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Self-injurious behaviour: limbic dysregulation and stress effects in an animal model

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Abstract

Background Self-injurious behaviour (SIB) is prevalent in neurodevelopmental disorders, but its expression is highly variable within, and between diagnostic categories. This raises questions about the factors that contribute to aetiology and expression of SIB. Expression of SIB is generally described in relation to social reinforcement. However, variables that predispose vulnerability have not been as clearly characterised. This study reports the aetiology and expression of self-injury in an animal model of pemoline-induced SIB. It describes changes in gross neuronal activity in selected brain regions after chronic treatment with pemoline, and it describes the impact that a history of social defeat stress has on the subsequent expression of SIB during pemoline treatment.

Methods *Experiment 1* – Male Long-Evans rats were injected on each of five consecutive days with pemoline or vehicle, and the expression of SIB was evaluated using a rating scale. The brains were harvested on the morning of the sixth day, and were assayed for expression of cytochrome oxidase, an index of sustained neuronal metabolic activity. *Experiment 2* – Male Long-Evans rats were exposed to a regimen of 12 daily sessions of social defeat stress or 12 daily sessions of handling (i.e. controls). Starting on the day after completion of the social defeat or handling regimen, each rat was given five daily injections of pemoline. The durations of self-injurious oral contact and other stereotyped behaviours were monitored, and the areas of tissue injury were quantified.

Results *Experiment 1* – Neuronal metabolic activity was significantly lower in a variety of limbic and limbic-associated brain structures in the pemoline-treated rats, when compared with activity in the same regions of vehicle-treated controls. In addition, neuronal activity was low in the caudate–putamen, and in subfields of the hypothalamus, but did not differ between groups for a variety of other brain regions, including nucleus accumbens, substantia nigra, ventral tegmentum, thalamus, amygdala, and cortical regions. *Experiment 2* – All the pemoline-treated rats exhibited SIB, and whereas the social defeat regimen did not alter the total amount of self-injurious oral contact or other stereotyped behaviours, it significantly increased the severity of tissue injury.

Conclusions A broad sampling of regional metabolic activity indicates that the pemoline regimen produces enduring changes that are localised to specific limbic, hypothalamic and striatal structures. The potential role of limbic function in aetiology of SIB is further supported by the finding that pemoline-induced self-injury is exacerbated by prior exposure to social defeat stress. Overall, the results suggest brain targets that should be investigated further, and increase our understanding of the putative role that stress plays in the pathophysiology of SIB.

Keywords animal model, Lesch–Nyhan syndrome, pemoline, self-injury, social defeat, stress

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Introduction

Self-injurious behaviour (SIB) is a debilitating characteristic that is exhibited by individuals with a broad variety of neurodevelopmental disorders (Rojahn & Esbensen 2002). It is seen in virtually all people with Lesch–Nyhan syndrome (Lesch & Nyhan 1964; Schretlen *et al.* 2005), most people with Prader–Willi syndrome (Symons *et al.* 1999) and approximately one-third of children with autism (Matson *et al.* 1996). Interestingly, it is not a definitive trait of most diagnostic categories. Rather, expression is heterogeneous within most of these groups, but shared across diagnoses. This suggests that characteristics that are symptomatic of multiple forms of intellectual disabilities may tend to be co-morbid with self-injury, or may even predispose vulnerability for the behavioural pathology.

Clinical studies reveal that SIB is particularly prevalent in disorders where ongoing distress and pathological irritability are prominent features (Anderson & Ernst 1994; Sovner & Fogelman 1996), and emotional stress may be a key trigger for SIB (Anderson & Ernst 1994; Lindauer *et al.* 1999). Furthermore, abnormal activity of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis is a common characteristic of intellectually disabled self-injurers (Verhoeven *et al.* 1999; Sandman *et al.* 2003, 2008; Symons *et al.* 2003; Kemp *et al.* 2008). However, it is not clear if LHPA axis dysregulation is a predisposing factor, or a consequence of SIB in these studies.

The relationship between emotional stress and self-injury is also seen in captive non-human primates. Self-injurious rhesus macaques display greater emotional responsiveness than their non-injurious counterparts do (Novak 2003), and the stress of relocation to novel housing produced long-lasting increases in self-biting behaviour in these animals (Davenport *et al.* 2008). In addition, we recently reported that individual differences in innate stress responsiveness predict vulnerability for induction of self-injury in the pemoline model of SIB (Muehlmann *et al.* 2011). Despite these intriguing implications for the role of stress in aetiology and expression of SIB, remarkably little study has been conducted on the manner in which stress and self-injury interact in intellectually disabled populations.

We have conducted an extensive characterisation of the pemoline model in rats, and our studies reveal several features that converge with observations from intellectually disabled self-injurers. For example, each pemoline-treated rat targets a specific tissue site (e.g. one forepaw, but not the other), and repeatedly injures that site (Kies & Devine 2004), in a manner that resembles the stereotypic body site preferences that are seen in human self-injurers (Symons & Thompson 1997). In addition, we found that pemoline-induced SIB is diminished by risperidone, valproate and topiramate (Muehlmann *et al.* 2008), drugs that are partially effective in human self-injurers (Ruedrich *et al.* 1999; McCracken *et al.* 2002; Shapira *et al.* 2002; Accardo 2003).

We now report two studies using the pemoline model of SIB in rats. In the first study, we used cytochrome oxidase (CO) histochemistry to identify brain regions that are impacted by chronic pemoline treatment in our model of SIB. The mitochondrial CO holoenzyme catalyses the final step in a process of electron transport that is tightly coupled to oxidative phosphorylation of adenosine diphosphate (yielding the highly energetic adenosine triphosphate) (Hatefi 1985). In brain tissue, this activity is required mostly for neuronal (as opposed to glial) activity, especially membrane repolarisation (Wong-Riley 1989). In contrast to more transient markers of neuronal activity (e.g. *c-fos*), the overall level of CO provides a reliable index of *sustained* neuronal function. Therefore, it has been described as a useful marker of neuroplasticity (Konkle & Bielajew 2004).

In the second study, we explored the impact of a prior history of stress exposure on the aetiology and expression of SIB, using the *social defeat* model. In this model, an experimentally naïve male rat (the *intruder*) is placed into the home cage of a larger male conspecific (the *resident*). The territorially dominant resident characteristically pins the intruder in a supine position (Miczek *et al.* 2004; Green & Devine 2009; Marcinkiewcz *et al.* 2009). This social defeat procedure activates limbic, hypothalamic and brainstem structures that are implicated in processing of stressful stimuli in the intruder rat (Martinez *et al.* 2002), and it is thought to emulate social stressors that rats may be exposed to in their natural habitats (Huhman 2006).

Methods

Experiment 1 – cytochrome oxidase

Animals

Sixteen male Long-Evans (LE) rats weighing 150–175 g (Charles River Laboratories, Raleigh, NC, USA) were housed in a climate-controlled vivarium with a 12 h/12 h light/dark schedule (lights on at 6:00 h). Standard laboratory rat chow (Lab Diet 5001) and tap water were available *ad libitum*. The rats were pair-housed in standard polycarbonate cages (43 cm × 21.5 cm × 25.5 cm) during 6 days of acclimation to the housing facility. Starting on the first day of pemoline treatment, each rat was individually housed, in order to ascertain that any injuries were self-inflicted. All the experimental procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida, and all the procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2011).

Drugs

The indirect monoamine agonist pemoline (Fuller *et al*. 1978; Gilbert *et al*. 1978) (2-amino-5-phenyl-1,3-oxazol-4-one; Spectrum Chemicals, New Brunswick, NJ, USA) was suspended at a concentration of 50 mg/ml in warm peanut oil (held at approximately 36°C), with constant stirring.

Pemoline treatment and assays of self-injury

On each of five consecutive mornings, approximately 2 h after the lights were turned on, each rat was examined for injuries, weighed and injected. Independent groups were treated with pemoline (200 mg/kg/day, s.c.; $n = 10$) or vehicle (4 ml/kg/day, s.c.; $n = 6$). The dose of pemoline was selected on the basis of our previous studies (Kies & Devine 2004), in which we found that reliable self-injury is produced by administration of 200 mg/kg/day, whereas a higher dose (300 mg/kg/day) produced very rapid onset of more severe self-injury, and a lower dose (100 mg/kg/day) did not produce reliable SIB.

The injections were delivered to the nape of the neck or either flank on a rotating basis. The examinations consisted of visual inspection of each rat's head, forepaws, ventrum, hindpaws and tail. Each rat was assigned a tissue damage score according to the presence and extent of injuries (see the rating scale in Table 1). The placement of each self-inflicted injury and the number of sites of tissue damage were also recorded. In addition, the length of each injury was measured. Injuries on the ventrum were consistently oval, and injury on the tail (only one rat) encompassed the circumference of the tail along a portion of its length. Accordingly, the length of each injury at these sites provided an approximation of the relative sizes of injuries. Injuries on the paws were less regular in shape, so the length of the injury along the paw and up the limb was taken as an overall approximation of the extent of the injury.

Table 1 Tissue injury rating scale (adapted from Kies & Devine 2004)

Score	Severity	Description
0	No injury	No tissue damage
1	Very mild injury	Slight oedema, pink moist skin, involves small area
2	Mild injury	Moderate oedema, slight erythema, slightly denuded skin, involves medium area and/or involves multiple sites
3	Moderate injury	Substantially denuded skin, substantial oedema and erythema, large area and/or involves multiple sites
4	Severe injury	Open lesion, requires immediate euthanasia

The rats were checked again for injuries every evening, but these scores were not included in the data analysis (i.e. one score was counted per day from each rat). The evening scores (which resembled the morning scores quite closely) were used to make certain that no animal was allowed to severely injure itself overnight without intervention. In any case where an open lesion was identified (score = 4 on the rating scale), the rat expressing the open lesion was immediately euthanised.

On the final morning of the experiment (day 6, 8:00 h–10:00 h), each rat was checked again for injuries, and then rapidly decapitated. Each brain

was rapidly removed, frozen in 2-methylbutane at –40°C, and stored at –80°C.

Cytochrome oxidase histochemistry

Each brain was cryosectioned at 20 μm in the coronal plane. Sections were mounted onto microscope slides on ice, and frozen at –20°C overnight. The slides were then stained for CO histochemistry according to methods that were modified slightly from those of Gonzalez-Lima & Jones (1994). Briefly, the frozen slides were fixed with 0.5% glutaraldehyde and 10% sucrose in 0.1 M PO_4 buffer (5 min), rinsed with 10% sucrose: 0.1 M PO_4 buffer (15 min), and incubated with stirring for 60 min in the dark in pre-oxygenated 0.1 M PO_4 buffer containing 5% sucrose, 0.05% diaminobenzidine, 0.0075% cytochrome c, 0.002% catalase and 0.25% dimethyl sulfoxide. The slides were then fixed in 10% buffered formalin with 10% sucrose for 30 min, dehydrated in graded EtOH (30, 50, 70, 90, 95, 100%) and immersed in xylene (20 min). The slides were coverslipped, and optical density (OD) was quantified for all regions of interest.

Quantification of cytochrome oxidase

Digital images of the sections were captured using a *Northern Light* R95 white trans-illuminator (Imaging Research Inc.) and a Photometrics *CoolSnap* CCD camera (Roper Scientific) connected to a personal computer. Semi-quantitative densitometry was performed using MCID Basic software (Imaging Research Inc.). Regions of interest were identified with reference to a standard rat brain atlas (Paxinos & Watson 1998), and a standard sampling box was used in the data collection window for each of these regions. Bilateral OD measures were sampled from three sections per region. External background measures were taken from outside each section, used to correct each sample OD, and the measures from the six samples per region were averaged. During this procedure, the experimenter was unaware of the experimental conditions associated with any section.

Data analyses

Two of the pemoline-treated rats were euthanised on day 4, because of injury. In these cases, the missing self-injury data were replaced by repeating the final score that was attained for each dependent measure through the end of the experiment. The brains were harvested and used for the CO assays, in the same manner as the brains of the rats that completed the experiment. This strategy was used to avoid the potential that the data would over- or underestimate the outcomes if the most severe self-injurers were eliminated. One additional rat was found dead on day 4. The cause was unknown, and it was removed from the experiment.

The percentage of rats that self-injured, mean tissue injury scores, numbers of injured sites and total length of injuries were plotted across days. Between-groups differences in tissue injury scores, number of injured sites and size of injuries were each evaluated using repeated-measures analyses of variance (RM-ANOVA). Effects were treated as statistically reliable when the *P*-values were less than 0.05. All significant effects were further analysed with Bonferroni post-tests.

The corrected OD measures were compared between groups using unpaired *t*-tests for each selected brain region. Between-groups differences were treated as statistically reliable when the *P*-values were less than 0.05. In order to present the outcomes of all the 38 anatomical regions that were assayed, the averaged OD scores for the pemoline-treated rats were subtracted from the scores for the vehicle-treated rats, and the data were then graphed as difference scores.

Experiment 2 – social defeat stress

Animals

Twenty-three male LE rats weighing 150–175 g (Charles River Laboratories) were pair-housed in the same manner as the rats in Experiment 1, with a 12 h/12 h light/dark schedule (lights on at 6:00 h) and *ad libitum* food and water. These were the experimental rats and were used as the 'intruders'. An additional six vasectomised male LE rats weighing 400–450 g were pair-housed with six female LE rats weighing 200–225 g in a separate housing room with an opposite 12 h/12 h light/dark schedule (lights off at 6:00 h). These rats were used as the 'residents'.

Social defeat

Each male resident was trained to exhibit dominance behaviour by repeatedly removing the female and introducing a smaller male rat into the cage. All the residents used in this study consistently demonstrated dominance behaviour in pre tests of social dominance. Social defeat sessions with the experimental intruders were then run during the dark phase of the resident rats' light cycle, starting at approximately 7:00 h. At the beginning of each social defeat session, the female resident was removed from the home cage. Ten minutes later, an intruder rat was placed into the home cage with the resident rat. The rats were then allowed to interact for 5 min or until the intruder displayed a submissive behaviour three times. Submissive behaviour was defined as supine posture, with the resident male rat on top, for at least 2 s. After this direct interaction phase, each intruder was removed, placed into a 10 cm × 10 cm × 15 cm double-layered wire mesh cage, and returned to the home cage of the resident male. This indirect interaction phase allowed the intruder rat to be out of physical contact but still experience stressful sensory stimuli. The intruder was maintained in the wire mesh cage until 10 min had elapsed from the start of the direct interaction phase, equalising the total duration of the stress session across rats. After the 10-min social defeat session concluded, both the female resident and the intruder were returned to their respective home cages. The intruder rats were subjected to social defeat once daily for 12 consecutive days, seeing each resident twice in that period, 6 days apart. Control rats were handled for 2 min each day for 12 days to ascertain that group differences could not be attributed to additional handling stress in the defeated rats.

Drugs

Pemoline was suspended at a concentration of 50 mg/ml in peanut oil. In order to get the pemoline into suspension, the solution was stirred overnight.

Pemoline treatment and assays of self-injury

Following the 12-day social defeat regimen, the rats were inspected, weighed and injected with pemoline at 150 mg/kg (s.c.) each morning for five consecutive days. In this experiment, a slightly lower dose was used than in the CO experiment, because the dose in that experiment (200 mg/kg/day) produced substantial SIB, and we anticipated that the stress exposure would exacerbate the expression of pemoline-induced SIB. These injections were administered at the nape of the neck and either flank on a rotating basis in the same manner as in Experiment 1. The inspections were conducted in the same manner as in Experiment 1, and like in Experiment 1, they were repeated in the evening.

In this experiment, the inspections were videorecorded, still images of the injured tissue were captured, and the MCID Basic software was used to outline the injured tissue, and to calculate the area of injury in mm^2. In addition, night-vision cameras were focused on the cages of the rats (one camera per cage), and 5-min time samples were recorded once every 3 h over the entire day. The duration of self-injurious oral contact and stereotypy were quantified during each videotaped interval by a trained observer. Self-injurious oral contact was defined as oral contact that stayed fixed on any specific site (e.g. forepaw) for longer than 2 s. This was differentiated from grooming, which is oral contact that continues to move along a body part or from one body part to another. The stereotypy measure is a compilation of the duration of stereotyped bobbing and licking, wherein the rat either bobbed its head or licked the side or floor of the cage repeatedly, and the duration of stereotyped digging, sniffing, or burrowing through the bedding. The duration (in seconds) of self-injurious oral contact and stereotypy were each summed over the entire day (i.e. from the eight video samples) and divided by the total number of seconds recorded.

Data analyses

Six rats were euthanised before the end of the experiment (four stressed rats and two control rats) because of injury. In these cases, the missing data were replaced in the same manner as in Experiment 1.

Between-groups differences in size of injuries, duration of self-injurious oral contact and duration of stereotypy were each evaluated using RM-ANOVA. Effects were treated as statistically reliable

when the *P*-values were less than 0.05. All significant effects were further analysed with pre-planned Fisher's least significant difference post-tests.

Results

Experiment 1 – cytochrome oxidase

Tissue injury was observed by day 4 in all the pemoline-treated rats (Fig. 1A). In the latter half of the experiment, the tissue injury scores ($F_{5,65} = 22.48$, $P < 0.0001$), number of injured sites ($F_{5,65} = 18.97$, $P < 0.0001$) and total size of injuries ($F_{5,65} = 15.63$, $P < 0.0001$) were greater than the corresponding measures in the control group (Fig. 1B–D).

Cytochrome oxidase expression was significantly lower in the pemoline-treated rats in the caudate–putamen (CPu), ventral pallidum (Fig. 2A), subregions of the septum and bed nucleus of stria terminalis (BNST) (Fig. 2B), specific hippocampal fields and hypothalamic nuclei (Fig. 2C), and the periaqueductal grey (PAG) (Fig. 2D). CO expression did not differ between the pemoline- and vehicle-treated groups in the substantia nigra, ventral tegmentum, core or shell of the nucleus accumbens septi (NAS), globus pallidus, limbic cortex, amygdaloid nuclei, subiculum, motor cortex, thalamic nuclei, or dorsal raphe (Fig. 2).

Experiment 2 – social defeat stress

All the pemoline-treated rats self-injured (Fig. 3A), and so the history of social defeat stress did not significantly affect the incidence of pemoline-

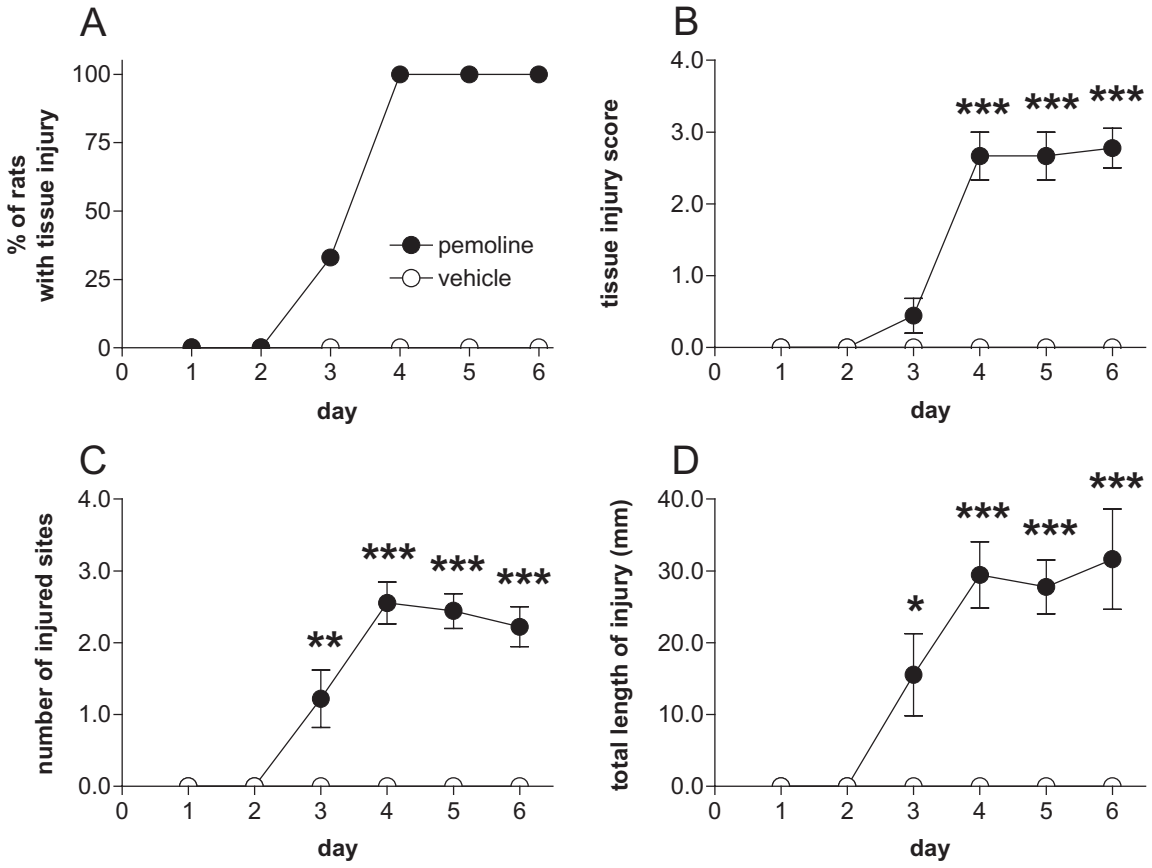

Figure 1 Incidence of pemoline-induced self-injury, tissue injury scores, number of injured sites and total length of injuries. Each measured increased across days. Self-injurious behaviour was observed in all the pemoline-treated rats, and in none of the vehicle-treated rats (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

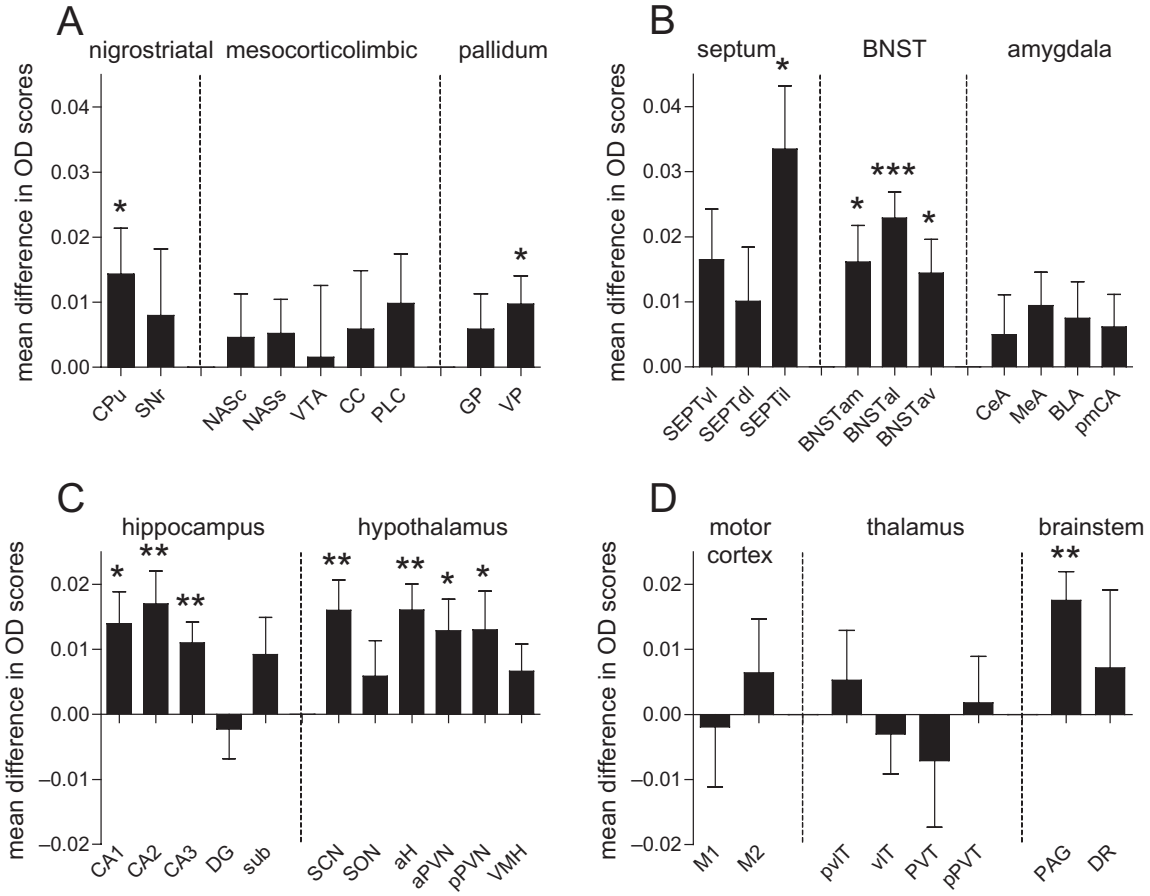

Figure 2 Cytochrome oxidase activity expressed as mean difference between the pemoline-treated and vehicle-treated rats. OD, optical density; CPu, caudate–putamen; SNr, substantia nigra (pars reticulata); NASc, nucleus accumbens septi (core); NASs, nucleus accumbens septi (shell); VTA, ventral tegmental area; CC, cingulate cortex; PLC, prelimbic cortex; GP, globus pallidus; VP, ventral pallidum; SEPTvl, ventral lateral septum; SEPTdl, dorsal lateral septum; SEPTil, interior lateral septum; BNST, bed nucleus of stria terminalis; BNSTam, anteromedial BNST; BNSTal, anterolateral BNST; BNSTav, anteroventral BNST; CeA, central amygdala; MeA, medial amygdala; BLA, basolateral amygdala; pmCA, post-medial cortical amygdala; CA1–CA3, cornu ammonis 1–3; DG, dentate gyrus; sub, subiculum; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; aH, anterior hypothalamus; aPVN, anterior paraventricular nucleus; pPVN, posterior paraventricular nucleus; VMH, ventromedial hypothalamus; M1 and M2, M1 and M2 motor cortex; pvlT, posterior ventrolateral thalamus; vlT, ventrolateral thalamus; PVT, paraventricular thalamus; pPVT, posterior paraventricular thalamus; PAG, periaqueductal grey; DR, dorsal raphe. All values are expressed as group means ± SEM (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

induced SIB. However, the sizes of the injuries were significantly greater in the rats that had a history of social defeat stress (Fig. 3B). The RM-ANOVA revealed a significant main effect of time ($F_{10,210} = 39.91$, $P < 0.01$) and a significant stress × time interaction ($F_{10,210} = 2.095$, $P < 0.05$), wherein rats first displayed injured tissue around day 2 or 3, and the size of injured tissue reached asymptote on day 4 or 5. There were no significant differences in the amount of time spent in self-injurious oral contact between stressed and control rats (Fig. 3C). There was a significant main effect of time ($F_{4,84} = 46.47$, $P < 0.01$) as all the rats began to show self-injurious oral contact on day 2, which peaked on day 3, and continued throughout the experiment; however, there was no significant main effect of stress nor a stress × time interaction effect. There were also no differences in the duration of other pemoline-induced stereotypies (Fig. 3D). The RM-ANOVA revealed a significant main effect of time

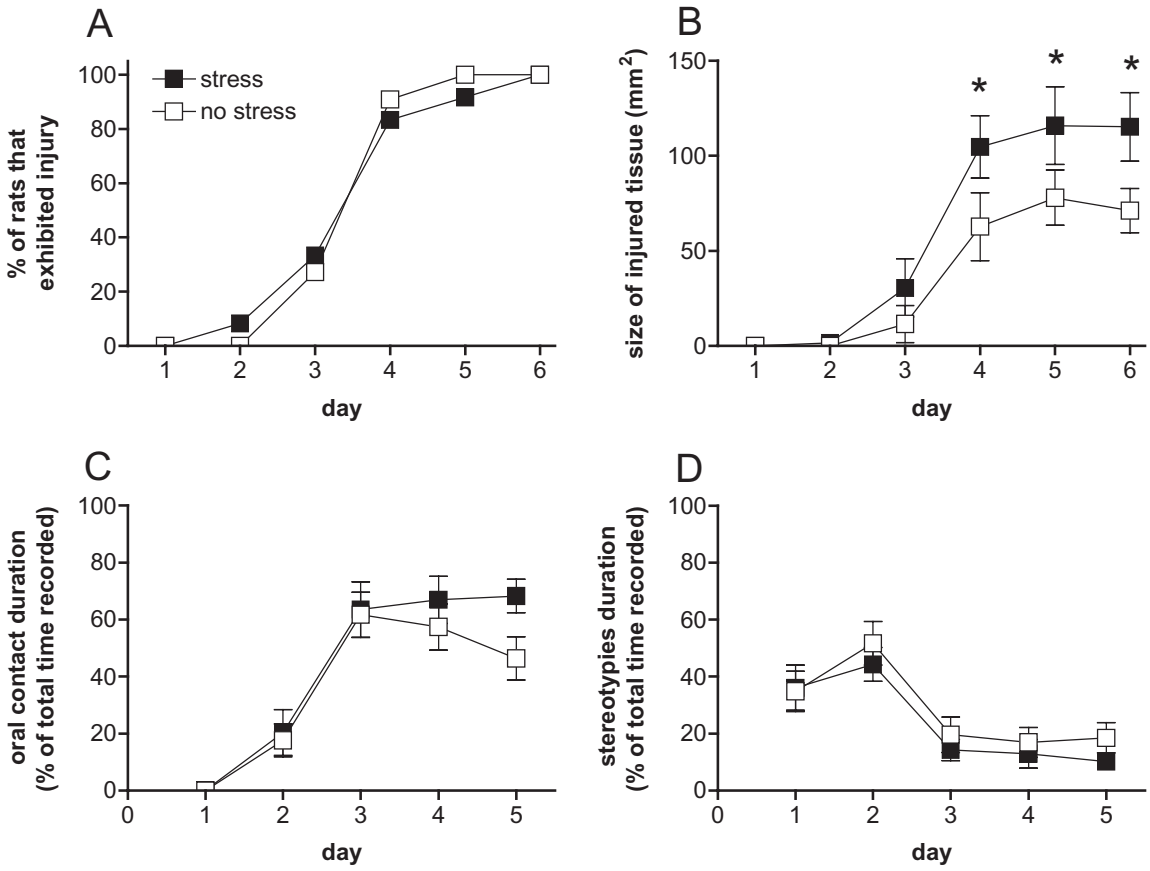

Figure 3 Effects of repeated social defeat stress on pemoline-induced self-injury and stereotypy. All the rats exhibited pemoline-induced self-injury (A); however, the rats with a history of repeated social defeat stress had larger areas of tissue damage (B). Repeated social defeat stress did not have any significant effect on pemoline-induced self-injurious oral contact (C) or whole body stereotypies (D). All values are expressed as group means ± SEM (*$P < 0.05$).

($F_{4,84} = 14.35$, $P < 0.01$), as all the rats showed moderate amounts of stereotypy on days 1 and 2, which lessened on days 3–5 as self-injurious oral contact duration increased. No significant main effect of stress or a stress × time interaction effect was found.

Discussion

In the CO histochemistry study, all the pemoline-treated rats exhibited self-injury, whereas none of the vehicle-treated rats injured (as expected). The anatomical assays revealed that sustained metabolic activity was suppressed in many of the 38 structures that were sampled from the self-injurious pemoline-treated rats, when compared with measures from the vehicle-treated rats. This suppression of neuronal activity was significant in one structure of the basal ganglia, and in a variety of limbic and limbic-associated structures. Although CO histochemistry provides only a gross characterisation of the regional effects of pemoline, it raises the possibility that direct or indirect impacts of the pemoline regimen in these regions may be associated with the induction of SIB.

Chronic pemoline treatment produced a statistically significant, but modest reduction in neuronal activity in the CPu, and the effect was not significant in the NAS or frontal cortex. The paucity of effects in the striatum and frontal cortex may seem surprising, as pemoline is an indirect monoamine agonist (Fuller *et al.* 1978; Gilbert *et al.* 1978), and

these regions are targets of extensive dopaminergic innervation (Graybiel & Ragsdale 1979). Moreover, all measures of dopamine function were decreased in samples of CPu and NAS taken from individuals with Lesch–Nyhan syndrome (Lloyd *et al.* 1981; Ernst *et al.* 1996), and these biomarkers are generally ascribed a critical role in the aetiology of SIB in both Lesch–Nyhan syndrome (Schretlen *et al.* 2005) and animal models of the disorder (Breese *et al.* 1984). However, postsynaptic neurons expressing excitatory D_1 and inhibitory D_2 dopamine receptors are intermingled in the striatum and frontal cortex (Gaspar *et al.* 1995; Le Moine & Bloch 1995), so pemoline-stimulated dopamine efflux would have opposing actions on these cells. As CO is most responsive to increases in membrane repolarisation (Wong-Riley 1989), the opposing actions of dopamine on D_1- and D_2-expressing cells could obscure the physiological effects of pemoline in these parts of the rat brain. CO histochemistry may be better suited to examine indirect impacts of pemoline on neurons that are downstream from these initial effects.

The other sites that exhibited significant suppression of neuronal metabolic activity were the ventral pallidum, interior lateral septum, BNST, subfields of the hippocampus, hypothalamic nuclei, and the PAG. On the other hand, other septal nuclei, dentate gyrus and subiculum, and the amygdala were not significantly impacted by chronic pemoline treatment. Thus, it seems that neuronal activity was suppressed only in a select subset of limbic-associated structures. Neuronal activity was not systematically altered in motor cortex or thalamus, suggesting a lack of involvement of these structures. Overall, the limbic/hypothalamic structures that exhibited significant pemoline-induced changes in neuronal metabolic activity, and the nigrostriatal and mesocorticolimbic systems, should each be examined more closely. The potential that these structures participate in the induction of SIB, and the specific biochemical alterations that occur in these structures require further analysis.

The suggestion that limbic structures may participate in pemoline-induced SIB is supported by evidence that emotional stress contributes to the *expression* of SIB in clinical populations (Favell *et al.* 1982; Anderson & Ernst 1994; Lindauer *et al.* 1999) and animal models (Stodgell *et al.* 1998; Davenport *et al.* 2008). For example, escape from or avoidance of aversive situations (i.e. stressors) is recognised as a basic communicative function that drives SIB in intellectually disabled self-injurers (Favell *et al.* 1982), and footshock stress increases the expression of SIB in the neonatal 6-hydroxydopamine (6-OHDA) lesion model in rats (Stodgell *et al.* 1998). These observations led us to examine whether a background of emotional distress might influence the *aetiology* of SIB in the pemoline model. We selected the social defeat model of emotional stress because it activates the aforementioned limbic, hypothalamic and brainstem mechanisms (Martinez *et al.* 2002). Additionally, repeated social defeat increases basal plasma corticosterone concentrations (Covington & Miczek 2001), and this is consistent with reports that LHPA axis function is dysregulated in intellectually disabled self-injurers (Verhoeven *et al.* 1999; Sandman *et al.* 2003, 2008; Symons *et al.* 2003; Kemp *et al.* 2008) [although it should be noted that both elevated and suppressed basal corticosterone concentrations have been found (Verhoeven *et al.* 1999; Symons *et al.* 2003)].

In our study of the impact of social defeat on subsequent acquisition of pemoline-induced SIB, all the pemoline-treated rats self-injured, regardless of whether they were pre-exposed to social defeat stress or not. Therefore, it was not possible to see any stress-induced increase in the incidence of self-injury. Likewise, there were no significant differences in the daily durations of self-injurious oral contact – perhaps also owing to a ceiling effect. However, the severity of tissue injury was significantly greater in the previously stressed rats, as indicated by the overall sizes of injured tissue in these rats. Thus, a history of psychosocial stress appears to contribute to the aetiology of SIB. When considered along with our recent finding that stress-hyper-responsive rats are particularly vulnerable for pemoline-induced SIB, the results indicate that stress reduction may be an important intervention in populations that are at risk for development of SIB. These findings may also have implications for the role that impoverished institutional environments have played in the aetiology of SIB among the intellectually disabled.

Studies of the brain structures that are impacted by social defeat may also help to focus the search for neuroanatomical substrates that contribute to the aetiology of SIB. Interestingly, *c-fos* expression is

activated in many limbic, hypothalamic and brainstem structures after acute defeat. The response habituates in some of these structures (e.g. septum, lateral hypothalamus, central amygdala), but enduring effects are found in others (BNST, paraventricular nucleus, medial amygdala, PAG, raphe) (Martinez *et al.* 1998). As social defeat stress enhanced the severity of self-injury in rats that were subsequently treated with pemoline, the brain regions in which there is overlap between the persistent actions of social defeat, and the brain regions where pemoline produces reductions in sustained neuronal activity may be particularly important in mediating the contribution of stress to the aetiology of pemoline-induced SIB. These areas (BNST, paraventricular nucleus and PAG) may be particularly interesting targets for additional investigation.

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