Influence of Administration of the β-Adrenoceptor Antagonist Propranolol on Anxiety-Like Behaviours in Mice Exposed to Early Life Stress

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ABSTRACT

Traumatic experiences in childhood and adolescence can result in sensitization of stress responses, increasing the risk of developing post-traumatic stress disorder (PTSD) in adulthood. A hallmark of PTSD is anxiety caused by the over-consolidation of traumatic memories as a result of stress-related increases in norepinephrine. We conducted two experiments to test the effect of the β-adrenoceptor antagonist propranolol on anxiety-like behaviours (measured in an elevated plus-maze) in mice exposed to a three-day juvenile stress paradigm before being re-exposed to stress 30 days later. Experiment 1 comprised injections immediately following juvenile stress while Experiment 2 consisted of post-re-exposure injections. Anxiety levels were tested 2 hours and 2 weeks after re-exposure. While juvenile injections did not produce meaningful changes in anxiety, post-re-exposure administration of propranolol resulted in reduced anxiety and increased impulsivity, albeit short-lived. Therefore, propranolol appears to exert meaningful short-term effects on anxiety when administered soon after the re-experiencing of trauma.

ACKNOWLEDGEMENTS

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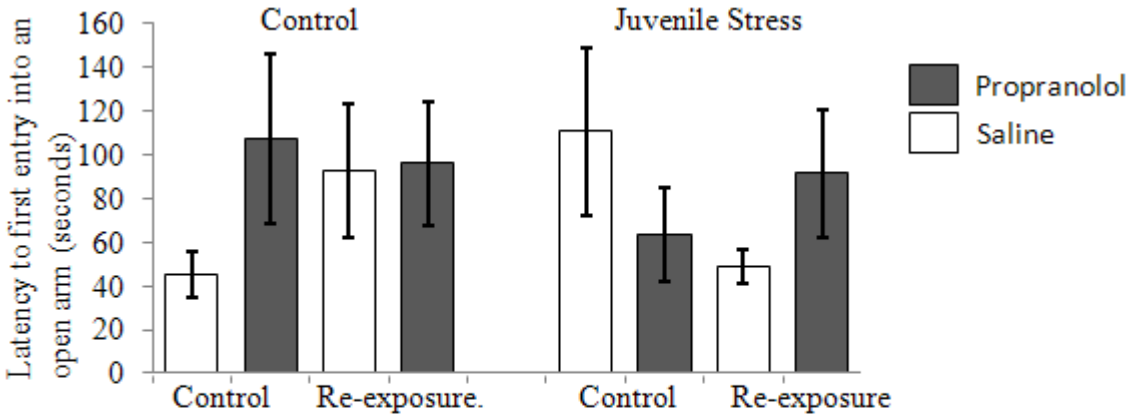

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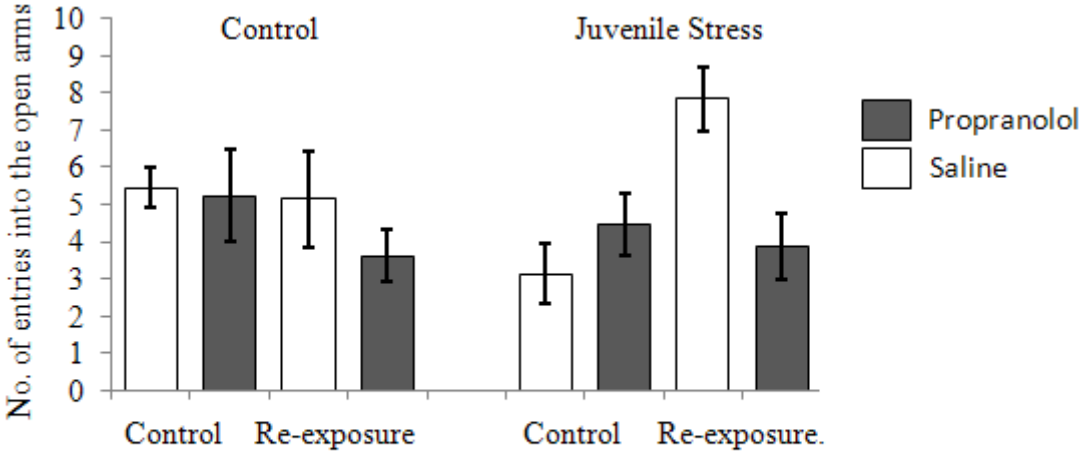

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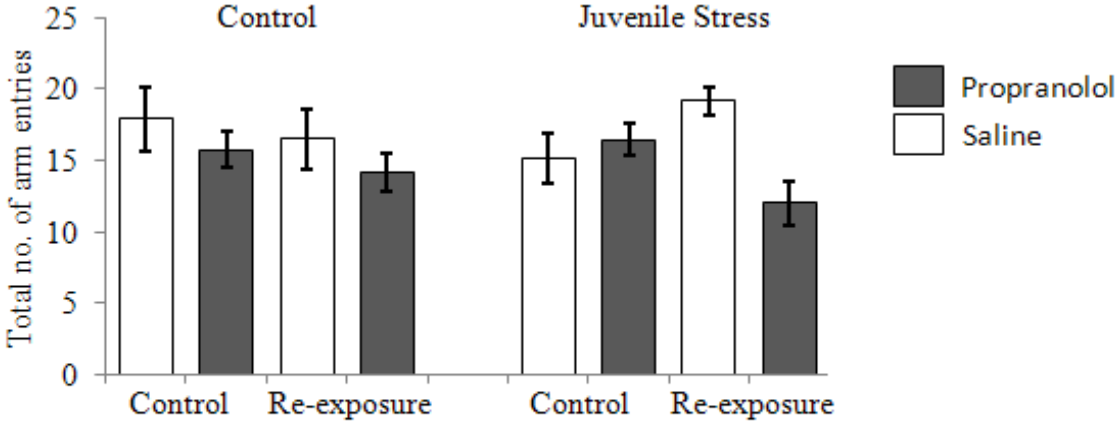

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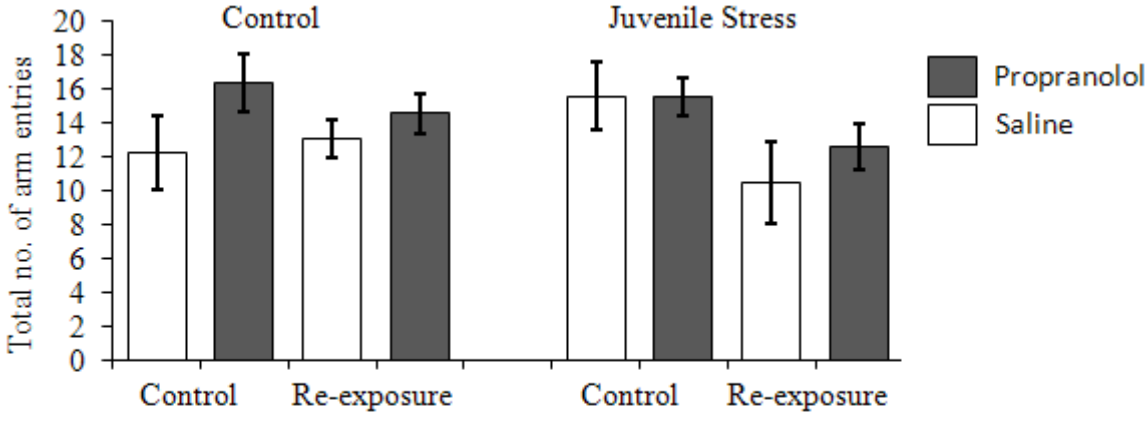

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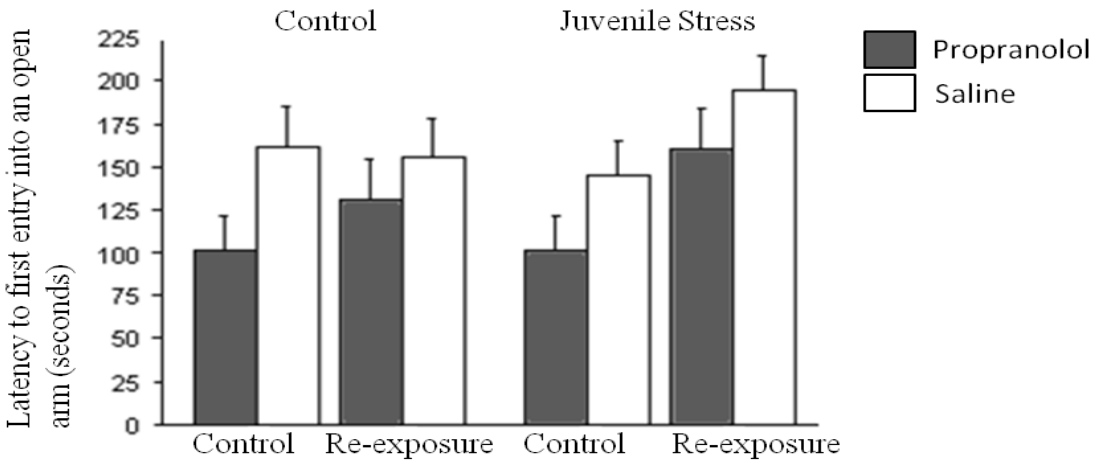

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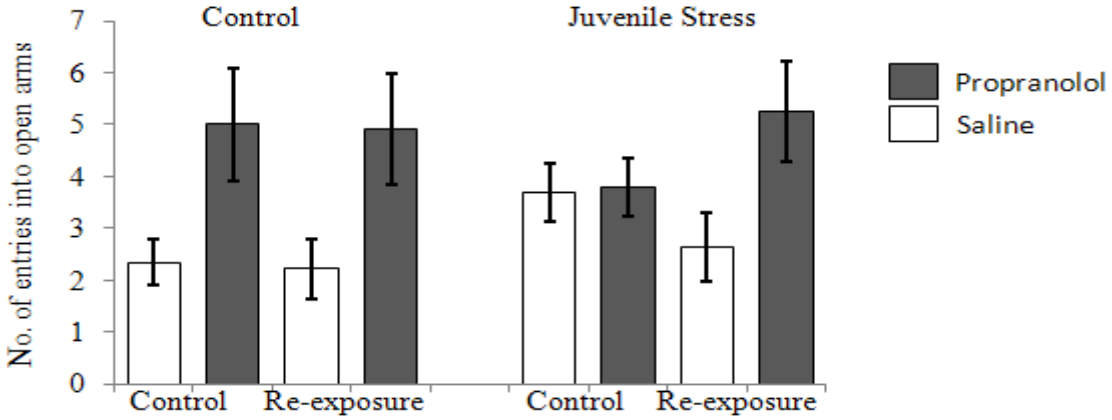

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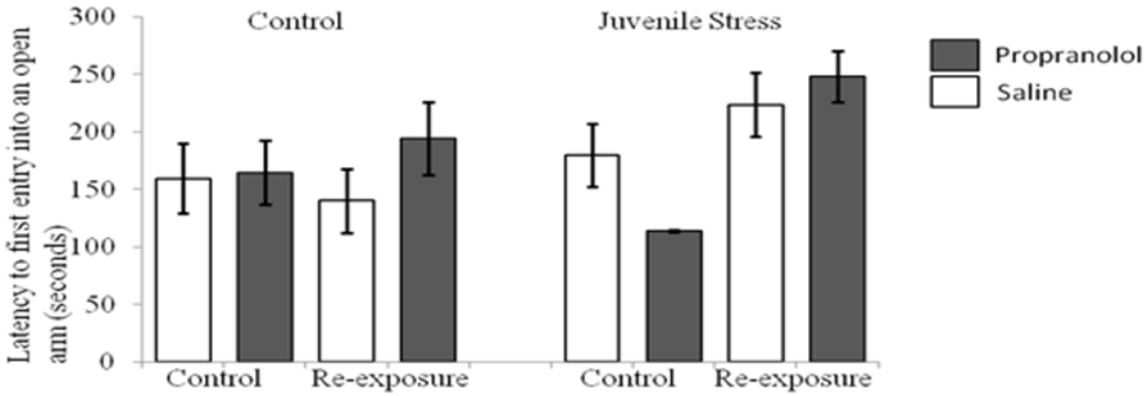

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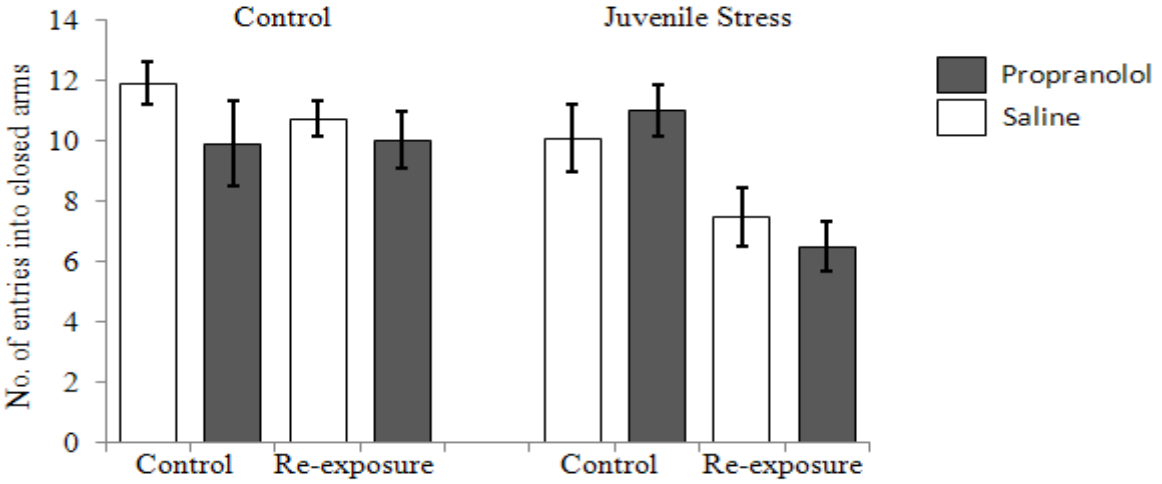

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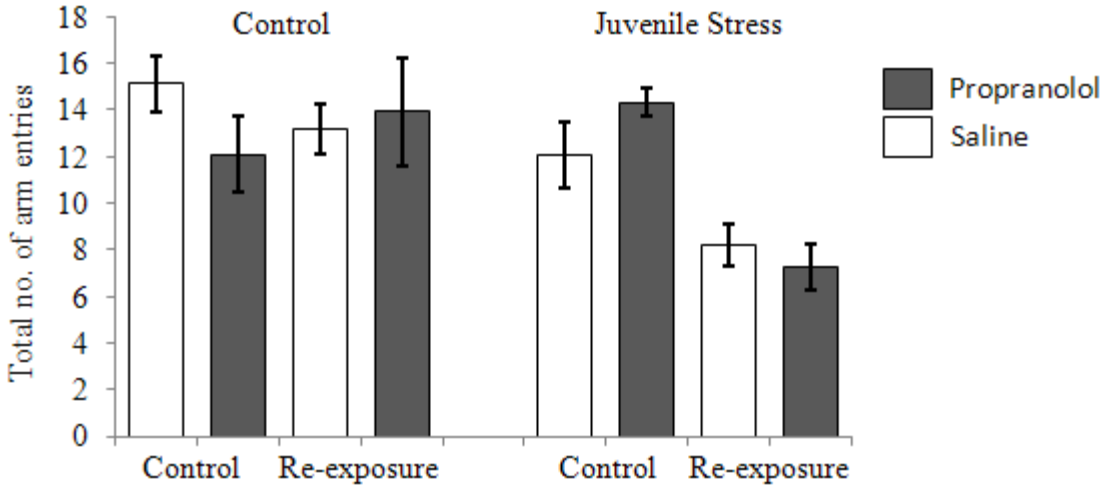

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INTRODUCTION

There is considerable evidence that adverse early life events have profound and long-lasting effects on brain functions, and may represent a risk factor for the development of psychopathologies later in life (Fumagalli, Molteni, Racagni and Riva, 2007) Empirical observations derived from psychoanalytic, psychotherapeutic and general psychiatric clinical settings have suggested that childhood maltreatment has long-term consequences on mental health in adulthood (Mello, Faria, Mello, Carpenter, Tyrka and Price, 2009), with studies showing increasing evidence for an important role of adverse early life experiences in the development of psychiatric disorders in adulthood (Coplan, Andrews, Rosenblum, Owens, Friedman, Gorman et al., 1996). More methodologically rigorous studies carried out in the more recent past have confirmed these earlier findings, while advances in basic and applied neuroscience have led to greater insights into the underlying mechanisms (Mello et al.).

The importance of research on development of psychiatric disorders as a consequence of abuse and maltreatment in juvenility is highlighted by the high incidence of child maltreatment. During 2007, an estimated 794,000 children were determined by Child Protective Services to be victims of abuse and neglect in the U.S.A. (United States Department of Health & Human Services [USDHHS], 2007). The problem of violence is particularly alarming, with between 500 million and 1.5 billion children estimated to experience violence annually worldwide. The consequences of such violence are highly damaging, with many child victims experiencing long-standing physical and mental health difficulties later in life (United Nations International Children's Fund [UNICEF], 2009).

1. The Link between Early Life Stress and Adult Psychopathology

An extensive body of data has revealed a pre-eminent role for early untoward experience in the vulnerability for major psychiatric disorders. These data are provided both from animal models of stressful early life experiences as well as clinical studies with human subjects (Nemeroff, 1999).

Stressful events have been implicated as an important element in the provocation and exacerbation of a wide range of physiological and psychological disturbances, including the classical psychosomatic disorders and affective disorders such as depression (Anisman, Zaharia, Meaney and Merali, 1998). In humans, a childhood history of abuse, neglect or trauma is known to increase later susceptibility to affective disorders when compared to those adults who were reared in a nurturing environment (Ladd, Huot, Thrivikraman, Nemeroff, Meaney and Plotsky, 2000). The emergence of such psychological disturbances may be due to the fact that traumatic experiences in the form of acute or chronic stressors in early life challenge the capacity of an individual to cope, and if coping fails, various events occur that result in a long lasting state of distress (de Kloet, Joëls and Holsboer, 2005). The susceptibility of childhood maltreatment victims to develop psychopathology in adulthood is not surprising when one considers that early life experiences not only attenuate responses to stressors experienced during adulthood, but can even induce behavioural changes not attainable by powerful pharmacological treatments. This is a testament to the profound impact of even mild early life experiences (Anisman et al.).

Over 30% of children exposed to traumatic experiences – physical and psychological – proceed to develop a clinical syndrome with emotional, behavioural, cognitive, social and physical symptoms called post-traumatic stress disorder (PTSD) (Perry & Azad, 1999). Numerous studies have provided support for a causal link between adverse early life

experiences in the form of childhood abuse and maltreatment and the development of depression, anxiety and PTSD in adolescence and adulthood (Spertus, Yehuda, Wong, Halligan and Semeretis, 2003; Sachs-Ericsson, Blazer, Plant and Arnow, 2005; Collishaw, Pickles, Messer, Rutter, Shearer and Maughana, 2007; McCabe, Miller, Laugesen, Antony and Young, 2009; Gaudiano & Zimmerman, 2009; Neigh, Gillespie and Nemeroff, 2009; Elzinga, Spinhoven, Berretty, de Jong and Roelofs, 2010). In fact, the ability of early life environments to modify adult neuroendocrine, behavioural, emotional, metabolic and cognitive functions has been described in animal models since the 1950s (Weaver, 2009). Recent animal studies have underscored the strength of early life stress' effect on psychological functioning. For example, Wei, David, Duman, Anisman and Kaffman (2010) found that experimental conditions that induce high and prolonged levels of stress during the post-natal period override the effects of high levels of post-natal maternal care in mice, resulting in increased anxiety-like behavior in the offspring.

While diseases of the central nervous system (CNS) are uniquely human in nature, making it problematic to draw strict correlations between animal models and humans, we speculate that early life adversities may prompt high-risk individuals to develop psychiatric illnesses later in life, likely due to their inability to cope appropriately with challenging environmental conditions (Fumagalli et al., 2007). Therefore, while children who have been maltreated have a greater likelihood of displaying negative developmental outcomes and psychopathology (Cicchetti & Toth, 2005), not every child exposed to abuse will go on to develop adult disease, raising the possibility that individuals who fall ill possess a pre-existing genetic vulnerability that interacts with early adverse experiences, increasing the risk of developing stress-related pathology (Neigh et al., 2009).

1.1 Early Life Stress and PTSD

Early life trauma can increase an individual's risk of developing PTSD in response to traumas experience in adulthood; indeed childhood abuse may be a root cause for development of PTSD later in life (Heim et al., 2000). Widom (1999) documented a significant increase in risk for PTSD for abused and neglected children who were followed into young adulthood, in contrast to a matched comparison group. The increased risk for lifetime PTSD was evident for subjects who had experienced all three specific types of abuse – physical abuse, sexual abuse and neglect. Crucially, childhood experiences of abuse and neglect also contributed independently to a person's risk of PTSD, even when known risk factors were controlled (Widom). Lansford, Dodge, Pettit, Bates, Crozier and Kaplow (2002) found that adolescents maltreated early in life had significantly higher levels of PTSD symptoms than their non-maltreated counterparts. The findings held after controlling for family and child characteristics correlated with maltreatment, proving that early physical maltreatment predicts adolescent psychological and behavioural problems beyond the effects of other factors associated with maltreatment. Nearly a decade earlier, Bremner, Southwick, Johnson, Yehuda and Charney (1993) found that patients seeking treatment for combat-related PTSD have higher rates of childhood physical abuse than combat veterans without PTSD, implying that childhood physical abuse may have preceded the development of combat-related PTSD in Vietnam War veterans.

These findings are in harmony with more than a decade of research using animal models that has shown that early life experiences shape the neurobiological systems involved in stress reactivity and regulation and that some of the effects appear permanent (Gunnar & Quevedo, 2007). The underlying physiological processes appear to involve persistent sensitization by early life stress of central nervous system (CNS) circuits involved in the regulation of stress

and emotion (Heim & Nemeroff, 2001). Thus, early life stress may alter the vulnerability threshold of brain systems, leading to sensitization to subsequent stressors and resulting in the increased vulnerability to subsequent stressors that might be required for a given disease to emerge (Heim & Nemeroff, 2001; Fumagalli et al., 2007). The brain systems in question include the central biogenic amines, circulating catecholamines and hypothalamic-pituitary-adrenal hormones, all of which are known to play a fundamental role in the stress response (Anisman et al., 1998). It follows that these different components of the stress response must also play a prominent role in stress-induced pathogenesis and subsequent development of psychopathology.

2. Neurobiology and Neuroendocrinology of the Mammalian Stress System

The mammalian stress system interacts with, influences and is influenced by several systems in the brain that serve cognitive and/or executive, fear, anger and reward functions; these systems form a complex, integrated, feedback system consisting of positive and negative feedback loops (Chrousos, 2009). The various behavioural, endocrine, autonomic and immune responses to stress are a result of the interplay among and between these systems (Tsigos & Chrousos, 2002).

The central control stations of the stress system include the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) neurons of the paraventricular nuclei of the hypothalamus, the CRH neurons of the nuclei of the medulla and the norepinephrine (NE) system located in the locus coeruleus (LC). Other prominent neurotransmitter systems in the brain involved in the stress response include the peripheral limbs of the hypothalamic-pituitary-adrenal (HPA) axis, the catecholaminergic neurons of the medulla and the opioid peptide systems. Mutual interactions of these different central stress stations influence and

affect the initiation, propagation and termination of stress system activity (Stratakis & Chrousos, 1995; Tsigos & Chrousos, 2002).

2.1 CRH/AVP

CRH is the principal hypothalamic regulator of the pituitary-adrenal axis (Charmandari, Tsigos and Chrousos, 2005) and has been implicated in various components of the stress response, including arousal and autonomic activity (Tsigos & Chrousos, 2002). Along with CRH, AVP is also a key regulator of the HPA axis (Lolait, Stewart, Jessop, Young and O'Carroll, 2007). Stress is a potent activator of CRH release from the hypothalamus and extrahypothalamic sites (Bale & Vale, 2004); during stress, the relative proportion of the subset of neurons that secrete CRH and AVP increase significantly (Charmandari et al.). CRH and AVP stimulate secretion of adrenocorticotropin-releasing hormone (ACTH) (Stratakis & Chrousos, 1995; Lolait et al.) and in doing so, govern the HPA axis (de Kloet et al., 2005).

2.2 HPA Axis

Work in animal models of stress has shown the HPA axis to be especially involved in the promotion of adaptive responses to stress, anxiety and fear (Sapolsky, Armanini, Packan, Sutton and Plotsky, 1990). The HPA axis represents a major counter-regulatory system activated upon exposure to many stressors (Ladd et al., 2000). In this system, the hypothalamus controls secretion of ACTH – primarily via release of CRH – from the anterior pituitary, which in turn stimulates the secretion of glucocorticoid hormones (mainly cortisol in humans and corticosterone in rodents, amphibians, reptiles and birds) by the adrenal cortex (Tsigos & Chrousos, 2002). Glucocorticoids are the final effectors of the HPA axis and regulate a broad spectrum of physiological functions essential for life (Tsigos & Chrousos). They also act back on the hypothalamus and pituitary to suppress CRH and ACTH production in a negative feedback cycle (Chrousos, 2009).

In non-stressful situations, both CRH and AVP are secreted by the hypothalamus in a consistent, rhythmic fashion (Engler, Pham, Fullerton, Ooi, Funder and Clarke, 1989). However, stress disrupts diurnal variations in the amplitude of CRH and AVP pulses and, consequently, influences the occurrence of ACTH secretions. Acute stress results in increased amplitude and synchronization of CRH and AVP pulses and increases in ACTH and cortisol secretions (Tsigos & Chrousos, 2002).

Glucocorticoids participate in the control of homeostasis and the stress response (Tsigos & Chrousos, 2002). The acute secretion of glucocorticoids in response to a stressor is in essence the primary mediator in the chain of hormonal events triggered in response to stress. Glucocorticoids have a range of effects in target systems which increase the availability of energy substrates in different areas of the body, allowing the organism to adapt to changing demands of its environment. In doing so, glucorticoids give rise to the fight-or-flight response (Lupien, Maheu, Tu, Fiocco and Schramek, 2007). As stated earlier, glucocorticoids act back on the hypothalamus and pituitary to suppress CRH and ACTH production. This negative feedback serves to limit the exposure of tissues to glucocorticoids, thus mitigating their catabolic, lipogenic, anti-reproductive and immunosuppressive effects (Chrousos, Kinob and Charmandari, 2009).

2.3 LC/NE System

NE-containing axons are distributed widely throughout the CNS, suggesting a prominent role of NE in CNS function and behavior (Berridge & Waterhouse, 2003). NE is secreted by the sympathetic nervous system within seconds of perceiving a stressor, and along with epinephrine (Epi), increases blood glucose levels through a variety of mechanisms, fuelling the fight-or-flight response (Nelson, 2005). NE is also known to play a role in emotional memory. Emotional experience activates the adrenergic system, thus modulating encoding so

that the incident may be better remembered; stimulation of the central NE system results in enhancement, and blockade in a reduction, of memory for emotional material (O'Carroll, Drysdale, Cahill, Shajahan and Ebmeier, 1999). As O'Carroll et al. point out, one potential down-side of this modulatory mechanism is that extremely emotional events may be remembered too well by some individuals, leading to intrusive, distressing recollections and flashbacks, central clinical features of PTSD.

Most noradrenergic neurons in the brain are concentrated in the locus coeruleus (LC) (Berridge & Waterhouse, 2003), the largest of seven brainstem nuclei from which all noradrenergic projections in the brain emanate (Ladd et al., 2000). Evidence suggests that CRH inputs from the central nucleus of the amygdala to the LC may mediate activation of the latter by environmental stressors, and disruption of the LC/NE system may result in stress-related disorders (Berridge & Waterhouse).

2.4 Effects of Early Life Stress on the Functioning of the Stress System

Over half a century ago, Levine (1957) wrote that "the effects of early life experience modify later reactivity of the central nervous system under stress conditions". Today, there is an expanding body of literature strongly suggesting that early adverse experience, including prenatal stress and childhood stress, has profound and long-lasting effects on the development of neurobiological systems, thereby influencing subsequent reactivity to stress and vulnerability to develop PTSD (Heim & Nemeroff, 2009).

Early life experiences are known to program HPA axis functioning, including negative feedback, to modify the response to subsequent stressor experiences (Anisman et al., 1998). Indeed the responsiveness of adult organisms to psychological stressors is altered as a result of the significant impact exerted by early environmental factors on the developing HPA axis (Ladd et al., 2000).

Heim et al. (2000), in one of the first human studies to report persistent changes in stress reactivity in adults who suffered early life trauma, found that severe early life stress is associated with persistent sensitization of the pituitary-adrenal and autonomic stress response, and that this sensitization is likely responsible for an increased risk of developing psychopathology in adulthood. These findings were impressively consistent with findings from laboratory animal studies (Heim et al., 2000).

The increased reactivity to stress and subsequent vulnerability to mood and anxiety disorders appears to be mediated, at least in part, by persistent activation and hyper-responsiveness of hypothalamic and extra-hypothalamic CRH circuits (Nemeroff, 1999). Considerable evidence indicates that early life stress affects the central CRH system, resulting in long-term alterations in the stress-responsive neuroendocrine system (Heim, Owens, Plotsky and Nemeroff, 1997). Untoward early life events induce a persistent increase in CRH neuronal activity, paralleling findings of elevated cerebrospinal fluid CRH concentrations in depression and PTSD. CRH hypersecretion seems to be accompanied by sensitization of the stress response, a finding that is aligned with the concept of sensitization of the HPA axis in PTSD, which allows the organism to maximally respond to stress (Heim et al., 2007). Moreover, early life stress, like PTSD, is associated with behavioral sensitization to noradrenergic (NE) stimulation (Heim et al., 2007).

3. PTSD

The Diagnostic and Statistical Manual of Mental Disorders-IV-TR (American Psychiatric Association, 2000) describes the essential feature of PTSD as being:

the development of certain characteristic symptoms following exposure to an extreme traumatic stressor involving direct personal experience of an event involving actual or threatened death or serious injury or learning about the unexpected or violent death, injury or

threat of death of injury experienced by a family member or other close associate. The person's response to the event must involve intense fear, horror or helplessness (or disorganized/agitated behaviour in the case of children) in order for a PTSD diagnosis to be made.

According to Yehuda, the onset of PTSD is facilitated by a failure to contain the biological stress response at the time of the trauma, resulting in a cascade of alterations that lead to the characteristic symptoms of PTSD (as cited in de Kloet et al., 2005). These symptoms may be clustered into three groups – re-experiencing the traumatic event, avoidance of stimuli that resemble the event and numbing of emotional responsiveness, and increased arousal – and are defined in terms of their connection with the traumatic event that is the presumed cause of the disorder (Breslau, 2009). The vulnerability of an individual to PTSD is defined at three different levels: the traditional clinical phenotype (emotional reactivity and personality), the functional phenotype (neuroendocrine reactivity to stimuli) and the genotype (for example, polymorphisms in genes involved in HPA signaling) (de Kloet et al.).

Since 1980, research on PTSD has focused chiefly on Vietnam War veterans and to a lesser degree on victims of specific types of traumas, such as natural disaster or rape. With the growth of psychiatric epidemiology, PTSD has been studied in samples of the general population in various countries. In the latest edition of the DSM, the definition of traumatic events that can potentially cause PTSD has been enlarged to include a wider range of events than the typical traumatic events of the initial definition (Breslau, 2009). Davidson, Stein, Shalev and Yehuda estimate that 10-40% of individuals who have been exposed to extreme trauma such as the holocaust, combat, rape, abuse or a traffic accident develop PTSD (as cited in de Kloet et al., 2005).

3.1 Early Life Trauma and PTSD in Humans

A frequently replicated finding is the enhanced probability of PTSD in stress-exposed persons who had experienced prior traumatic events (Breslau, 2009). A study of Vietnam War veterans found that patients seeking treatment for combat-related PTSD have higher rates of childhood physical abuse than combat veterans without PTSD, implying that childhood physical abuse may have been an antecedent to the development of combat-related PTSD (Bremner et al., 1993).

Furthermore, recent studies suggest a strong relation between early adverse life events, i.e. childhood physical abuse and negative parenting behaviour and the development of PTSD in response to combat exposure (Heim et al., 1997). The increased likelihood of developing PTSD among child maltreatment sufferers is not confined to those who developed it as a result of exposure to the stresses of war, but is also seen in victims of non-combat trauma. For example, studies of general population samples carried out by Breslau, Chilcoat, Kessler and Davis as well as Galea et al. have reported higher rates of prior trauma (including childhood maltreatment) among stress-exposed persons who succumbed to PTSD than among exposed persons who did not (as cited in Breslau, 2009).

These findings have been interpreted by Post & Weiss as supporting a ‘sensitization’ process, i.e. greater responsiveness to subsequent stressors (as cited in Breslau, 2009). The neurobiological and neuroendocrinological basis of this sensitization – as has been alluded to in previous sections – appears to involve phenomena ranging from persistent activation and hyper-responsiveness of CRH circuits (Nemeroff, 1999) and programming of the HPA axis including feedback (Anisman et al., 1998; Ladd et al., 2000) to sensitization to noradrenergic stimulation (Morgan, Krystal and Southwick, 2003) and sensitization of the autonomic stress response (Heim et al., 2000).

3.2 Neurobiology and Neuroendocrinology of PTSD

The role of the neurobiological systems involved in PTSD is summarized thusly by Heim & Nemeroff (2009):

Neurobiological systems that have been implicated in that pathophysiology of PTSD include the HPA axis, as well as various neurotransmitters and neuropeptides that comprise a network of brain regions that regulate fear and stress responses, including the prefrontal cortex, hippocampus, amygdala, and brainstem nuclei. More recently, there have been attempts to link the identified neurobiological changes to the specific features that constitute PTSD, such as altered mechanisms of learning and extinction, sensitization to stress, and arousal.

With regards to the HPA axis, although it is activated by acute stressors, studies conducted by Yehuda with combat veterans with PTSD revealed counter-intuitive decreases in cortisol concentrations – a paradoxical finding that has been replicated in Holocaust survivors, refugees and abused persons with PTSD, although findings are not uniformly consistent across studies (as cited in Heim & Nemeroff, 2009). Indeed cortisol levels of PTSD patients may also be similar to or greater than those of comparison subjects (Yehuda, 2006). While studies using the Dexamethasone Suppression Test (DST) have shown that there is enhanced negative feedback inhibition of cortisol at the level of the pituitary, the suggestion that this is responsible for other HPA axis alterations in PTSD is largely descriptive and offers little explanation as to why some individuals show such alterations following exposure to trauma while others do not (Yehuda).

3.3 Role of NE and Epi in PTSD Pathophysiology

An expanding body of literature comprising neuroendocrine, pharmacologic challenge, brain imaging and other studies has provided compelling evidence for increased noradrenergic activity in humans with PTSD (Southwick, Bremner, Rasmusson, Morgan, Anset and

Charney, 1999). This increased activity is generally observed in response to various stressors and has been suggested by Southwick et al. (1997) to be associated with a variety of hyperarousal and re-experiencing symptoms reminiscent of PTSD (as cited in Southwick et al., 1999).

Redmond conducted a series of studies that showed that acute stress and fear activate the LC/NE system (as cited in Southwick et al., 1999). Stress and fear-related activation of the LC results in increased release of NE in brain regions involved in perceiving, evaluating and responding to potentially threatening stimuli, such as the amygdala, hippocampus, striatum and prefrontal cortex (Southwick et al.). Rapid activation of the LC/NE system facilitates an organism's ability to respond effectively in dangerous situations, but is detrimental when arousal occurs to such an extent that it starts to have undesirable effects on the organism's normal functioning, as seen in PTSD (Charney et al., 1995)

Similarly, with regards to memory, although remembering dangerous situations may protect one from similar potentially dangerous situations in the future, trauma survivors find these memories to be distressing and tormenting (Southwick et al., 1999). McGaugh and Roozendal have both hypothesized that traumatic events stimulate the release of NE and Epi, causing an over-consolidation of memory for the stressful event (as cited in Southwick et al.). The result is a deeply engraved traumatic memory expressed in the form of intrusive recollections, nightmares and flashbacks (Pitman, 1989), a typical sign of PTSD. This consolidated traumatic memory, when re-experienced alongside release of NE and Epi, further strengthens the memory trace, causing an even greater likelihood of subsequent intrusive recollections (Southwick et al.).

The role of Epi in the consolidation of recently formed memories was explored by Gold & Van Buskirk, who demonstrated that post-trial injections of Epi facilitated retention of

inhibitory avoidance training with effects that were dose-dependent (intermediate doses enhanced retention) and time-dependent (memory enhancing effects of Epi were inversely related to the time between training and Epi administration) (as cited in Southwick et al., 1999). Epi does not readily cross the blood brain barrier and, according to Introini-Collison, is thought to affect memory storage by activating peripheral β-adrenergic receptors on afferent fibers that project to the nucleus solitary tract before releasing NE in the amygdala (as cited in Southwick et al.). This explains the potential of the β-adrenergic blocker Propranolol as an agent that could weaken consolidation of traumatic memories. According to Gold & McCarty, Epi may also enhance memory by increasing circulating levels of glucose, which readily crosses the blood brain barrier (as cited in Southwick et al.).

In addition to their effects on memory consolidation, Epi and NE have also been shown to enhance memory retrieval when administered at the time of memory testing (Southwick et al., 1999). Liang, Juler and McGaugh found that retention for an inhibitory avoidance task is enhanced by intra-amygdala infusions of NE immediately after training (as cited in Southwick et al.).

Human investigations have reported a relationship between NE and intrusive traumatic memories (Southwick et al., 1999). In studies of combat veterans with PTSD (Yehuda et al.), 24-hour urinary excretion of NE has been positively correlated with intrusive traumatic memories (as cited in Southwick et al.). In a study comparing women with histories of childhood sexual abuse, Lemieux & Coe reported significantly higher NE and Epi levels in women with PTSD compared to controls (as cited in Southwick et al.). Previously, Yehuda et al. found significantly elevated 24-hour NE and Epi excretion among veterans with PTSD compared to control subjects (as cited in Southwick et al.).

3.4 Existing and Emerging Pharmacological Interventions for PTSD.

While recent guidelines advise against drug treatments as a routine first-line treatment for PTSD in preference to psychological therapy (National Collaborating Centre for Mental Health [NCCMH], 2005), medications are often necessary to mitigate symptoms and the pursuit of more effective medication is essential to developing a range of effective treatment options (Cukor, Spitalnick, Difede, Rizzo and Rothbaum, 2009). A number of pharmacologic agents have shown promise in alleviating PTSD symptomatology. These include the N-methyl-D-aspartate (NMDA) receptor partial agonist D-cycloserine, which may facilitate fear extinction and reduce post-treatment relapse, Prozasin, an α-1 adrenergic blocker which may target specific sleep-related disturbances in PTSD patients, and ketamine, an anesthetic NMDA receptor antagonist which could aid in the disruption of memory processes of traumatic experience (Cukor et al.). That being said, pharmacologic modulation of the stress reactivation process to alter subsequent recall is not fully understood, despite its potential as a feasible therapeutic target (Cai, Blundell, Han, Greene and Powell, 2006).

The present investigation will test the efficacy of propranolol, a non-selective β-adrenergic blocker that blocks both the β1 and β2 receptors (Southwick et al., 1999) and has been used with psychiatric patients to treat social phobia, disorders of aggression and violence, resistant mania, akasthesia and hypertension (Southwick et al.; Cukor et al., 2009).

As stated in Section 3.3, Epi and NE are released during traumatic events, resulting in an over-consolidation of memory for the stressful event and subsequent intrusive recollections and flashbacks (Southwick et al., 1999; Pitman, 1989). This appears to be a deleterious consequence of what is actually an adaptive mechanism whereby the significance of an experience facilitates its remembrance (Pitman et al., 2002). Indeed according to Vaiva et al., prolonged adrenergic activation in the aftermath of a trauma has been linked to increased risk

for PTSD (as cited in Cukor et al., 2009). It follows that the β-adrenergic blocker propranolol, if administered before or immediately after a traumatic event, could prevent or diminish the sensitization of catecholamine systems and associated PTSD symptoms or, when administered early enough, might prevent the over-encoding of traumatic memories that results from stress-related increases in Epi and NE (Southwick et al.), consequently preventing the development of PTSD (Pitman et al.). It is thought that since propranolol is lipid soluble and readily crosses the blood brain barrier, its effects on emotional memory could be mediated by both central and peripheral actions of the drug (Southwick et al.).

Numerous studies have been carried out to assess propranolol's efficacy in modulating traumatic memories. Cahill, Prins, Weber and McGaugh (1994) found that subjects who received placebo an hour before viewing a series of slides depicting either neutral or emotionally stressful scenes displayed significantly better memory for emotional slides than neutral slides a week after exposure, while subjects who received propranolol did not remember the emotional slides significantly better, suggesting that β-activation may be involved in the enhanced memory associated with arousing or emotional experiences. These results suggest that memory storage is modulated by β-adrenergic systems and that the enhanced memory for events associated with emotional arousal involves activation of β-adrenergic receptors (Cahill et al.). Brunet, Orr, Tremblay, Robertson, Nader and Pitman (2008) asked individuals with PTSD to write a script of their traumatic experience before administering either propranolol or placebo. A week later, psychophysiologic reactivity was measured while the subjects listened to a recording of a script of their traumatic experience. Subjects who received post-retrieval placebo showed responses typical of trauma victims with PTSD whereas those who received propranolol showed responses typical of trauma victims without PTSD (Brunet et al.). Famularo, Kinscherff and Fenton conducted open treatment

trials of propranolol with children and reported a decrease in nightmares, explosiveness, exaggerated startle, insomnia and hyper-alertness (as cited in Morgan et al., 2003), symptoms associated with alterations in NE and Epi (Southwick et al., 1999).

Thus, propranolol clearly holds some potential in alleviating memory-related symptoms of PTSD. However, much research still needs to be conducted to truly gauge the extent of understanding of propranolol's effects on the adrenergic systems involved in the encoding, processing and recollection of traumatic memories. Moreover, propranolol has barely been tested on mice models of PTSD, hence the present study in which the effects of propranolol on anxiety-like behaviour in mice exposed to juvenile stress will be assessed.

4. Toward an Animal Model of PTSD

Cohen, Zohar, Matar, Zeev, Loewenthal and Richter-Levin (2004) provide a thoughtful summary of the goals and validity of animal models:

In order for the animal model of a human condition to be useful in advancing clinical understanding, it must be as valid as possible an approximation of the human disorder it is modeling. The rationale behind the use of animal models for human conditions is to enable experimentation in ways and with sample sizes, which are many times impossible in humans, for ethical/moral or practical/technical reasons… In order for an animal behavioral model to serve its purpose and help to elucidate certain aspects of complex human clinical conditions comprising emotional, cognitive, existential, behavioral and physiological components, such as PTSD, the design of the model must strive to parallel the clinical condition as closely as possible.

With regards to PTSD, several animal models have been deployed in which intense stressful experiences, aversive challenges and situational reminders of a traumatic stress have been shown to result in long-term effects on behavioural, autonomic and hormonal responses

mimicking many of the changes seen in human subjects with PTSD (Cohen, Zohar and Matar, 2003). There is variability with regards to the models used; indeed there may be more animal models of anxiety disorders than of any other psychiatric condition (Lister, 1990). To date, much animal research on PTSD has utilized rat models (e.g. Paré, 1996; Levy, Kadar and Dachir, 2001; Cohen & Zohar, 2004; Rau, De Cola & Fanselow, 2005; Wakizono et al., 2007). This is not surprising because rats perform well in many of the cognitive and operant tasks that are the foundation of modern behavioural pharmacology (Cryan & Holmes, 2005). However in recent times, there has been an explosion in the use of mice in neuropsychiatric research, partly because mice are uniquely amenable to gene targeting and alteration techniques, and also because they have the practical and economic advantage of being relatively easy to breed and house in large numbers (Cryan & Holmes). Therefore, while there is presently a relative paucity of PTSD research with mice, this could well change in coming years.

4.1 Mouse Models of PTSD

The dearth of PTSD research using mice means that there is no single widely accepted model for testing the efficacy of pharmacological agents in impairing consolidation of traumatic memories. One PTSD study somewhat similar in investigative angle to our own study was conducted by Cai et al. (2006), in an experiment which tested whether the endogenous stress hormone corticosterone could reduce recall of an established contextual fear memory in mice. The researchers utilized a Pavlovian fear conditioning model, known to provide one of the best rodent models of acquired anxiety disorders (Cai et al.) Mice were trained in a classical fear-conditioning paradigm before being re-exposed to the training environment forty eight hours later, after which they were injected with various doses of corticosterone, anisomycin or vehicle solution prior to being tested for contextual fear

memory. It was found that corticosterone impaired subsequent recall of the established fear memory, an effect that may be interpreted as inhibition of reconsolidation (Cai et al.).

5. Goal and Hypotheses of the Present Study

The present study was performed to test the efficacy of propranolol in reducing post-traumatic stress and anxiety (ostensibly by impairing over-consolidation of traumatic memories). To this end, we used a model of PTSD in which mice were subjected to stress in juvenility, resulting in sensitization of responses to future trauma. The mice were then re-exposed to stress in adulthood, thus providing a model of PTSD based on sensitization to stress. This allowed for the testing of the efficacy of propranolol (administered either following juvenile stress or following re-exposure to stress in adulthood) in reducing fear and anxiety measures in these mice.

To accomplish this, we conducted two experiments to assess the efficacy of propranolol in attenuating fear and anxiety responses in mice subjected to a three-day variable stress paradigm in juvenility. The sole difference between the two experiments was the time of injection: Experiment 1 comprised injection in juvenility while Experiment 2 comprised injection in adulthood. In both cases, it was hypothesized that propranolol would be associated with decreased levels of fear and anxiety in the mice, as measured in the elevated plus maze. We hypothesized that propranolol, whether administered after juvenile stress or following re-exposure to stress in adulthood, would sufficiently interfere with the over-consolidation of traumatic memories so as to reduce anxiety levels initially boosted by juvenile stress exposure.

EXPERIMENT 1

Methods

Subjects

Male CD-1 mice (N = 69) were randomly assigned to one of eight experimental groups outlined below. All animals were locally bred in the Carleton University Institute of Neuroscience vivarium. Post-weaning, the mice were group-housed in cages of 3-4 with their same-sex siblings and housed in Plexiglas cages with ad libitum access to food pellets and water. Mice were maintained in the colony room with a 12:12 hour light-dark cycle. All experimental procedures complied with the guidelines set out by the Canadian Council on Animal Care (CACC) and were approved by the Carleton University Animal Care Committee (ACC).

Apparatus

Juvenile Stressors

Restraint.

Cone-shaped plastic baggies (decapicones) were used for baggy restraint, with adhesive tape used to seal the decapicones and prevent escape. Each decapicone had a hole at its narrow end, enabling the restrained mouse to breathe.

Forced swim stress.

Plastic buckets filled with water measured to be between 20-21° were used.

Wet bedding.

Mice' home cages and bedding were flooded by water to a depth of approximately one inch. After the procedure, the animals were moved to clean cages furnished with dry bedding and a nestlet.

Injections

Injections of propranolol and saline were delivered intraperitoneally (i.p.) using 30-gauge needles. Mice were weighed on each day that they were to be injected, and injection amounts of propranolol were selected at a proportion of 5mg/kg of body weight.

Elevated Plus Maze

A standard elevated plus maze such as that employed by Pellow et al. (1985) was used consisting of two open arms, 50 x 10cm and two enclosed arms, 50 x 10 x 40 cm, with an open roof and arranged such that the two open arms are opposite to each other. The maze was elevated to a height of 50 cm. Measures indicated in the procedure section were recorded by an observer sitting in the same room as the maze.

Procedure

Juvenile Stress and Injection

An unpredictable three-day variable stress paradigm was used consisting of 10 minutes of decapicone restraint on the 27th PND, 10 minutes of forced swim on the 28th PND and 1 hour of wet bedding on the 29th PND. Each mouse was injected with either propranolol or saline at 5mg/ kg (i.p.) following each stressor.

Decapicone restraint.

The mice were coaxed headfirst into the cone-shaped decapicone, which was then tightened so as to restrain movements of the mice but still permit breathing. Decapicones were then fastened onto a wooden table using adhesive tape. Mice were monitored throughout the procedure to ensure that they were able to breathe at all times and did not suffocate. After 10 minutes, the mice were released back into their cages.

Forced swim.

Mice were placed in a plastic bucket filled three quarters of the way up with water measured to be between 20-21° at the time of filling. The animals were monitored as a precautionary measure against drowning. After 10 minutes, the mice were retrieved from the bucket and returned to their cages.

Wet bedding.

Mice' home cages were flooded with water to a depth of approximately 1 inch. After 1 hour, the mice were shifted to clean cages furnished with dry bedding and nestlets.

Following completion of the juvenile stress paradigm, animals were returned to their home cages and were not disturbed for 30 days save for routine cage maintenance.

Stress Re-exposure

On the 57th PND, mice were re-exposed to stress via a social interaction with a retired breeder. Individual mice were placed in the cage of a resident aggressive retired breeder (Ref). Cages were fitted with transparent glass lids to facilitate a clear view of the encounter and prevent serious injury to the animals. After 15 minutes, the intruder mouse was returned to its home cage.

Behavioural Testing

Anxiety levels of the mice were assessed in an elevated plus maze 2 hours and 2 weeks after the social interaction. Each mouse was placed in the plus maze for 5 minutes, during which the latency to first entry into an open arm, number of entries into the open and closed arms, total number of arm entries and frequency of stretch attended postures (where the mouse stretches towards an open arm before retracting to its original position) were recorded manually by an observer present in the room.

Groups

The present 2 x 2 x 2 design consisted of three treatments - juvenile stress (juvenile stress vs. control), stress re-exposure (re-exposure vs. control and drug treatment (propranolol vs. saline). A detailed description of the groups is offered below.

Juvenile Stress– Re-exposure – Propranolol

These animals underwent the variable stress paradigm in juvenility accompanied with propranolol injections, and were re-exposed to stress in adulthood.

Juvenile Stress – Re-exposure – Saline

These animals underwent the variable stress paradigm in juvenility accompanied with saline injections, and were re-exposed to stress in adulthood.

Juvenile Stress – Control – Propranolol

These animals underwent the variable stress paradigm in juvenility accompanied with propranolol injections, but were not re-exposed to stress in adulthood.

Juvenile Stress – Control – Saline

These animals underwent the variable stress paradigm in juvenility accompanied with saline injections, but were not re-exposed to stress in adulthood.

Control – Re-exposure – Propranolol

These animals did not undergo the in variable stress paradigm in juvenility, were injected with propranolol on PNDs 27-28 and were re-exposed to stress in adulthood.

Control – Re-exposure – Saline

These animals did not undergo the variable stress paradigm in juvenility, were injected with saline on PNDs 27-28 and were re-exposed to stress in adulthood.

Control – Control – Propranolol

These animals did not undergo the variable stress paradigm in juvenility, were injected with propranolol on PNDs 27-28 and were not exposed to stress in adulthood.

Control – Control – Saline

These animals did not undergo the variable stress paradigm in juvenility, were injected with saline on PNDs 27-28 and were not exposed to stress in adulthood.

Statistical Analyses

The effects of juvenile stress, drug treatment and re-exposure to stress on anxiety levels were analyzed by analyses of variance (ANOVAs) using a significance level of $\alpha = 0.05$. Follow-up comparisons comprising Bonferroni/Dunn tests were conducted.

Results

Two Hours after Re-exposure to Stress

A factorial ANOVA was conducted to gauge the effects of propranolol on various aspects of mouse behaviour in the elevated plus maze. The interaction between juvenile stress, stress re-exposure and drug (propranolol vs. saline) was found to have a significant effect on latency to first entry into an open arm ($F (1, 61) = 4.54$, $p < .05$). However, propranolol did not show a significant main effect ($F (1, 61) = 0.67$, ns).

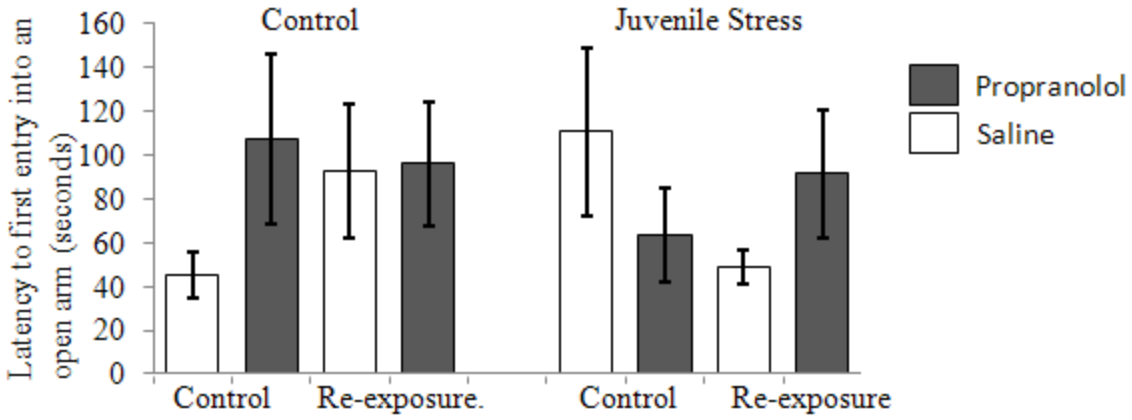

Figure 1 . Latency to 1st entry into an open arm measured 2 hours after re-exposure to stress. X-axis represents re-exposure condition.

The interaction between juvenile stress and stress re-exposure significantly affected entries into the open arms ($F (1, 61) = 5.46$, $p < .05$), as did the interaction between re-exposure and injection ($F (1, 61) = 5.87$, $p < .05$). However, juvenile stress and re-exposure did not exert any significant simple effects ($p > .05$ in both cases). Among juvenile-stressed animals re-exposed to stress in adulthood (the 'PTSD-like' group), saline appeared to be associated with more entries into the open arms ($M = 7.73$, $SD = 2.14$) than propranolol ($M = 3.89$, $SD = 2.67$)

(Figure 2). Propranolol itself was not found to exert a significant main effect (F (1, 61) = 1.978, ns).

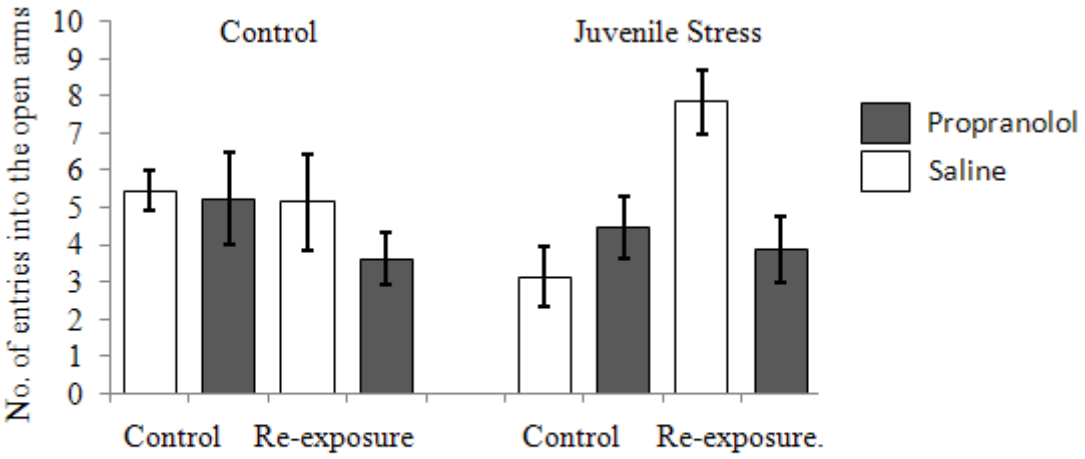

Figure 2. Number of entries into open arms measured 2 hours after re-exposure to stress. The X-axis represents the re-exposure condition.

The interaction between re-exposure and drug exerted a significant effect on total number of arm entries (F (1, 61) = 4.35, p < .05). Propranolol was found to have a significant effect on total arm entries (F (1, 61) = 4.41, p < .05). Follow up tests showed the difference between propranolol and saline as being marginally short of significant (p = .051, ns). Among mice in

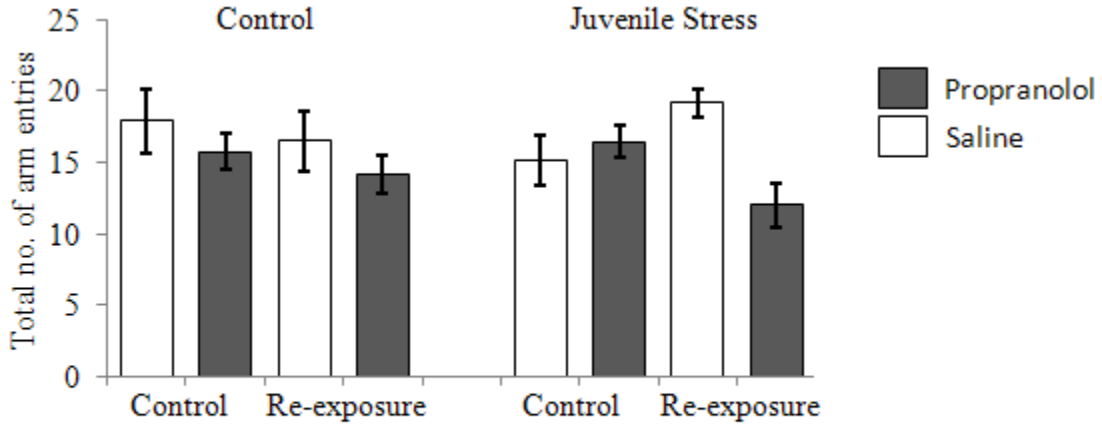

Figure 3. Total number of arm entries measured 2 hours after re-exposure to stress. The X-axis represents re-exposure condition.

the PTSD-like group, those injected with saline made more total arm entries (M = 19.17, SD = 2.32) than those injected with propranolol (M = 12.00, SD = 4.61) (Figure 3).

Two Weeks after Re-exposure to Stress

The ANOVA revealed that significant interaction effects noted two hours after re-exposure were no longer present two weeks after re-exposure. For instance, the interaction between juvenile stress, stress re-exposure and drug no longer exerted a significant effect on latency to first entry into an open arm two weeks after stress re-exposure (F (1, 61) = .41, ns). Moreover, the interaction between juvenile stress and stress-re-exposure (F (1, 61) = .77, ns) and the interaction between re-exposure and drug (F (1, 61) = 1.13, ns) were not found to significantly affect number of entries to the open arms. However, the interaction between juvenile stress and re-exposure did significantly affect entries to the closed arms (F (1, 61) = 5.57, $p < .05$), although no significant simple effects were found for either juvenile stress ($p > .05$) or re-exposure ($p > .05$). The interaction between re-exposure and drug no longer exerted a significant effect on total number of arm entries (F (1, 61) = .01, ns). Also, the effect of propranolol observed after two hours was blunted after two weeks (F (1, 61) = 2.62, ns) (Figure 4**)**; among mice in the PTSD-like group, there was no meaningful difference in total arm entries between injection of saline (M = 10.5, SD = 5.89) and propranolol (M = 12.67, SD

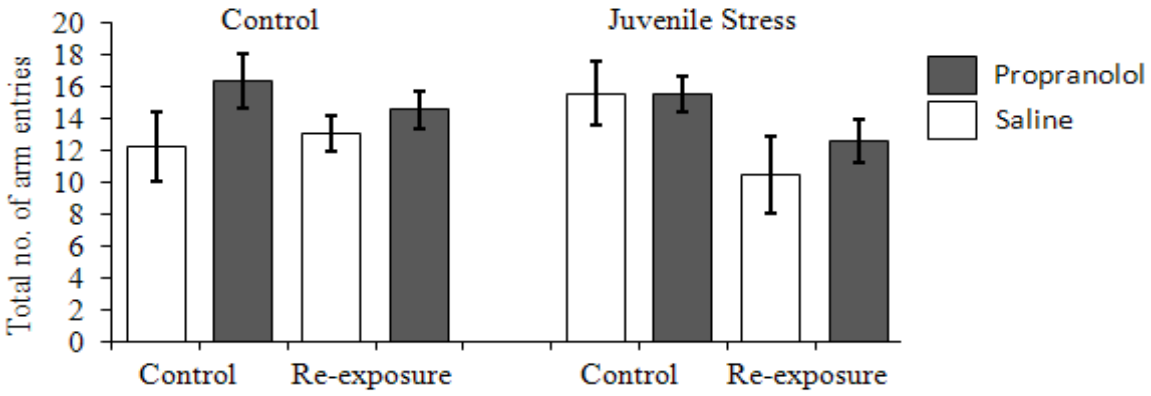

Figure 4. Total number of arm entries measured 2 weeks after re-exposure to stress. The X-axis represents re-exposure condition.

= 4.24). Follow-up tests confirmed that the effects of propranolol and saline on total arm entries did not significantly differ (p = .14, ns).

EXPERIMENT 2

Methods

Subjects

Male CD-1 mice (N = 84) were randomly assigned to one of eight experimental groups outlined below. All animals were locally bred in the Carleton University Institute of Neuroscience vivarium. Post-weaning, the mice were group-housed in cages of 3-4 with their same-sex siblings and housed in Plexiglas cages with ad libitum access to food pellets and water. Mice were maintained in the colony room with a 12:12 hour light-dark cycle. All experimental procedures complied with the guidelines set out by the Canadian Council on Animal Care (CACC) and were approved by the Carleton University Animal Care Committee (ACC).

Apparatus

The apparatus was the same as that of Experiment 1.

Procedure

Mice in Experiment 2 underwent juvenile stress, adulthood stress re-exposure and behavioural testing procedures that were identical to those in Experiment 1. However, they were not injected in juvenility but were injected immediately after re-exposure to stress.

Groups

The present 2 x 2 x 2 design consisted of three treatments - juvenile stress (juvenile stress vs. control), stress re-exposure (re-exposure vs. control and drug treatment (propranolol vs. saline). A detailed description of the groups is offered below.

Juvenile Stress – Re-exposure – Propranolol

These animals underwent the variable stress paradigm in juvenility and were re-exposed to stress in adulthood prior to being injected with propranolol.

Juvenile Stress – Re-exposure –Saline

These animals underwent the variable stress paradigm in juvenility and were re-exposed to stress in adulthood prior to being injected with saline.

Juvenile Stress – Control – Propranolol

These animals underwent the variable stress paradigm in juvenility and were not re-exposed to stress in adulthood prior to being injected with propranolol.

Juvenile Stress – Control – Saline

These animals underwent the variable stress paradigm in juvenility and were not re-exposed to stress in adulthood prior to being injected with saline.

Control – Re-exposure – Propranolol

These animals did not undergo the variable stress paradigm in juvenility but were exposed to stress in adulthood prior to being injected with propranolol.

Control – Re-exposure – Saline

These animals did not undergo the variable stress paradigm in juvenility but were exposed to stress in adulthood prior to being injected with saline.

Control – Control – Propranolol

These animals did not undergo the variable stress paradigm in juvenility and were not exposed to stress in adulthood prior to being injected with propranolol.

Control – Control –Saline

These animals did not undergo the variable stress paradigm in juvenility and were not exposed to stress in adulthood prior to being injected with saline.

Statistical Analyses

The effects of juvenile stress, drug treatment and re-exposure to stress on anxiety levels were analyzed by analyses of variance (ANOVAs) using a significance level of $\alpha = 0.05$. Follow-up comparisons comprising Bonferroni/Dunn tests were conducted.

Results

Two Hours after Re-exposure to Stress and Injection

A factorial ANOVA was conducted to ascertain the effects of juvenile stress, adulthood stress re-exposure and subsequent injection of either saline or propranolol on anxiety levels. Despite the absence of any significant interaction effects, propranolol exerted a hugely significant main effect on latency to first entry into an open arm ($F (1, 76) = 28.56$, $p < .05$). For instance, among mice exposed to neither juvenile nor adult stress, propranolol showed a significant simple effect in reducing latency to first open arm entry ($p < .05$). While propranolol did not display a significant simple effect in the PTSD-like group ($p > .05$, ns), it was nevertheless associated with a lower latency to first open arm entry ($M = 73.42$, $SD = 68.59$) in comparison to saline ($M = 158.91$, $SD = 107.17$) (Figure 5). Follow-up tests vouched for the significance of injection of propranolol versus saline in influencing latency to first entry into an open arm ($p < .05$).

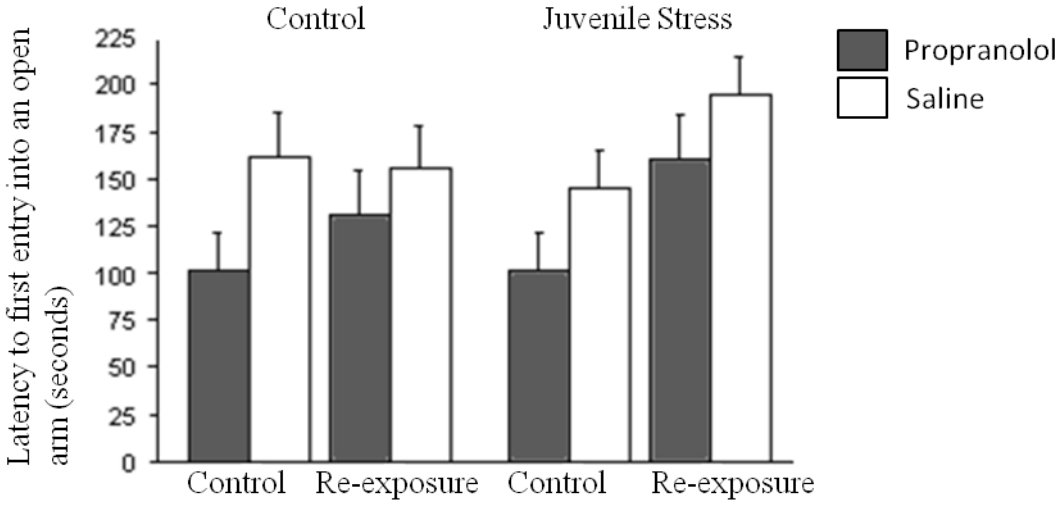

Figure 5. Latency to first open arm entry measured 2 hours after stress re-exposure and injection. X-axis represents re-exposure condition.

The interaction between re-exposure and drug significantly affected number of entries into the open arms ($F (1, 76) = 4.58$, $p < .05$). Propranolol exerted a significant main effect on

number of open arm entries ($F(1, 76) = 15.66$, $p < .05$). Figure 6 shows that propranolol is associated with more open arm entries in all the groups within the exception of mice that were juvenile-stressed but not re-exposed to stress in adulthood. Propranolol was found to exert a significant simple effect on mice that were not stressed in juvenility but were exposed to adulthood stress. Among these animals, those injected with propranolol made on average more than twice as many entries to the open arms ($M = 5.90$, $SD = 3.25$) as their saline-injected counterparts ($M = 2.22$, $SD = 1.72$). Follow-up tests affirmed that whether animals were injected with propranolol or saline strongly influenced the number of times they entered the open arms ($p < .05$).

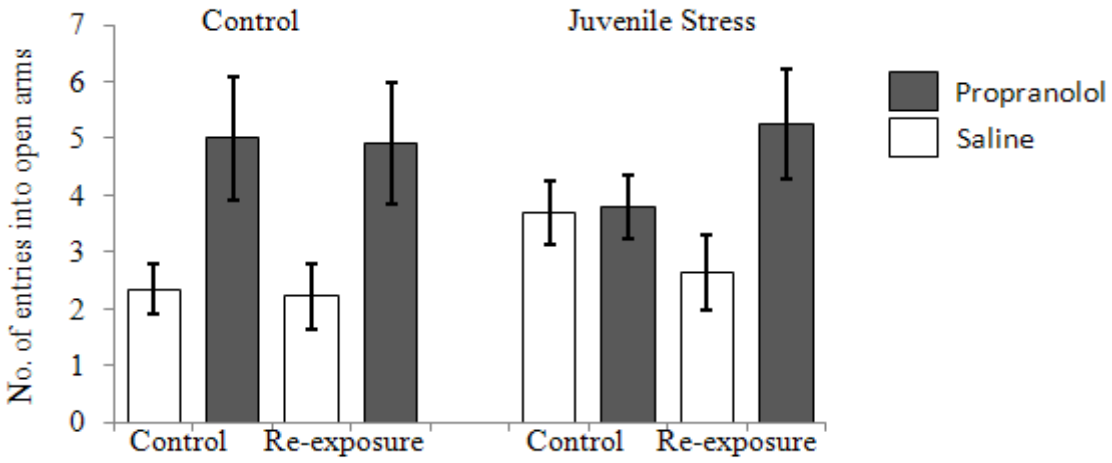

Figure 6. Number of entries into open arms measured 2 hours after stress re-exposure and injection. The X-axis represents re-exposure condition.

Two Weeks after Re-exposure to Stress and Injection

Although the interaction between juvenile stress and stress re-exposure did not significantly affect latency to first entry into an open arm (F (1, 76) = 2.22, ns), Figure 7 suggests that there may have been a tendency for the interaction to affect latency. This is most noticeable among juvenile stressed mice injected with propranolol in adulthood. Among these animals, those that were re-exposed to stress took an average of 248 seconds to make their first open arm entry (SD = 77.25) while those that weren't re-exposed to stress only took an average of 160.62 seconds (SD = 96.67) to make their first venture into an open arm. Meanwhile, propranolol no longer exerted a significant main effect on latency to first entry into an open arm (F (1, 76) = 0.00, ns) or for that matter – number of entries into the open arms (F (1, 76) = .10, ns) – two weeks after stress re-exposure and injection as it did two hours after.

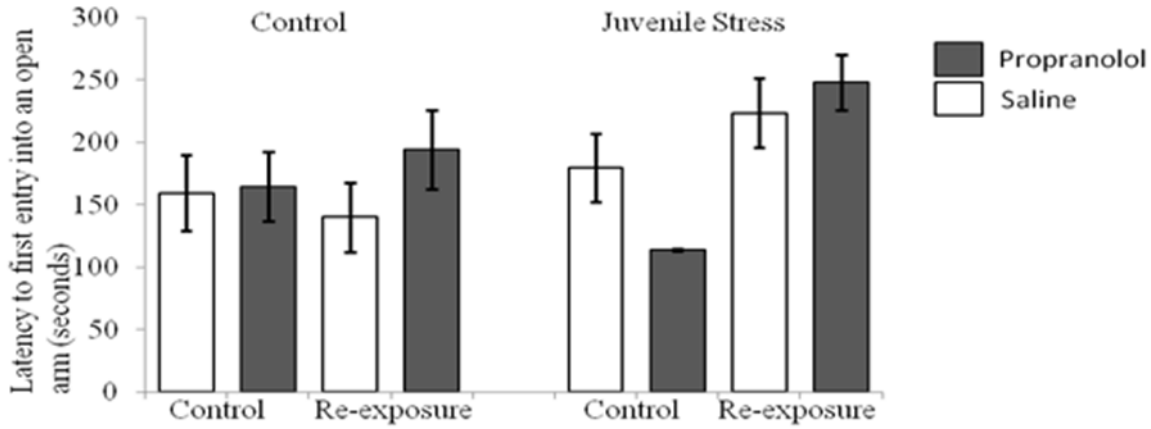

Figure 7. Latency of first entry to an open arm measured 2 weeks after re-exposure and injection. X-axis represents re-exposure condition.

The interaction between juvenile stress and stress re-exposure significantly affected entries to the closed arms (F (1, 76) = 4.22, $p < .05$). Juvenile stress had a significant main effect on closed arm entries (F (1, 76) = 12.46, $p < .05$), while the main effect of stress re-exposure fell

just short of significance (F (1, 76) = 3.70, p = .05, ns). Follow-up tests indicated that both juvenile stress and re-exposure stress significantly affected closed arm entries (p < .05 in both cases). The effect of juvenile stress was most pronounced among animals that were re-exposed to stress (Figure 8**)**.

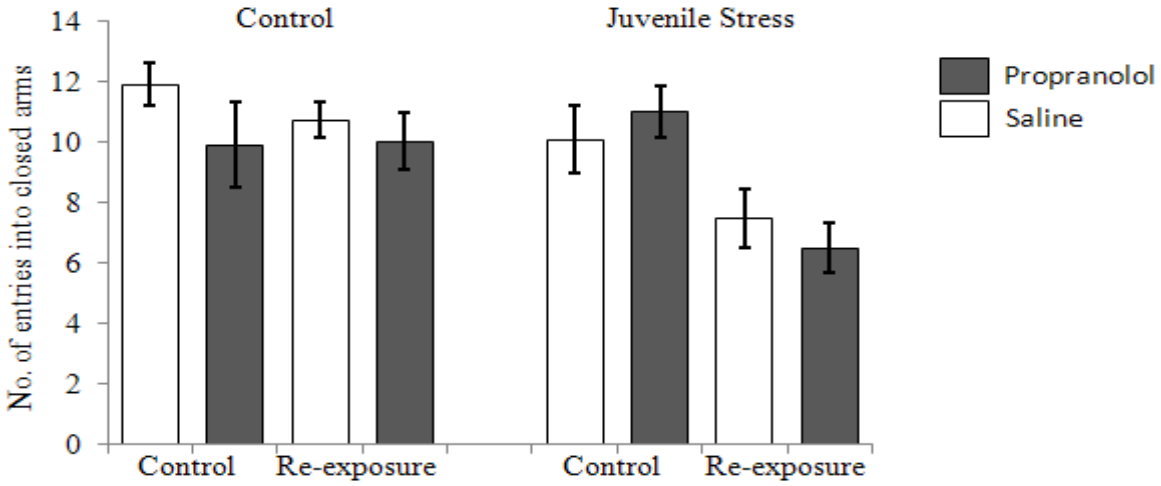

Figure 8. Number of entries into closed arms measured 2 weeks after stress re-exposure and injection. X-axis represents re-exposure condition.

The effects of stress exposure on total arm entries were similar to their effects on closed arm entries – the interaction between juvenile stress and adult re-exposure was found to significantly affect total number of arm entries (F (1, 76) = 7.53, p < .05), while juvenile stress was found to exert a significant main effect on total arm entries (F (1, 76) = 15.77, p < .05). Follow-up analyses re-affirmed that whether animals were exposed to stress in juvenility or not significantly determined the total number of arm entries they made two weeks after adulthood stress re-exposure and injection (p < .05), or roughly six weeks following exposure to juvenile stress. Juvenile stress was found to exert a significant simple effect among mice that were re-exposed to stress and then injected with propranolol (p < .05). Within this group, animals that were not exposed to stress in juvenility made on average over twice as many total

arm entries (M = 16, SD = 7.96) as their juvenile-stressed counterparts (M = 7.25, SD = 3.41) (Figure 9).

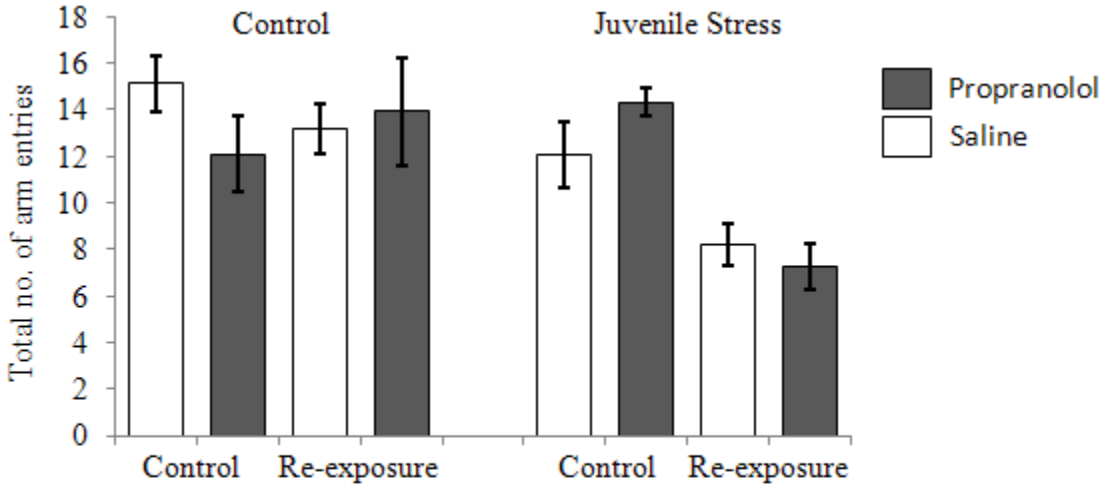

Figure 9. Total number of arm entries measured 2 weeks after stress re-exposure and injection. The X-axis represents re-exposure condition.

DISCUSSION

The present investigation assessed the efficacy of the β-adrenergic antagonist propranolol in attenuating over-consolidation of traumatic fear memories, ostensibly by diminishing the sensitization of catecholamine (Epi and NE) systems (Southwick et al., 1999). It is believed that prevention of the over-encoding of traumatic memories, known to occur as a result of stress-related increases in Epi and NE (Southwick et al., 1999), could play a role in preventing the development of PTSD (Pitman et al., 2002). In Experiment 1, mice were injected with either propranolol or saline solution following exposure to stress in juvenility; in Experiment 2, injections took place following exposure to stress in adulthood. In both cases, we hypothesized that propranolol would result in less anxiety and fear, constructs measured through observation of mice in an elevated plus-maze.

Experiment 1 – Injection in Juvenility

Propranolol injection in juvenility did not reduce fear related to the initial exploration of a novel and anxiety-inducing stimulus, as indicated by the fact that it did not reduce latency to first entry into an open arm. While juvenile stress and adult stress interacted to affect emotional reactivity (as indicated by the number of open arm entries), propranolol failed to reduce emotional reactivity in the PTSD-like group. Saline injection among PTSD-like mice was associated with a very high number of open arm entries, possibly indicative of hyper-arousal among these animals. It may be that the combination of juvenile stress and adult stress abolished the expected differences in anxiety-related behaviours among mice injected with propranolol and mice injected with saline. Indeed saline injection itself may have exacerbated the effects of juvenile stress (injection essentially being a stressor), bearing in mind that animals were injected three times in juvenility (once after each stressor). In effect, it is possible that the experience of undergoing three injections in juvenility may have aggravated

the effects of the juvenile stressors to the extent that this treatment precluded potential effects of propranolol to reduce anxiety levels in adulthood

Propranolol was associated with fewer open arm entries than saline. Given that locomotion has been used as an index of exploratory anxiety and that reduced locomotion has been interpreted as indicating greater anxiety or fear (Tang & Sanford, 2004), this paradoxical finding may support the argument that the stress associated with juvenile stressing procedures and subsequent injections may have masked or skewed the effects of propranolol on anxiety. Indeed it has been shown that the experience of injection by needle is a stressful one (Ryabinin, Wang and Finn, 1999) and that injection stress may be a manipulation in its own right (Raap, Morin, Medici and Smith (2000). In fact, Robertson, Shelton and Athar found that intraperitoneal injection of saline evoked the release of AVP (cited in Wright & Lincoln, 1984) and increased CRH levels on the paraventricular nucleus of the hypothalamus (cited in Kiss & Aguilera, 1993). CRH and AVP stimulate the secretion of ACTH to govern the HPA axis (de Kloet et al., 2005), resulting in release of corticosterone.

Given that acute administration of propranolol does not alter plasma corticosterone levels in mice (Murchison, Zhang, Zhang, Ouyang, Lee and Thomas, 2004), it is a valid concern that the experience of injection during juvenility may have aggravated the stress response and resulted in levels of NE activity that were not anticipated and therefore could not be satisfactorily controlled by a 5mg/ kg dose of propranolol. This could possibly explain why juvenile injections of propranolol did not reduce anxiety in adulthood.

Unlike what was observed two hours after re-exposure, juvenile stress and adulthood stress no longer interacted to affect emotional reactivity when anxiety levels were measured two weeks after re-exposure. However, they did affect activity levels as measured by closed arm entries. Propranolol no longer affected total activity levels two weeks after stress re-exposure.

Thus, neither stress nor propranolol exerted any meaningful effects on anxiety levels two weeks after adult re-exposure (i.e. six weeks after injection of propranolol in juvenility).It may be that propranolol did not exert effects due to injection stress as previously speculated, but it could also be that propranolol simply does not exert long-term effects on anxiety. A discussion of the effects of propranolol in Experiment 2 – in which animals were not injected three times in juvenility but were injected just once in adulthood – could therefore shed some light on the timeframe of possible anxiolytic effects of propranolol. As will be detailed shortly, propranolol was found to not reduce anxiety two weeks after injection in Experiment 2. It is therefore not surprising that propranolol did not exert effects on anxiety levels two weeks after stress re-exposure (i.e. six weeks after injection) in Experiment 1, regardless of whether the experience of injection was inherently stressful.

Experiment 2 – Injection in Adulthood

When injections took place in adulthood (following re-exposure to stress), propranolol significantly reduced anxiety as indicated by how hesitant animals were to make their first foray into the open arms of the elevated plus maze two hours after stress re-exposure and injection. Seeing as how latency to open arms is reflective of avoidance behaviour (Redolat et al., 2002), the reduced latency observed among mice injected with propranolol shows that propranolol worked to reduce exploration anxiety when injected two hours before behavioural testing. In addition to attenuating exploration anxiety, propranolol also reduced emotional reactivity and increased impulsivity (and as a consequence, activity levels), as indicated by the higher number of open entries made by propranolol-injected mice compared to their saline-injected counterparts.

However, propranolol no longer induced a decrease in either latency to first open arm entry or number of open arm entries two weeks after stress re-exposure and injection. Essentially,

these data suggest that propranolol's ability to reduce anxiety and emotional reactivity diminished after two weeks. Thus, it is worth considering whether this may be due to the half-life and metabolic properties of propranolol. In humans, the half-life of propranolol is known to be short, reflecting a high rate of clearance (Evans, Nies and Shand, 1973). Recent reports on the metabolism of propranolol have revealed that the half-life of propranolol is lower in mice than it is in humans (Baughman, Talarico and Soglia, 2009). Given that the half-life of propranolol is dose-dependent (Staniforth, Yorkston and Zaki, 1982), it is quite conceivable that the 5mg/kg dose of propranolol used in the present study may not have been sufficient to permit the drug to remain the system for a long enough period of time so as to affect anxiety two weeks after injection.

Interestingly, juvenile stress and adult re-exposure interacted to significantly reduce total activity levels two weeks after re-exposure and injection. Among animals that were exposed to adulthood stress, those that were also juvenile-stressed displayed much lower activity levels than juvenile controls. This may imply that stress in juvenility continued to affect activity levels in adulthood despite the intervening adulthood stress.

Limitations

In any experiment dealing with the developmental sequelae of early life stress, the selection of the time period during which experimental subjects are to be exposed to stress is of critical importance. Rice & Barone state that over the course of ontogeny, there are time windows of increased and decreased stress system reactivity, which often correlate with an increased vulnerability to disruption of the stress system (as cited in Schmidt, 2010). This is not surprising given that different parts of the brain develop at different times and have different windows of vulnerability (Rice & Barone, 2000).

One particularly vulnerable time period is adolescence, the final developmental period before adulthood and a time of enhanced brain architecture plasticity (Schmidt, 2010). As Rice & Barone (2000) point out, although the developmental timing and patterns of this and other periods differ between humans and rodents, there are also substantial similarities that support using rodents as a developmental model for humans (as cited in Schmidt). This has important implications for any experiment looking at PTSD, the onset for which is rarely before the early teens (Paus, Keshavan and Giedd, 2008). However, adolescence is a difficult period to define in absolute margins as no single event signals its onset or termination (Schmidt). A conservative and generally accepted estimate for the rodent is post-natal days (PND) 28-42, a range derived by considering the age range during which age-specific behavioural discontinuities from younger and older animals are evident (Spear, 2000). Therefore, we settled on 27 PND as the age at which juvenile stress exposure would begin.

Aside from selection of an appropriate age range, the type of stress paradigm to be used also holds much importance. The secretions of different neuromodulators can vary depending on the extent and level of stress exposure (McEwen & Sapolsky, 1995). Short inescapable stress is a useful component of stress paradigms as it produces long-lasting changes in the HPA axis of rodents (van Dijken et al., 1993), including long-lasting increases of vasopressin stores in hypothalamic CRH stores (Schmidt, Binnekade, Janszen and Tilders, 1996). It has been widely suggested that repeated exposure to one particular stressor might gradually produce habituation, consequently diminishing the physiological stress response (Zucchi et al., 2009). This has been demonstrated in experiments such as that conducted by Gądek-Michalska & Bugajski (2003), who showed that rodents repeatedly exposed to restraint stress exhibited a habituated corticosterone response when they were subsequently challenged with an acute exposure to restraint. However, while repeated stress might allow for habituation as

reflected by a decline in plasma glucocorticoid levels, a variable stress paradigm combines different stressors in an unpredictable sequence to maintain elevated glucocorticoid levels (Zucchi et al.). Indeed Magarinos & McEwen (1995) found that exposure to a variable stress paradigm produced continued elevation in corticosterone levels when the animals were subsequently exposed to an acute restraint stress session. Therefore, variable stress seems to promote more intense physiological changes probably by hampering habituation to the aversive situation (Marin et al., 2007), making it ideal for the present study.

With regards to the selection of stressing methods, it is important that deprivation and pain be minimized through the use of ethological based aversive situations (Hascoët, Bourin and Dhonnchadea, 2001). One commonly used means of stressing animals is restraint, largely because it is straightforward, painless and without lasting debilitation (Buynitsky & Mostofsky, 2009) and also because it is known to elicit a variety of physiological stress responses (Grissom, Kerr and Bhatnagar, 2008). Methods of restraint such as placing the entire animal in a Decapicone (a tapered, conical, plastic film tube) produce the restriction and immobilization of movement (Buynitsky & Mostofsky).

Importantly, Grandin & Deesing note that the psychological and physiological changes associated with restraint appear to result from the distress and aversive nature of having to remain immobile, rather than any concurrent activation of pain mechanisms or irreversible discomfort (as cited in Buynitsky & Mostofsky, 2009). With respect to duration of restraint stress, at times, a researcher interested in social interactions may decide that 5 minutes of restraint is sufficient while at other times, restraint may continue for 6 hours (Buynitsky & Mostofsky). It is known that habituation occurs to mild continuous restraint, but the impact of full body restraint, as conducted in the present investigation, is not known.

In general, it seems that selection of duration of restraint stress largely depends on the goals

of the particular experiment. Lastly, unlike "pure" psychogenic stressors, restraint also involves a physical manipulation that limits the defensive style of the animal and thus may be considered a neurogenic stressor (McIntyre, Kent, Hayley, Merali and Anisman, 1999). Given that many stimuli have elements of both psychogenic and neurogenic stressors, restraint is a useful means of stressing animals like mice.

In PTSD, a traumatic event activates the stress response, which is re-activated upon re-experiencing events similar to the trauma or less intense stressors, eventually leading to a dysregulated response that has a lower threshold of activation (Rau et al., 2005). Thus, prior exposure to potentially traumatic experiences represents a significant risk factor for PTSD following subsequent exposure to a potentially traumatic experience, as has been shown in clinical studies as well as animal models (Cohen, Geva, Matar, Zohar and Kaplan, 2008).

Acute social defeat has been shown to elevate both corticosterone and ACTH and enhance CRH mRNA expression in the parvocellular paraventricular nucleus to an extent that it can be considered a severely stressful stimulus for the sub-ordinate animal (Keeney, Jessop, Harbuz, Marsden, Hogg and Blackburn-Munro, 2006). Depending on the stress parameter, the stress response induced by a single social defeat may last from hours to days and weeks (Koolhaas, De Boer, De Rutter, Meerlo and Sgoifo, 1997). A single social defeat also results in the reduction of amplitude of circadian body temperature rhythms and loss of body weight (Stam, Akkermans and Wiegant, 1997). Thus, the experience of social defeat was sufficiently stressful so as to serve as a re-exposure stressor in our mouse model of PTSD.

Animal models of anxiety disorders have tapped into knowledge of the natural behavioural patterns of rats and mice to develop ethologically based behavioural tasks, most popular among which are the exploratory 'approach-avoidance' tasks (Cryan & Holmes, 2005) based on the innate aversion of rodents to brightly illuminated areas and on their spontaneous

exploratory behaviour (Hascoët et al., 2000). Mice are a naturally foraging and exploratory species, and exploration-based tasks exploit the conflicting tendencies to approach versus avoid a potentially dangerous area (Cryan & Holmes). The aversive area takes different forms in different tests, such as the open, elevated arms in the case of the elevated plus-maze (Cryan & Holmes).

The elevated plus-maze test is probably the most popular animal model of anxiety and is based on the study of unconditioned, or spontaneous, behaviour (Rodgers & Dalvi, 1997). The apparatus consists of a maze elevated to a height of about 50 cm and consisting of two open and two enclosed arms with an open roof, arranged such that the two open arms are opposite to each other (Pellow et al., 1985). Over a typical test session, non-drug-treated mice are expected to avoid the aversive open arms and prefer to remain in the protected zones of the apparatus for most of the testing period, a pattern of behaviour that has face validity as a measure of anxiety-like behaviour given that anxiety disorders like PTSD are often typified by a pervasive avoidance of a feared object or situation (Cryan & Holmes, 2005). The critical determinants considered to be correlated with anxiety are the entries made into the open arms and time spent on these arms (Hogg, 1996).

Behavioural changes such as extremely compromised exploratory behaviour on the elevated plus maze reflect anxiety-like behaviours, i.e. fearfulness and hypervigilance (Cohen et al., 2004). In fact, voluntary passage onto the open arms of the elevated plus maze is associated with hormonal (elevated plasma corticosterone concentrations) and behavioural changes indicative of increased anxiety (Hogg, 1996). However, while normal exploratory behaviour favours the closed arms, this tendency can be decreased by administration of anxiolytic compounds, which reduce the natural aversion to the open arms and promote exploration thereof (Hogg; Hascoët et al., 2000).

Thus, the elevated plus maze been shown to be a valid measure of behavioural stress responses (Cohen et al., 2004). Moreover, it has been demonstrated to hold superior predictive validity for anxiolytic compounds over other tests of anxiety-like behaviour such as the mirror chamber test, which, like the elevated plus maze, is based on the conflict between rodents' predisposition to explore novel environments and their fear of exposed, well-lit spaces (Paterson, Iwunze, Davis, Malekiani and Hanania, 2010)

To sum up the advantages of the elevated plus maze, it is a fast and simple procedure not involving the use of expensive equipment; it is based on spontaneous behaviour and does not necessitate lengthy training nor the use of noxious stimuli, and it is able to identify both anxiolytic and anxiogenic drug effects under identical conditions (Pellow et al., 1985). Crucially, acute stressors employed in the present study such as forced swim (Britton, Page, Baldwin and Koob), restraint (Albonetti & Farabollini) and social defeat (Heinrichs, Pith, Miczek, Britton and Koob; Rodgers & Cole) have been reported to be influential on behaviour exhibited by animals on the elevated plus maze (as cited in Hogg, 1996), indicating high face validity of the elevated plus maze as a test of anxiety brought about as a consequence of these stressors. Most importantly, the patterns of results obtained using the elevated plus-maze task have been shown to be replicable across other species, anxiety/affective behaviour measures, studies and laboratories (Walf & Frye, 2007). At the same time, there is currently no validated model of PTSD; hence there is much uncertainty as to whether the elevated plus maze test captures anxiety related to PTSD or anxiety in general. Future studies need to establish a valid paradigm for PTSD in order to evaluate the effects of propranolol on outcomes beyond its anxiolytic effects.

Furthermore, it might be useful to do away with injections as a mode of drug delivery – given its stressor effects – and instead use an alternate route of drug administration such as

oral administration, which is not uncommon in mouse studies involving propranolol (Narimatsu, Mochida, Ueno, Horie, Yamamoto and Suzuki, 2002; Hagesawa & Saiki, 2002; Pierroz, Bouxsein, Rizzoli and Ferrari, 2006).

Additionally, it might also be worth experimenting with different stress-injection interval lengths. In Experiment 1, injections took place immediately following juvenile stress but in reality, children and adolescents involved in stressful and traumatic events will rarely receive treatment for post-traumatic stress right after ceasing of the traumatic episode. Experiment 2 modelled a more realistic situation in which a PTSD patient receives treatment soon after re-experiencing trauma. This could explain why propranolol was found to reduce anxiety in this experiment, at least when anxiety was tested two hours after injection.

Aside from expanding the interval between juvenile stress and injection, future studies could also consider choosing a different age interval for juvenile stressing. We began our three-day stress paradigm at an age of 27 PND, which falls at the start of the generally accepted estimated period of rodent adolescence (28-42 PND) (Spear, 2000). By beginning juvenile stress procedures at a later age, adolescent stress could be modelled more accurately, while the resulting shrinkage of the interval between juvenile stress and adulthood behavioural testing might lead propranolol to produce more pronounced anxiolytic effects. Castleden, Kaye and Parsons (1975) have reported that the pharmacokinetics of propranolol are age-dependent (as cited in Staniforth et al., 1982), making it well worthwhile to experiment with later age intervals for juvenile stressing and injection.

Conclusions

The two experiments comprising the present investigation aimed to assess the efficacy of propranolol in reducing anxiety levels in adulthood of mice exposed to stress in juvenility. Based on our results, it appears that propranolol is more efficacious in reducing anxiety when it is administered following the re-experiencing of stress as opposed to when it is administered after the initial juvenile stress. This implies that while propranolol diminished PTSD symptoms when administered after adulthood stress re-exposure, it was not as effective in preventing the over-encoding of traumatic memories resulting from juvenile stress-induced increases in Epi and NE.

These findings are far from conclusive however, given the possible existence of confounds (injection as a stressor) in our experimental methodology and the considerable room for experimentation with regards to timing and dosage of injection and the selection of time periods for stressing procedures. The testing of anxiolytic effects of propranolol in mouse models of PTSD is a fairly new and novel direction of research and one that still requires much refinement before it can produce conclusive and potentially, practical findings with applicability to clinical settings. Nevertheless, preliminary studies such as this one suggest that propranolol does indeed hold some potential in alleviating anxiety produced as a consequence early life stress.

REFERENCES

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders* (Revised 4th ed.) Washington, DC: Author.

Anisman, H., Zaharia, M.D., Meaney, M.J., & Merali, Z. (1998). Do early-life events permanently alter behavioural and hormonal responses to stressors? [Electronic version]. *International Journal of Developmental Neuroscience, 16,* 149-164.

Bale, T.L. & Vale, W.W. (2004). CRF and CRF receptors: role in stress responsivity and other behaviour [Electronic version]. *Annual Reviews of Pharmacology & Toxicology, 44,* 525-557.

Baughman, T.M., Talarico, C.L. & Soglia, J.R. (2009). Evolution of the metabolism of propranolol by linear ion trap technology in mouse, rat, dog, monkey and human cryopreserved hepatocytes [Electronic version]. *Rapid Communications in Mass Spectrometry, 23,* 2146-2150.

Berridge, C.W. & Waterhouse, B.D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes [Electronic version]. *Brain Research Reviews, 42,* 33-84.

Bremner, J.D., Southwick, S.M., Johnson, D.R., Yehuda, R. & Charney, D.S. (1993). Childhood physical abuse and combat-related posttraumatic stress disorder in Vietnam veterans [Electronic version]. *American Journal of Psychiatry, 150(2),* 235-239.

Breslau, N. (2009). The epidemiology of trauma, PTSD and other posttrauma disorders [Electronic version]. *Trauma, Violence & Abuse, 10(3),* 198-210.

Brunet, A., Orr, S.P., Tremblay, J., Robertson, K., Nader, K. & Pitman, R. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-

driven traumatic imagery in post-traumatic stress disorder [Electronic version]. *Journal of Psychiatric Research, 42,* 503-506.

Buynitsky, T. & Mostofsky, D.I. (2009). Restraint stress in biobehavioral research: recent developments [Electronic version]. *Neuroscience and Biobehavioral Reviews, 33,* 1089-1098.

Cahill, L., Prins, B., Weber, M. & McGaugh, J.L. (1994). β-adrenergic activation and memory for emotional events [Electronic version]. *Nature, 371,* 702-704.

Cai. W., Blundell, J., Han, J., Greene, R.W. & Powell, C.M. (2006). Postreactivation glucocorticoids impair recall of established fear memory [Electronic version]. *The Journal of Neuroscience, 26(37),* 9560-9566.

Charmandari, E., Tsigos, C. & Chrousos, G. P. (2005). Endocrinology of the stress response [Electronic version]. *Annual Reviews of Physiology, 67,* 259-284.

Charney, D.S., Deutch, A.Y., Southwick, S.M. & Krystal, J.H. (1995). Neural circuits and mechanisms of post-traumatic stress disorder. In Friedman, M.J., Charney, D.S. & Deutch, A.Y. (Ed.), *Neurobiological and Clinical Consequences of Stress: From Normal Adaptation to Post Traumatic Stress Disorder* (pp. 271-287). Philadelphia: Lippincott-Raven.

Chrousos, G.P. (2009). Stress and disorders of the stress system [Electronic version]. *Nature Reviews Endocrinology, 5,* 374-381.

Chrousos, G.P., Kinob, T. & Charmandari, E. (2009). Evaluation of the hypothalamic-pituitary-adrenal axis function in childhood and adolescence [Electronic version]. *Neuroimmunomodulation, 16(5),* 272-283.

Cicchetti, D. & Toth, S.L. (2005). Child Maltreatment [Electronic version]. *Annual Review of Clinical Psychology, 1,* 409-438.

Cohen, H., Geva, A.B., Matar, M.A., Zohar, J. & Kaplan, Z. (2008). Post-traumatic stress behavioural responses in inbred mouse strains: can genetic predisposition explain phenotypic vulnerability [Electronic version]. *International Journal of Neuropsychopharmacology, 11,* 331-349.

Cohen, H. & Zohar, J. (2004). An animal model of posttraumatic stress disorder: the use of cut-off behavioral criteria [Electronic version]. *Annals of the New York Academy of Sciences, 1032,* 167-168.

Cohen, H., Zohar, J. & Matar, M. (2003). The relevance of differential response to trauma in an animal model of posttraumatic stress disorder [Electronic version]. *Biological Psychiatry, 53,* 463-473.

Cohen, H., Zohar, J., Matar, M., Zeev, K., Loewenthal, U. & Richer-Levin, G. (2004). Setting apart the affected: the use of behavioural criteria in animal models of post traumatic stress disorder [Electronic version]. *Neuropsychopharmacology, 29,* 1962-1970.

Collishaw, S., Pickles, A., Messer, J., Rutter, M., Shearer, C. & Maughana, B. (2007). Resilience to adult psychopathology following childhood maltreatment: Evidence from a community sample [Electronic version]. *Child Abuse & Neglect, 31,* 211-229.

Coplan, J.D., Andrews, M.W., Rosenblum, L.A., Owens, M.J., Friedman, S., Gorman, J.M. & Nemeroff, C.B. (1996). Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders [Electronic version]. *Proceedings of the National Academy of Sciences, 93,* 1619-1623.

Cryan, J. & Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety [Electronic version]. *Nature Reviews Drug Discovery, 4,* 775-790.

Cukor, J., Spitalnick, J., Difede, J., Rizzo, A. & Rothbaum, B.O. (2009). Emerging treatments for PTSD [Electronic version]. *Clinical Psychology Review, 29,* 715-726.

Dal-Zotto, S., Marti, O. & Armario, A. (2000). Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor [Electronic version]. *Behavioural Brain Research, 114 (1-2),* 175-181.

Harro, J., Tõnissaar, M., Eller, M., Kask, A. & Oreland, L. (2001). Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry [Electronic version]. *Brain Research, 899(1-2),* 227-239.

de Kloet, R., Joëls, M. & Holsboer, F. (2005). Stress and the brain: from adaptation to disease [Electronic version]. *Nature Reviews Neuroscience, 6,* 463-475.

Elzinga, B.M., Spinhoven, P., Berretty, E., de Jong, P. & Roelofs, K. (2010). The role of childhood abuse in HPA-axis reactivity in Social Anxiety Disorder: A pilot study [Electronic version]. *Biological Psychology, 83,* 1-6.

Engler, D., Pham, T., Fullerton, M.J., Ooi, G., Funder, J.W. & Clarke, I.J. (1989). Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. I. Effect of an audiovisual stimulus and insulin-induced hypoglycemia [Electronic version]. *Neuroendocrinology, 49(4),* 367-381.

Evans, G.H., Nies, A.S. & Shand, D.G. (1973). The disposition of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat [Electronic version]. *The Journal of Pharmacology and Experimental Therapeutics, 186(1),* 114-122.

Fumagalli, F., Molteni, R., Racagni, G. & Riva, M.A. (2007). Stress during development: Impact on neuroplasticity and relevance to psychopathology [Electronic version]. *Progress in Neurobiology, 81,* 197-217

Gądek-Michalska, A. & Bugajski, J. (2003). Repeated handling, restraint, or chronic crowding impair the hypothalamic-pituitary-adrenocortical response to acute restraint stress [Electronic version]. *Journal of Physiology and Pharmacology, 54(3),* 449-459.

Gaudiano, B.A. & Zimmerman, M. (2009). The relationship between childhood trauma history and the psychotic subtype of major depression. *Acta Psychiatrica Scandinavica.* Epub ahead of print retrieved March 29, 2010, from http://www.ncbi.nlm.nih.gov/pubmed/19764926?ordinalpos=&itool=EntrezSystem2.PEntrez. Pubmed.Pubmed_ResultsPanel. SmartSearch&linkpos=1&log$=citationsensor

Grissom, N., Kerr, W. & Bhatnagar, S. (2008). Struggling behavior during restraint is regulated by stress experience [Electronic version]. *Behavioral Brain Research, 191,* 219-226.

Gunnar, M. & Quevedo, K. (2007). The neurobiology of stress and development [Electronic version]. *Annual Review of Psychology, 58,* 145-173

Hascoët, M., Bourin, M. & Dhonnchadea, B.A.N. (2001). The mouse light-dark paradigm: a review [Electronic version]. *Progress in Neuropsychopharmacology and Biological Psychiatry, 25,* 141-166.

Hasegawa, H. & Saiki, I. (2002). Psychosocial stress augments tumor development through beta-adrenergic activation in mice [Electronic version]. *Japanese Journal of Cancer Research, 93(7),* 729-735.

Heim, C. & Nemeroff, C.B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies [Electronic version]. *Society of Biological Psychiatry, 49,* 1023-1039.

Heim, C. & Nemeroff, C.B. (2009). Neurobiology of posttraumatic stress disorder [Electronic version]. *CNS Spectrums, 14(1),* 13-24

Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., Miller, A.H., Nemeroff, C.B. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood [Electronic version]. *Journal of the American Medical Association,284(5),* 592-597.

Heim, C., Owens, M.J., Plotsky, P.M. & Nemeroff, C.B. (1997). The role of early adverse life events in the etiology of depression and posttraumatic stress disorder: focus on corticotropin-releasing factor [Electronic version]. *Annals of the New York Academy of Sciences, 821,* 194-207.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety [Electronic version]. *Pharmacology Biochemistry and Behaviour, 54(1),* 21-30.

Keeney, A., Jessop, D.S., Harbuz, M.S., Marsden, C.A., Hogg, S. & Blackburn-Munro (2006). Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice [Electronic version]. *Journal of Neuroendocrinology, 18,* 330-338.

Kiss, A. & Aguilera, G. (1993). Regulation of the hypothalamic pituitary adrenal axis during chronic stress: responses to repeated intraperitoneal hypertonic injection [Electronic version]. *Brain Research, 630,* 262-270.

Koolhaas, J.M., De Boer, S.F., De Rutter, A.J., Meerlo, P. and Sgoifo, A. (1997). Social stress in rats and mice [Electronic version]. *Acta physiologica Scandinavica, 640,* 69-72.

Ladd, C.O., Huot, R.L., Thrivikraman, K.V., Nemeroff, C.B., Meaney, M.J. & Plotsky, P.M. (2000). Long-term behavioural and neuroendocrine adaptations to adverse early experience [Electronic version]. *Progress in Brain Research, 122.*

Lansford, J.E., Dodge, K.A., Pettit, G.S., Bates, J.E., Crozier, J., Kaplow, J. (2002). A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence [Electronic version]. *Archives of Pediatrics &Adolescent Medicine, 156(8),* 824-830.

Léonhardt, M., Matthews, S.G., Meaney, M.J. and Walker, C. (2007). Psychological stressors as a model of maternal adversity: diurnal modulation of corticosterone responses and changes in maternal behavior [Electronic version]. *Hormones and Behavior, 51(1),* 77-88.

Lepsch, L.B., Gonzalo, L.A., Magro, F.J.B., Delucia, R., Scavone, C. & Planeta, C.S. (2005). Exposure to chronic stress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats [Electronic version]. *Addiction Biology, 10(3),* 251-256.

Levine, S. (1957). Infantile experience and resistance to physiological stress [Electronic version]. *Science, New Series, 126(3270),* 405.

Levy, A., Kadar, T. & Dachir, S. (2001). An animal model for studying therapeutic drugs against post-traumatic stress disorder [Electronic version]. *Military Medicine, 166(12),* 74-75.

Lister, R.G. (1990). Ethologically-based animal models of anxiety disorders [Electronic version]. *Pharmacology & Therapeutics, 46,* 321-340.

Lolait, S.J., Stewart, L.Q., Jessop, D.S., Young, W.S. & O'Carroll, A. (2007). The Hypothalamic-Pituitary-Adrenal Axis Response to Stress in Mice Lacking Functional Vasopressin V1b Receptors [Electronic version]. *Endocrinology, 148(2),* 849-856.

Lupien, S.J., Maheu, F., Tu, M., Fiocco, A. & Schramek, T.E. (2007). The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition [Electronic version]. *Brain and Cognition, 65,* 209-237.

Magarinos, A.M. & McEwen, B.S. (1995). Stress-induced hippocampal atrophy of apical dendrites of CA3c neurons: comparison of stressors [Electronic version]. *Neuroscience, 69(1),* 83-88.

Marin, M.T., Cruz, F.C. & Planeta, C.S. (2007). Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats [Electronic version]. *Physiology & Behaviour, 90,* 29-35.

McCabe, R.E., Miller, J.L., Laugesen, N., Antony, M.M. & Young, L. (2010). The relationship between anxiety disorders in adults and recalled childhood teasing [Electronic version]. *Journal of Anxiety Disorders, 24,* 238-243.

McEwen, B.S. & Sapolsky, R.M. (1995). Stress and cognitive function [Electronic version]. *Current Opinion in Neurobiology, 5,* 205-216.

McIntyre, D.C., Kent, P., Hayley, S., Merali, Z. & Anisman, H. (1999). Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats [Electronic version]. *Brain Research,840,* 65-74.

Mello, M.F., Faria, A.F.., Mello, A.F., Carpenter, L.C., Tyrka, A.R. & Price, L.H. (2009). Childhood maltreatment and adult psychopathology: pathways to hypothalamic-pituitary-

adrenal axis dysfunction [Electronic version]. *Brazilian Journal of Psychiatry, 31(Suppl. 2)*, 41-48.

Morgan, C.A., Krystal, J.H. & Southwick, S.M. (2003). Toward early pharmacological posttraumatic stress intervention [Electronic version]. *Biological Psychiatry, 53, 834-843.*

Murchison, C.F., Zhang, X., Zhang, W., Ming, O. & Lee, A. (1995). A distinct role for norepinephrine in memory retrieval [Electronic version]. Cell, 117, 131-143.

Narimatsu, S., Mochida, M., Ueno, K., Horie, T., Yamamoto, S. and Suzuki, T. (2002). Induction of cytochrome P450 1A1 in mice by repeated oral administration of propranolol [Electronic version]. *Drug Metabolism and Pharmacokinetic, 17(1)*, 54-59.

National Collaborating Centre for Mental Health (2005). Post-traumatic stress disorder: the management of PTSD in adults and children in primary and secondary care. *National Clinical Practice Guideline, 26.* London: Gaskell and the British Psychological Society.

Neigh, G.N., Gillespie, C.F. & Nemeroff, C.B. The neurobiological toll of child abuse and neglect [Electronic version]. *Trauma, Violence & Abuse, 10(4)*, 389-410.

Nelson, R.J. (2005). *An introduction to behavioral endocrinology* (3rd ed.). Sunderland: Sinauer.

Nemeroff, C.B. (1999). The preeminent role of early untoward experience on vulnerability to major psychiatric disorders: the nature-nurture controversy revisited and soon to be resolved [Electronic version]. *Molecular Psychiatry, 106,* 106-08.

O'Carroll, R.E., Drysdale, E., Cahill, L., Shajahan, P. And Ebmeier, K.P. (1999). Stimulation of the noradrenergic system enhances and blockade reduces memory for emotional material in man [Electronic version]. *Psychological Medicine, 29,* 1083-1088.

Paré, W.P. (1996). Enhanced retrieval of unpleasant memories influenced by shock controllability, shock sequence, and rat strain [Electronic version]. *Biological Psychiatry, 39(9),* 808-813.

Paterson, N.E., Iwunze, M., Davis, S.F., Malekiani, S.A. & Hanania, T. (2010). Comparison of the predictive validity of the mirror chamber and elevated plus maze [Electronic version]. *Journal of Neuroscience Methods, 188,* 62-70.

Paus, T., Keshavan, M. & Giedd, J.N. (2008). Why do many psychiatric disorders emerge during adolescence? [Electronic version]. *Nature Reviews Neuroscience, 9,* 947-957.

Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985). Validation of open-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat [Electronic version]. *Journal of Neuroscience Methods, 14,* 149-167.

Perry, B.D. & Azad, I. Posttraumatic stress disorders in children and adolescents [Electronic version]. *Current Opinion in Pediatrics, 11(4):* 310-316.

Pierroz, D.D., Bouxsein, M.L., Rizzoli, R. & Ferrari, S.L. (2006). Combined treatment with a beta-blocker and intermittent PTH improves bone mass and microarchitecture in ovariectomized mice [Electronic version]. *Bone, 39(2),* 260-267.

Pitman, R.K. (1989). Post-traumatic stress disorder, hormones and memory [Electronic version]. *Biological Psychiatry, 26,* 221-223.

Pitman, R.K., Sanders, K.M., Zusman, R.M., Healy, F.C., Lasko, N.B., Cahill, L. et al. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with Propranolol [Electronic version]. *Biological Psychiatry, 51,* 189-192.

Raap, D.K., Morin, B., Medici, C.N. & Smith, R.F. (2000). Adolescent cocaine and injection stress effects on the estrous cycle [Electronic version]. Physiology & Behaviour, 70, 417-424.

Rau, V., De Cola, J.P. & Fanselow, M.S. (2005). Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder [Electronic version]. *Neuroscience and Biobehavioural Reviews, 29,* 1207-1223.

Redolat, R., Vicens, P. & Carrasco, M.C. (2002). Effects of nicotine on motor activity, passive avoidance and plus-maze behavior in aged NMRI and C57BL mice [Electronic version]. *Neuroscience Research Communications, 30(2),* 91-101.

Rice, D. & Barone, S., Jr. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models [Electronic version]. *Environmental Health Perspectives,108(3),* 511-533.

Rodgers, R.J. & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze [Electronic version]. *Neuroscience and Biobehavioral Reviews, 21(6),* 801-810.

Ryabinin, A.E., Wang, Y.M. & Finn, D.A. (1999). Different levels of Fos immunoreactivity after repeated handling and injection stress in two inbred strains of mice [Electronic version]. *Pharmacology Biochemistry and Behaviour, 63(1),* 143-151.

Sachs-Ericsson, N., Blazer, D., Plant, E.A. & Arnow, B. Childhood sexual and physical abuse and the 1-year prevalence of medical problems in the National Comorbidity Survey [Electronic version]. *Health Psychology, 24(1),* 32-40.

Sapolsky, R.M., Armanini, M.P., Packan, D.R., Sutton, S.W. & Plotsky, P.M. (1990). Glucocorticoid feedback inhibition of adrenocorticotropic hormone secretagogue release. Relationship to corticosteroid receptor occupancy in various limbic sites [Electronic version]. *Neuroendocrinology, 51(3), 328-36*

Schmidt, E.D., Binnekade, R., Janszen, A.W.J.W. and Tilders, F.J.H. (1996). Short stressor induced long-lasting increases of vasopressin stores in hypothalamic corticotropin-

releasing hormone (CRH) neurons in adult rats [Electronic version]. *Journal of Neuroendocrinology, 8,* 703-712.

Schmidt, M.V. (2010). Molecular mechanisms of early life stress – lessons from mouse models [Electronic version]. *Neuroscience and Biobehavioral Reviews, 34,* 845-852.

Southwick, S.M., Bremner, J.D., Rasmusson, A., Morgan, C.A., Arnset, A. and Charney, D.S. (1999). Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder [Electronic version]. *Biological Psychiatry, 46,* 1192-1204.

Spear, L.P. The adolescent brain and age-related behavioral manifestations [Electronic version]. *Neuroscience and Biobehavioral Reviews, 24,* 417-463.

Spertus, I.L., Yehuda, R., Wong, C.M., Halligan, S. & Seremetis, S.V. (2003). Childhood emotional abuse and neglect as predictors of psychological and physical symptoms in women presenting to a primary care practice [Electronic version]. *Child Abuse & Neglect, 27(11),* 1247-1258

Stam, R., Akkermans, L.M.A. & Wiegant, V.M. (1997). Trauma and the gut: interactions between stressful experience and intestinal function [Electronic version]. *Gut, 40,* 704-709.

Staniforth, D.H., Yorkston, N.J. & Zaki, S.A. (1982). Dose dependent changes in propranolol half life when used as an adjunct to neuroleptic treatment [Electronic version]. *Psychopharmacology, 76,* 62-65.

Stratakis, C.A. & Chrousos, G.P. (1995). Neuroendocrinology and pathophysiology of the stress system [Electronic version]. *Annals of the New York Academy of Sciences, 771,* 1-18.

Tang, X. & Sanford, L.D. (2005). Home cage activity and activity-based measures of anxiety in 129P3/J, 129X1/SvJ and C57BL/6J mice [Electronic version]. *Physiology & Behaviour,84,* 105-115.

Tõnissaar, M., Herm, L., Eller, M., Koiv, K., Rinken, A. & Harro, J. (2008). Rats with high or low sociability are differently affected by chronic variable stress [Electronic version]. *Neuroscience, 152,* 867-876.

Tsigos, C. & Chrousos, G.P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress [Electronic version]. *Journal of Psychosomatic Research, 53,* 865-871.

U.S. Department of Health and Human Services (2007). *Child Maltreatment*. Washington, D.C.: U.S. Government Printing Office.

United Nations International Children's Fund (UNICEF) (2009). *The state of the world's children: special edition.* New York, NY: Brodock.

van Dijken, H.H., de Goiej, D.C., Sutanto, W., Mos, J., de Kloet, E.R. & Tilders, F.J. (1993). Short inescapable stress produces long-lasting changes in the brain pituitary-adrenal axis of adult male rats [Electronic version]. *Neuroendocrinology, 58(1),* 57-64.

Wakizono, T., Sawamura, T., Shimizu, K., Nibuya, M., Suzuki, G., Hirano, J. et al. (2007). Stress vulnerabilities in an animal model of post-traumatic stress disorder [Electronic version]. *Physiology & Behaviour, 90(4),* 687-695.

Walf, A. & Frye, C. (2007). The use of the elevated plus maze as an assay of anxiety-related behaviour in rodents [Electronic version]. *Nature Protocols, 2(2),* 322-328.

Weaver, I.C.G. (2009). Shaping adult phenotypes through early life environments [Electronic version]. *Brain Defects Research, C (87),* 314-326.

Wei, L., David, A., Duman, R.S., Anisman, H. & Kaffman, A. (2010). Early life stress increases anxiety-like behavior in Balbc mice despite a compensatory increase in levels of postnatal maternal care. *Hormones and Behaviour.* Epub ahead of print retrieved March 29, 2010 from http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WGC-4Y700C9H-1&_user=10&_coverDate=01%2F22%2F2010&_rdoc=1_fmt=high&_orig=search&

sort=d&_docanchor=&vvie=c&_acct=C000050221&_version=1&_urlVersion=0&_userID=10&md5+bd6053bee501e3239d930930b2dd6d8f

Widom, C.S. (1999). Posttraumatic stress disorder in abused and neglected children grown up [Electronic version]. *American Journal of Psychiatry, 156, 1223-1229.*

Wright, D.M. & Lincoln, D.W. (1984). Stress-induced analgesia evoked by intraperitoneal injection of hypertonic saline: evidence for its occurrence in vasopressin deficient rats [Electronic version]. Physiology & Behaviour, 34, 691-695.

Yehuda, R. (2006). Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications [Electronic version]. *Annals of the New York Academy of Sciences, 1071*, 137-166.

Yehuda, R., Southwick, S.M., Giller, E.L., Xiaowan, M.A. & Mason, J.W. (1992). Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans [Electronic version]. *Journal of Nervous Mental Disorders, 180,* 321-325.

Zucchi, F.C.R., Kirkland, S.W., Jadavji, N.M., van Waes, L.T., Klein, A., Supina, R.D. et al. (2009). Predictable stress versus unpredictable stress: a comparison in a rodent model of stroke [Electronic version]. *Behavioural Brain Research, 205*, 67-75.