2000) found that the dorsal and anterior pons activated during the experience of anger of sadness, but that the anterior pons was relatively deactivated during the experience of happiness and fear. A study of grief also found dorsal pons activation during the experience of this emotion (Gundel et al., 2003). Pons activation is also, however, associated with laughter and feelings of happiness (Wild, Rodden, Grodd & Ruch, 2003). Pontine activation thus

appears to occur during the experience of various emotions, but there is no clear overarching theoretical framework to explain the pons' function in this regard.

The third contrast tested the prediction that the trauma group would show greater responsivity to negative than positive social signals, this time on medication. The aim was to establish whether any negativity bias evident in the trauma group on placebo would remain evident on the opioid medication. This contrast was thus conducted only in the six regions where significant between-group differences were found on placebo. No significant effects were found.

What the second and third planned contrasts illustrate is that perhaps buprenorphine attenuates the exaggerated cortical-limbic responsivity to negative social signals present in the trauma group. I qualify this statement because this is an indirect contrast – the comparison is so complicated that I could not set up a direct contrast in BrainVoyager. What these contrasts also illustrate is that, once again, the effect of the opioid manipulation on the two groups differs. If both groups changed equally on the medication, the effects should still be present. However, this was not the case. The fact that the trauma and control groups responded differently to the opioid manipulation supports the idea that exposure to early social trauma may lead to long-term dysregulation of opioid function.

Interim summary: Impact of opioid manipulation on neural activation. I was able to demonstrate that the low dose of buprenorphine resulted in observable changes in neural activation, many of these occurring in brain areas marked as theoretically significant in the literature review. In the region of interest analysis, I was able to demonstrate between-group differences in the response to the opioid manipulation. Most interestingly, buprenorphine had a greater impact in reducing response to negative social signals in the trauma group, in anterior regions of the insula, which appear to be part of the social pain, or SEPARATION-DISTRESS system in humans. A further set of contrasts indicated that buprenorphine was able to attenuate a negativity bias (greater response to negative than positive social signals) evident in the trauma group on placebo. Although obviously preliminary, these findings are very encouraging.

Limitations of the neuroimaging component of this study. The research reported here constituted an exploratory pharmacological challenge study, using a low-dose buprenorphine manipulation in combination with a small sample size: There was thus a real risk of obtaining null findings. The reasons for using a low dose of buprenorphine have been discussed above, and in opiate-naïve individuals small amounts of buprenorphine seem capable of having marked effects (see, e.g., Bodkin et al., 1995; Emrich et al., 1982; Escher et al., 2007; McAleer et al., 2003).

The fact that a group-by-medication interaction was not evident in the whole brain random effects analysis could well be due to the small sample size (n = 11 per group). The random effects analysis method is the preferred method in fMRI research, largely because although fixed effects analysis is more powerful and better suited to small samples, it does not permit generalization beyond the sample used. Twelve participants per group are the minimum recommended number when using random effects analysis (Savoy, 1999), and 15 per group is becoming the new minimum guideline in the field (E. Meintjes, personal communication, 11 March 2011). Given the extremely stringent inclusion/exclusion criteria employed and hence the difficulty finding appropriate participants, ongoing recruitment would have been an extremely time-consuming process, and of course the financial implications would have been substantial. Most importantly, a number of pharmacological challenge studies using only 12 participants per group had been published, indicating that it should be possible to see effects at the sample size reported here (see, e.g., Harmer et al., 2003; McKie et al., 2005; Miskowiak et al., 2007; Norbury et al., 2007). For future work, a larger group size would be most beneficial, of course.

It was possible to demonstrate effects resulting from the opioid manipulation, but not between-groups effects in the whole brain analysis; hence the need for the ROI analysis. Because of concerns regarding the small sample, and the exploratory nature of this research, I did not correct for Type 1 error, which represents a very real threat in fMRI work (Amaro & Barker, 2006; Poldrack et al., 2008). The fMRI results must thus be regarded as tentative, requiring further confirmation. However, I regard the results from the region of interest analysis as most encouraging, and with larger groups in the follow-up study (discussed below), I hope to demonstrate robust effects.

There are also concerns around whether or not fMRI is suited to capturing the (probably) relatively slow and large scale dynamics that represent emotional changes in the brain (Liotti & Panksepp, 2004). For this reason, a repeated block-design protocol was selected above an event-related design. Although event-related designs allow better temporal resolution, they are best suited to capturing rapid neural responses. Block designs allow a stable neural state to be set up, which to my thinking was more likely to represent the sort of emotional changes that are of interest. Furthermore, block designs are much more sensitive to small effects, establishing a far better signal-to-noise ratio than event-related designs (Amaro & Barker, 2006; Cacioppo et al., 2003; Friston, Zarahn, Josephs, Henson & Dale, 1999; Savoy, 1999). They are generally regarded as yielding robust results. One of their flaws is setting up neural expectancy or habituation patterns, and I attempted to avoid this by using random (within set limits) epoch timing, and limiting the block repeats to two (Liu, 2004; Savoy, 2005). It seems that, for capturing responses to emotional facial expressions, this was the best format choice.

In terms of interpreting activations, it would be most helpful in future to include an emotion check after each block of faces. This would provide information on the actual feeling states invoked in participants while watching the different emotions, and thus add to the interpretation. Another concern is that in passive tasks, there is no measure of the participants' attention to the stimuli. This could also perhaps be checked by a simple post-block question assessing to what extent the stimuli demanded attention, or if the participant was thinking about something else. This would enable exclusion of data where participants were not attentive.

One final concern, and one that has no simple resolution, is that of a possible cross-race effect in processing faces. The marked benefit for own-race face recognition is a very strong finding in cognitive psychology (for a review, see Meissner & Brigham, 2001), and recent neuroimaging work has indicated that processing other-race faces may elicit different neural activity that represents automatic, unconscious responses. For instance, in the United States, enhanced amygdala response has been documented in response to other-race faces (see, e.g., Cunningham et al., 2004; Lieberman, Hariri, Jarcho, Eisenberger & Bookheimer, 2005; Phelps et al., 2000). There is also recent evidence suggesting that different neural responses are elicited when processing emotions

in own- versus other-race faces (Lee et al., 2008). The set of emotional faces that we used featured only Caucasian faces, while our participants were of various races. What impact this could have on our results is unknown, given the findings mentioned above. Fortunately, the groups in the fMRI subsample were well matched on race (no significant difference), so whatever effect may have been present, would have been present to a similar extent in both groups, and thus would not have impacted unduly on the results.

There are also indications in the literature that the effects seen with other-race stimuli may be linked to social categorization and prejudice, and can be attenuated by increased contact between races (see, e.g., Bernstein, Young & Hugenberg, 2007; Hancock & Rhodes, 2008). It is therefore very difficult to predict how young South African university students might respond to faces of different races. Moreover, how to control for these effects is also not clear: would using a set of emotional faces featuring a variety of races be able to statistically 'shuffle out' the effect across participants of different races, or, given the small samples typically used in neuroimaging, would it simply increase the confound? Recruiting participants only from one race is an obvious solution, but in the long term this has ethical implications, particularly in the South African context, where broad concerns around race and knowledge production remain highly pertinent. It is not clear to me how best to address this problem. Despite its obvious relevance, it does not seem to be a factor that is generally given consideration in social- affective neuroscience research, possibly due to the complexities it introduces.

General Discussion

Overview of Results

This study constitutes a first step in examining the broad thesis that early social trauma may lead to depression, at least in part, because it results in opioid dysregulation. I examined certain baseline characteristics, and the response to a once-off pharmacological challenge using the opioid buprenorphine, in medically and psychiatrically healthy young adults who differed only in terms of exposure to early social trauma. With regard to baseline characteristics, the groups did not differ on

general experience of positive or negative effect, or on depression scores. They did, however, differ significantly and substantially on scores reflecting a trait disposition on the SEEKING system. This result indicates that even psychiatrically healthy young adults who have been exposed to early social trauma show reduced tendencies to engage with the world in a positive, expectant, and motivated way. The neurochemical and psychological links between social bonding/attachment and SEEKING system activation will be discussed further below.

Importantly, this study demonstrated clearly that trauma-exposed and control participants responded differently to the opioid manipulation, at all the levels measured (affective, cognitive, and neural). With regard to the measures of subjective affect and social cognition, this altered response in the trauma group was not straightforward, and did not directly match the predictions made. However, this was not entirely unanticipated. As stated in the Introduction, at this initial, exploratory stage of the investigation, it was difficult to predict the precise form that opioid dysregulation would take, and the specific predictions made in this study, particularly for the affective and social cognitive domains, were heuristic, based on what is known regarding affect and cognition in depression. The key finding is that the groups responded differently: with regard to subjective affective states, buprenorphine reduced the experience of both positive and negative affect in the trauma group, but had little impact on affect in the control group. With regard to social cognition, the trauma group showed a negativity bias in interpreting ambiguous social information, perceiving neutral faces as sad. This bias was not attenuated by buprenorphine administration. However, buprenorphine enhanced a positivity bias in the control group – these participants were more likely to interpret neutral faces as happy. There was no sign of a positivity bias, or of enhancement of positive interpretations on medication, in the trauma group. These markedly different patterns of response to the opioid manipulation suggest that the opioid system was functioning differently in the two groups.

As mentioned above, it is possible that longer periods of consistent buprenorphine administration might be required to restore the opioid system to normal functioning, at which point equivalent affective experience and social cognition might be seen across the trauma and control groups. However, given the clear changes in neural activation

demonstrated in the fMRI analysis, we can be certain that the medication did have immediate effects – these may simply need more time and amplification in order to manifest at the affective and social cognitive levels.

The fMRI analysis indicated that even a low dose of buprenorphine was able to produce observable alterations in neural activity. Due to the small group sizes, the critical group by medication interaction was not evident in the whole brain analysis. However, in the more powerful ROI analysis, this study was able to demonstrate important effects. Most critically, it demonstrated reduced activation on medication in the anterior insula in response to negative social stimuli. This reduction was significantly greater in the trauma group than in controls. The anterior insula has been shown to be critically involved in both physical and social pain, and should perhaps be considered part of the SEPARATION-DISTRESS system in humans. This result is in line with Panksepp's model (Panksepp et al., 1997; Watt & Panksepp, 2009), which proposes that social pain mechanisms evolved from pre-existing mechanisms for physical pain; it is also consistent with a body of human neuroimaging work demonstrating that physical and social pain share neural substrates, and are both mediated by mu-opioids (DeWall et al., 2010; Eisenberger et al., 2006; Eisenberger et al., 2003; Kennedy et al., 2006; Liberzon et al., 2007; Masten et al., 2009; Zubieta et al., 2005; Zubieta et al., 2003; Zubieta et al., 2001). Moreover, this analysis, indicating a greater sensitivity to the opioid manipulation in the trauma group in such a key region, is most encouraging, as it supports the contention that opioid function is dysregulated subsequent to early social trauma. The final two ROI analyses again provided evidence of a greater sensitivity to opioid manipulation in the trauma group. The two contrasts indicated that buprenorphine appears able to attenuate heightened responses to negative social signals in the trauma group. An equivalent change in the controls was not evident.

In total, the results from this first study provide critical proof of the key concept under investigation – the opioid system does seem to be functioning differently in medically and psychiatrically healthy young adults who have been exposed to early social trauma. I now turn back to the literature to consider the implications of these findings in light of recent contributions to the field of depression research.

Understanding Depression

An interactive matrix of chemistries underlies the disorder. As emphasized in the literature review, depression remains poorly understood. Despite the identification of multiple neuroanatomical and neurochemical correlates of depression (or perhaps because of it), a single coherent unifying explanation of major depression has yet to emerge. Since the commencement of the study reported here, there has been a move in the literature towards developing more complex, systems-level models of the disorder (see, e.g., Anisman, 2009; Giacobbe et al., 2009; Mayberg, 2009; Northoff et al., in press). It seems clear that single factor theories are unlikely to be able to explain depression (Watt & Panksepp, 2009). Increasingly, major depression is seen as a multifactorial disorder, manifesting in different clinical presentations depending on which factors are most strongly implicated. Several authors are focusing on more pluralistic modeling of the neurochemistries involved, with an emphasis on the way in which these chemistries interact with and regulate each other. These inter-connections have been conceptualized in terms of a matrix of interactive factors (see, for e.g., Table 17). The argument is that without such a multifactorial understanding, it is unlikely that the etiology of depression will be able to be explained (Panksepp & Watt, 2011; Zellner et al., in press).

Table 17

Neurobiological Factors Forming an Interactive Depressive Matrix

Depressive factor	**Driven by**	**Producing**	**Behavioral and symptomatic correlates**
Increased CRF, hypercortisolemia, cholecystokinin and reduced BDNF	Multifactorial limbic influences on paraventricular nucleus promoting activation of HPA stress axis.	Increased dynorphin, decreased 5-HT, reduced neuroplasticity/HC atrophy. Intensification of separation distress. Disrupted ventral HC feedback on core affective regions.	Dysphoria, sleep and appetite loss. Reduced short-term memory, and other cognitive deficits.
Increased acetylcholine	Reduction of social and other rewards, declining opioid tone, and any other social punishment.	Facilitation of separation distress circuitry and other negative emotions. Effects on other core variables.	Negative affect and excess attention to negativistic perceptions and thoughts.
Decreased mu opioids and oxytocin	Separation distress, other stressors, including physical illness and pain.	Disinhibition/release of stress cascades; decreased 5-HT and DA; overdriven NE. Promotion of pro-inflammatory cytokine generation.	Anhedonia and sadness, reduced positive affect and reduced sense of connection. Suicidality.
Increased dynorphin in accumbens/VTA	Stress cascades.	Down regulation of VTA and mesolimbic DA system.	Anhedonia, dysphoria, loss of motivation.
Increased pro-inflammatory cytokines	Acute but probably not chronic stress, acute reduction of opioids.	Promotion of stress cascades, decreased serotonergic and increased glutamatergic tone. Impairment of HPA axis negative feedback.	Fatigue, malaise and appetitive losses. Increased cognitive disruption. Anhedonia.
Reduced serotonergic drive/vulnerability	Stress, increased corticosteroids, cytokines, decreased mu opioids.	Lowered dopaminergic and increased noradrenergic drive. Less functional segregation among brain systems.	Poor affective regulation. Impulsivity. Obsessive thought, suicidality.
Diminished catecholaminergic (DA and NE) tone	Constitutional vulnerability, stress and poor reward availability.	Reduced "signal-to-noise" processing in all sensory-perceptual and motor/executive systems.	Fatigue, diminished psychic "energy": appetitive sluggishness, dysphoria. Impaired coordination of cognitive and emotional information processing.

Note: Reproduced with permission from Zellner et al. (in press).

The separation-distress model of depression. Examining the role of endogenous opioids in this multifactorial complex is a vital task. Opioids remain integral to newer articulations of the separation-distress hypothesis of depression. As is evident from Table 17, most of the neurochemical changes that exert depressive influences are driven by stress. The links between stress and depression are clear and convincing, but although opioids are clearly implicated in regulating stress responses, their role with regard to depression is not well articulated.

The literature on stress and depression has struggled to account for the variability of psychiatric responses to stress, with depression being just one possible outcome. Other difficulties in this literature include the question of why some individuals are more vulnerable to depression regardless of stressor severity, and of exactly how the various affective changes that characterize depression follow from stress (Slavich et al., 2010; Watt & Panksepp, 2009). The separation-distress model of depression, which focuses on a very particular kind of stressor, social loss, embedded in a framework of core emotion systems, can make a significant contribution in this regard.

The separation-distress model seeks to explain depression at both the neurobiological level, and at that of subjective feeling states, with both linked to the central concepts of social attachment and social loss. The positive feeling states of comfort and security associated with social attachment are peptide mediated, involving increased levels of mu-opioids. In contrast, separation-distress is characterized by reduced levels of mu-opioids, by increased stress chemistries, and elevated kappa-opioids (Panksepp & Watt, 2011).

This model is based on the idea that for social brains, separation-distress constitutes a prototypic stress state. The survival of infant mammals is dependent on intact primary attachment bonds. The mammalian brain has clear links between attachment mechanisms and stress physiology. Any threat to, or disturbance of, these social attachment bonds elicits immediate and marked distress. Separation-distress powerfully activates the HPA axis, and, as reviewed above, early attachment experiences exert a permanent effect on stress neurophysiology. The model thus proposes that failure of attachment, or social loss, activates chronic stress cascades that can lead to depression. It is thought that the trajectory to development of depression involves not only mu-opioid

mediated separation-distress/social pain, but also dysregulation of the kappa-opioid mediated shutdown mechanism for the original panic or protest phase. Moreover, if critical attachment failures/social losses and the resultant stress cascades occur early in development, the model predicts that this will result in long-term vulnerability to depression via permanent changes in stress responsivity, including changes in opioid function.

There is mounting evidence that social stressors are particularly depressogenic. The idea that social loss is a potent cause of depression has a long history, beginning with Freud's conceptualization of depression being related to mourning (Freud, 1917/1950). Compelling recent evidence indicates that personal loss is more depressogenic than other, equivalently severe, stressors. A study contrasting targeted interpersonal rejection with equivalently severe stressors found that social rejection was three times more likely to lead to depression (Slavich, Thornton, Torres, Monroe & Gotlib, 2009). These authors argue that the specific characteristics of social rejection are more important than simple stressor severity. Similarly, investigators examining HPA axis development in human children state that it is particularly sensitive to social challenges (Flinn, 2006; Flinn et al., 2009). A longitudinal study (Slavich et al., 2010) demonstrated that participants who experienced early parental loss later developed depression following less severe interpersonal losses; this pattern was not seen in participants who had been exposed to other equivalently severe early stressors. This indicates that disruption of early attachment bonds acts to sensitize individuals to loss and rejection, making them far more vulnerable to depression, as is predicted by the separation-distress model of depression.

The current study has provided evidence of opioid system dysregulation subsequent to early social trauma, and it has also demonstrated reduced SEEKING in trauma-exposed participants. As indicated previously, recent articulations of the separation-distress model propose that dysregulated kappa-opioid mediated downregulation of SEPARATION-DISTRESS mechanisms play a critical role in the development of anhedonia and depression. The animal literature indicates that mesolimbic dopamine levels and SEEKING behavior are reduced in depression (Anisman & Matheson, 2005; Harro, Kanarik, Matrov & Panksepp, in press; Nestler & Carlezon, 2006; Pereira Do Carmo, Stevenson, Carlezon & Negus, 2009). The

separation-distress model proposes that the stress of social loss recruits kappa-opioids, upregulating prodynorphin, especially in the nucleus accumbens (Berton & Nestler, 2006; Mu, Neumann, Panksepp, Schluter & Dong, 2011). Kappa-opioids lead to changes in motivation and to dysphoria (Bals-Kubik, Ableitner, Herz & Shippenberg, 1993; Carlezon et al., 2006; Hasebe et al., 2004; Walsh, Strain, Abreu & Bigelow, 2001), reducing SEEKING by inhibiting dopamine release in the ventral tegmental area (Panksepp & Watt, 2011). The results of the current study are in line with the arguments regarding the long-term impact of social trauma on the SEEKING system.

It is important to provide a psychological-level account of the reciprocal relationship between the social bonding and SEEKING systems. It must be remembered that the separation-distress model of depression is an extension of Bowlby's original account of attachment. In this account, attachment's chief function is to provide a safe base, and a sense of self-esteem or efficacy. Crucially, when attachment is successful ('secure' in Bowlby and Ainsworth's terms), the infant is able to respond adaptively to novel environments, exploring confidently and with interest. The secure base provided by this social attachment thus promotes adaptive SEEKING behavior. Infants who are insecurely attached are far less willing to explore, apparently lacking the sense of security and confidence, or sense of agency, imparted by secure social attachment. These well-established behavior patterns yield insight into the reciprocal relationship between attachment/social bonding and SEEKING at the psychological level, and hence into the fact that SEEKING is impacted when social bonds are lost.

In terms of associated feeling states, the model postulates that social comfort and social loss are characterized by inverse patterns of mu- and kappa-opioids (Panksepp & Watt, 2011; Zellner et al., in press). Feelings of social comfort are created by high levels of mu-, and low levels of kappa-opioids; whereas feelings of social loss are characterized by low mu- and high kappa-opioids. Social loss is thus argued to feel bad in a very particular way. The shutdown of the separation-distress protest phase is also associated with quite specific feeling states. Whereas activation of SEEKING is associated with dopamine mediated positive affect, including feelings of motivation, agency, engagement and positive expectancy states, shutdown of SEEKING is associated kappa-opioid mediated reductions in dopamine, and with feelings of hopelessness, loss of interest and

lack of motivation – essentially by the characteristics of anhedonia. Moreover, the transition from protest to despair may thus involve a new feeling state; one that might comprise a combination of the pain of social loss, in addition to feelings of lassitude and despair.

It must be admitted that the current study, in line with the clinical literature on buprenorphine, focused only the mu-opioid agonist effects of this opioid medication. This focus was due to the known and marked effects of full mu-opioid agonists on mood; the mu-opioid effects of buprenorphine thus seemed most relevant. However, buprenorphine also impacts on kappa-opioids, acting as an antagonist at these receptors. This new idea in the separation-distress model, that inhibiting kappa-opioid effects may be as important as enhancing mu-opioid effects in treating the symptoms of depression, only increases the importance of investigating the actions and efficacy of buprenorphine.

The results of the current study are consistent with these most recent formulations of the separation-distress hypothesis of depression, where both mu- and kappa-opioids are implicated, and both the SEEKING and SEPARATION-DISTRESS systems are involved. This study demonstrated reduced SEEKING in trauma-exposed participants, as well as a marked effect for buprenorphine in this group in reducing response to negative social signals in the anterior insula – a region known to be implicated in both physical and social pain, and thus a candidate region for the SEPARATION-DISTRESS system in humans.

There are very exciting prospects ahead, both in terms of research and in terms of implications for treatment. The importance of investigating buprenorphine's efficacy has been stated; clearly there is hope that this medication can give relief to some individuals with refractory major depression. In addition, one of the strengths of this model of depression is that its focus extends beyond neurobiology. Incorporating an understanding of emotion enables the development of therapeutic interventions at multiple levels. In addition to its implications regarding the need to raise mu-opioids and reduce kappa-opioids, the model also suggests that promotion of prosocial activities that impact on these chemistries (e.g., CARE and PLAY) should be effective interventions.

Perhaps a key understanding that emerges from this perspective is that the mammalian brain treats social threats similarly to the way in which it treats physical

threats/stressors. The fact that social threats elicit such strong physiological stress responses, and that social challenges exert a disproportionate effect on HPA axis development, indicate that mammalian brains treat these threats as highly salient. This is indicative of the fact that, for mammals, social needs are as critical as physical needs; thus the need for evolution to wire in a system that signals both physical and social pain. In fact, in infant mammals, social needs take precedence, because if the bond with the primary caregiver is not intact, the infant's physical needs will not be met (Lieberman & Eisenberger, 2009). The adaptive importance of mammalian social bonds, and the neurobiological response to threats to these bonds, both support the idea that early social loss serves as a prototypic stressor for mammalian young. One critical point emphasized in this separation-distress model is that depression seems inherently linked to our capacity for social attachment – it is the price we pay for being such highly social creatures (Panksepp & Watt, 2011; Watt & Panksepp, 2009).

Considerations for Future Studies

The placebo effect. A potential problem with using a placebo control condition in the investigation of mu-opioid effects came to light towards the end of the data collection period. The design used in this study was chosen because placebo controlled designs are the gold standard in medical research. Although knowledge of placebo effects is longstanding, these effects were thought to result from psychological expectancies. Placebo was considered physiologically inert, and therefore the best contrast when examining the effects of active agents. However, research around placebo effects is currently posing a major threat to this traditional gold-standard medical research design (Diederich & Goetz, 2008; Posternak & Zimmerman, 2007). It has been shown that placebo exerts its effects through the opioid system – in other words, the placebo effect *is* an opioid effect.

The relationship between placebo and opioid effects is obviously highly relevant to the line of research I am pursuing. Early on, a landmark study demonstrated that placebo effects could be completely blocked by the mu-opioid antagonist, naloxone (Levine, Gordon & Fields, 1978). Very recently, researchers have directly shown that

placebo activates mu-opioid transmission in regions associated with modulating both pain and emotion, and with reward processing, including the periaqueductal grey, nucleus accumbens, rostral anterior cingulate and insula (Scott et al., 2008; Wager, Scott & Zubieta, 2007; Zubieta & Stohler, 2009).

Using a placebo control may thus not be ideal when attempting to examine mu-opioid effects, as subtracting the placebo effect may actually remove part of the very effect I am seeking to examine. It must be noted that most of the experimental work cited above examined placebo effects in relation to physical pain, where participants perhaps specifically expected pain to reduce. In contrast, my work did not set up any clear expectancies. It is thus not entirely clear what impact placebo effects would have in this context. Nonetheless, the possibility that placebo may elicit mu-opioid activity and thus mimic at least part of buprenorphine's effect, is a problem I intend to avoid in future work. I have designed and received funding for a follow-up study, where the effect of buprenorphine will be contrasted with that of a mu-opioid receptor blockade, created by the agent naltrexone. This design should yield a clearer contrast. Use of this mu-opioid blockade could also permit direct examination of buprenorphine's kappa-opioid effects, if buprenorphine is administered once the mu-opioid blockade is in place. Given the importance of kappa-opioid effects in the newer articulations of the separation-distress hypothesis of depression, this is a very important line of investigation to pursue.

Adapting the neuroimaging protocols. For the fMRI component, this study used a standard echoplanar functional imaging protocol. There are two main problems with this: First, the areas in which I am most interested are not best imaged by default scanner protocols – specialized protocols are required to reduce artifact from mouth and sinus areas, and properly image deep midbrain and medial forebrain structures in any detail. I will use such adapted protocols in the follow-up study. Second, there is a recent trend in imaging to use arterial spin labeling (ASL) rather than BOLD imaging to examine small regions in detail (E. Meintjes, personal communication, 11 March 2011). ASL does yield better specificity, so it would seem better suited to imaging the small, deep brain regions that are of key interest here. However, BOLD imaging yields a far stronger signal than ASL. Given this trade-off between specificity and power, I am

considering using both imaging methods in the neuroimaging investigation that forms part of the follow-up study.

Another consideration related to the neuroimaging component is how best to approach region of interest analyses. Given the theoretical base on which my research is grounded, there are very specific regions that are of interest, so whole brain analysis is of limited relevance to this work. However, defining regions of interest is no simple matter. Using functionally defined regions of interest from whole brain analyses (as was done in the current study) can be problematic, especially if the contrasts in these ROI analyses are not independent of those in the whole brain analysis. However, mask-based region of interest analysis may have limited success. It involves creating an anatomical mask that includes a specific area (e.g. amygdala, or a section of the anterior cingulate cortex), and conducting the analysis only within that area. Difficulties may occur because most often the size of an activation cluster is far smaller than that of the entire masked region, but as all the voxels in the mask are included in the analysis, this activation may not be detected. Some researchers use functional localizers in separate imaging sessions to define their regions of interest, but this approach is also open to criticism (Friston et al., 2006). It is possible that using masks for very small deep brain regions of interest would be the best approach, in combination with either functional localizers or functional definition for larger cortical regions.

In terms of functional tasks used in the scanner, it would be helpful to investigate activation in response to social rejection, and how this is mediated by opioid manipulation. I have obtained a version of the cyberball task that has been used in a number of imaging studies (e.g., Eisenberger et al., 2003; Masten et al., 2009; Way et al., 2009). In this task, participants are initially included in, and are then excluded from a social computer game, thus allowing online tracking of the response to social rejection.

Additional physiological and emotional measures. It would be most helpful to include measures of cortisol and cytokine activity in the comparison groups, to illustrate more clearly the interplay between these factors and the opioid system. Physiological measures such as skin-conductance and heart-rate during the social rejection scanner task

would also contribute to a fuller picture of the response to social stress in trauma-exposed versus control participants.

Obtaining more differentiated measures of emotional response would help to refine our understanding of affective responses in these participants. Positive and negative affect are broad constructs; measuring various emotions more specifically would yield a more nuanced picture.

Adjusting the sample composition. For the follow-up study I intend to have a minimum of 15 participants per comparison group. I also intend to recruit only females. There are clear gender differences in both the incidence of depression, and in some opioid-mediated effects. Critically, gender differences in the functioning of the mu-opioid system have been reported; both in terms of baseline receptor binding potential, and in terms of response to stressors, including physical pain (Liberzon et al., 2002; Zubieta et al., 1999; Zubieta et al., 2002). There are also indications that women respond more strongly to social pain, showing an enhanced inflammatory response that is associated with increased activation in the dorsal anterior cingulate and anterior insula (Eisenberger, Inagaki, Rameson, Mashal & Irwin, 2009). Including both genders may thus introduce confounds into the data. A better strategy is to include only one gender, or to recruit sufficient males and females to permit a comparison across the sexes. Investigating gender differences is going to be imperative in the long term, as any explanatory theory of depression must be able to account for the greater prevalence of the disorder in women.

Examining genetic factors. Another factor that might contribute to risk for depression is the polymorphism on the mu-opioid receptor gene. Carriers of the G variant (A118G) have reduced mu-opioid receptor messenger RNA, and seem to experience more physical pain (Coulbault et al., 2006; Klepstad et al., 2004; Landau, Kern, Columb, Smiley & Blouin, 2008; Lotsch & Geisslinger, 2006; Sia et al., 2008; Zhang, Wang, Johnson, Papp & Sadee, 2005). A recent study investigated if this polymorphism was also associated with increased sensitivity to social pain. The authors found that the G variant was associated with dispositional sensitivity to rejection, and that

when experiencing social rejection, carriers showed greater reactivity in regions known to be implicated in both physical and social pain – the dorsal anterior cingulate and anterior insula (Way et al., 2009).

In the long term, examining the impact of different kinds of social trauma at different ages in childhood, along with how these variables interact with genetic factors is going to be required. Examining what factors may buffer these effects will also be imperative – information around resilience and protective interventions is certainly needed.

Conclusion

This initial exploratory study has indicated that even healthy normal individuals who have been exposed to early social trauma show evidence of altered opioid function. Although these individuals did not differ from controls on baseline measures of negative emotion, they did differ on SEEKING, a crucial primary process appetitive, motivational emotion system, that is strongly implicated in the mechanism of depression in new articulations of the separation-distress model. Moreover, in response to opioid manipulation these trauma-exposed participants consistently demonstrated a different pattern of reaction to that seen in controls who had no exposure to early social trauma. When given a once-off low dose of buprenorphine, trauma-exposed participants responded differently to controls in terms of subjective affect, social cognition, and neural activation. These findings add to a growing body of evidence implicating the endogenous opioids in depression, via their role as mediators of emotional responses to social stressors.

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APPENDIX A

PARTICIPANT TELEPHONIC SCREENING QUESTIONAIRE

DATE: _____

A. Personal Details:

Full Name: _____
Date of Birth: _____
Gender: _____
Occupation: _____
Student number: _____

B. Contact Details:

Address: _____
_____
_____

Tel. number (h) : _____
(w): _____
(c) : _____

Email Address: _____

C. Medical Details: ***Please circle***

1. Are you right-handed? Yes / No

2. Do you take any kind of medication on a regular basis? Yes / No
   If yes, please specify what kind
_____

3. Are you allergic to any medication? Yes / No
   If yes, please specify what kind
_____

4. Have you ever had a head injury? Yes / No
   If yes, describe most severe:_____

Were you knocked unconscious? Yes / No
If yes; how long? _____

Any surgery/hospitalisation as a result of your head injury? Yes / No
If yes; please specify:_____

5. Do you have a metal object in your body (eg. aneurysm clip)? Yes / No
If yes, please specify:_____

6. Do you wear a metal prosthesis (eg. artificial leg)? Yes / No
If yes, please specify: _____

7. Do you have a pace-maker? Yes / No

8. Have you ever been diagnosed with asthma? Yes / No

9. Have you ever been diagnosed with chronic bronchitis, emphysema, or any other respiratory problems? Yes / No

10. Have you ever been diagnosed with a hepatic (liver) problem/disorder? Yes / No

11. If you are female, are you currently pregnant? Yes / No

- If you are female and answered no to question 10, are you planning on becoming pregnant within the next year? Yes / No

12. If you are female, are you currently a breastfeeding mother? Yes / No

13. Have you ever been diagnosed with a renal problem/disorder? Yes / No

14. Have you ever had seizures or an epileptic fit? Yes / No

15. Has anyone in your immediate family (siblings, parents) ever been diagnosed with epilepsy? Yes / No
If yes, please specify who: _____

16. Have you ever been diagnosed with a psychiatric illness? Yes / No
If yes, please specify: _____

17. Have you ever had any neurological condition? Yes / No
If yes, please specify: _____

18. Other notes:

_____

_____

_____

_____

_____

Brief Telephonic Verbal Consent Form

This study is entitled 'Functional brain imaging in healthy participants with a history of early adversity'. It will look at the effects of the opioid, buprenorphine, on brain function. You will be administered a small dose of buprenorphine on 2 occasions. You will also perform neuropsychological tasks, undergo brain imaging, and have blood drawn for genetic testing.

Please circle

1. At this stage, do you consent to participate in this study? Yes / No

2. Do you acknowledge that all of the details (eg age & medical details) given to the researcher by you are correct? Yes / No

3. Are you satisfied that any questions that you may have at this stage have been appropriately answered? Yes / No

Signature of research assistant:

Date:

APPENDIX B:

PARTICIPANT INFORMATION AND CONSENT DOCUMENTS FOR BEHAVIORAL AND SCANNER SESSIONS

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM
FOR BEHAVIORAL SESSIONS

TITLE OF THE RESEARCH PROJECT: Functional brain imaging in healthy subjects with a history of early adversity

US PROTOCOL NUMBER: OP-0307

UCT REFERENCE NUMBER: 018/2009

PRINCIPAL INVESTIGATOR: Professor Dan J Stein

ADDRESS: MRC Anxiety & Stress Research Unit, University of Stellenbosch Department of Psychiatry/ University of Cape Town Department of Psychiatry

CONTACT NUMBER: +27 21 938-9228

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Committee for Clinical Trials at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

This trial is being run at the Department of Psychiatry, University of Cape Town. We aim to recruit a total of 40 participants over a period of 2 years

What is this research study all about?

The purpose of this study is to find out what effects Buprenorphrine has on individuals who have experienced a childhood traumatic event **versus those who have not**. This study will make comparisons between treatments. Randomisation means that the participants are put into treatment groups by chance. The groups are selected by a computer that has no information about the individual participants. Participants in each group (Buprenorphrine or placebo) then have a different treatment and their responses are then compared.
In a blinded study the treatment provided will be hidden or concealed so you will not know which treatment you will receive. This research study is called "double blind". In a

double blind research study neither you nor your study doctor will know in which treatment group you are (although if your doctor needs to find out he/she can do so).

A placebo is a dummy treatment such as a pill or a capsule, which looks like the real thing but is not. Placebo medications do not contain active ingredients.

In a crossover study you will first receive one treatment and then the other. If you agree to take part in this study you will be given either Buprenorphrine or placebo on one visit and the other (either Buprenorphrine or placebo) on the following visit.

Procedures

If you agree to take part in the study and if you meet all of the conditions required to enter the study, you will have the following tests and procedures:
At the first clinic visit your study doctor will ask you some questions to confirm whether you have been exposed to a traumatic event in your childhood.
If you are eligible and agree to participate in the study you will be asked to attend the clinic on up to 2 more occasions. You will receive study medication from your doctor which you will be given in the morning of your visit. You will then be asked to complete questionnaires and do some tasks as well as have a brain image scan done (if you agree to do so).

At each visit your past and current medical conditions together with any medications that you are taking currently or have taken recently will be recorded. A record will also be taken of any side effects that you may be experiencing.

You will be given a physical examination and your weight, blood pressure and heart rate will be measured at each visit.

Blood samples (about 30 ml [6 tablespoons]) will be collected for routine laboratory testing, for a pregnancy test (if you are female) and for possible future genetic studies. You will be asked to have your blood drawn on the first day of attendance. Approximately 30ml of blood will be drawn from your arm. We may need to contact you again to get another blood sample should we fail to get a DNA sample from your blood. The blood sample you give may be used to create a cell line. This is done by changing some of your blood cells so that they can grow forever. The cell line is living tissue and it can be used to make more of your DNA at any time in the future. Candidate polymorphisms identified to be associated with anxiety or depression and possibly playing a role in explaining variance in the fMRI results will be investigated later on. This process will take place at the MRC Centre for Molecular and Cellular Biology and the Division of Medical Biochemistry, Faculty of Health Sciences, at the University of Stellenbosch. The DNA will then be taken from the cell line and saved for scientific analyses which will be performed now, and possibly in the future.

We may contact you later for further information, or request you to complete another interview at a later date, in order to obtain follow-up information that may be of use in our genetic analyses. This may involve an assessment similar to the current assessment, including a series of interviews and/or another blood sample. Your current participation is in no way binding to your future participation.

Your cell line and DNA will be maintained permanently, unless you request to have it removed. If at any time in the future you wish to have your DNA, cell lines or clinical data removed from the storage site, you may do so by contacting the researchers

conducting this study.

What will your responsibilities be?

Your doctor will be required to ask you about medications that you may be taking currently or that you may have taken recently.

Your doctor will also advise you on which prescription or over-the-counter medications or any other remedies or foods that you will be required to either stop or restrict your consumption of during the entire length of the study. This will include a restriction on the amount of alcohol that can be consumed.

At each visit you may be asked to complete questionnaires or tasks to check the status of your symptoms. These will measure your mood, emotional responses, trust, sociability and emotional resilience.

Will you benefit from taking part in this research?

If you agree to participate in the study, no direct benefit to you can be guaranteed, but you will be seen by the study doctor or staff at regular intervals.
Your participation in this study will add to the medical knowledge about the use of this medicine.

If you agree to participate in this study Buprenorphine may or may not be beneficial in treating or improving your symptoms. However, the information learned from this study may help to establish a new medication for the treatment of people who have been exposed to trauma in their childhoods.

Are there in risks involved in your taking part in this research?

All drugs and even placebos may cause side effects in some people. There may be risks, inconveniences or side effects that are not known at this time.

The most commonly reported adverse reactions of Buprenorphrine administration are constipation, headaches, insomnia, drowsiness, nausea and vomiting, fainting and dizziness, orthostatic hypotension, sweating.

Special caution should be exercised when driving or using machinery since the study medication may cause drowsiness.

You will receive both buprenorphrine and placebo, each at a separate visit. At the visit that you receive placebo (the medically inactive substance), your symptoms may not improve or may worsen. Even if you receive active medication during the study, your symptoms may not improve or may worsen. As mentioned earlier this research study is called "double blind". In a double blind research study neither you nor your study doctor will know in which treatment group you are. It is possible that you may not report any side effects when receiving treatment. This does not mean that you have received placebo. Similarly, even if you do receive placebo you may experience some side effects which you and your doctor feel could be associated with the study drug.

Because the effects of Buprenorphrine on the unborn foetus (child/baby) or nursing baby/infant are uncertain, you will not be allowed to enter this study if you are pregnant or breastfeeding or planning to become pregnant within 6 weeks of your screening visit.

If you choose to participate in this study, you must use one of the allowed contraceptive methods (a way to prevent you from becoming pregnant) for the specified period of time before and after you enter the study. Ask your doctor if you have any questions about these choices and which might be best for you.
Acceptable contraceptive methods, when used consistently and in accordance with both the product label and the instructions of your doctor, are as follows:

a Non-childbearing potential (i.e., physiologically incapable of becoming pregnant, including being post-menopausal. For purposes of this study, postmenopausal is defined as one year without menses); or

b Child-bearing potential, you must agree to one of the following:
- Male partner who is sterile prior to your entry into the study and is your sole sexual partner;
- Oral contraceptives (either combined or progestogen only);
- Double-barrier method of contraception consisting of spermicide with either condom or diaphragm;
- IUD with a documented failure rate of less than 1% per year; or

Even when you use one of the allowed contraceptive methods, there may be a small risk that you could become pregnant. Because of this, you will be tested during the study to see if you are pregnant. If one of these tests shows that you have become pregnant, your unborn baby may have been exposed to Buprenorphrine even if you stop taking the drug right away. So, if you think you are pregnant or may become pregnant, you must tell Dr_____ at the earliest opportunity. If you should become pregnant during the study you will be asked, required or requested to, stop taking the study drug immediately. You will also be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy must be reported to your study doctor.

There may be other risks, inconveniences and side effects to the embryo, foetus (unborn child), or nursing infant that are unknown at this time.

Who will have access to your medical records?

Maintaining your confidentiality is important. Your personal information (for example your gender, age, the details of your medical conditions) and other information (the data collected by the investigators as part of the study) will be identified by a number (i.e., coded). Your name will not appear in any publications or reports produced from this study. The investigators will keep the information and the results collected about you in this study. This information about you will be kept in a secure place. By agreeing to take part in this study, you will be allowing certain persons to see the information about you (both personal, including your name, and other information) held by the study doctor. You have the right to withdraw your consent to participate in this study at any time. If you withdraw your consent to participate in this study no new information will be collected from you and added to existing data or to a database. Your information will be processed electronically (i.e., by a computer) or manually and analysed to determine the outcome of this study. Your information may/could be sent to regulatory authorities and to the Ethics Committees. You have the right to ask the study doctor about the data being collected on you for the study and about the purpose of this data. You have the right to ask the study doctor to allow you to see your personal information and to have any necessary corrections made to it.

What will happen in the unlikely event of some form of injury occurring as a direct result of your taking part in this research study?

If you become ill or injured as a result of participation in this clinical study, you will be referred for appropriate medical treatment. The University of Stellenbosch's insurance policy will cover the costs of such treatment. If you have any questions concerning the availability of compensation/medical care or if you think you have experienced a research-related illness or injury, contact details are below.

Your right at law to claim compensation for injury where you can prove negligence is not affected. For medicines that have already been approved by the Medical Authorities to treat this condition, normal legal rules on compensation will apply.
If you have any questions about your rights as a research subject, you should contact the Committee for Pharmaceutical Trials of the University of Stellenbosch, Tel: (021) 938 9075, Fax: (021) 933-6330.

If you have questions about this trial you should first discuss them with your study doctor or the Committee for Pharmaceutical Trials of the University of Stellenbosch.
Dr S Seedat: Tel (24hr contact number): 082-784-8148
Dr P Carey: Tel (24hr contact number): 083-700-0046
Dr D Stein: Tel (24hr contact number): 083- 263-9679

After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at: The Registrar, SA Medicines Control Council, Department of Health, Private Bag x 828, Pretoria, 0001, Fax: (012) 323 4474

Will you be paid to take part in this study and are there any costs involved?

No you will not be paid to take part in the study but your transport and meal costs (R100) will be covered for each study visit. There will be no costs involved for you, if you do take part.

Is there any thing else that you should know or do?

- You can contact Dr ……..….. at tel …… if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...……...

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..…….. on (*date*) ……...……...

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) …… to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

....

Signature of interpreter **Signature of witness**

F-MRI PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT: Functional brain imaging in healthy subjects with a history of early adversity.

US PROTOCOL NUMBER: OP-0307

UCT REFERENCE NUMBER: 018/2009

PRINCIPAL INVESTIGATOR: Professor Dan J Stein

ADDRESS: MRC Anxiety & Stress Research Unit, University of Stellenbosch Department of Psychiatry/ University of Cape Town Department of Psychiatry

CONTACT NUMBER: +27 21 938-9228

Dear Volunteer

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Committee for Clinical Trials at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

If you receive this invitation to participate, you will already have given your consent/assent to participate in the part of this study that is assessing the effect that Buprenorphrine has on individuals who have experienced a childhood traumatic event.

STUDY PROCEDURES

At each visit, besides getting the study medication from your doctor and being asked to complete the questionnaires and tasks, if you are agreeable you will have a type of brain scan, called an fMRI (functional magnetic resonance imaging) scan. As the scan is done in a relatively confined space, occasionally people become anxious. This does not happen often, and if you feel anxious, we will spend time allowing you to get used to the surroundings. During the scan, you will be asked to perform some simple tasks of memory and attention, which will enable the investigators to determine your brain function. The scan will require you to lie on your back on a table that will move into the scanning machine for the 30 minutes it will take for the scan. During this time you will be able to close your eyes and rest. You will also be able to talk to the study doctor/assistant at all times during the scan if you should experience any discomfort. The scan is a safe procedure if you have been screened correctly for the presence of any magnetic material on or inside you such as pace-makers, surgical clips and metal objects in the eyes. A formal screen for this will be done at the screening visit by a member of the study team. When the magnet in the machine is switched on, it will make

some loud banging noises, but you will be clearly warned when this will take place. At this time you will feel nothing and the noise is not harmful to you in any way. To minimise the possible discomfort associated with this, we will give you some soft earplugs to put in and will also put earphones on so that you can listen to music if you so choose.

DISCOMFORT ASSOCIATED WITH THE STUDY
There are only low or minimal risks associated with your participation in this study. If you feel tired at any point in any of the visits, you should please ask your study doctor/psychologist for a rest. If for some reason you are unable to complete a visit on a particular day we may reschedule to complete the assessments at another time.

POTENTIAL BENEFITS
There may be no direct benefits to you for participating in this study. However, you will be making an important contribution to this research that may benefit others in the future. We expect that the results of this study will help us understand the effects of apathy and depression on brain function as well as memory and concentration.

COMPENSATION FOR STUDY PARTICIPATION
While you will not be paid to take part in this study, all evaluations will be provided at no cost to you or your medical aid. You will be compensated for travel and meal costs for each session (R100 per session).

CONFIDENTIALITY
Your participation is regarded as strictly confidential. The results of the study will be published in the professional literature and made available to of the Committee for Human Research of Subcommittee C at the University of Stellenbosch, but your identity will not be revealed at any time to people outside of the study team.

THE RIGHT TO ASK QUESTIONS/WITHDRAW FROM THE STUDY
You have the right to ask questions at any time about any aspect of the study. If you have any queries, you can contact:
Dr S Seedat: Tel (24hr contact number): 082-784-8148
Dr P Carey: Tel (24hr contact number): 083-700-0046
Dr D Stein: Tel (24hr contact number): 083- 263-9679

Your participation in the study is entirely voluntary. You have the right to withdraw at any time. If you decide to withdraw from the study, it will not jeopardize you or any future treatment you may require in any way.

You are entitled to a signed copy of this document.

If you agree to take part, please complete the following section.

I)……. have been invited to take part in the above research project entitled Functional brain imaging in healthy subjects with a history of early adversity.

The study doctor/nurse has explained the details of the study to me and I understand what they have said to me.
They have also explained that this study will involve up to 3 assessments which include interviews, filling questionnaires, a physical examination including a blood test, and brain scan.
I also know that I am free to withdraw from the study at any time if I am unhappy.
By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced in any way or by anyone to take part.

Name of Participant (printed)

Signature of Participant Dated

Declaration by investigator

I (name) ……..…… declare that:

I explained the information in this document to ……..
I encouraged him/her to ask questions and took adequate time to answer them.
I am satisfied that he/she adequately understand all aspects of the research, as discussed above
I did/did not use an interpreter (if an interpreter is used, then the interpreter must sign the declaration below).

Signed at (place) .....…..…….. on (date) ……...……...

Signature of investigator

Declaration by interpreter

I (name) ……..…… declare that:

I assisted the investigator (name) ……..……. to explain the information in this document to ……..……... using the language medium of Afrikaans.
We encouraged him/her to ask questions and took adequate time to answer them.
I conveyed a factually correct version of what was related to me.
I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (place) .....….....…….. on (date) ……...……...

Signature of interpreter

APPENDIX C

Guidelines for Interpretation of CTQ-SF Scores

Table C1

	Severity Rating			
CTQ-SF Subscale	None or minimal	Low to moderate	Moderate to severe	Severe to extreme
Emotional Abuse	5-8	9-12	13-15	16 and above
Physical Abuse	5-7	8-9	10-12	13 and above
Sexual Abuse	5	6-7	8-12	13 and above
Emotional Neglect	5-9	10-14	15-17	18 and above
Physical Neglect	5-7	8-9	10-12	13 and above

Guidelines for Interpretation of BDI Scores

Table C2

Total BDI Score	Interpretation
0 - 13	No or Minimal symptoms of depression
14 - 19	Mild symptoms of depression
20 - 28	Moderate symptoms of depression
29 - 63	Severe symptoms of depression

Appendix D

Results for the fMRI subset

In the text of the thesis I present the results for the full sample. However, only a subset of these participants' data was usable for the fMRI analysis. In this appendix, I present the results of the same analyses as were conducted on the full sample, to demonstrate that the results of the sub-sample used for the fMRI analysis were no different from those of the full sample.

Participant Characteristics

Beck Depression Inventory – 2nd Ed. (BDI-II). The trauma and control groups did not differ significantly on depression scores (see Table D1). Participants' scores all fell within the *None–Minimal* range of depressive symptoms.

Table D1

BDI-II: Group means for the fMRI subset

Trauma group	Control group	t	p	d
(*n* = 9)	(*n* = 10)			
6.00 (4.40)	4.40 (5.28)	0.72	.24	.33

Note: Means, with standard deviations in brackets, are presented.
Data were missing for two trauma group participants and one control group participant.

PANAS – general ratings.

The same pattern of results as seen in the full sample was evident for the fMRI subset, with no interaction between trauma status and affect, $F(1,20) = 0.31$, $p = .59$, *partial eta*^{2} = .02. Both the trauma and control groups reported far more positive than negative general affect, $F(1,20) = 308.34$, $p < .0001$, *partial eta*^{2} =.94.

Table D2

PANAS – general ratings: Group means for the fMRI Subset

Trauma group (*n* = 11)		Control group (*n* = 11)	
Positive affect	Negative affect	Positive affect	Negative affect
35.46 (4.63)	14.55 (4.23)	34.46 (4.32)	12.18 (2.68)

Note: Means, with standard deviations in brackets are presented.

Affective Neuroscience Personality Scales (ANPS).

A significant group difference (in the same direction as that seen in the full sample) on the SEEKING subscale was also evident in the fMRI subset, $t(20) = 1.99$, $p < .03$, $d = .85$. Control participants scored significantly higher on this subscale than trauma participants.

As was seen for the full sample, the prediction that the trauma group would score higher on the SADNESS subscale was not supported, $t(20) = 0.22$, $p < .41$, $d = .09$, respectively.

Table D3

ANPS subscales: Group means for fMRI subset

ANPS Subscale	Control group (*n* = 11)	Trauma group (*n* = 11)
SEEKING	22.46 (1.97)	20.46 (2.70)
FEAR	16.09 (2.91)	16.73 (1.85)
CARE	18.64 (4.74)	20.09 (3.65)
ANGER	16.00 (2.61)	15.77 (4.78)
PLAY	20.73 (2.33)	20.36 (4.23)
SADNESS	16.66 (2.01)	16.91 (3.56)

Note: Means, with standard deviations in brackets, are presented.

Impact of Opioid Manipulation on Subjective Experience of Affect

PANAS – moment ratings.

The same pattern of results as seen for the full sample was evident for the fMRI subset: both the trauma and control groups reported far more positive than negative affect, $F(2.21, 44.23) = 77.53$, $p < .0001$, *partial eta*^{2} = .16, and there was a significant interaction between trauma status and medication-PANAS ratings, $F(2.21, 44.23) = 5.29$, $p < .003$, *partial eta*^{2} = .21. For a graphical representation of this interaction, see Figure 1. The data patterns illustrated here are very similar to those shown for the full sample.

Table D4

PANAS current affect ratings: Group means for the fMRI subset on and off buprenorphine

	Trauma group (*n* = 11)	Control group (*n* = 11)
Placebo - positive affect	29.10 (9.17)	28.91 (5.21)
Placebo – negative affect	15.36 (5.35)	11.19 (2.96)
Medication – positive affect	25.18 (6.29)	32.00 (4.98)
Medication – negative affect	13.55 (4.63)	11.09 (1.05)

Note: Means, with standard deviations in brackets, are presented.

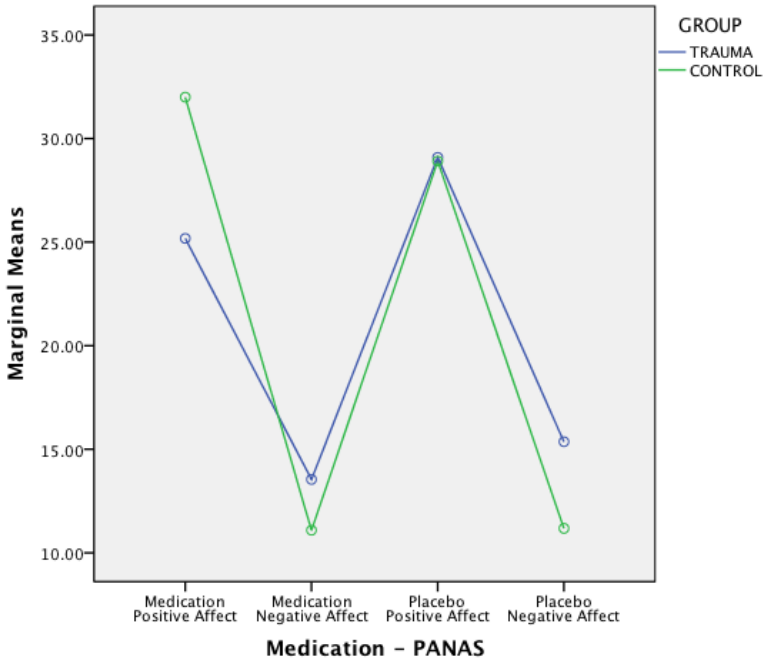

Figure1:

Interaction of trauma status with medication – PANAS ratings of current affect

Simple effects analysis of the interaction indicated the same patterns as those seen for the full sample: There were no significant within-groups effects. That is, within the trauma and control groups, no significant change in either positive or negative affect on medication versus placebo was observed. The opioid manipulation was not sufficiently powerful to result in statistically significantly altered experience of emotion within the groups.

In terms of positive affect, participants in both the trauma and control groups reported very similar scores on placebo ($p < .96$). However, on the opioid medication, the control group reported significantly more positive affect than the trauma group ($p < .011$).

In terms of negative affect, on placebo the trauma group reported significantly more negative affect than the control group ($p < .035$). However the between group difference was non-significant in the medication condition ($p < .10$).

Impact of Opioid Manipulation on Behavior

Response bias task. A 2 (group) x 8 (medication condition – emotion) mixed-design ANOVA indicated a significant interaction between trauma status and types of errors made for neutral faces on and off medication, for both the full sample and the fMRI subset, $F(2.93, 85.02) = 3.61$, $p < .017$, *partial eta*^{2} = .11 and $F(2,31, 46.11) = 3.48$, $p < .033$, *partial eta*^{2} = .15, respectively.

Table D5

Response Bias: Group means for the fMRI subset; % of total choices for neutral faces on and off buprenorphine

		Trauma group	Control group
		(n =11)	*(n =11)*
Placebo	Anger	23.2 (12.6)	26.0 (10.3)
	Happy	8.7 (8.9)	17.8 (14.5)
	Sad	56.6 (19.9)	46.6 (12.1)
	Fear	11.5 (8.6)	9.3 (5.7)
Medication	Anger	19.7 (10.8)	27.4 (9.5)
	Happy	9.1 (7.3)	22.0 (15.7)
	Sad	60.6 (20.4)	40.2 (20.3)
	Fear	10.6 (9.4)	8.7 (6.1)

Note: Means, with standard deviations in brackets, are presented.

Simple effects analysis of the interaction indicated the following: for the full sample, on placebo, the trauma group was significantly more likely than the control group to judge neutral faces as sad ($p < .049$). Although this trend was present in the fMRI subset, this contrast was not significant.

On medication two group differences were found for both the full sample and the fMRI subset: firstly, the control group was significantly more inclined to judge neutral expressions as showing happiness than was the trauma group ($p < .022$); and secondly, the trauma group was significantly more inclined to judge neutral expressions as showing sadness than was the control group ($p < .029$).