Disambiguating the Effects of Social Instability Stress in Adolescence on Learning and Memory Tasks that Involve the Medial Prefrontal Cortex and Hippocampus

by

Matthew Green

A thesis
submitted in partial fulfilment
of the requirements for the degree of
Master of Arts

Department of Psychology
BROCK UNIVERSITY
St. Catharines, Ontario

April, 2013

Abstract

Exposure to chronic stress can alter the structure and function of brain regions involved in learning and memory, and these effects are typically long-lasting if the stress occurs during sensitive periods of development. Until recently, adolescence has received relatively little attention as a sensitive period of development, despite marked changes in behaviour, heightened reactivity to stressors, and cognitive and neural maturation. Therefore, the purpose of the present study was to investigate the long-term effects of chronic stress in adolescence on two spatial learning and memory tasks (Morris water maze and Spatial Object Location test) and on a working memory task (Delayed Alternation task). Male rats were randomly assigned to chronic social instability stress (SS; daily 1 hour isolation and subsequent change of cage partner between postnatal days 30 and 45) or to a no-stress control group (CTL). During acquisition learning in the Morris water maze task, SS rats demonstrated impaired long-term memory for the location of the hidden escape platform compared to CTL rats, although the impairment was only seen after the first day of training. Similarly, SS rats had impaired long-term memory in the Spatial Object Location test after a long delay (240 minutes), but not after shorter delays (15 or 60 minutes) compared to CTL rats. On the Delayed Alternation task, which assessed working memory across delays ranging from 5 to 90 seconds, no group differences were observed. These results are partially in line with previous research that revealed adult impairment on spatial learning and memory tasks after exposure to chronic social instability stress in adolescence. The observed deficits, however, appear to be limited to long-term memory as no group differences were observed during brief periods of retention.

Acknowledgments

First and foremost I would like to thank my supervisor, Dr. Cheryl McCormick, whose continuous guidance and support has helped me to mature as a student and as an individual. Cheryl, you're not nearly as menacing as you think and I appreciate everything you've done for me. I would also like to thank members of the McCormick lab who participated in the scoring of my data, challenged me intellectually, or coached me through the tough times, thank you. Additionally, to my committee members, Dr. Tim Murphy and Dr. Cameron Muir, I thank you for your time and expertise during the preparation of my thesis research.

There are a number of people outside of Brock to whom I owe a debt of gratitude. I am extremely grateful to my parents and two sisters, all of whom have provided encouragement, support, and guidance from the beginning. Each one of you is an inspiration to me and I thank you for being there during the good times and bad. Last, but certainly not least, I would like to thank Sierra because I could not have done this without you, nor would I have wanted to. I hope you know how much I appreciate your help and how much I value our time together.

Table of Contents

List of Figures

Introduction

The use of animal models has uncovered much information regarding the deleterious consequences of exposure to stressors on cognitive performance and its neural underpinnings. In rodents, exposure to repeated (or chronic) stressors in adulthood produces deficits on tasks that involve working memory (Hains et al., 2009; Mika et al., 2012; Mizoguchi et al., 2000), cognitive flexibility (Lapiz-Bluhm, Soto-Piña, Hensler, & Morilak, 2009; Liston et al., 2006), and spatial learning and memory (Conrad, Grote, Hobbs, & Ferayorni, 2003; Hoffman et al., 2011; Luine, Villegas, Martinez, & McEwen, 1994). These stress-induced deficits are accompanied by dendritic atrophy in the medial prefrontal cortex (implicated in working memory and cognitive flexibility) (Goldwater et al., 2009; Hains et al., 2009) and the hippocampus (implicated in spatial learning and memory) (Conrad, Magariños, LeDoux, & McEwen, 1999; Hoffman et al., 2011; Luine et al., 1994). Thus, chronic stress has deleterious effects on the structure and function of brain regions that are critically involved in learning and memory processes.

In adulthood, the effects of chronic stress are typically short-lived (1-3 weeks), often dissipating after cessation of the stressor (Conrad et al., 1999; Goldwater et al., 2009; Heine, Maslam, Zareno, Joels, & Lucassen, 2004; Hoffman et al., 2011; Luine et al., 1994). Conversely, the effects of chronic stress during prenatal and neonatal life on cognitive functioning are long-lasting and often observable well-into adulthood (Brunson et al., 2005; Lui et al., 2011; Wu et al., 2007). Such findings are consistent with evidence that times of rapid development in ontogeny are particularly vulnerable to environmental factors (reviewed in Lupien, McEwen, Gunnar, & Heim, 2009). Nevertheless, adolescence, which is characterized by increased brain development and concomitant

changes in behaviour and cognitive function, has received relatively little attention as a sensitive period of development (reviewed in McCormick, Mathews, Thomas, & Waters, 2010).

Some recent research has shown an enduring effect of chronic stress experienced in adolescence on anxiety-like and depressive behaviours (Green, Barnes, & McCormick, in press; McCormick, Smith, & Mathews, 2008; Sterlemann et al., 2008) and cognitive functioning (Isgor, Kabbaj, Akil, & Watson, 2004; McCormick, et al., 2012; Morrissey, Mathews, & McCormick, 2011). Notably, male rats exposed to social instability stress in adolescence demonstrate impaired performance in adulthood on several hippocampal-dependent tasks (spatial object location test; memory of contextual fear conditioning) (McCormick, Nixon, Thomas, Lowie, & Dyck, 2010; McCormick et al., 2012; Morrissey et al., 2011). One objective of this research was to examine the effects of social instability stress in adolescence on adult spatial learning and memory in the Morris water maze. A second objective was to investigate whether adult performance on a medial prefrontal cortical-dependent task (Delayed Alternation test of working memory; reversal learning in the Morris Water Maze) is altered by social instability stress in adolescence, because of the prolonged development of this brain region (Markham, Morris, & Juraska, 2007) and its rich expression of corticosteroid receptors (reviewed in Herman, Ostrander, Mueller, & Figueiredo, 2005; Herman & Cullinan, 1997). The literature background and rationale for these objectives are described next.

Stress and the Hypothalamic-Pituitary-Adrenal (HPA) axis

Stress is a non-specific response to a perceived challenge, whether physical, psychological, or both, that depends on an individual's perception of their ability to cope

with that challenge (Selye, 1973). The hypothalamic-pituitary-adrenal (HPA) axis is one of the main stressor-responsive systems of the body that help organisms meet the challenges of their environment. In response to a perceived challenge, the paraventricular nucleus (PVN) of the hypothalamus induces the release of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophyseal portal veins, which travel to the anterior pituitary to cause the release of adrenocorticotropic releasing hormone (ACTH) into the bloodstream. At the adrenal gland, ACTH causes the release of glucocorticoids (cortisol in humans; corticosterone in rats) into the circulation. Glucocorticoids bind to two main corticosteroid receptor types; mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). In the brain, MR expression is restricted to the limbic regions, whereas GR expression is much more diffuse, with the highest densities found in the hippocampus and medial prefrontal cortex (reviewed in Herman et al., 2005).

The affinity of MR for glucocorticoids is significantly greater than that of GR, and at basal glucocorticoid concentrations approximately 80% and 15% of receptors are occupied, respectively (Reul & de Kloet, 1985), which renders GR more sensitive to fluctuations in glucocorticoid concentrations. Increased concentrations of glucocorticoids in the bloodstream are detected at all levels of the HPA axis, and in several brain regions (notably, the hippocampus, prelimbic prefrontal cortex, and amygdala), which inhibit, or cease further activation, of the HPA axis. Thus, a built in negative feedback system regulates glucocorticoid concentrations (reviewed in Herman et al., 2005).

Glucocorticoids exert a number of effects on target cells, including influencing energy metabolism, immune function, cardiovascular tone, appetite, and sexual behaviour (reviewed in McEwen, 2006; Sapolsky, Romero, & Munck, 2000). Thus, glucocorticoids help mobilize resources and suppress activation of non-vital systems during times of stress, which promotes homeostasis and the survival of the organism. Conversely, chronic activation of the HPA axis or failure of the negative feedback system to inhibit further release of glucocorticoids results in prolonged elevations, which can have potentially deleterious effects on the body. For example, chronic stress can lead to cardiovascular disease, immunosuppression, and inflammation (reviewed in McEwen, 1998). Additionally, acute and chronic activation of the HPA axis has been shown to influence learning and memory, although the effects typically depend on the intensity and duration of the stressor and the stage of ontogeny at which it occurs (reviewed in McCormick & Mathews, 2010).

Acute Stress, Learning and Memory

The hippocampus is critically involved in the formation of episodic memories (memory for past experiences; reviewed in Eichenbaum, 2001; reviewed in Tulving & Markowitsch, 1998) as well as spatial learning and memory (reviewed in Jarrard, 1993; O'Keefe & Dostrovsky, 1971). The high density of glucocorticoid receptors in the hippocampus is important for their ability to regulate HPA axis activity, but also allows for glucocorticoids to influence learning and memory function. For example, small doses of glucocorticoids enhance the consolidation of emotionally-salient information, which is likely pertinent for survival (Barsegyan, Mackenzie, Kurose, McGaugh, & Roozendaal, 2010; Buchanan & Lovallo, 2001; Okuda, Roozendaal, & McGaugh, 2004; Roozendaal,

Quirarte, & McGaugh, 2002). The relationship between stress and memory formation, however, follows a dose-dependent inverted U function, such that if glucocorticoid concentrations are too high (Pugh, Fleshner, & Rudy, 1997 ; Joëls, 2006) or too low (Pugh et al., 1997; Roozendaal, Portillo-Marquez, & McGaugh, 1996; Roozendaal et al., 2002), memory consolidation is impaired. Conversely, memory retrieval is most efficient during times of low glucocorticoid receptor binding and is impaired dose-dependently by glucocorticoids (de Quervain, Roozendaal, & McGaugh, 1998; Diamond et al., 2006; Roozendaal, Griffith, Buranday, de Quervain, & McGaugh, 2003).

The medial prefrontal cortex, which also regulates HPA activity and richly expresses corticosteroid receptors, is involved in working memory (the ability to hold and manipulate information in mind; Dunnett, Nathwani, & Brasted, 1999; Sloan, Good, & Dunnett, 2006) and reversal learning (the ability to extinguish previously learned information in lieu of new information; Lacroix, White, & Feldon, 2002; Salazar, White, Lacroix, Feldon, & White, 2004). Basal concentrations of glucocorticoids are important for the maintenance of working memory, as evidenced by impairment of memory performance following adrenalectomy and restoration of memory performance with physiological doses of corticosterone (Mizoguchi, Ishige, Takeda, Aburada, & Tabira, 2004). Nevertheless, acute stress or glucocorticoid administration is known to impair working memory (Barsegyan et al., 2010), probably at least in part due to altered dopamine release, which is critically involved in working memory (Butts, Weinberg, Young, & Phillips, 2011; Mizoguchi et al., 2004). Furthermore, the effects of acute glucocorticoid administration on memory processes (consolidation, retrieval, and working memory) are mediated by activation of β-adrenoceptors in the basolateral

amygdala, which interacts with and alters the functioning of the hippocampus and medial prefrontal cortex (Barsegyan et al., 2010; Roozendaal et al., 2002). Taken together, acute stress differentially affects memory processes and these effects depend on circulating concentrations of glucocorticoids and are thus short-lived.

Chronic Stress, Learning and Memory

Rodents exposed to chronic stress or repeated glucocorticoid administrations in adulthood demonstrate marked deficits on a variety of spatial learning and memory tasks, including the Y-maze (Conrad, Galea, Kuroda & McEwen, 1996; Conrad et al., 2003), the Morris water maze (Hoffman et al., 2011; Yau et al., 2012), and the radial arm maze (Luine et al., 1994) when tested 24 hours to one week after the last stress exposure. Moreover, chronic stress has been shown to cause apical dendritic atrophy of pyramidal neurons in the CA3 subregion of the hippocampus (Conrad et al., 1999; Magariños, Deslandes, & McEwen, 1999; Watanabe, Gould, & McEwen, 1992) as well as reduced cell proliferation and survival of neurons in the dentate gyrus, part of the hippocampal formation (Heine et al., 2004; Pham, Nacher, Hof, & McEwen, 2003), when tested 24 hours to one week after the last stress exposure. Thus, chronic stress in adulthood negatively affects the structure and functioning of the hippocampus even days after the last stress exposure. Nevertheless, the effects of chronic stress in adulthood are typically reversed after two to three weeks of recovery (Conrad et al., 1999; Heine et al., 2004; Hoffman et al., 2011; Luine et al., 1994).

Performance on medial prefrontal cortical-dependent tasks is also affected by adult chronic stress or prolonged glucocorticoid administration. Working memory on the Radial Arm maze (Hoffman et al., 2011) and Delayed Alternation task (Hains et al.,

2009), as well as extra-dimensional rule-switching (Liston et al., 2006) and reversal learning (Lapiz-Bluhm et al., 2009) on the Attention Set-shifting task is significantly impaired after prolonged exposure to stressors. Like the effects of chronic stress on hippocampal-dependent tasks, there is evidence that performance on medial prefrontal cortical-dependent tasks normalizes weeks after cessation of the stressor (Hoffman et al., 2011). Additionally, exposure to chronic stress in adulthood induces apical dendritic atrophy of pyramidal neurons in the medial prefrontal cortex (Cook & Wellman, 2004; Goldwater et al., 2009; Liston et al., 2006; Radley et al., 2005, 2006, 2008) and decreases expression of dopamine receptors (DR; subtype 1) and DR1-mediated long-term potentiation[1] (Goldwater et al., 2009), both of which are reversed after 21 days of recovery (Goldwater et al., 2009; Radley et al., 2005). Taken together, the structure and function of the hippocampus and medial prefrontal cortex are vulnerable to the effects of chronic stress, which typically last up to a week after cessation of the stressor, but tend to dissipate thereafter.

Stress during Perinatal Life

Whereas the effects of chronic stress in adulthood on brain regions critically involved in learning and memory are typically short-lived, stressors can have a more enduring impact when experienced early in life. Exposure to chronic stress in utero or in the first weeks of life impairs spatial (Brunson et al., 2005; Huang et al., 2010; Lemaire, Koehl, Le Moal, & Abrous, 2000; Vallée et al., 1999; Wang et al., 2011) and working memory (Son et al., 2006; Vallée et al., 1999) performance in adulthood. Cognitive

[1] Long-term potentiation is considered to be one of the neurological mechanisms underlying learning and memory and involves the strengthening of synaptic connections in the brain through experience-related activation.

deficits are also accompanied by decreased hippocampal long-term potentiation (Brunson et al., 2005; Son et al., 2006), NMDA receptor (NR1 and NR2B) expression (Huang et al., 2010; Son et al., 2006), and neurogenesis (Huang et al., 2010; Lemaire et al., 2000) as well as reduced extracellular dopamine (Mokler, Torres, Galler, & Morgane, 2007) in the medial prefrontal cortex, all of which are associated with learning and memory processes. Similar to the effects in adulthood, prolonged stress in early-life also alters the dendritic complexity of pyramidal neurons in the hippocampus and prefrontal cortex, only the effects persist long after cessation of the stressor (Monroy, Hernandez-Torres, & Flores, 2010; Pascual & Zamora-Leon, 2007). Such findings are consistent with basic developmental principles whereby organisms are most susceptible to environmental influences during phases of rapid development (Andersen, 2003; Rice & Barone, 2000). Although adolescence, the transitional period between childhood and adulthood, is also a period of ontogeny marked by brain and neuroendocrine maturation and concomitant changes in behaviour and cognition, the impact of prolonged stress during this time has received relatively little attention until recently.

Adolescent Development

In rats, adolescence is broadly defined as ranging from weaning (postnatal day [PND] 21) to sexual maturity (approximately PND 60) (Lupien et al., 2009). Although there is no clear onset or offset of adolescence, the behavioural repertoire of adolescents is different than that of juvenile or adult rats. For example, adolescent rats engage in increased rough and tumble play, genital self-grooming, risk taking, and novelty seeking (Doremus-Fitzwater, Varlinskaya, & Spear, 2009; Hernandez-Gonzalez, 2000; Meaney & Stewart, 1981; Pellis, Field, Smith, & Pellis, 1997). Performance on cognitive tasks is

also developing during this time, as adolescence demonstrate immature spatial memory (Morris water maze), working memory (Delayed Alternation task), and extinction learning (operant conditioning chamber) as well as increased contextual fear generalization compared to adults (Andrzewski et al., 2011; Koss, Franklin, & Juraska, 2011; Ito, Pan, Yang, Thakur, & Morozov, 2009; Spreng, Rossier, & Schenk, 2002). Changes in behaviour and cognitive function reflect ongoing brain development, some of which drives, is driven by, or is independent of adolescent hormonal changes.

Maturation of the hypothalamic-pituitary-gonadal (HPG) axis (or gonadarche) that occurs around the time of puberty (preputial separation in males PND 42 ± 2) marks the beginning of sexual maturation and, in males, an increase in circulating testosterone that prompts a second-wave of sexual differentiation of the brain (Sisk & Zehr, 2005). Conversely, maturation of the adrenal gland (or adrenarche) begins before weaning and ends at approximately mid-adolescence when sex differences in circulating adrenal steroids emerge (Pignatelli, Xiao, Gouveia, Ferreira, & Vinson, 2006). In male rats, basal plasma concentrations of corticosterone increase gradually between PND 20 and 45, the age at which adult concentrations are reached (Pignatelli et al., 2006). In response to stress, adolescent male rats have a more pronounced release of ACTH and corticosterone as well as a prolonged increase in corticosterone compared to adults (Foilb, Lui, & Romeo, 2011). Whether the heightened and prolonged stress response in adolescence is because of greater activation or because of reduced negative feedback of the HPA axis is unclear, but heightened HPA activity may confer greater risk for developing brain regions in individuals exposed to prolonged stress during this time.

Immature spatial learning, working memory, and impulse inhibition during adolescence suggests that the hippocampus and medial prefrontal cortex are undergoing structural and functional changes. Indeed, neuron number in the medial prefrontal cortex decreases during adolescence, whereas glial number increases, reflecting ongoing myelination of axons (Markham et al. 2007). Moreover, the expression of the activity regulated cytoskeletal-associated gene (Arc), involved in synaptic plasticity, is higher in the medial prefrontal cortex of adolescents than of adults and may reflect ongoing synaptogenesis (the formation of new connections between neurons) (Schochet, Kelley, & Landry, 2005). Connectivity between the medial prefrontal cortex and subcortical regions also changes during adolescence. For example, glutamatergic projections from the basolateral amygdala to the medial prefrontal cortex (Cunningham, Bhattacharyya, & Benes, 2002), and from the medial prefrontal cortex to the nucleus accumbens (NAc) (Brenhouse, Sonntag, & Andersen, 2008), increase throughout adolescence. Similarly, dopaminergic projections from the ventral tegmental area to the medial prefrontal cortex increase in density throughout adolescence before reaching adult levels by PND 60 (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988). Adolescent rats also have altered dopaminergic modulation of GABAergic interneurons compared to adult rats (Tseng & O'Donnell, 2007), which may be because of down regulation of dopamine receptors (subtype D1 and D2) after PND 40 (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Teicher, Andersen, & Hostetter, 1995). As D1 receptor binding in the medial prefrontal cortex influences working memory (D1 agonists impair, whereas D1 antagonist block impairment) (Zahrt, Taylor, Mathew, & Arnsten, 1997) and is influenced by stress (Mizoguchi et al., 2000; Murphy, Arnsten, Jentsch, & Roth, 1996),

environmental influences during adolescence may alter their D1 expression and working memory performance later in life.

Like the medial prefrontal cortex, the hippocampus continues to develop throughout adolescence. In rats, soma size of cells in, and the overall volume of, the dentate gyrus, CA1, and CA3 subregions of the hippocampus increases between late-adolescence and early-adulthood (Isgor et al., 2004). Neurogenesis in the dentate gyrus is also higher in adolescent compared to adult mice (He & Crews, 2007) and, in hamsters, dendritic length decreases whereas dendritic spine density increases in the lower blade of the dentate gyrus during the adolescent period (Zehr, Nichols, Schulz, & Sisk, 2008). There is also evidence of linear increases in dopamine receptor (D1, D2, and D4) expression in the hippocampus from juvenility to mid-adolescence when it reaches adult levels (Tarazi & Baldessarini, 2000). Expression of neural cell adhesion molecules[1] increases in the hippocampus of male rats during adolescence, whereas its polysialylated[2] form decreases (Tsoory, Guterman, & Richter-Levin, 2008). Taken together, there is a high rate of development that occurs in the hippocampus and medial prefrontal cortex during adolescence and, as a target of glucocorticoids during a period of prolonged release in response to stressors, these regions may be particularly vulnerable to chronic stress.

---

[1] The neural cell adhesion molecule provides neuronal stability, influences intracellular signalling cascades and neurite outgrowth and is believed to be critical in learning and memory.

[2] Attachment of polysialic acid to neural cell adhesion molecules reduces its adhesive properties and is believed to promote plasticity by permitting structural remodelling.

Adolescent Stress, Learning and Memory

Recent research has shown that chronic social instability stress (daily 1 hour of isolation and change of cage partner) in adolescence (PND 30-45) causes spatial memory deficits on the Spatial Object Location task in adulthood in both male and female rats (McCormick, Nixon et al., 2010; McCormick et al., 2012). Adult spatial memory is also impaired in the Morris water maze in male rats subjected to variable physical stress, but not variable social stress, in adolescence (PND 28-56) (Isgor et al., 2004). Similarly, mice exposed to mild social stress (change of cage partners once per week) beginning in adolescence (PND 30) and ending in early adulthood (PND 80) did not show deficits on the Morris water maze in later adulthood (PND 270), but did demonstrate spatial impairment on both the Morris water maze and the Y maze in old age (PND 450) (Sterlemann et al., 2010). Furthermore, adult male rats have impaired contextual fear memory compared to control rats, which, like spatial learning and memory, critically involves the hippocampus, when they are tested weeks after chronic social instability stress in adolescence, but not if the stress occurred in adulthood (PND 70-85) (Morrissey et al., 2011). Therefore, the effects of chronic stress are more enduring if administered in adolescence compared to adulthood. Additionally, most of the studies mentioned above found that chronic stress in adolescence did not cause immediate impairment, only delayed, which is in contrast to the effects of chronic stress in adulthood, which are immediate and short-lived (Green & McCormick, in press).

The enduring effects of chronic stress in adolescence also extend to neural measures in the hippocampus. For example, adult rats exposed to chronic stress in adolescence have altered expression of markers of neurogenesis (e.g. doublecortin)

compared to controls (McCormick, Nixon, et al., 2010; McCormick et al., 2012). Additionally, variable physical, but not social, stress between PND 28 and 56 caused a reduction in the overall volume of the hippocampus of rats in adulthood (approximately PND 80), despite increased neuronal density and soma size (Isgor et al., 2004). Chronic stress in adolescence also decreases long-term potentiation and increases long-term depression induction in the dorsal hippocampus of adult rats after they were exposed to an acute stressor (15 minutes forced swim) (Maggio & Segal, 2011). Neural cell adhesion molecule (L1) and its polysialylated form, which increase and decrease, respectively, throughout adolescence, are also altered in adult rats weeks after exposure to a brief chronic stress procedure in adolescence (PND 28-30) (Tsoory, Guterman, & Richter-Levin, 2010).

In sum, repeated exposure to stressors in adolescence, and not adulthood, appear to alter the trajectory of hippocampal development and later functioning. Little is known, however, about the influences of adolescent stress on medial prefrontal cortical development and later functioning. A study by Toledo-Rodriguez and Sandi (2007) found that exposure to variable stressors for a brief period in early adolescence (PND 28-30) impaired fear extinction learning (a task critically dependent on the integrity of the medial prefrontal cortex; Milad & Quirk, 2002; Morgan, Romanski, & LeDoux, 1993) when rats were tested as adults. Additionally, temporal order recognition was impaired in male rats immediately after exposure to one week of restraint stress (PND 21-28), which appears to be mediated by altered NMDA and AMPA receptor-induced long-term potentiation (Yuen et al., 2012). Nevertheless, the behavioural effects disappeared less than a week after the last stress exposure. Other studies have examined the effects of

social deprivation in early-adolescence (PND 21-34) and found that weeks later rats had increased brain derived neurotropic factor and D2 receptors in the medial prefrontal cortex, which was accompanied by impaired reversal learning and latent inhibition (Han Li, Xue, Shao, & Wang, 2011, 2012). Nonetheless, social deprivation likely alters development differently than repeated stress as it denies the opportunity for critical social experiences rather than causing stress, per se, and the research conducted thus far has not examined stress effects in mid-adolescence when endocrine changes are associated with heightened brain development (Green & McCormick, in press). Thus, there is a paucity of research on the effects of stress in adolescence on medial prefrontal cortical function. Therefore, the purpose of the current study was to investigate whether chronic social instability stress during mid-adolescence alters adult performance on medial prefrontal cortical-dependent cognitive tasks (delayed alternation and reversal learning in the Morris water maze). Additionally, the current study sought to replicate and extend previous research regarding the long lasting effect of social instability stress on spatial learning and memory tasks (Morris water maze and spatial object location task). The rationale for using social instability stress and choosing the cognitive tasks mentioned above will be discussed in the next section.

Adolescent Social Instability Stress

Social instability stress consists of two components, daily 1 hour of isolation in a small ventilated container and subsequent pairing with a new cage partner. The stress procedure is carried out during mid-adolescence (PND 30-45) to span puberty and peripubertal brain organization. After the isolation component, rats have elevated corticosterone concentrations in the bloodstream compared to rats that remained with

their cage partner in the home cage (McCormick, Merrick, Secen, & Helmreich, 2007). The social instability component was originally included because recovery from isolation stress is reduced in rats that are paired with a new cage partner compared to rats that are returned to their original cage partner (McCormick et al., 2007). Also, pairing rats with a new cage partner altered habituation to repeated isolation stress. Furthermore, social stress paradigms during adolescence may be particularly effective because adolescence is a period during which much social learning occurs and is a time when social interactions are more rewarding than in adulthood (Douglas, Varlinskaya, & Spear, 2004). In support of this possibility, social instability stress has been shown to have long lasting influences on fear conditioning (Morrissey et al., 2011), spatial memory (McCormick, Nixon et al., 2010; McCormick et al., 2012), social behaviour (Green et al., 2012), anxiety and mood disorders (McCormick et al., 2008; Mathews, Wilton, Styles, & McCormick, 2008), and sensitivity to drugs of abuse (Mathews, Mills, & McCormick, 2008) when animals are tested in adulthood. Importantly, social instability stress in adolescence does not affect HPA function in adulthood (McCormick et al., 2008), which suggests that the enduring effects mentioned above are caused by organizational (in adolescence) rather than activational effects of stress on performance in adulthood.

Morris Water Maze

A variety of paradigms have been developed to assess spatial learning and memory in rodents, the most frequently used being the Morris water maze, which was first described by Morris (1981). Rodents can be trained on a variety of tasks in the water maze that are designed to examine various theoretical questions, neurological mechanisms and functions. Acquisition (or place) learning, whereby a test animal is

placed in a circular arena that is partially filled with an opaque liquid and is expected to learn the location of a submerged escape platform using distinct extra-maze visual cues, is the most frequently used task. Providing extra-maze visual cues allows the animal to create a cognitive map and to successfully navigate to the hidden escape platform. Removal of visual cues impairs place learning in the Morris water maze (Morris, 1984).

The standard protocol for Morris water maze testing includes three phases; acquisition learning, a probe trial, and reversal learning (reviewed in Vorhees & Williams, 2006). During acquisition learning sessions, test subjects undergo a series of trials in which they are placed randomly in one of three equally sized quadrants that do not contain the hidden escape platform. A trial ends when the escape platform has been reached or when the trial duration has elapsed (typically 1-2 minutes), in which case the test animal is placed on the platform. At the end of each trial the test animal remains on the platform (typically 15-30 seconds) so that it can learn its location in the maze relative to extra-maze visual cues. The latency to reach the escape platform as well as the distance travelled to the escape platform are the most commonly used measures of spatial learning. The latter, however, provides a measure of spatial learning that is independent of differences in motor function that may arise due to the experimental manipulation (pharmacological treatment, brain lesion, age at testing, etc.).

After acquisition learning, a probe trial is typically conducted to assess spatial memory retrieval and thus, should occur at least 24 hours after the last acquisition trial for the performances to be independent of one-another (reviewed in Vorhees & Williams, 2006). The probe trial differs from an acquisition trial in that the escape platform has been removed from the arena and thus the trial ends only when its duration has elapsed

(typically 30-60 seconds). The number of crosses over the platform's former location as well as the amount of time spent in the region that previously contained the platform is used as a measure of spatial memory. Longer probe trials are discouraged because the absence of an escape platform may extinguish spatial memory or lead to the abandonment of a spatial-mapping strategy altogether (reviewed in Vorhees & Williams, 2006). Moreover, rodents' motivation to actively seek the escape platform on subsequent trials may become diminished if this behaviour goes unreinforced over repeated or lengthy probe trials.

After the probe trial, reversal learning is assessed by returning the platform to the maze and placing it in a new location, usually opposite to the platform's original location. The procedures used for reversal learning are usually identical to those used for acquisition learning, although it assesses a different function; reversal learning involves extinguishing the memory for the platform's former location as well as learning the new location. Rodents will typically search in the original location to find the platform on the first several trials, but will readily abandon swimming to this location shortly after (reviewed in Vorhees & Williams, 2006). Similar to reversal learning, the delayed-matching-to-place task requires test animals to learn a new platform location at the beginning of each session or block. On the first trial of a session, the escape platform can be found through trial and error, however, the latency to reach, and distance travelled to, the escape platform typically decreases on the second trial, which suggests intact memory for the 'last location' of the escape platform. Both reversal learning and delayed-matching-to-place are believed to rely, at least in part, on working memory function as

the test animal must correctly remember the last location of the escape platform (Sloan et al., 2006; Lacroix et al., 2002; Salazar et al., 2004).

The Morris water maze is not the only tool for assessing spatial learning and memory in rodents, although it is widely utilized because of its numerous strengths. For example, rodents require very little training in the water maze because they are good swimmers and eager to escape an enclosed arena filled with water (Morris, 1984). Additionally, researchers can tease apart whether deficits in performance are because of spatial learning and memory deficits or because of sensorimotor impairment or motivation by conducting a cued (or visible) platform test. Unlike tests on land, the water maze largely prevents test animals' from using olfactory cues to find the escape platform as opposed to spatial mapping strategies. Lastly, water maze training does not require stressful food restriction, because the refuge on the platform provides sufficient reinforcement to induce learning. Nevertheless, some researchers have argued that Morris water maze testing itself is inherently stressful (Engelmann, Ebner, Landgraf, & Wotjak, 2006) and ways to minimize stress have been proposed (maintaining temperature of water and test room at 25 degrees Celsius and allowing rats to habituate to the arena beforehand; reviewed in D'Hooge & De Deyn, 2001).

Lesions to the hippocampus, particularly the dorsal subregion (reviewed in D'Hooge & De Deyn, 2001), impair acquisition learning, probe trial performance (Sloan et al., 2006; Morris, Schenk, Tweedie, & Jarrard, 1990), and savings on the delayed matching-to-place task (irrespective of short or long inter-trial intervals) (Morris et al., 1990; Steele & Morris, 1999). Hippocampal lesions, however, do not affect swim speed or ability to reach the platform on visible platform trials (reviewed in Sloan et al., 2006),

which suggests that lesion-induced impairment is specific to spatial learning and memory and is independent of sensorimotor function and motivation. Although the hippocampus plays a crucial role, other brain regions are involved and likely contribute to a complex neural network that underlies spatial learning and memory in the water maze. The entorhinal cortex, which provides major input to the hippocampus through the alvear and perforant pathway, is involved in place learning as evidenced by lesion-induced deficits in acquisition learning (Schenk & Morris, 1985). Moreover, a lesion to the subiculum produces acquisition learning and probe trial deficits, which are comparable to rats with hippocampal lesions, and impairment on delayed matching-to-place savings (irrespective of inter-trial interval; 5 seconds, 3 or 23 minutes), that are less severe compared to hippocampal lesions or lesions to both structures (Morris et al., 1990). Overtraining, by performing frequent and inter-mixed hidden and cued platform trials, marginally reduces hippocampal and subiculum lesion-induced deficits, but remains severely impaired in rats with damage to both regions. Conversely, lesions to the medial prefrontal cortex were shown to have no effect on acquisition learning and probe trial performance (Sloan et al., 2006), although there is evidence of impaired reversal learning (Salazar et al., 2004).

Spatial Object Location Test

The Spatial Object Location test is believed to assess hippocampal-dependent memory in rodents (Ennaceur, Neave, & Aggleton, 1997). Briefly, test animals undergo a familiarization phase whereby they are individually exposed to two identical objects in an arena that is surrounded by distinct, distal visual cues (similar to the Morris water maze test). In the test phase the same two objects are presented, but one object is moved to a new location. Because rats have an innate preference for novelty (Bardo, Neisewander, &

Pierce, 1989) they will spend more time with the object that was moved to the new location than the object that remained in the familiar location, assuming their spatial memory is intact. Indeed, animals do spend more time with the object in the novel location if the familiarization phase and the test phase are separated by brief delays (1 minute to 1 hour; Ennaceur et al., 1997; McCormick Nixon et al., 2010; McCormick et al., 2012), but they show no preference for either object after longer delays (24 hours; Ennaceur et al., 1997) or after damage to the hippocampus (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). Moreover, if rats undergo a version of the task that assesses object recognition and not object location recognition then task performance remains intact, indicating that the hippocampus is involved in processing the spatial location of the object.

The Spatial Object Location test is relatively easy to administer in that it does not require task training and can be conducted within a single day. Because the task uses rats' preference for novelty and does not require training, food or water restriction is not required to motivate the animals to learn the task. Furthermore, the Spatial Object Location test does not involve aversively motivated behaviour, such as the Morris water maze task, and therefore is believed to be less stressful.

Delayed Alternation Task

The Delayed Alternation Task, sometimes referred to as the delayed non-matching-to-place task, assesses working memory, the ability to hold information in mind. The Delayed Alternation Task is performed typically in a T-maze or in an operant chamber with two levers. In either apparatus, rats are trained to alternate responses (left and right) on successive trials (typically 10 or more per block). In the Delayed

Alternation task, animals only receive reinforcement after performing a response that was different from that of the previous trial. The Morris water maze is different from the Delayed Alternation task in that the former is trial-independent (animals use spatial cues on each trial to locate a platform that does not move), whereas the latter is trial-dependent (a correct response depends on the response on the previous trial) and thus assesses working memory. Once animals are proficient on the task (approximately 80% of responses are alternations) researchers typically implement inter-trial delays to increase task difficulty. Longer inter-trial delays have been shown to decrease accuracy on the Delayed Alternation Task (Koss et al., 2011; Sloan et al., 2006) presumably because test animals have to maintain the last response in working memory for a greater period of time without distraction or confusion with past trials.

One advantage of the operant version of the Delayed Alternation Task is that it allows for the collection of a large number of trials. In the operant version, however, rats (and monkeys) may use a strategy other than working memory, such as practice or placement (standing in front of lever that needs to be pressed next; Dunnett et al., 1999; Kojima, Kojima, & Goldman-Rakic, 1982), which can be avoided by testing rats in a T-maze. One disadvantage of the Delayed Alternation Task in general is that it requires test animals to be food restricted prior to training to motivate them to learn the task. Under extreme conditions, such as 24 hour food deprivation, rats will have elevated concentrations of corticosterone (albeit less so than a stressor-induced increase) and disrupted HPA functioning if deprivation continues (reviewed in Toth & Gardiner, 2000). Nevertheless, moderate increases in corticosterone concentrations during restriction or brief deprivation likely reflect increased energy needs and one month of food restriction

has been shown to not alter the stress profile of adult male rats (Stewart, Meaney, Aitkin, Jensen, & Kalant, 1988). Despite food restriction, the Delayed Alternation Task is widely used in rats (Koss et al., 2011; Kinoshita et al., 2008), mice (Rossi et al., 2012), and monkeys (Kojima et al., 1982) to assess working memory and prefrontal functioning.

The integrity of the medial prefrontal cortex is critical for working memory and, as such, Delayed Alternation task performance as evidenced by lesion studies in rats (Bubser & Schmidt, 1990; Dias & Aggleton, 2008; Granon, Vidal, Thimus-Blanc, Changeux, & Poucet, 1994; Sloan et al., 2006) and mice (Rossi et al., 2012). Whereas some studies have shown that medial prefrontal lesions produce deficits at longer delays (Rossi et al., 2012; Sloan et al., 2006), other studies have shown impairment to be delay-independent (Yoon, Okada, Jung, & Kim, 2008; Kinoshita et al., 2008), although the delays utilized often differ between studies. Importantly, deficits on the Delayed Alternation task after medial prefrontal lesion are not due to altered motor behaviour or side biases (Dunnett et al., 1999). There is also evidence of recovery of task performance if the lesion occurs in early in life (PND 10), but this takes several weeks to occur and likely reflects the high rate of plasticity during this period (Freeman & Stanton, 1992). Furthermore, there is evidence of hippocampal involvement in the Delayed Alternation Task, possibly related to the spatial component (left and right), although the literature is mixed with some studies showing both performance impairment (Ainge, van der Meer, Langston, & Wood, 2007; Hock & Bunsey, 1998; Maruki, Izaki, Hori, Nomura, & Yamauchi, 2001) and other studies finding no such effect (Lipska, Aultman, Verma, Weinberger, & Moghaddam, 2002; Sloan et al., 2006).

Dopamine receptor binding (mainly the D1 subunit, although there is evidence for a role of D2) in the medial prefrontal cortex influences Delayed Alternation task in an inverted U fashion (Zahrt et al., 1997; reviewed in Seamans & Yang, 2004), which is believed to be the result of an altered signal to noise ratio (Kroener, Chandler, Phillips, & Seamans, 2009; Dreher, Guigon, & Burnod, 2002). NMDA receptor binding also affects Delayed Alternation Task performance, as evidenced by deficits after prefrontal injections of non-competitive antagonists (Verma & Moghaddam, 1996). Importantly, NMDA receptor blockade caused increased extracellular dopamine in the medial prefrontal cortex and performance deficits could be blocked with a dopamine antagonist, once again pointing to the crucial role of dopamine transmission in working memory and, as such, Delayed Alternation task performance.

Hypotheses

Based on the ongoing development of the hippocampus during adolescence, the rich expression of corticosteroid receptors in this brain region, and the ability of chronic stressors to alter developmental trajectories, it is hypothesized that social instability stress in adolescence will impair male rats' performance on both the Morris water maze and the Spatial Object Location test in adulthood. It is also hypothesized that social instability stress during adolescence will cause enduring deficits on the Delayed Alternation Task, because the medial prefrontal cortex, like the hippocampus, is a primary target for stress hormones and continues to develop during this period of ontogeny.

Methods

Animals

Male Long-Evans rats (n = 64) were obtained from Charles River, St. Constant, Quebec, Canada, at 22 days of age and given one week to acclimate to the animal colony. Animals were housed in pairs in plastic (polycarbonate) cages, kept on a 12:12 hour light/dark cycle (lights on at 8am), and food and water was made available ad libitum. All experimental procedures were in concordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Canadian Council on Animal Care guidelines and were approved by the Animal Care and Use Committee at Brock University.

Social Instability Stress

Animals were randomly assigned to the social instability stress (SS) group or to the no stress control (CTL) group and housed in same condition pairs. Beginning on postnatal day (PND) 30, SS rats were isolated in a small ventilated container (approximately 14 cm in diameter and 10 cm in height) for 1 hour each day until PND 45 (for a review of the SS model see McCormick, 2010). Immediately after isolation each day, rats were returned to the animal colony and housed with a new cage partner that had also undergone the 1 h isolation. Importantly, SS rats were never paired with the same cage partner more than once, except on PND 45 when they were returned to their original cage partner. The SS procedure was always conducted during the lights on phase of the light/dark cycle, but the time it occurred varied to minimize habituation. CTL rats remained undisturbed except for feeding and cage maintenance. After the stress procedure half of the SS and CTL rats were assigned to

Morris water maze and Spatial Object Location testing and the other halves were assigned to Delayed Alternation Task testing.

Morris Water Maze

Apparatus. A circular arena (140 cm diameter, 67 cm height) was filled with water (25 ± 2°C) to a depth of 26 cm and the water was made opaque using powdered coffee whitener. The pool was divided into four equal size quadrants and a submerged escape platform (12 cm diameter, 24 cm height) was placed in the center of the north quadrant (approximately 13 cm off the wall). The temperature of the testing room was maintained at approximately 27 ± 2°C, and distinct visual cues were placed on each of the four walls. Behaviour in the water maze was measured using Smart tracking software, which received visual input from a Sony (model DCR-SR47) video-camera positioned overhead (approximately 2.5 meters from the floor). Throughout water maze testing, feces were removed from the pool after trials and the water was stirred to prevent the use of odour trails.

Acquisition Learning. Beginning on PND 85, each rat (n = 16/group) underwent three acquisition learning test sessions (separated by 48 hours), which consisted of 4 trials each. A trial began by placing a test rat in one of the three quadrants (randomly chosen) that did not contain the escape platform and ended when the test rat located the escape platform. If the test rat did not locate the escape platform within 60 seconds, it was placed on the platform by the experimenter. At the end of each trial the test rat remained on the platform for 30 seconds to learn its location relative to the extra-maze visual cues. The location of the escape platform stayed the same throughout acquisition learning. Between trials test rats were placed in a holding cage that contained dry towels and was next to a

heater for two minutes. After each session rats were returned to their home cage once dry. The distance travelled to the escape platform on both the first and last trials of each session was used to measure performance.

Probe Trial. The escape platform was removed from the arena and each animal underwent a single 60 second probe trial 48 hours after the last acquisition session. Each rat was placed in one of the three quadrants (randomly chosen) that did not previously contain the escape platform. The percent of time spent in the target area (a circle, approximately 38 cm in diameter, positioned over the platform's former location) and in an equally sized area located at the opposite side of the maze was used to measure rats' spatial bias.

Reversal Learning. The escape platform was returned to the water maze and placed in the center of the quadrant opposite its original location. Two days after the probe session, two sessions of reversal learning were conducted (separated by 48 hours), which consisted of 4 trials each. Similar to acquisition learning and probe trials, test animals were placed in one of the three quadrants (randomly chosen) that did not contain the escape platform for each trial. The distance travelled to the escape platform was measured to assess performance.

Spatial Object Location Test

Apparatus. Two identical open-top arenas (60 cm X 60 cm X 60 cm) made of white melamine were used. Distinct visual cues were placed on the walls of the testing room and a Sony digital camera (model DCR-SR47) video-camera was positioned overhead (approximately 2.5 meters from the floor) to record animals' interactions with the objects during both the familiarization and test phase described below. Scents were

not removed from the arena, although after habituation, between phases, and after each session feces and urine were removed. All test sessions were conducted during the lights on phase of the light/dark cycle. The procedures were as used previously in our lab (McCormick, Nixon et al., 2010; McCormick et al., 2012) and based on those of Ennaceur and colleagues (Ennaceur & Meliani, 1992).

Habituation. SS and CTL rats were habituated to the test arena for 15 min the day before testing commenced. Cage partners were habituated at the same time to avoid the sequential removal of an animal from the home cage, and this was consistent throughout testing. Similarly, the order that rats were habituated and tested was counterbalanced across groups and the experimenter remained blind to condition.

Familiarization Phase. SS and CTL rats underwent 3 spatial object location test sessions separated by 24 hours. Each test session consisted of a 3 minute familiarization phase and a 3 minute test phase, which were separated by a delay (60 minutes, 15 minutes, 240 minutes, respectively). During the familiarization phase, a test animal was placed in the arena facing the wall closest to the experimenter. Two identical objects made of metal or plastic were located in the corners of the arena furthest from the experimenter and the time spent investigating each object was measured. A rat was considered to be exploring an object when it was directing its nose at a distance of less than 2.5 cm to the object and/or touching it with its nose (Ennaceur & Delacour, 1988; Ennaceur & Meliani, 1992). The procedures were the same for each familiarization phase, except that rats saw a different pair of identical objects during each session. Test animals were always returned to the home cage immediately after familiarization.

Test Phase. After the delay that followed familiarization, rats underwent a 3

minute test phase. During the test phase, SS and CTL rats were returned to the same arena, except that one of the two objects seen before was moved to one of the previously unoccupied corners. The object that was moved and the corner that it was moved to were counterbalanced across condition and test sessions, such that rats never experienced the same object relocation twice. The amount of time that animals spent investigating each object was measured. Because rats have an innate preference for novelty, if their spatial memory is intact they will spend more time with the object in the new location compared to the object that has not moved.

Delayed Alternation Task

Rats assigned to the Delayed Alternation Task were tested in two batches. The first batch began testing at 130 days of age and the second batch began testing at 180 days of age. Both batches contained an equal number of SS and CTL rats (n = 16/batch).

Apparatus. A T-maze was constructed from grey polyvinyl chloride foam sheets and consisted of 3 equally sized arms (45 cm length, 10 cm width, 16 cm height) and a start box (26cm length, 15cm height) that was attached to the base of the center arm. Guillotine-style doors were used to separate the start box from the center arm as well as the center arm from the two choice arms. Transparent lids with hinges prevented the test animal from escaping the apparatus during testing, but afforded access to the experimenter. A small ceramic ramekin (6.5 cm diameter, 3.5 cm height) was placed at the end of either choice arm and was used to keep the reinforcement (non-salted, hulled sunflower seed) out of the test rat's sight. To prevent the use of olfactory cues, sunflower seeds were scattered around the entire outside of the T-maze.

Food Restriction. Animals were food restricted to 85-90% their original body weight over 2.5 weeks beginning at either PND 130 (batch 1; *n* = 8/group) or 180 (batch 2; *n* = 8/group). Each animal was weighed every 2 days to ensure that cage partners were losing approximately the same weight percentage and that no animal lost more than 10% their original body weight within 1 week.

Habituation. Test animals underwent 10 minutes of habituation each day for 6 consecutive days. Each rat was habituated individually and the guillotine-style doors remained open for all three sessions so that the animal could explore the T-maze. On the first three sessions, sunflower seeds were placed throughout the apparatus to encourage exploration and so that the animal could get use to consuming the reinforcement in the maze. On the last three sessions, sunflowers seeds were placed in the ramekins only so that test animals could get use to eating the reinforcement at the end of the choice arms. One animal (CTL) failed to consume the sunflower seeds throughout habituation and was therefore dropped from testing and another (CTL) was removed from the experiment due to health complications.

Shaping. Shaping involved six sessions consisting of 10 trials/session, with sessions separated by 24 h. At the beginning of each trial the rat was placed in the start box for three seconds before the door was opened. In these sessions, one choice arm was baited with a reinforcer and access to the other choice arm was blocked. Once the test rat's whole body was inside the choice arm the door behind him was closed and he was given 3 seconds to consume the reward. The baited and blocked arms alternated from trial to trial, thus teaching the rats to alternate between left and right. The choice arm that was baited on the first trial alternated for each session.

Training. Rats began training 24 hours after their last shaping session. During training sessions (separated by 24 hours), both choice arms were accessible, thus allowing the rat to choose between left and right. The first trial of each training session was a free choice trial, meaning that a response of either left or right was rewarded. On the next 10 trials, however, the test rat was only rewarded if he chose the arm that was not chosen on the previous trial, thus rats were rewarded for alternating between left and right. A rat was considered to have made a response when his whole body was inside the choice arm at which point the door was closed behind him and he was given approximately 3 seconds to consume the food or to punish him for an incorrect response. Training ended after 13 sessions or, to prevent over-training, if a rat completed 8 out of 10 correct alternations on 3 consecutive sessions. The number of trials to reach criterion was measured to assess task learning.

Pretesting. 24 hours after a test animal completed his training he began pretesting. The purpose of pretesting sessions was to reduce the decrease in performance that occurs on the first couple sessions once longer delays are introduced (Koss et al., 2011). Similar to training sessions, pretesting sessions consisted of a free choice trial followed by 10 choice trials, however, between trials, a semi-random delay (0, 5, 10, or 15 seconds) was incorporated, which began when the rat was placed in the start box. Each animal received the same delay schedule and no delay was used on consecutive trials or more than 3 times in a session. All animals underwent two pretesting sessions, which were separated by 24 hours.

Testing. The first of 15 testing sessions (separated by 24 hours) began 24 hours after pretesting. Testing sessions included a single free choice trial and 10 choice trials,

which were separated by a semi-random delay (5, 10, 15, 30, 60, or 90 seconds). On each session, all delays were used, but no delay was used more than twice and the same delay was never used on consecutive trials (e.g. 30, 60, 5, 30, 90, 10, 15, 60, 5, 90 or 5, 15, 90, 30, 10, 30, 60, 15, 10, 60). The number of correct alternations that was made for each delay on all sessions was used as a measure of performance. Because delays were semi-randomized their frequency across the 15 test sessions varied (the lowest frequency being 22 trials), thus we only analyzed the first 22 trials for each delay. Throughout the entire procedure the order that rats were tested was counterbalanced across condition. Further, because rats show a small stress response to the removal of their cage partner, cage partners were tested at least 1.5 hours apart and the rat that was tested first alternated each day.

Statistical Analyses

Statistical analyses involved between and within group analysis of variance (ANOVA). Post hoc analyses involved F tests for simple effects and Bonferroni corrected t-tests, where appropriate. An alpha level of $p < 0.05$, two-tailed, was used to determine statistical significance.

Results

Weight

A mixed factor [Group (SS, CTL) X Age (30, 45)] ANOVA on rats' weight revealed a significant main effect of Group ($F_{1,63} = 4.30$, $p = 0.04$) and of Age ($F_{1,63} = 4.53$, $p < .001$) as well as a significant interaction between the two variables ($F_{1,63} = 4.38$, $p = .04$). Simple effects analyses indicated that on PND 30, prior to the stress procedure, SS and CTL rats did not differ in weight ($t_{63} = 1.52$, $p = .13$), however, on PND 45, after the stress procedure, SS rats weighed significantly less compared to CTL rats ($t_{63} = 2.22$, $p = .03$) (see Figure 1).

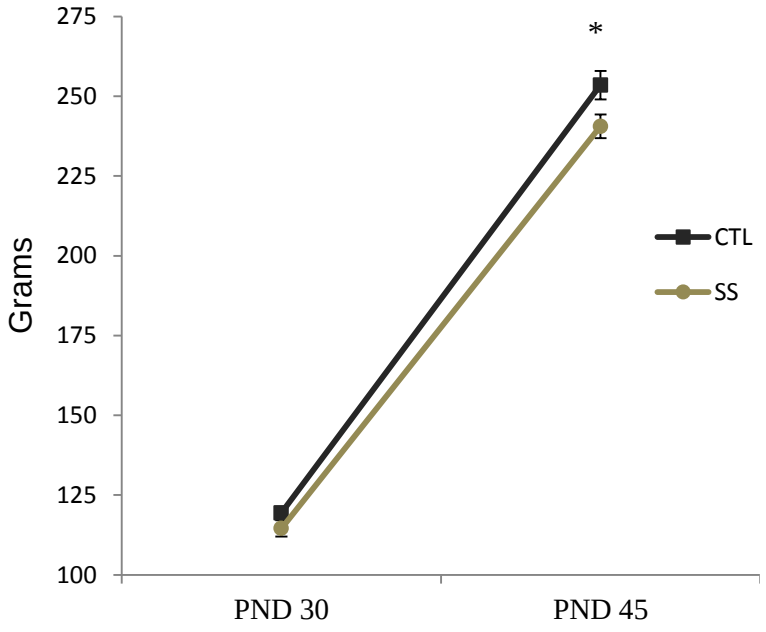

*Figure 1. Average weight of CTL and SS rats before (PND 30) and after (PND 45) the adolescent stress procedure. Error bars represent standard error of the mean. At PND 30, there were no group differences, but at PND 45 SS rats weighed significantly less than CTL rats. * p < .05.*

Morris Water Maze

Acquisition Learning

Short-term memory. A mixed factor [Group (SS,CTL) X Trial (First,Last) X Session (1,2,3)] ANOVA on distance travelled to the platform revealed a significant main effect of Trial ($F_{1,30} = 111.15$, $p < .001$) and of Session ($F_{2,60} = 65.90$, $p < .001$) as well as an interaction between Group and Trial ($F_{1,30} = 7.90$, $p = .009$) (see Figure 2). Simple effects analyses revealed no differences between SS and CTL rats in the distance

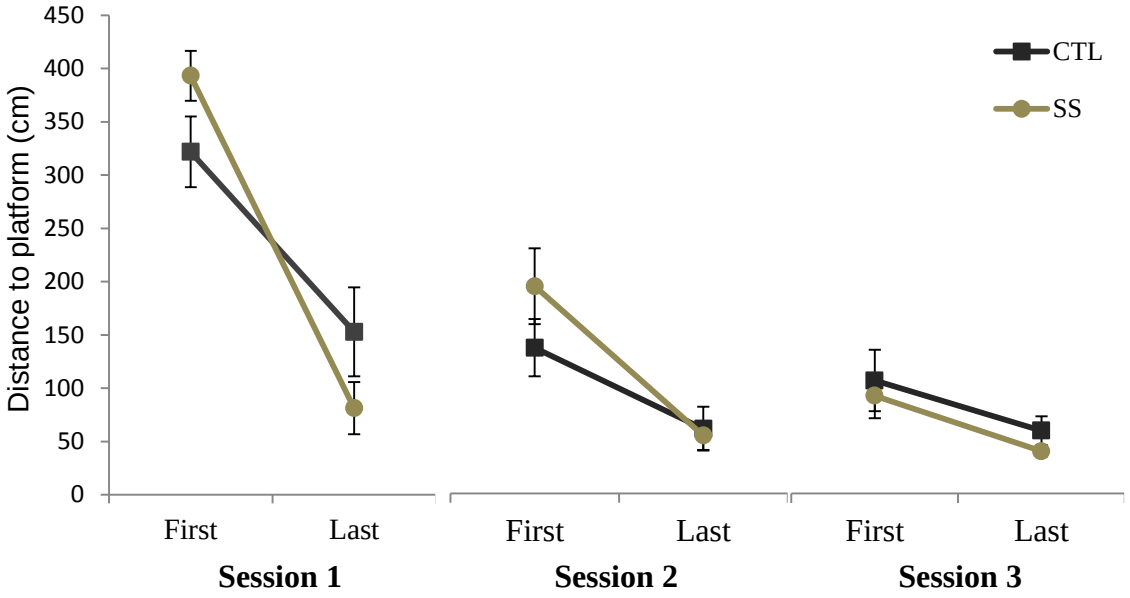

Figure 2. Average distance travelled to the escape platform on the first and last trials of three acquisition learning sessions. Error bars represent standard error of the means. Significant interaction between Group (CTL, SS) and Trial (First, Last).

travelled to the platform on first ($t_{30} = 1.38$, $p = .18$) and last ($t_{30} = 1.54$, $p = .14$) trials collapsed across sessions. Separate analyses were conducted for each group, which demonstrated that both SS ($t_{15} = 4.68$, $p < .001$) and CTL rats ($t_{15} = 11.88$, $p < .001$)

swam a greater distance to the platform on the first compared to last trials collapsed across sessions (see Figure 3). Nevertheless, the significant interaction indicates that SS rats had better short-term spatial learning as evidenced by more improvement from first to last trials collapsed across sessions.

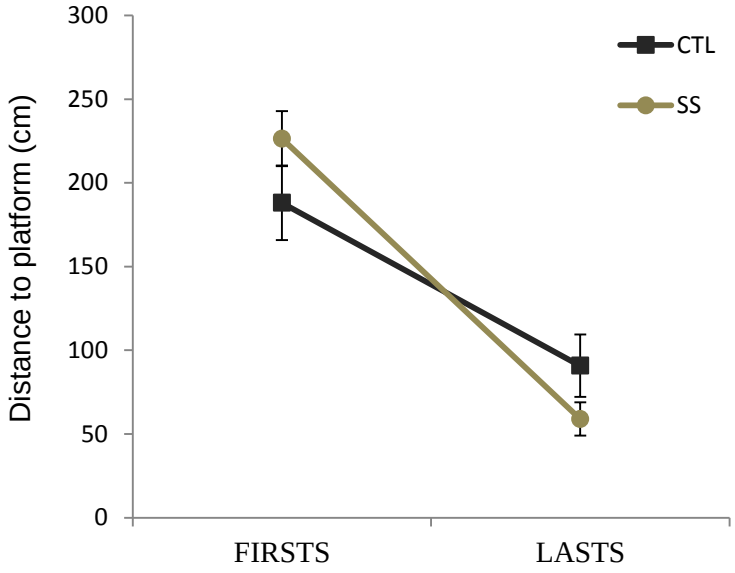

Figure 3. Average distance travelled to the escape platform on the first and last trials collapsed across three sessions of acquisition learning. Error bars represent standard error of the mean. There were no differences between CTL and SS rats on first or last trials collapsed across sessions, ps > .05.

To determine whether the significant Trial X Group interaction was accounted for by heightened anxiety of SS rats on the first trial of sessions 1 and 2, swim strategy (the amount of time spent swimming near the wall compared to away from the wall) was analyzed, without having an a priori hypothesis. Past research has shown that the tendency of rats to stay near the perimeter of a test arena (also known as thigmotaxis) is reduced after administration of anxiolytic drugs (Treit & Fundytus, 1988). A mixed factor [Group (SS,CTL) X Trial (First, Last) X Session (1,2)] ANOVA on the amount of the total distance travelled that was spent near the wall revealed a significant main effect of

Trial ($F_{1,30}$ = 147.86, p < .001) and of Session ($F_{1,30}$ = 74.52, p < .001) as well as an interaction between Day and Trial ($F_{1,30}$ = 26.43, p < .001) and Trial and Group ($F_{1,30}$ = 12.58, p = .001). Simple effects analyses revealed that SS and CTL rats did not differ on the first trials (t_{30} = 1.62, p = .12), but that SS rats swam significantly less near the wall than CTL rats on the last trials (t_{30} = 2.26, p = .03), collapsed across session. Differences on the last session suggest that SS rats swam more directly to the escape platform than CTL rats at the end of each session but not at the beginning, which provides evidence that group differences in acquisition learning were not likely accounted for by differences in anxiety.

Long-term memory. To investigate group differences in spatial memory over a longer-term than the 20 min test session, group differences in performance were analyzed between sessions (Inter-day Session; distance travelled to the platform on the last trial of a session compared to the first trial of the next session). A mixed factor [Group (SS,CTL) X Trial (Last,First) X Inter-day Session (1,2)] ANOVA on distance travelled to the platform revealed a significant main effect of Trial ($F_{1,30}$ = 6.29, p = .02) and of Inter-day Session ($F_{1,30}$ = 16.50, p < .001) as well as a three way interaction between Group, Trial, and Inter-day Session ($F_{1,30}$ = 5.03, p = .03). To examine the interaction, follow-up analyses were split by Inter-day Session, which revealed a significant interaction of Trial and Group for Inter-day Session 1 ($F_{1,30}$ = 5.00, p = .03), but not Inter-day Session 2 ($F_{1,30}$ = 0.06, p = .81). Therefore, separate analyses were conducted for each group to determine whether performance decreased between session 1 and 2. The results revealed that distance travelled to the platform did not differ for CTL rats (t_{15} = 0.35, p = .73), but SS rats swam a significantly greater distance on the first trial of day 2 compared to the

last trial of day 1 (t_{15} = 3.22, p = .006), which may reflect long-term memory decay (see Figure 4).

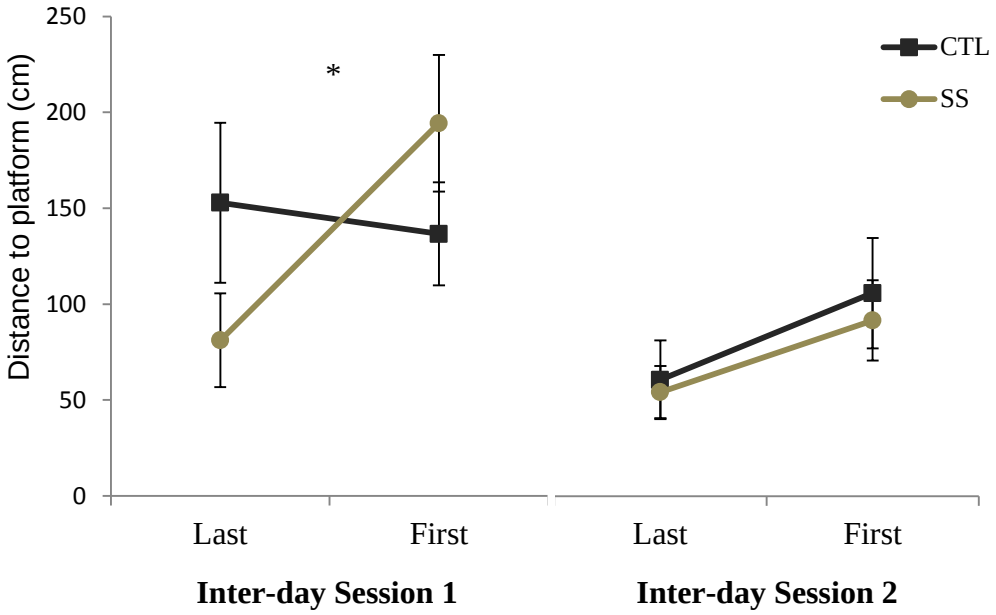

*Figure 4. Average distance travelled to the escape platform on the last trial of day 1 and the first trial of day 2 (left pane), and their performance on the last trial of day 2 and the first trial of day 3 (right pane). Error bars represent standard error of the mean. SS rats' performance decreased between days 1 and 2, * p < .01.*

Probe Trial

A mixed factor [Group (SS,CTL) X Zone (Target,Opposite)] ANOVA on the percent of time spent in regions of the water maze during the 1 minute probe trial revealed a significant effect of Zone ($F_{1,30}$ = 15.64, p < .001), but not an effect of Group ($F_{1,30}$ = 0.21, p = .65) nor an interaction between Group and Zone ($F_{1,30}$ = 1.15, p = .29). Follow-up analyses on the main effect of Zone revealed a spatial bias of SS and CTL rats for the target zone compared to opposite zone (t_{31} = 3.95, p < .001) (see Figure 5). The spatial bias of rats may change throughout the probe trial, however, because rats cannot

locate the escape platform. Thus, a second analysis was conducted examining group differences in spatial bias in 15 second intervals. A mixed factor [Group (SS,CTL) X Zone (Target,Opposite) X Interval (1,2,3,4)] on time spent in locations of the water maze revealed a significant interaction of Zone and Interval ($F_{3,90} = 9.39$, $p < .001$), but not a three way interaction including Group. Simple effects analyses indicated that SS and CTL rats spent more time in the target zone compared to the opposite zone during the first 15 second interval ($t_{31} = 6.36$, $p < .001$), but not the second, third, or fourth 15 second intervals ($p = .36$, $p = .27$, $p = .18$, respectively), which indicates that both groups showed a strong spatial bias initially, but that strategy was soon abandoned (after 15 seconds) once they could not locate the platform (see Figure 5).

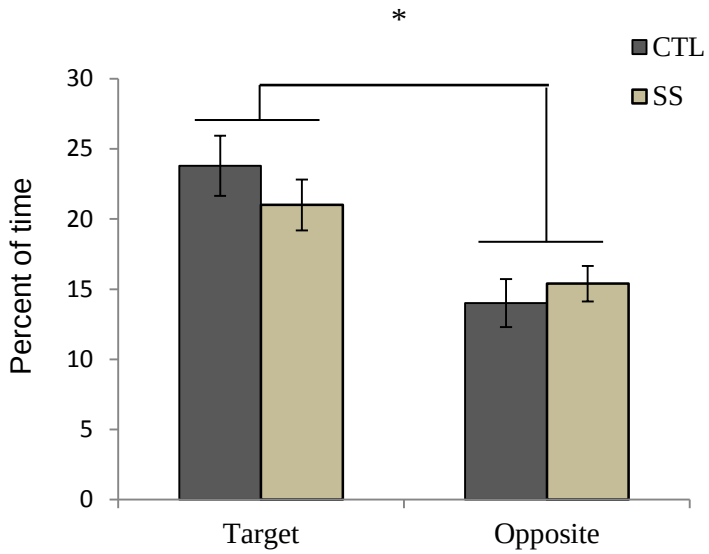

*Figure 5. Average percent of time spent in the zone that previously contained the hidden escape platform (Target) compared to an equally sized zone on the opposite side of the arena. Error bars represent standard error of the means. There were no group differences in the amount of time spent in either zone. All rats demonstrated a spatial bias for the target zone compared to the opposite zone, * p < .001.*

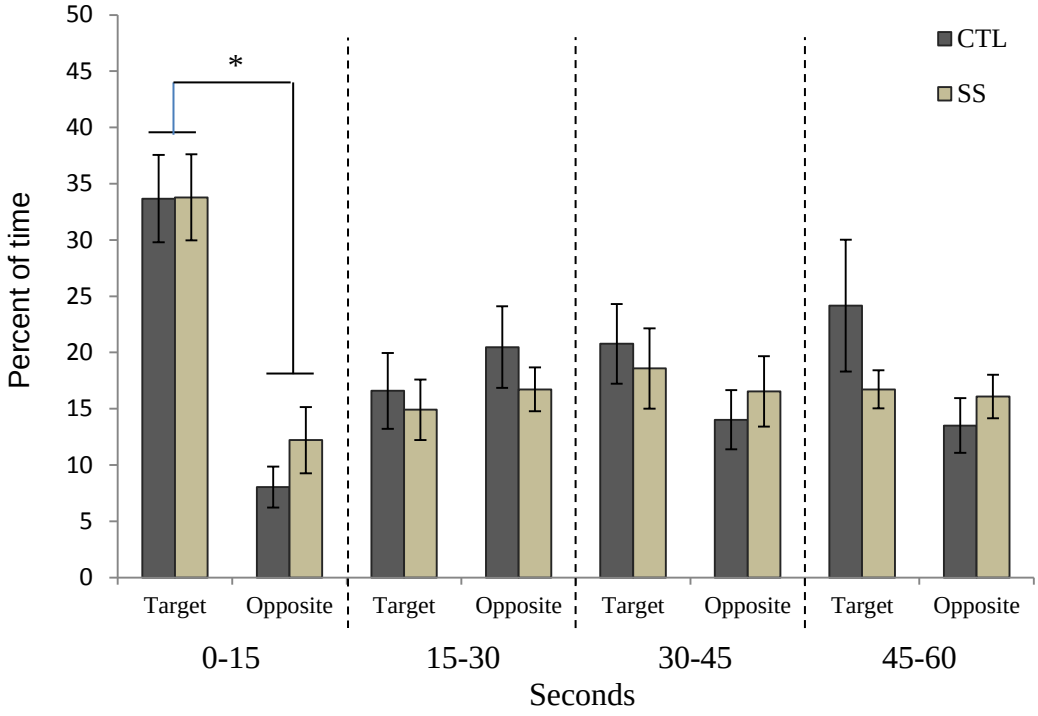

*Figure 6. Average percent of time spent in the zone that previously contained the escape platform (Target) or an equally sized zone at the opposite side of the arena during 15 second intervals of the Probe trial. Error bars represent standard error of the means. There were no group differences during any of the intervals, but all rats showed a clear spatial bias during the first 15 seconds only, * $p < .001$.*

Reversal Learning

Short-term Memory. A mixed factor [Group (SS,CTL) X Trial (First,Last) X Session (1,2,)] ANOVA on distance travelled to the platform revealed a significant main effect of Trial ($F_{1,30} = 37.39$, $p < .001$) and of Session ($F_{1,30} = 27.15$, $p < .001$) as well as an interaction of Trial and Session ($F_{1,30} = 24.88$, $p < .001$), but no effect of, or interaction involving, Group ($ps > .05$) (see Figure 7).

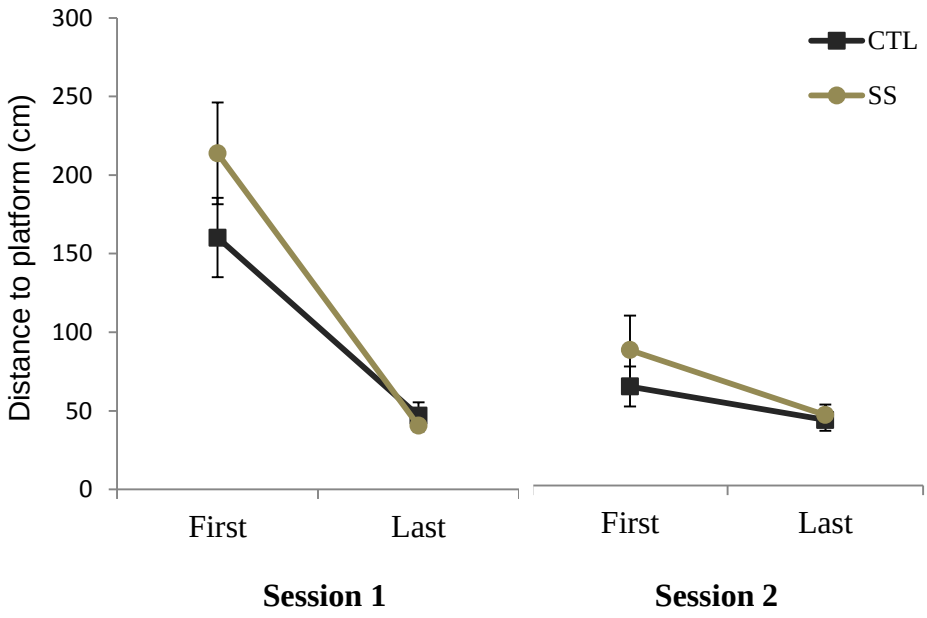

Figure 7. Average distance travelled before reaching the hidden escape platform during two sessions of reversal learning sessions. Error bars represent standard error of the means. There was no significant main effect of group or interactions involving group.

Long-term Memory. To determine whether there were Group differences in long-term memory for the platform's new location a mixed factor [Group (SS,CTL) X Trial (Last,First)] on distance travelled to the platform. The results of the test revealed a significant effect of Trial ($F_{1,30} = 5.30$, $p = .028$), but no effect of Group or interaction of Trial and Group ($ps > .05$). Nevertheless, examination of the groups separately indicated that CTL rats performance did not differ from the last trial of session 1 to the first trial of session 2 ($t_{15} = 1.05$, $p = .31$), whereas SS rats showed somewhat worse performance on the first trial of session 2 compared to the last trial of session 1 ($t_{15} = 2.08$, $p = .06$), consistent with the decrement in performance seen after the first session of acquisition learning, although it failed to reach statistical significance (see Figure 8).

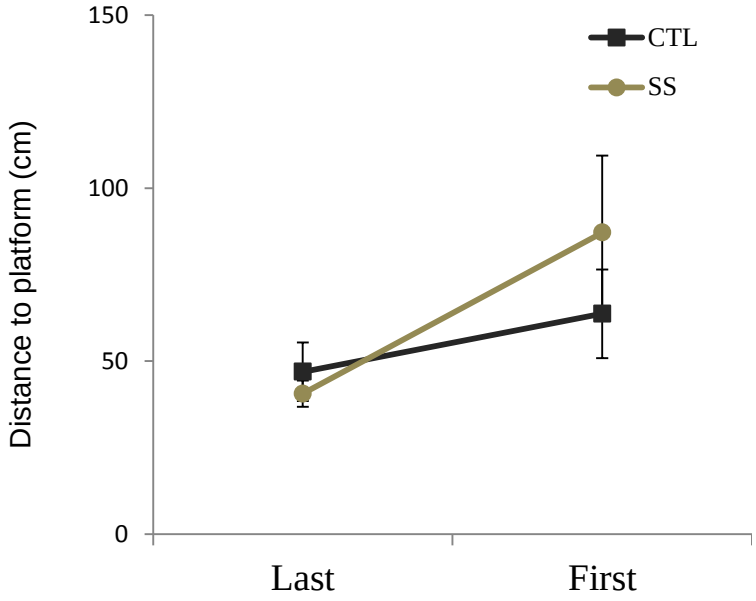

Figure 8. Average distance travelled to the hidden escape platform on the last trial of session 1 and the first trial of session 2 during reversal learning. Error bars represeent standard error of the means. SS rats showed a nonsignificant trend toward impaired performance at the beginning of session 2 compared to the last trial of session 1.

Spatial Object Location Test

Familiarization Phase. A mixed factor [Group (SS,CTL) X Object (1,2) X Delay (15,60,240 minutes)] on time (seconds) spent interacting with objects revealed a significant effect of Delay ($F_{2,60} = 12.52$, $p < .001$). As expected, no significant effect of Group, Object or interaction between the variables was observed ($ps > .05$), which indicates that there were no differences between groups in time spent interacting with the objects or in preference for one object over the other. Post hoc comparisons of the main effect of Delay revealed that time spent interacting with both objects was highest in the first test session (Delay: 60 minutes), which did not differ from the second test session (Delay: 15 minutes; p = .13), although time interacting during both test sessions 1 and 2

was greater than test session 3 (Delay: 240 minutes; p < .001 and p = .02, respectively). Thus, rats appeared to lose interest in investigating objects with repeated testing (see Figure 9).

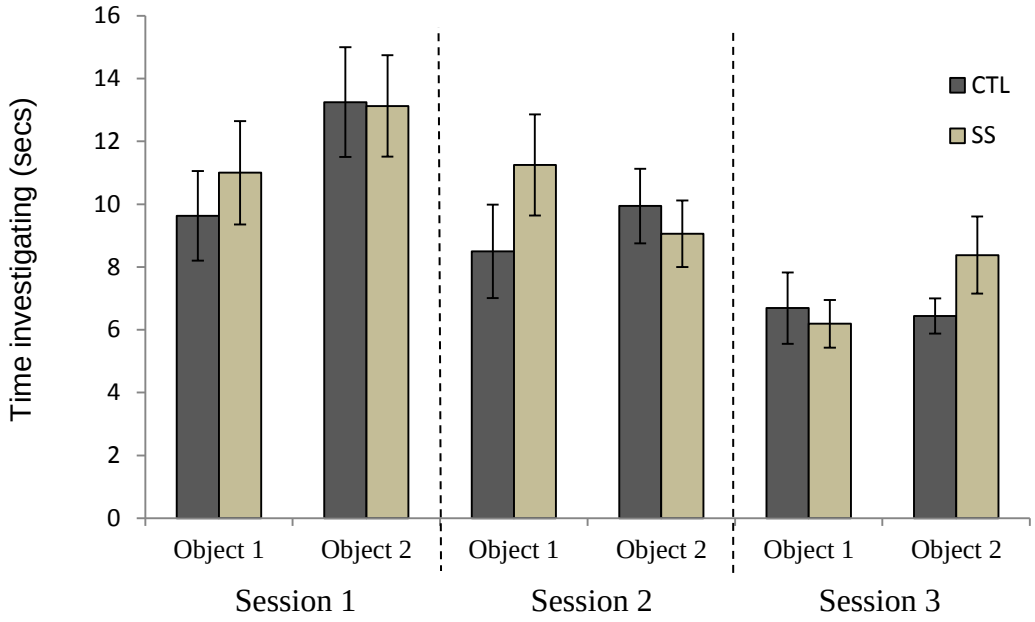

Figure 9. Average time spent with objects during the familiarization phase of three sessions of Spatial Object Location testing. Error bars represent the standard error of the means. There were no group differences in time spent investigating the objects.

Test Phase. A mixed factor [Group (SS,CTL) X Object (Novel,Familiar) X Delay (15,60,240 minutes)] ANOVA on time spent interacting with objects revealed a significant main effect of Object ($F_{1,28} = 22.18$, $p < .001$) and of Delay ($F_{2,56} = 22.37$, $p < .001$), but no main effect or interactions with Group (ps > .05). Follow-up analyses demonstrated that SS and CTL rats spent more time with the object in the novel location compared to the object in the familiar location ($t_{93} = 5.30$, $p < .001$) collapsed across delays. Additionally, similar to the familiarization phase, SS and CTL rats spent significantly more time investigating the objects during the first test session (Delay: 60 minutes) compared to the second test session (Delay: 15 minutes; $p = .007$) and the

second test session compared to the third test session (Delay: 240 minutes; $p < .001$). Despite no significant effect of group, a paired samples t-test was conducted comparing time spent with each object during the 240 minute delay, where group differences were hypothesized, separately for SS and CTL rats. The results indicated that CTL rats spent significantly more time with the object in the novel location compared to the object in the familiar location ($t_{15} = 4.46$, $p < .001$), whereas SS rats did not demonstrate a preference for either object ($t_{15} = 1.35$, $p = .20$). Thus, an overall preference for the object in the novel location was observed for both groups, however, SS rats showed no preference for the object in the novel location after the longest (240 minute) delay (see Figure 10).

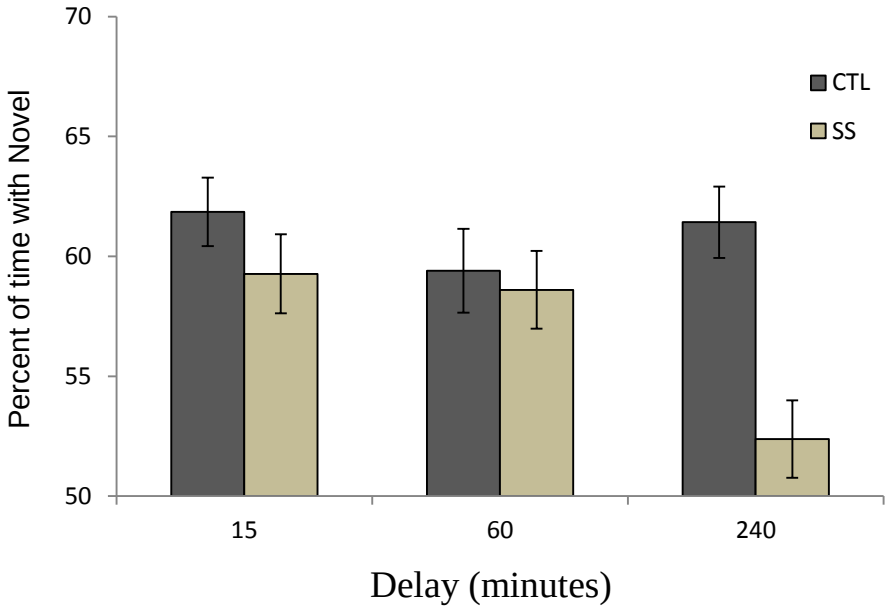

Figure 10. Percentage of time investigating objects that was spent with the object in the novel location during the test phase of the Spatial Object Location test. Error bars represent standard error of the means. Post hoc analyses revealed that CTL rats spent significantly more time with the object in the novel location compared to the object in the familiar location after a 240 minute interval, whereas SS rats did not demonstrate a preference.

Delayed Alternation Task

Training. An independent samples t-test on the number of trials required to reach criterion for task learning revealed no differences between SS and CTL rats (t_{27} = 0.02, p = .98). Furthermore, there was no effect of batch on the number of trials required to reach criterion for task learning (t_{27} = 1.60, p = .12) (see Figure 11).

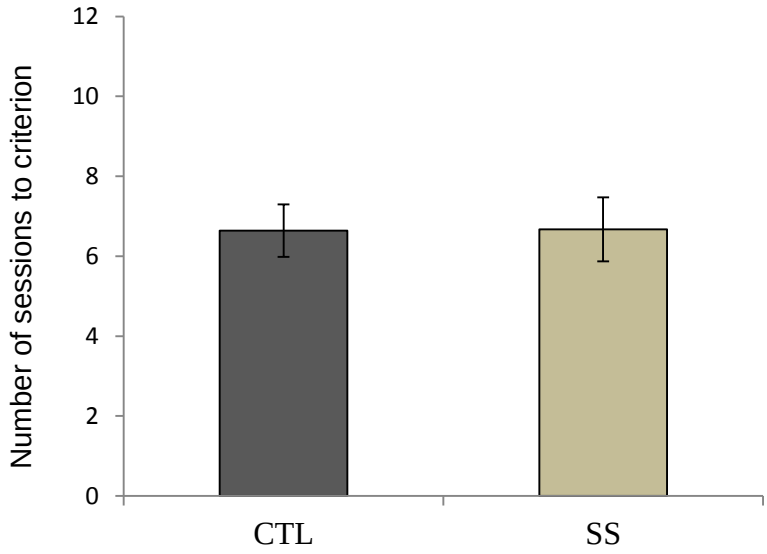

Figure 11. Average number of training sessions required for CTL and SS rats to reach criterion for testing. Error bars represent standard error of the means.

Testing. A mixed factor [Group (SS,CTL) X Delay (5,10,15,30,60,90)] ANOVA on the number of correct alternations revealed a significant main effect of Delay ($F_{5,135}$ = 29.10, p < .001), but not of Group ($F_{1,27}$ = 0.01, p = .987) nor an interaction of Group and Delay ($F_{5,135}$ = 0.16, p = .98). Post hoc comparisons revealed that fewer correct alternations were made for the longer delays (60 and 90 seconds) compared to all other delays (ps ≤ . 001) and that performance on the 10 second delay was better than performance on the 15 and 30 second delay (ps = .007 and .032, respectively). Thus,

overall SS and CTL rats' performance generally decreased as delay length increased, although no group differences were observed (see Figure 12).

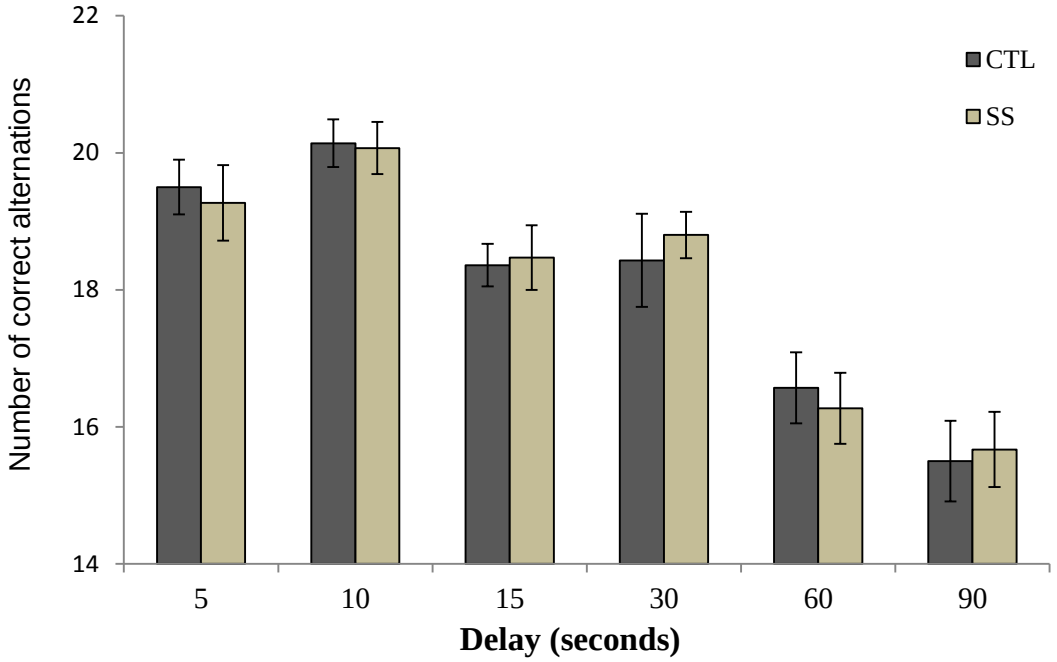

Figure 12. Average number of correct alternations (out of 22) for each delay interval. Error bars represent the standard error of the means. There were no significant group differences.

Discussion

Social instability stress (SS) in adolescence (PND 30-45) produced a modest impairment in long-term, but not short-term or working, spatial memory when tested in adulthood. SS rats' ability to locate the escape platform in the Morris water maze decreased from the first to the second session (48 hrs apart) of acquisition learning and showed a similar trend in decline between the first two sessions of reversal learning (48 hrs apart), whereas control rats' performance did not change between sessions. Furthermore, on a Spatial Object Location test, SS rats were able to discriminate between an object in a novel location and an object in a familiar location after short-term delays (15 and 60 minutes), but not after a longer delay (240 minutes), whereas control rats showed evidence of spatial memory after both short and long delays. On the Delayed Alternation task, which assesses working memory over brief retention periods (from 5-90 seconds), no group differences were observed. Taken together, these results suggest that exposure to chronic social stressors in adolescence causes an enduring deficit on long-term memory formation and/or retrieval, which persists into adulthood.

In the Morris water maze, all rats' performance improved both within a day and across days for both acquisition learning and reversal learning. During the probe trial, rats in both conditions demonstrated a clear preference for the zone that previously contained the escape platform compared to an equally sized zone at the opposite side of the arena. Thus, rats appeared to be learning a spatial strategy (navigating using the extra-maze visual cues) to locate the escape platform during Morris water maze testing rather than a sequence of behaviours (e.g. swimming in concentric circles). As mentioned above, although SS rats did not retain the acquired performance from the first to the second

session of acquisition learning, they performed similarly to controls and showed no decline in performance between days 2 and 3, and on the probe trial. Previous studies have shown that extensive training on hippocampal-dependent tasks can attenuate the effect of various experimental manipulations on long-term memory formation, which may have reduced the effects of SS. For example, mice with long-term spatial memory impairment in the Morris water maze and in the Barnes maze caused by knocking out adenylyl cyclases 1 and 8[1] or overexpression of hippocampal calcineurin[2], respectively, demonstrated partial recovery from their deficits when overtrained (Mansuy, Mayford, Jacob, Kandel, & Bach, 1998; Zhang, Storm, & Wang, 2011). Similarly, cyclic adenosine monophosphate response element binding (CREB)[3] deficient mice with severely impaired long-term, but intact short-term, memory for place learning and contextual fear were less impaired after undergoing extensive training (Bourtchuladze et al. 1994). In contrast, mice with long-term spatial memory impairment caused by reduced protein kinase A activity demonstrated prolonged deficits in the Morris water maze under training protocols with fewer trials (2 trials per day) (Abel et al., 1997). Thus, long-term memory deficits are typically observed when the acquisition phase for learning of the relevant information is brief. Therefore, the fact that in the present study rats stressed during adolescence showed long-term spatial memory impairment between days 1 and 2 of acquisition learning, but not between days 2 and 3 or on the probe trial may be related

---

[1] Adenylyl cyclases 1 and 8 play an important role in coupling activity-dependent cellular calcium influxes with cyclic adenosine monophosphate (cAMP) increases and are crucial for long-term potentiation and long-term memory formation.

[2] Calcineurin is protein phosphatase, which can interfere with the intermediate phase of long-term potentiation and long-term memory formation if overexpressed.

[3] CREB is a family of transcription factors that activate gene transcription in response to increases in cAMP and intracellular calcium. Interfering with CREB impairs long-term potentiation and long-term memory formation.

to overtraining. Indeed, when the location of the platform was moved to assess reversal learning adolescently stressed rats showed a similar trend toward impaired long-term spatial memory the next day, although the difference did not reach statistical significance.

Although testing in the Morris water maze can increase concentrations of glucocorticoids, which are known to affect learning processes (reviewed in McCormick & Mathews, 2010), the long-term spatial memory deficits observed in adolescently stressed rats were not likely caused by differential reactivity of the HPA axis during testing. In the present study, the temperature of the test room and water were maintained at 25 degrees Celsius, which has been found to minimize increases in corticosterone as well as its effect on learning processes (Sandi, Loscertales, & Guaza, 1997). Furthermore, previous studies have found that social instability stress in adolescence does not affect the corticosterone response to acute stressors (swim stress and restraint stress) in adulthood (Mathews, Wilton et al., 2008; McCormick, Robarts, Kopeikina, & Kelsey, 2005).

SS rats did not discriminate between novel and familiar locations of objects after a 4 hour delay in the Spatial Object Location test, which also suggests reduced long-term spatial memory. These findings also counter the possibility that the differences in performance between SS and CTL rats in the Morris water maze are because of the stressful nature of the task; the Spatial Object Location test relies on inherent preferences of the rats to explore novel versus familiar rather than aversive properties to motivate performance. Further, the difference in SS appears to be related to memory rather than encoding, because SS and CTL did not differ in time spent investigating the objects during the familiarity phase. The spatial memory deficit of SS rats after 4 hours, but not 15 or 60 minutes, is consistent with past research that shows process-specific (long-term

memory) impairment. For example, mice with impaired long-term, but intact short-term, memory perform comparable to controls on memory for conditioned fear 30 and 60 minutes after training, but not after delays of two hours or longer (Bourtchuladze et al., 1994). Similarly, object memory was intact 30 minute after a familiarization phase, but showed evidence of decline after inter-trial intervals of 2 hours or longer in mice overexpressing hippocampal calcineurin (Mansuy et al., 1998). Therefore, the effects of social instability stress on spatial learning and memory appear to be related specifically to long-term memory and are not likely mediated by altered HPA reactivity in adulthood.

I had not hypothesized, however, that the deficits after SS would be limited to long-term memory. Rather than showing deficits in learning and memory involving brief periods of retention, the data provide evidence of enhanced short-term spatial learning and memory in SS rats during Morris water maze testing, as the distance travelled to the hidden escape platform decreased between the first and last trials more so for SS rats than for CTL rats. On the Spatial Object Location test, there were no group differences in the percent of time spent with the object in the novel location compared to the object in the familiar location after 15 minutes (CTL = 61.9%, SS = 59.3%) or 60 minutes (CTL = 59.4%, SS = 58.6%). Moreover, there were no group differences on the Delayed Alternation task, which assessed working memory across brief delays ranging from 5 to 90 seconds, and this can be interpreted in several ways. The findings may suggest that the hippocampus is more susceptible to the effects of social instability stress in adolescence than is the medial prefrontal cortex. Nevertheless, the idea that one type of performance is related to one brain region is simplistic; although there are regional differences in the involvement with spatial and working memory, both the hippocampus and medial

prefrontal cortex are part of a complex network that is involved in learning and memory (Chiba, Kesner, & Reynolds, 1994). Thus, the reduced performance of SS rats compared to CTL rats might be process-specific (long-term memory) rather than brain region specific. Additionally, the reduced performance of SS in some tasks may reflect task difficulty. For example in the DAT task, all rats performed well above chance after 5, 10, 15, 30, 60, and 90 second delays (88%, 91%, 84%, 85%, 75%, and 71% accuracy, respectively). Indeed, past research has shown that the effects of experimental manipulations on rats' learning and memory can be evident on difficult tasks and not easier versions (Messing & Sparber, 1985).

Despite a possible attenuating influence of overtraining, the effect of social instability stress during adolescence on learning and memory function was modest and process specific (related to long-term, not short-term, memory). The modest effects on learning and memory performance may reflect the mild nature of the stress procedure and the long recovery period between stress exposure and time of testing. Previous research investigating the impact of chronic stress in adulthood on tests of cognitive function and various neural correlates typically involves 6 hours of restraint stress daily for 21 days, the effects of which tend to diminish after several weeks (e.g. Conrad et al., 1996, 1999, 2003; Goldwater et al., 2009; Luine et al., 1994). Conversely, the stress procedure in the present study involved 1 hour of isolation and a subsequent change of cage partner for 16 days and, despite its` milder intensity, caused significant impairment more than five weeks later on two indices of long-term spatial memory. Thus, the present study provides further evidence that the developing hippocampus is particularly vulnerable to environmental influences during adolescence. In contrast, performance on the Delayed

Alternation task, which relies more heavily on the medial prefrontal cortex, was not affected by adolescent social instability stress and may show enduring dysfunction only after more severe stress procedures or on different tasks (such as attentional set-shifting).

These results of the present study, that social instability stress in adolescence causes long-term spatial memory deficits are partially in line with previous research that investigated memory function after exposure to chronic stress in adolescence. For example, male rats subjected to the same adolescent stress procedure had impaired contextual and cued fear memory 24 hours after training when tested 4 weeks after the last stress exposure, indicative of long-term memory impairment (Morrissey et al., 2011). Another study found that SS rats demonstrated impaired memory for object location 4 hours after training when tested in adulthood (McCormick et al., 2012). SS rats in the same study, however, did not show a preference for the object in the novel location compared to the object in the familiar location after 15 or 60 minute delays, whereas control rats did, suggesting that spatial deficits are not limited to long-term memory. Taken together, past research shows that social instability stress in adolescence has deleterious effect on multiple learning and memory processes in adulthood, whereas the results of the present study suggest that deficits may be more specific.

Other adolescent chronic stress procedures have been investigated in relation to learning and memory function in adulthood. For example, male rats exposed to variable physical stress (forced swim, restraint, loud noise, ether, and cold), but not variable social stress (isolation, novel environment, litter-shifting, crowding, and subordination), between 28-56 days of age demonstrated impaired acquisition learning and probe trial performance in the Morris water maze (Isgor et al., 2004). Whether the impaired

acquisition learning reflected short-term or long-term memory deficits is unclear (trials were averaged for each session), but as stressed rats performed slightly better on the first session and worse on subsequent sessions compared to controls the impairment seems to be at least in part related to long-term memory. Similarly, male rats subjected to repeated stress (elevated platform stress) during early adolescence (PND 26-28) had impaired Morris water maze performance when tested in young adulthood (beginning PND 60) (Avital & Richter-Levin, 2005). Conversely, acquisition learning in the Morris water maze was unaffected in adult (PND 270) male mice after a mild social stress procedure (change of cage partners every 7 days between 32 and 80 days of age) (Sterlemann et al, 2010). When tested in old age (PND 450), however, adolescently stressed mice demonstrated spatial learning and memory impairment in both the Morris water maze and a Y-maze, which suggests that the effects of adolescent stress may be more readily detectable during cognitive decline in old age. Conversely, adult long-term memory for a conditioned stimulus was not impaired in rats that underwent three days of predator scent and elevated platform stress during early-adolescence, although stressed males did show impairment on fear extinction compared to controls, which is indicative of medial prefrontal cortex dysfunction (Toledo-Rodriguez & Sandi, 2007). Taken together, adolescent stress procedures tend to negatively affect learning and memory function in adulthood, but the effect often depends on the duration and intensity of the stressor, the age of stress exposure, the age at testing, and the type of tests used.

The effects of chronic stress are long-lasting when the stress occurs in adolescence, but not if it occurs in adulthood, which suggests that adolescence is a particularly vulnerable period of ontogeny (reviewed in Green & McCormick, in press).

Few studies to date, however, have provided a direct comparison of adolescent and adult chronic stress on measures of learning and memory. Morrissey and colleagues (2011) found that social instability stress in adolescence altered long-term memory for conditioned cue and contextual fear in adulthood, whereas the same stress procedure administered in adulthood had no effect when male rats were tested weeks later. Additionally, three days of elevated platform stress in adolescence, but not in adulthood, impaired Morris water maze performance 4 weeks later (Avital & Richter-Levin, 2005). Although future research is needed to directly compare the effects of chronic stress in adolescence and adulthood, the evidence to date suggests that adolescence is a vulnerable period of ontogeny during which repeated stress alters future learning and memory function.

Social instability stress in adolescence may be altering adult long-term spatial memory in a number of ways. Various correlates of long-term memory formation have been identified, but researchers generally agree that one mechanism underlying learning and memory is long-term potentiation (reviewed in Lynch, 2004). Long-term potentiation is most readily detectible in the hippocampus and can be broken down into two main phases; an early phase (lasting up to 2-3 hours) and a late phase (lasting from hours to days), which serve short-term and long-term memory, respectively. Whereas short-term memory and the early phase of long-term potentiation involve modification of existing proteins, long-term memory and late phase long-term potentiation involves altered gene transcription and de novo protein synthesis (reviewed in Bliss & Collingridge, 1993). Both forms of long-term potentiation are induced by activity-dependent activation of N-Methyl-D-aspartate (NMDA) receptors, causing an influx of calcium in the postsynaptic

cell and, if sufficiently depolarized, the release of a magnesium molecule from its receptor, which is presumed to act as a coincidence detector. An influx of calcium triggers the extracellular signal-regulated kinase (ERK) pathway, which culminates in the translocation of ERK to the nucleus of the cell where it influences gene transcription via cyclic adenosine monophosphate response element binding (CREB) and various immediate early genes. Previous studies have provided evidence that various correlates of long-term memory formation are altered in adulthood in rats that were exposed to chronic stress in adolescence. For example, Maggio and Segal (2011) found that adult male rats that were repeatedly stressed in adolescence had altered long-term potentiation and long-term depression in the hippocampus. Additionally, expression of glutamate and GABA receptors (Sterlemann et al., 2010), markers of neurogenesis (McCormick et al., 2012; Sterlemann et al., 2010), and neural cell adhesion molecules (Tsoory et al., 2008) is altered in adulthood after exposure to chronic stress in adolescence. Future studies should examine the effects of social instability stress on other behavioural measures of long-term memory and investigate a possible mediating role of various proteins involved in the signalling pathways that regulate transcription factors. For example, it may be worthwhile to examine adult long-term spatial memory using a less intensive training protocol (1 or 2 trials per session) as well as the expression of CREB or other upstream proteins known to be involved in long-term memory. Additionally, future studies should include an adult comparison group to investigate whether the effects are specific to stress in adolescence.

In conclusion, the present study provides evidence that exposure to chronic social instability stress in adolescence impairs male rats' long-term spatial memory in

adulthood. Conversely, rats' performance was not affected on spatial and working memory tasks that involved brief delays, indicating that impairment was process specific and possibly region specific (related to the hippocampus and not the medial prefrontal cortex). Moreover, the present results contribute to a growing body of literature that identifies the hippocampus and related learning and memory functions to be particularly vulnerable to environmental influences during this period of ontogeny.

References

Abel, T., Nguyen, P. V., Barad, M., Deuel, T. A., Kandel, E. R., & Bourtchouladze, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell, 88,* 615-626.

Ainge, J. A., van der Meer, M. A. A., Langston, R. F., & Wood, E. R. (2007). Exploring the role of context-dependent hippocampal activity in spatial alternation behaviour. *Hippocampus, 17,* 988-1002.

Andersen S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioural Reviews, 27,* 3-18.

Andersen, S. L., Thompson, A. T., Rutstein, M., Hostetter, J. C., & Teicher, M. H. (2000). Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse, 37,* 167–169.

Andrzejewski, M. E., Schochet, T. L., Feit, E. C., Harris, R., Mckee, B. L., & Kelley, A. E. (2011). A comparison of adult and adolescent rat behavior in operant learning, extinction, and behavioral inhibition paradigms. *Behavioral Neuroscience, 125,* 93-105.

Avital, A. & Richter-Levin, G. (2005). Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. *International Journal of Neuropsychopharmacology. 8,* 163–173.

Bardo, M. T., Neisewander, J. L., & Pierce, R. C. (1989). Novelty-induced place preference behavior in rats: Effects of opiate and dopaminergic drugs. *Pharmacology Biochemistry and Behavior, 32,* 683-689.

Barsegyan, A., Mackenzie, S. M., Kurose, B. D., McGaugh, J. L., & Roozendaal, B. (2010). Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. *Proceedings of the National Academy of Sciences, 107,* 16655-16660.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361,* 31-39.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., & Silva, A. J. (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell, 79,* 59-68.

Brenhouse, H. C., Sonntag, K. C., & Andersen, S. L. (2008). Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: Relationship to enhanced motivational salience of drug cues in adolescence. *The Journal of Neuroscience, 28,* 2375-2382.

Brunson, K. L., Kramar, E., Lin, B., Chen, Y., Colgin, L. L., Yanagihara, T. K., Lynch, G., & Baram, T. (2005). Mechanisms of late-onset cognitive decline after early-life stress. *The Journal of Neuroscience, 25,* 9328-9338.

Bubser, M. & Schmidt, W. J. (1990). 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behavioural Brain Research, 37,* 157-168.

Buchanan, T. W. & Lovallo, W. R. (2001). Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology, 26,* 307-317.

Butts, K. A., Weinberg, J., Young, A. H., & Phillips, A. G. (2011). Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *Proceedings of the National Academy of Sciences, 108,* 18459-18464.

Chiba, A. A., Kesner, R. P., & Reynolds, A. M. (1994). Memory for spatial location as a function of temporal lag in rats: Role of hippocampus and medial prefrontal cortex. *Behavioral and Neural Biology, 61,* 123-131.

Conrad, C. D., Galea, L. A. M., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze and this effect is blocked by tianeptine pretreatment. *Behavioural Neuroscience, 110,* 1321–1334.

Conrad, C. D., Grote, K. A., Hobbs, R. J., & Ferayorni, A. (2003). Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiology of Learning and Memory, 79,* 32-40.

Conrad, C. D., Magariños, A. M., LeDoux, J. E., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioural Neuroscience, 113,* 902–913.

Cook, S. C. & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of Neurobiology, 60,* 236-248.

Cunningham, M. G., Bhattacharyya, S., & Benes, F. M. (2002). Amygdalo-cortical sprouting continues into early adulthood: Implications for the development of normal and abnormal function during adolescence. *The Journal of Comparative Neurology, 453,* 116-130.

de Quervain, D. J. F., Roozendaal, B., & McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature, 394,* 787-790.

D'Hooge, R. & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews, 36,* 60-90.

Diamond, D. M., Campbell, A. M., Park, C. R., Woodson, J. C., Conrad, C. D., Bachstetter, A. D., & Mervis, R. F. (2006). Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. *Hippocampus, 16,* 571-576.

Dias, R. & Aggleton, J. P. (2008). Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching-and matching-to-place in the T-maze in the rat: Differential involvement of the prelimbic–infralimbic and anterior cingulate cortices in providing behavioural flexibility. *European Journal of Neuroscience, 12,* 4457-4466.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2009). Social and non-social anxiety in adolescent and adult rats after repeated restraint. *Physiology and Behavior, 97,* 484-494.

Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2004). Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate
housing of subjects and partners. *Developmental Psychobiology, 45,* 153–162.

Dreher, J. C., Guigon, E., & Burnod, Y. (2002). A model of prefrontal cortex dopaminergic modulation during the delayed alternation task. *Journal of Cognitive Neuroscience, 14,* 853-865.

Dunnett, S. B., Nathwani, F., & Brasted, P. J. (1999). Medial prefrontal and neostriatal lesions disrupt performance in an operant delayed alternation task in rats. *Behavioural Brain Research, 106,* 13-28.

Eichenbaum, H. (2001). The hippocampus and declarative memory: Cognitive mechanisms and neural codes. *Behavioural Brain Research, 127,* 199-207.

Engelmann, M., Ebner, K., Landgraf, R., & Wotjak, C. T. (2006). Effects of Morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. *Hormones and Behavior, 50,* 496-501.

Ennaceur, A. & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research, 31,* 47-59.

Ennaceur, A. & Meliani, K. (1992). A new one-trial test for neurobiological studies of memory in rats. III: Spatial vs. non-spatial working memory. *Behavioural Brain Research, 51,* 83-92.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, *113*, 509-519.

Foilb, A. R., Lui, P., & Romeo, R. D. (2011). The transformation of hormonal stress responses throughout puberty and adolescence. *Journal of Endocrinology*, *210*, 391-398.

Freeman, J. H. & Stanton, M. E. (1992). Medial prefrontal cortex lesions and spatial delayed alternation in the developing rat: Recovery or sparing? *Behavioural Neuroscience*, *106*, 924–932.

Goldwater, D. S., Pavlides, C., Hunter, R. G., Bloss, E. B., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2009). Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience*, *164*, 798-808.

Granon, S., Vidal, C., Thinus-Blanc, C., Changeux, J. P., & Poucet, B. (1994). Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. *Behavioral Neuroscience*, *108*, 883-891.

Green, M. R., Barnes, B., & McCormick, C. M. (in press). Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long–Evans rats. *Developmental Psychobiology*.

Green, M. R. & McCormick, C. M. (in press). Effects of stressors in adolescence on learning and memory in rodent models. *Hormones and Behaviour.*

Hains, A. B., Vu, M. A. T., Maciejewski, P. K., Van Dyck, C. H., Gottron, M., & Arnsten, A. F. (2009). Inhibition of protein kinase C signaling protects prefrontal cortex dendritic spines and cognition from the effects of chronic stress. *Proceedings of the National Academy of Sciences*, *106*, 17957-17962.

Han, X., Li, N., Xue, X., Shao, F., & Wang, W. (2011). Brief social isolation in early adolescence affects reversal learning and forebrain BDNF expression in adult rats. *Brain Research Bulletin, 86,* 173–178.

Han, X., Li, N., Xue, X., Shao, F., Wang, W. (2012). Early social isolation disrupts latent inhibition and increases dopamine D2 receptor expression in the medial prefrontal cortex and nucleus accumbens of adult rats. *Brain Research, 1447,* 38–43.

He, J. & Crews, F. T. (2007). Neurogenesis decreases during brain maturation from adolescence to adulthood. *Pharmacology Biochemistry Behaviour*, *86,* 327–33.

Heine, V. M., Maslam, S., Zareno, J., Joels, M., & Lucassen, P. J. (2004). Suppressed proliferation and apoptotic changes in the rat dentate gyrus after acute and chronic stress are reversible. *European Journal of Neuroscience, 19,* 131–144.

Herman, J. P. & Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in Neurosciences, 20,* 78-84.

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Progress in Neuropsychopharmacology and Biological Psychiatry, 29,* 1201–1213.

Hernandez-Gonzalez, M. (2000). Prepubertal genital grooming and penile erections in relation to sexual behavior of rats. *Physiology and Behaviour, 71,* 51–56.

Hock, B. J., & Bunsey, M. D. (1998). Differential effects of dorsal and ventral hippocampal lesions. *The Journal of Neuroscience*, *18,* 7027-7032.

Hoffman, A. N., Krigbaum, A., Ortiz, J. B., Mika, A., Hutchinson, K. M., Bimonte-Nelson, H. A., & Conrad, C. D. (2011). Recovery after chronic stress within spatial reference and working memory domains: correspondence with hippocampal morphology. *European Journal of Neuroscience, 34,* 1023-1030.

Huang, Y., Shi, X., Xu, H., Yang, H., Chen, T., Chen, S., & Chen, X. (2010). Chronic unpredictable stress before pregnancy reduce the expression of brain-derived neurotrophic factor and N-methyl-D-aspartate receptor in hippocampus of offspring rats associated with impairment of memory. *Neurochemical Research, 35,* 1038-1049.

Isgor, C., Kabbaj, M., Akil, H., & Watson, S. J. (2004). Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus, 14,* 636–648.

Ito, W., Pan, B. X., Yang, C., Thakur, S., & Morozov, A. (2009). Enhanced generalization of auditory conditioned fear in juvenile mice. *Learning and Memory, 16,* 187–192.

Jarrard, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. *Behavioral & Neural Biology, 60,* 9-26

Jarrard, L. E. & Davidson, T. L. (1990). Acquisition of concurrent conditional discriminations in rats with ibotenate lesions of hippocampus and of subiculum. *Psychobiology, 18,* 68-73.

Joëls, M. (2006). Corticosteroid effects in the brain: U-shape it. *Trends in Pharmacological Sciences, 27,* 244-250.

Juraska, J. M., & Markham, J. A. (2004). The cellular basis for volume changes in the rat cortex during puberty: White and gray matter. *Annals of the New York Academy of Sciences, 1021,* 431–435.

Kalsbeek, A., Voorn, P., Buijs, R. M., Pool, C. W., & Uylings, H. B. M. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *The Journal of Comparative Neurology, 269,* 58-72.

Kinoshita, S., Yokoyama, C., Masaki, D., Yamashita, T., Tsuchida, H., Nakatomi, Y., & Fukui, K. (2008). Effects of rat medial prefrontal cortex lesions on olfactory serial reversal and delayed alternation tasks. *Neuroscience Research, 60,* 213-218.

Klug, M., Hill, R. A., Choy, K. H. C., Kyrios, M., Hannan, A. J., & van den Buuse, M. (2012). Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice. *Neurobiology of Disease, 46,* 722-731.

Kojima, S., Kojima, M., & Goldman-Rakic, P. S. (1982). Operant behavioral analysis of memory loss in monkeys with prefrontal lesions. *Brain research, 248,* 51-59.

Koss, W. A., Franklin, A. D., & Juraska, J. M. (2011). Delayed alternation in adolescent and adult male and female rats. *Developmental Psychobiology, 53,* 724–731.

Kroener, S., Chandler, L. J., Phillips, P. E., & Seamans, J. K. (2009). Dopamine modulates persistent synaptic activity and enhances the signal-to-noise ratio in the prefrontal cortex. *PLoS One, 4,* e6507.

Lacroix, L., White, I., & Feldon, J. (2002). Effect of excitotoxic lesions of rat medial prefrontal cortex on spatial memory. *Behavioural Brain Research.* 133, 69–81.

Lapiz-Bluhm, M. D. S., Soto-Piña, A. E., Hensler, J. G., & Morilak, D. A. (2009). Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats. *Psychopharmacology, 202,* 329-341.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences, 97,* 11032-11037.

Lipska, B. K., Aultman, J. M., Verma, A., Weinberger, D. R., & Moghaddam, B. (2002). Neonatal damage of the ventral hippocampus impairs working memory in the rat. *Neuropsychopharmacology, 27,* 47-54.

Liston, C., Miller, M. M., Goldwater, D. S., Radley, J. J., Rocher, A. B., Hof, P. R., Morrison, J. H., & McEwen, B. S. (2006). Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *The Journal of Neuroscience, 26*, 7870-7874.

Lui, C. C., Wang, J. Y., Tain, Y. L., Chen, Y. C., Chang, K. A., Lai, M. C., & Huang, L. T. (2011). Prenatal stress in rat causes long-term spatial memory deficit and hippocampus MRI abnormality: Differential effects of postweaning enriched environment. *Neurochemistry International*, *58*, 434-441.

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research, 639*, 167-170.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*, 434-445.

Lynch, M. A. (2004). Long-term potentiation and memory. *Physiological Reviews*, *84*, 87-136.

Magariños, A. M., Deslandes, A., & McEwen, B. S. (1999). Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. *European Journal of Pharmacology, 371*, 113-122.

Maggio, N., & Segal, M. (2011). Persistent changes in ability to express long-term potentiation/depression in the rat hippocampus after juvenile/adult stress. *Biological Psychiatry*, *69*, 748-753.

Mansuy, I. M., Mayford, M., Jacob, B., Kandel, E. R., & Bach, M. E. (1998). Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. *Cell*, *92*, 39-49.

Markham, J. A., Morris, J. R., & Juraska, J. M. (2007). Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. *Neuroscience, 144*, 961-968.

Maruki, K., Izaki, Y., Hori, K., Nomura, M., & Yamauchi, T. (2001). Effects of rat ventral and dorsal hippocampus temporal inactivation on delayed alternation task. *Brain Research*, *895*, 273-276.

Mathews, I. Z., Mills, R. G., & McCormick, C. M. (2008). Chronic social stress in adolescence influenced both amphetamine conditioned place preference and locomotor sensitization. *Developmental Psychobiology*, *50*, 451-459.

Mathews, I. Z., Wilton, A., Styles, A., & McCormick, C. M. (2008). Increased depressive behaviour in females and heightened corticosterone release in males to swim stress after adolescent social stress in rats. *Behavioural Brain Research, 190,* 33-40.

McCormick, C. M. (2010). An animal model of social instability stress in adolescence and risk for drugs of abuse. *Physiology & Behavior, 99,* 194-203.

McCormick, C. M., & Mathews, I. Z. (2010). Adolescent development, hypothalamic-pituitary-adrenal function, and programming of adult learning and memory. *Progress in Neuro-psychopharmacology and Biological Psychiatry, 34,* 756-765.

McCormick, C. M., Mathews, I. Z., Thomas, C., & Waters, P. (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain and Cognition, 72,* 73-85.

McCormick, C. M., Merrick, A., Secen, J., & Helmreich, D. L. (2007). Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. *Journal of Neuroendocrinology, 19,* 116-126.

McCormick, C. M., Nixon, F., Thomas, C., Lowie, B., & Dyck, J. (2010). Hippocampal cell proliferation and spatial memory performance after social instability stress in adolescence in female rats. *Behavioural Brain Research, 208,* 23-29.

McCormick, C. M., Robarts, D., Kopeikina, K., & Kelsey, J. E. (2005). Long-lasting, sex-and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Hormones and Behavior, 48,* 64-74.

McCormick, C. M., Smith, C., & Mathews, I. Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behavioural Brain Research, 187,* 228-238.

McCormick, C. M., Thomas, C. M., Sheridan, C. S., Nixon, F., Flynn, J. A., & Mathews, I. Z. (2012). Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. *Hippocampus, 22,* 1300-1312.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences, 840,* 33-44.

McEwen, B. S. (2006). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences, 840,* 33-44.

Meaney, M. J. & Stewart, J. (1981). A descriptive study of social development in the rat (Rattus norvegicus). *Animal Behaviour, 29,* 34-45.

Messing, R. B., & Sparber, S. B. (1985). Greater task difficulty amplifies the facilitatory effect of des-glycinamide arginine vasopressin on appetitively motivated learning. *Behavioral Neuroscience, 99*, 1114.

Mika, A., Mazur, G. J., Hoffman, A. N., Talboom, J. S., Bimonte-Nelson, H. A., Sanabria, F., & Conrad, C. D. (2012). Chronic stress impairs prefrontal cortex-dependent response inhibition and spatial working memory. *Behavioral Neuroscience, 126,* 605-619.

Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature, 420*, 70-74.

Mizoguchi, K., Ishige, A., Takeda, S., Aburada, M., & Tabira, T. (2004). Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. *The Journal of Neuroscience, 24*, 5492-5499.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D. H., & Tabira, T. (2000). Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *The Journal of Neuroscience, 20*, 1568-1574.

Mokler, D. J., Torres, O. I., Galler, J. R., & Morgane, P. J. (2007). Stress-induced changes in extracellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatally malnourished rats. *Brain Research, 1148*, 226-233.

Monroy, E., Hernández-Torres, E., & Flores, G. (2010). Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. *Journal of Chemical Neuroanatomy, 40,* 93-101.

Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neuroscience Letters, 163,* 109-113.

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12*, 239-260.

Morris, R. G. M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 11*, 47-60.

Morris, R. G. M., Schenk, F., Tweedie, F., & Jarrard, L. E. (1990). Ibotenate lesions of hippocampus and/or subiculum: Dissociating components of allocentric spatial learning. *European Journal of Neuroscience, 2,* 1016-1028.

Morrissey, M. D., Mathews, I. Z., & McCormick, C. M. (2011). Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats. *Neurobiology of Learning and Memory, 95,* 46-56.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. *Learning and Memory, 9,* 49-57.

Murphy, B. L., Arnsten, A. F., Jentsch, J. D., & Roth, R. H. (1996). Dopamine and spatial working memory in rats and monkeys: Pharmacological reversal of stress-induced impairment. *The Journal of Neuroscience, 16,* 7768-7775.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from unit activity in the freely-moving rat. *Brain Research. 34,* 171-175.

Okuda, S., Roozendaal, B., & McGaugh, J. L. (2004). Glucocorticoid effects on object recognition memory require training-associated emotional arousal. *Proceedings of the National Academy of Sciences of the United States of America, 101,* 853-858.

Pascual, R. & Zamora-León, S. P. (2007). Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. *Acta Neurobiologiae Experimentalis, 67,* 471-479.

Pellis, S. M., Field, E. F., Smith, L. K., & Pellis, V. C. (1997). Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. *Neuroscience & Biobehavioral Reviews, 21,* 105-120.

Pham, K., Nacher, J., Hof, P. R., & McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *European Journal of Neuroscience, 17,* 879-886.

Pignatelli, D., Xiao, F., Gouveia, A. M., Ferreira, J. G., & Vinson, G. P. (2006). Adrenarche in the rat. *Journal of Endocrinology, 191,* 301-308.

Pugh, C. R., Fleshner, P., & Rudy, J. W. (1997). Type II glucocorticoid receptor antagonists impair contextual but not auditory-cue fear conditioning in juvenile rats. *Neurobiology of Learning and Memory, 67,* 75–79.

Radley, J. J., Rocher, A. B., Janssen, W. G., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2005). Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. *Experimental Neurology, 196,* 199-203.

Radley, J. J., Rocher, A. B., Miller, M., Janssen, W. G., Liston, C., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2006). Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral Cortex, 16,* 313-320.

Radley, J. J., Rocher, A. B., Rodriguez, A., Ehlenberger, D. B., Dammann, M., McEwen, B. S., Morrison, J. H., Wearne, S. L., & Hof, P. R. (2008). Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *The Journal of Comparative Neurology, 507*, 1141-1150.

Reul, J. M. H. M. & De Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology*, *117*, 2505-2511.

Rice, D. & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environmental Health Perspective, 108,* 511–533.

Rossi, M. A., Hayrapetyan, V. Y., Maimon, B., Mak, K., Je, H. S., & Yin, H. H. (2012). Prefrontal cortical mechanisms underlying delayed alternation in mice. *Journal of Neurophysiology*, *108*, 1211-1222.

Roozendaal, B., Griffith, Q. K., Buranday, J., de Quervain, D. J. F., & McGaugh, J. L. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: Dependence on the basolateral amygdala. *Proceedings of the National Academy of Sciences, 100*, 1328-1333.

Roozendaal, B., Portillo-Marquez, G., & McGaugh, J. L. (1996). Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. *Behavioral Neuroscience, 110,* 1074-1083.

Roozendaal, B., Quirarte, G. L., & McGaugh, J. L. (2002). Glucocorticoids interact with the basolateral amygdala β-adrenoceptor–cAMP/cAMP/PKA system in influencing memory consolidation. *European Journal of Neuroscience*, *15*, 553-560.

Salazar, R. F., White, W., Lacroix, L., Feldon, J., & White, I. M. (2004). NMDA lesions in the medial prefrontal cortex impair the ability to inhibit responses during reversal of a simple spatial discrimination. *Behavioural Brain Research*, *152*, 413-424.

Sandi, C., Loscertales, M., & Guaza, C. (1997). Experience-dependent Facilitating Effect of Corticosterone on Spatial Memory Formation in the Water Maze. *European Journal of Neuroscience*, *9*, 637-642.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*, 55-89.

Schenk, F. & Morris, R. G. M. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Experimental Brain Research, 58,* 11-28.

Schochet, T. L., Kelley, A. E., & Landry, C. F. (2005). Differential expression of Arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. *Neuroscience, 135,* 285-297.

Seamans, J. K. & Yang, C. R. (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Progress in Neurobiology, 74,* 1-58.

Selye, H. (1973). The Evolution of the Stress Concept: The originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. *American Scientist, 61,* 692-699.

Sisk, C. L. & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology, 26,* 163-174.

Sloan, H. L., Good, M., & Dunnett, S. B. (2006). Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task and a water maze reference memory task. *Behavioural Brain Research, 171,* 116-126.

Son, G. H., Geum, D., Chung, S., Kim, E. J., Jo, J. H., Kim, C. M., Lee, K. O., Kim, H., Choi, S., Kim, H. T., Lee, K. J., & Kim, K. (2006). Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. *The Journal of Neuroscience, 26,* 3309-3318.

Spreng, M., Rossier, J., & Schenk, F. (2002). Spaced training facilitates long-term retention of place navigation in adult but not in adolescent rats. *Behavioural Brain Research, 128,* 103-108.

Steele, R. J., & Morris, R. G. M. (1999). Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. *Hippocampus, 9,* 118-136.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M. B., & Schmidt, M. V. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. *Hormones and Behavior, 53,* 386-394.

Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Müller, M. B., & Schmidt, M. V. (2010). Chronic social stress during adolescence induces cognitive impairment in aged mice. *Hippocampus, 20,* 540-549.

Stewart, J., Meaney, M. J., Aitken, D., Jensen, L., & Kalant, N. (1988). The effects of acute and life-long food restriction on basal and stress-induced serum corticosterone levels in young and aged rats. *Endocrinology, 123*, 1934-1941.

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D1, D2 and D4 receptors in rat forebrain. *International Journal of Developmental Neuroscience*, *18*, 29-37.

Teicher, M. H., Andersen, S. L., & Hostetter, J. C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Developmental Brain Research, 89*, 167-172.

Toledo-Rodriguez, M. & Sandi, C. (2007). Stress before puberty exerts a sex-and age-related impact on auditory and contextual fear conditioning in the rat. *Neural Plasticity, 2007*, 1-12.

Toth, L. A. & Gardiner, T. W. (2000). Food and water restriction protocols: Physiological and behavioral considerations. *Journal of the American Association for Laboratory Animal Science, 39*, 9-17.

Treit, D., & Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology Biochemistry and Behavior, 31*, 959-962.

Tseng, K. Y. & O'Donnell, P. (2007). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cerebral Cortex, 17*, 1235-1240.

Tsoory, M., Guterman, A., & Richter-Levin, G. (2008). Exposure to stressors during juvenility disrupts development-related alterations in the PSA-NCAM to NCAM expression ratio: potential relevance for mood and anxiety disorders. *Neuropsychopharmacology, 33*, 378-393.

Tsoory, M. M., Guterman, A., & Richter-Levin, G. (2010). "Juvenile stress" alters maturation-related changes in expression of the neural cell adhesion molecule L1 in the limbic system: Relevance for stress-related psychopathologies. *Journal of Neuroscience Research, 88*, 369-380.

Tulving, E. & Markowitsch, H. J. (1998). Episodic and declarative memory: Role of the hippocampus. *Hippocampus, 8*, 198-204.

Vallée, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999), Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. *European Journal of Neuroscience, 11*, 2906–2916.

Verma, A. & Moghaddam, B. (1996). NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: Modulation by dopamine. *The Journal of Neuroscience, 16*, 373-379.

Vorhees, C. V. & Williams, M. T. (2006). Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*, 848-858.

Wang, X. D., Rammes, G., Kraev, I., Wolf, M., Liebl, C., Scharf, S. H., Rice, C. J., Wurst, W., Holsboer, F., Deussing, J. M., Baram, T. Z., Stewart, M. G., Müller, M., & Schmidt, M. V. (2011). Forebrain CRF1 modulates early-life stress-programmed cognitive deficits. *The Journal of Neuroscience*, *31*, 13625-13634.

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research*, *588*, 341-345.

Wu, J., Song, T. B., Li, Y. J., He, K. S., Ge, L., & Wang, L. R. (2007). Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats. *Brain Research*, *1141*, 205-213.

Yau, S. Y., Lau, B. W. M., Zhang, E. D., Lee, J. C. D., Li, A., Lee, T., Ching, Y. P., Xu, A. M., & So, K. F. (2012). Effects of voluntary running on plasma levels of neurotrophins, hippocampal cell proliferation and learning and memory in stressed rats. *Neuroscience*. *222*, 289-301

Yoon, T., Okada, J., Jung, M. W., & Kim, J. J. (2008). Prefrontal cortex and hippocampus subserve different components of working memory in rats. *Learning and memory*, *15*, 97-105.

Yuen, E. Y., Wei, J., Liu, W., Zhong, P., Li, X., & Yan, Z. (2012). Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*, *73*, 962-977.

Zahrt, J., Taylor, J. R., Mathew, R. G., & Arnsten, A. F. (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *The Journal of Neuroscience*, *17*, 8528-8535.

Zehr, J. L., Nichols, L. R., Schulz, K. M., & Sisk, C. L. (2008). Adolescent development of neuron structure in dentate gyrus granule cells of male Syrian hamsters. *Developmental Neurobiology*, *68*, 1517-1526.

Zhang, M., Storm, D. R., & Wang, H. (2011). Bidirectional synaptic plasticity and spatial memory flexibility require Ca2+-stimulated adenylyl cyclases. *The Journal of Neuroscience*, *31*, 10174-10183.