Effects of social context on endocrine function and Zif268 expression in response to an acute stressor in adolescent and adult rats

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Psychology

Submitted in partial fulfillment
of the requirements for the degree of

Master of Arts

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St. Catharines, Ontario

Abstract

There is a paucity of studies comparing social buffering in adolescents and adults, despite their marked differences in social behaviour. I investigated whether greater effects of social buffering on plasma corticosterone concentrations and expression of Zif268 in neural regions after an acute stressor would be found in adolescent compared with adult rats. Samples were obtained before and after one hour of isolation stress and after either one or three hours of recovery back in the colony with either a familiar or unfamiliar cage partner. Adolescent and adult rats did not differ in plasma concentrations of corticosterone at any time point. Corticosterone concentrations were higher after one hour isolation than at baseline ($p < 0.001$), and rats with a familiar partner during the recovery phase had lower corticosterone concentrations than did rats with an unfamiliar partner ($p = 0.02$). Zif268 immunoreactive cell counts were higher in the arcuate nucleus in both age groups after isolation ($p = 0.007$) and higher in the paraventricular nucleus of adolescents compared with adults during the recovery phase irrespective of partner familiarity. There was a significant decrease in immunoreactive cell counts after one hour isolation compared to baseline in the basolateral amygdala, central nucleus of the amygdala, and in the pyramidal layer of the hippocampus (all $p < 0.05$). An effect of partner familiarity on Zif268 immunoreactive cell counts was found in the granule layer of the dentate gyrus irrespective of age (higher in those with a familiar partner, $p = 0.03$) and in the medial prefrontal cortex in adolescents (higher with an unfamiliar partner, $p = 0.02$). Overall, the acute stress and partner familiarity produced a similar pattern of results in adolescents and adults, with both age groups sensitive to the social context.

Acknowledgments

Firstly, I would like to thank my supervisor Dr. Cheryl McCormick for always being there for me and for making sure that I was always at my best throughout my journey as a graduate student. Cheryl, you are one of the most amazing influences in my life and I look forward to everything I will learn from you in the future. I would like to thank Feather Nixon for not only being a magnificent help to me during sample collection but also for keeping me smiling and laughing no matter the situation. I must thank the members of the McCormick lab that helped me get through all of my data collection. Also, I thank my committee members, Dr. Stephen Emrich and Dr. Cameron Muir, for all their input and expertise throughout the preparation and completion of my thesis research.

In addition, I give so much thanks to my parents that continue to believe in me and support me no matter what. I cannot thank my housemates enough for helping me get through the good times and the hard times and being there whenever I needed them. Lastly, I am forever grateful to my grandmother, the most wonderful personality I have ever known, for helping mold me into the person I am today.

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Introduction

In social species, social bonds are able to facilitate learning (reviewed in Galef & Laland, 2005), health (Yee, Cavigelli, Delgado, & McClintock, 2008), and recovery from aversive experiences (reviewed in Kikusui, Winslow, & Yuji Mori, 2006). Similar to humans, many mammals, such as rodents and non-human primates, are highly social and benefit from social experiences (reviewed in Kikusui et al., 2006). Several studies have demonstrated an enhancement in neural and behavioural function for animals that are exposed to social interactions compared to those deprived of a social environment (reviewed in Vanderschuren, Niesink, & Van Pee, 1997). For example, rats raised in a social environment after weaning had an increase in dendritic spine density in the medial prefrontal cortex and in the hippocampus compared with rats that are socially deprived for a similar amount of time (Silva-Gomez, Rojas, Juarez, & Flores, 2003). Further, rats that are deprived of social interactions after weaning demonstrate impaired social activity (Hol, Van den Berg, Van Ree, & Spruijt, 1999) and social behaviours (both sexual and agnostic; van den Berg et al., 1999) in adulthood. However, exposing socially deprived rats to a social environment in early adolescence, but not mid-adolescence, restores regular social behaviours in adulthood (e.g. Hol et al., 1999). Thus, early social experiences are critical determinants of subsequent social behaviour.

Social bonds not only shape future social interactions, they can also influence the physiological and behavioural effects of stressors (reviewed in Kikusui et al., 2006; Hennessy, Kaiser, & Sachser, 2009). Further, the quality of social interactions influences their effects in positive or negative ways. Positive social experiences (maternal behaviours, interactions with familiar peers) have been found to buffer stressor induced

glucocorticoid release from the hypothalamic-pituitary-adrenal (HPA) axis, while negative social experiences (social defeat) enhance glucocorticoid release and can increase an animal's susceptibility to disease (reviewed in Kikusui et al., 2006; Hennessy et al., 2009). Moreover, the status of the peer interacted with can change a rat's physiological and behavioural responses to stressors (reviewed in DeVries, 2002).

Along with the quality of social experiences (positive or negative), the developmental stage of a social animal might also play a role in the effects of social experiences. Besides sexual maturation, adolescence is an important period of social learning, neural development, and physiological development (reviewed in Blakemore & Mills, 2014).The developmental stage of adolescence is more sensitive to social input compared with other developmental stages (childhood and adulthood) in many social species (reviewed in Spear, 2000). During adolescence, neural substrates involved in HPA axis reactivity and social function undergo structural and functional modification. The developmental changes occurring during adolescence might increase this age group's sensitivity to stressors (reviewed in Spear, 2000), and this might affect the buffering effects of social experiences. Moreover, the effects of social buffering are also moderated by the peer an animal interacts with. Not many studies, however, have examined the effects of peers (such as peer familiarity) on the social buffering of adolescent HPA reactivity to stressors. My thesis research sought to explore how HPA axis reactivity and neural responses to stressors are differently affected by social buffering in adolescent compared to adult rats.

Theoretical context

Critical periods in the lifespan of an organism are defined as periods of time during which an organism is more sensitive to environmental experiences and requires a specific stimulus, or the absence of a specific stimulus, to develop or learn a particular response (reviewed in Bornstein, 1989). Failure to be exposed to, or not exposed to, a required stimulus during a critical period interferes with or abolishes the response being developed (reviewed in Immelmann, 1975; Bornstein, 1989). Several examples of critical periods have been described, such as food preferences in birds before the 7th day of life (as reviewed in Hess, 1964), altered cortical responses to light in a mouse eye if the eye is deprived of light between 19 and 32 days after birth (Gordon & Stryker, 1996), and social attachment in the early life of dogs (reviewed in Scott, Stewart, & De Ghett, 1974). Critical periods for skills or internal mechanisms are not the same across species, but several species have critical periods for certain behaviours (reviewed in Immelmann, 1975).

Early evidence of critical periods comes from imprinting/social binding (the attraction to any living or non-living object present during a period in early life) research on newly hatched goslings by Konrad Lorenz (reviewed in Tzschentke & Plagemann, 2006). Past research has demonstrated that imprinting within a specific period of time in young animals has lasting effects (reviewed in Tzschentke & Plagemann, 2006). For example, ducklings were reported to imprint to a wooden model of an adult duck within 12 and 17 hours after hatching, whereas ducklings exposed to the stimulus less than 9 hours or more than 17 hours after hatching did not form as strong of a bond (as reviewed in Hess, 1958; Ramsay & Hess, 1954). Moreover, the lack of sensory stimulation during critical periods of imprinting have been reported to impair synaptic pruning in neural sites

such as the dorsocaudal neostriatum and result in the alteration of future learning (reviewed in Tzschentke & Plagemann, 2006; Bock & Braun, 1999).

More evidence of critical periods comes from past research of hormone dependent sexual differentiation, such that the failure to develop masculine or feminine neural phenotypes is dependent on testosterone exposure in prenatal and early postnatal life (as reviewed in Schulz, Molenda-Figueira, & Sisk, 2009). For example, a transient rise in testosterone during prenatal and early postnatal life masculinizes neural sites in males, whereas the absence of testosterone in females results in the development of the female neural phenotype (as reviewed in Resko & Roselli, 1997).

Few interactions between the environment and central nervous system (CNS), however, involve strictly defined windows of time, as suggested by the term critical period, and thus the term sensitive period applies to a broader scope of environmental effects on CNS development (reviewed in Knudsen, 2004). Sensitive periods are limited periods in development during which experience has a greater effect on the brain (reviewed in Bornstein, 1989; Knudsen, 2004). For example, barn owls have a high degree of plasticity in auditory and spatial processing in early life (Brainard & Knudsen, 1998), and impairing the hearing and vision of the barn owl during the sensitive period will impair their representation of auditory cues, but restoring the senses and providing a more enriched environment for the barn owl after the sensitive period will restore regular auditory and spatial function (Brainard & Knudsen, 1998). Other research demonstrates that in rats there are a number of sensitive periods in early life for the development of future social behaviours (reviewed by Blass, 1987). Juvenile rats should experience suckling behaviours (nipple attachment), huddling behaviours (sibling contact), and

exposure to the odor of the mother and nest to have regular social function in adulthood (reviewed by Blass, 1987). However, exposure to some of these experiences and not others during sensitive periods throughout juvenile life can still result in regular adult social function (reviewed by Blass, 1987).

Only in the last ten years has adolescence been considered another sensitive period of development. Sex hormone exposure during adolescence also has organizational effects of adult male and female social behaviours (reviewed in Schulz et al., 2009). For example, male hamsters that are castrated before puberty have reduced sexual behaviour and aggressive behaviours compared with rats with testicular hormones present throughout puberty (reviewed in Schulz & Sisk, 2006). Further, female rats ovariectomized before adolescence demonstrate a higher preference for male rats than female rats that is not found in females ovariectomized after adolescence (de Jonge, Muntjewerff, Louwerse, & Van de Poll, 1988). Abnormalities in sex hormone concentrations during puberty also affects the adult stress response. For example, testosterone injection did not lower corticosterone concentrations after restraint stress in adult male rats castrated just before puberty, whereas testosterone did decrease corticosterone concentrations after restraint stress in adult male rats castrated after puberty (Evuarherhe, Leggett, Waite, Kershaw, & Lightman, 2009). These results demonstrate that sensitivity to androgens during puberty changes how the stress response of adult male rats responds to testosterone in the future. The basis of differential sensitivity in adolescence compared with other age groups is unknown, although many researchers have proposed the differential sensitivity in adolescence is tied to HPA function (reviewed in McCormick & Mathews, 2010; Green & McCormick, 2013;

McCormick & Green, 2013; Eiland & Romeo, 2013; Blakemore & Mills, 2013; Holder & Blaustein, 2014).

The hypothalamic-pituitary-adrenal (HPA) axis

When a stressor is perceived or anticipated, signals from sensory pathways and/or higher neural regions are relayed to the paraventricular nucleus of the hypothalamus (PVN) to activate the main stress pathway, the hypothalamic-pituitary-adrenal (HPA) axis. Stimulation of the paraventricular nucleus of the hypothalamus brings about the release of secretagogues, primarily corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), that act on the anterior pituitary to promote the synthesis and release of adrenocorticotropic hormone (ACTH), which in turn leads to the release of glucocorticoids into the bloodstream by acting on the adrenal cortex (reviewed in Herman, Ostrander, Mueller, & Figueiredo, 2005; See figure 1). The HPA axis is also under circadian control, with the peak of glucocorticoid release found near the beginning

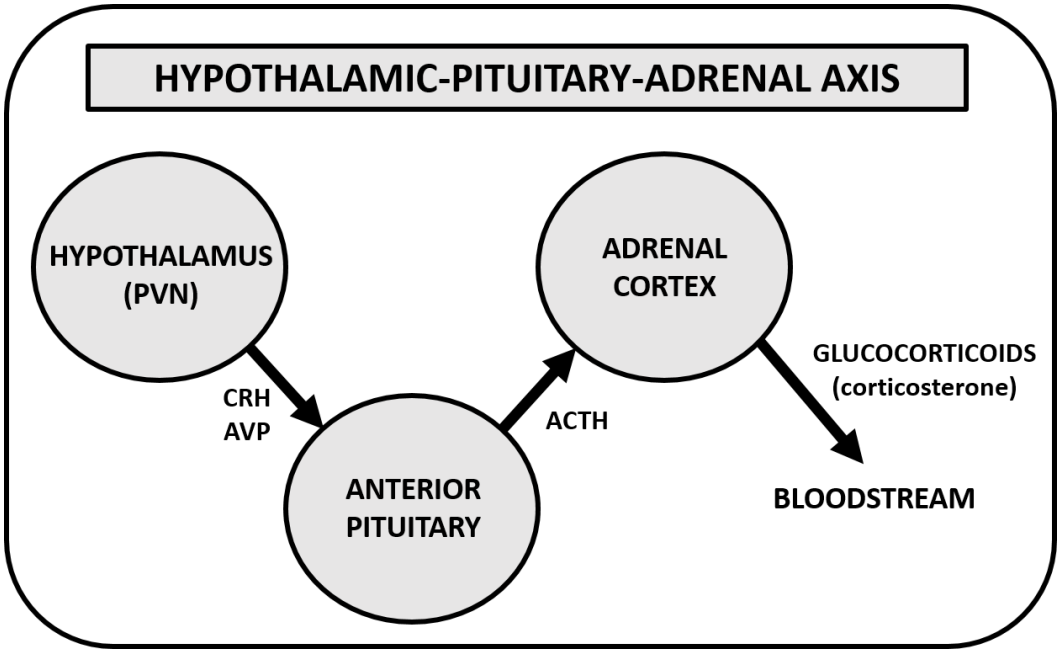

Fig. 1. A depiction of the hypothalamic-pituitary-adrenal (HPA) axis.

of the wake cycle and the nadir of glucocorticoid release near the beginning of the sleep cycle for both humans (Spiegel, Leproult, & Van Cauter, 1999) and rodents (Atkinson & Waddell, 1997).

The role of glucocorticoids, primarily cortisol in humans and corticosterone in rodents, are to help an organism adapt to and recover from the effects of a stressor through their influence on the metabolic, cardiovascular, immune, and neurological systems (reviewed in De Bosscher, Van Craenenbroeck, Meijer, & Haegeman, 2008; McEwen, Eiland, Hunter, & Miller, 2012). For example, glucocorticoids act to replenish the energy that is expended when responding to stressors and aid in immune system defence by enhancing cytokine function (reviewed in McEwen, 2004; Dhabhar, 2009). Glucocorticoids also mediate transitions between developmental stages and elicit programming effects that can change how an organism later releases and responds to glucocorticoids (reviewed in Wada, 2008). Rises in glucocorticoids promote the development of organs and behavioural function during critical periods of development (reviewed in Wada, 2008). However, the prolonged release of, and exposure to, glucocorticoids in response to repeated (or chronic) stressors can have several deleterious effects. For example, both glucocorticoid treatments and exposure to chronic stressors have been found to impair memory and alter hippocampal plasticity in rats depending on the amount of glucocorticoids administered or length and type of stressor exposure (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003; McCormick et al., 2012).

Glucocorticoids exert their effects by binding onto corticosteroid receptors found throughout the organism (reviewed in De Bosscher et al., 2008). Corticosteroid receptors

consist of both glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), which have different binding affinities for glucocorticoids. MRs have a lower affinity for corticosterone and thus are bound maximally at low concentrations, whereas GRs have a higher affinity and require higher concentrations for maximal binding, such as the high concentrations that come with exposure to a stressor (reviewed in Herman, McKlveen, Solomon, Carvalho-Netto, & Myers, 2012). GRs are found at all levels of the HPA axis and have high densities in several other neural sites, most notably in the hippocampus and medial prefrontal cortex, which are important sites of negative feedback (reviewed in Herman et al., 2012). The ability of glucocorticoids to inhibit their own release through a negative-feedback loop helps to defend an organism from the many deleterious effects of prolonged glucocorticoid release (reviewed in De Bossher, Van Craenenbroeck, Meijer, & Haegeman, 2008). GRs, however, can be down regulated by exposure to chronic stressors (Herman, Adams, & Prewitt, 1995), which may affect glucocorticoid-dependent functioning and reduce negative feedback.

HPA reactivity and social input

Glucocorticoid release can also be moderated by different types of social input, such as social context and the quality of social interactions. Negative social interactions promote glucocorticoid release and other known stress responses (increased heart rate, increased body heat) in humans and other social mammals (reviewed in Kikusui et al., 2006). For example, humans show a potentiated release of cortisol when experiencing social-evaluative threat (presenting on a subject while being watched) compared to presenting on a subject when no one is watching (Dickerson, Mycek, & Zaldivar, 2008). Rodents that experience social defeat (placed into a cage with a larger aggressive male

conspecific) exhibit various stressor related reactions (weight loss, high heart rate, high temperature) (de Jong et al., 2005). Nevertheless, rodents that experience social defeat and a return to social housing show an attenuation of stress responses compared to those returned to single housing (de Jong et al., 2005). Thus, supportive social interactions can dampen glucocorticoids release and other stress responses.

The buffering effects of social interactions on the physiological and behavioural responses to stressors in social animals paired with a conspecific (a member of the same species) compared to when alone have also been well documented. For example, men and women given social support have attenuated cortisol concentrations and lower heart rate when performing a demanding task than those who performed the task alone (Thorsteinsson, James, & Gregg, 1998). In rodents, animals paired with a conspecific during fear conditioning or during recovery from a fear conditioning paradigm displayed decreased freezing (a behavioural measure of fear in rodents), an attenuated increase in temperature, and a dampened corticosterone release compared with rats that experienced the fear conditioning paradigm without a conspecific (Kiyokawa, Takeuchi, & Mori, 2007; Kiyokawa, Hiroshima, Takeuchi, & Mori, 2014a). Moreover, rats had lower prolactin concentrations (another indicator of stress) in an open field test (a common test for anxiety in rodents) when paired with a same-sex conspecific than rats placed in the open field test alone (Wilson, 2000). Nevertheless, research has also reported a potentiated corticosterone response to a novel box when with a peer than when alone (Armario, Luna, & Balasch, 1983), which might have to do with the perception of the stressor or the peer with which the rat is paired.

The attenuating properties of social housing and paired-exposure to stressors show mixed results depending on the conspecific with which the subject is paired (reviewed in DeVries, 2002). Some studies found a potentiated corticosterone release in response to a stressor when with a familiar partner compared with an unfamiliar one (Armario & Balasch, 1981; Armario et al., 1983), whereas others found an attenuated response to a stressor when paired with a familiar conspecific compared with an unfamiliar conspecific (Hennessy, Zate, & Maken, 2008) and a faster return to baseline corticosterone when paired with a familiar peer compared with an unfamiliar peer after exposure to a novel environment (Terranova, Cirulli, & Laviola, 1999). Further, the psychological/physiological state of the peer is an important moderator of the effects of social buffering (reviewed in Kikusui et al., 2006). For example, rats with non-stressed partners displayed reduced physiological and behavioural responses to a stressor than did rats paired with a stressed partner (Kiyokawa, Kikusui, Takeuchi, & Mori, 2004; Davitz & Mason, 1955). The buffering effects of social interactions, however, might also depend on the nature of the stressor and may be greater in certain stages of development than others.

Adolescence and social buffering

The developmental stage of adolescence is a period of transition from childhood to adulthood, encompassing sexual maturation in both humans and rodents (Sisk & Foster, 2004). The boundaries of adolescence are difficult irrespective of the species, but adolescence always involves puberty and the associated increase in physical, cognitive and social changes (reviewed in Blakemore & Mills, 2014). In rodents, puberty in males and females has been defined by physical changes in the animals towards sexual

maturity, such as vaginal opening in females (postnatal day (PND) 35±2) and preputial separation in males (PND 42±2) (reviewed in McCormick & Mathews, 2010). An often used classification of age for adolescence in rodents is within postnatal days 28 and 45, but adolescence can also be divided into early adolescence (PND 21-34), mid-adolescence (PND 34-46), and late adolescence (PND 46-59) with adulthood beginning around postnatal day 60 (reviewed in McCormick & Mathews, 2010).

Throughout adolescence, an increase in the preference for, and the amount of social interactions with, peers is demonstrated in both humans and rodents (reviewed in Spear, 2000). Whereas early- and mid-adolescent rats are reported to initiate and display more play behaviours with conspecifics, late-adolescent and adult rats display less social play (Klein, Padow, & Romeo, 2010) and more agonistic behaviours (Meaney & Stewart, 1981) towards conspecifics. Mid-adolescent rats that are social deprived (housed singly) engage in more social play behaviours (chasing, wrestling, pinning) when later paired with a conspecific than do rats left in a social environment (Holloway & Suter, 2004). Socially deprived adolescent rats also prefer the side of a cage that was earlier paired with a conspecific more than do similarly socially deprived adults (Douglas, Varlinskaya, & Spear, 2004). Another study by Varlinskaya and Spear (2008) compared social activity in single and group housed rats paired with a conspecific during early adolescence (PND 28), mid-adolescence (PND 35), late adolescence (PND 42), and adulthood (PND 70). After 30 minutes of isolation, adolescent male rats of all ages (PND 28, 35, 42) engaged in more social interactions and social play behaviours with a conspecific than similarly isolated adults (Varlinskaya & Spear, 2008).

Brain regions that are sensitive to social experiences (eg. medial prefrontal cortex; Wall, Fischer, & Bland, 2012) and that regulate social behaviours (eg. medial amygdala; Weathington, Strahan, & Cooke, 2012), undergo structural and functional development during adolescence (reviewed in Sturman & Moghaddam, 2011). In addition, brain regions that influence HPA reactivity (medial prefrontal cortex, and areas of the limbic system such as the hippocampus and the amygdala) also undergo restructuring during the adolescent stage (reviewed in Sturman & Moghaddam, 2011). The neural changes that occur throughout adolescence may underlie both the increased vulnerability to stressors and an increased sensitivity of adolescents to social stimuli that is found during this period. A greater sensitivity to social experiences might help defend adolescents against the effects of stressors, which is of great importance due to differences in endocrine responses to stressors found between adolescents and other age groups (discussed in the next section). Because of the changes in brain regions governing social behaviour during adolescence, the period of adolescence may be more sensitive to the buffering effects of social interactions on HPA reactivity and other stress responses than is the period of adulthood.

Age differences in HPA responses to stressors

Several studies have reported a more prolonged release of corticosterone in response to an acute stressor in pre-pubertal adolescent rats compared with similarly stressed adult rats (Foilb, Lui, & Romeo, 2011; Lui et al., 2012; Romeo et al., 2006a; Romeo, Karatsoreos, & McEwen, 2006b; Hall & Romeo, 2013). Moreover, a recent study reported a prolonged corticosterone release in adolescent rats paired with a previously stressed peer compared with a non-stressed peer but no differences in

similarly paired adult rats (Hall & Romeo, 2013). A faster return to baseline corticosterone concentrations in adult rats exposed to an acute stressor might signify a difference in negative feedback or another functional difference in the HPA axis of adolescent and adult rats. However, although adolescents show a more prolonged corticosterone release to stressors compared with adults, the two groups do not differ in GR receptor densities (reviewed in Vázquez, 1998). Thus, the basis for the prolonged glucocorticoid release in response to stressors in adolescents compared with adults is unknown, although a recent study suggests adrenal sensitivity to ACTH may be greater in adolescents than in adults (Romeo et al., 2014). Further, some studies have not found adolescents and adults to differ in HPA responses to stressors (Schramm-Sapyta et al., 2007), and others have found greater HPA responses to stressors in adults compared with adolescents (Willey, Anderson, Morales, Ramirez, & Spear, 2012; Cao et al., 2010). Measuring neural activation in adolescent and adult rats in response to stressors may provide information about the basis for age differences in HPA function.

Immediate early genes and neural activation

Immediate-early genes (IEGs) are widely used as functional anatomical markers of brain activity in animal models. These genes are rapidly and transiently activated in response to stimuli (reviewed in Kovács, 2008). Stimuli that activate IEGs range from internal disruptions of homeostasis to disruptions caused by several types of stressors, such as lipopolysaccharide injection or restraint (reviewed in Kovács, 2008). In response to such stimuli, the time point of peak IEG expression varies depending on several factors (IEG type, stimulus type) but is usually around 30 min for IEG mRNA and 60-120 min for the IEG protein product (reviewed in Kovács, 1998; Kovács, 2008). IEG mRNA and

synthesized proteins are numerous in type and in the stimuli to which they respond. Thus, different IEGs can be activated by different stimuli and the lack of one IEG in a neural site does not mean that there is not another IEG being activated to the same stimulus; the cells in question might be involved in the functional process in question by some other means or another IEG might be involved. The IEG c-fos and its protein product Fos are the most widely used markers of neural function in non-human animal research (reviewed in Kovács, 2008). Nevertheless, there are several other IEGs that can be used (e. g., c-Jun, ARC, zif268).

Measuring IEGs for neural activation is also popular because of their ease of detection (reviewed in Kovács, 2008). IEG detection is possible through methods such as immunohistochemistry and western blotting that involve using antibodies to target antigens on IEG protein products, as well as the quantification of IEG mRNA by methods such as in situ hybridization.

Neural responses to stressors

Age differences in HPA axis reactivity may be because of differences in how the adolescent and adult brains respond to stressors. For instance, several studies have found greater Fos expression in the paraventricular nucleus of the thalamus in adolescent rats than in adult rats in response to both acute stressors (Viau, Bingham, Davis, Lee & Wong, 2005) and repeated stressors (Lui et al., 2012; Romeo et al., 2006a). In addition, the paraventricular nucleus has been shown to have similar volumes and number of cells in prepubertal adolescents and adults rats, indicating that the age difference in hormone secretion is not because of differences in gross morphology (Romeo, Karatsoreos, Jasnow, & McEwen, 2007). Greater activity of the paraventricular nucleus in adolescence

than in adults may underlie the age differences in glucocorticoid release in response to stressors.

Moreover, neural sites that are activated by stressors also are activated by social experiences, some of which are social stressors (reviewed in Martinez, Calvo-Torrent, & Herbert, 2002). For example, losing aggressive social interactions in rodents (social defeat) has been found to increase c-fos expression in the paraventricular nucleus, and the amygdala (medial and central parts), among other areas (Martinez, Phillips, & Herbert, 1998). Another study has reported an increase in Fos expression in the paraventricular nucleus and medial amygdala to both agonistic and copulatory behaviours (Kollack-Walker & Newman, 1995). An increase in IEG expression has also been reported in response to maternal behaviours (Numan, Numan, Marzella, & Palumbo, 1998). Further, a recent study by Kiyokawa et al. (2014b) measured Fos expression in adult rats exposed to a context previously paired with foot shocks either alone, with an unfamiliar peer, or with a familiar peer. This study reported decreased Fos expression in the PVN and central amygdala for rats placed in the context with either a familiar or unfamiliar peer compared to alone and decreased Fos expression in the PVN for rats paired with a familiar versus an unfamiliar peer (Kiyokawa, Honda, Takeuchi, & Mori, 2014b). Nevertheless, these studies did not measure neural activity to social experiences or partner familiarity in both adolescent and adult rats.

The adolescent and adult brains also respond differently to social experiences. For example, in a study by Varlinskaya et al. (2013), adolescent and adult rats were injected intraperitoneally with either saline or ethanol and placed in a Plexiglas chamber with either an unfamiliar partner or alone. In result, the extended amygdala (central amygdala,

basolateral amygdala, bed nucleus of stria terminalis), lateral septum, and lateral hypothalamus all expressed more activation in adolescent rats paired with an unfamiliar conspecific than in adults placed in a similar social environment (Varlinskaya, Vogt, & Spear, 2013). Adult rats placed in a Plexiglas chamber alone expressed more activation in the anterior cingulate cortex, the ventromedial orbitofrontal cortex, the nucleus accumbens (shell and core), the lateral parabrachial nucleus, the substantia nigra, and the locus coeruleus than adults paired with an unfamiliar conspecific or similarly tested adolescent rats (Varlinskaya et al., 2013). Another study found greater brain-derived neurotrophic factor (BDNF) regulated immediate early gene activity in the adolescent rat hippocampus after social defeat but no difference in similarly defeated adults (Coppens et al., 2011). Thus, the adolescent and adult brains respond differently to social experiences.

The immediate early gene Zif268 has been discussed as a marker for neural plasticity, which may be particularly relevant considering our focus on adolescence and it being an important period for social learning. Zif268 regulates the expression of several genes that regulate long term memory consolidation and has been linked to long-term potentiation, the increase in synaptic efficacy in response to stimulus evoked synaptic activity (reviewed in Knapska & Kaczmarek, 2004). The immediate early gene Zif268 is also expressed in response to stressors. For example, an increase in zif268 expression in response to acute swim and restraint stress has been described in the rat prefrontal cortex, hippocampus (CA1, CA3, dentate gyrus), amygdala (MeA, CeA), paraventricular nucleus, arcuate nucleus, and several other cortical areas (Cullinan, Herman, Battaglia, Akil, & Watson, 1995). Similar to earlier discussion, Cullinan et al. (1995) found high

Zif268 expression at around 30 to 60 minutes following the stressors, and a return to baseline levels of Zif268 expression 120 minutes following the stressors.

Thesis questions

Previous research in our lab has found impaired social interactions (Green, Barnes, & McCormick, 2013a; McCormick et al., 2012; McCormick et al., 2013a), increased anxiety (Green et al., 2013a; McCormick, Smith, & Mathews, 2008), and impaired memory (Green & McCormick, 2013b) in adult rats that were exposed to social instability stress (daily one hour isolation and return to a new cage partner for 16 days during adolescence). In addition, adolescent rats exposed to the social instability stress procedure demonstrated an impaired memory of fear in adulthood that was not found in adult rats exposed to the procedure and tested in later adulthood (Morrissey, Mathews, & McCormick, 2011). These findings support the hypothesis that adolescence is a period of heightened sensitivity to stressors. The McCormick lab previously reported an increase in corticosterone immediately following one hour isolation and an impeded return to baseline concentrations in rats after a one hour recovery period if paired with an unfamiliar peer compared to those paired with a familiar cage partner (McCormick, Merrick, Secen, & Helmreich, 2007). These additional findings support the hypothesis that partner familiarity can affect the social buffering of a stress response, however, they did not include an adult rat comparison.

The goal of my Master's thesis research was to replicate the findings of the adolescent stress response to social instability stress, discover the neural sites involved, and extend the findings to adults. I tested the hypothesis that adolescent HPA axis reactivity and neural activity would be more sensitive to the effects of social buffering

than adults. I measured corticosterone and Zif268 expression of adolescent (postnatal day 30) and adult (postnatal day 70) male rats in response to, and during recovery from, one hour of isolation stress. During the recovery period, rats were paired with a familiar or unfamiliar same age peer and respective cage. I predicted that compared to adults, adolescents would show a more prolonged stress response and differential activation of the associated stress response nuclei, such as the paraventricular nucleus of the hypothalamus, arcuate nucleus, and the basolateral and central nuclei of the amygdala (as reviewed in Herman & Cullinan, 1997), and in brain regions that are important for regulating social behaviours, such as the medial prefrontal cortex (Wall et al., 2012), hippocampus (Calfa, Bussolino, & Molina, 2007), and medial amygdala (Samuelsen & Meredith, 2009). Testosterone concentrations were also measured in all rats throughout the procedure because of its importance in reducing adult male rodent HPA reactivity (as reviewed in Romeo, 2010) and because of testosterone reactivity to stressors and social context (as reviewed in Gleason, Fuxjager, Oyegbile, & Marler, 2009).

Methods

Animals

Male Long-Evans rats (N = 96) were obtained from Charles River, St. Constant, Quebec, at 22 days of age. Rats were housed in pairs and maintained under a 12 h light-dark cycle (lights on at 08:00 h) with food and water available *ad libitum*. Use of animals in this experiment was approved by the Brock University Institutional Animal Care and Use Committee (ACUC) and was carried out in adherence to the Canadian Council of Animal Care guidelines.

Stress conditions

Rats were randomly assigned to be tested either as adolescents (PND 30) or as adults (PND 70) and to experimental conditions, which were the time points at which blood samples were collected and the animals were perfused for brain collection. Two time points were used to determine the stress response of adolescents and adults: (i) baseline (immediately after removal from the home cage), and (ii) after isolation for 1 h (isolation). To determine the influence of partner familiarity on recovery from the isolation stress, two time points were used: (i) after isolation for 1 h and a 1 h return to either the original cage partner (familiar partner) or to a new cage partner (unfamiliar partner) and new cage, and (ii) after isolation for 1 h and a 3 h return to either the familiar partner or to a new cage partner. Isolation consisted of removing rats from their home cage and confining them in ventilated, round plastic containers for 1 h during the light phase of the diurnal cycle. Adolescent isolation containers were 14 cm in diameter and 10 cm in height, whereas adult isolation containers were 20 cm in diameter and 12 cm in height. Isolation stress is similar to restraint stress in that both involve restricting movements, with the main difference between the two being the shape of containers (round for our isolation procedure, and tubular for restraint procedures). The cage partners to which rats returned after isolation had also undergone isolation at the same time (cage partners were both returning to a familiar partner or an unfamiliar partner at the same time). Experiments involved two cohorts of rats and experiments were conducted between 09:00 and 13:30 h to minimize the influence of circadian variation. All rats were housed in the same room of the animal care facility.

Plasma corticosterone and testosterone assays

A blood sample (approximately 200 µl) was obtained from a tail nick and collected in ice chilled blood collection tubes (Sarstedt) within three minutes. Samples were centrifuged at 1730 x g and 4°C for 10 min, and plasma was collected and stored at -20°C until the assays. Plasma corticosterone and testosterone concentrations were determined using enzyme-linked immunosorbent assay kits (Neogen, Lansing, MI) and a Biotek Synergy plate reader. To extract the steroids before assays, plasma was pipetted into individual culture tubes with 2 ml of ethyl ether and vortexed. Solids in the plasma and ethyl ether mixture were then frozen in a dry ice and acetone bath and the liquid phase was collected in a new culture tube. After evaporation of the liquid phase, samples were then reconstituted in 100 µl of the extraction buffer provided in the kit, then further diluted (20 µl in 980 extraction buffer). Duplicate aliquots (50 µl) of the final dilution were used for the assay. Intra-assay variance was under 10% and inter-assay variance was under 15%. Assay sensitivity was 0.05ng/mL for corticosterone and 0.006 ng/mL for testosterone.

Zif268 immunohistochemistry

Immediately after blood sampling, rats were deeply anaesthetized by an overdose of sodium pentobarbital (150 mg / kg) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS; pH 7.4). Brains were removed from the skulls and post-fixed in a 30% sucrose and 4% paraformaldehyde solution until equilibrated. Coronal sections (37 µm; sections separated by 222 µm) were sliced on a cryostat and collected throughout the medial prefrontal cortex, the hypothalamus, and the hippocampus and were stored in cryoprotectant at -20°C until the time of assay.

Free-floating coronal sections were washed thoroughly in 0.1 M PBS, then in PBS-X (0.1 M PBS with 3% Triton X-100), and incubated at room temperature in a 0.3% H_2O_2 in 0.1 M PBS-X solution for 30 min. Sections were then washed in PBS-X, blocked at room temperature in 10% goat serum (Sigma) solution for 1 h, and incubated at 4°C overnight in primary anti-body (1:10,000; zif-268 rabbit mAb; 15F7; Cell Signaling Technology, Inc.) in PBS-X. The next day, sections were washed in PBS-X and then incubated for 2 h at room temperature in secondary antibody (biotinylated goat anti-rabbit IgG; 1:400; Vector Laboratories, Inc.). After another series of washes in PBS-X, sections were incubated in an avidin-biotin horseradish peroxidase complex (Vector Laboratories, Inc.) for 1.5 h at room temperature. Horseradish peroxidase was visualized with 3,3'diaminobenzidine (DAB) in a 3 M sodium acetate buffer containing 0.05% H_2O_2 (Vector Laboratories, Inc.). After a final series of washes in PBS-X, sections were mounted on Superfrost Plus slides (Fisher Scientific, Inc.), dried, dehydrated in increasing concentrations of ethanol (70%, 95%, 100%), placed in xylenes, and coverslipped using Permount mounting medium (Fisher Scientific, Inc.). To facilitate identification of subregions of the medial prefrontal cortex and amygdala, those sections were lightly counterstained with neutral red before they were coverslipped. No immunoreactive cells were detected in control sections that were not treated with the primary antibody.

Microscopy and cell counting

Immunostained sections were analyzed using a Nikon Eclipse 80i microscope equipped with a digital camera (Nikon DXM1200F) and Nikon ACT-1 software. Immunoreactive (ir) cell counts were conducted blind to experimental condition and at

400X magnification in a 250 μm^2 area in each hemisphere of the paraventricular nucleus (PVN), the arcuate nucleus, the medial prefrontal cortex (mPFC; anterior cingulate cortex, infralimbic cortex, prelimbic cortex), the pyramidal layer of the hippocampus (CA1, CA2, & CA3), the granule layer of the dentate gyrus, the basolateral amygdala (BLA), the central nucleus of the amygdala (CeA), the medial posterodorsal amygdala (MePD), and the medial posteroventral amygdala (MePV). These areas were chosen for examination based on their roles in social behaviour and HPA function (Martinez et al., 2002). Regions were identified according to the atlas of Paxinos and Watson (Paxinos and Watson, 2005): mPFC sections used for counting were within the coordinates from bregma of 3.00 mm and 2.52 mm; for PVN, within bregma -1.44 and -1.72; for arcuate nucleus, within bregma -2.52 and -2.76; for regions of the hippocampal formation, within bregma -2.64 and -3.24; for CEA and BLA, within bregma -2.52 and -2.92; for MePD and MePV, within bregma -2.76 and -3.24. The mean number of ir-cells per hemisphere per brain region per rat was used for analysis for all regions of interest except the hippocampal formation. For the hippocampal formation, in each hemisphere of each of three sections, ir-cells were counted in five separate regions, the CA1, CA2, and CA3, which were averaged and labeled as the pyramidal layer, and in the suprapyramidal and infrapyramidal blades of the dentate gyrus, which were averaged and labeled as the granule layer.

Statistical analyses

Statistical analyses were performed using SPSS version 20 software and consisted of between group analysis of variance with the factors of Age (adolescent, adult) and Time (baseline, after 1 h isolation) for the Stress analyses, and with the factors of Age

(adolescent, adult), Partner Familiarity (familiar, unfamiliar), and Time (after 1 h isolation and 1 h return to the colony, after 1 hour isolation and 3 h return to the colony) for the Post-stress recovery analyses. Whenever multiple regions were counted within the same sections, brain region was included as a within-subject factor in analyses. Post hoc test analyses consisted of F tests for simple effects and/or between group t-tests, where appropriate. A between group t-test also was used to determine whether testosterone concentrations differed between baseline and 1 h isolation stress adult groups. Although the experiments initially involved n = 8 per age for each time point, final sample sizes were reduced to n = 5 – 8 for each measure for several reasons (e.g., unable to obtain blood sample; damaged sections; sections not within a region of interest). An alpha level of $p \leq 0.05$ was used to determine statistical significance, although values of $p \leq 0.10$ are noted.

Results

Table 1 provides a summary of the results.

Corticosterone

Stress: An Age x Time ANOVA indicated that corticosterone concentrations were higher after isolation than at baseline ($F_{1, 22} = 27.14$, $p < 0.001$). There was no effect of age ($p = 0.63$) or interaction of Age and Time ($p = 0.93$). Post-stress: An Age x Time (1 or 3 h after isolation) x Partner Familiarity (familiar or unfamiliar) ANOVA indicated that corticosterone concentrations were lower when returned to a familiar partner than to an unfamiliar partner ($F_{1, 44} = 5.75$, $p = 0.02$; all other main effects and interactions were $p > 0.50$; see Figure 2).

Testosterone

Table 1
Summary of the results.

	Stress		**Post-Stress**		
	Age effect (adolescent compared to adult)	**Isolation effect** (compared to baseline)	**Age effect** (adolescent compared to adult)	**Partner familiarity effect** (Unfam. compared to fam. partner)	**Time effect** (1 h compared to 3 h post-stress)
Endocrine					
Corticosterone	n.s.	↑	n.s.	↑	n.s.
Testosterone	↓	n.s.	↓	n.s.	↓
Zif268-ir					
PVN	n.s.	n.s.	↑	n.s.	↓
Arcuate nucleus	n.s.	↑	n.s. (↑)	n.s.	n.s.
mPFC	n.s.	n.s. (↓)	n.s	↓ (adol. only)	n.s.
Pyramidal layer	n.s.	↓	n.s.	n.s.	n.s. (↑)
Granule layer	n.s.	n.s.	n.s.	↓	↑
BLA	n.s.	↓	n.s.	n.s.	n.s.
CEA	n.s.	↓	n.s.	n.s.	n.s.
MePD	n.s.	n.s.	n.s.	n.s. (↑)	n.s.
MePV	n.s.	n.s.	n.s.	n.s.	n.s. (↓)

Arrows indicate the direction of an effect of $p \leq 0.05$; Arrows within brackets indicate the direction of an effect of $p \leq 0.10$. n.s. = no statistically significant effect, $p > 0.05$).

Testosterone concentrations were below the limit of detection of the assay for the adolescents. Stress: In adults, testosterone concentrations did not differ significantly between baseline and 1 h isolation groups ($t_8 = 0.82$, $p = 0.44$). Post-stress: Testosterone concentrations were higher 1 h than 3 h after isolation ($F_{1, 16} = 10.40$, $p = 0.004$). There was no effect of Partner Familiarity ($p = 0.54$). The interaction of the main effects was not significant ($p = 0.25$), although exploratory post hoc comparisons indicated that

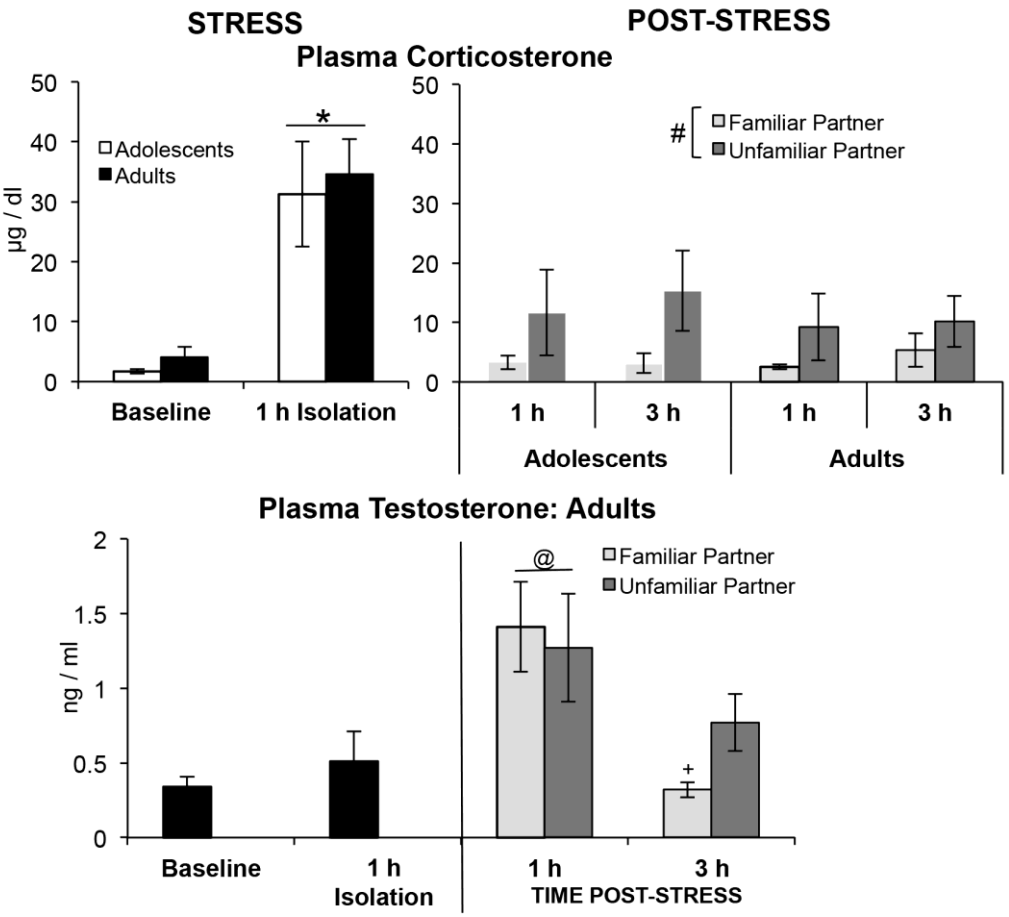

*Fig. 2. Mean (±S.E.M.) concentration of plasma corticosterone for adolescent and adult, and of plasma testosterone for adult before and after 1 h isolation stress and after1 and 3 h of recovery post-stress with either a familiar or unfamiliar cage partner (n = 5–8 per group). *Higher than baseline, collapsed across Age (main effect of Time, p <0.001); #higher than in rats with a familiar partner, collapsed across Age and Time Post-Stress (main effect of Partner Familiarity, p = 0.02);@higher than 3 h post-stress (main effect of Time Post-Stress, p = 0.004);+post hoc comparison showed decline from 1 to 3 h significant only in those with a familiar cage partner, p = 0.02.*

the decrease in testosterone concentrations from 1 h to 3 h after isolation stress was significant in those with a familiar partner only (p = 0.02; p = 0.24 for unfamiliar partners), and as a result testosterone concentrations were moderately higher when with an unfamiliar cage partner (p = 0.06) than with a familiar cage partner (p = 0.78) for three hours (see Figure 2).

Zif268 expression

PVN. Stress: An Age x Time (baseline, 1 h isolation) ANOVA on Zif268-ir cell counts in the PVN found no significant group differences or interaction (all $p > 0.30$). Post-stress: An Age x Time (1 or 3 h after isolation) x Partner Familiarity on Zif268-ir cell counts in the PVN found that adolescents had higher ir-cell counts in the PVN than did adults ($F_{1, 38} = 4.40$, $p = 0.04$) and ir-cell counts were higher 1 h after the end of isolation than 3 h after ($F_{1, 38} = 6.52$, $p = 0.02$). Neither the effect of Partner Familiarity nor any of the interactions were significant (all $p > 0.10$; see Figure 3).

Arcuate nucleus. Stress: Zif268-ir cell counts in the arcuate nucleus were higher after 1 h isolation compared to baseline ($F_{1, 21} = 8.78$, $p = 0.007$). There was no effect of Age ($p = 0.79$), although the Age X Time interaction approached significance ($p = 0.10$). Post-stress: An Age x Time (1 or 3 h after isolation) x Partner Familiarity on Zif268-ir cell counts in the arcuate nucleus found no significant main effects or interactions (all $p > 0.15$, except for trend for higher counts in adolescents than in adults $p = 0.10$; see Figure 3).

mPFC regions. Stress: An Age x mPFC region (ACC, Prelimbic, Infralimbic) X Time (baseline, 1 h isolation) ANOVA on Zif268-ir cell counts found an interaction of mPFC region and Time ($F_{2, 42} = 4.66$, $p = 0.02$). The difference between baseline and isolation was not significant for any region of the mPFC, although the lower ir-cell counts in the ACC after isolation compared to baseline approached significance ($p = 0.07$). Post-stress: An Age X Time (1 or 3 h after isolation) x Partner familiarity (familiar or unfamiliar) X PFC region (ACC, Prelimbic, Infralimbic) ANOVA on Zif268- ir cell

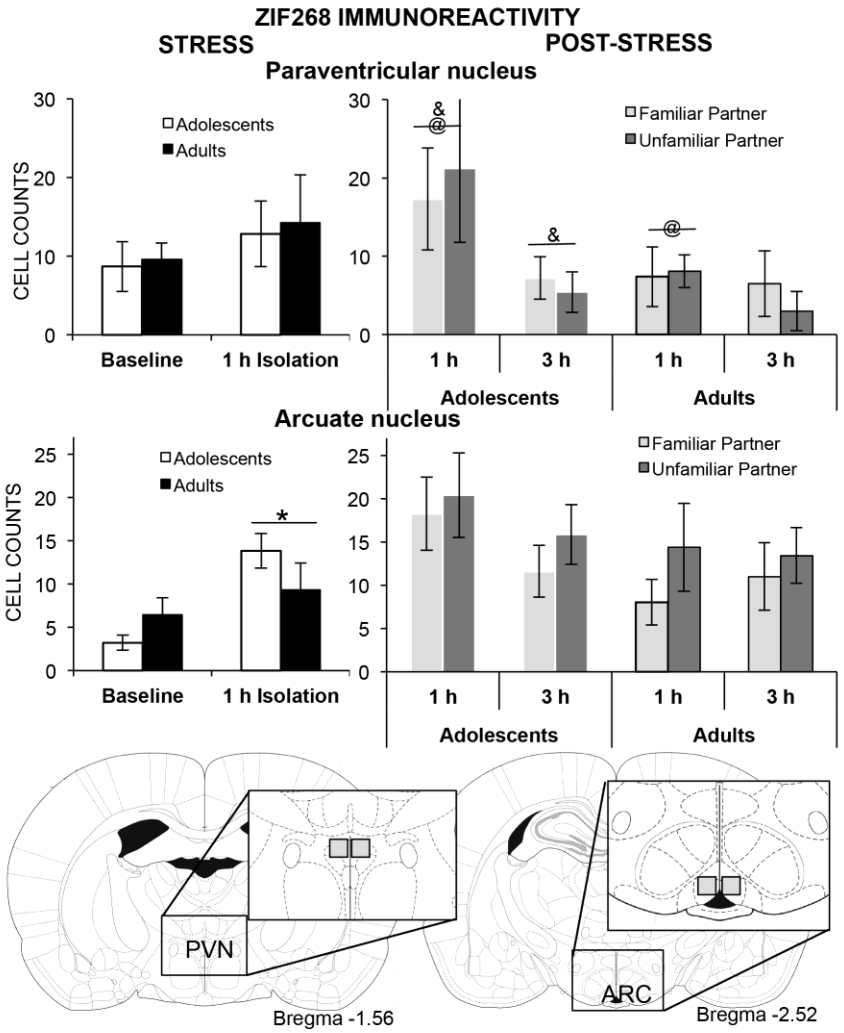

*Fig. 3. Mean (±S.E.M.) Zif268 immunoreactive cell counts in brain regions for adolescent and adult rats before and after 1 h isolation stress and after 1 and 3 h of recovery post-stress with either a familiar or unfamiliar cage partner (n = 5–8 per group).@Higher than 3 h post-stress, collapsed across Age (main effect of Time Post-Stress, p = 0.02);& higher in adolescents (main effect of Age, p = 0.04); *higher than baseline, collapsed across Age (main effect of Time, p = 0.007). Atlas images used for selection of regions of interest (images are used with permission from Paxinos & Watson, The Rat Brain in Stereotaxic Coordinates, 5/e, 2005, Elsevier). Boxes depict the areas with regions of interest located under low magnification that were then photographed at 400× magnification.*

counts found an interaction of PFC region and Age ($F_{2, 86} = 6.03$, $p = 0.006$), and near significant interaction of Age and Partner Familiarity ($F_{1, 43} = 3.35$, $p = 0.08$) and of Age x Partner Familiarity x Time ($F_{1, 43} = 2.95$, $p = 0.09$). Thus, separate analyses were

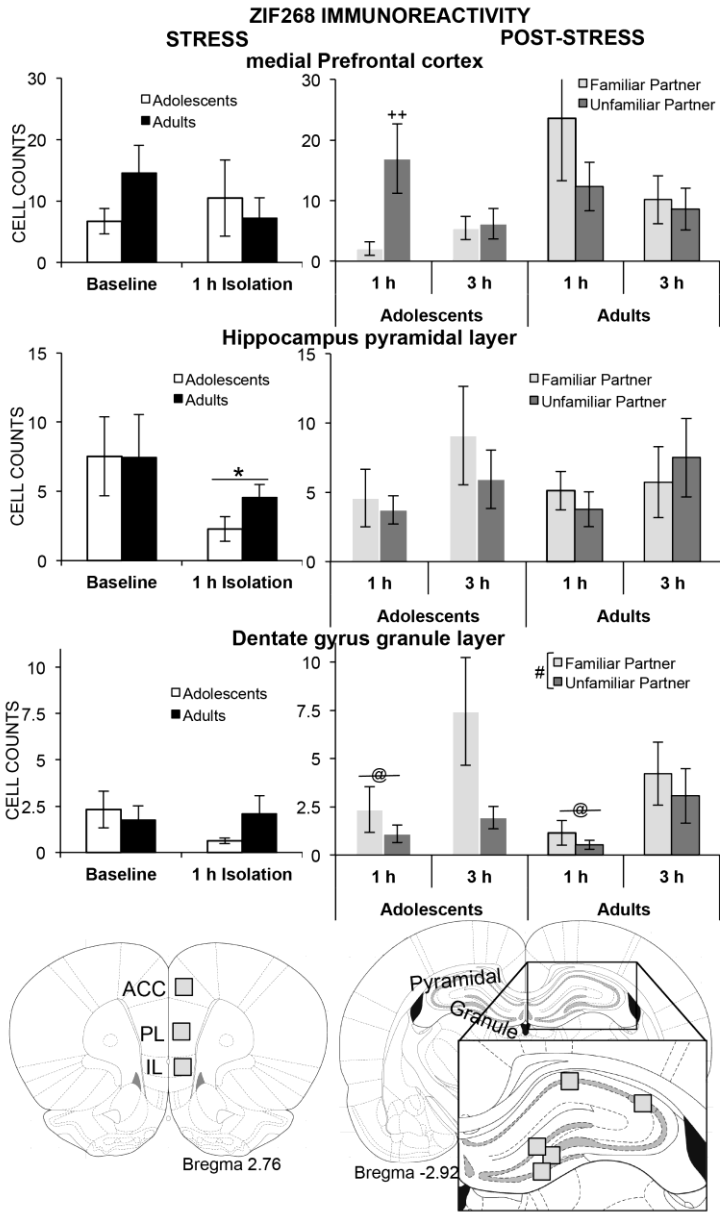

*Fig. 4. Mean (±S.E.M.) Zif268 immunoreactive cell counts in brain regions for adolescent and adult rats before and after 1 h isolation stress and after 1 and 3 h of recovery post-stress with either a familiar or unfamiliar cage partner (n = 5–8 per group).++Higher than in rats with a familiar partner, in adolescents only (p = 0.05); *lower than baseline, collapsed across Age (main effect of Time, p = 0.05);#higher than in rats with an unfamiliar partner, collapsed across Age and Time Post-Stress (main effect of Partner Familiarity, p = 0.03);@lower than 3 h post-stress, collapsed across Age (main effect of Time Post-Stress, p = 0.005). Atlas images used for selection of regions of interest (images are used with permission from Paxinos & Watson, The Rat Brain in Stereotaxic Coordinates, 5/e, 2005, Elsevier). Boxes depict the areas with regions of interest located under low magnification that were then photographed at 400× magnification.*

conducted for each region of the mPFC. Because interactions with age were still significant for the separate regions with those analyses, analyses then were conducted for the age groups separately.

In adolescents, there were higher ir-cell counts in the ACC than in the prelimbic and infralimbic regions ($F_{2, 48} = 9.69$, $p < 0.001$), ir-cell counts were higher in those with an unfamiliar partner than a familiar partner ($F_{1, 24} = 6.52$, $p = 0.02$), and there was an interaction of Partner Familiarity and Time ($F_{1, 24} = 5.38$, $p = 0.03$), with the effect of Partner Familiarity driven by the 1 h after isolation time point ($p = 0.02$, and $p = 0.83$ at 3 h time point). In adults, there were higher ir-cell counts in the ACC than in the prelimbic and infralimbic regions ($F_{2, 38} = 21.47$, $p < 0.001$), but no other main effect or interaction was significant ($p > 0.30$; see Figure 4).

Hippocampal regions. Stress: An Age x Hippocampal Region (Pyramidal, Granule) X Time (baseline, 1 h isolation) ANOVA on Zif268-ir cell counts found an interaction of Hippocampal Region and Time ($F_{1, 25} = 4.62$, $p = 0.04$). Zif268-ir cell counts were lower after 1 h isolation than at baseline in the pyramidal layer ($p = 0.05$) and did not differ by time in the granule layer ($p = 0.34$). Post-stress: There was no main effect of Age, Partner Familiarity (familiar or unfamiliar), Time (1 or 3 h after isolation), or significant interaction among the main effects for cell counts in the pyramidal layer, although the higher ir-cell counts 3 h after isolation compared to 1 h after isolation approached significance ($F_{1, 53} = 3.03$, $p = 0.09$; all other $p > 0.39$). In the granule layer, cell counts were higher 3 h after isolation compared to 1 h after isolation ($F_{1, 53} = 8.75$, $p = 0.005$), and higher when with a familiar cage partner than with an unfamiliar cage

partner ($F_{1, 53} = 4.78$, $p = 0.03$). No other main effect or interaction was significant ($p > 0.20$; see Figure 4).

CeA and BLA. Stress: An Age x Amygdala Region (BLA, CeA) X Time (baseline, 1 h isolation) ANOVA on Zif268-ir cell counts found higher counts in the BLA than in the CeA ($F_{1, 24} = 5.66$, $p = 0.03$) and higher counts at baseline than after isolation ($F_{1, 24} = 4.20$, $p = 0.05$). Post-stress: There was no main effect of Age, Partner Familiarity (familiar or unfamiliar), or Time (1 or 3 h after isolation), or significant interaction among the main effects for cell counts in either the BLA or CeA ($p > 0.14$, except for the interaction of Time and Partner Familiarity = 0.09 in the BLA; see Figure 5).

MeA. Stress: An Age x Medial Amygdala Region (MePD, MePV) X Time (baseline, 1 h isolation) ANOVA on Zif268-ir cell counts found higher counts in the MePV than in the MePD ($F_{1, 23} = 35.41$, $p < 0.001$). Post-stress: The effect of Partner Familiarity approached significance in the MePD ($F_{1, 48} = 3.00$, $p = 0.09$), and the effect of Time approached significance in the MePV ($F_{1, 48} = 2.78$, $p = 0.10$) (all other main effects and interactions $p > 0.25$; see Figure 5).

Discussion

The main results of my thesis are that adolescent and adults did not differ in the effects of social buffering on corticosterone release after an acute stress exposure, although modest age differences were observed in Zif268 expression in response to stress in some brain regions

Corticosterone

Adolescent and adult rats did not differ in baseline corticosterone concentrations. Both groups showed a significant increase in corticosterone concentrations in response to

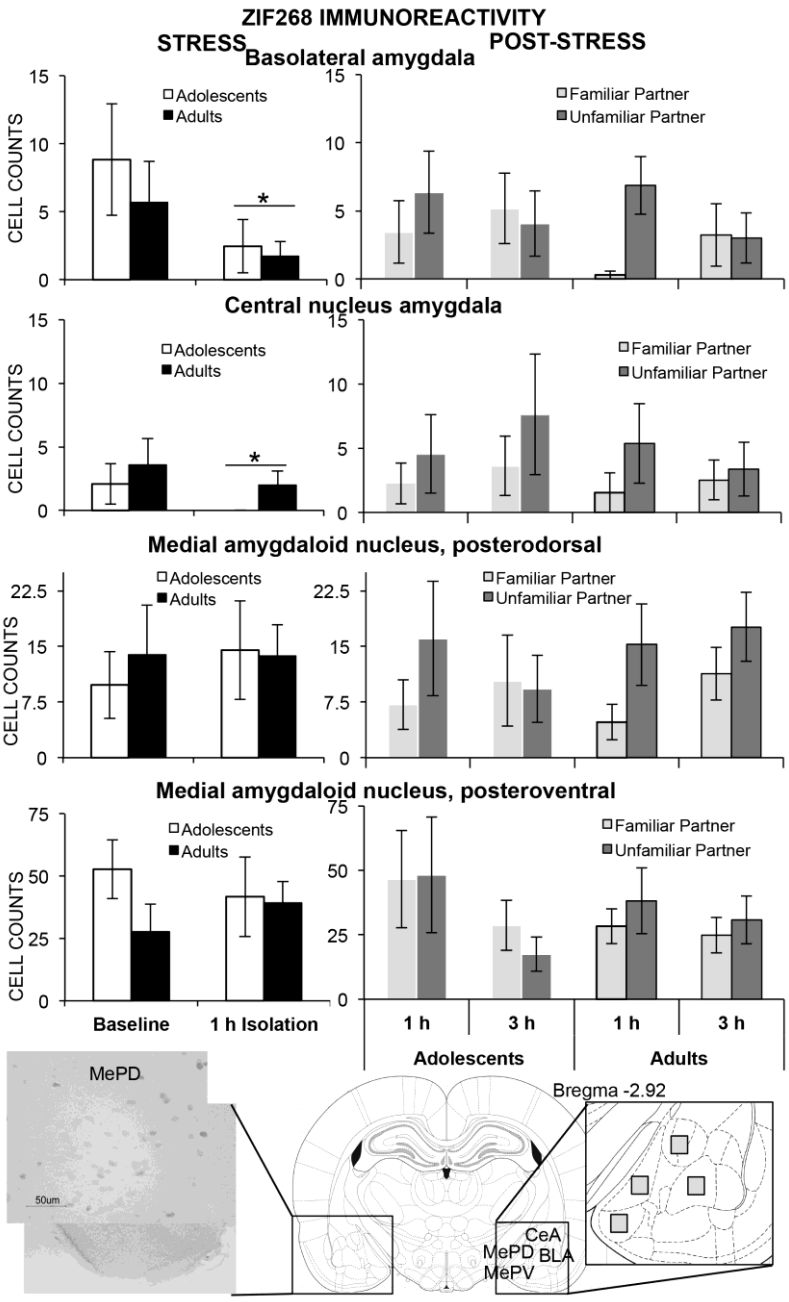

*Fig. 5. Mean (±S.E.M.) Zif268 immunoreactive cell counts in brain regions for adolescent and adult rats before and after 1 h isolation stress and after 1 and 3 h of recovery post-stress with either a familiar or unfamiliar cage partner (n = 5–8 per group). *Lower than baseline, collapsed across Age (main effect of Time, p = 0.05). Atlas image used for selection of regions of interest (images are used with permission from Paxinos & Watson, The Rat Brain in Stereotaxic Coordinates, 5/e, 2005, Elsevier) and an image of aZif268 region of interest at 400× magnification overlaying an image at 100× magnification comparable to the boxed region of the atlas image. Boxes depict the areas with regions of interest located under low magnification that were then photographed at 400× magnification.*

1 hour of isolation stress, with no age difference at this time point either. These findings replicate the results of McCormick et al. (2007) in adolescent rats of an increase in corticosterone immediately after isolation and extend these results by demonstrating a similar increase in adult concentrations. The lack of age differences in the present experiment are in keeping with some previous research using 30 minutes of restraint stress as the stressor (Romeo, Lee, Chhua, McPherson, & McEwen, 2004; Romeo et al., 2006a; Romeo et al., 2006b; Viau et al., 2005; Lui et al., 2012). In contrast, other studies have reported higher corticosterone in adolescent than adult rats 30 minutes after restraint stress (Foilb et al., 2011) and higher corticosterone in adult than adolescent rats 30 minutes after an ethanol injection (Willey et al., 2012). Thus, it is possible that an age difference would have been found had I used an earlier blood sampling time point in my Masters research.

In contrast to my predictions, adolescents and adults did not differ in corticosterone during recovery from the stressor. Many studies have demonstrated a prolonged release of corticosterone following 30 minutes of restraint stress in adolescent rats compared with adult rats (Romeo et al., 2004; Romeo, Lee, & McEwen, 2005; Romeo et al., 2006a; Romeo et al., 2006b; Lui et al., 2012; Foilb et al., 2011; Doremus-Fitzwater, Varlinskaya, & Spear, 2009; Hall & Romeo, 2013). Other studies, however, have reported a greater corticosterone response in adult compared with adolescent rats 30 minutes after an ethanol (Willey et al., 2012) or nicotine injection (Cao et al., 2010) and a more prolonged corticosterone response in adult rats compared with adolescent rats following an injection of lipopolysaccharide (Goble et al., 2011). Thus, differences in adolescent and adult HPA reactivity might have been found if I had used a different

stressor. Moreover, studies that have demonstrated a more prolonged corticosterone release to acute restraint stress in adolescent than adult rats report these results 5-30 minutes following the cessation of the stressor (Lui et al., 2012; Romeo et al., 2005; Romeo et al., 2006a; Romeo et al., 2006b; Hall & Romeo, 2013) and I did not measure corticosterone at those time points. Nevertheless, studies have also reported higher corticosterone concentrations in adolescent than adult rats 60 minutes after the cessation of restraint stress (Romeo et al., 2004; Foilb et al., 2011), which was not the case in my results. Thus, reports on adolescent and adult corticosterone responses to stressors appear to be mixed depending on the type of stressor as well as the time points used for data collection. The mixed results suggest that the important age differences may be in sensory and cognitive neural centres that activate the HPA axis rather than in the HPA axis itself.

My prediction that partner familiarity would facilitate recovery from stress was supported, replicating the results of McCormick et al. (2007). Specifically, adolescents had higher concentrations of corticosterone one hour into the recovery period when paired with an unfamiliar peer than a familiar peer. My results also extended the findings of McCormick et al. (2007) by demonstrating that this effect was observable three hours after isolation and not only one hour after isolation. In addition, I extended these results to adults, and found no difference between adolescents and adults in the effects of partner familiarity. The slower return to baseline corticosterone concentrations in rats paired with an unfamiliar peer than a familiar peer may be because of increased social interactions in rats paired with an unfamiliar peer. For example, past research has reported an increase in corticosterone concentrations for adolescent rats that engaged in social play compared to

adolescents that did not engage in social play (Gordon, Kollack-Walker, Akil, & Panksepp, 2002). Further, another study reported more rough-and-tumble play in adolescent rats paired with an unfamiliar peer than with a familiar peer following 24 hours isolation (Cirulli, Terranova, & Laviola, 1996). Further, McCormick et al. (2007) found that adolescent rats were more active when returned to an unfamiliar partner than to a familiar partner after one hour isolation. Thus, the social buffering effect on corticosterone concentrations may involve reduced levels when with a familiar partner than with an unfamiliar partner.

Testosterone

Consistent with their pre-pubertal status (reviewed in Romeo, 2010), testosterone concentrations were below the limit of detection of the assay in the adolescent rats. Thus, analyses were conducted for adults only. Adult rats did not differ in testosterone concentrations immediately after one hour isolation compared to baseline. They did, however, show an increase in testosterone concentrations one hour into the recovery period compared to the other time points, irrespective of whether rats were with a familiar or unfamiliar peer. Past studies have reported a potentiated release of testosterone in mammals exposed to an acute stressor (reviewed in Chichinadze & Chichinadze, 2008). For example, a study by Foilb et al. (2011) reported an increase in adult testosterone concentrations one hour following the cessation of restraint stress. Thus, in my results, the increase in testosterone one hour into the recovery period might be in response to the stressor.

The greater release of testosterone following stressors may also be driven by the social interactions that take place after adult rats are returned to a cage partner (also

reviewed in Chichinadze & Chichinadze, 2008). Past studies have reported an increase in testosterone concentrations in animals following an agonistic encounter (reviewed in Gleason et al., 2009; Hirschenhauser & Oliveira, 2006; Wingfield, Hegner, Dufty, & Ball, 1990), and attacks on intruders in rodents have been well documented (reviewed in Blanchard & Blanchard, 1977; Blanchard, McKittrick, & Blanchard, 2001; Blanchard, Wall, & Blanchard, 2003). Higher testosterone concentrations were found in rodents that won agonistic encounters than rodents that did not compete in agonistic encounters (Oyegbile & Marler, 2005) and increased plasma testosterone from baseline 10 minutes to 60 minutes after an aggressive encounter has been reported in birds (Wingfield & Wada, 1989). When observing adult rats returned to their unfamiliar or familiar peers after one hour isolation, however, aggressive behaviours were rarely exhibited (Hodges, unpublished observations). This result suggests that something other than agonistic behaviours were increasing testosterone in adult rats.

Adult rats also demonstrated an impeded return to baseline testosterone concentrations three hours into the recovery period when paired with an unfamiliar peer than with a familiar peer. As mentioned above, testosterone concentrations increase in response to agonistic encounters within species (reviewed in Gleason et al., 2009; Hirschenhauser & Oliveira, 2006; Wingfield et al., 1990; Oyegbile & Marler, 2005; Wingfield & Wada, 1989). The effect of partner familiarity three hours into the recovery period, therefore, may be because of more social interactions in adult rats paired with an unfamiliar peer than a familiar peer. Once again, however, not many agonistic behaviours were observed in adult rats during the recovery period (Hodges, unpublished observations) suggesting another mechanism for testosterone release is more likely.

Zif268 expression

Consistent with previous reports of immediate-early-gene activation in adolescent and adult rats exposed to acute stressors (e.g., Romeo et al., 2006a), both adolescent and adult rats showed similar Zif268 expression to one hour isolation compared to baseline in several neural regions. Higher Zif268 ir-cell counts were found in the arcuate nucleus immediately after one hour isolation in both adolescent and adult rats. These findings are consistent with reports of increased Fos expression in the arcuate nucleus after exposure to an acute stressor (Kwon et al., 2006). The arcuate nucleus has projections to the paraventricular nucleus that influence HPA reactivity to stressors (reviewed in Herman & Cullinan, 1997; Senba & Ueyama, 1997).

In contrast with the increase in Zif268 expression in the arcuate nucleus after one hour isolation, my results demonstrated a decrease in ir-cell counts in the pyramidal layer of the hippocampus, in the basolateral amygdala, and in central nucleus of the amygdala of both adolescent and adult rats. A study by Shoji and Mizoguchi (2010) reported lower Fos ir-cell counts in the medial prefrontal cortex, nucleus accumbens, bed nucleus of stria terminalis and amygdala after acute restraint stress in previously stressed aged rats than aged rats that were not previously disturbed. Another study reported a decrease in Fos expression in the amygdala, bed nucleus of stria terminalis, and periaqueductal gray after an acute fluoxetine injection in rats (Lino-de-Oliveira, Sales, Aparecida Del Bel, Silveira, & Guimarães, 2001). Rather than stress effects, the reduced expression may reflect the decrease in sensory stimulation when rats are isolated (reviewed in Lapiz et al., 2003). Moreover, additional brain sampling time points during the one hour isolation might have elucidated more information about Zif268 expression in these neural sites. For example, a

study reported a decrease in Fos expression in the amygdala, pyriform cortex, and tenia tecta of adult rats after two hours of restraint compared to after 15 minutes of restraint (Kellogg, Awatramani, & Piekut, 1998). It should be noted, however, that an increase in immediate-early-gene expression has been reported in the hippocampus and amygdala in different strains of rat exposed to social interactions (Salchner et al., 2004), a different type of restraint (Meyza, Boguszewski, Nikolaev, & Zagrodzka, 2007), and an open field test (Boguszewski & Zagrodzka, 2005). These findings are in keeping with evidence that immediate-early-gene expression depends on the type of, and duration of, the stressor (as reviewed in Kovács, 2008).

Consistent with my predictions and previous reports (Romeo et al., 2006a; Lui et al., 2012), my results did reveal an effect of age on Zif268 expression in the PVN during the recovery period. Past studies measuring Fos have reported higher Fos ir-cell counts in adolescent than adult rats following a stressor (Romeo et al., 2006a; Lui et al., 2012; Novak, Parfitt, Sisk, & Smale, 2007). Zif268 ir-cell counts in the arcuate nucleus followed a pattern similar to the PVN in the recovery period (higher counts in adolescent rats one hour into recovery than adults), which is consistent with reports of similar immediate-early-gene activation patterns to stressors in the PVN and arcuate nucleus (Kwon et al., 2006; Meyza et al., 2007; Cullinan et al., 1995). Both the PVN and arcuate nucleus are important mediators of HPA reactivity to stressors (reviewed in Herman & Cullinan, 1997). The PVN is responsible for regulating the HPA axis when exposed to a stressor whereas the arcuate nucleus has been shown to have inhibitory effects on HPA reactivity and extend neural projections into the PVN (reviewed in Senba & Ueyama, 1997; Kalra et al., 1999). During the recovery period, however, I did not find a difference

in Zif268 expression in the PVN for adolescent and adult rats after isolation when paired with an unfamiliar peer compared with a familiar peer. A study by Kiyokawa et al. (2014b) reported greater Fos expression in the PVN to a fear conditioned stimulus in adult rats paired with an unfamiliar peer than a familiar peer. My results of no difference in Zif268 expression in the PVN during recovery when paired with a familiar or unfamiliar peer, however, may reflect the different stressor used (isolation stress) and the similarities I found in the adolescent and adult rat corticosterone concentrations during recovery.

Inconsistent with my predictions, adolescent and adult rats did not differ in Zif268 ir-cell counts in neural sites other than the PVN and arcuate nucleus during the recovery period. These results may reflect the similarities found in the adolescent and adult rat corticosterone concentrations during recovery. An age difference, however, was found for partner familiarity effects in the medial prefrontal cortex. Only adolescent rats had higher Zif268 ir-cell counts in the medial prefrontal cortex one hour into the recovery period when paired with an unfamiliar peer compared with a familiar peer. A study by Wall et al. (2012) reported an increase in Fos ir-cell counts in the medial prefrontal cortex of group-housed rats exposed to a novel rat compared to an empty cage. Another study reported an increase in c-fos ir-cell counts in the medial prefrontal cortex of rats that were first isolated then exposed to social play than rats alone (van Kerkhof et al., 2013). A decrease in social play and other impairments in social interactions have been reported in pre-pubertal adolescent rats with mPFC lesions than both sham and non-lesioned rats (Schneider & Koch, 2005). Increased Zif268 expression in adolescent rats paired with an unfamiliar peer compared with a familiar peer one hour into the recovery period might

signify exposure to a novel rat or more social interactions, such as social play, with an unfamiliar rat than a familiar rat. Moreover, in the same study, mPFC lesions did not decrease play behaviours in adult rats but did in adolescents (Schneider & Koch, 2005). These findings are consistent with my findings of a partner familiarity effect on mPFC Zif268 expression in adolescent and not in adult rats.

During the recovery period, another effect of partner familiarity was found in the granule layer of the dentate gyrus (higher Zif268 ir-cell counts when paired with a familiar than unfamiliar peer) regardless of age and time after isolation. Past studies have reported inhibited cell proliferation in the dentate gyrus in rodents after social defeat (reviewed in Buwalda et al., 2005). For example, a study reported lower cell proliferation in the dentate gyrus of mice exposed to daily single social defeats than mice either exposed to an unfamiliar peers with no defeat or mice that experienced multiple social defeats daily (Yap et al., 2006). Thus, decreased Zif268 ir-cell counts in the granule layer of the dentate gyrus may be because of more agonistic behaviours in adolescent and adult rats paired with unfamiliar peers than with familiar peers during the recovery period.

In summary, Zif268 expression in response to one hour isolation and after returning to an unfamiliar or familiar peer did not differ between adolescent and adult rats. In response to one hour isolation, Zif268 expression in both adolescent and adult rats decreased in the basolateral amygdala, central nucleus of the amygdala, and pyramidal layer of the hippocampus, while increasing in the arcuate nucleus. In the recovery period, an effect of partner familiarity was found in both adolescent and adult rats (higher Zif268 ir-cell counts in the granule layer of the dentate gyrus for rats returned to a familiar peer than an unfamiliar peer). There were, however, age differences in Zif268 ir-cell counts in

the paraventricular nucleus (higher Zif268 ir-cell counts one hour into the recovery period in adolescent than adult rats) and only an effect of partner familiarity in the medial prefrontal cortex for adolescents (higher Zif268 ir-cell counts one hour into the recovery period with an unfamiliar peer than a familiar peer). Thus, there are slight differences in adolescent and adult neural function in response to one hour isolation and recovery with a familiar or unfamiliar partner regardless of their similar corticosterone concentrations throughout the procedure.

Conclusion

The majority of investigations on social buffering have focused on the maternal buffering of neonatal stress responses, but a number of social interactions (such as interactions with peers) have buffering effects on hypothalamic-pituitary-adrenal function in social species (reviewed in Hennessy et al., 2009; Hostinar, Sullivan, & Gunnar, 2014). One exception is a recent study by Hall and Romeo (2013) that reported that adolescents showed no effect of social buffering on stress responses; a similar recovery was found in ACTH and corticosterone concentrations whether with or without a partner after restraint stress. In contrast, another study by Kiyokawa et al. (2014b) reported less fear (freezing to a tone previously paired with a shock) in adult rats paired with a familiar peer than with an unfamiliar peer at the time of testing. Neither the study by Hall and Romeo (2013) nor the study by Kiyokawa et al. (2014b) involved adults, and so possible differences between adolescents and adults could not be tested. My Master's thesis research indicated that adolescent HPA function is sensitive to the familiarity of the cage partner during stress recovery, replicating the previous report of McCormick et al. (2007), and extending that report by demonstrating similar social buffering effects on

corticosterone concentrations after an acute stressor in both adolescent and adult rats. Although my predictions of an increased expression of Zif268 in the neural sites of adolescents than adults in response to the one hour isolation and return to an unfamiliar or familiar peer were not supported in most neural sites, my results are consistent with reports of similar immediate-early-gene expression in adolescent and adult rats in several neural sites in response to stressors and with my results of similar corticosterone concentrations in response to the procedure in both age groups. It remains possible, though, that differences between adolescents and adults in social buffering effects would emerge under conditions of chronic stress and/or chronic social instability, given the reports of long-lasting detrimental effects of such experiences when encountered in adolescence but not when encountered in adulthood (reviewed in Green & McCormick, 2013c; McCormick & Green, 2013b). I am currently investigating this possibility.

Nevertheless, my findings cast some doubt with regard to the extent to which HPA function is immature in adolescence. In light of growing evidence that the direction of differences in HPA stress responses between adolescents and adults is stressor-specific (reviewed in McCormick, Hodges, & Simone, in press) as well as the extensive evidence of ongoing brain maturation in adolescence (reviewed in Blakemore & Mills, 2014), any differences in stress responses between adolescents and adults more likely reflect age differences in perceptions of the stressor.

References

Armario, A., & Balasch, J. (1981). Corticosterone response in group-housed rats exposed to psychogenic stresses in different social conditions. *Physiology & behavior, 27*(1), 179-181.

Armario, A., Luna, G., & Balasch, J. (1983). The effect of conspecifics on corticoadrenal response of rats to a novel environment. *Behavioral and neural biology, 37*(2), 332-337.

Atkinson, H. C., & Waddell, B. J. (1997). Circadian Variation in Basal Plasma Corticosterone and Adrenocorticotropin in the Rat: Sexual Dimorphism and Changes across the Estrous Cycle 1. *Endocrinology, 138*(9), 3842-3848.

Blakemore, S. J., & Mills, K. L. (2014). Is Adolescence a Sensitive Period for Sociocultural Processing?. *Annual review of psychology, 65*, 187-207.

Blanchard, R. J., & Blanchard, D. C. (1977). Aggressive behavior in the rat. *Behavioral biology, 21*(2), 197-224.

Blanchard, R. J., McKittrick, C. R., & Blanchard, D. C. (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology & Behavior, 73*(3), 261-271.

Blanchard, R. J., Wall, P. M., & Blanchard, D. C. (2003). Problems in the study of rodent aggression. *Hormones and behavior, 44*(3), 161-170.

Blass, E. M. (1987). Critical events during sensitive periods of social development in rats. In M. H. Bornstein (Ed.), *Sensitive periods in development: Interdisciplinary perspectives* (pp. 81-98). Hillsdale, NJ: Erlbaum.

Bock, J., & Braun, K. (1999). Filial imprinting in domestic chicks is associated with spine pruning in the associative area, dorsocaudal neostriatum. *European Journal of Neuroscience*, *11*(7), 2566-2570.

Boguszewski, P., & Zagrodzka, J. (2005). Expression of c-Fos in response to stressogenic stimuli in the amygdala of old vs. young rats—a preliminary study. *Acta Neurobiologiae Experimentalis*, *65*(2), 191-194.

Bornstein, M. H. (1989). Sensitive periods in development: structural characteristics and causal interpretations. *Psychological Bulletin*, *105*(2), 179.

Brainard, M. S., & Knudsen, E. I. (1998). Sensitive periods for visual calibration of the auditory space map in the barn owl optic tectum. *The Journal of Neuroscience*, *18*(10), 3929-3942.

Buwalda, B., Kole, M. H., Veenema, A. H., Huininga, M., De Boer, S. F., Korte, S. M., & Koolhaas, J. M. (2005). Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. *Neuroscience & Biobehavioral Reviews*, *29*(1), 83-97.

Calfa, G., Bussolino, D., & Molina, V. A. (2007). Involvement of the lateral septum and the ventral hippocampus in the emotional sequelae induced by social defeat: role of glucocorticoid receptors. *Behavioural brain research*, *181*(1), 23-34.

Chichinadze, K., & Chichinadze, N. (2008). Stress-induced increase of testosterone: contributions of social status and sympathetic reactivity. *Physiology & behavior*, *94*(4), 595-603.

Cirulli, F., Terranova, M. L., & Laviola, G. (1996). Affiliation in periadolescent rats: behavioral and corticosterone response to social reunion with familiar or unfamiliar partners. *Pharmacology Biochemistry and Behavior, 54*(1), 99-105.

Coburn-Litvak, P. S., Pothakos, K., Tata, D. A., McCloskey, D. P., & Anderson, B. J. (2003). Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. *Neurobiology of learning and memory, 80*(1), 11-23.

Cullinan, W. E., Herman, J. P., Battaglia, D. F., Akil, H., & Watson, S. J. (1995). Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience, 64*(2), 477-505.

Davitz, J. R., & Mason, D. J. (1955). Socially facilitated reduction of a fear response in rats. *Journal of Comparative and Physiological Psychology*, *48*(3), 149.

De Bosscher, K., Van Craenenbroeck, K., Meijer, O. C., & Haegeman, G. (2008). Selective transrepression versus transactivation mechanisms by glucocorticoid receptor modulators in stress and immune systems. *European journal of pharmacology, 583*(2), 290-302.

de Jonge, F. H., Muntjewerff, J. W., Louwerse, A. L., & Van de Poll, N. E. (1988). Sexual behavior and sexual orientation of the female rat after hormonal treatment during various stages of development. *Hormones and behavior*, *22*(1), 100-115.

DeVries, A. C. (2002). Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. *Hormones and Behavior, 41*(4), 405-413.

Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation, 16*(5), 300-317.

Dickerson, S. S., Mycek, P. J., & Zaldivar, F. (2008). Negative social evaluation, but not mere social presence, elicits cortisol responses to a laboratory stressor task. *Health Psychology, 27*(1), 116.

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 & behavior, 97*(3), 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*(3), 153-162.

Evuarherhe, O., Leggett, J., Waite, E., Kershaw, Y., & Lightman, S. (2009). Reversal of the hypothalamo-pituitary–adrenal response to oestrogens around puberty. *Journal of Endocrinology*, *202*(2), 279-285.

Foilb, A. R., Lui, P., & Romeo, R. D. (2011). The transformation of hormonal stress responses throughout puberty and adolescence. *Journal of Endocrinology, 210*(3), 391-398.

Galef, B. G., & Laland, K. N. (2005). Social learning in animals: empirical studies and theoretical models. *Bioscience, 55*(6), 489-499.

Gleason, E. D., Fuxjager, M. J., Oyegbile, T. O., & Marler, C. A. (2009). Testosterone release and social context: when it occurs and why. *Frontiers in neuroendocrinology, 30*(4), 460-469.

Goble, K. H., Bain, Z. A., Padow, V. A., Lui, P., Klein, Z. A., & Romeo, R. D. (2011). Pubertal-Related Changes in Hypothalamic-Pituitary-Adrenal Axis Reactivity and Cytokine Secretion in Response to an Immunological Stressor. *Journal of neuroendocrinology, 23*(2), 129-135.

Gordon, J. A., & Stryker, M. P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. *The Journal of neuroscience, 16*(10), 3274-3286.

Gordon, N. S., Kollack-Walker, S., Akil, H., & Panksepp, J. (2002). Expression of c-fos gene activation during rough and tumble play in juvenile rats. *Brain Research Bulletin, 57*(5), 651-659.

Green, M. R., Barnes, B., & McCormick, C. M. (2013a). Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long–Evans rats. *Developmental psychobiology, 55*(8), 849-859.

Green, M. R, & McCormick, C. M (2013b). Effects of social instability stress in adolescence on long-term, not short-term, spatial memory performance. *Behavioural Brain Research, 256*, 165-171.

Green, M. R., & McCormick, C. M. (2013c). Effects of stressors in adolescence on learning and memory in rodent models. *Hormones and Behavior, 64*, 365-379.

Hall, B. S. and Romeo, R. D. (2013). The influence of poststress social factors on hormonal reactivity in prepubertal male rats. *Developmental Psychobiology*. doi: 10.1002/dev.21190

Hennessy, M. B., Zate, R., & Maken, D. S. (2008). Social buffering of the cortisol response of adult female guinea pigs. *Physiology & behavior*, *93*(4), 883-888.

Hennessy, M. B., Kaiser, S., & Sachser, N. (2009). Social buffering of the stress response: diversity, mechanisms, and functions. *Frontiers in neuroendocrinology*, *30*(4), 470-482.

Herman, J. P., Adams, D., & Prewitt, C. (1995). Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology*, *61*(2), 180-190.

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in neurosciences*, *20*(2), 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 Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201-1213.

Herman, J. P., McKlveen, J. M., Solomon, M. B., Carvalho-Netto, E., & Myers, B. (2012). Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Brazilian Journal of Medical and Biological Research*, *45*, 292-298.

Hess, E. H. (1958). Imprinting in animals. *Scientific American*, *198*(3), 81-90.

Hess, E. H. (1964). Imprinting in Birds Research has borne out the concept of imprinting as a type of learning different from association learning. *Science, 146*(3648), 1128-1139.

Hirschenhauser, K., & Oliveira, R. F. (2006). Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Animal Behaviour*, *71*(2), 265-277.

Hol, T., Van den Berg, C. L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation during the play period in infancy decreases adult social interactions in rats. *Behavioural brain research*, *100*(1), 91-97.

Holloway, K. S., & Suter, R. B. (2004). Play deprivation without social isolation: housing controls. *Developmental psychobiology, 44*(1), 58-67.

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: A review of animal models and human studies across development. *Psychological Bulletin, 140*(1), 256-282.

Immelmann, K. (1975). Ecological significance of imprinting and early learning. *Annual Review of Ecology and Systematics*, *6*(1), 15-37.

Kalra, S. P., Dube, M. G., Pu, S., Xu, B., Horvath, T. L., & Kalra, P. S. (1999). Interacting Appetite-Regulating Pathways in the Hypothalamic Regulation of Body Weight 1. *Endocrine reviews, 20*(1), 68-100.

Kellogg, C. K., Awatramani, G. B., & Piekut, D. T. (1998). Adolescent development alters stressor-induced Fos immunoreactivity in rat brain. *Neuroscience, 83*(3), 681-689.

Kikusui, T., Winslow, J. T., & Mori, Y. (2006). Social buffering: relief from stress and anxiety. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1476), 2215-2228.

Kiyokawa, Y., Kikusui, T., Takeuchi, Y., & Mori, Y. (2004). Partner's stress status influences social buffering effects in rats. *Behavioral neuroscience, 118*(4), 798.

Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2007). Two types of social buffering differentially mitigate conditioned fear responses. *European Journal of Neuroscience, 26*(12), 3606-3613.

Kiyokawa, Y., Hiroshima, S., Takeuchi, Y., & Mori, Y. (2014a). Social buffering reduces male rats' behavioral and corticosterone responses to a conditioned stimulus. *Hormones and behavior, 65*(2), 114-118.

Kiyokawa, Y., Honda, A., Takeuchi, Y., & Mori, Y. (2014b). A familiar conspecific is more effective than an unfamiliar conspecific for social buffering of conditioned fear responses in male rats. *Behavioural Brain Research, Behavioural brain research, 267*, 189-193.

Klein, Z. A., Padow, V. A., & Romeo, R. D. (2010). The effects of stress on play and home cage behaviors in adolescent male rats. *Developmental psychobiology, 52*(1), 62-70.

Kollack-Walker, S., & Newman, S. W. (1995). Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. *Neuroscience, 66*(3), 721-736.

Kovács, K. J. (1998). Invited review c-Fos as a transcription factor: a stressful (re) view from a functional map. *Neurochemistry international, 33*(4), 287-297.

Kovács, K. J. (2008). Measurement of Immediate-Early Gene Activation-c-fos and Beyond. *Journal of neuroendocrinology, 20*(6), 665-672.

Knapska, E., & Kaczmarek, L. (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK?. *Progress in neurobiology, 74*(4), 183-211.

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience, 16*(8), 1412-1425.

Kwon, M. S., Seo, Y. J., Shim, E. J., Choi, S. S., Lee, J. Y., & Suh, H. W. (2006). The effect of single or repeated restraint stress on several signal molecules in paraventricular nucleus, arcuate nucleus and locus coeruleus. *Neuroscience, 142*(4), 1281-1292.

Lapiz, M. D. S., Fulford, A., Muchimapura, S., Mason, R., Parker, T., & Marsden, C. A. (2003). Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. *Neuroscience and behavioral physiology, 33*(1), 13-29.

Lino-de-Oliveira, C., Sales, A. J., Aparecida Del Bel, E., Silveira, M. C. L., & Guimarães, F. S. (2001). Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression. *Brain research bulletin, 55*(6), 747-754.

Lui, P., Padow, V. A., Franco, D., Hall, B. S., Park, B., Klein, Z. A., & Romeo, R. D. (2012). Divergent stress-induced neuroendocrine and behavioral responses prior to puberty. *Physiology & behavior, 107*(1), 104-111.

Oyegbile, T. O., & Marler, C. A. (2005). Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Hormones and behavior, 48*(3), 259-267.

Martinez, M., Phillips, P. J., & Herbert, J. (1998). Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *European Journal of Neuroscience, 10*(1), 20-33.

Martinez, M., Calvo-Torrent, A., & Herbert, J. (2002). Mapping brain response to social stress in rodents with c-fos expression: a review. *Stress: The International Journal on the Biology of Stress, 5*(1), 3-13.

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*(2), 116-126.

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*(2), 228-238.

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*(5), 756-765.

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*(6), 1300-1312.

McCormick, C. M., Green, M. R., Cameron, N. M., Nixon, F., Levy, M. J., & Clark, R. A. (2013a). Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats. *Hormones and behavior, 63*(1), 5-12.

McCormick, C. M., & Green, M. R. (2013b). From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. *Neuroscience, 249*, 242-257.

McCormick, C. M., Hodges, T. E., & Simone, J. J. (2014). Peer pressures: Social instability stress in adolescence and social deficits in adulthood in a rodent model. *Developmental Cognitive Neuroscience*. In press.

McEwen, B. S. (2004). Protection and Damage from Acute and Chronic Stress: Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. *Annals of the New York Academy of Sciences, 1032*, 1-7.

McEwen, B. S., Eiland, L., Hunter, R. G., & Miller, M. M. (2012). Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology, 62*(1), 3-12.

Meaney, M. J., & Stewart, J. (1981). A descriptive study of social development in the rat (Rattus norvegicus). *Animal Behaviour, 29*(1), 34-45.

Meyza, K. Z., Boguszewski, P. M., Nikolaev, E., & Zagrodzka, J. (2007). The effect of age on the dynamics and the level of c-Fos activation in response to acute restraint in Lewis rats. *Behavioural brain research, 180*(2), 183-189.

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*(1), 46-56.

Novak, C. M., Parfitt, D. B., Sisk, C. L., & Smale, L. (2007). Associations between behavior, hormones, and Fos responses to novelty differ in pre-and post-pubertal grass rats. *Physiology & behavior, 90*(1), 125-132.

Numan, M., Numan, M. J., Marzella, S. R., & Palumbo, A. (1998). Expression of c-fos, fos B, and egr-1 in the medial preoptic area and bed nucleus of the stria terminalis during maternal behavior in rats. *Brain research, 792*(2), 348-352.

Ramsay, A. O., & Hess, E. H. (1954). A laboratory approach to the study of imprinting. *Wilson Bull, 66*, 196-206.

Resko, J. A., & Roselli, C. E. (1997). Prenatal hormones organize sex differences of the neuroendocrine reproductive system: observations on guinea pigs and nonhuman primates. *Cellular and molecular neurobiology, 17*(6), 627-648.

Romeo, R. D., Lee, S. J., Chhua, N., McPherson, C. R., & McEwen, B. S. (2004). Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology, 79*(3), 125-132.

Romeo, R. D., Lee, S. J., & McEwen, B. S. (2005). Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology, 80*(6), 387-393.

Romeo, R. D., Bellani, R., Karatsoreos, I. N., Chhua, N., Vernov, M., Conrad, C. D., & McEwen, B. S. (2006a). Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal axis plasticity. *Endocrinology, 147*(4), 1664-1674.

Romeo, R. D., Karatsoreos, I. N., & McEwen, B. S. (2006). Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. *Hormones and behavior, 50*(3), 463-468.

Romeo, R. D., Karatsoreos, I. N., Jasnow, A. M., & McEwen, B. S. (2007). Age-and stress-induced changes in corticotropin-releasing hormone mRNA expression in the paraventricular nucleus of the hypothalamus. *Neuroendocrinology, 85*(4), 199-206.

Romeo, R. D. (2010). Pubertal maturation and programming of hypothalamic–pituitary–adrenal reactivity. *Frontiers in neuroendocrinology, 31*(2), 232-240.

Romeo, R. D., Minhas, S., Svirsky, S. E., Hall, B. S., Savenkova, M., & Karatsoreos, I. N. (2014). Pubertal Shifts in Adrenal Responsiveness to Stress and Adrenocorticotropic Hormone in Male Rats. *Psychoneuroendocrinology*. In press.

Samuelsen, C. L., & Meredith, M. (2009). Categorization of biologically relevant chemical signals in the medial amygdala. *Brain research, 1263*, 33-42.

Schneider, M., & Koch, M. (2005). Deficient social and play behavior in juvenile and adult rats after neonatal cortical lesion: effects of chronic pubertal cannabinoid treatment. *Neuropsychopharmacology, 30*(5), 944-957.

Schramm-Sapyta, N. L., Cha, Y. M., Chaudhry, S., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. *Psychopharmacology, 191*(4), 867-877.

Schulz, K. M., Molenda-Figueira, H. A., & Sisk, C. L. (2009). Back to the future: the organizational–activational hypothesis adapted to puberty and adolescence. *Hormones and behavior*, *55*(5), 597-604.

Schulz, K. M., & Sisk, C. L. (2006). Pubertal hormones, the adolescent brain, and the maturation of social behaviors: lessons from the Syrian hamster. *Molecular and cellular endocrinology*, *254*, 120-126.

Scott, J. P., Stewart, J. M., & De Ghett, V. J. (1974). Critical periods in the organization of systems. *Developmental Psychobiology*, *7*(6), 489-513.

Senba, E., & Ueyama, T. (1997). Stress-induced expression of immediate early genes in the brain and peripheral organs of the rat. *Neuroscience research*, *29*(3), 183-207.

Shoji, H., & Mizoguchi, K. (2010). Acute and repeated stress differentially regulates behavioral, endocrine, neural parameters relevant to emotional and stress response in young and aged rats. *Behavioural brain research*, *211*(2), 169-177.

Silva-Gomez, A. B., Rojas, D., Juarez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain research*, *983*(1), 128-136.

Sisk, C. L., & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature neuroscience*, *7*(10), 1040-1047.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, *24*(4), 417-463.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet*, *354*(9188), 1435-1439.

Sturman, D. A., & Moghaddam, B. (2011). The neurobiology of adolescence: changes in brain architecture, functional dynamics, and behavioral tendencies. *Neuroscience & Biobehavioral Reviews*, *35*(8), 1704-1712.

Terranova, M. L., Cirulli, F., & Laviola, G. (1999). Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. *Psychoneuroendocrinology, 24*(6), 639-656.

Thorsteinsson, E. B., James, J. E., & Gregg, M. E. (1998). Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology*, *17*(5), 436.

Tzschentke, B., & Plagemann, A. (2006). Imprinting and critical periods in early development. *World's poultry science journal*, *62*(04), 626-637.

Van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental psychobiology, 34*(2), 129-138.

Vanderschuren, L. J., Niesink, R. J., & Van Pee, J. M. (1997). The neurobiology of social play behavior in rats. *Neuroscience & Biobehavioral Reviews, 21*(3), 309-326.

van Kerkhof, L. W., Trezza, V., Mulder, T., Gao, P., Voorn, P., & Vanderschuren, L. J. (2013). Cellular activation in limbic brain systems during social play behaviour in rats. *Brain Structure and Function*, 1-31.

Varlinskaya, E. I., & Spear, L. P. (2008). Social interactions in adolescent and adult Sprague–Dawley rats: impact of social deprivation and test context familiarity. *Behavioural brain research, 188*(2), 398-405.

Varlinskaya, E. I., Vogt, B. A., & Spear, L. P. (2013). Social context induces two unique patterns of c-Fos expression in adolescent and adult rats. *Developmental psychobiology, 55*(7), 684-697.

Vázquez, D. M. (1998). Stress and the developing limbic–hypothalamic–pituitary–adrenal axis. *Psychoneuroendocrinology, 23*(7), 663-700.

Viau, V., Bingham, B., Davis, J., Lee, P., & Wong, M. (2005). Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology, 146*(1), 137-146.

Wada, H. (2008). Glucocorticoids: mediators of vertebrate ontogenetic transitions. *General and comparative endocrinology, 156*(3), 441-453.

Wall, V. L., Fischer, E. K., & Bland, S. T. (2012). Isolation rearing attenuates social interaction-induced expression of immediate early gene protein products in the medial prefrontal cortex of male and female rats. *Physiology & behavior, 107*(3), 440-450.

Weathington, J. M., Strahan, J. A., & Cooke, B. M. (2012). Social experience induces sex-specific fos expression in the amygdala of the juvenile rat. *Hormones and behavior, 62*(2), 154-161.

Willey, A. R., Anderson, R. I., Morales, M., Ramirez, R. L., & Spear, L. P. (2012). Effects of ethanol administration on corticosterone levels in adolescent and adult rats. *Alcohol, 46*(1), 29-36.

Wilson, J. H. (2000). A conspecific attenuates prolactin responses to open-field exposure in rats. *Hormones and behavior, 38*(1), 39-43.

Wingfield, J. C., & Wada, M. (1989). Changes in plasma levels of testosterone during male-male interactions in the song sparrow, Melospiza melodia: time course and specificity of response. *Journal of Comparative Physiology A, 166*(2), 189-194.

Wingfield, J. C., Hegner, R. E., & Dufty, A. M. AM Jr., and GF Ball. (1990). The ‘‘challenge hypothesis’’: Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist, 136*, 829-846.

Yap, J. J., Takase, L. F., Kochman, L. J., Fornal, C. A., Miczek, K. A., & Jacobs, B. L. (2006). Repeated brief social defeat episodes in mice: effects on cell proliferation in the dentate gyrus. *Behavioural brain research, 172*(2), 344-350.

Yee, J. R., Cavigelli, S. A., Delgado, B., & McClintock, M. K. (2008). Reciprocal affiliation among adolescent rats during a mild group stressor predicts mammary tumors and lifespan. *Psychosomatic medicine, 70*(9), 1050-1059.