International Journal of Neuropsychopharmacology (2010), **13**, 361–372. Copyright © CINP 2009
doi:10.1017/S1461145709990174

ARTICLE

CINP

Neuroendocrine and neurochemical impact of aggressive social interactions in submissive and dominant mice: implications for stress-related disorders

Marie-Claude Audet and Hymie Anisman

Institute of Neurosciences, Carleton University, Ottawa, Ontario, Canada

Abstract

Social conflicts may engender stress-related behavioural and physiological disturbances in the victims of aggression. In addition, stress-like neurochemical changes and ensuing depressive and anxiety symptoms might also be evident in the perpetrators of aggressive acts. The present investigation assessed basal levels of circulating corticosterone and of brain serotonin (5-HT) and norepinephrine (NE) in pre-identified submissive and dominant mice. In addition, brain neurochemical changes were determined following a single or three 15-min aggressive episodes both in submissive mice and in those that dominated the aggressive interplay. Three minutes after single and repeated confrontations, plasma corticosterone levels and 5-HT utilization within the prefrontal cortex (PFC) and hippocampus were increased to a comparable extent in submissive and dominant animals. Interestingly, however, NE utilization within the PFC and hippocampus was augmented to a greater level in submissive mice. These results suggest that 5-HT neuronal functioning was generally responsive to aggressive events, irrespective of social rank, whereas NE neuronal activity within the PFC and hippocampus was more sensitive to the submissive/dominance attributes of the social situation. It is possible that NE and 5-HT variations associated with an aggressive experience contribute to depressive- and anxiety-like manifestations typically observed after such psychosocial stressors, particularly in submissive mice. However, given that 5-HT changes occur irrespective of social rank, these data suggest that a toll is taken on both submissive and dominant mice, leaving them vulnerable to stress-related pathology.

Received 30 October 2008; Reviewed 21 January 2009; Revised 1 May 2009; Accepted 21 May 2009; First published online 23 June 2009

Key words: Aggression, depression, norepinephrine, psychosocial stressors, submission/dominance status, serotonin.

Introduction

Studies in both humans and animals have indicated that psychosocial stressors were particularly potent in promoting behavioural disturbances and eliciting neurochemical variations that have been implicated in several psychopathologies. In this regard, victims of harassment, bullying or aggression reported considerable distress and developed maladaptive social behaviours (e.g. poor self-esteem, submissiveness, social withdrawal) as well as depressive and anxiety symptoms (Bjorkqvist, 2001; Hawker & Boulton, 2000). Although most studies have focused on victims, perpetrators of intimidation and aggression might also display stress-related disturbances (Matthiesen & Einarsen, 2007; Seals & Young, 2003). Given the difficulties of evaluating brain chemical variations associated with social conflicts in humans, there have been repeated calls for the analyses of neurochemical changes associated with defensive and offensive behaviours in animal models of aggression (Bjorkqvist, 2001; de Almeida *et al*. 2005). Although social defeat and aggression in rodents are not equivalent to submissiveness and bullying in humans, nevertheless, stress outcomes associated with agonistic encounters in animals may be instructive with respect to the physiological disturbances provoked by psychosocial

Address for correspondence: Dr M.-C. Audet, Institute of Neurosciences, Carleton University, Ottawa, Ontario K1S 5B6, Canada.
Tel.: (613) 520-2600 (ext. 1694) *Fax*: (613) 520-4052
Email: mcaudet@connect.carleton.ca

stressors in humans (Miczek, 1979; Miczek & de Wit, 2008).

Aggressive encounters in submissive animals induce behavioural changes reminiscent of depression, including a general decrease in exploratory activity, motivational disturbances and anhedonia, as well as increased anxiety (Becker *et al*. 2008; Berton *et al*. 1999; Keeney *et al*. 2001; Rygula *et al*. 2005). Similarly, elevated circulating levels of stress hormones adrenocorticotropic hormone (ACTH) and corticosterone were evident following defeat (Bhatnagar *et al*. 2006; Keeney *et al*. 2001, 2006). Such hormonal responses were also observed in animals that dominated an agonistic encounter (Bartolomucci *et al*. 2001; Covington & Miczek, 2005), although diminished pituitary-adrenal activity was also reported (Cacho *et al*. 2003). Interestingly, in high-ranking male infrahuman primates and rodents, elevated glucocorticoid levels may also reflect the distress associated with preserving their social rank or the costs of maintaining reproductive strategies (Barrett *et al*. 2002; Bartolomucci *et al*. 2001; Sapolsky, 2005).

Beyond behavioural and neuroendocrine changes, aggressive interactions in animal models provoke brain serotonin (5-HT) abnormalities like those elicited by other relatively strong stressors. For example, acute social defeat in mice enhanced *in-vivo* hippocampal 5-HT release (Keeney *et al*. 2006). After repeated confrontations, tissue levels of 5-HT, its metabolite 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan hydroxylase were also increased within several stress-related brain regions (Amstislavskaya & Kudryavtseva, 1997; Devoino *et al*. 2003). It may be particularly significant that abnormally low cerebrospinal fluid (CSF) concentrations of 5-HIAA were reported in humans and non-human primates with a history of initiating severe offensive and violent acts (Brown *et al*. 1979; Higley *et al*. 1996; Lee & Coccaro, 2001), thus providing the impetus for the notion that low 5-HT metabolism was a trait marker for aggression and violence. In line with this view, compared to low-aggressive mice, those that had been selected for high aggressiveness displayed lower 5-HIAA and 5-HT levels within the prefrontal cortex (PFC) after aggressive experiences (Caramaschi *et al*. 2007, 2008). In addition to the low baseline 5-HT functioning associated with an aggressive disposition, diminished extracellular 5-HT measured *in vivo* at the PFC (van Erp & Miczek, 2000) and in CSF (van der Vegt *et al*. 2003*a*) was observed in rats during and after a display of aggressive behaviours.

Aggressive experiences have also been related to variations of norepinephrine (NE) activity, although the available data are less extensive and consistent, particularly with respect to defeated and submissive states (Lee & Coccaro, 2001). Nonetheless, elevated CSF NE levels were reported in rats both before and shortly after they dominated an agonistic encounter (van der Vegt *et al*. 2003*a*), and low prefrontal NE levels were found in high-compared to low-aggressive mice after repeated victories (Caramaschi *et al*. 2008). However, considerably less attention has been devoted to NE utilization in relation to aggressive behaviours in rodent models, whether in defeated or in victorious animals.

The present study examined basal levels of circulating corticosterone and of 5-HT and NE and their metabolites in several stressor-sensitive limbic brain regions in mice that had been identified as being submissive or dominant. In addition, the direct impact of single and repeated aggressive experiences on corticosterone as well as on monoamine levels and utilization was assessed. Finally, given the limited data available regarding potential changes provoked by aggression in relation to social status, these outcomes were determined in both mice that had lost (submissive) or won (dominant) the agonistic encounter.

Methods

Animals

In a first experiment naive male CD-1 mice (Charles River Canada), aged 6–8 wk, were housed in groups of four in $27 \times 21 \times 14$ cm polypropylene cages. Mice that displayed aggressive behaviours towards their cagemates (i.e. chasing or biting) were removed from the cage and singly housed, thus forming a group of preselected dominant animals ($n = 20$). The remaining three mice of the cage in which a dominant mouse was selected were not used for the purpose of this experiment. In addition, 40 naive male CD-1 mice of the same age were isolated upon arrival at the vivarium. These mice were never put in direct contact with another animal prior to a social stressor test and thus were not pre-categorized on the basis of their dominance/submissiveness. In a second experiment, 20 dominant mice were pre-selected from a new pool of animals. In this study, mice that had exhibited submissive postures (e.g. defensive upright posture) when confronted by an aggressor during home-cage interactions were individually housed, thus constituting a group of pre-screened submissive mice ($n = 20$).

After the initial isolation or selection procedures, mice were allowed 2 wk to acclimatize to their surroundings. They were maintained in a controlled 12-h

light/dark cycle (lights on 08:00 hours) with temperature (22 °C) and humidity (63%) kept constant and with free access to food and 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.

Behavioural testing

Social stressor procedure

The first experiment was aimed at testing the immediate outcomes of aggressive interactions experienced either on a single or on three occasions. All procedures were conducted between 08:30 and 13:00 hours to minimize effects related to diurnal factors. During a stressor session, a 'socially naive' mouse was introduced, individually, to the home cage of a pre-selected dominant mouse and direct interactions were permitted for 15 min. Thereafter the intruder mouse, that had now acquired the status of 'submissive' as a result of the agonistic encounter, was returned to its home cage. This social stressor procedure was undertaken either on a single occasion (single) or on each of three consecutive days (repeated). New pairs of submissive/dominant mice were formed for each session in the repeated condition. Excessive aggressive behaviours (e.g. incessant biting) were interrupted by lightly shaking or gently knocking the cage. Social interactions were videotaped for further scoring. Behavioural indices collected were (1) latency to initiate the first aggressive episode, and (2) frequency of aggressive episodes. Mice that had not fought during the first 5 min were excluded from the further analyses as were animals that showed clear signs of having been bitten. On two occasions in the single condition the presumed 'submissive' mouse dominated the social confrontation. In these instances, the predetermined submissive/dominant classifications were reversed. Similarly, the 'socially naive' mice that were not subjected to the social stressor were brought to the testing area once or on three occasions, in a separate room, but were not handled or put into direct contact with another mouse. Submissive and dominant mice were sacrificed by rapid decapitation 3 min after the end of the single or the third 15-min aggressive encounter, as was a non-stressed mouse.

Basal measurements and response to a mild stressor in dominant and submissive mice

In the preceding experiment, dominant mice had been pre-selected for their propensity to aggressiveness and then tested in a social dominance situation with 'socially naive' mice. However, previous social experiences and/or development of a dominant social rank might have contributed to basal levels of the physiological variables assessed even in the absence of a further social stressor test. Moreover, because 'socially naive'/submissive mice were handled and introduced to the dominant mouse's home cage, handling, cage transfer, or exposure to novelty (a stressor in its own right) might also have influenced the expected outcomes. Thus, an additional experiment was conducted in which pre-selected dominant and submissive mice either remained undisturbed in their home cages (basal) or were introduced to a novel clean cage with clean bedding for 15 min (novelty) ($n=10$ per group). Three minutes afterwards, mice were decapitated and physiological samples collected as in the first experiment. Thus, it could be determined whether neuroendocrine and neurochemical differences accompanied social status in the absence of a further aggressive encounter, and whether these mice differed as a function of a mild stressor unrelated to aggressive acts.

Blood collection and brain removal

Immediately after decapitation trunk blood was collected in tubes containing 10 μg EDTA, centrifuged, and the plasma stored at -80 °C for subsequent corticosterone determination. Brains were rapidly removed and placed on a stainless-steel brain matrix ($2.5 \times 3.75 \times 2.0$ cm) positioned on a block of 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 medial prefrontal cortex (mPFC), hippocampus, paraventricular nucleus of the hypothalamus (PVN), and locus coeruleus 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 5-HT and NE and that of their respective metabolites, 5-HIAA and 3-methoxy-4-hydroxyphenylglycol (MHPG).

Corticosterone determination

Plasma corticosterone levels were determined, in duplicate, using a commercial radioimmunoassay (RIA) kit (ICN Biomedicals Inc., USA). Assays were conducted in a single run to prevent inter-assay variability; the intra-assay variability was $<10\%$.

Central monoamine and metabolite determination

Levels of 5-HT and 5-HIAA, as well as NE and MHPG, were determined using high-performance liquid chromatography (HPLC) as previously described (Hayley *et al*. 1999). Briefly, tissue punches were sonicated in a solution obtained from a stock solution containing 500 ml HPLC grade water, 5.0 ml methanol, 0.0186 g EDTA, and 14.17 g monochloroacetic acid. The PVN and locus coeruleus were sonicated in 300 μl of this solution, whereas the mPFC and hippocampus were sonicated in 500 μl. After centrifugation, 20 μl of the supernatant was passed at a flow rate of 1.5 ml/min (1400–1600 p.s.i.) through a system equipped with a M-600 pump (Milford, USA), a guard column, a radial compression column (5 m, C18 reverse phase, 8 mm $\times$ 10 cm), and a 3-cell coulometric electrochemical detector (ESA model 5100A). The mobile phase used for separation comprised 1.3 g heptane sulfonic acid, 0.1 g disodium EDTA, 6.5 ml triethylamine, and 35 ml acetonitrile that had been filtered using 0.22-mm filter paper, degassed, and the pH levels adjusted to 2.5 using phosphoric acid. A Hewlett-Packard integrator determined the height and area of the peaks. The protein content of each sample was measured using bicinchoninic acid with a protein analysis kit (Pierce Scientific, Canada), and a spectrophotometer (Brinkman, PC800 colorimeter). Neurotransmitter concentrations were based on protein levels. The lower limit of detection for the monoamines and metabolites was 5.0 pg/ml.

Data analyses

Submissive and dominant mice that had experienced the social stressor were compared to their non-stressed counterparts. Plasma corticosterone levels as well as monoamine and metabolite concentrations in each brain region were analysed through a 3 (status: non-stressed, submissive, and dominant mice) $\times$ 2 (repetition: single and repeated) between-group analysis of variance (ANOVA). A second analysis determined whether submissive and dominant mice that were challenged by a 15-min aggressive encounter test (first experiment) would differ from the pre-selected submissive and dominant mice that had been left in their home cages without any further manipulation or exposed to a novel cage (second experiment). Plasma corticosterone as well as monoamine and metabolite concentrations in each brain region were analysed through a 2 (status: submissive and dominant) $\times$ 4 (stressor condition: single aggression, repeated aggression, basal, and novelty) ANOVA. Follow-up comparisons for both analyses were performed using *t* tests with a Bonferroni correction to maintain the α-level at 0.05. In addition to between-group comparisons, Pearson's correlation coefficients were determined between both the frequency of aggressive episodes and the latencies to the first aggressive episode with plasma corticosterone, monoamine, and metabolite levels. Several samples were lost over the course of the study, thus the degrees of freedom differed across the outcome measures.

Results

Each of the figures provides the data for both the first and second experiments (left and right panels, respectively). The left panels depict the neuroendocrine or neurochemical effects of single or repeated aggressive encounters in submissive and dominant animals relative to mice that had not engaged in such an encounter either during the pre-selection procedure or on the test day. The right panels show the corticosterone and monoamine changes in mice that had been pre-selected for dominant *vs*. submissive behaviour during home-cage interactions, and then left undisturbed (basal levels) or subsequently challenged by a mild stressor (novel surrounding).

Relation between latencies to first attack and frequencies of aggressive episodes with outcome variables

Aggressive behaviours began shortly after the introduction of the 'socially naive' mouse to the home cage of the pre-selected dominant mouse (85.25 ± 22.05 s). In submissive mice exposed to a single aggressive encounter, latencies to the first aggressive episode were negatively correlated with corticosterone levels ($r = -0.81$, $p < 0.05$), but this correlation was not apparent in the dominant mice ($r = -0.10$) or in those that had repeatedly experienced aggressive interactions ($r = -0.33$ and 0.27). Latencies to the first attack were not significantly correlated to monoamine and metabolite concentrations ($r = -0.46$ to 0.58 across brain regions).

Similarly, the number of aggressive episodes (20.81 ± 3.93) was not significantly correlated to either corticosterone levels ($r = 0.12$), or the monoamine and metabolite concentrations ($r = -0.43$ to 0.37 across brain regions).

Plasma corticosterone

Aggressive encounters influenced circulating corticosterone levels ($F_{2,42} = 38.26$, $p < 0.0001$). As depicted in Fig. 1 (left panel), concentrations were increased to a comparable extent in submissive and dominant

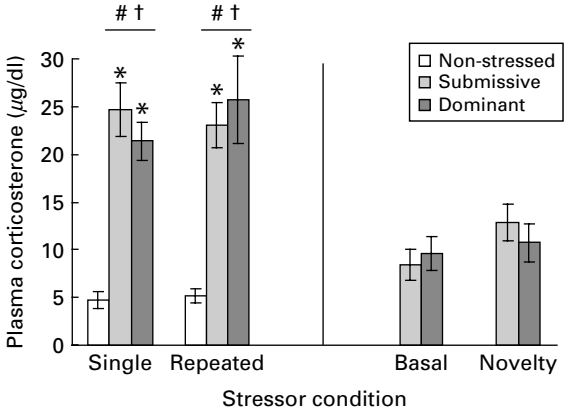

Fig. 1. Plasma corticosterone levels (μg/dl) in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.0001$ relative to non-stressed mice. # $p<0.0001$ relative to pre-selected submissive and/or dominant mice in the basal condition. † $p<0.0001$ relative to pre-selected submissive and/or dominant mice in the novelty condition.

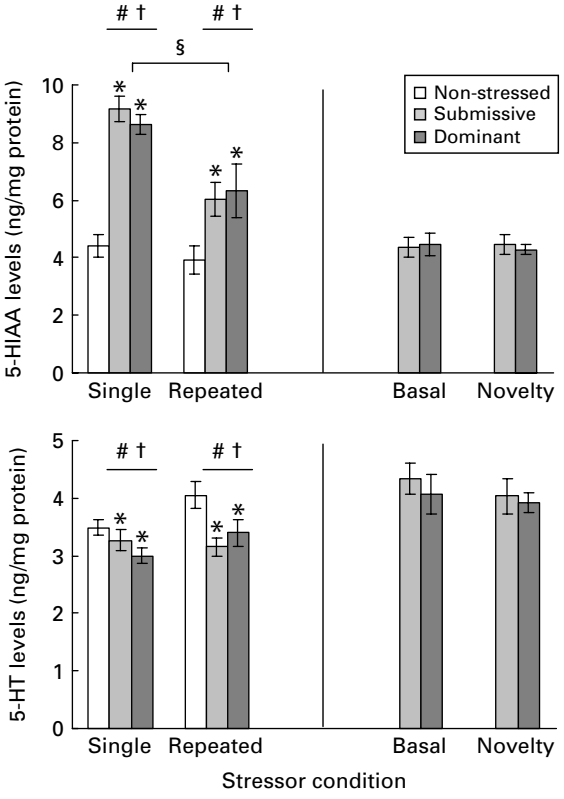

Fig. 2. Concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT within the medial prefrontal cortex in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.05$ relative to non-stressed mice. § $p<0.05$ relative to repeated condition. # $p<0.01$ relative to pre-selected submissive and/or dominant mice in the basal condition. † $p<0.05$ relative to pre-selected submissive and/or dominant mice in the novelty condition.

compared to non-stressed mice. Plasma corticosterone also varied as a function of whether mice had been subjected to aggressive interactions on the test day relative to their submissive and dominant counterparts that had remained undisturbed or that had been exposed to a mild stressor (right panel; $F_{3,72}=23.32$, $p<0.0001$). The follow-up tests indicated that corticosterone levels after single and repeated aggressive encounters were higher than in mice that had not undergone a challenge or those exposed to the novel cage; these stressor-related variations were comparable in submissive and dominant animals.

Brain monoamine levels and utilization

mPFC

Figure 2 shows the mean concentrations of 5-HIAA (upper panel) and 5-HT (lower panel) within the mPFC. The accumulation of 5-HIAA and the levels of 5-HT within the mPFC were both altered after aggressive interactions (F's$_{2,42}=10.41$ and 3.56, p's <0.0001 and 0.05, respectively). Follow-up comparisons indicated that relative to non-stressed animals, 5-HIAA levels were increased to a comparable extent in submissive and dominant mice, whereas 5-HT levels were reduced. It was further observed that elevations of 5-HIAA concentrations were more pronounced in mice exposed to an aggressive encounter on a single occasion than after repeated sessions (F's$_{1,42}=6.39$, $p<0.05$).

Comparison of submissive and dominant mice subjected to the social stressor (Fig. 2, left panels) and those at basal level or exposed to novelty (Fig. 2, right panels) revealed that both 5-HIAA and 5-HT levels differed across the stressor conditions ($F_{3,72}=37.16$ and 9.62, respectively, p's <0.0001). In particular, the 5-HIAA accumulation was higher and the 5-HT concentrations were lower after one or three aggressive encounters than they were in submissive and dominant mice that had not been stressed (basal levels) or

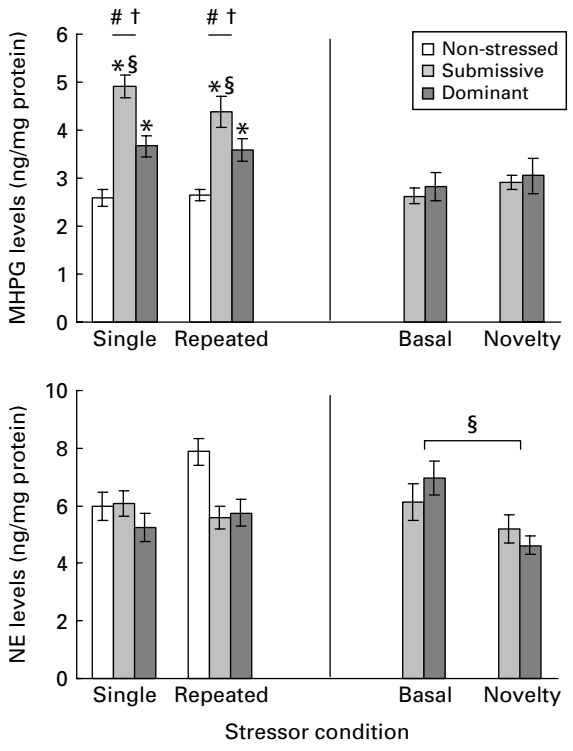

Fig. 3. Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE) within the medial prefrontal cortex in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.01$ relative to non-stressed mice. *§ $p<0.001$ relative to non-stressed and dominant mice. # $p<0.0001$ relative to pre-selected submissive and/or dominant mice in the basal condition. † $p<0.001$ relative to pre-selected submissive and/or dominant mice in the novelty condition. § $p<0.01$ relative to the basal *vs.* novelty comparison.

that had been exposed to novelty. These stressor-related changes were comparable in submissive and dominant mice.

Aggressive interactions influenced MHPG concentrations within the mPFC ($F_{2,42}=22.12$, $p<0.0001$), irrespective of whether mice experienced a single or three such interactions. Relative to non-stressed controls, MHPG accumulation was increased both in submissive and in dominant mice, and this effect was greater in the submissive animals (see Fig. 3). In contrast, prefrontal NE levels were not affected by aggressive interactions, although concentrations in non-stressed mice in the repeated condition (transported to the testing area three times without being stressed further) appeared somewhat higher, but not significantly so ($p=0.07$).

The comparison between mice that had encountered aggressive acts on the test day and their submissive and dominant controls indicated that prefrontal MHPG levels varied as a function of the status × stressor condition interaction ($F_{3,72}=3.80$, $p<0.01$). Follow-up tests showed that in the submissive mice, MHPG concentrations after single or repeated aggressive encounters were higher than at basal levels or after introduction to the novel cage. However, no such differences were apparent in the dominant mice. It also appeared that stressor-related NE levels differed between submissive and dominant mice ($F_{3,72}=3.86$, $p<0.05$). Specifically, NE concentrations after novelty were, unexpectedly, lower than those evident in mice that had not been stressed.

Hippocampus

Hippocampal 5-HIAA, shown in Fig. 4, was altered after aggressive interactions ($F_{2,42}=4.44$, $p<0.05$), but did not vary as a function of the number of aggressive encounters mice experienced. The follow-up comparisons confirmed that 5-HIAA concentrations were elevated to a comparable extent in submissive and in dominant mice compared to non-stressed mice. Unlike for 5-HIAA, the levels of 5-HT in dominant and submissive mice were unaffected by aggressive encounters.

Analysis of 5-HIAA levels as a function of the aggressive encounters, the novelty situation or the basal level condition indicated that these groups did not differ from one another with respect to metabolite accumulation ($F<1$). However, a significant effect of the stressor condition was apparent with respect to 5-HT levels ($F_{3,72}=24.18$, $p<0.0001$) (see Fig. 4). Specifically, 5-HT levels in submissive and dominant mice that had experienced a single or repeated aggressive interaction were lower than at basal levels or after the novel-cage exposure. The submissive and dominant mice did not differ from one another in this regard.

Accumulation of MHPG within the hippocampus (see Fig. 5) varied as a function of the status × repetition interaction ($F_{2,42}=5.20$, $p<0.01$). Follow-up comparisons confirmed that in the single condition, MHPG accumulation was increased in submissive mice relative to both non-stressed and dominant mice, whereas in the repeated condition, the metabolite accumulation was increased to a comparable extent in submissive and in dominant mice (Fig. 5). It was also observed that aggressive interactions influenced NE levels ($F_{2,42}=7.44$, $p<0.01$). This stemmed from a small

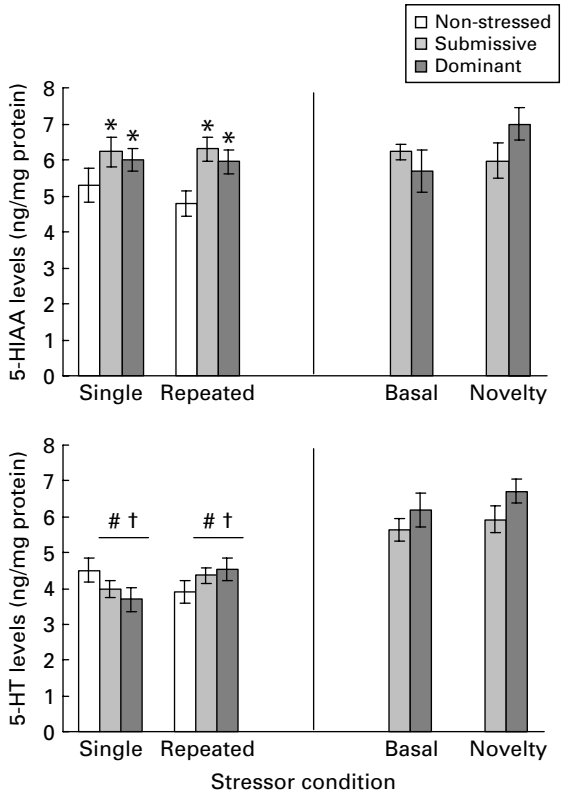

Fig. 4. Concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT within the hippocampus in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.05$ relative to non-stressed mice. # $p<0.0001$ relative to pre-selected submissive and/or dominant mice in the basal condition. † $p<0.0001$ relative to pre-selected submissive and/or dominant mice in the novelty condition.

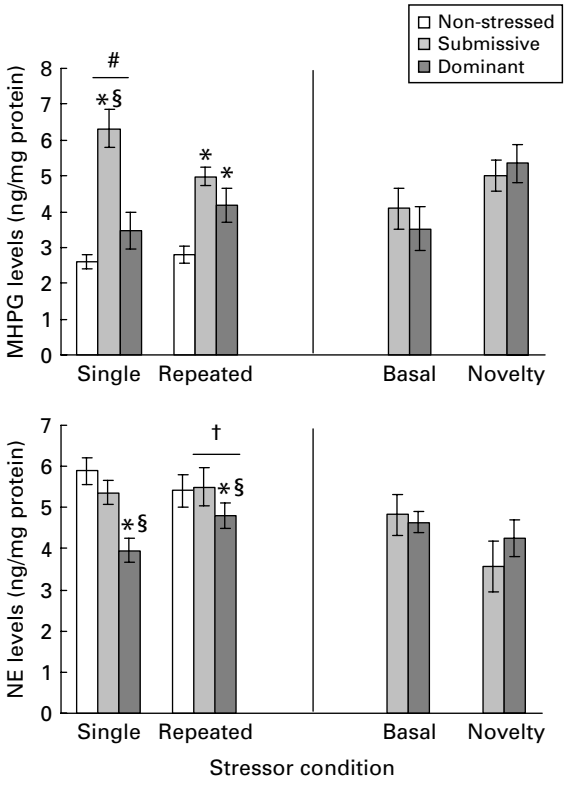

Fig. 5. Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE) within the hippocampus in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.05$ relative to non-stressed mice. *§ $p<0.01$ relative to non-stressed and dominant mice (MHPG levels) or relative to non-stressed and submissive mice (NE levels). # $p<0.01$ relative to pre-selected submissive and/or dominant mice in the basal condition. † $p<0.05$ relative to pre-selected submissive and/or dominant mice in the novelty condition.

but significant decline of NE in dominant mice, irrespective of whether they were exposed to a single or three aggressive encounters.

The MHPG levels in mice that had been subjected to aggressive interactions and their submissive and dominant counterparts in the basal and novelty conditions varied as a function of the status × stressor constressor condition interaction ($F_{3,72}=3.52$, $p<0.05$). Follow-up tests indicated that in the submissive mice, MHPG concentrations after a single aggressive encounter were higher than in the basal condition, but did not differ significantly from mice in novelty condition. No differences between stressor conditions were apparent in the dominant mice.

The NE levels in submissive and dominant mice across the stressor conditions revealed a significant main effect of the stressor condition ($F_{3,72}=3.13$, $p<0.05$). It appeared that NE concentrations were higher after three aggressive encounters, regardless of dominance status, than after novelty.

PVN and locus coeruleus

Figures 6 and 7 illustrate the levels of MHPG (upper panels) and NE (lower panels) within the PVN and locus coeruleus, respectively. Concentrations of MHPG within the PVN and locus coeruleus were

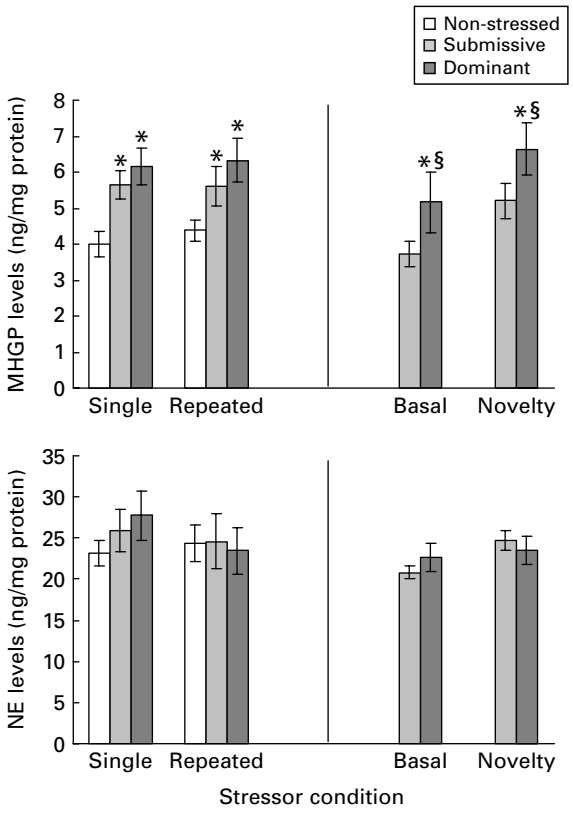

Fig. 6. Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE) within the paraventricular nucleus of the hypothalamus in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.01$ relative to non-stressed mice. *§ $p<0.05$ relative to pre-selected submissive mice.

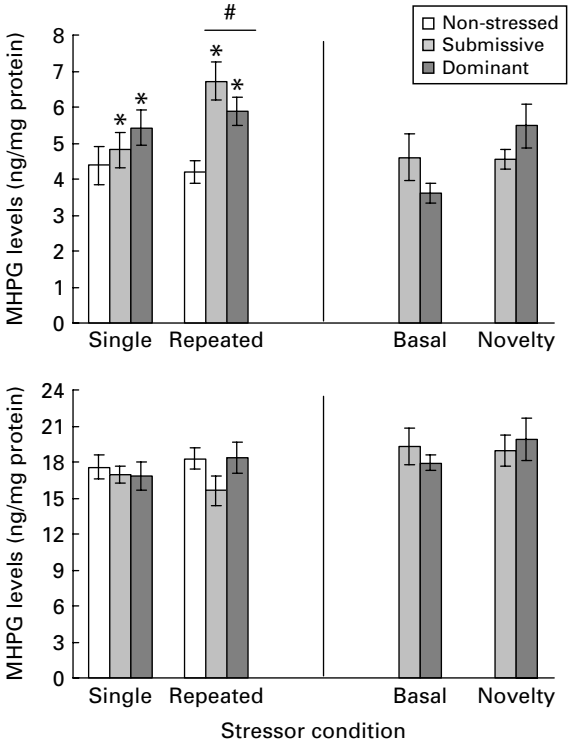

Fig. 7. Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) and norepinephrine (NE) within the locus coeruleus in non-stressed mice and in submissive and dominant mice engaged in one (single) or three (repeated) aggressive encounters (left panel) as well as in pre-selected submissive and dominant mice in the absence of an aggressive encounter on test day (basal) or exposed to a novel cage (novelty) (right panel). Data represent means ± S.E.M. * $p<0.05$ relative to non-stressed mice. # $p<0.0001$ relative to the basal condition.

influenced by aggressive encounters ($F's_{2,42}=10.01$ and 4.36, $p's<0.0001$ and 0.05, respectively), but NE levels were unaffected. Relative to non-stressed mice, MHPG accumulation within both these regions was increased to a comparable extent in submissive and in dominant mice, regardless of whether they were subjected to aggressive interactions on a single or repeated basis. Although the rise of MHPG within the locus coeruleus was actually modest in the mice stressed on a single occasion, the interaction between social status and the repetition of the stressor was not statistically significant.

When contrasting submissive and dominant mice in the stressor conditions, MHPG levels within the PVN were influenced by both the status ($F_{1,68}=5.97$, $p<0.05$), and the stressor condition ($F_{3,68}=3.22$, $p<0.05$). Specifically, MHPG concentrations were higher in dominant than in submissive mice, and elevated in the stressor groups (single and repeated encounter, novelty) relative to basal values; however, the latter effects were weak and actually non-significant when considering the Bonferroni correction. Finally, MHPG concentrations within the locus coeruleus varied across the stressor conditions ($F_{3,68}=6.42$, $p<0.001$). Relative to the basal level, MHPG levels in dominant and submissive mice were elevated after repeated aggressive encounters. Unlike MHPG accumulation, NE levels within the two brain regions in submissive and dominant mice did not vary across stressor conditions.

Discussion

Intimidation and aggression may engender adverse emotional and physiological repercussions in victims

and may favour the development of neuropathological disturbances, such as depression and anxiety (Bjorkqvist, 2001; Hawker & Boulton, 2000). These outcomes are not restricted to victims of aggression and bullying, but may also be evident in the perpetrators (Matthiesen & Einarsen, 2007; Seals & Young, 2003). The present investigation, conducted in mice, assessed (1) whether submissive and dominant animals would exhibit different basal corticoid and monoamine profiles in stressor-sensitive limbic brain regions, and (2) whether these physiological processes would be differentially affected directly after single and repeated aggressive encounters in mice that were victims of aggression (submissive mice) relative to mice that initiated aggressive acts and dominated the social interaction (dominant mice).

As previously reported, shortly after aggressive interactions the levels of corticosterone increased in both submissive (Bhatnagar *et al*. 2006; Keeney *et al*. 2001, 2006) and dominant (Bartolomucci *et al*. 2001; Covington & Miczek, 2005) mice. These elevations were not attributable to handling, cage transfer, exposure to novelty, or the social status previously attained, since low basal and novelty-related corticosterone levels were found in pre-selected submissive and dominant mice. The fact that corticosterone variations were comparable in the submissive and dominant mice is not surprising as the corticosterone increases probably represent an adaptive physiological response to stressors, regardless of how they came about (Sapolsky *et al*. 2000). Yet, as blood samples were only taken at a single time after the aggressive encounter, it is uncertain whether the duration of the elevation differed in dominant and submissive mice. Nevertheless, these findings are consistent with those in infrahuman primates indicating that HPA functioning may be elevated in dominant males as they are in submissive males (Sapolsky, 2005).

Like the corticosterone changes, after aggressive confrontations 5-HT utilization within the PFC and hippocampus, reflected by 5-HIAA accumulation, was increased to a comparable extent in the dominant and submissive mice. Moreover, as observed following strong uncontrollable stressors (Anisman & Zacharko, 1990), the increased prefrontal 5-HIAA accumulation was accompanied by modest reductions in the parent amine levels, possibly reflecting 5-HT utilization exceeding its synthesis. Within the hippocampus, the increased 5-HIAA accumulation was somewhat less marked than within the PFC, particularly after the single aggressive encounter, and levels of 5-HT were not affected. It is interesting that within the PFC the 5-HIAA variations apparent after three aggressive experiences were less pronounced than after a single confrontation. It is uncertain whether this reflects an adaptation like that frequently observed in connection with other psychogenic and neurogenic stressors (Anisman & Zacharko, 1990).

The adaptation-like effect was not apparent within the hippocampus, raising the possibility that the PFC and hippocampus might play very different roles in relation to stressful, aggressive experiences. Indeed, within the PFC, both submissive and dominant mice had higher 5-HIAA and lower 5-HT concentrations after aggressive encounters relative to the levels observed in the absence of such an encounter. Thus, the animal's previously established social status itself does not appear responsible for the altered PFC 5-HT levels and utilization observed immediately after the encounters. In contrast, within the hippocampus, basal and novelty-related 5-HIAA accumulation in dominant and submissive mice was comparable to the levels that followed aggressive experiences. As such, it is possible that hippocampal 5-HIAA variations associated with the submissive/dominant status was a pre-existing condition, rather than one instigated specifically by an aggressive encounter on the test day, as observed in the case of the PFC.

The enhanced 5-HT utilization provoked by aggressive encounters in submissive mice is in line with the general view that 5-HT systems are activated in response to social defeat or chronic subordination (Blanchard *et al*. 1991, 2001; Devoino *et al*. 2003; Keeney *et al*. 2006). However, the increased 5-HIAA accumulation in dominant animals is in apparent contrast with the diminished prefrontal and CSF 5-HT activity determined in rats *in vivo* during and shortly after aggressive confrontations (van der Vegt *et al*. 2003*a*; van Erp & Miczek, 2000). Specifically, it had been reported in rats that during engagement in offensive acts, 5-HT neurotransmission is rapidly and transiently enhanced (van der Vegt *et al*. 2003*b*), and that subsequent reductions of *in-vivo* 5-HT release may reflect a compensatory inhibition of amine functioning (van der Vegt *et al*. 2003*a*). Although speculative, it is possible that aggressive experiences in the present study elicited a similar, rapid increase of 5-HT neurotransmission, particularly at the PFC, and as a consequence, elevated metabolite concentrations were present in post-mortem brain tissue collected 3 min after confrontations. It remains to be determined whether 5-HT and 5-HIAA levels would vary at lengthier intervals following an aggressive encounter.

It has been proposed that low and high 5-HT activation profiles in animals might be related to distinct aggressive phenotypes. Specifically, low 5-HT

functioning has been associated with the disposition to engage in impulsive aggression or violence, but not necessarily to instrumental aggression aimed at dominance or reproductive success (Berman *et al*. 1997; de Almeida *et al*. 2005; van der Vegt *et al*. 2003*a*). In the present investigation the increased 5-HIAA concentrations in dominant mice after aggressive encounters might not reflect the features associated with excessive and impulsive aggression, but instead might be indicative of the stress associated with functional dominance intended to protect territory from intruders and/or to reassert or maintain dominance status (van der Vegt *et al*. 2003*a*). The fact that basal hippocampal 5-HIAA concentrations in dominant mice that had not been challenged to an aggressive encounter on the test day were comparable to levels observed immediately after aggressive interactions suggests that our aggressive mice were unlike those presenting the typical low 5-HT profile associated with pathological aggression. However, as amines were determined in post-mortem tissue, rather than *in vivo*, aggressive behaviours could not be related to baseline levels of 5-HT release. Moreover, as 5-HT levels and utilization were only measured at a single time following the social stressor test, it is uncertain whether variations of amine turnover persist for lengthy intervals as observed in relation to instrumental aggression (see Summers & Winberg, 2006), nor is it known whether 5-HT turnover would further stabilize at low levels, as thought to occur with pathological forms of aggression (Brown *et al*. 1979; Caramaschi *et al*. 2007, 2008; Lee & Coccaro, 2001).

Psychosocial stressors, such as social disruption (regrouping with cage-mates after social isolation) and chronic subordination were also associated with increased prefrontal NE utilization (Gibb *et al*. 2008) and elevated locus coeruleus tyrosine hydroxylase levels (Watanabe *et al*. 1995). However, limited data are available concerning NE variations that immediately follow social defeat or aggressive acts. Given the role of the PFC and hippocampus in cognitive processing (Hynes *et al*. 2006), PFC moderation of amygdala-mediated anxiety (Berkowitz *et al*. 2007), and the role of NE in emotional memory (van Stegeren, 2008), it might be expected that NE neurotransmission in these regions would be particularly sensitive to psychological factors related to defeat or submissiveness. Indeed, in contrast to the 5-HT variations, the enhanced prefrontal and hippocampal NE utilization engendered by aggressive encounters was more pronounced in submissive than in dominant mice, pointing to a role for NE neurotransmission in the early stress response associated with submission or defeat states. Moreover, MHPG accumulation in submissive mice subjected to aggressive confrontations was notably increased compared to that evident in their counterparts that had not undergone this stressor, particularly within the PFC, suggesting that NE utilization was directly tied to defeat experiences.

Unlike the effects evident in submissive mice, elevated prefrontal and hippocampal MHPG concentrations in dominant mice after aggressive encounters were comparable to those observed in the absence of this experience (i.e. basal NE utilization). Although these data do not permit causal conclusions to be drawn concerning the relation between basal NE functioning and dominance/aggressiveness traits, they do distinguish between basal and aggression-related MHPG accumulation in dominant and submissive mice, wherein aggressive encounters primarily affect NE utilization in the submissive mice.

Increased hippocampal NE utilization was engendered in dominant mice given a sufficient number of aggressive experiences. Unexpectedly, it was only in the dominant animals that hippocampal NE levels were reduced, even though elevated MHPG was not evident after a single encounter. It is conceivable that the NE reduction in dominant mice occurred very quickly during the single 15-min confrontation, and as a result NE availability was insufficient to permit augmented release to continue (and hence be detected), as observed in the case of 5-HT release measured *in vivo* (van der Vegt *et al*. 2003*a*, *b*). This supposition is clearly speculative and *in-vivo* studies are needed to assess the dynamic NE changes that accompany aggressive and submissive behaviours.

Single and repeated confrontations also increased MHPG accumulation within the PVN and locus coeruleus. Interestingly, PVN accumulation of MHPG on the test day was greater in dominant than in submissive mice, and this difference was particularly notable in those animals that had not been exposed to an aggressive encounter. Thus, it seems that basal NE utilization within this brain region might be elevated in dominant mice prior to an encounter, although as in the case of hippocampal NE utilization, the present findings do not address the direction of causality between social status and NE activity.

Limitations and conclusion

In the present investigation neurochemical functioning was inferred from the accumulation of monoamine metabolites, rather than directly measuring the *in-vivo* release of these amines. Thus, a static measure was obtained for a dynamic process that changes during

social interactions. It was also the case that the data in the basal and novelty conditions were not obtained at the same time as in mice that experienced aggressive encounters on one or three occasions. Accordingly, conclusions pertaining to basal neurochemical differences associated with social status and the direct impact of aggressive experiences ought to be considered cautiously.

These limitations notwithstanding, the data of the present investigation indicated that being the recipient of intimidating or aggressive attacks provoked marked stress-related neuroendocrine and neurochemical changes. Interestingly, being the initiator of aggressive acts also engendered multiple biological alterations that could be viewed either as adaptive changes or as reflections of distress that could potentially provoke adverse psychological repercussions (Miczek *et al*. 2008; Sapolsky, 2005; Summers & Winberg, 2006). In particular, circulating corticosterone and 5-HT utilization, particularly at the PFC, appeared to be responsive to aggressive events, in general, whereas NE neurotransmission within the PFC and hippocampus was aligned with the psychological attributes of the aggressive encounter (being dominant or submissive). As the PFC and hippocampus are thought to be fundamental in the evolution of depression, it is possible that variations of NE and 5-HT neurotransmission within these regions may contribute to depressive- and anxiety-like behaviours elicited by social stressors (Becker *et al*. 2008; Berton *et al*. 1999; Keeney *et al*. 2001; Rygula *et al*. 2005).

Acknowledgements

This research was supported by the Canadian Institutes of Health Research (CIHR). H. Anisman holds a Canada Research Chair in Neuroscience. M.-C. Audet is funded by Fonds de la recherche en sante du Quebec (FRSQ).

Statement of Interest

None.

References

Amstislavskaya TG, Kudryavtseva NN (1997). Effect of repeated experience of victory and defeat in daily agonistic confrontations on brain tryptophan hydroxylase activity. *FEBS Letters* **406**, 106–108.

Anisman H, Zacharko RM (1990). Multiple neurochemical and behavioral consequences of stressors: implications for depression. *Pharmacology & Therapeutics* **46**, 119–136.

Barrett GM, Shimizu K, Bardi M, Asaba S, Mori A (2002). Endocrine correlates of rank, reproduction, and female-directed aggression in male Japanese macaques (*Macaca fuscata*). *Hormones and Behavior* **42**, 85–96.

Bartolomucci A, Palanza P, Gaspani L, Limiroli E, *et al.* (2001). Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiology & Behavior* **73**, 401–410.

Becker C, Zeau B, Rivat C, Blugeot A, *et al.* (2008). Repeated social defeat-induced depression-like behavioral and biological alterations in rats: involvement of cholecystokinin. *Molecular Psychiatry* **13**, 1079–1092.

Berkowitz RL, Coplan JD, Reddy DP, Gorman JM (2007). The human dimension: how the prefrontal cortex modulates the subcortical fear response. *Review in Neuroscience* **18**, 191–207.

Berman ME, Tracy JI, Coccaro EF (1997). The serotonin hypothesis of aggression revisited. *Clinical Psychology Review* **17**, 651–665.

Berton O, Durand M, Aguerre S, Mormede P, Chaouloff F (1999). Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience* **92**, 327–341.

Bhatnagar S, Vining C, Iyer V, Kinni V (2006). Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *Journal of Neuroendocrinology* **18**, 13–24.

Bjorkqvist K (2001). Social defeat as a stressor in humans. *Physiology & Behavior* **73**, 435–442.

Blanchard DC, Cholvanich P, Blanchard RJ, Clow DW, *et al.* (1991). Serotonin, but not dopamine, metabolites are increased in selected brain regions of subordinate male rats in a colony environment. *Brain Research* **568**, 61–66.

Blanchard RJ, McKittrick CR, Blanchard DC (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology & Behavior* **73**, 261–271.

Brown GL, Goodwin FK, Ballenger JC, Goyer PF, Major LF (1979). Aggression in humans correlates with cerebrospinal fluid amine metabolites. *Psychiatry Research* **1**, 131–139.

Cacho R, Fano E, Areso P, Garmendia L, *et al.* (2003). Endocrine and lymphoproliferative response changes produced by social stress in mice. *Physiology & Behavior* **78**, 505–512.

Caramaschi D, de Boer SF, de Vries H, Koolhaas JM (2008). Development of violence in mice through repeated victory along with changes in prefrontal cortex neurochemistry. *Behavioural Brain Research* **189**, 263–272.

Caramaschi D, de Boer SF, Koolhaas JM (2007). Differential role of the 5-HT1A receptor in aggressive and non-aggressive mice: an across-strain comparison. *Physiology & Behavior* **90**, 590–601.

Covington 3rd HE, Miczek KA (2005). Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. *Psychopharmacology* (*Berlin*) **183**, 331–340.

de Almeida RM, Ferrari PF, Parmigiani S, Miczek KA (2005). Escalated aggressive behavior: dopamine, serotonin and GABA. *European Journal of Pharmacology* **526**, 51–64.

Devoino LV, Al'perina EL, Podgornaya EK, Polyakov OV, *et al.* (2003). Nature of the distribution of serotonin and a serotonin metabolite in brain structures and the development of immunosuppression in submissive mice. *Neuroscience and Behavioral Physiology* **33**, 473–477.

Franklin KBJ, Paxinos G (1997). *A Stereotaxic Atlas of the Mouse Brain*. San Diego, CA: Academic Press.

Gibb J, Hayley S, Gandhi R, Poulter MO, Anisman H (2008). Synergistic and additive actions of a psychosocial stressor and endotoxin challenge: circulating and brain cytokines, plasma corticosterone and behavioral changes in mice. *Brain, Behavior, and Immunity* **22**, 573–589.

Hawker DS, Boulton MJ (2000). Twenty years' research on peer victimization and psychosocial maladjustment: a meta-analytic review of cross-sectional studies. *Journal of Child Psychology and Psychiatry* **41**, 441–455.

Hayley S, Brebner K, Lacosta S, Merali Z, Anisman H (1999). Sensitization to the effects of tumor necrosis factor-alpha: neuroendocrine, central monoamine, and behavioral variations. *Journal of Neuroscience* **19**, 5654–5665.

Higley JD, Mehlman PT, Poland RE, Taub DM, *et al.* (1996). CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. *Biological Psychiatry* **40**, 1067–1082.

Hynes CA, Baird AA, Grafton ST (2006). Differential role of the orbital frontal lobe in emotional versus cognitive perspective-taking. *Neuropsychologia* **44**, 374–383.

Keeney A, Jessop DS, Harbuz MS, Marsden CA, *et al.* (2006). Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *Journal of Neuroendocrinology* **18**, 330–338.

Keeney AJ, Hogg S, Marsden CA (2001). Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiology & Behavior* **74**, 177–184.

Lee R, Coccaro E (2001). The neuropsychopharmacology of criminality and aggression. *Canadian Journal of Psychiatry* **46**, 35–44.

Matthiesen SB, Einarsen S (2007). Perpetrators and targets of bullying at work: role stress and individual differences. *Violence and Victims* **22**, 735–753.

Miczek KA (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology* (*Berlin*) **60**, 253–259.

Miczek KA, de Wit H (2008). Challenges for translational psychopharmacology research-some basic principles. *Psychopharmacology* (*Berlin*) **199**, 291–301.

Miczek KA, Yap JJ, Covington 3rd HE (2008). Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacology & Therapeutics* **120**, 102–128.

Rygula R, Abumaria N, Flugge G, Fuchs E, *et al.* (2005). Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behavioural Brain Research* **162**, 127–134.

Sapolsky RM (2005). The influence of social hierarchy on primate health. *Science* **308**, 648–652.

Sapolsky RM, Romero LM, Munck AU (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* **21**, 55–89.

Seals D, Young J (2003). Bullying and victimization: prevalence and relationship to gender, grade level, ethnicity, self-esteem, and depression. *Adolescence* **38**, 735–747.

Summers CH, Winberg S (2006). Interactions between the neural regulation of stress and aggression. *Journal of Experimental Biology* **209**, 4581–4589.

van der Vegt BJ, Lieuwes N, Cremers TI, de Boer SF, Koolhaas JM (2003*a*). Cerebrospinal fluid monoamine and metabolite concentrations and aggression in rats. *Hormones and Behavior* **44**, 199–208.

van der Vegt BJ, Lieuwes N, van de Wall EH, Kato K, *et al.* (2003*b*). Activation of serotonergic neurotransmission during the performance of aggressive behavior in rats. *Behavioral Neuroscience* **117**, 667–674.

van Erp AM, Miczek KA (2000). Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *Journal of Neuroscience* **20**, 9320–9325.

van Stegeren AH (2008). The role of the noradrenergic system in emotional memory. *Acta Psychologica* (*Amsterdam*) **127**, 532–541.

Watanabe Y, McKittrick CR, Blanchard DC, Blanchard RJ, *et al.* (1995). Effects of chronic social stress on tyrosine hydroxylase mRNA and protein levels. Brain Research. *Molecular Brain Research* **32**, 176–180.