International Journal of Neuropsychopharmacology (2013), **16**, 351–363. © CINP 2012
doi:10.1017/S1461145712000120

ARTICLE

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The differential impact of social defeat on mice living in isolation or groups in an enriched environment: plasma corticosterone and monoamine variations

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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. Although environmental enrichment may protect against some of the adverse behavioural and biological effects of social defeat, it seems that, among male group-housed mice maintained in an enriched environment (EE), aggressive behaviours may be more readily instigated, thus promoting distress and exacerbating psychopathological features. Thus, although an EE can potentially have numerous beneficial effects, these may depend on the general conditions in which mice were raised. It was observed in the current investigations that EE group-housed BALB/cByJ mice displayed increased anxiety-like behaviours compared to their counterparts maintained in a standard environment (SE). Furthermore, in response to social defeat, EE group-housed male mice exhibited decreased weight gain, exaggerated corticosterone elevations and altered hippocampal norepinephrine utilization compared to their SE counterparts. These effects were not apparent in the individually housed EE mice and, in fact, enrichment among these mice appeared to buffer against serotonin changes induced by social defeat. It is possible that some potentially beneficial effects of enrichment were precluded among group-housed mice, possibly owing to social disturbances that might occur in these conditions. In fact, even if social interaction is an essential feature of enrichment, it seems that some of the positive effects of this housing condition might be optimal when mice are housed individually, particularly with regard to buffering the effects of social defeat.

Received 10 November 2011; Reviewed 13 December 2011; Revised 12 January 2012; Accepted 18 January 2012; First published online 21 March 2012

Key words: Corticosterone, environmental enrichment, norepinephrine, serotonin, social defeat.

Introduction

Animal models of psychopathologies have increasingly focused on the impact of psychosocial stressors, including social defeat, to identify their biological correlates. In this regard, rodents that had experienced social defeat exhibited elevated anxiety (Buwalda *et al.* 2005) as well as depressive-like behaviours, such as motivational disturbances and anhedonia (Becker *et al.* 2008). Moreover, relative to non-stressed mice, the defeated mice displayed elevated serotonin (5-HT) and norepinephrine (NE) utilization in the prefrontal cortex (PFC) and hippocampus (Audet & Anisman, 2010), increased mesolimbic dopamine (DA) activity (Miczek *et al.* 2008) and down-regulation of hippocampal brain-derived neurotrophic factor (BDNF) transcripts (Tsankova *et al.* 2006).

Environmental enrichment has traditionally been thought to buffer the adverse effects of stressors and to limit the development of fear and anxiety (Benaroya-Milshtein *et al.* 2004; Chapillon *et al.* 1999; Fox *et al.* 2006), as well as to attenuate depressive-like behaviours elicited by chronic social defeat (Schloesser *et al.* 2010). In line with a positive role for enrichment in contending with stressors, housing rodents in an enriched environment (EE) also increased levels of 5-HT in the PFC and hippocampus (Brenes *et al.* 2008, 2009), NE within the hippocampus (Brenes *et al.* 2009), mesolimbic DA activity (Segovia *et al.* 2010) and increased neurogenesis or cell survival (Hendriksen *et al.* 2010).

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In contrast to reports of beneficial effects attributable to enrichment, this treatment has also been found to promote aggressive behaviours, particularly among group-housed male mice, causing severe wounding in subordinates and ultimately reducing the well-being of these animals (Haemisch *et al.* 1994; Howerton *et al.* 2008; van Loo *et al.* 2002). In this regard, we have shown that housing male CD-1 mice (known to be relatively aggressive; Howerton *et al.* 2008) in groups of three to four in an EE promoted aggression between cage mates and exaggerated corticosterone and brain monoamine responses to a subsequent mild stressor (McQuaid *et al.* 2011).

In evaluating the effects of an EE on behavioural outcomes, several investigators housed male mice individually (Lehmann & Herkenham, 2011; Schloesser *et al.* 2010), possibly to avoid aggression that might otherwise occur within enriched conditions. However, social interaction may be an important component of enrichment (van Praag *et al.* 2000) and housing animals in isolation may obfuscate positive effects that might otherwise emerge. Furthermore, individual housing itself may be stressful for mice and may induce symptoms reminiscent of depression in animal models of the disorder (Saenz *et al.* 2006). Indeed, when given the choice between an empty or an inhabited cage, mice preferred the proximity of another male, regardless of their social status (van Loo *et al.* 2001).

The current investigation examined the behavioural and neurochemical effects associated with enriched housing. Given the propensity for severe aggression in CD-1 male mice, we assessed the effects of enrichment in BALB/cByJ mice, a highly anxious strain (Anisman *et al.* 1998) that is not known to be very aggressive. Thus, we could determine whether housing male mice in groups in an EE *vs.* a standard environment (SE) would influence anxiety-like behaviours under conditions in which severe aggression would be absent (expt 1). Further, we evaluated whether enrichment in group- and individually housed mice (expts 2 and 3, respectively) would differentially influence corticosterone and monoamine responses to a social defeat stressor.

Materials and methods

Animals and housing procedures

Eighty-five naive male BALB/cByJ mice (Jackson Laboratory, USA), aged 6–8 wk, were housed three mice/cage (expts 1 and 2) or individually (expt 3) in either an EE or a SE. The EE consisted of polypropylene rat maternity cages ($50 \times 40 \times 20$ 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. 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 ($27 \times 21 \times 14$ cm) with only one cotton nestlet. Mice were left undisturbed in their respective environments (EE or SE) for 4 wk, with the exception of weighing, weekly routine cage cleaning and the scoring of aggressive behaviours.

In addition to the experimental mice, 17 singly housed CD-1 retired breeders (aged 9–12 months), expected to be relatively aggressive, were used as social stressors during the social defeat procedure. Mice were kept on a 12-h light/dark cycle (lights on 08:00 hours) 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 of the Canadian Council on Animal Care.

In expt 1, anxiety-like behaviours were measured in the elevated plus-maze after 4 wk of living in EE or SE conditions. Inasmuch as aggression might influence the effects of enriched housing, in expts 2 and 3 we evaluated the influence of EE and SE conditions among mice housed in groups or individually, respectively. Thus, in these studies we assessed the protracted effects of 4 wk of housing in EE *vs.* SE and that of grouped (expt 2) *vs.* individual (expt 3) housing on corticosterone and monoamine responses to social defeat stress that occurred each day during the fourth week of the housing conditions. Characteristics of the mice (e.g. age) as well as experimental conditions (e.g. time of assignment to respective environments, duration of enrichment prior to the stressor procedure, duration of the stressor procedure, experimenter cleaning cages and the stressor procedure itself) were identical for expts 2 and 3.

Scoring aggressive behaviours

Home-cage aggressive behaviours in SE and EE group-housed mice were scored 3 d/wk (Monday, Wednesday and Friday) for 4 wk, commencing immediately upon arrival of mice to the laboratory. Prior to scoring, mice were tail marked to allow for individual identification within a cage. On these occasions the frequency and duration of aggressive interactions were scored in

real time over a 5-min interval. These interactions were categorized as attacks, aggressive chasing or aggressive grooming, all resulting in submissive behaviours in the targeted mouse.

Expt 1

Elevated plus-maze

Mice that had been housed in groups for 4 wk in the EE or SE conditions ($n=8$–9 per group respectively) were tested for anxiety-like behaviours in the elevated plus-maze. The elevated plus-maze (60 cm above the floor) consisted of a wooden maze that comprised two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm) with an open roof, arranged such that the two open arms were opposite each other. Mice were brought to the testing room to acclimatize to the new environment 1 h prior to testing and were then placed, individually, into the maze facing a closed arm for 5 min. Entries into the open and closed arms, time spent in these arms, latency to enter into the open arms and the number of stretch attempts into the open arms were scored.

Expts 2 and 3

Social stressor procedure

Testing occurred between 08:30 and 13:00 hours to minimize effects related to diurnal factors. After living in their assigned environments for 3 wk, half of the EE and SE mice (expt 2: $n=36$ EE or SE mice; expt 3: $n=32$ EE or SE mice) were exposed to a retired breeder CD-1 mouse for 15 min on each of seven consecutive days, whereas the other half (non-stressed controls) remained undisturbed in their home cages. Specifically, mice were introduced, individually, into the home cage of a retired breeder and direct interactions were permitted for 15 min. Each mouse was confronted with a different retired breeder on each of the seven defeat sessions, so that each BALB/cByJ mouse was exposed to seven different retired breeders across the course of the stressing period. Excessive aggressive behaviours were interrupted by inserting a wire mesh partition that allowed for auditory and visual exchange between the two mice, but prevented physical contact. The criterion used to stop interactions was the persistence of aggressive attacks from the retired breeder (e.g. chasing/biting) and the display of defeat by the BALB/cByJ mouse (submissive posture accompanied by vocalizations). Due to the very aggressive nature of the CD-1 retired breeders and the smaller size of the BALB/cByJ mice in comparison to the CD-1 mice, to prevent injury a partition was inserted for every social defeat session. Following each stressor exposure, mice were returned to their assigned environments. During each social defeat session, defensive behaviours were scored. Mice were categorized as being passively or actively defensive according to the display of aggressive behaviours in response to the retired breeder's attacks. In addition, the issue of aggressive encounters was determined (social defeat *vs*. non-defeated/non-victorious mice), and only mice that had been defeated at least four times over the seven sessions and defeated on the seventh session were included in further analyses.

Blood collection and brain removal

Three minutes after the seventh defeat, mice were rapidly decapitated and trunk blood was 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 × 3.75 × 2.0 cm) positioned on a block of ice that rested on dry ice. The matrix had a series of slots spaced 500 μm apart that guided razor blades to provide coronal brain sections. Once the brains were sliced, tissue from the PFC, 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 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 <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 a 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 × 10 cm) and a three-cell coulometric electrochemical detector (ESA model 5100A; Thermo Scientific, USA). For separation, a mobile phase was used, comprising 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 (USA) integrator. A protein analysis kit (Fisher Scientific, Canada) and a spectrophotometer (PC800 colorimeter; Brinkmann Instruments Inc., USA) 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

Anxiety-like behaviours in the elevated plus-maze for expt 1 were analysed through a one-way analysis of variance (ANOVA; enrichment: standard *vs.* enriched). Plasma corticosterone concentrations as well as concentrations of NE, 5-HT and their metabolites in the PFC, hippocampus and CeA were analysed through a series of 2 (enrichment) × 2 (stressor: non-stressed *vs.* social defeat) between-groups ANOVAs for expts 2 and 3 separately. Follow-up comparisons comprised *t* tests with Bonferonni's correction to maintain the α level at 0.05.

Results

Aggressive behaviours during grouped and enriched housing

As expected, aggression levels were generally low in BALB/cByJ mice. In fact, of the 53 group-housed mice of expts 1 and 2, only one EE mouse was removed from the study for displaying overly aggressive behaviours (defined as continuous attacking so that injury had occurred) and very few aggressive encounters were witnessed during the 5-min/cage scoring sessions. Nevertheless, even among this relatively non-aggressive strain of mouse, across expts 1 and 2, there were eight EE and seven SE mice that bore wounds. Thus, the aggressive behaviour in BALB/cByJ mice was lower than that seen in strains such as CD-1, where we previously found that >45% were wounded by conspecifics in the enriched condition *vs.* <1% in the standard-housed mice (McQuaid *et al.* 2011). This does not imply that social conditions were not disrupted among enriched BALB/cByJ mice housed in groups, but simply points to the limited aggression that occurs in this strain.

Defensive behaviours during social defeat sessions

In both expts 2 and 3, enriched animals displayed fewer active defensive behaviours in response to the retired breeders' attacks compared to SE animals. In expt 2, only two of nine EE mice fought back (i.e. active defence), whereas seven of the nine SE mice did ($\chi^2 = 5.56$, $p < 0.05$). In expt 3, only two of nine EE mice fought back, whereas eight of the nine SE mice did ($\chi^2 = 8.10$, $p < 0.01$).

Expt 1

Anxiety-like behaviours

As shown in Fig. 1, EE animals displayed significantly more anxiety-like behaviours than SE mice in the elevated plus-maze. Compared to their SE counterparts, the enriched mice displayed longer latencies to enter the open arms ($F_{1,15} = 9.57$, $p < 0.01$). In addition, the ratios of time spent in open arms ($F_{1,15} = 6.71$, $p < 0.05$) and of entries made into open arms ($F_{1,15} = 9.41$, $p < 0.01$), were much lower in EE than in SE mice. There were no differences between EE and SE mice with regard to the time spent or entries into the closed arms and the number of stretch attempts made (data not shown).

Expts 2 and 3

Weight changes

As seen in Fig. 2, over the course of the stressor regimen, group-housed mice that experienced defeat gained significantly less weight than the non-stressed animals ($F_{1,32} = 8.96$, $p < 0.01$). Although the enrichment × stressor interaction was not significant, based on *a priori* predictions the simple effects comprising the interaction were examined. These comparisons confirmed that the enriched group-housed mice gained significantly less weight after stressor exposure compared to enriched animals that did not experience social defeat ($p < 0.05$), an effect that was not found in SE mice (Fig. 2*a*). Unlike these effects, weight change did not differ as a function of the stressor condition among individually housed EE and SE mice.

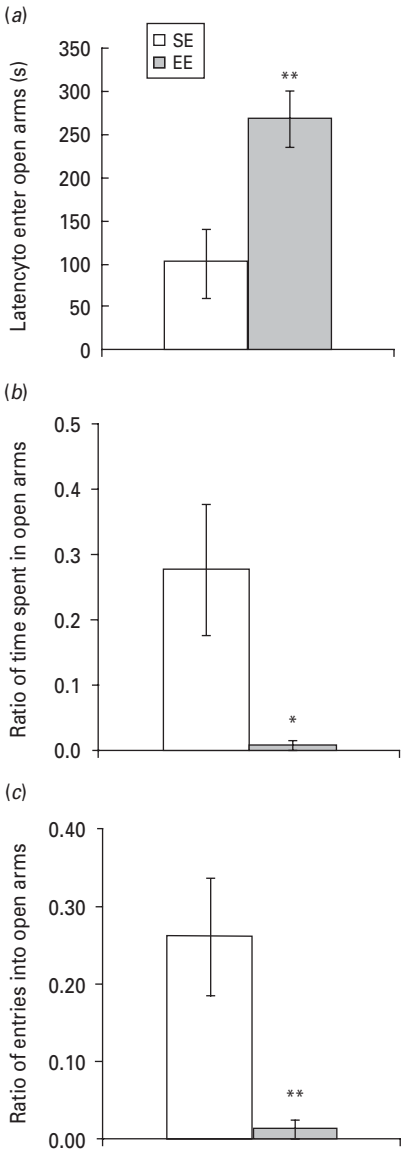

Fig. 1. (*a*) Latency to enter the open arms (s), as well as (*b*) ratio of time spent in the open arms (s) divided by (time spent in the open arms + time spent in the closed arms) and (*c*) ratio of entries into the open arms divided by (entries into the open arms + entries into the closed arms) in the elevated-plus maze in an enriched environment (EE) and standard environment (SE) mice. Data are represented by means ± S.E.M. ** $p<0.01$ and * $p<0.05$ relative to mice that had been housed in standard conditions.

Plasma corticosterone levels

Among group-housed mice of expt 2, plasma corticosterone levels varied as a function of the enrichment × stressor interaction ($F_{1,28}=4.59$, $p<0.05$). Follow-up comparisons of the simple effects comprising this interaction indicated that, in the absence of defeat, corticosterone levels were comparable in EE and SE mice. However, in response to repeated defeat, corticosterone levels were elevated and this outcome was significantly higher in EE mice compared to their SE counterparts ($p<0.01$) (Fig. 3*a*). In contrast, among individually housed mice, corticosterone levels were significantly higher in defeated mice compared to their non-stressed counterparts ($F_{1,28}=41.32$, $p<0.001$), but the corticosterone elevations did not differ as a function of the EE *vs*. SE conditions (Fig. 3*b*).

Monoamine variations within the PFC

Among group-housed mice, 5-HIAA and 5-HT concentrations in the PFC were unaffected by the stressor or enrichment treatments (Fig. 4*a*), whereas social defeat in individually housed mice increased 5-HIAA accumulation compared to the non-stressed mice ($F_{1,28}=7.24$, $p<0.05$) (Fig. 4*b*). The enrichment × stressor interaction for individually housed mice was not significant but, based on *a prior* predictions, the simple effects that comprised this interaction were examined. The follow-up comparisons confirmed that, in the absence of a stressor, the levels of 5-HIAA were comparable for EE and SE mice. However, after defeat, the utilization of 5-HT was higher among SE than in EE mice ($p<0.05$).

Unlike the 5-HT changes, prefrontal NE and MHPG variations did not vary with the enriched or stressor treatments. Among mice housed individually, there was a modest rise of MHPG ($p=0.08$), but this outcome was not statistically significant (data not shown).

Monoamine variations within the hippocampus

Hippocampal 5-HIAA concentrations were increased after repeated defeat in group-housed mice ($F_{1,32}=7.61$, $p<0.05$) (Fig. 5*a*). In this instance, however, 5-HIAA elevations were not moderated by whether mice had been housed in the SE *vs*. EE conditions. In contrast, in individually housed mice, neither 5-HT nor 5-HIAA concentrations were significantly affected by any treatments (Fig. 5*b*), although the 5-HIAA changes approached significance ($F_{1,28}=3.46$, $p=0.07$).

Among group-housed mice of expt 2, hippocampal MHPG concentrations varied as a function of the enrichment × stressor interaction ($F_{1,32}=5.51$, $p<0.05$) (Fig. 6*a*). Follow-up comparisons of the simple effects comprising this interaction indicated that NE utilization in the absence of a further stressor was comparable in the two housing conditions. However,

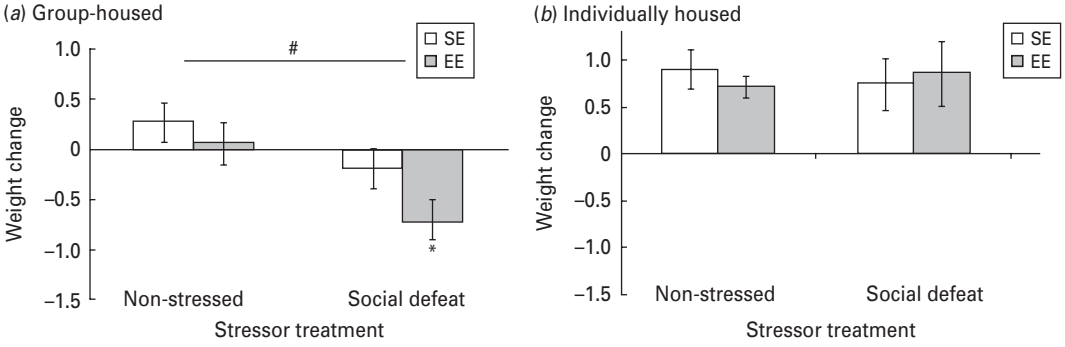

Fig. 2. Changes in weight over the course of seven repeated exposures to social defeat stress (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. (*a*) Represents group-housed mice and (*b*) corresponds to individually housed mice. Data are represented by means ± S.E.M. # $p<0.01$ relative to non-stressed mice; * $p<0.05$ relative to EE non-stressed mice.

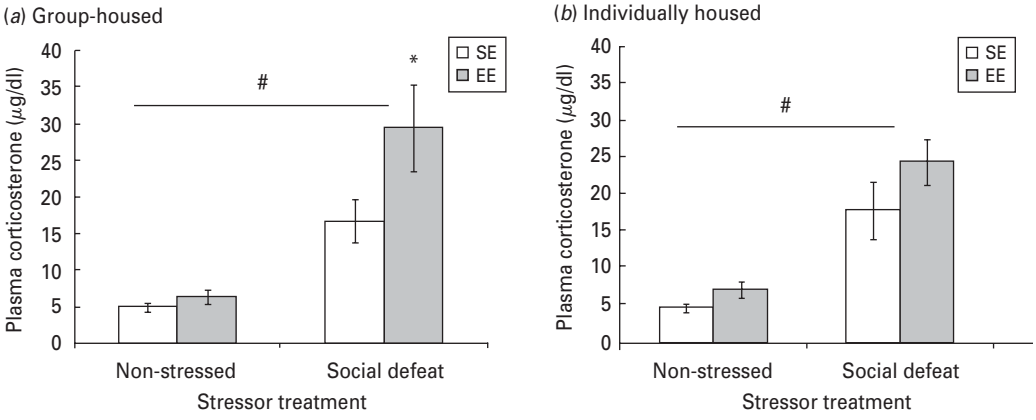

Fig. 3. Plasma corticosterone concentrations (μg/dl) collected 3 min after the final social defeat stressor (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. (*a*) Shows the data of group-housed mice, whereas (*b*) provides the data for individually housed mice. Data represent means ± S.E.M. * $p<0.01$ relative to SE stressed mice; # $p<0.001$ relative to non-stressed mice.

following social defeat, MHPG elevations were apparent in EE mice relative to the non-stressed mice housed in this condition ($p<0.001$), whereas this increase did not occur in SE mice ($p=0.33$). This said, the magnitude of the MHPG increase was relatively small (~25%), but the variance accounted for was actually relatively substantial ($\eta^2=0.15$).

Among individually housed mice that experienced social defeat, MHPG levels were increased compared to their non-stressed counterparts, irrespective of the housing conditions ($F_{1,28}=4.00$, $p=0.05$) (Fig. 6*b*). Despite the altered utilization, the hippocampal NE concentrations in both group- and individually housed mice were not altered by the housing or stressor conditions, although once again this outcome was just shy of statistical significance among individually housed mice ($F_{1,28}=3.51$, $p=0.07$).

Monoamine variations within the CeA

As depicted in Fig. 7*a*, in response to defeat, the group-housed mice displayed markedly increased 5-HIAA CeA concentrations ($F_{1,32}=14.56$, $p<0.01$), whereas 5-HT levels were unaffected by the treatments. In contrast, among individually housed mice, 5-HIAA accumulation was unaltered, although concentrations of 5-HT varied as a function of the enrichment × stressor interaction ($F_{1,27}=5.19$, $p<0.05$) (Fig. 7*b*). The follow-up tests confirmed that, in the absence of stress, 5-HT levels were lower in EE mice compared to SE mice, $p<0.05$ (an effect that was not due to an increase in 5-HT levels in SE mice, as the 5-HT levels were similar to those of SE group-housed mice in expt 2). Furthermore, in SE mice, 5-HT levels were modestly reduced after stressor exposure

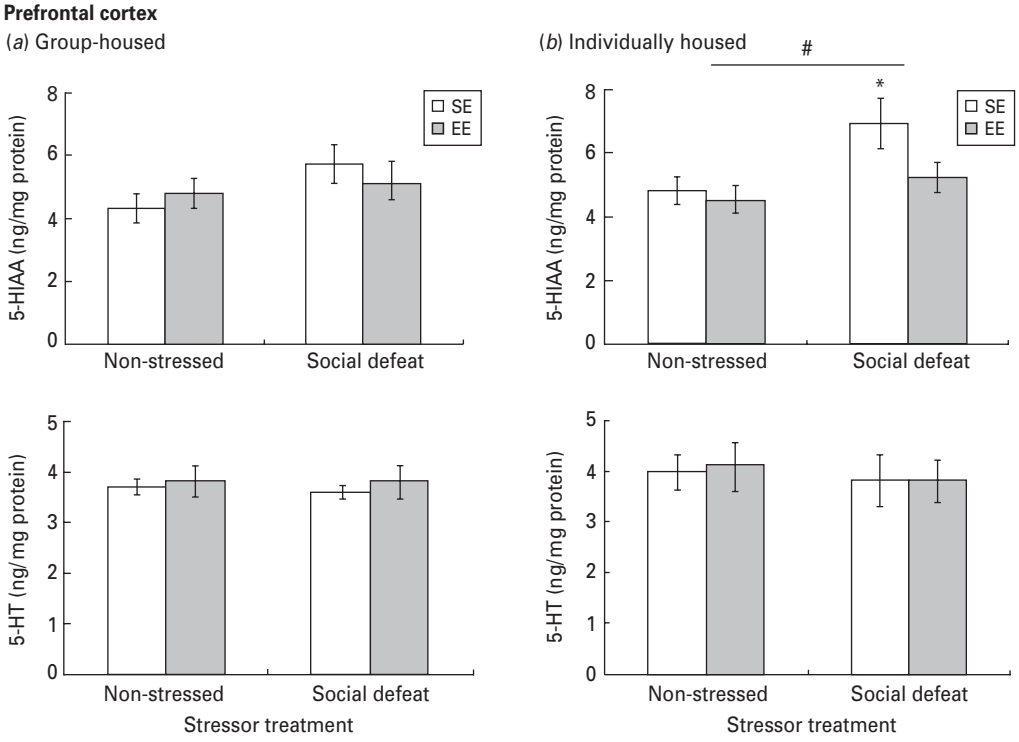

Fig. 4. Prefrontal cortex concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT; ng/mg protein) collected 3-min following the last social defeat session (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. Data for group-housed mice are shown in (*a*), while data for individually housed mice are shown in (*b*). Data represent means ± S.E.M. * $p < 0.05$ relative to EE stressed mice; # $p < 0.05$ relative to non-stressed mice.

compared to control levels, $p = 0.06$, a trend that was not found for EE mice ($p = 0.28$).

The MHPG accumulation in the CeA was affected by stressor exposure and did not differ between enriched *vs*. standard conditions. Specifically, following defeat, group-housed mice exhibited unaltered NE utilization; however, NE levels were elevated ($F_{1,32} = 4.36$, $p < 0.05$) (Fig. 8*a*). In contrast to these effects, MHPG concentrations among individually housed mice was increased following social defeat relative to that evident in non-stressed mice ($F_{1,27} = 5.16$, $p < 0.05$) and levels of NE were unaffected by the treatments that the mice received (Fig. 8*b*).

Discussion

As expected, based on earlier studies with BALB/c substrains (van Loo *et al.* 2003), in the current investigations severe aggression was not evident in BALB/cByJ mice. This contrasts with the aggressive behaviour associated with enriched housing in CD-1 mice (McQuaid *et al.* 2011). This does not imply, however, that the social conditions among EE mice were not disrupted or stressful. Indeed, given the increased anxiety-like behaviours associated with enriched conditions, as well as decreased weight gain and exaggerated corticosterone levels in response to social defeat in EE animals, it seemed that the EE among group-housed mice was relatively stressful. These effects might have resulted from the complex social interactions among group-housed mice that could have occurred in the EE. In fact, the availability of highly desired components of the EE may elicit territorial behaviours (Nevison *et al.* 1999) and, ultimately, certain animals may be denied access to these resources (Howerton *et al.* 2008). Furthermore, enrichment may promote a less stable social hierarchy, which has been associated with higher levels of distress (Haemisch *et al.* 1994).

A potential additional indication of increased vulnerability associated with enrichment was provided by the finding that EE mice were less likely to actively defend themselves (or fight back) when attacked by the retired breeder. It might be that previous experience of being dominated by a cage mate (or more frequent territorial behaviours) that had occurred in the

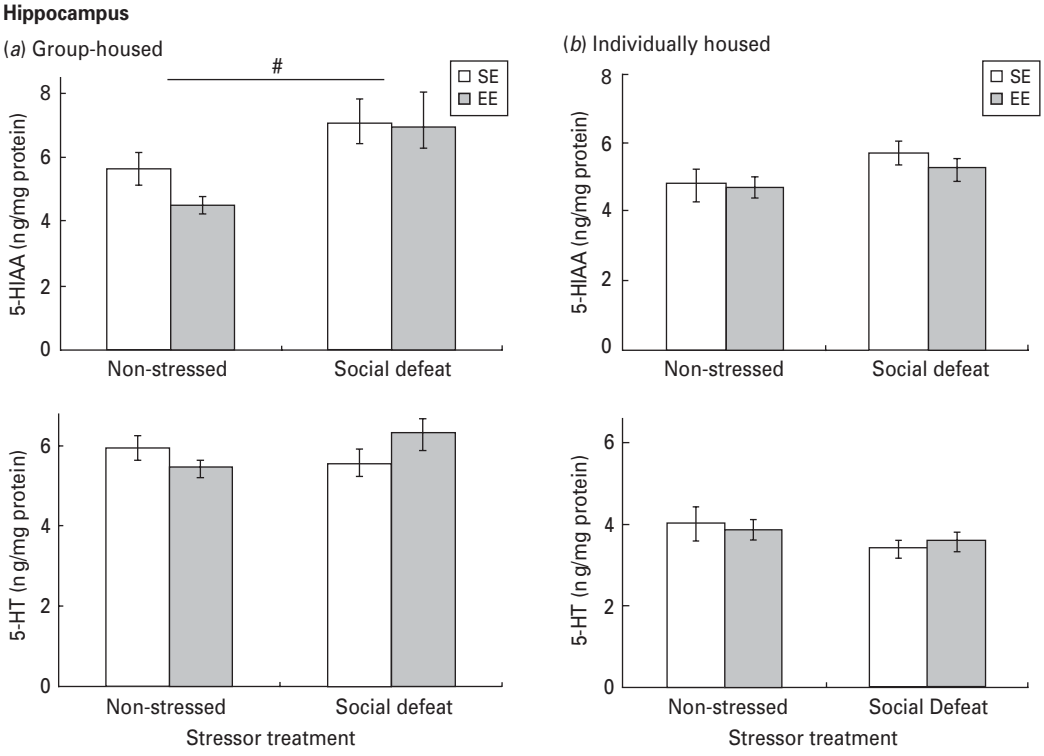

Fig. 5. Hippocampal concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT; ng/mg protein) collected 3-min following the last exposure to social defeat (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. Data for group-housed mice are shown in (*a*) while (*b*) shows data for individually housed mice. Data represent means ± S.E.M. $^{\#}p < 0.05$ relative to non-stressed mice.

EE might have encouraged the submissive behaviours that were more pronounced in the EE mice. However, this profile was also observed in EE mice that were housed alone. The source for this outcome is not immediately apparent, although it should be noted that it seems a reproducible effect as we have observed the same outcome in another recent experiment. Specifically, using the same procedure, we found that none of 10 group-housed EE mice displayed active defensive behaviours during social defeat sessions, whereas 11 of 12 mice showed these behaviours after being housed in a SE.

The greater anxiety-like behaviours in EE relative to SE mice in the elevated plus-maze was manifested by the increased latencies to enter into the open arms, as well as the decreased ratios of time spent and entries made into the open arms compared to the closed arms (Pellow *et al.* 1985). In fact, mice housed in EE conditions barely explored the open arms of the plus-maze. In contrast, EE and SE animals made a comparable number of stretch attempts, (risk assessment behaviour) and exhibited comparable entries into the closed arms, indicating that the EE mice were not immobile in the plus-maze and appraised the open and closed arms just as the SE animals did. In contrast to the present findings, it was previously shown that enriched mice housed in groups displayed fewer risk assessment behaviours (Roy *et al.* 2001) and decreased anxiety in the plus-maze (Chapillon *et al.* 1999; Friske & Gammie *et al.* 2005). It was also reported that enriched mice were more active in the plus-maze and made more closed arm entries (Roy *et al.* 2001), suggesting increased arousal. The source for the different outcomes across studies is uncertain given the numerous procedural differences that existed (i.e. sex, age, strain/species of rodents and stability of the environment; Simpson & Kelly, 2011). It is possible that the current method of enrichment, which did not include changing items weekly, might have enhanced territorial behaviours, thus contributing to the anxiogenic effects observed among the enriched animals 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, possibly owing to the heightened aggression in these mice (McQuaid *et al.* 2011). In the current investigation, corticosterone

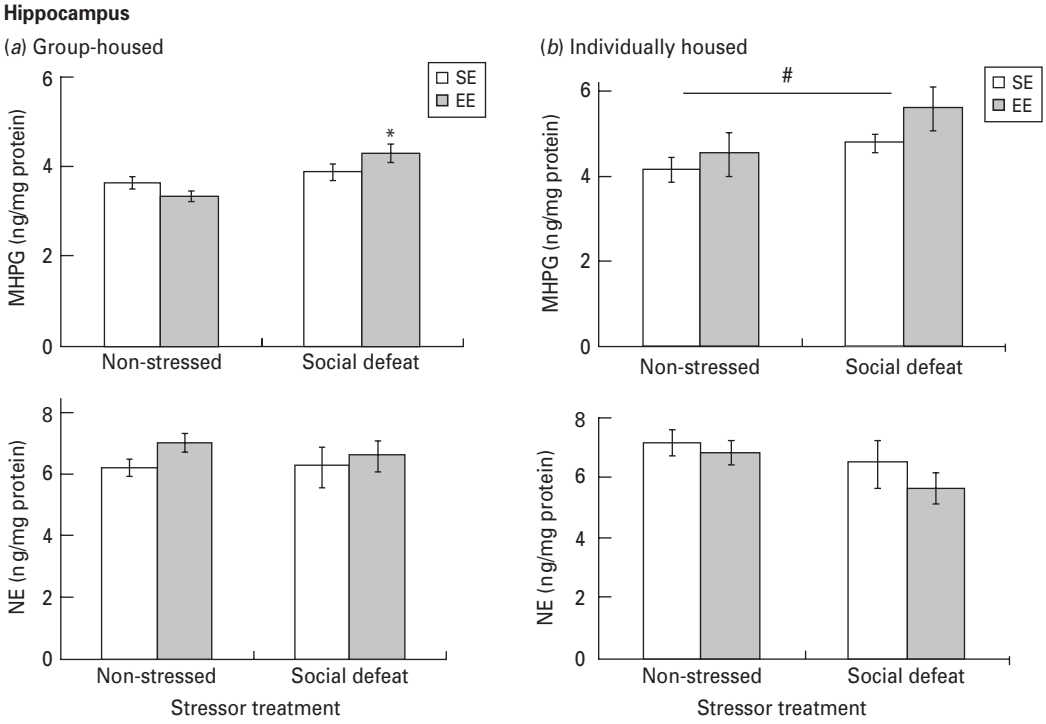

Fig. 6. Hippocampal concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE; ng/mg protein) collected 3-min after the final social defeat stressor (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE). Data in (*a*) represent group-housed mice and (*b*) correspond to individually housed mice. Data represent means ± S.E.M. * $p < 0.001$ relative to EE non-stressed mice; # $p = 0.055$ relative to non-stressed mice.

elevations elicited by repeated social defeat were also more pronounced in group-housed EE mice compared to SE mice. A similar profile was also apparent in individually housed mice, although this outcome did not reach statistical significance and was less pronounced than in group-housed mice. Nevertheless, because the effect of defeat in isolated mice was somewhat elevated in the EE relative to the SE condition, it may be premature to conclude that the observed effects in group-housed mice were related to aggression. Yet, after social defeat, enriched group-housed mice also gained less weight, an effect not seen in their SE counterparts or in individually housed mice under EE conditions. The fact that both the enhanced hormone response and weight changes associated with social defeat were less evident in isolated EE mice suggests that grouping male mice in enriched conditions may be stressful. Indeed, increased basal corticosterone levels and decreased weight gain have previously been observed in enriched male rodents compared to SE counterparts (Moncek *et al.* 2004; van Loo *et al.* 2002) and these effects were attributed to elevated aggression associated with enrichment (van Loo *et al.* 2002).

Brain monoamine activity was influenced by the social stressor, the housing conditions and by whether mice had been housed in groups or individually and these neurochemical changes varied with the specific brain region assessed. Specifically, 5-HT activity in EE and SE mice was differentially affected in group- *vs.* individually housed mice. The prefrontal 5-HIAA elevations normally elicited by social defeat in individually housed SE mice (e.g. Audet & Anisman, 2010) were not apparent among SE group-housed mice. Interestingly, among individually housed mice, 5-HIAA accumulation after social defeat was higher in SE than in EE mice, possibly indicating that enrichment among individually housed mice acted to buffer against the rise of prefrontal 5-HIAA levels ordinarily associated with defeat. Furthermore, among group-housed mice, 5-HIAA levels in the hippocampus and CeA were elevated in both EE and SE mice after repeated defeat, whereas the 5-HT levels were unaffected. In contrast, in individually housed mice, 5-HT utilization in these regions was not influenced by social defeat, although a modest decline in amygdala 5-HT levels was apparent in SE mice only. Once more, this might again be indicative of a buffering effect, in

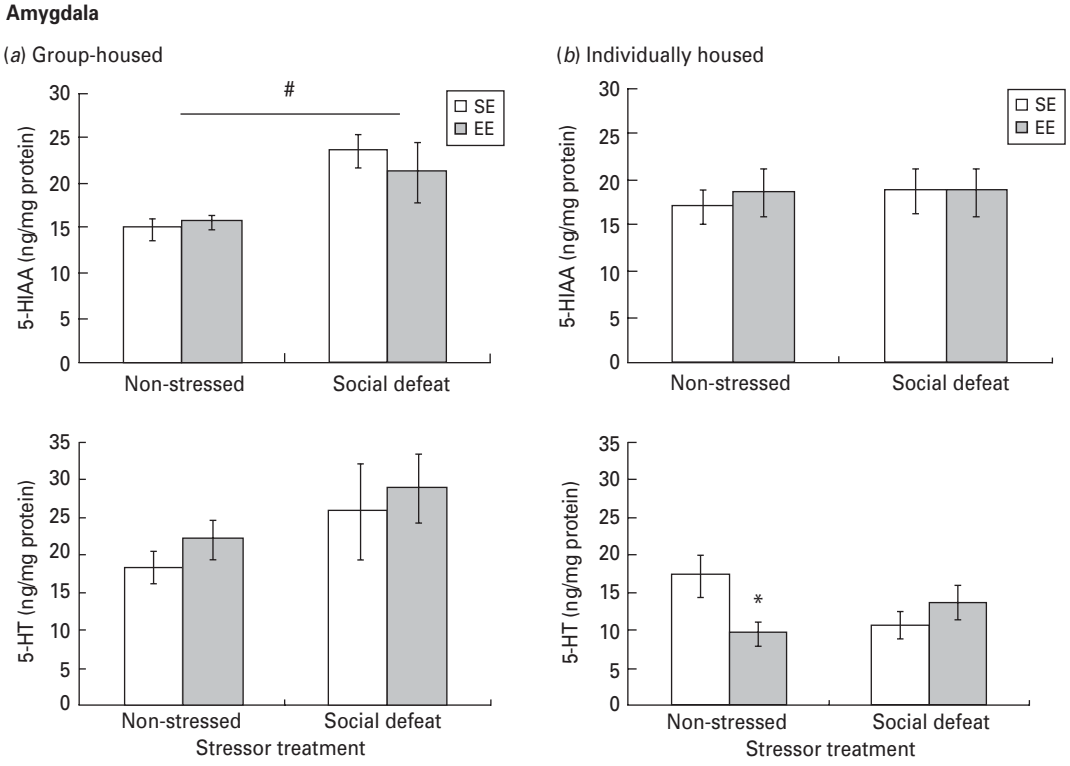

Fig. 7. Central amygdala concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT; ng/mg protein) collected 3-min after the final social defeat stressor (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. Data for mice that had been group-housed are shown in (*a*) or individually housed, shown in (*b*). Data represent means ± S.E.M. # $p < 0.01$ relative to non-stressed mice; * $p < 0.05$ relative to non-stressed SE mice.

which enrichment among individually housed mice prevented the decline of amygdala 5-HT in response to the stressor. It should be noted that 5-HT concentrations in the CeA were reduced in EE mice in the absence of defeat, possibly accounting, in part, for these effects. Overall, these outcomes are consistent with the view that, among individually housed mice, environmental enrichment might serve as a buffer that limits specific variations of 5-HT activity that are otherwise associated with social defeat. It is interesting that these 5-HT alterations among individually housed enriched mice occurred only in the PFC and CeA and were not found in the hippocampus, possibly indicating a degree of specificity regarding the effects of enrichment on 5-HT variations.

The finding that prefrontal NE activity was seemingly unaffected by the stressor or housing conditions is not entirely surprising. Although it has frequently been observed that NE neuronal activity is elevated by acute stressors, we observed an adaptation-like effect within the PFC in response to repeated exposure to psychogenic and neurogenic stressors (Anisman & Zacharko, 1990). It might similarly be the case that the NE variations associated with a single defeat episode were attenuated with repeated defeat experiences. In contrast to the effects evident within the PFC, NE utilization in the hippocampus was enhanced after repeated defeat in group-housed EE mice, an effect that was not found in individually housed EE mice. Although the enhanced NE utilization was modest, these data again point to the enriched group-housed environment being a potentially stressful one.

The amygdala is thought to be highly involved in stress-related pathologies, such as post-traumatic stress disorder (PTSD; Yehuda & LeDoux, 2007). Furthermore, NE enhancement in the amygdala has been implicated in the development of PTSD (Debiec *et al.* 2011). Thus, in view of the potential involvement of amygdala NE functioning associated with fear and anxiety, it might have been expected that NE in the CeA would be especially sensitive to social defeat. This seemed apparent among the individually housed mice, in which MHPG concentrations increased following defeat, as well as among group-housed mice that displayed increased NE concentrations in response to social defeat. Thus, the enhanced NE

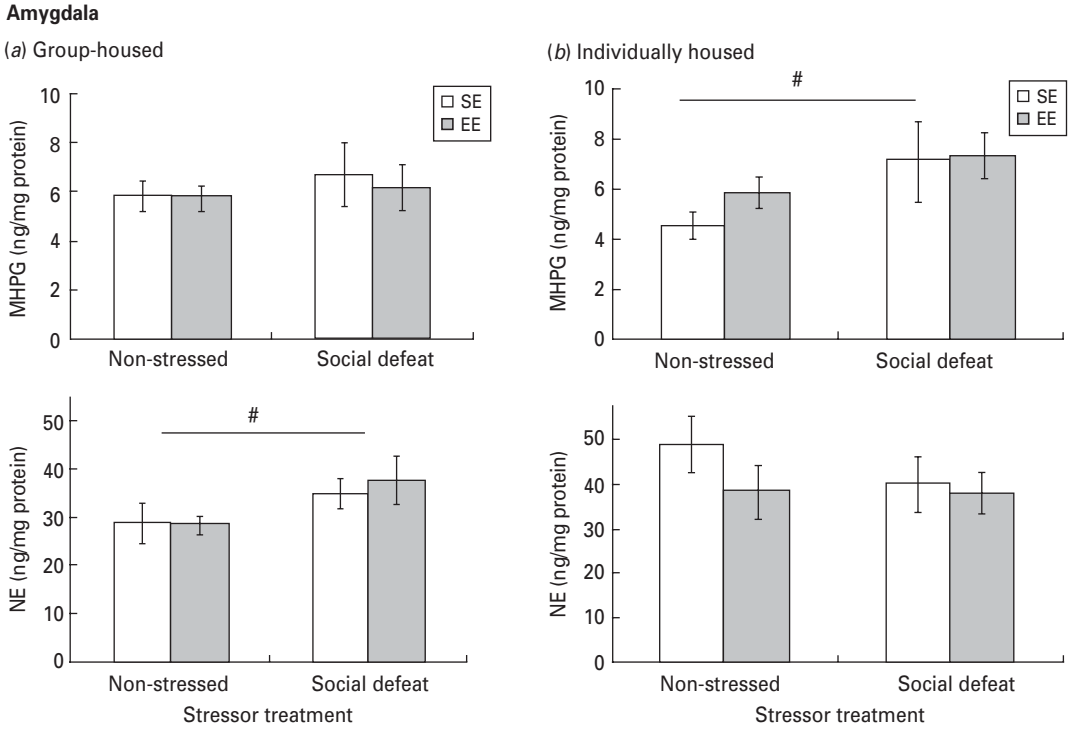

Fig. 8. Central amygdala concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE; ng/mg protein) collected 3-min following the last social defeat session (social defeat) or at corresponding times in controls (non-stressed) among enriched environment (EE) and standard environment (SE) mice. (*a*) Shows data for group-housed mice, whereas (*b*) shows data for individually housed mice. Data represent means ± S.E.M. # $p < 0.05$ relative to non-stressed mice.

concentrations in the amygdala displayed by group-housed mice after repeated social defeat may be particularly important, especially considering its involvement in certain stress-related pathologies.

Taken together, it appeared that enrichment among group-housed mice led to distress, reflected by increased anxiety in the plus-maze, as well as decreased weight gain and exaggerated corticosterone elevations and hippocampal NE utilization in response to social defeat. In contrast, among individually housed mice, there was no indication that the EE was stressful, as weight was not altered and the corticosterone variations were modest. It thus seems that the social component of enrichment in mice might not be protective with regard to the outcomes ordinarily associated with repeated social defeat, which contrasts with reports from experiments conducted with rats (Ruis *et al.* 1999). These species-related differences might be related to differences in social structure, social development and typical behavioural patterns (e.g. agonistic interactions) exhibited by rats *vs*. mice (Scott, 1966). In this regard, it seems that in mice the social aspect of the EE might promote territorial and competitive behaviours.

The combination of social and physical enrichment in the current experiment might have created a somewhat stressful environment rather than a supportive and beneficial one that would buffer the effects of subsequent social defeat. However, as indicated earlier, social interaction may be an important component of an EE (van Praag *et al.* 2000) and depriving mice of social contact, despite the otherwise enriched housing, might have precluded still greater beneficial effects from emerging. Consistent with this perspective, it has been suggested that the positive impact of enrichment is not simply due to any single element, but reflects the interaction of multiple components (socialization and physical activity) that comprise this environment (van Praag *et al.* 2000). This said, in the current investigation, it seems that enrichment among individually housed mice acted to buffer against altered 5-HT activity in the PFC and CeA in response to social defeat. Several investigators have, indeed, reported that enrichment using singly housed mice protects against the stress effects of chronic social defeat, particularly with regard to anxiety and depressive-like behaviours (Lehmann & Herkenham, 2011; Schloesser *et al.* 2010). Although this outcome

was observed in the present investigation with regard to brain 5-HT variations elicited by repeated defeat, this does not imply that EE among individually housed mice was beneficial in other respects (e.g. in preventing the reduced BDNF levels that accompany social defeat). Indeed, as indicated earlier, it is possible that some of the effects of enrichment would be absent when an essential element, namely, one involving social interaction, was eliminated from the enrichment experience.

Acknowledgements

This research was supported by the Canadian Institutes of Health Research (CIHR). H.A. holds a Canadian Research Chair in Neuroscience. R.M. is funded by the Natural Sciences and Engineering Council of Canada (NSERC). M.-C.A. is funded by Fonds de la recherche en santé du Québec (FRSQ).

Statement of Interest

None.

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