DIFFERENTIAL INTERACTIONS BETWEEN ENVIRONMENTAL ENRICHMENT AND STRESSOR EXPERIENCES IN MICE: IMPACT ON BEHAVIOUR, NEUROENDOCRINE AND NEURCHEMICAL FUNCTIONS

by

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Abstract

Environmental enrichment is associated with numerous beneficial effects, including protection against the adverse behavioural and biological effects of stressors. However, enrichment has also been shown to induce aggression in male mice, reducing the well being of these animals. The current investigations examined the impact of enrichment on subsequent stressor exposure in two different strains of male mice. An aggressive strain, (CD-1) and a less aggressive, but highly anxious strain (BALB/cByJ) of mice were used. The impact of enrichment on locomotor and anxiety-like behaviours, corticosterone levels and monoamine concentrations in response to stress were measured. It appeared that enrichment in group-housed male mice promotes aggression, and may exaggerate biological processes in response to stress, possibly increasing vulnerability to stressor-related outcomes. In contrast, enriched mice housed individually do not display stress-induced exaggerated responses, further supporting the conclusion that adverse effects of enrichment develop owing to the aggressive behaviours elicited by that treatment.

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Introduction

Stressful life events can alter neuroendocrine, neurotransmitter, and immune functioning, thus contributing to the precipitation and development of psychopathologies, such as anxiety and depressive illnesses (Anisman & Merali, 1999, 2003; McEwen, 2000). Conversely, it is thought that a positive or enriched environment may have the opposite effect, by acting as a protective factor against the harmful consequences of stressors (Fox, Merali & Harrison, 2006).

Environmental enrichment in humans includes early nutritional, educational and physical exercise programs. It has been shown that children who partake in enrichment display fewer pathological traits as adults (for example; lower scores for schizotypal personality and antisocial behaviours) compared to control children who did not receive enrichment (Raine, Mellingen, Liu, Venables, & Mednick, 2003). Furthermore, early childhood intervention programs (which involved emotional and educational support) were found to buffer against the negative effects on intellectual and emotional development associated with family stress (Ramey & Ramey, 1998).

The positive effects of environmental enrichment are not restricted to humans. Enrichment paradigms have frequently been used in animal studies, revealing beneficial effects on both behavioural and physiological parameters. For example, rodents raised in an enriched environment display enhanced learning and memory (Pacteau, Einon, & Sinden, 1989), are more resistant to various brain insults that otherwise promote seizures (Young, Lawlor, Leone, Dragunow, & During, 1999), and exhibit facilitated recovery from brain lesions (Will, Rosenzeig, Bennett, Hebert, & Morimoto,1977). These differences in behaviour are associated with changes in brain anatomy, such as increased

dendritic branching (Pham, Winblad, Granholm, & Mohammed, 2002) and enhanced neurogenesis (Kempermann, Kuhn, & Gage, 1998). Interestingly, many of the brain changes elicited by enrichment during development have also been detected when the enrichment is provided to an adult animal (van Praag, Kempermann, & Gage, 2000).

Although the enrichment effects on the brain have been well established, the impact of environmental enrichment on emotionality and the stress response are still somewhat questionable (Roy, Belzung, Delarue, & Chapillon, 2001). The preponderance of evidence has suggested that environmental enrichment does protect animals from the adverse effects of stressors and can limit the development of stress-induced behavioural changes, including decreased fear and anxiety-like behaviours (Benarolya-Milshtein et al., 2004; Chapillon, Manneche, Belzung, & Caston, 1999; Fox et al., 2006). However, it has also been reported that enrichment in male mice might induce aggression, causing severe wounding in subordinates, and ultimately reducing the well being of these animals (Hamesish, Voss, & Gartner, 1994; Howerton, Garner, & Mench, 2008; Van Loo et al., 2002). Similarly, the impact of enrichment on the stressor hormone corticosterone has also proven inconsistent: some studies reported that enrichment increased resting corticosterone levels (Marashi, Barnekow, Ossendorf, & Sachser, 2003; Van Loo et al., 2002), whereas others indicated that there were no differences in basal corticosterone levels between enriched and non-enriched animals (Roy et al., 2001).

What is enrichment?

In laboratory animals, an environment considered 'enriched' typically consists of group housing in large cages which affords the opportunity for complex social interaction. The environment is also highly structured, with tunnels, nesting materials,

shelters, as well as running wheels that can provide an opportunity for physical and cognitive activity (van Praag et al., 2000). It was Hebb, in the late 1940's who first demonstrated that rats raised in an enriched environment showed improved maze learning in adulthood compared to their littermates who were housed in standard laboratory conditions (Hebb, 1947).

Different aspects of enrichment

In humans, social support is an important factor in reducing vulnerability to affective disorders, as it can minimize psychological distress (Lepore, 1992). It has similarly been demonstrated that housing rats in groups diminished the anxiogenic effects ordinarily elicited by a stressor (Andrade & Guimaraes, 2003). In addition to social forms of enrichment, material providing nestling, shelter opportunities, and exercise have also been found to be important components of enrichment in animals.

For instance, mice have a strong preference for nesting material when given the choice between this and other forms of enrichment (Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1998). Nesting material allows mice to have some control over their living conditions which may lead to more effective coping, i.e., engagement in behavioural responses that reduce the effect of stressful situations (Wechsler, 1995). Furthermore, having nesting material was also found to reduce agonistic behaviours between group-housed mice (Van Loo et al., 2002). Interestingly, although it affects animal behaviours, nesting material does not appear to alter physiological parameters such as urine and plasma corticosterone, and only slightly impacted body weight (Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997). Providing a 'super-enriched' environment (i.e., a more complex cage with extra

shelters and toys) increased play behaviour compared to enriched or standard housed conditions (Marashi et al., 2003). In fact, mice were found to be willing to work for access to a number of different resources, such as a structured cage as well as a cage that contains a running wheel (Olsson & Dahlborn, 2002). Indeed, it has been reported that simply having access to a running wheel was sufficient to enhance neurogenesis, doubling the total number of surviving newborn cells in the dentate gyrus of the hippocampus (van Praag, Kempermann, Gage, 1999). Interestingly, enhanced neurogenesis has recently been shown to be a critical feature of environmental enrichment which might be responsible for alleviating depressive-like behaviours in response to a chronic social stressor (Schloesser, Lehmann, Martinowich, Manji, & Hekenham, 2010).

Effect of Enrichment on Emotionality

Although environmental enrichment may prevent the adverse outcomes of stressors, its specific effects on emotionality are uncertain (Roy et al, 2001). In the elevated plus-maze, a paradigm where behaviours displayed in the stressful open arms versus the protective enclosed arms of the maze are measured to reflect anxiety levels (Pellow, Chopin, File, & Briley, 1985), enriched animals spent more time in the open arms, and more frequently entered into the open arms relative to controls, indicating lower anxiety levels (Benarolya-Milshtein et al., 2004; Chapillon et al., 1999; Friske & Gammie, 2005). Contrary to this finding, however, the opposite result has also been reported; that enrichment mice tended to spend more time in the enclosed arms and less in the open arms compared to standard-housed mice (Roy et al., 2001), and exhibited higher activity levels compared to standard housed mice when tested in an open field

(Roy et al., 2001). Further, when exposed to a stressor involving reminders of foot shock, enriched mice displayed reduced freezing compared to standard-housed mice (Benarolya-Milshtein et al., 2004).

The effect of exposure to a predator or predator odor, which has been shown to elicit strong fearful and anxiety-like behaviours (Zandrossi & File, 1992), has also been examined in conjunction with environmental enrichment. In line with the preceding reports, enriched rats spent an increased amount of time in proximity to a predator (cat) compared to the animals housed in standard cages, which was indicative of reduced anxiety (Klein, Lambert, Durr, Schaefer, & Waring, 1994). In contrast, however, enriched and standard mice showed no differences in stress or anxiety-related behaviours when exposed to cat feces (Roy et al., 2001).

The effects of enrichment on emotionality have also been examined using the forced-swim test (FST) (Brenes, Rodriguez, & Fornaguera, 2008). The FST has been used to gauge depression-related responses by quantifying immobility time in an inescapable water environment as a measure of 'behavioural despair', a symptom observed in human depression (Krishnan & Nestler, 2008). It was found that enriched rats showed the lowest immobility and the highest levels of swimming in the FST when compared to rats living in group housing and isolation. In contrast, isolated animals displayed the highest immobility and lowest swimming levels. It was thus suggested that environmental enrichment produces an antidepressive-like effect when compared to the isolated group of rats (Brenes et al., 2008). However, it should be noted that the FST is more appropriately used as a screen for antidepressant drugs, rather than a test of

depression (Petit-Demouliere, Chenu, & Bourin, 2005) and hence these data ought to be considered cautiously.

Effects of Enrichment on circulating corticosterone

Like emotionality, the impact of enrichment on basal corticosterone levels has proven inconsistent. It has been reported that basal corticosterone levels among enriched and standard housed mice did not differ from one another; however, after exposure to a stressor such as cat feces odour, standard-housed mice had higher corticosterone levels compared to their enriched counterparts (Roy et al., 2001). Other researchers, however, demonstrated that enriched mice had higher basal corticosterone levels compared to standard-housed mice (Benarolya-Milshtein et al., 2004; Marashi et al., 2003; Van Loo et al., 2002), but, after exposure to cues associated with previous electric shock (reminder stimuli), the enriched mice did not exhibit an increase of corticosterone levels as the stressed standard mice did (Benarolya-Milshtein et al., 2004). It was suggested that the higher basal corticosterone levels for the enriched mice may be a result of increased activity in a complex cage (Olsson & Dahlborn, 2002), and that the absence of a corticosterone increase in enriched mice after the stressor may reflect a diminished general reactivity to stressful experiences (Benarolya-Milshtein et al., 2004).

Effect of enrichment on the Brain

Effect of enrichment on brain neurotrophins. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) are important for neuronal development and survival, and it may be that they act as a protector by slowing down cell loss (Alderson, Alterman, Barde, & Lindsay, 1990). Interestingly, while psychological stressors have been associated with reductions of BDNF in the hippocampus (Kozlovsky, et al., 2007),

environmental enrichment was shown to increase BDNF as well as nerve growth factor (NGF) in the hippocampus (Pham et al., 2002). Exercise might be a crucial component of enrichment involved in the BDNF increases, as rats given access to only a running wheel showed upregulation of BDNF mRNA expression within the hippocampus (Neeper, Gomez-Pinilla, Choi, & Cotman, 1995). Interestingly, it was also shown that abrupt deprivation of habitual running in rats can lead to long-lasting reductions of both mRNA expression of BDNF and its receptor, TrkB, in the hippocampus (Widenfalk, Olson, & Thoren, 1999). Variations of BDNF mRNA levels have also been found in enriched environments that do not include a running wheel. Rats group-housed in large cages with various toys showed enhanced cognitive performance in the Morris water maze and increased expression of BDNF mRNA in the hippocampus compared to rats subjected to impoverished/isolated housing (Falkerberg et al., 1992).

It appears that BDNF upregulation might be important to the enhanced hippocampal neurogenesis (Rossi et al., 2006), which is known to occur following exposure to an enriched environment (Kempermann et al., 1998). Enriched mice which were BDNF knockouts failed to show the two-fold increase in hippocampal neurogenesis apparent in their enriched wild-type counterparts, indicating that BDNF is necessary for hippocampal neurogenesis to take place in enriched mice (Rossi et al., 2006).

Effects of enrichment on brain monoamine levels. Although the many positive effects environmental enrichment has on brain morphology have been well established (Pham et al., 2002), the impact of enrichment on brain monoamines has been less understood. Monoamine levels and utilization are altered in response to stressors (Anisman & Zacharko, 1992; Hayley, Borowski, Merali, & Anisman, 2001), and it might

thus be of importance to determine the impact of enrichment in modulating stressor-provoked monoamine variations. One investigation revealed that mice living in enrichment had increased norepinephrine (NE) concentrations in the parieto-temporo-occipital cortex, cerebellum, and pons/medulla oblongata, whereas no changes in the concentrations of NE, serotonin (5-HT), or dopamine (DA) occurred in the hippocampus and prefrontal cortex (PFC) (Naka, Shiga, Yaguchi, & Okado, 2002). It was likewise reported that rats housed in an enriched environment did not differ from standard and isolated animals in levels of NE in the PFC; however, increased levels of NE were evident in the ventral striatum (VS). Furthermore, elevated levels of 5-HT were found in the PFC, although no differences were found with its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in this same brain region, and there were no variations with respect to 5-HT and 5-HIAA in the VS (Brenes et al., 2008). It was suggested that the augmented levels of 5-HT in the PFC and NE levels in the VS may be implicated in antidepressant-like effects associated with environmental enrichment (Brenes et al., 2008).

It is not entirely clear whether exercise is an important component of enrichment involved in monoamine changes or, if other features of enrichment play a role as well (van Praag et al., 2000). It has been found that NE levels in the PFC were comparable at baseline for rats with and without access to running wheels; however, after footshock, rats with access to running wheels had lower NE levels, indicating that exercise might have blunted NE release in response to stress (Soares et al., 1999). Thus it seems likely that running may be an important contributor to the monoamine variations elicited by environmental enrichment.

The effects of Enrichment on the Immune System

Different housing conditions have also proven to influence immune functioning (Rabin, Lyte, & Hamil, 1987), such as natural killer cell activity (Benarolya-Milshtein et al., 2004), which play an important role in host resistance to viral infections (Shavit et al., 1985). Cytokines (immune signalling molecules) are of particular interest because pro-inflammatory cytokines can elicit stressor-like effects and have been implicated in the development of major depressive disorder (Anisman & Merali, 2003; Anisman, Merali, & Hayley, 2008). A super-enriched environment has been shown not to influence levels of IL-2, IL-4, IL-10, and IFN-gamma in the spleen relative to that evident in standard-housed mice. However, the super-enriched mice had lower ratios of pro- and anti-inflammatory cytokines (e.g., IFN-gamma/IL-10) compared to the standard housed mice, reflecting a skewed immune response, which may be less adequate for protection against viral infections (Marashi et al., 2003). Unexpectedly, few studies have examined the effects of enrichment on cytokines in either blood or brain, a fact that is surprising given that enrichment has been implicated as a protective factor against stressors, and stressors are known to elicit variations of both peripheral and central pro-inflammatory cytokines (Audet, Mangano, & Anisman, 2010; Anisman & Merali, 2003).

Taken together, enrichment seems to have many beneficial effects that, alone or in combination, may help prevent or limit stressor-induced behaviours or pathologies. However, as will be discussed in the next sections, enrichment in some circumstances may also negatively impact behaviours and biological systems, particularly in male mice.

With Good comes Bad: Does Enrichment promote Aggression?

It has been shown that enrichment in group-housed males can promote aggression which can be relatively severe and may negatively affect the well being of animals (Haemisch et al., 1994; Howerton et al., 2008; Van Loo et al., 2002). However, this appears to be relevant only for male mice and not male rats housed in enrichment. For example, mice experiencing aggression in enriched environments were shown to display an increased number of wounds and decreased weight gain (Van Loo et al., 2002). Interestingly, a significant increase in agonistic encounters has not only been reported in an enriched environment but also in super-enriched housing compared to standard housing conditions (Marashi et al., 2003). Aggression promoted by enrichment may be due, in part, to competition for highly preferred or valuable components of the enriched environment, such as running wheels, in which case certain animals may be denied access (Nevison, Hurst, & Barnard, 1999). Specifically, giving mice a shelter with a running wheel attached significantly increased the amount of escalated aggression compared to the standard housed mice, and this effect was apparent irrespective of whether the wheel was fixed in place or used for running (Howerton et al., 2008). Another explanation for enrichment-induced aggression may relate to divisions created by rigid forms of enrichment and the resulting creation of territories that mice might strive to defend (Sandnabba, 1997).

The aggression promoted by environmental enrichment not only differs between specific forms of enrichment but also among different strains of mice. Pronounced strain differences were found between BALB/cN and CD-1 male mice placed in enriched environments, wherein the CD-1 mice were more aggressive (e.g., increased frequency

and duration of agonistic encounters, shorter latency until first agonistic encounter), and had higher testosterone levels than the BALB/cN mice (Van Loo et al., 2003). The BALB/cN mice, however, had more injuries due to fighting, although the wounds were smaller and less severe than among the CD-1 mice (Van Loo et al., 2003).

Should enrichment include group housing for male mice? As enrichment that includes group-housing promotes aggression, the question remains as whether male mice living in an enriched environment should be housed alone or in groups. It is possible that for particularly aggressive strains, enrichment may only be beneficial for singly housed mice (Howerton et al., 2008). However, individual housing has also been shown to be stressful for rodents (Saenz, Villagr, & Trias, 2006). Further analysis has been conducted to determine if male mice would have a preference for social contact or prefer to live alone. Mice that were given the choice between an empty or an inhabited cage showed a clear preference for the proximity of another male. Even subordinate mice preferred their dominant cage-mate over an empty cage, as did dominant mice for their subordinate cage-mate, confirming that male mice prefer to live with other male mice, regardless of their social status (Van Loo, De Groot, Van Zutphen, & Baumans, 2001).

Modulation of Aggression. Aggression in group housed male mice can be influenced by different factors such as cage dimensions and group size. It appeared that aggression escalated with increasing number of mice housed together and with decreasing floor area per animal (Butler, 1980; Poole & Morgan, 1976). In contrast to these findings, it was reported that although reducing group size is an effective way of decreasing aggression, a larger cage may actually promote a moderate increase in aggressive behaviours. Group size; however, seems to be a much more influential factor

on aggressive behaviours when compared to cage dimension. Therefore, housing male mice in smaller groups (three to five) may be an effective way of decreasing wound severity and chances of encountering stressful aggressive interactions (Van Loo, Mol, Koolhaas, Van Zutphen, & Baumans, 2001).

Enrichment and Social Defeat Stress

Stressors of a social nature have very powerful effects, including the development of psychopathology in humans (Miczek, Yap, & Covington III, 2008). Consequently, animal models of depression and anxiety disorders have increasingly focused on psychosocial stressors. In particular, rodents that have been exposed to a social conflict and defeated displayed behavioural alterations similar to depression, such as motivational disturbances and anhedonia (Becker et al., 2008). Moreover, after one or three social defeat episodes, defeated mice displayed increased plasma corticosterone as well as elevated 5-HT and NE utilization in the PFC and hippocampus compared to non-stressed mice (Audet & Anisman, 2010). In addition, plasma levels and brain expression of pro-inflammatory cytokines were altered in defeated mice (Audet et al., 2010). If enrichment has positive effects on an organism's well being, defined in terms of attenuation of stressor-provoked biological changes, then it might be expected that enrichment would act to buffer against the effects of social defeat in mice. However, it is also possible that enrichment, if it promotes aggression, could sensitize the stressor-related systems so that the known effects of social defeat may be enhanced in enriched animals that have been aggressed during housing.

The Present Investigations

The first study (Manuscript 1) was conducted with CD-1 male mice, a relatively

aggressive strain (Parmigiani, Palanza, Rodgers, & Ferrari, 1999) in which dominance hierarchies are readily evident (Audet & Anisman, 2010). These mice were group-housed in either an enriched environment (EE) or a standard environment (SE) and we examined whether enrichment would, in fact, increase aggressive behaviours (Experiment 1). Experiment 2 assessed whether housing in an enriched environment would alter plasma corticosterone, as well as monoamine concentrations in the PFC and hippocampus in response to a mild stressor applied 4 weeks after housing conditions commenced. It was predicted that male CD-1 mice housed in an enriched environment would show increased aggressiveness in their home-cage as well as elevated corticosterone and NE activity in response to a mild stressor compared to mice housed in a standard environment. In fact, these experiments revealed that the CD-1 mice housed in enrichment were very aggressive towards one another, resulting in significant wounding and the removal of many overly aggressive mice from the studies. For these reasons, subsequent experiments used BALB/cByJ mice, a highly stress-vulnerable strain (Anisman, Lacosta, McIntyre, Kent, & Merali, 1998) and one that is only moderately aggressive (Van Loo et al., 2003).

The following investigations (Manuscript 2) commenced with Experiment 1, designed to examine whether group-housed male BALB/cByJ mice living in environmental enrichment for 4 weeks would influence basal anxiety-like behaviours in the elevated plus-maze. Furthermore, we examined whether housing male mice in groups (Experiment 2) in an enriched versus a standard environment would influence plasma corticosterone levels as well as hippocampal and amygdala monoamine variations in response to a more potent stressor, social defeat. We predicted that group-housing male mice in enrichment would increase aggressive behaviours, and thus result in exaggerated

stressor-related outcomes in response to social defeat stress. Lastly, Experiment 2 was repeated; however, all mice were housed individually in enriched or standard environments to eliminate the possibility of aggression in the enriched enrichment (Experiment 3). We predicted that enriched mice housed individually would not reveal the exaggerated stress responses and instead, enrichment in this case might act to buffer against the effects of social defeat.

Environmental enrichment in mice promotes aggressive behaviors and elevated corticosterone and brain norepinephrine activity in response to a mild stressor

MANUSCRIPT

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Abstract

Housing rodents in an enriched environment has typically been thought to have positive effects on an animal's well being and cognitive functioning. However, in some strains of mice, enriched environments have also been reported to elicit aggression and to promote stress-related outcomes. In the current investigations we examined whether an enriched environment would elicit aggression among CD-1 male mice and thus sensitize responses to a subsequent mild stressor. It was first observed that mice housed in an enriched environment (EE) for 2 weeks displayed more aggressive behaviors than did mice that had been housed in a standard environment (SE). In a second experiment it was noted that after 4 weeks of housing in an EE or SE, mice exhibited comparable plasma corticosterone concentrations as well as levels of brain norepinephrine and its metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG) in the absence of a challenge. However, upon exposure to mild stressor (placement in a novel cage), EE mice were more active and displayed higher corticosterone concentrations as well as enhanced MHPG accumulation in the prefrontal cortex (PFC) and hippocampus relative to their SE counterparts. It seems that enrichment in male mice promotes aggression, and may sensitize biological processes possibly increasing vulnerability to stressor-related outcomes.

Keywords: *Aggression, corticosterone, environmental enrichment, norepinephrine, sensitization, stress*

INTRODUCTION

Stressful events influence neuroendocrine and brain neurotransmitter activity, and might thus contribute to the precipitation and exacerbation of psychopathologies, including anxiety and depression (Anisman et al., 2008; McEwen, 2008). Some of the adverse effects of stressors, however, can be attenuated among animals housed in an enriched environment (Fox et al., 2006). In this respect, the beneficial effects of enrichment have been reported in relation to enhanced cognitive performance and increased expression of brain-derived neurotrophic factor (BDNF) mRNA (Falkerberg et al., 1992; van Praag et al., 2000), dendritic branching (Pham et al., 2002), and neurogenesis (Kempermann et al., 1998) in the hippocampus.

The impact of enrichment on emotionality (Roy et al., 2001) and markers of distress (Fox et al., 2006) appear to be less consistent. For example, resting corticosterone levels among enriched mice have been reported to be either increased (Van Loo et al., 2002; Marashi et al., 2003; Benarolya-Milshtein et al., 2004), decreased, (Belz et al., 2003) or unchanged (Van de Weerd et al., 1997; Roy et al., 2001) compared to their standard-housed counterparts. Furthermore, enrichment in male mice was shown to promote aggression, causing wounding and stress-related physiological changes, thus creating a potentially stressful situation and reducing the well being of these animals (Van Loo et al., 2002; Howerton et al., 2008).

Of the varied neurochemical changes elicited by stressors, elevated turnover of norepinephrine (NE) within the prefrontal cortex (PFC) and hippocampus is particularly reliable and has been related to several behavioral disturbances (Anisman et al., 2008). Specifically, in response to acute stressors the concentrations of NE and of its metabolite,

3-methoxy-4-hydroxyphenylglycol (MHPG) increase appreciably, and if the stressor is sufficiently severe, NE concentrations may decline owing to synthesis of the amine not keeping pace with its utilization (Anisman et al., 2008). Although stress-provoked NE and MHPG changes are relatively transient, the processes underlying monoamine functioning is subject to sensitization, so that subsequent exposure to mild stressors may provoke more pronounced amine variations than in animals that had not previously been stressed. Indeed, it seems that even mild stressors that ordinarily have little effect may elicit marked monoamine variations in previously stressed animals (Anisman et al., 2003; Maier & Watkins, 2005).

The current investigations examined whether housing male mice in an enriched environment would, in fact, increase aggressive behaviours (Experiment 1). Moreover, we assessed whether relatively protracted housing in an enriched environment would alter basal plasma corticosterone, as well as NE and MHPG concentrations in the PFC and hippocampus, and whether this housing condition would exacerbate the effects of a mild stressor applied later (Experiment 2). Specifically, it was hypothesized that CD-1 male mice housed in an enriched environment would display more aggressive behaviors than mice housed in a standard environment. Further, it was predicted that basal corticosterone and NE activity would be elevated among enriched mice, and that these outcomes would be enhanced in response to a mild stressor.

MATERIALS AND METHODS

Animals and housing procedures

For both experiments naïve male CD-1 mice, aged 6-8 weeks, were obtained from Charles River Canada (St. Constant, QC, Canada) and housed (3-4/cage) upon arrival in

either an enriched environment (EE) or a standard environment (SE). The EE consisted of large rat maternity polypropylene cages (50 x 40 x 20 cm) equipped with two running wheels, one red polypropylene shelter, one orange polypropylene shelter with an angled running wheel, as well as three yellow polypropylene tunnels and two cotton nestlets. The SE consisted of standard polypropylene cages (27 x 21 x 14 cm) with only one cotton nestlet. All mice were kept in the same room, on a 12-hr light-dark cycle (lights on: 0800-2000 hr) in a temperature-(21°C) and humidity-controlled (63%) room and given *ad libitum* access to food and tap water.

In an attempt to minimize stress associated with novel objects (Lehman & Herkenham, 2011), enrichment items were not changed over the course of the experiment. In Experiment 1, home-cage aggressive behaviours in SE and EE were scored daily for 2 weeks and commenced immediately upon arrival of mice. In Experiment 2, mice were left undisturbed in their respective environments for 4 weeks, with the exception of routine cage cleaning once/week. All experimental procedures were approved by the Carleton University Animal Care Committee and met the guidelines set out by the Canadian Council on Animal Care.

Experiment 1: Behavioral scoring

Mice were identified by tail markings. Frequency and duration of aggressive interactions were scored daily over a 5-min interval in real-time. A description of each aggressive encounter was also documented so that the interactions could be categorized as attacks, aggressive chasing, or aggressive grooming, all resulting in submissive behaviors in the targeted mouse. Attacks included biting, sometimes accompanied by tail rattling. Aggressive chasing included rushing or leaping at another mouse. Finally,

aggressive grooming was defined as one mouse vigorously licking and nipping a second mouse (n=31 mice/group).

Experiment 2: Behavioural testing

Testing procedures were conducted between 08:30 and 13:00 hours to minimize effects related to diurnal factors. After 4 weeks of living in their assigned environments, mice were brought to the testing area to acclimate for a 24-hr period. On the test day, half of the mice in the EE and SE were exposed to a mild stressor that consisted of being taken from their home-cage and placed individually in a clear novel cage (standard size: 27 x 21 x 14 cm) with fresh bedding. The remaining mice were not disturbed and stayed in their home-cage (n=10/group). During the novel cage stressor, motor activity was monitored through a MicroMax system (AccuScan Instruments Inc, OH, USA) composed of metal frames equipped with 16 pairs of infrared photobeam sensors (Model 20-MMAM), a distribution hub (Model 20-MMDB), and an analyzer (Model 20-MMA). The total number of beam interruptions during the 45-min period was used as the activity index.

Blood collection and brain removal

Immediately after the 45-min novel cage exposure (or at corresponding times for mice in the no stressor conditions), mice were brought to a different room where they underwent rapid decapitation without anaesthesia. Trunk blood was then immediately collected in tubes containing 10 mg EDTA, centrifuged, and the plasma stored at -80 °C for subsequent corticosterone determination.

Brains were rapidly removed, flash-frozen in isopropanol and placed on dry ice for 20 seconds. Frozen brains were then placed on a stainless-steel brain matrix (2.5 x

3.75 x 2.0 cm) positioned on a block of ice that rested on dry ice. The matrix has a series of slots spaced 500 μm apart that guide razor blades to provide coronal brain sections. Once the brains were sliced, tissue from the PFC and hippocampus was collected following the mouse atlas of Franklin & Paxinos (1997). Tissue punches were placed in 0.3 M monochloroacetic acid containing 10% methanol and internal standards and were stored at -80 °C for subsequent determination of NE and MHPG.

Corticosterone determination

A commercial radioimmunoassay (RIA) kit (ICN Biomedicals Inc., USA) was used to determine plasma corticosterone concentrations (in duplicate). Assays were performed in a single run to prevent inter-assay variability; the intra-assay variability was less than 10%. The assay sensitivity was 1.7 ng/ml.

Determination of NE and MHPG concentrations

High-performance liquid chromatography (HPLC) was used to determine concentrations of NE and MHPG. Tissue punches were sonicated in solution obtained from a stock solution which contained 14.17 g monochloroacetic acid, 0.0186 g EDTA, 5.0 ml methanol and 500 ml HPLC grade water. Following centrifugation, a 20 μl aliquot of the supernatant was passed at a flow rate of 1.5 ml/min (1400–1600 p.s.i.) through a system containing a M-600 pump (Milford, USA), guard column, radial compression column (5 m, C18 reverse phase, 8 mm x10 cm), and a 3-cell coulometric electrochemical detector (ESA model 5100A). For separation, a mobile phase was used comprising of 1.3 g heptane sulfonic acid, 0.1 g disodium EDTA, 6.5 ml triethylamine, and 35 ml acetonitrile. The mobile phase was then filtered using 0.22-mm filter paper, degassed, and the pH level was adjusted to 2.5 with phosphoric acid. The area and height

of the peaks were determined using a Hewlett-Packard integrator. A protein analysis kit (Pierce Scientific, Canada), and a spectrophotometer (Brinkman, PC800 colorimeter) in conjunction with bicinchoninic acid were used to measure protein concentrations of each sample. Neurotransmitter concentrations were based on protein levels. The lower limit of detection for the monoamines and metabolites was 5.0 pg/ml.

Data Analyses

For Experiment 1, aggressive outcomes were analysed through a one-way analysis of variance (ANOVA) (Housing: Standard vs. Enriched). In Experiment 2, locomotor activity was analysed through a one-way ANOVA (Housing) and plasma corticosterone concentrations as well as NE and MHPG concentrations in the PFC and hippocampus were analysed through a 2 (Housing) x 2 (Stressor: No stressor vs. Novel cage stressor) between-groups ANOVAs. Follow-up comparisons comprised of t-tests with a Bonferonni correction to maintain the alpha level at 0.05. In addition, Pearson's correlation coefficients were determined between plasma corticosterone, NE and MHPG concentrations within PFC and hippocampus.

RESULTS

Experiment 1

For ethical reasons, highly aggressive mice, defined as those that repeatedly attacked other mice to the extent that injury occurred or was imminent, were removed from both EE and SE cages. When this was done, a new dominant mouse typically emerged and on some occasions, this mouse became excessively aggressive and also had to be removed from the study. After just 5 days, of the 31 mice in each condition, more of the EE mice (11) than the SE mice (4) were removed from the study ($\chi^2 = 4.31, p < 0.05$),

attesting to the aggressive behavior associated with the enriched conditions (see Table 1). Because a high number of aggressive mice had been removed at the beginning of Day 5, aggressive scores were analyzed for Days 1 to 4 only. Specifically, each category of aggressive encounters was scored every day and then averaged. Analyses of aggressive encounters during the 5-min daily assessment period indicated that more aggressive encounters were scored in EE (7.75 ± 3.25) than SE mice (3.75 ± 1.25), but this difference was not significant, $F(1, 6) = 1.32$. Aggressive chases, however, were significantly more common in EE mice than in SE mice, $F(1, 6) = 6.82$, $p < 0.05$. Furthermore, EE mice displayed significantly more wounds than did their SE counterparts, $F(1, 6) = 7.56$, $p < 0.05$.

Table 1.

Mean ± SEM Number of Aggressive Encounters in the Home-cages of EE (Enriched Environment) and SE (Standard Environment) mice.

	Agonistic Encounters	Attacks	Aggressive Chases*	Aggressive Grooms	Wounds*
EE	7.75 ± 3.25	6.00 ± 2.90	1.25 ± 0.48*	0.25 ± 0.25	5.50 ±1.89*
SE	3.75 ±1.25	3.25 ±1.25	0.00 ±0.00	0.00 ± 0.00	0.25 ± 0.25

* $p < .05$ relative to mice housed in standard environments.

Experiment 2

As depicted in Figure 1, enriched mice appeared more active than their SE counterparts during exposure to the novel cage, although this effect was not significant $F(1, 16) = 3.39, p = 0.08$.

Plasma corticosterone concentrations varied as a function of the interaction between Housing and Stressor, $F(1, 37) = 5.88, p < 0.05$. Follow-up comparisons of the simple effects comprising this interaction indicated that after 30 days of EE and SE housing, hormone concentrations in the absence of a further stressor were comparable in the two conditions. However, placement in the novel cage markedly increased corticosterone in EE mice compared to their SE counterparts, $p < 0.01$ (see Figure 2). In contrast, after the 45-min novel cage stressor, plasma corticosterone concentrations were not changed in mice that had been housed in standard cages.

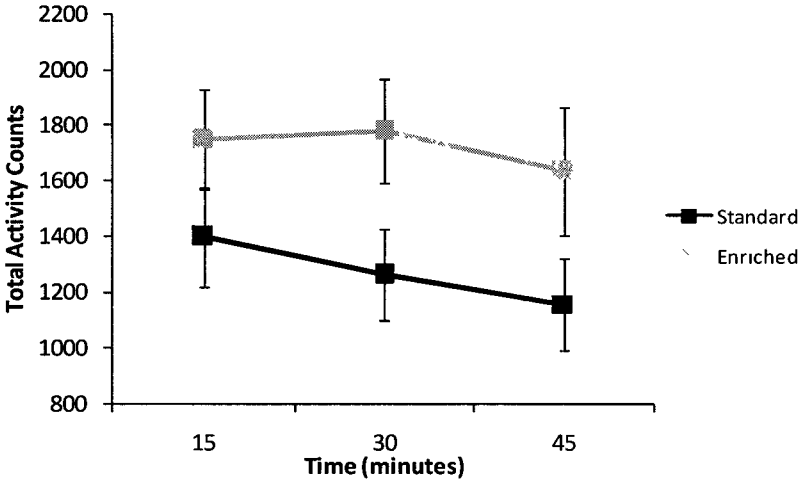

Figure 1. Home-cage locomotor activity over 15-min blocks of time (B1 to B3) in EE (Enriched Environment) and SE (Standard Environment) mice that had been exposed to a novel cage stressor for 45-min. Data are represented by means $\pm$ S.E.M.

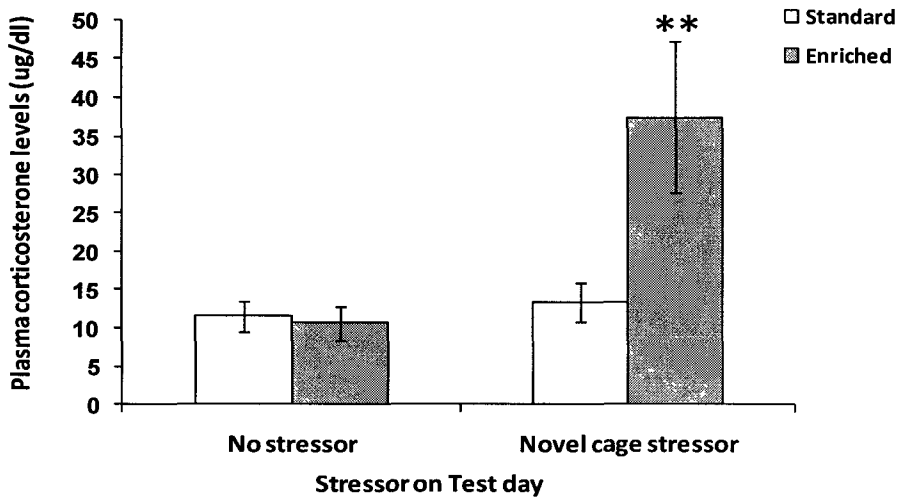

Figure 2. Plasma corticosterone concentrations (μg/dl) collected immediately after a 45-min novel cage stressor experience (Novel cage stressor) or at a corresponding time in controls (No stressor) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means $\pm$ S.E.M. ** $p < 0.01$ relative to mice that had been housed in standard conditions.

Variations of MHPG concentrations (top panels) in the PFC and hippocampus were strikingly similar (Figures 3 and 4, respectively). Within both the PFC and hippocampus, MHPG accumulation varied as a function of the Housing x Stressor interaction, $F(1, 36) = 3.96$ and 3.87, $p = 0.05$ and 0.058, respectively. Based on *a priori* predictions, follow-up comparisons of MHPG changes were conducted of the simple effects comprising these interactions. These analyses paralleled those evident with respect to corticosterone which indicated that in the absence of a further treatment, MHPG concentrations within the two brain structures were comparable in EE and SE mice. After the 45-min novel cage stressor, however, MHPG concentrations within both regions were elevated in EE mice relative to SE mice (p's < 0.05). Unlike MHPG variations, concentrations of the monoamine NE within the PFC and hippocampus (middle panels) were not significantly affected by the housing or stressor conditions.

Within the PFC, MHPG/NE ratio (bottom panels) varied as a function of the Housing x Stressor interaction, $F(1, 36) = 7.29$, $p < 0.01$. Follow-up comparisons of the simple effects comprising this interaction showed that NE turnover among SE mice was not significantly affected by the novel cage stressor, whereas among EE mice a significant increase of MHPG/NE ratio was apparent in stressed relative to non-stressed mice. Thus, after the novel cage stressor, the MHPG/NE ratio was increased in EE mice compared to SE mice ($p < 0.01$). Within the hippocampus, in contrast, MHPG/NE ratio was only increased by the stressor administered on test day, $F(1, 36) = 3.95$, $p < 0.05$, irrespective of whether mice had been housed in SE or EE.

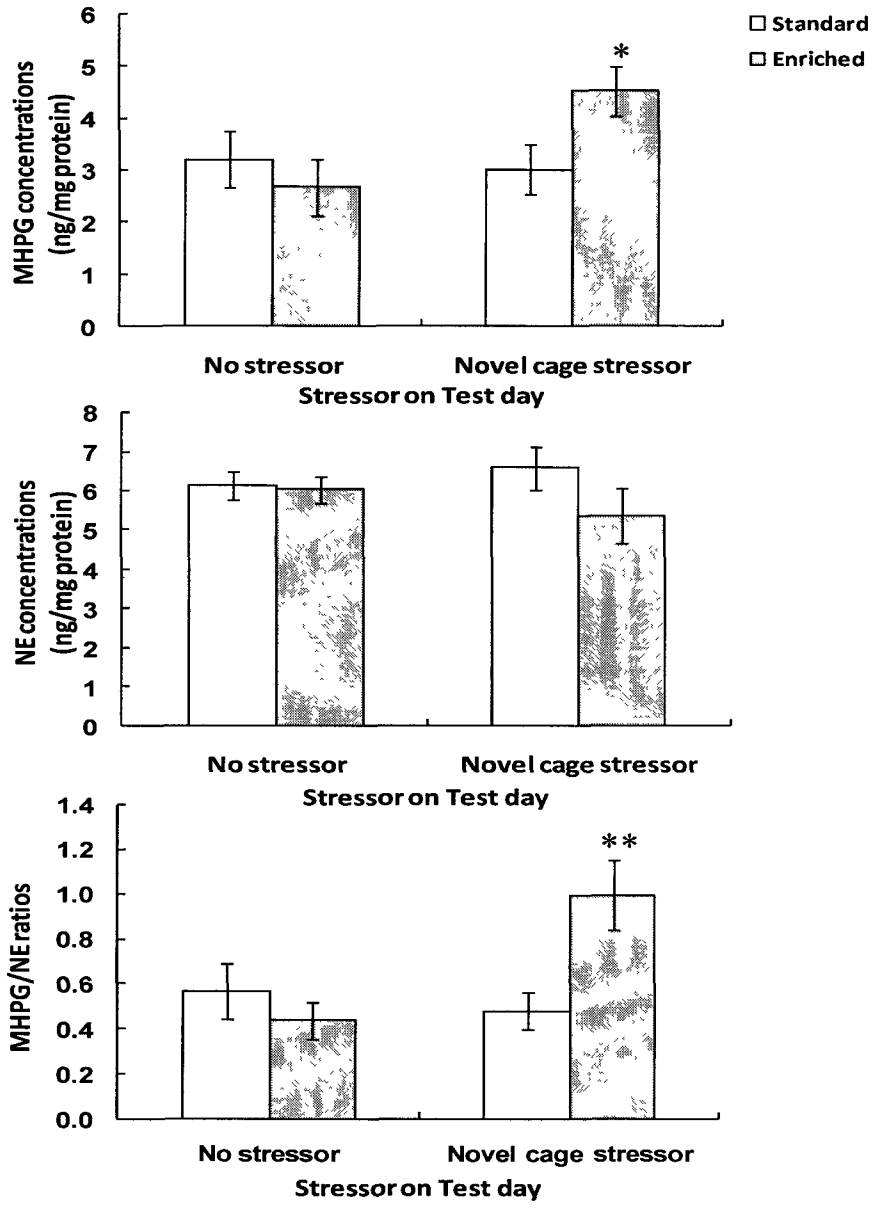

Figure 3. Concentrations of MHPG and NE as well as MHPH/NE ratios within the prefrontal cortex collected immediately after a 45-min novel cage stressor experience (Novel cage stressor) or at a corresponding time in controls (No stressor) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means $\pm$ S.E.M. * $p < 0.05$ and ** $p < 0.01$ relative to mice that had been housed in standard conditions.

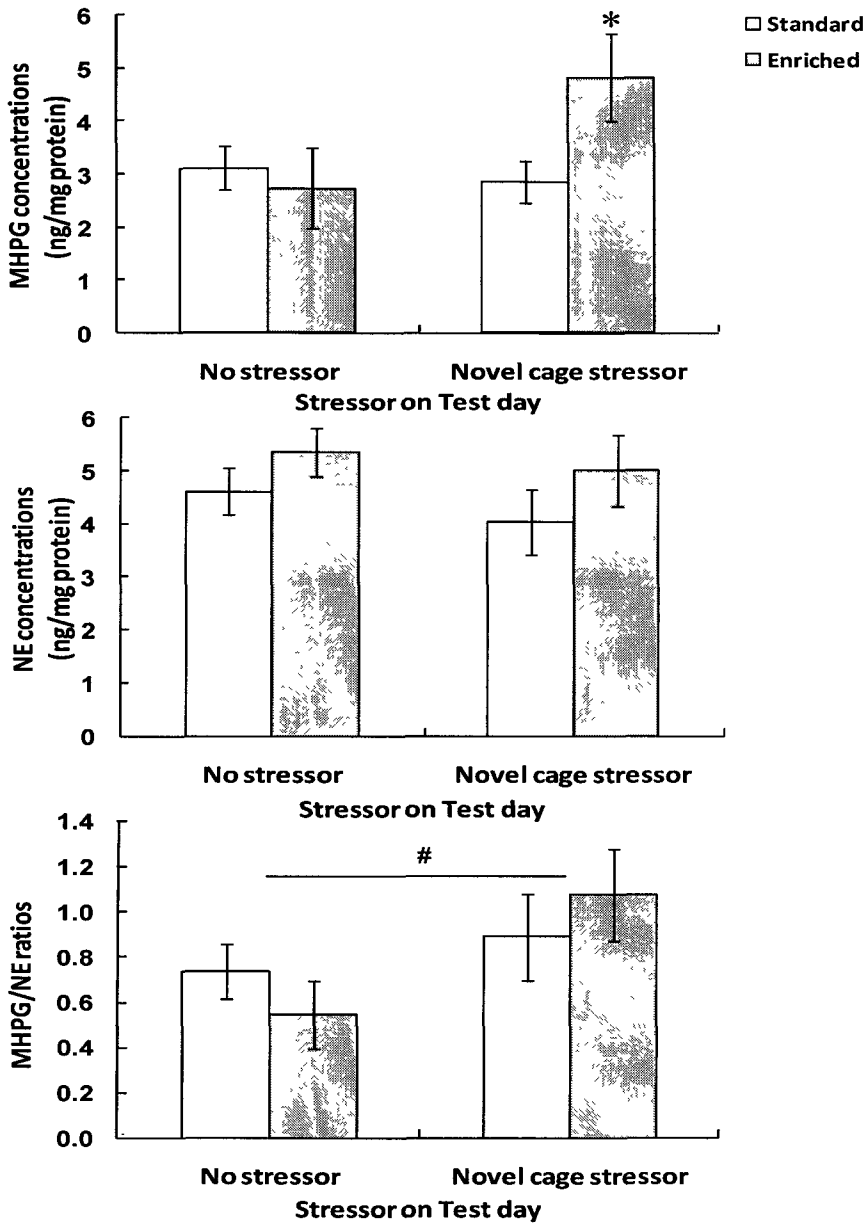

Figure 4. Concentrations of MHPG and NE as well as MHPH/NE ratios within the hippocampus collected immediately after a 45-min novel cage stressor (Novel cage stressor) or at a corresponding time in controls (No stressor) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means $\pm$ S.E.M. * $p < 0.05$ relative to mice that had been housed in standard conditions. # $p < 0.05$ relative to mice that had not been stressed on test day.

Correlation analyses performed on all groups indicated that plasma corticosterone concentrations were positively correlated with MHPG concentrations in both the PFC ($r = 0.43, p < 0.01$) and hippocampus ($r = 0.33, p < 0 .05$), likely suggesting that these reflected general stress responses. As well, MHPG concentrations in the PFC was negatively correlated with NE concentrations in the same brain region ($r = -0.36, p < 0.05$). Although these data are correlational, they are consistent with the view that high levels of utilization elicited by a stressor, reflected by MHPG accumulation, favoured the concentration of NE declining. Interestingly, when the correlation analyses included only EE mice exposed to the novel cage stressor, the correlations between MHPG and NE concentrations within the PFC were particularly strong ($r = -0.85, p < 0.01$).

DISCUSSION

Consistent with previous reports (Marashi et al. 2003; Howerton et al., 2008), environmental enrichment in the current investigation promoted high levels of aggression among male mice, as reflected by the increased number of aggressive chases that occurred in EE mice, the elevated number of highly aggressive EE mice that were removed during the observation period of Experiment 1, and the wounding reported almost exclusively in the EE mice. Unfortunately, it was not possible to conduct an analysis between social status, (i.e. submissive/dominant mice) corticosterone and monoamine concentrations due to the very unstable social hierarchies observed, especially as several overly aggressive mice were necessarily removed from the study. A less stable social hierarchy has previously been reported in EE mice compared to their SE counterparts, and has been associated with both higher levels of stress and aggression (Haemisch et al., 1994). It is thus possible that in the present investigation, the unstable

hierarchy apparent in EE cages was stressful and contributed to increased levels of aggression in enriched mice.

Housing mice in enriched conditions did not alter basal circulating corticosterone or NE utilization and turnover within the PFC and hippocampus. However, when confronted with a mild stressor, these stress markers appeared sensitized in mice that had been previously housed in EE conditions. As well, EE mice appeared slightly more active when they were placed in the novel environment. It thus seems that EE mice might be more reactive to the effects of a subsequent stressor than mice that had been housed in standard conditions. This might be particularly pertinent considering that sensitization of neuroendocrine and neurochemical processes have been implicated in the development and the recurrence of anxiety- and depressive-like symptoms (Post, 1992; Anisman et al., 2003).

Interestingly, within the PFC, NE utilization and turnover in EE mice was elevated after mild stressor exposure and actual transmitter concentrations appeared slightly lower compared to their SE counterparts. Consistent with the notion that a stressor might reduce NE concentrations if utilization levels are excessive, prefrontal MHPG accumulation was inversely related to NE concentrations, and this was especially notable among EE mice. In the present investigation the stressor administered on the test day was relatively mild, however, if a more intense stressor (e.g., social defeat) had been used, prefrontal NE functioning might have been overly taxed (i.e., allostatic overload; McEwen et al., 2008) leading to significant amine reductions. Interestingly, NE turnover in the hippocampus was influenced only by the novel cage stressor, and was not affected by whether mice had been housed in EE or SE conditions. This raises the possibility that

the PFC, at least in relation to NE neurotransmission, might be more sensitive to aggression, as previously suggested (Audet & Anisman, 2010).

Mice have been reported to have a strong liking for enrichment items, especially for running wheels, and to be willing to work to access this resource (Olsson & Dahlborn, 2002). As already suggested, in the present investigation, aggression promoted by enrichment may be due, in part, to competition for highly preferred or valued components of the enriched environment, so that certain animals may have been denied access to these resources (Nevison et al., 1999). The unstable hierarchy and high levels of aggression in the current investigation were likely stressful and hence resulted in sensitized corticosterone and NE neuronal processes, so that the subsequent mild challenge elicited enhanced hormone and NE activity. As previously suggested (Howerton et al., 2008), it is possible that enriched conditions involving group-housing, at least in CD-1 male mice, might not constitute a positive environment (as competitive access to resources promoted aggression rather than play behaviors). It is possible, however, that by increasing the duration of the enrichment period, more stable social hierarchies would have been established, thus eliciting more positive outcomes. This said, in the present report mice were placed in their respective cages once they arrived from the breeding farm. It is possible that the advantageous effects of enriched housing, without the adverse effects comprising aggressive behaviors, might have been evident had enrichment been instituted at weaning.

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Conflict-of-Interest Statement

The authors report no conflicts of interest.

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Enrichment in group-housed versus individually-housed male mice differentially impacts behaviour, corticosterone and monoamine activity

MANUSCRIPT

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Abstract

Social defeat in mice is a potent stressor that promotes the development of depressive and anxiety-like behaviours, as well as variations of neuroendocrine and brain neurotransmitter activity. Environmental enrichment, which is typically associated with positive effects, has been shown to protect against some of the adverse behavioural effects of social defeat. In contrast, male mice group-housed in an enriched environment display more aggressive behaviours, causing wounding which may reduce the well being of these animals. In the current investigations, it was observed that enriched group-housed mice displayed increased anxiety-like behaviours compared to their SE counterparts. Furthermore, in response to social defeat stress, EE group-housed male mice showed decreased weight, exaggerated corticosterone levels, and altered hippocampal norepinephrine (NE) utilization compared to SE counterparts. Interestingly, these responses were not apparent in the individually-housed enriched mice. These findings suggest that providing enriched conditions to group-housed male mice may promote a stressful environment, possibly due to aggression between cage mates. This conclusion is further supported as these exaggerated stressor-related outcomes were not apparent in enriched mice housed individually.

INTRODUCTION

Animal models of depression and anxiety disorders have increasingly focused on psychosocial stressors, including social defeat, to identify biological correlates of these disorders. In particular, rodents that had experienced social defeat exhibited depression-like behavioural alterations, such as motivational disturbances and anhedonia (Becker et al., 2008) as well as increased general anxiety-like behaviours (Buwalda et al., 2005). Furthermore, relative to non-stressed mice, the defeated mice also displayed elevated serotonin (5-HT) and norepinephrine (NE) utilization in the prefrontal cortex (PFC) and hippocampus (Audet & Anisman, 2010) as well as increased mesolimbic dopamine (DA) activity (Miczek et al., 2008).

Environmental enrichment has traditionally been thought to protect animals from the adverse effects of stressors and to limit the development of fear and anxiety (Chapillon et al., 1999; Benarolya-Milshtein et al., 2004; Fox et al., 2006), and was found to attenuate depressive-like behaviours elicited by chronic social defeat (Schloesser et al., 2010). Interestingly, housing animals in an enriched environment also increased levels of 5-HT in the PFC (Brenes et al., 2008), cortical NE (Naka et al., 2002) and mesolimbic DA activity (Segovia et al., 2010).

In contrast to reports of beneficial effects attributable to enrichment, it has also been reported to promote aggressive behaviours, particularly in group-housed male mice, causing severe wounding in subordinates, and ultimately reducing the well being of these animals (Haemisch et al., 1994; Van Loo et al., 2002; Howerton et al., 2008). In this regard, we have shown that housing CD-1 male mice (known to be relatively aggressive; Howerton et al., 2008) in groups of 3-4 in an enriched environment induced aggression

between cage mates and sensitized corticosterone and brain monoamine responses so that subsequent exposure to a mild stressor elicited exaggerated outcomes (McQuaid et al., in press).

Several investigators have housed male mice individually in enrichment paradigms (Schloesser et al., 2010; Lehmann & Herkenham, 2011), possibly to avoid aggression. However, the social interaction has traditionally been considered an important component of enrichment (van Praag et al., 2000). Furthermore, individual housing itself may be stressful for mice, and may induce symptoms reminiscent of depression ordinarily seen in animal models of the disorder (Saenz et al., 2006). Indeed, when given the choice between an empty or an inhabited cage, mice showed a preference for the proximity of another male, regardless of their social status (Van Loo et al., 2001). Therefore, it may be desirable to use less aggressive strains of mice in enrichment paradigms, where group housed male mice are required.

The current investigations were conducted to assess several behavioural and neurochemical effects associated with enrichment housing. To this end, instead of using CD-1 mice, which have been shown to injure each other in an enriched environment, BALB/cByJ mice a highly anxious strain (Anisman et al., 1998) were assessed. Furthermore, BALB/c substrains of mice have been found to be only moderately aggressive (Van Loo et al., 2003). In the current investigation we examined whether housing male mice in groups of 3 in an enriched versus a standard environment would influence anxiety-like behaviours in an elevated plus-maze test (Experiment 1). Further, we examined whether enriched versus standard environments would influence corticosterone levels and brain monoamine variations elicited by social defeat and

whether housing mice in their respective environments either in groups of 3 (Experiment 2) versus individually (Experiment 3) would influence these outcomes. As in our studies with male CD-1 mice (McQuaid et al., in press), we predicted that enrichment in group-housed mice would increase aggression, and thus result in exaggerated corticosterone and monoamine responses to social defeat (Experiment 2). In contrast, there would be no exaggerated responses in enriched individually-housed mice in response to the social stressor (Experiment 3) and that enrichment would act to buffer against the effects of social defeat.

MATERIALS AND METHODS

Animals and housing procedures

One hundred naïve male BALB/cByJ mice, aged 6-8 weeks, were obtained from Jackson Laboratory (Bar Harbor, Maine). Upon arrival, they were housed 3 mice/cage (Experiments 1 and 2) or individually (Experiment 3) in either an enriched environment (EE) or a standard environment (SE). The EE consisted of polypropylene rat maternity cages (50 x 40 x 20 cm) equipped with two running wheels, one red polypropylene shelter, one orange polypropylene shelter with an angled running wheel, as well as three yellow polypropylene tunnels and two cotton nestlets. In an attempt to minimize stress associated with novel objects (Lehmann & Herkenham, 2011), enrichment items were not changed throughout the experiment. The SE consisted of standard polypropylene cages (27 x 21 x 14 cm) with only one cotton nestlet. Mice were left undisturbed in their respective environments (EE or SE) for 4 weeks, with the exception of weighing, routine cage cleaning once/week and the scoring of aggressive behaviours.

In addition to the experimental mice, 17 CD-1 retired breeders, 9-12 months of age, were singly-housed upon their arrival. These mice had been exposed to females and thus were expected to exhibit relatively marked aggressiveness compared to naïve mice that had never experienced prior male-female interactions. These retired breeders were not included as experimental subjects, and were used only as social stressors during the repeated social defeat procedure. Mice were kept on a 12-hr light-dark cycle (lights on: 0800-2000 hr) in a temperature-(21°C) and humidity-controlled (63%) room and given *ad libitum* access to food and tap water. All experimental procedures were approved by the Carleton University Animal Care Committee and met the guidelines set out by the Canadian Council on Animal Care.

In Experiment 1, anxiety-like behaviours were measured in the elevated plus-maze after 4 weeks of living in EE or SE conditions. Experiment 2 and 3 assessed the protracted effects of 4 weeks of housing in EE versus SE on corticosterone and monoamine responses to social defeat stress which occurred each day during the 4th week of housing conditions.

Behavioral scoring

Home-cage aggressive behaviours in SE and EE group-housed mice were scored three days per week (Monday, Wednesday, and Friday) for 4 weeks and commenced immediately upon arrival of mice. Prior to scoring, mice were tail marked to allow for individual identification within a cage. Specifically, frequency and duration of aggressive interactions were scored 3 times/week over a 5-min interval in real-time. A description of each aggressive encounter was also documented so that the interactions could be categorized as attacks, aggressive chasing, or aggressive grooming, all resulting in

submissive behaviours in the targeted mouse. Attacks included biting, sometimes accompanied by tail rattling. Aggressive chasing included rushing or leaping at another mouse. Finally, aggressive grooming was defined as one mouse vigorously licking and nipping a second mouse.

Experiment 1

Elevated plus-maze

Mice that had been housed in the SE or EE conditions were tested for anxiety-like behaviours in the elevated plus-maze after living in their assigned environments for 4 weeks. The elevated plus-maze consisted of a wooden maze that comprised two open arms (50cm x 10cm) and two enclosed arms (50cm x 10cm) with an open roof, arranged such that the two open arms were opposite to each other. The entire maze was elevated 60cm from the floor. The mice were brought to the testing room to acclimate to the new environment 1 hour prior to testing and were then placed, individually, into the maze facing a closed arm for 5-min. Entries to the open and closed arms, time spent in the open and closed arms, latency to enter into the open arms and the number of stretch attempts into the open arms were scored in real-time.

Experiments 2 and 3

Social stressor procedure

Testing procedures were conducted between 08:30 and 13:00 hours to minimize effects related to diurnal factors. After living in their assigned environments for 3 weeks, half of EE and SE mice of both the grouped and isolated conditions were exposed to a 15-min social defeat episode once a day on each of 7 consecutive days, whereas the other half (non-stressed controls) remained undisturbed in their EE or SE home-cages (n =

10/group). Specifically, mice were introduced, individually, into the home-cage of a retired breeder and direct interactions were permitted for 15-min. Each mouse was exposed to a different retired breeder on the 7 days of the stressing period. Excessive aggressive behaviours were interrupted by inserting a wire-mesh partition which allowed for auditory and visual exchange between the two mice, but inhibited physical contact. During the social defeat session a number of behavioural measures were determined in real-time: occurrence of aggressive encounters (social defeat vs. no aggressive interaction vs. no defeated/no victorious mice), latency for the 1st aggressive episode, and latency to defeat. Following each stressor exposure, mice were placed back into their assigned environments.

Blood collection and brain removal

Three minutes after the 7th social defeat session (or at corresponding times for mice in the non-stressed conditions); mice were brought to a different room and underwent rapid decapitation. Trunk blood was then collected in tubes containing 10 mg EDTA, centrifuged, and the plasma stored at -80 °C for subsequent corticosterone determination.

Brains were immediately removed and placed on a stainless-steel brain matrix (2.5 x 3.75 x 2.0 cm) positioned on a block of ice that rested on dry ice. The matrix has a series of slots spaced 500 μm apart that guide razor blades to provide coronal brain sections. Once the brains were sliced, tissue from the hippocampus and central amygdala (CeA) was collected by micro-punch using a hollow 20-gauge microdissection needle, following the mouse atlas of Franklin & Paxinos (1997). Tissue punches were placed in 0.3 M monochloroacetic acid containing 10% methanol and internal standards and were

stored at -80 °C for subsequent determination of NE and 5-HT, as well as their respective metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleacetic acid (5-HIAA).

Corticosterone determination

A commercial radioimmunoassay (RIA) kit (ICN Biomedicals Inc., USA) was used to determine plasma corticosterone concentrations (in duplicate). Assays were performed in a single run to prevent inter-assay variability; the intra-assay variability was less than 10%.

Determination of monoamine and metabolite concentrations

High-performance liquid chromatography (HPLC) was used to determine concentrations of the monoamines and their metabolites. Tissue punches were sonicated in solution obtained from a stock solution which contained 14.17 g monochloroacetic acid, 0.0186 g EDTA, 5.0 ml methanol and 500 ml HPLC grade water. Tissue punches from the CeA were sonicated in 300 µl of this stock solution, whereas tissue punches from hippocampus were sonicated in 500 µl. Following centrifugation, a 20 µl aliquot of the supernatant was passed at a flow rate of 1.5 ml/min (1400–1600 p.s.i.) through a system containing a M-600 pump (Milford, USA), guard column, radial compression column (5 m, C18 reverse phase, 8 mm x10 cm), and a 3-cell coulometric electrochemical detector (ESA model 5100A). For separation, a mobile phase was used comprising of 1.3 g heptane sulfonic acid, 0.1 g disodium EDTA, 6.5 ml triethylamine, and 35 ml acetonitrile. The mobile phase was then filtered using 0.22-mm filter paper, degassed, and the pH level was adjusted to 2.5 with phosphoric acid. The area and height of the peaks were determined using a Hewlett-Packard integrator. A protein analysis kit

(Pierce Scientific, Canada), and a spectrophotometer (Brinkman, PC800 colorimeter) in conjunction with bicinchoninic acid were used to measure protein levels of each sample. Neurotransmitter concentrations were based on protein levels. The lower limit of detection for the monoamines and metabolites was 5.0 pg/ml.

Data Analyses

For Experiment 1 and 2, in which mice had been housed in groups of 3, aggressive behaviors within the home cage were analysed through a one-way analysis of variance (ANOVA) (Housing: Standard vs. Enriched). Anxiety-like behaviours in the elevated plus-maze for Experiment 1 were analysed through a one-way ANOVA (Housing: Standard vs. Enriched). Plasma corticosterone concentrations as well as NE, 5-HT and their metabolites in the hippocampus and CeA were analysed through a 2 (Housing) x 2 (Stressor: Non stressor vs. Social defeat stressor) between-groups ANOVA (Experiments 2 and 3). Follow-up comparisons comprised t-tests with a Bonferonni correction to maintain the alpha level at 0.05.

RESULTS

Aggressive outcomes

Aggression levels appeared low in the current experiments. In fact out of the 60 group-housed mice for Experiment 1 and 2, only one EE mouse had to be removed from the study for displaying *overly* aggressive behaviours and very few aggressive encounters were witnessed during the 5min/cage scoring sessions. However, across the course of the group-housed experiments, 16 wounds in EE cages and 9 wounds in SE cages were recorded attesting to the presence of aggressive behaviours.

Experiment 1

Anxiety-like behaviours

As shown in Figure 1, EE animals displayed significantly more anxiety-like behaviours than their SE counterparts in the elevated plus-maze. Compared to their SE counterparts, the enriched mice spent less time in the open arms, $F(1, 15) = 4.62, p < 0.05$, emitted fewer entries to open arms, $F(1, 15) = 7.16, p < 0.05$, and displayed increased latency to enter the open arms, $F(1, 15) = 9.57, p < 0.01$. There were no differences between EE and SE mice with respect to the time spent in the closed arms, number of entries to closed arms and the number of stretch attempts made (data not shown).

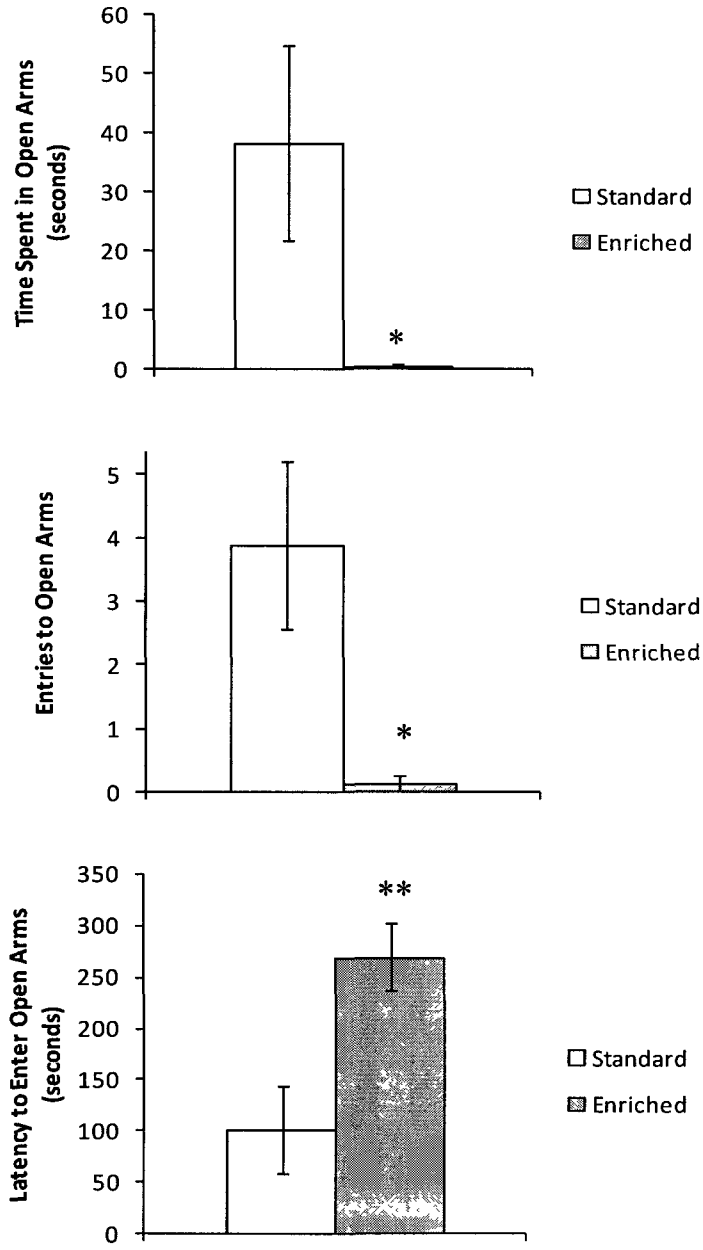

Figure 1. Time spent in the open arms (seconds), number of entries into the open arms and latency to enter into the open arms (seconds) in EE (Enriched Environment) and SE (Standard Environment) mice that had been exposed to the elevated plus-maze. Data are represented by means $\pm$ S.E.M, * $p < 0.05$ and ** $p < 0.01$ relative to mice that had been housed in standard conditions.

Experiments 2 and 3

Weight changes

As depicted in Figure 2A, after living in EE or SE conditions for 4 weeks, group-housed mice that experienced social defeat weighed less than non-stressed mice, $F(1, 33) = 4.13, p = 0.05$, and enriched mice weighed less than their SE counterparts, $F(1, 33) = 6.25, p < 0.05$. Individually-housed mice did not reveal any weight variations (Figure 2B). Furthermore, over the course of stressor exposure, group-housed mice who experienced social defeat gained significantly less weight than the non-stressed animals, $F(1, 33) = 10.43, p < 0.01$. In fact, during this period enriched mice gained significantly less weight than their SE counterparts, $F(1, 33) = 4.05, p = 0.052$ (Figure 2C). Weight change did not differ between EE and SE mice who had been individually housed.

Plasma corticosterone levels

Relative to non-stressed mice, plasma corticosterone levels 3-min after the last social defeat session were increased in EE and SE mice irrespective of whether they were housed in groups or individually, *F's* (1, 29 and 1, 32) = 34.92 and 51.46, respectively, *p*'s < 0.001. The interaction between Housing condition and the stressor treatment was not significant, but as *a priori* predictions had been made in this regard, comparisons were nonetheless conducted of the simple effects comprising this interaction. These comparisons indicated that among group-housed mice, the corticosterone elevations elicited by social defeat were significantly higher in EE than in SE mice, $p < 0.05$ (Figure 3A). In contrast, EE and SE mice housed individually did not differ in their corticosterone response to the social stressor (Figure 3B).

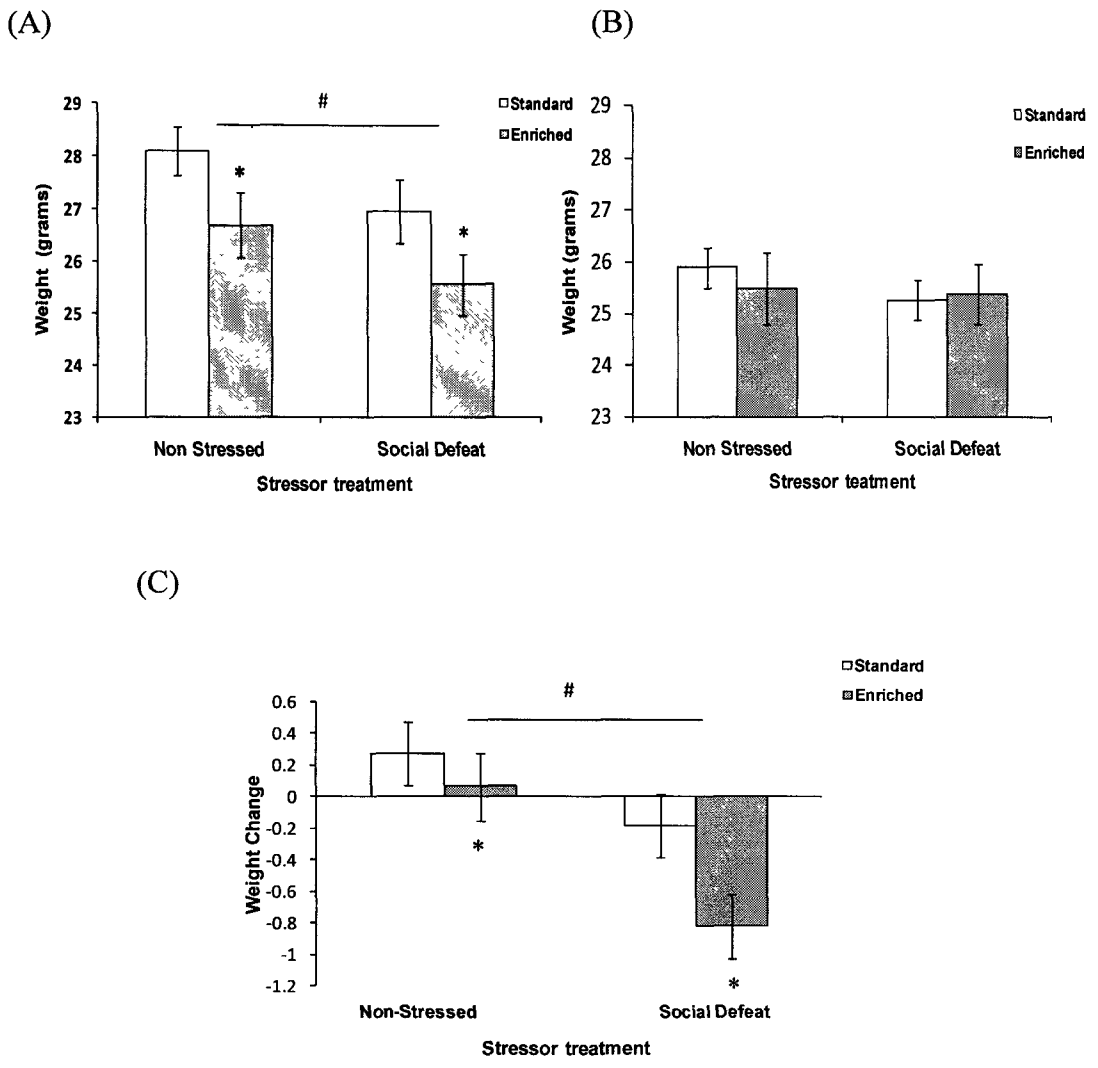

Figure 2. Weight (grams) just before the final social defeat exposure (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed either in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data are represented by means $\pm$ S.E.M, # $p = 0.05$ relative to non-stressed mice, and * $p < 0.05$ relative to mice that had been housed in standard conditions. (C) Change in Weight over the course of social defeat exposure or at corresponding times in controls among EE and SE mice that had been housed in groups. Data are represented by means $\pm$ S.E.M, # $p < 0.01$ relative to non-stressed mice, * $p = 0.05$ relative to SE mice.

(A) (B)

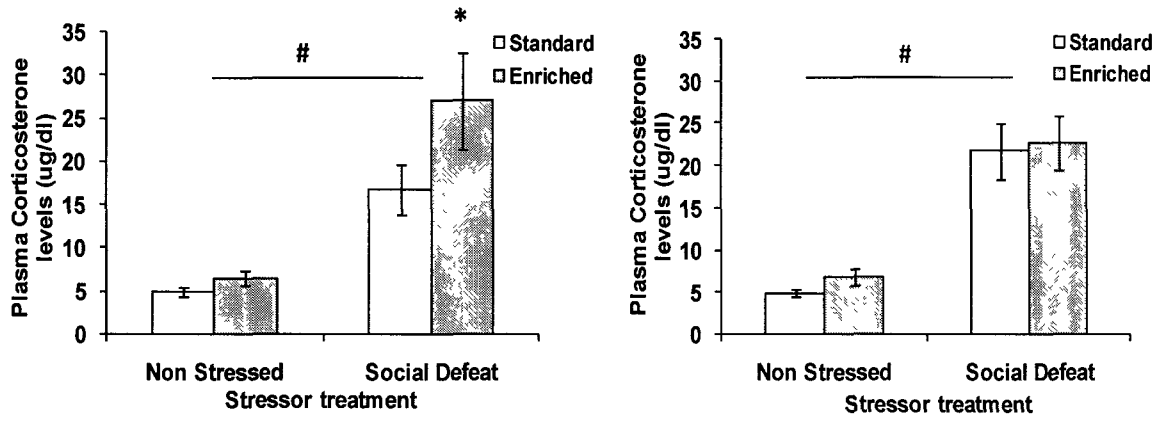

Figure 3. Plasma corticosterone concentrations (μg/dl) collected 3-min after the final social defeat stressor (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data represents means $\pm$ S.E.M. # $p < 0.001$ relative to non-stressed mice, and * $p < 0.05$ relative to SE stressed mice.

Monoamine variations within the hippocampus

Among group-housed mice, hippocampal MHPG concentrations varied as a function of the Housing x Stressor interaction, $F(1, 33) = 6.11, p < 0.05$ (Figure 4A). Follow-up comparisons of the simple effects comprising this interaction indicated that after 30 days of EE and SE housing, NE utilization in the absence of a further stressor was comparable in the two conditions. However, MHPG elevations elicited by social defeat exposure were apparent in EE mice, $p < 0.001$, whereas this increase did not reach significance in SE mice, $p = 0.32$. In contrast, among individually-housed mice who experienced social defeat, MHPG levels were increased compared to non-stressed counterparts irrespective of whether mice had been housed in SE versus EE conditions, $F(1, 32) = 4.95$, $p < 0.05$ (Figure 4B). Despite the altered utilization, the hippocampal NE concentrations in both grouped- and individually-housed mice were not altered in response to the housing or stressor conditions (bottom panels).

Hippocampal 5-HIAA concentrations were increased after repeated social defeat in mice living in groups and individually, $F(1, 33$ and $1, 32) = 7.59$ and $6.28, p < 0.01$ and $p < 0.05$ respectively (Figure 5A and 5B). In this instance, however, 5-HIAA elevations were not moderated by whether mice had been housed in SE versus EE conditions. Once again, 5-HT levels were not significantly affected by any treatments (bottom panels).

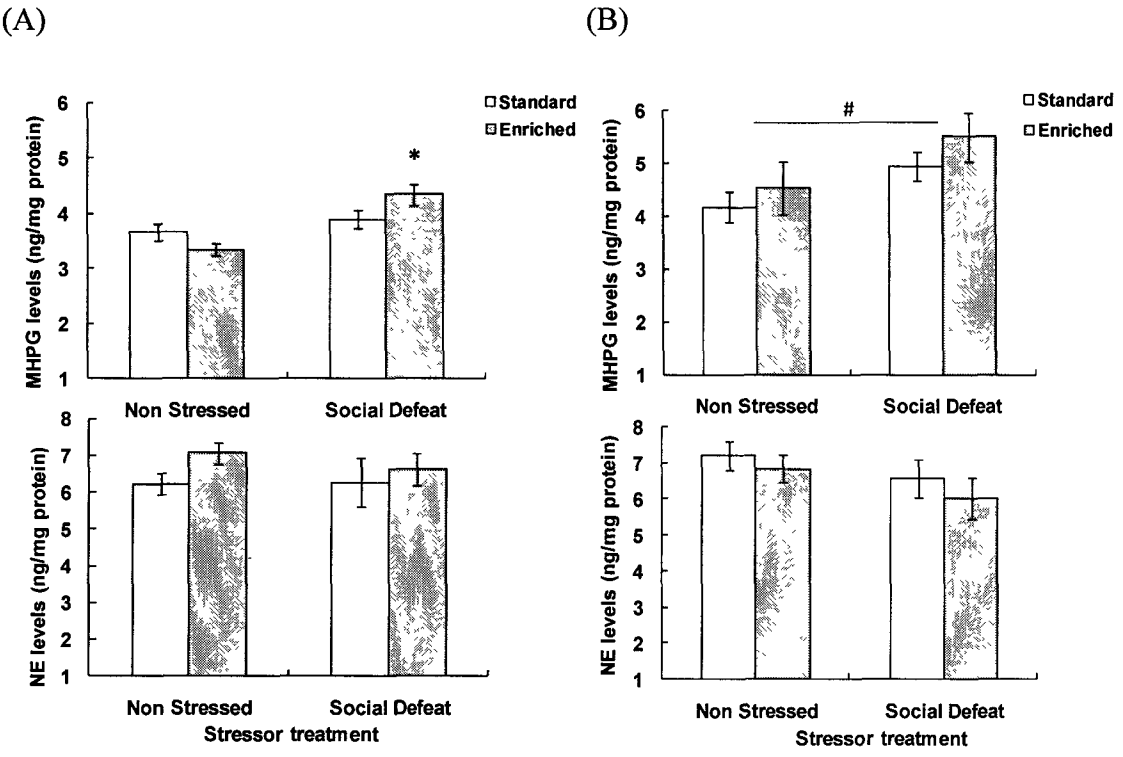

Figure 4. Hippocampal concentrations of MHPG and NE (ng/mg protein) collected 3-min after the final social defeat stressor (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data represents means $\pm$ S.E.M. * $p < 0.001$ relative to EE non-stressed mice, # $p < 0.05$ relative to non-stressed mice.

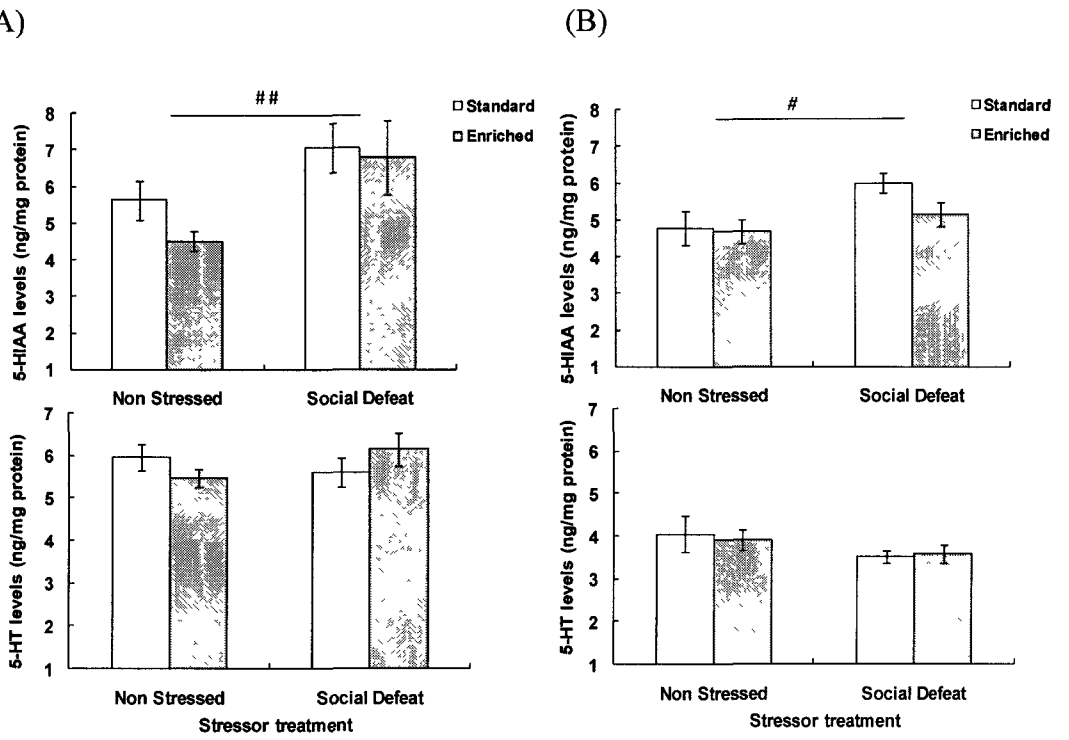

Figure 5. Hippocampal concentrations of 5-HIAA and 5-HT (ng/mg protein) collected 3-min after the final social defeat stressor (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data represents means $\pm$ S.E.M. ## $p < 0.01$ and # $p < 0.05$ relative to non-stressed mice.

Monoamine variations within the central amygdala

In the CeA, MHPG accumulation was affected only by stressor exposure, which varied as a function of whether mice had been housed in groups or individually. Specifically, following social defeat, group-housed mice revealed unaltered NE utilization, however, NE levels were increased compared to non-stressed mice, $F(1, 33) = 3.93, p = 0.056$ (Figure 6A). In contrast, individually-housed mice displayed higher NE utilization after stressor exposure, $F(1, 32) = 6.63, p < 0.05$, but did not show altered levels of NE (Figure 6B).

As depicted in Figure 7A, group-housed mice displayed markedly increased 5-HIAA (top panel) and 5-HT (bottom panel) concentrations in the CeA in response to social defeat, $F(1, 33) = 14.62, p < 0.01$, and $F(1, 33) = 4.00, p = 0.05$, respectively, and this was irrespective of whether they had been housed in EE or SE conditions. Serotonin utilization was unaltered in individually-housed mice, however, 5-HT concentrations among individually-housed mice, although non-significant ($p = 0.10$), appeared altered (shown in Figure 7B). Because an *a prior* prediction had been made in regards to 5-HT variations in CeA, comparisons of the simple effects comprising the interaction were assessed. These comparisons confirmed that 5-HT levels were reduced after social defeat in SE mice only, $p < 0.05$.

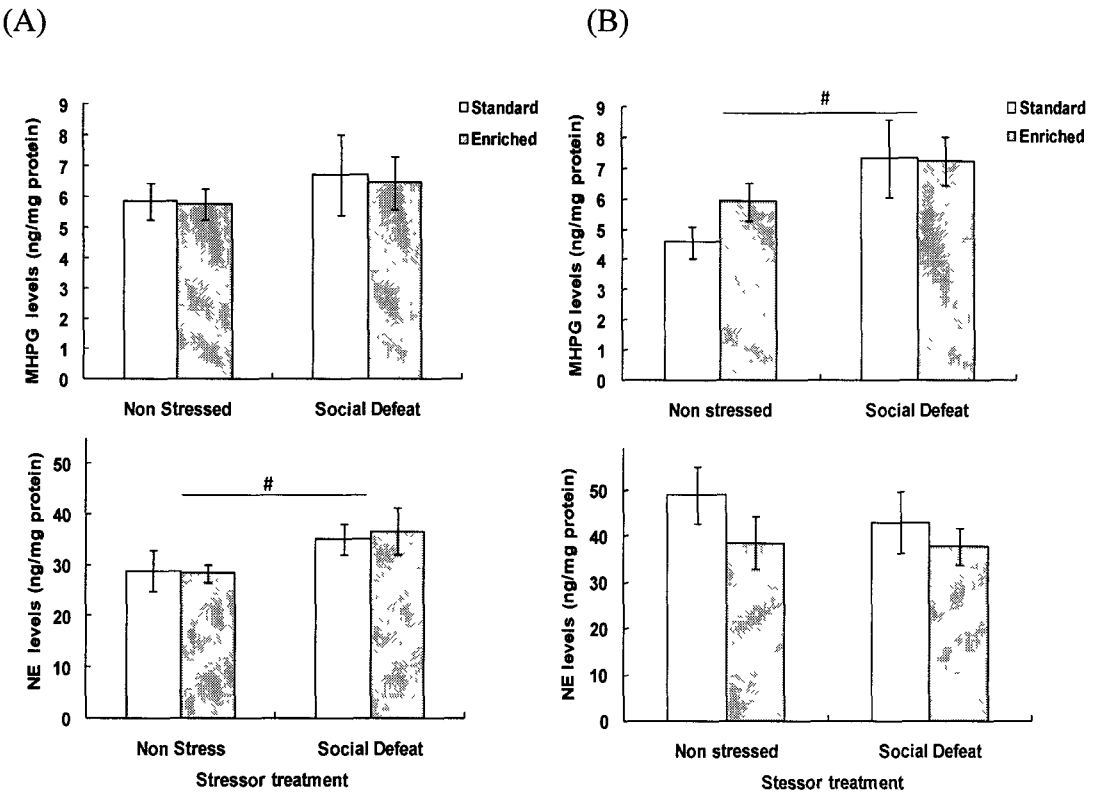

Figure 6. Central amygdala concentrations of MHPG and NE (ng/mg protein) collected 3-min after the final social defeat stressor (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data represents means $\pm$ S.E.M. # $p = 0.05$ or < 0.05 relative to non-stressed mice.

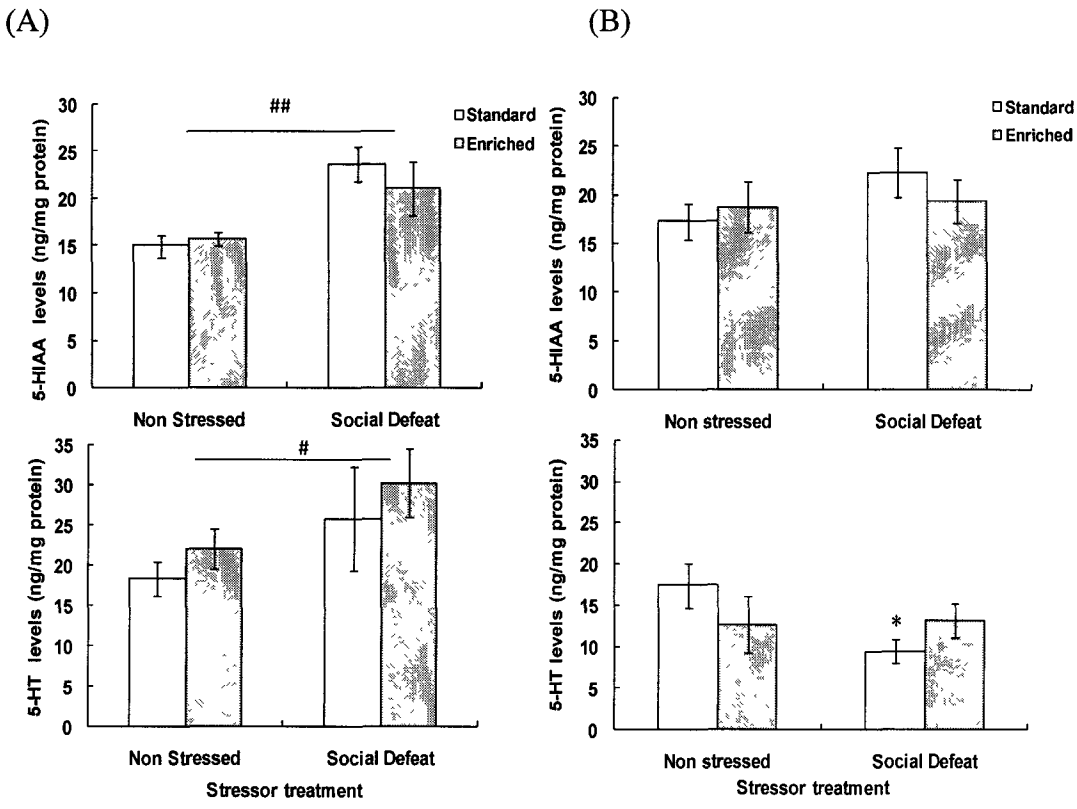

Figure 7. Central amygdala concentrations of 5-HIAA and 5-HT (ng/mg protein) collected 3-min after the final social defeat stressor (Social Defeat) or at corresponding times in controls (Non Stressed) among EE (Enriched Environment) and SE (Standard Environment) mice that had been housed in groups (Group-Housed) (A) or individually (Individually-Housed) (B). Data represents means $\pm$ S.E.M. ## $p < 0.01$ and # $p = 0.05$ relative to non-stressed mice, * $p < 0.05$ relative to non-stressed SE mice.

DISCUSSION

In contrast to previous experiments using CD-1 mice, in the current investigations using BALB/cByJ mice it was not possible to conclude that aggression was elevated in the enriched versus standard cages using the current method of scoring aggression (5 min/cage every second day). This said, there were more wounds found in the enriched mice relative to those that were housed in a standard environment. The difference between the two studies, is likely a reflection of the lower aggression levels typically observed in the BALB/c substrains compared to the CD-1 strain (Van Loo et al., 2003). Although speculative, it is possible that some of the effects observed in the current investigation, such as increased anxiety-like behaviours, and exaggerated corticosterone and NE levels in response to social defeat, may be due, in part to aggression between group-housed enriched cage-mates. Alternatively, it might also be the case that the social interaction (i.e. without overt aggression) in the enriched environment resulted in the current findings.

Enriched mice displayed greater anxiety-like behaviours than SE mice in the elevated plus maze, as indicated by the increased latency to enter into the open arms as well as the decreased time spent and reduced number of entries made into the open arms (Pellow et al., 1985). In fact, mice that had been housed in EE conditions barely explored the open arms of the plus maze. In contrast, EE and SE animals made comparable number of stretch attempts, described as a risk assessment behaviour in the plus-maze (Rodgers & Johnson, 1995). In effect, the EE mice appeared to be reluctant to enter into the open arms compared to SE mice, but they were not immobile in the plus maze and were appraising the open arms just as the SE animals were. In contrast to the present findings, it was previously shown that mice maintained in the enriched condition in

groups displayed fewer risk assessment behaviours (Roy et al., 2001) and decreased anxiety in the plus maze (Chapillon et al., 1999; Benaroya-Milshtein et al., 2004; Friske & Gammie et al., 2005). It is possible that aggression in our enrichment paradigm among group-housed mice, combined with other protocol differences (i.e., sex, age, and strain/type of rodents), might explain these two opposite anxiety profiles in the plus maze.

We recently reported that the plasma corticosterone response to a mild stressor (novel cage exposure) was more pronounced in group-housed CD-1 male mice living in enriched conditions, probably due to the heightened aggression recorded in these mice (McQuaid et al., in press). Consistent with this, corticosterone elevations elicited by repeated social defeat in the present investigation were also more pronounced in group-housed EE mice compared to SE mice. Interestingly, this exaggerated corticosterone response was not apparent in the individually housed animals. Furthermore, after social defeat, enriched group-housed mice also gained less weight than their SE counterparts, but a similar outcome was not observed for mice housed singly. The fact that the enhanced hormone response and weight changes were not seen in isolated EE mice suggests that grouping male mice in EE conditions may be stressful, and aggression might contribute in this regard, along with other factors (such as dominance hierarchies, even if overt aggression was not documented). Increased basal corticosterone levels and decreased weight gain have previously been observed in enriched male rodents compared to standard-housed counterparts (Van Loo et al., 2002; Moncek et al., 2004), and were attributed to elevated aggression levels in the enriched condition (Van Loo et al., 2002).

Like the corticosterone variations, among group-housed mice NE utilization in the hippocampus was enhanced after repeated social defeat in EE mice, whereas among individually-housed mice NE utilization was increased in both EE and SE animals. It has been shown that NE utilization can be activated simply by running on wheels provided (Chaouloff 1989; Soares et al., 1999), an important component of enrichment (van Praag et al., 2000). In the current experiment, however, EE and SE mice that were housed individually did not differ in their levels of NE utilization, suggesting that the increased NE activity was, in fact, not due simply to the availability of the running wheels. Instead, the social interactions (either aggression or simply social interactions) between enriched cage-mates may have impacted hippocampal NE activity. Furthermore, exaggerated hippocampal NE response to the social stressor, although modest, may again be suggestive of the enriched group-housed environment as being a potentially stressful one. In contrast, NE activity in the CeA followed a much different trend than that evident in the hippocampus as there were no differences between SE and EE animals. Specifically, among group-housed mice NE levels increased following defeat, but utilization was unaltered, whereas among individually-housed mice the stressor provoked increased NE utilization, but without the level of the parent amine being affected.

After experiencing repeated social defeat, hippocampal 5-HIAA concentrations (reflecting utilization of 5-HT) increased to a similar extent in enriched and standard-housed mice living either in groups or individually. It seems, at least in the current study, that serotonergic activity in the hippocampus, although readily impacted by the social defeat, was not altered by enriched housing conditions or by individual vs. grouped housing conditions. These results were consistent with previous findings indicating that

5-HT activity in the PFC and hippocampus was increased after aggressive encounters (i.e. involving social defeat stress), but was not influence by additional factors such as dominance status (Audet & Anisman, 2010). As observed in the hippocampus, no differences were evident regarding 5-HT activity within the CeA between EE and SE group-housed mice; both 5-HT utilization and levels were increased after repeated social defeat in SE and EE mice. However, 5-HT levels declined (without a change of the metabolite) in individually-housed SE animals after repeatedly experiencing defeat. This was only found in SE mice and may be indicative of a 'buffering effect' of enrichment among the EE mice. Furthermore, it appeared that the serotonergic activity was differentially affected in the two stress-sensitive brain regions. Specifically, the 5-HT activity in the hippocampus appeared to be more robust relative to the changes within the amygdala, although it is uncertain how these relative differences translate into behavioral outcomes. In this regard, although these structures serve different functions, both structures are highly involved in emotional memory (Richter-Levin, 2004).

Finally, enrichment in the individually-housed mice did not affect weight, corticosterone levels, or hippocampal monoamine activity, begging the question of whether enrichment is beneficial for individually-housed mice. However, amygdala 5-HT levels were reduced following defeat in individually-housed SE mice but not in their enriched counterparts. This might suggest that enrichment, including individual housing, acted to limit the 5-HT decline. Several investigators reported that enrichment using singly housed mice is able to protect against the effects of chronic social defeat stress (Schloesser et al., 2010; Lehmann & Herkenham, 2011). However, the findings in the current experiment were not fully consistent with these earlier reports. It has been

suggested that it is not one element of enrichment, but the interaction of multiple components (socialization and physical activity), that is essential to elicit the impact of enrichment in rats (van Praag et al., 2000). It likewise appears in the current experiments, that in mice, enrichment which does not allow social interaction/stimulation does not lead to adverse outcomes. In contrast, the effects of social defeat were particularly notable among mice that also experienced the social aspect of enrichment (by group-housing male mice).

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Conflict-of-Interest Statement

The authors report no conflicts of interest.

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General Discussion

Environmental enrichment is widely recognized as having numerous beneficial behavioural and neurobiological effects (Fox et al., 2006; Simpson & Kelly, 2011; van Praag et al., 2000). However, it has been reported that male rodents living in enrichment displayed signs of chronic stress, (i.e. larger adrenals, and increased corticosterone concentrations) (Moncek, Duncko, Johansson, & Jezova, 2004), as well as increased aggression levels which may be harmful to these animals (Hamesish et al., 1994; Howerton et al., 2008; Van Loo et al., 2002). In spite of these negative reports, reviews in this area tend to emphasize the therapeutic and protective effects of environmental enrichment (Fox et al., 2006).

To explain the heightened aggression levels sometimes demonstrated in environmental enrichment, it has been suggested that enrichment promotes an unstable social hierarchy which is stressful for animals and may contribute to increased aggressive behaviours (Haemisch et al., 1994). In addition, enrichment may also promote competition for highly preferred components of the environment, (such as running wheels) in which certain animals are denied access to these resources (Nevison et al., 1999). In the current investigations, at least with CD-1 mice, the observed hierarchies were very unstable (as social status appeared to change frequently). It was further observed that the dominant mice prevented their submissive cage-mates from running on the wheels. In fact, if the submissive animals attempted to run on the wheels, they would immediately be aggressively chased or attacked. Taken together, it appeared that in CD-1 mice both unstable hierarchies and competition contributed to stress in the enriched housing conditions and thus led to the exaggerated responses to stressor exposure.

In the current experiments, aggression levels differed between male CD-1 and BALB/cByJ mice housed in enrichment. Specifically, enriched male CD-1 mice readily demonstrated aggressive behaviours and wounding, making it clear that enrichment had increased aggressive interactions compared to standard housing conditions. Although enriched male BALB/cByJ mice also displayed more wounds than their standard-housed counterparts, very few aggressive acts were scored, thus aggression levels were less clear in the BALB/cByJ strain. It was likewise reported that CD-1 mice displayed greater aggressive behaviours than BALB/cN mice (a substrain of BALB/cByJ mice), reflected by both behavioural measures and testosterone levels (Van Loo et al., 2003). Irrespective of the differences in aggressive behaviours between these two strains, the current experiments seemed to both suggest that enrichment with group-housed male mice represent a stressful environment. This was reflected by: increased wounding and anxiety-like behaviours, as well as exaggerated corticosterone and hippocampal NE utilization levels in response to a stressor in group-housed enriched mice.

Interestingly, the corticosterone responses in group-housed CD-1 and BALB/cByJ enriched mice were strikingly similar even though the stressors employed were of a very different nature (i.e. single exposure to a mild novel cage stress versus repeated exposure to social defeat stress). In fact, the single exposure to a novel cage seemed to induce a more profound corticosterone and NE response in enriched CD-1 mice than did the repeated social defeat exposure in the BALB/cByJ animals. It may be possible that there was an adaptation to the repeated social stressor, an effect which has previously been reported in response to other psychogenic and neurogenic stressors (Anisman & Zacharko, 1990). Thus it is possible that a single social defeat experience might have

elicited more pronounced stressor-induced outcomes in the BALB/cByJ mice compared to a repeated exposure paradigm. This said, the procedures, including the time of tissue sampling, was very different in the two studies and hence direct comparisons between these experiments requires further experimental analysis.

Although the group-housed enriched mice displayed exaggerated corticosterone and norepinephrine responses to stress, these effects were not apparent in the mice housed individually in enriched conditions. In fact, the mice housed individually in enrichment did not differ from their standard-housed counterparts in terms of corticosterone and monoamines responses, with the exception that SE animals displayed lower 5-HT amygdala concentrations following social defeat. Therefore, these results suggest that unlike group-housing, enrichment which includes individually-housed mice does not appear to create a stressful environment, and instead, may buffer against some effects of stress (although this attenuated effect was modest and very specific).

Limitations and future directions

In the current investigations, plasma corticosterone and neurochemical measurements were taken at the time of sacrifice (i.e. immediately after 45-min of stress in the CD-1 experiment and 3-min after the last of 7 social defeat sessions in the BALB/cByJ experiments). Therefore, the data represent a static measure of a dynamic process and thus it is not known whether EE and SE mice would differ in these measures over time.

Furthermore, in the current investigations an accurate account of aggression in the home-cages was not possible with the current scoring method employed. Therefore, longer durations of videotaped sessions immediately after cage-cleaning is necessary, a

method that has previously been used and appears to be more reliable (Van Loo et al., 2003). This will allow for a more detailed assessment of social interactions within the environments so that correlations can be made between social status in the home-cage with neuroendocrine and neurochemical outcomes in response to stressors. As previously mentioned, correlations between status and outcomes were not possible with CD-1 mice due to very unstable hierarchies which prevented the determination of clear status. However, it has been suggested that longer durations of enrichment may allow a stable hierarchy to develop between cage-mates (Van Loo et al, 2002), thus enabling stable statuses to develop.

In addition to the studies described, a complementary experiment was conducted examining pro-inflammatory cytokines and BDNF mRNA expression within the PFC and hippocampus. This investigation is very similar to experiments conducted using male BALB/cByJ mice that had been group-housed in enriched and standard environments and exposed to repeated social defeat stress for 7 days. Several findings from this experiment can be seen in Appendix A. Briefly, compared to their SE counterparts, enriched mice displayed decreased basal IL-1r1 mRNA expression but sensitized IL-6 mRNA expression in the hippocampus following repeated social defeat. Stressors can induce brain pro-inflammatory cytokine responses, which have been implicated in the development of major depressive disorder. Moreover, administration of pro-inflammatory cytokines can induce depressive-like symptoms similar to those observed in response to stressors (Anisman & Merali, 2003; Anisman et al., 2008). Taken together with previous results, the IL-6 response to social defeat in EE mice is very similar to the sensitized corticosterone and NE responses in enriched mice, supporting the contention that

enrichment involving group-housed male mice seems to be indicative of a stressful environment and may lead to increased vulnerability to stress-related pathologies.

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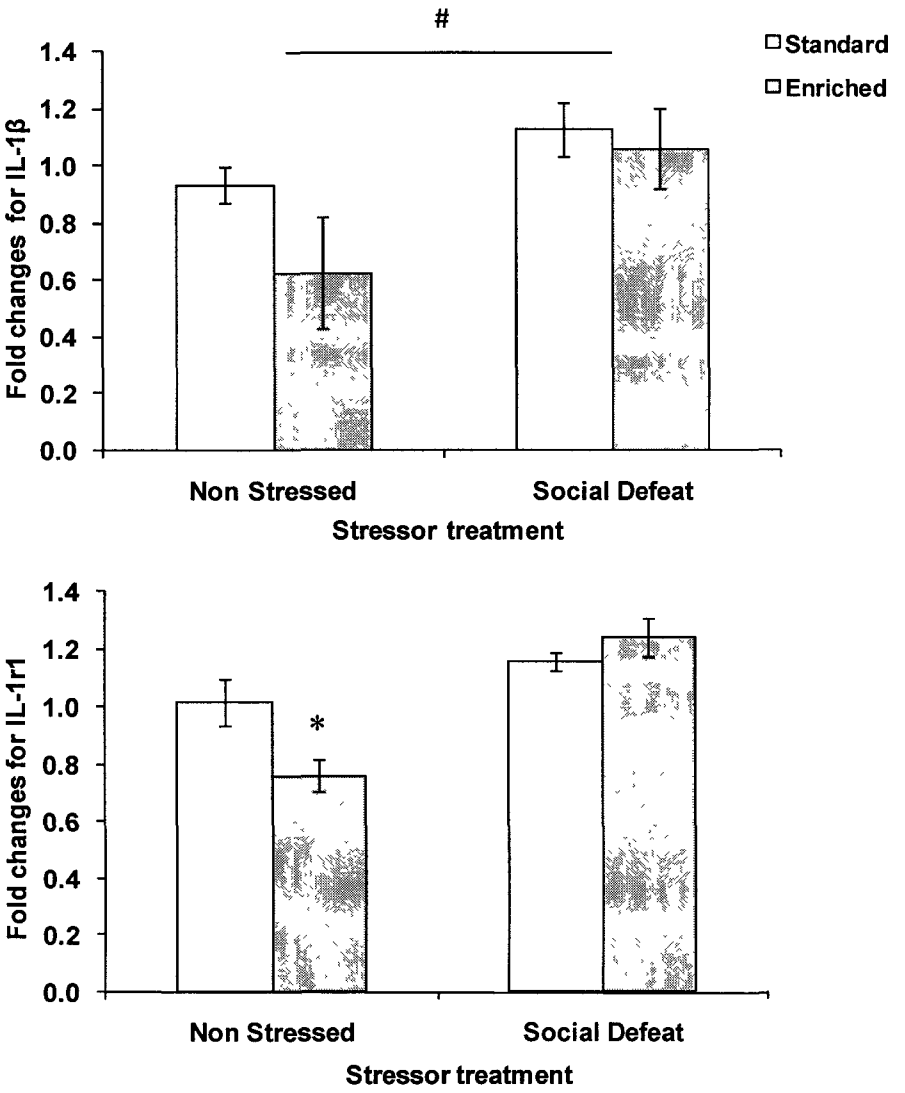
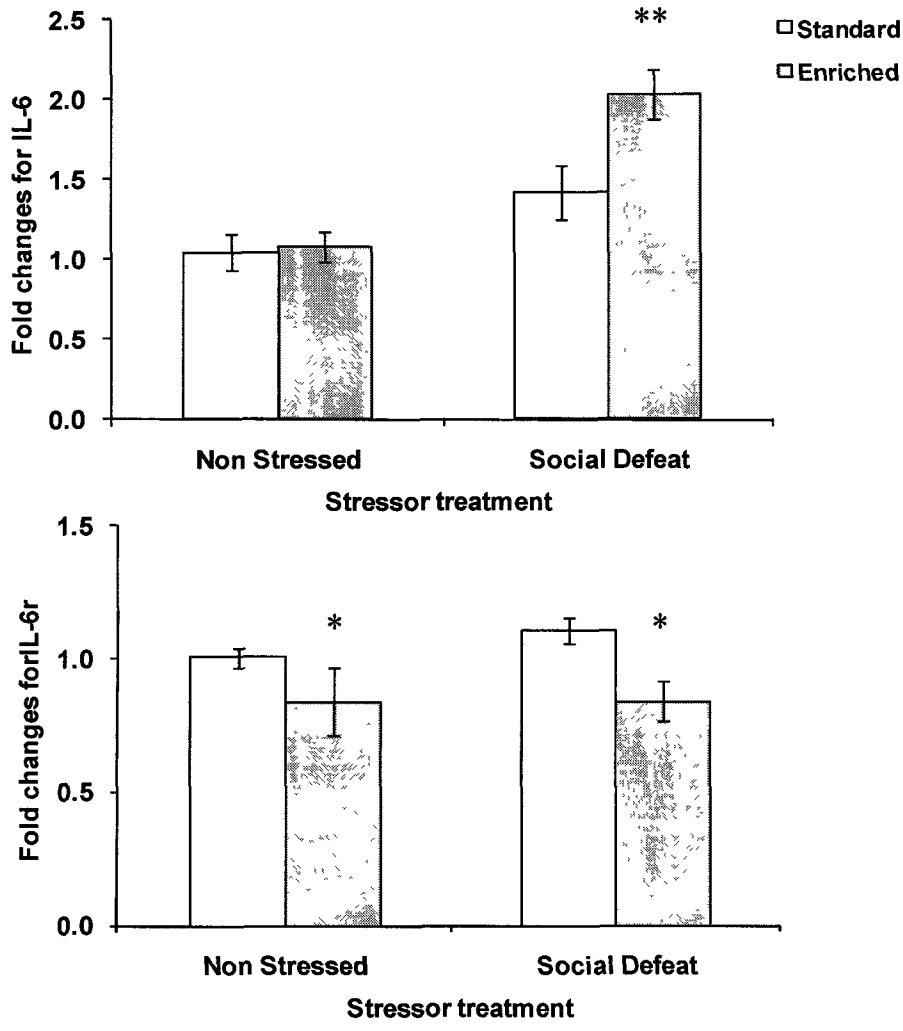
Appendix A

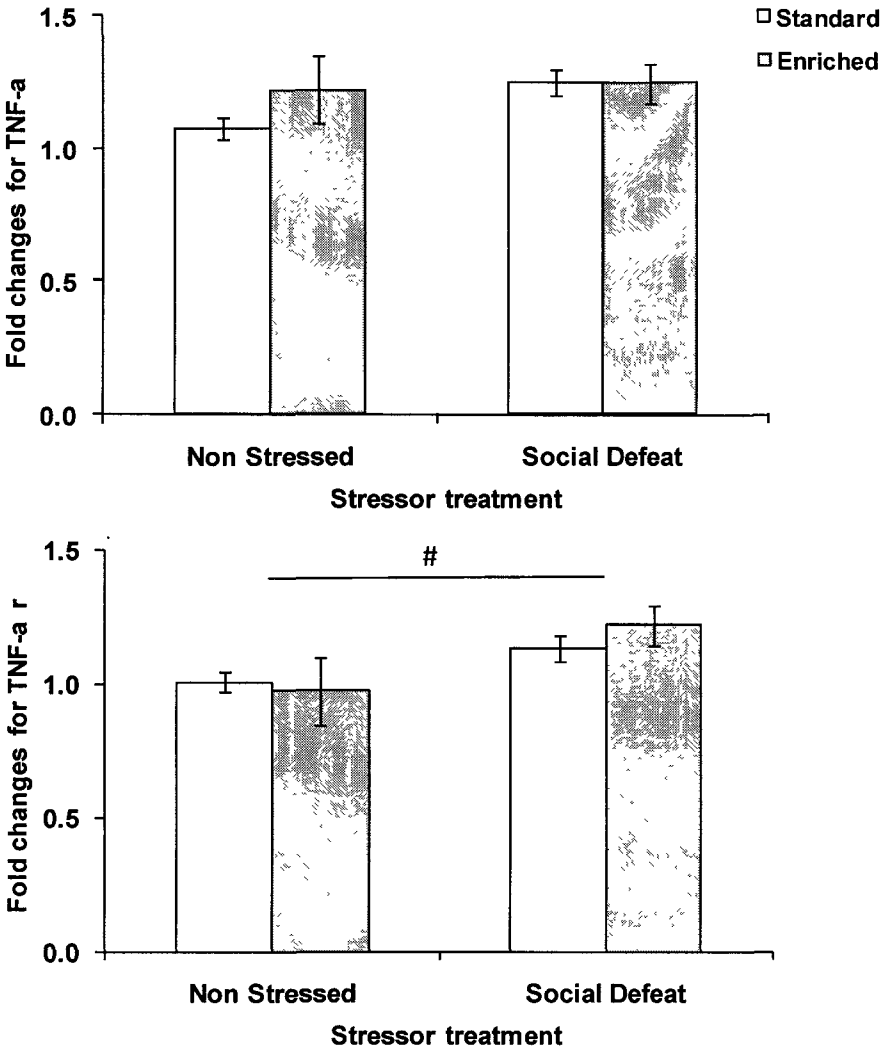
Hippocampal Cytokine Fold Changes



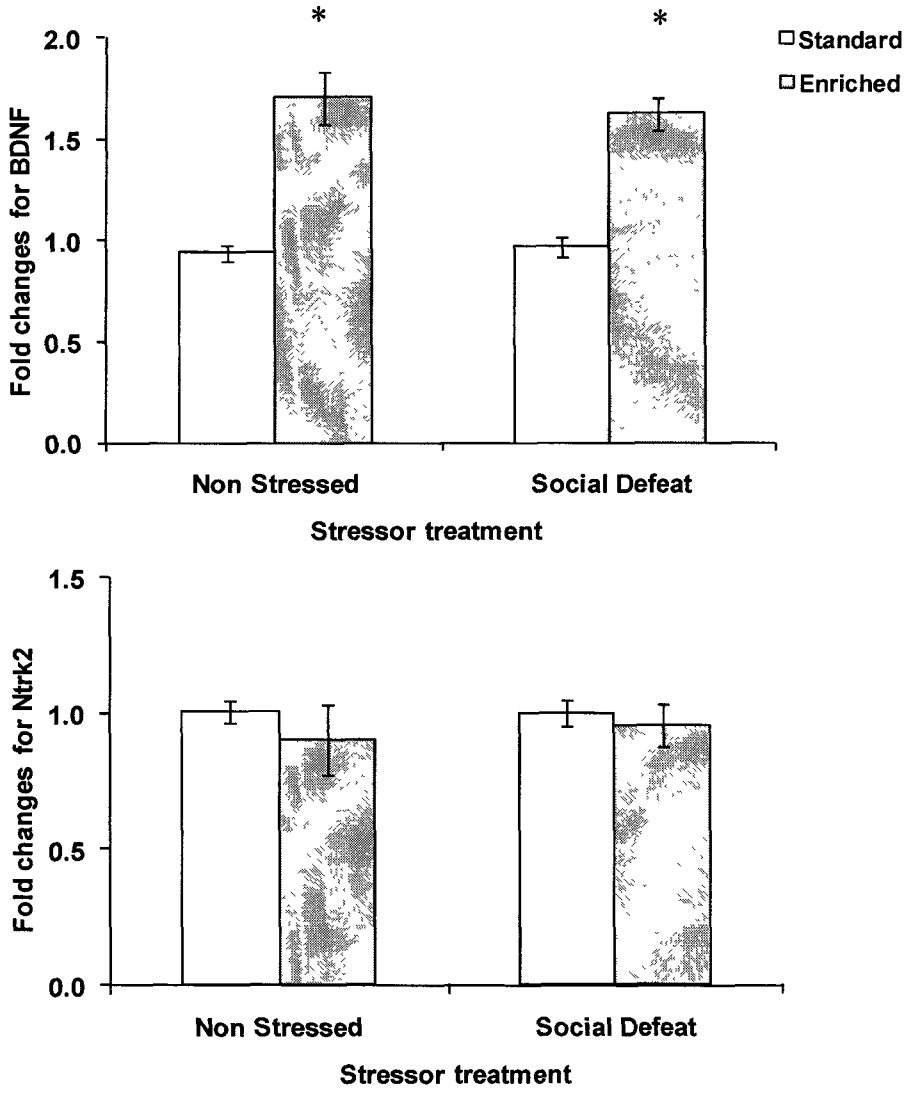
Fold changes of Interleukin (IL)-1β and IL-1r1 within the hippocampus collected 75-min after social defeat stress (Social Defeat) or at a corresponding time in controls (Non Stressed) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means ± S.E.M., # $p < 0.05$ relative to non-stressed mice, * $p < 0.01$ relative to non-stressed SE mice.



Fold changes of IL-6 and IL-6r within the hippocampus collected 75-min after social defeat stress (Social Defeat) or at a corresponding time in controls (Non Stressed) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means ± S.E.M. ** $p < 0.01$ relative to SE stressed mice, * $p < 0.01$ relative to mice housed in standard conditions.



Fold changes of TNF-α and TNF-α r within the hippocampus collected 75-min after social defeat stress (Social Defeat) or at a corresponding time in controls (Non Stressed) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means $\pm$ S.E.M. # $p < 0.01$ relative to non-stressed mice.



Fold changes of BDNF and Ntrk2 within the hippocampus collected 75-min after social defeat stress (Social Defeat) or at a corresponding time in controls (Non Stressed) among mice that had been housed in EE (Enriched Environment) or SE (Standard Environment) conditions. Data represents means $\pm$ S.E.M. * $p < 0.001$ relative to mice housed in standard conditions.