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Self-injurious behaviour: a comparison of caffeine and pemoline models in rats

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Abstract

Self-injurious behaviour (SIB) is a debilitating behaviour disorder that can have life-threatening consequences. It is often exhibited in intellectually handicapped and autistic populations, and it has been modeled with pharmacological manipulations in animals. We have characterized the induction of SIB using high doses of caffeine and pemoline in rats. Caffeine only produced very mild SIB in a small proportion of the rats, when administered repeatedly at very high doses (140–185 mg/kg/day). All the caffeine-treated rats showed profound signs of caffeine-toxicity at these doses, and lower doses did not induce any self-injury. On the other hand, pemoline was effective across a range of doses (100–300 mg/kg/day), including doses that did not produce overt signs of toxicity (100–200 mg/kg/day). The topography of the tissue injury sites (tail vs. paws and ventrum) differed between caffeine and pemoline treatments, and across doses of pemoline. The speed of onset, the incidence, and the severity of SIB occurred in a dose-orderly manner across the pemoline doses, and there was substantial individual variability in the induction of SIB when a moderately high dose (200 mg/kg/day) was used. These individual differences in vulnerability to self-injure are reminiscent of the fact that some humans with specific neurobiological disorders express SIB and some individuals with those same disorders do not. Accordingly, the pemoline model of SIB may be useful to investigate the neurobiological basis of factors that contribute to etiology of SIB.

Keywords: Caffeine; Pemoline; Self-injury; Self-injurious behaviour; Lesch–Nyhan syndrome; Autism; Animal model; Toxicity

1. Introduction

Self-injurious behaviour (SIB) is a devastating, chronic, and usually stereotyped behaviour disorder in which tissue damage is self-inflicted. It is commonly seen in intellectually handicapped and autistic populations, as well as populations with specific genetic syndromes. In these patient groups, the severity of the SIB can range from mild to life-threatening, and the forms and expressions of SIB are highly variable (e.g., head-banging, self-biting; for review, see Thompson and Caruso, 2002). Specific expressions of SIB predominate in particular groups. Stereotyped head-banging and face-hitting are often seen in autistic and other intellectually handicapped populations (Symons and Thompson, 1997), skin-picking is common in Prader–Willi syndrome (Schepis et al., 1994), and lip-, tongue-, and digit-biting is seen in Lesch-Nyhan syndrome (Nyhan, 1968a,b; Anderson and Ernst, 1994). In these groups, the behaviour disorder is often highly resistant to treatment (Lovaas, 1993; Gilbert et al., 1979; Maurice and Trudel, 1982; Anderson and Ernst, 1994).

Epidemiological studies have reported highly variable estimates of the incidence of SIB. These estimates range from 1.7% (Rojahn, 1986) to 65.9% (Rojahn, 1984) of general populations of intellectually handicapped individuals, depending upon factors like the definitions of self-injury, the residential settings (institutional vs. noninstitutional), and the degree of intellectual handicaps (for review, see Rojahn and Esbensen, 2002). Furthermore, the inci-

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dence of self-injury varies across patient groups. In Lesch–Nyhan patients, all or nearly all (Nyhan, 1968a,b; Mitchell and McInnes, 1984; Anderson and Ernst, 1994; Adler and Wrabetz, 1996) afflicted individuals exhibit self-biting behaviour, but the severity of the SIB varies from individual to individual, and appears to be related to the age of onset of the behaviour disorder (Anderson and Ernst, 1994). In Prader–Willi syndrome, skin-picking has been reported in 81% of individuals (Symons et al., 1999). Among individuals afflicted with Cornelia de Lange syndrome, approximately 44% self-injure (Berney et al., 1999), and as many as 34% of institutionalized autistic individuals exhibit some form of SIB (Matson et al., 1996). The high incidence of SIB in these various disorders suggests that there may be something about intellectual handicaps in general that predisposes individuals to exhibit SIB. However, there appear to be significant individual differences in vulnerability to acquire this devastating behaviour disorder, even within specific patient groups.

Neurobiological mechanisms that may participate in the development and expression of SIB have been examined in postmortem human brain tissue and in animal models. The human studies have revealed that disregulation of markers for dopamine neurotransmission is an important neurochemical feature in a variety of genetic disorders in which SIB is common. Dopaminergic innervation is reduced in the caudate, putamen, nucleus accumbens, globus pallidus, frontal cortex, substantia nigra, and ventral tegmental area of Lesch–Nyhan patients (Lloyd et al., 1981; Ernst et al., 1996). Furthermore, this reduced innervation in the caudate and putamen is accompanied by an increase in D1 and D2 receptor immunoreactivity (Saito et al., 1999). Accordingly, dopamine receptor supersensitivity may be involved in the expression of SIB. Additional neurochemical disregulation has been found in adenosine (Seegmiller et al., 1967; Rosenberger-Debiesse and Coleman, 1986; Page and Coleman, 1998), opioid (Coid et al., 1983; Gillberg et al., 1985; Sandman, 1988; Sandman et al., 1990; Willemsen-Swinkels et al., 1996; Saito et al., 1999), and serotonin (Castells et al., 1979; Jankovic et al., 1988) systems in Lesch–Nyhan syndrome, autism, and other disorders in which SIB is expressed.

Animal models of SIB have provided further evidence for neurochemical disregulation in SIB. Nonhuman primates that are socially isolated during early development exhibit stereotyped and self-injurious behaviours (Harlow and Harlow, 1962; Seay and Harlow, 1965; Harlow et al., 1965; Gluck and Sackett, 1974; Tiefenbacher et al., 2000; Novak, 2003), accompanied by diminished immunoreactivity for tyrosine hydroxylase and specific peptide neurotransmitters in the caudate and other areas (Seay and Harlow, 1965; Harlow et al., 1965; Martin et al., 1991). Neonatal 6-hydroxydopamine (6-OHDA) lesions followed by adult administration of dopamine agonists causes severe self-injury in rodents (Breese et al., 1984a,b), and administration of pharmacological agents that block adenosine receptors (Peters, 1967; Hoefnagel, 1968; Sakata and Fuchimoto, 1973; Mueller et al., 1982; Minana et al., 1984; Minana and Grisolia, 1986), augment dopamine function (Genovese et al., 1969; Mueller and Hsiao, 1980; Mueller and Nyhan, 1982; Mueller et al., 1982; Mueller et al., 1986; King et al., 1993; King et al., 1995; Turner et al., 1999; Cromwell et al., 1999; Shishido et al., 2000), or activate L-type calcium channels (Jinnah et al., 1999; Kasim et al., 2002; Kasim and Jinnah, 2003) also produce self-injury in rodents.

We have investigated the etiology of SIB in rats that were treated with caffeine (a nonselective adenosine receptor antagonist; Ally and Nakatsu, 1976), and in rats that were treated with pemoline (an indirect dopamine agonist; Everett, 1976). Furthermore, we have compared the efficacy of these two pharmacological models and we now report a characterization of the effects of chronic treatment with these two drugs during induction of SIB in rats. These studies will form the foundation for further investigations of the neurochemical basis of pharmacologically induced SIB in rats.

2. Methods

2.1. Animals

Male Long–Evans rats weighing 100–125 g were housed in a vivarium with a 12-h light/12-h dark cycle (lights on at 0700 h) where the temperature was maintained at 22–24 °C. Food and water were available ad libitum. The rats were housed in pairs for 1 week in standard polycarbonate cages (43×21.5×25.5 cm) prior to daily caffeine or pemoline administration. Starting on the first day of caffeine or pemoline treatment, each rat was individually housed to ascertain that any recorded injuries were self-inflicted. All the procedures in these experiments were preapproved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida, and all procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Caffeine (Sigma-Aldrich) was suspended in warm saline at a concentration of 20 mg/ml, and pemoline (Spectrum Chemicals) was suspended in warm peanut oil at a concentration of 50 mg/ml. Independent groups of rats were given daily subcutaneous (s.c.) injections of caffeine (140 or 185 mg/kg/day for 14 days) or pemoline (100 mg/kg/day for 14 days, or 200 mg/kg/day for 5 days, or 300 mg/kg/day for 4 days). Additional groups of rats were injected daily with saline (1.0 ml/kg/day for 14 days), or peanut oil (1.0 ml/kg/day for 5 days). All the injections were administered approximately 2 h after the lights were turned on each day, and the drug suspensions were kept warm during administration.

2.3. Experimental procedures

Each morning, the rats were examined for injuries, weighed, and injected with caffeine, pemoline, or vehicle. The examinations consisted of visual inspection of each rat's head, forepaws, ventrum (ventral thorax and abdomen), hindpaws, and tail, and these examinations were closely videotaped. The presence of injuries was scored by the experimenter, and then the videotapes were rescored by a trained observer who was blind to the treatment conditions. 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 with a ruler. Injuries on the ventrum were consistently oval, and injuries on the tail typically encompassed the tail along the length of the injury. 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.

Immediately after the drug injections, each rat was placed in a locomotor monitor, or back into its home cage, and activity was scored for 60 min. In the caffeine experiment, horizontal locomotion was measured every third day in 45×45 cm arenas (Coulborn Instruments). In the pemoline experiment, horizontal locomotion was recorded in the rats' home cages every day using a "Cage Rack" photocell system (San Diego Instruments). Since different monitors were used in the caffeine and pemoline experiments, the activity scores cannot be compared between these experiments, but they are compared across groups within each experiment.

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 euthanized. In the caffeine experiment, the expression of self-injury was minimal, and there was no need to euthanize any rats because of self-injury. This experiment continued until the morning of day 15. In the pemoline experiment, multiple rats simultaneously exhibited lesions on day 4 in the group that was treated daily with 300 mg/kg pemoline. Therefore, the entire group was euthanized on the evening of day 4. Similarly, the entire group that was treated daily with 200 mg/kg pemoline was euthanized on the morning of day 6. The peanut oil controls were also terminated on the morning of day 6. In light of the low expression of SIB in the group that was treated with pemoline at 100 mg/kg/day, the experimental procedures were continued until the morning of day 15 for this group.

On the final morning of the experiment (8:00–10:00 a.m.), each rat was checked again for injuries, and then rapidly decapitated (except the rats treated with pemoline at 300 mg/kg/day which were euthanized in the evening of day 4). The trunk blood was collected, and plasma was isolated by centrifugation at 1000×g for 5 min at 4 °C. The adrenal and thymus glands were removed from each rat. The isolated plasma and glands were frozen on dry ice, and stored at −80 °C.

Table 1
Tissue trauma rating scale, adapted from (Turner et al., 1999)

Score	Severity	Description
0	No SIB	No tissue damage
1	Very mild SIB	Slight edema, pink moist skin, involves small area
2	Mild SIB	Moderate edema, slight erythema, slightly denuded skin, involves medium area, and/or involves multiple sites
3	Moderate SIB	Substantial edema and erythema, large area, substantially denuded skin, and/or involves multiple sites
4	Severe SIB	Clear/open lesions, and/or amputation of digit, requires immediate euthanasia

2.4. Bioassays and histology

Plasma adrenocorticotrophic hormone (ACTH) concentrations were quantified by immunoradiometric assay (IRMA), using a kit from Nichols Institute Diagnostics, (San Juan Capistrano, CA, USA). This assay is highly specific for full-length ACTH using an ^{125}I-labelled monoclonal antibody directed at the N-terminal region, and a biotin-coupled polyclonal antibody directed at the C-terminal region. The interassay variability was less than 8%. Plasma corticosterone (CORT) concentrations were quantified by radioimmunoassay (RIA) using a kit from Diagnostic Products (Los Angeles, CA). The interassay variability for this kit increased with sample CORT concentrations, ranging from less than 5% for lower plasma CORT concentrations to less than 15% for higher plasma CORT concentrations. The adrenal and thymus glands were weighed to further assess the health status of the rats at the time of euthanasia.

2.5. Statistical analyses

The total expression of self-injury was determined for each rat by measuring the area under the curve (AUC) across days of treatment for each rat's tissue damage scores, total length of tissue damage, and total number of tissue damage sites. Between-groups differences in each of these dependent measures were analyzed using Kruskall–Wallis H-tests for both of the caffeine and pemoline experiments. In all cases where significant overall group differences were identified (i.e., $p<0.05$), pairwise Wilcoxon signed-rank tests were conducted to ascertain the source of differences between groups (i.e., comparing values for each drug-treated group, with the corresponding value for the vehicle-

treated control group, and comparing relevant between-groups differences among the drug doses). In light of the fact that the pemoline experiment had to be terminated on different days for the different dose groups, one set of analyses was conducted in which all four treatment groups were compared across the first four experimental days, and a second set of analyses was conducted in which the three remaining groups (vehicle, 100, and 200 mg/kg/day) were compared across the 6 days of their experiment. There was no appropriate comparison group for the 100-mg/kg/day group on days 7–15, so the data from this extended experiment are presented, but not statistically analyzed.

The correlation between tissue damage scores and the actual lengths and numbers of tissue damages was assessed by Spearman rho correlations for each of the caffeine and pemoline doses, to determine the validity of the tissue damage rating scale in relation to actual physical measures of tissue damage.

Between-groups differences in caffeine-induced horizontal locomotion scores and body weights were each assessed with 3×15 (group×day) repeated-measures analyses of variance (ANOVAs) comparing the saline, 140, and 185 mg/kg/day groups. Pemoline-induced differences in locomotor scores and body weights were each evaluated with 4×4 ANOVAs (comparing the vehicle, 100, 200, and 300 mg/kg groups that were tested on each of the first 4 days), and with 3×5 ANOVAs (comparing the vehicle, 100, and 200 mg/kg/day groups that were tested on each of the first 5 days). Once again, there was no appropriate comparison group for the 100-mg/kg/day group on days 7–15, so the data from this extended experiment are presented, but not statistically analyzed.

Between-groups differences in plasma ACTH and in plasma CORT concentrations were assessed with one-way ANOVAs for each of the caffeine and pemoline experiments. Adrenal and thymus weights were calculated as mg of gland weight per 100 g body weight. Drug-induced alterations in adrenal and thymus weights were also analyzed using one-way ANOVAs for each experiment. All significant locomotor, hormonal, body weight, and glandular weight effects ($p<0.05$) were further analyzed with Newman–Keul's post tests.

3. Results

3.1. Caffeine-induced SIB

Caffeine treatment produced signs of tissue damage in only 33% of the rats during the 15 days of treatment (Fig. 1a). The self-injury was slow to onset, beginning around day 4–7 of treatment, and it reached asymptote by days 8–12. Furthermore, the caffeine treatment did not produce clear dose-orderly differences in the incidence of SIB. Both doses ultimately induced SIB in 33% of the caffeine-treated rats, and there were no statistically significant between-groups differences in the total tissue damages, measured with the tissue damage rating scale ($H=2.566$, $p>0.05$), length of tissue damage ($H=2.523$, $p>0.05$), and number of injuries ($H=2.566$, $p>0.05$). The daily tissue damage scores, lengths of tissue damage, and number of tissue damage sites are depicted in Fig. 2a, b, and c.

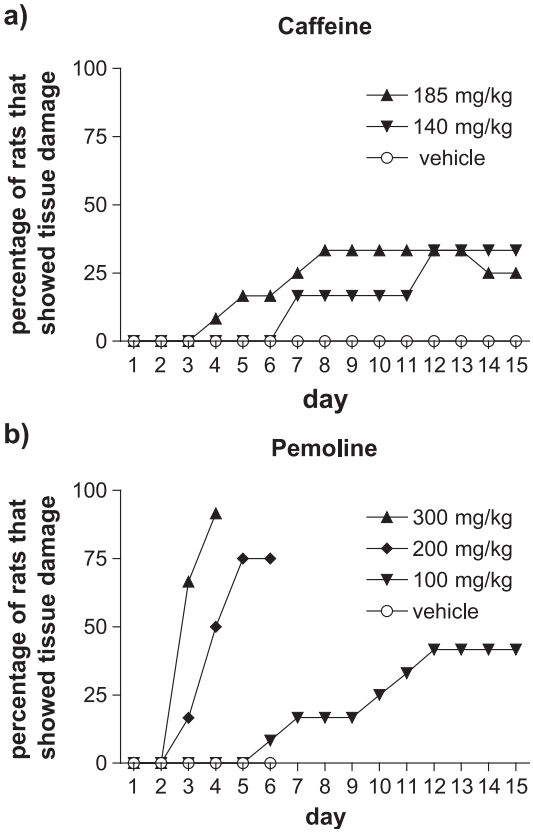

Fig. 1. Incidence of caffeine- and pemoline-induced SIB: (a) Caffeine induced self-injury in only about 1/3 of the rats, and the effects did not differ between the doses of caffeine. (b) On the other hand, dose-orderly expression of self-injury (scores of 1 or more on the tissue trauma scale) was exhibited in all the groups of pemoline-treated rats.

The caffeine-treated rats never exceeded a score of 2 (mild SIB) on the tissue damage rating scale, and the saline-treated controls never exhibited any self-injury (Fig 2a). However, the tissue damages in the caffeine-treated rats were occasionally large (up to 11 cm on the tail), and this was highly variable (Fig. 2b). These damages consisted only of mild erythema, and they appeared to result from excessive licking and grooming (casual observations). The experiment was terminated on the morning of day 16, owing to the deteriorating health of the caffeine-treated rats. Doses lower than 140 mg/kg/day were ineffective (data not shown), and the induction of SIB by doses higher than 185 mg/kg/day could not be evaluated, because these doses produced even more toxicity and substantial mortality. We immediately discontinued administration of caffeine doses higher than 185 mg/kg because many rats died within hours of the first injection, and all the rats exhibited profound signs of malaise. Accordingly, we did not fully evaluate the

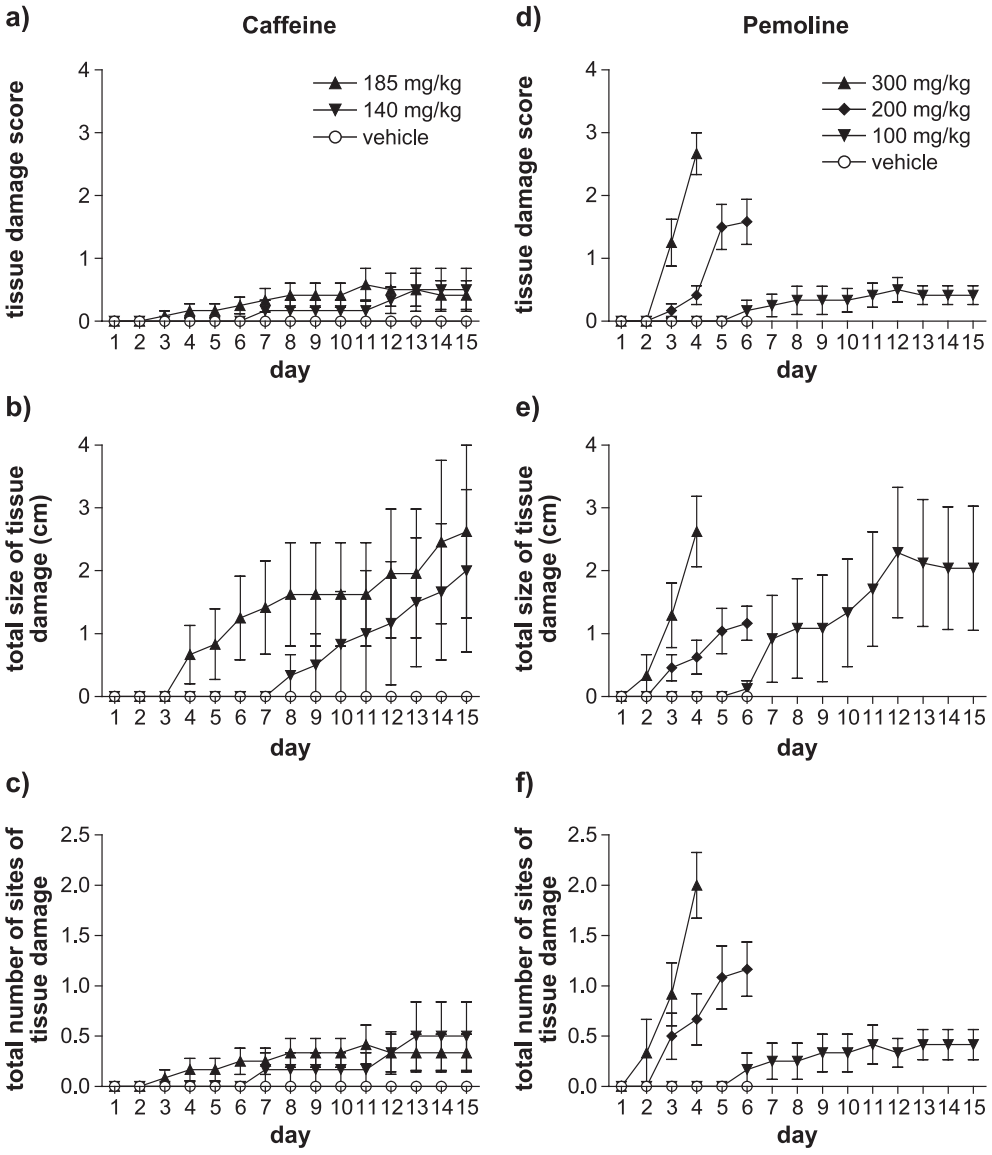

Fig. 2. Tissue damage scores, lengths, and numbers: Although mild expression of self-induced tissue damage was clearly observed in a small percentage of the caffeine-treated rats, overall the caffeine-treated groups did not differ significantly from the saline-treated controls in (a) tissue damage scores, (b) total length of injuries, or (c) total numbers of injury sites. The pemoline-treated rats exhibited dose-orderly elevations in tissue damage scores, (e) total length of injuries, and (f) total numbers of injury sites. (d) Wilcoxon tests revealed that the tissue damage scores were significantly higher in the group treated with 300 mg/kg/day than they were in the groups treated with 200 mg/kg/day (T=90.5, p<0.01), 100 mg/kg/day (T=84.0, p<0.01), or peanut oil vehicle (T=24.0, p<0.01) over the 4 days of the experiment. The tissue damage scores were significantly higher in the group treated with 200 mg/kg/day than they were in the groups treated with 100 mg/kg/day (T=91.0, p<0.01), or peanut oil vehicle (T=27.0, p<0.01) over the 6 days that these rats were tested. The tissue damage scores did not differ significantly between the rats treated with 100 mg/kg/day and the rats treated with peanut oil vehicle (T=54.0, p>0.05) over the 6 days that these rats were tested. (e) The tissue damage lengths were significantly higher in the group treated with 300 mg/kg/day than they were in the groups treated with 200 mg/kg/day (T=115.5, p<0.05), 100 mg/kg/day (T=84.0, p<0.01), or peanut oil vehicle (T=24.0, p<0.01) over the 4 days of the experiment. In addition, the tissue damage lengths were significantly higher in the group treated with 200 mg/kg/day than they were in the groups treated with 100 mg/kg/day (T=85.5, p<0.01), or peanut oil vehicle (T=3.0, p<0.01) over the 6 days that these rats were tested. The lengths of tissue damages did not differ significantly between the rats treated with 100 mg/kg/day and the rats treated with peanut oil vehicle (T=54.0, p>0.05) over the 6 days that these rats were tested. (f) The numbers of tissue damage sites did not differ significantly between the group treated with 300 mg/kg/day and the group treated with 200 mg/kg/day (T=136.0, p>0.05). However, the numbers of tissue damages did differ significantly between the rats treated with 300 mg/kg/day and the rats treated with 100 mg/kg/day (T=84.0, p<0.01), or peanut oil vehicle (T=24.0, p<0.01) over the 4 days of the experiment. The numbers of tissue damages were also significantly higher in the group treated with 200 mg/kg/day than they were in the groups treated with 100 mg/kg/day (T=86.0, p<0.01), or peanut oil vehicle (T=24.0, p<0.01) over the 6 days that these rats were tested. The numbers of tissue damages did not differ significantly between the rats treated with 100 mg/kg/day and the rats treated with peanut oil vehicle (T=54.0, p>0.05) over the 6 days that these rats were tested. The tissue damage scores, lengths and numbers of sites all increased later in the experiment for the rats that were treated with pemoline at 100 mg/kg/day. All values expressed are group means±S.E.M.

toxic and lethal effects of s.c. caffeine administration at these doses. It has previously been reported that the LD_{50} for oral caffeine administration is 264±10 mg/kg (Boyd et al., 1965).

3.2. Pemoline-induced SIB

In contrast to the inconsistent and mild expression of SIB in the caffeine-treated rats, pemoline produced self-injury in a substantial percentage of the rats, wherein the day of onset, and the peak percentage of self-injurious rats differed in dose-orderly fashion across the doses from 100 to 300 mg/kg/day. Nearly 100% of the rats exhibited self-injury within 4 days of treatment with 300 mg/kg/day. Seventy five percent exhibited self-injury within 6 days when treated with the 200 mg/kg/day dose, and approximately 40% exhibited self-injury within 12–15 days when treated with 100 mg/kg/day (Fig. 1b).

When all three pemoline-treated groups and the vehicle-treated group were compared for self-injury across the first 4 days of treatment, there were significant between-groups differences in scores on the tissue damage rating scale (H=28.47, p<0.01), length of tissue damage (H=25.71, p<0.01), and number of injuries (H=28.70, p<0.01). The daily tissue damage scores, lengths of tissue damage, and number of tissue damage sites are depicted in Fig. 2d, e, and f.

When the two remaining pemoline-treated groups and the vehicle-treated group were compared for self-injury across the first 6 days of treatment, there were significant between-groups differences in tissue damage scores (H=19.11, p<0.01), length of tissue damage (H=21.78, p<0.01), and number of injuries (H=21.57, p<0.01; Fig. 2d, e, and f).

3.3. Topography of self-inflicted tissue damage

The tissue-specific expression of SIB differed between the caffeine- and pemoline-treated rats, and differed across the doses of pemoline. The mild erythema that was observed in the caffeine-treated rats was almost exclusively expressed on the tail, for both of the doses that were tested. Only two rats slightly denuded a forepaw or hindpaw (Table 2). The rats that were treated with pemoline at 100 mg/kg/day also expressed self-injury that was primarily directed at the tail, but the rats that were treated with the higher doses of pemoline (200 and 300 mg/kg/day) preferentially targeted the forepaws and ventrum (Table 2), and expressed more severe tissue trauma at these sites (Fig. 2).

Table 2
Topography of expression of self-injury in caffeine- and pemoline-treated rats, depicting percentage of rats that injured at each site in each dose group (some rats injure at multiple sites)

Group	Forepaws	Hindpaws	Ventrum	Tail
Caffeine (140 mg/kg/day)	16.7	0	0	33.3
Caffeine (185 mg/kg/day)	0	16.7	0	25.0
Pemoline (100 mg/kg/day)	8.3	0	0	33.3
Pemoline (200 mg/kg/day)	33.3	16.7	58.3	8.3
Pemoline (300 mg/kg/day)	75	8.3	41.7	16.7

Table 3
Spearman Rho correlation matrix depicting correlations between tissue damage scores, and the total lengths/sites of tissue damage for the caffeine- and pemoline-treated rats

Treatment		Damage length	Number of sites
Caffeine	Correlation coefficient	0.998	1.000
(140 mg/kg/day)	Significance	p<0.01	p<0.01
Caffeine	Correlation coefficient	0.981	0.994
(185 mg/kg/day)	Significance	p<0.01	p<0.01
Pemoline	Correlation coefficient	0.979	0.963
(100 mg/kg/day)	Significance	p<0.01	p<0.01
Pemoline	Correlation coefficient	0.715	0.754
(200 mg/kg/day)	Significance	p<0.01	p<0.01
Pemoline	Correlation coefficient	0.850	0.846
(300 mg/kg/day)	Significance	p<0.01	p<0.01

3.4. Validation of the tissue damage scale

The tissue damage scores were significantly correlated with the measures of the lengths of the tissue damages, and with the numbers of sites of tissue damage for all the groups of rats that were treated with caffeine or pemoline. The Spearman Rho correlations are summarized in Table 3. Interobserver reliability for the tissue damage scores was greater than 95% for all the groups.

3.5. Locomotion

Both groups of caffeine-treated rats exhibited elevated locomotor scores across days in comparison with the scores of the saline vehicle-treated control [$F_{(10,105)}$=2.070, p<0.05]. The pemoline-treated rats exhibited elevated but highly variable locomotor counts across the 4 days that all four groups were tested [$F_{(9,111)}$=5.128, p<0.01], and across the 5 days that only the vehicle-, 100 and 200 mg/kg/day groups remained [$F_{(8,108)}$=5.721, p<0.01; Fig. 3].

3.6. Health of the rats

Both groups of caffeine-treated rats exhibited initial weight loss, followed by suppressed weight gain throughout the 15 days of the experiment [$F_{(28,294)}$=16.50, p<0.01]. The group of rats that were treated with pemoline at 300 mg/kg/day exhibited significant weight loss throughout the 4 days of the experiment [$F_{(9,114)}$=21.11, p<0.01], and the rats that were treated with pemoline at 100 or 200 mg/kg/day exhibited suppressed weight gain in comparison with the vehicle-treated group across the 5 days in which these 3 groups were assessed [$F_{(8,108)}$=20.31, p<0.01; Fig. 4].

Repeated administration of caffeine produced substantial alterations in functioning of the hypothalamic–pituitary–adrenal (HPA) axis (Fig. 5). Plasma ACTH [$F_{(2,35)}$=88.925,

$p<0.01$] and CORT [$F_{(2,35)}=8.40$, $p<0.01$] concentrations were elevated in the caffeine-treated rats. Adrenal gland weights were significantly elevated [$F_{(2,35)}=4.15$, $p<0.05$], and thymus weights were significantly depressed [$F_{(2,35)}=41.85$, $p<0.01$] in comparison with the measures in the vehicle-treated rats.

Significant alterations in HPA axis functioning were also observed in the pemoline-treated groups, although these alterations were less pronounced than they were in the caffeine-treated groups (Fig. 4). Plasma ACTH [$F_{(2,33)}=15.80$, $p<0.01$] and CORT [$F_{(2,33)}=7.14$, $p<0.01$] concentrations were elevated in the rats that were treated with the 100- and 200-mg/kg/day doses, when compared with the concentrations in the vehicle-treated rats. The ACTH and CORT concentrations could not be evaluated in the rats that were treated with pemoline at 300 mg/kg/day because they were euthanized in the evening, when circadian levels of circulating hormones are greatly elevated. The repeated administration of 300 mg/kg/day of pemoline produced hypertrophy of the adrenal glands [$F_{(3,44)}=11.66$, $p<0.01$], and this

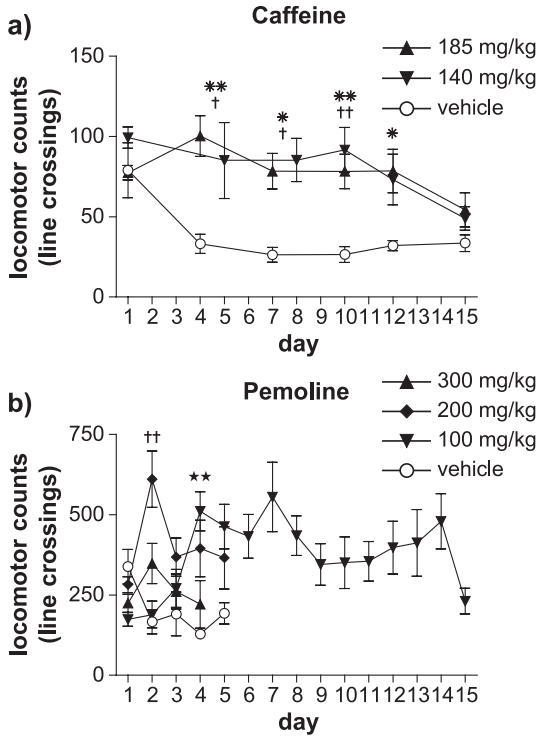

Fig. 3. Caffeine- and pemoline-induced horizontal locomotion: (a) All the rats exhibited high rates of locomotion after the initial injection of caffeine or vehicle on day 1 of the experiment. Subsequently, the caffeine-treated rats exhibited greater horizontal locomotion than did the vehicle-treated rats, and this effect disappeared by the last day of the experiment. Significant differences between saline- and caffeine-treated rats (Newman–Keul's tests) are depicted as follows: ✳ $p<0.05$, ✳✳ $p<0.01$ for comparisons of caffeine at 185 mg/kg/day with vehicle; † $p<0.05$, †† $p<0.01$ <0.01 for comparisons between caffeine at 140 mg/kg/day and vehicle. There were no significant differences between the groups treated with caffeine at 140 and 185 mg/kg/day. (b) The pemoline-treated rats exhibited significantly higher rates of horizontal locomotion than did the peanut oil vehicle-treated rats, and the locomotor scores were highly variable in these pemoline-treated rats. Significant differences between peanut oil- and pemoline-treated rats (Newman–Keul's tests) are depicted as follows: †† $p<0.01$ for comparisons of pemoline at 200 mg/kg/day with vehicle; ★★ $p<0.01$ for comparisons between pemoline at 100 mg/kg/day and vehicle. The locomotor counts did not differ significantly between the rats treated with 300 mg/kg pemoline and the rats treated with vehicle. All values expressed are group means±S.E.M.

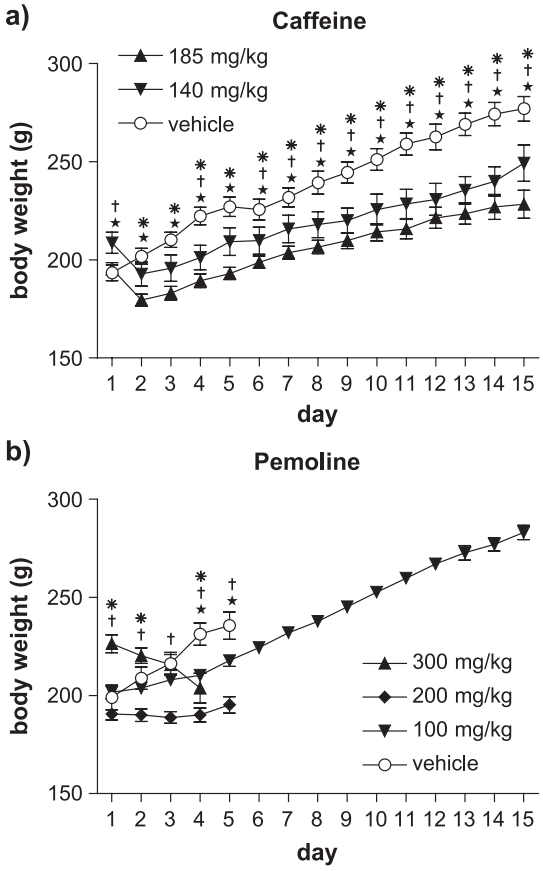

Fig. 4. Effects of caffeine and pemoline on body weight: (a) The caffeine-treated rats exhibited an initial drop in body weight followed by a steady gain, but both groups of caffeine-treated rats had lower weights throughout the experiment than did the vehicle-treated rats. Furthermore, the rats treated with the higher (185 mg/kg) dose exhibited lower body weights than did the rats treated with the lower (140 mg/kg) dose. Significant differences between saline- and caffeine-treated rats (Newman–Keul's tests) are depicted as follows: ✳ $p<0.05$ for comparisons of caffeine at 185 mg/kg/day with vehicle; † $p<0.05$ for comparisons between caffeine at 140 mg/kg/day and vehicle; ★ $p<0.05$, for comparisons between caffeine at 185 mg/kg/day and caffeine at 140 mg/kg/day. Many comparisons were significant at $p<0.01$ or less, but the additional levels of significance are not illustrated for the sake of clarity. (b) The body weights of the pemoline-treated rats differed significantly from the weights of the peanut oil vehicle-treated rats. The rats treated with 300 mg/kg/day lost weight throughout the experiment, and the rats treated with the lower doses (100 and 200 mg/kg/day) had lower weights than the vehicle-treated rats did. Significant differences between peanut oil- and pemoline-treated rats (Newman–Keul's tests) are depicted as follows: ✳ $p<0.05$ for comparisons of pemoline at 300 mg/kg/day with vehicle; † $p<0.05$ for comparisons of pemoline at 200 mg/kg/day with vehicle; ★ $p<0.05$ for comparisons between pemoline at 100 mg/kg/day and vehicle. Many comparisons were significant at $p<0.01$ or less, but the additional levels of significance are not illustrated for the sake of clarity. All values expressed are group means±S.E.M.

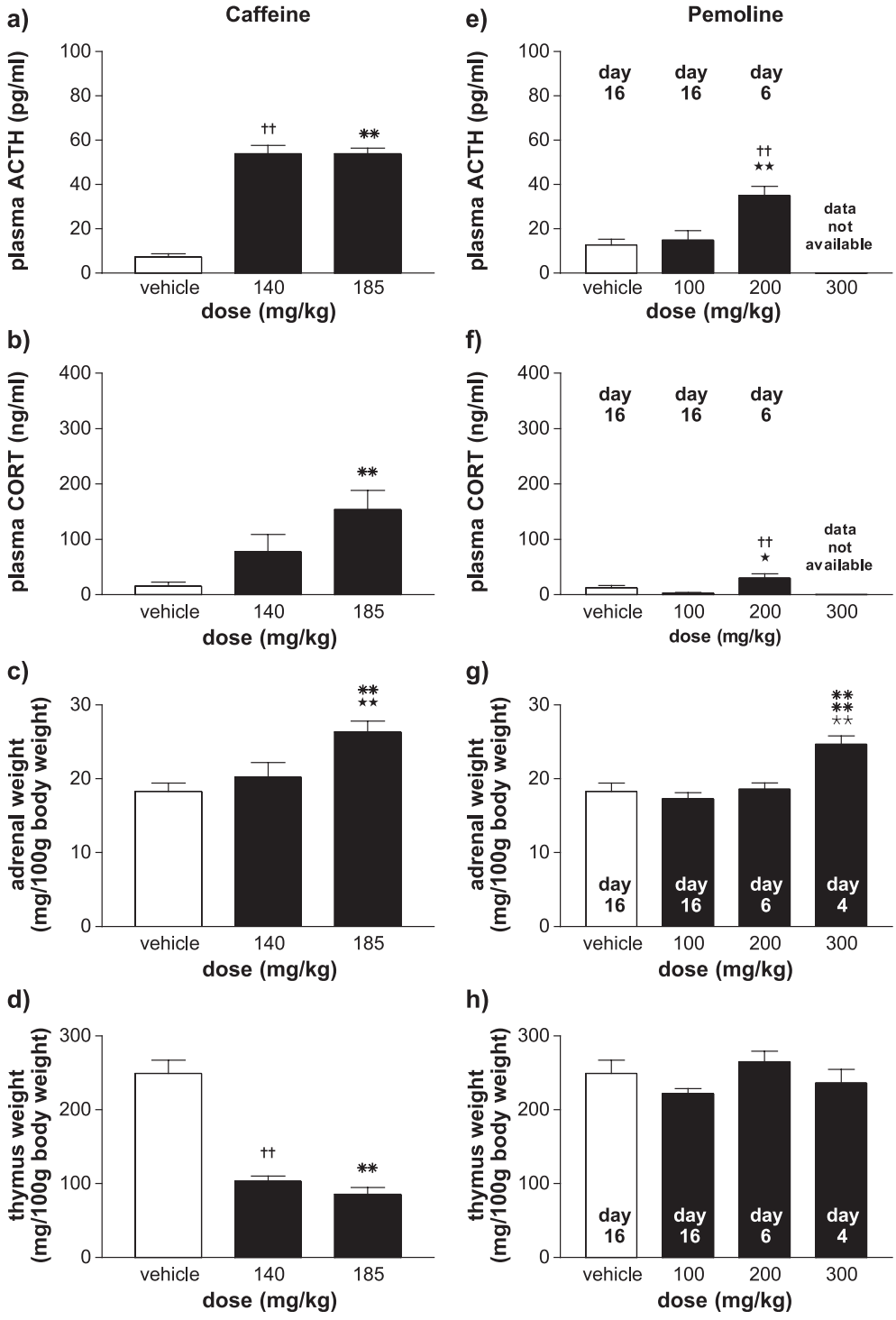

Fig. 5. Effects of caffeine and pemoline on HPA axis function: Elevated plasma ACTH (a) and CORT (b) concentrations were observed in the rats that were treated for 15 days with caffeine, in comparison with the rats that were treated with saline vehicle. Furthermore, the caffeine-treated rats exhibited adrenal hypertrophy (c) and thymus atrophy (d) in comparison with the vehicle-treated controls. Significant differences between groups (Newman–Keul's tests) are depicted as follows: ✳✳ $p<0.01$ for comparisons of caffeine at 185 mg/kg/day with vehicle; $^{\dagger\dagger}p<0.01$ for comparisons between caffeine at 140 mg/kg/day and vehicle; $^{\star\star}p<0.01$ for comparisons between caffeine at 185 mg/kg/day and caffeine at 140 mg/kg/day. Elevated plasma ACTH (e) and CORT (f) concentrations were also observed in some of the rats that were treated with pemoline, in comparison with the rats that were treated with peanut oil. The data are not available for ACTH and CORT concentrations for the group treated with pemoline at 300 mg/kg/day Furthermore, the pemoline-treated rats exhibited adrenal hypertrophy (g), but they did not exhibit thymus atrophy (h) in comparison with the vehicle controls. Significant differences between groups (Newman–Keul's tests) are depicted as follows: ✳✳ $p<0.01$ for pemoline at 300 mg/kg/day vs. vehicle; ✱✱ $p<0.01$ for pemoline at 300 mg/kg/day vs. pemoline at 200 mg/kg/day; ✶✶ $p<0.01$ for pemoline at 300 mg/kg/day vs. pemoline at 100 mg/kg/day; $^{\dagger\dagger}p<0.01$ for pemoline at 200 mg/kg/day vs. vehicle; $^{\star}p<0.05$, $^{\star\star}p<0.01$ for pemoline at 200 mg/kg/day vs. pemoline at 100 mg/kg/day. All values expressed are group means±S.E.M.

effect was not observed in the rats that were treated with the lower doses (100 and 200 mg/kg/day). The thymus weights were not significantly affected by pemoline administration [$F_{(3,44)}$=1.74, p>0.05].

All of the caffeine-treated rats exhibited chromodacryorrhea (blood-red porphyrin-containing secretions from the Harderian glands; Payne, 1994) around the eyes and snout, starting after the fourth or fifth drug administration, and persisting throughout the experiment. The rats that were treated with 300 mg/kg/day of pemoline also exhibited chromodacryorrhea starting after the second or third injection, but these secretions were never observed in the rats that were treated with pemoline at 100 or 200 mg/kg/day.

4. Discussion

In this investigation, we examined the caffeine and pemoline models of SIB as a foundation for future experiments in which we planned to compare and contrast these models to study potential neurobiological mechanisms that underlie the induction of self-injury. However, our characterization of caffeine and pemoline effects across days of treatment revealed serious problems with the caffeine model. Caffeine treatment only produced mild self-injury, and this mild injury occurred only in a small number of the rats. Self-biting behaviour was never observed in casual observations in the caffeine-treated rats. The induction of self-injury was not dose-orderly, and only occurred when caffeine was administered repeatedly at doses that are highly toxic—doses that induced substantial disregulation of the HPA axis. In fact, chromodacryorrhea was observed within the first few days of caffeine treatment (i.e., long before the appearance of self-injury), and these crusty "red tears" persisted throughout the experiment. Chromodacryorrhea has been used as an indicator of toxic actions of a variety of drugs and other substances (e.g., amitraz, amsacrine, L-nitronaphthalene, misonidazole; Moser, 1991; Sauer et al., 1995; Pegg et al., 1996; Graziano et al., 1996; Sauer et al., 1997) as a marker of naloxone-precipitated withdrawal from opiates (Buccafusco, 1990), and as an indicator of severe stress exposure (Harkness and Ridgway, 1980; Ross, 1994; Chen et al., 1997). We and others have also observed these secretions in rats with bacterial infections (unpublished observations).

Caffeine injections produced hyperlocomotion throughout most of the experiment (i.e., locomotor scores were significantly higher than the scores of the vehicle-treated controls)—except for the first and last locomotor testing sessions. The initial lack of between-groups differences in locomotor scores appears to be due to the stress-induced elevation in scores in the saline-treated rats after their first injection. The reason for the lack of group differences on the last day of treatment is unclear. It could have occurred due to tolerance to the psychomotor stimulant actions of caffeine (Holtzman and Finn, 1988), or it could have resulted from the deteriorating health of the rats. In addition to the Harderian secretions, the poor health of the caffeine-treated rats is further evident in the body weights and in the markers of HPA axis function. These rats exhibited substantial elevations in circulating ACTH and CORT concentrations measured 24 h after the final caffeine injections. The elevations in basal hormone concentrations were accompanied by substantial adrenal hypertrophy and thymus atrophy. These alterations in HPA axis function indicate that these rats were severely physiologically compromised (for review, see Maier and Watkins, 1998) by the repeated injections. Overall, these observations suggest that caffeine has severely limited utility because the toxic actions of caffeine may obscure the neurobiological mechanisms that underlie the etiology of the behaviour disorder.

The minimal expression of caffeine-induced self-injury in the current study does not concur with previous reports wherein caffeine administration was reported to produce moderate to severe self-injury in rats (Pfeiffer and Gass, 1962; Peters, 1967; Hoefnagel, 1968; Mueller et al., 1982; Ferrer et al., 1982; Mueller and Nyhan, 1983; Minana et al., 1984; Casas-Bruge et al., 1985; Minana and Grisolia, 1986). The reason for this discrepancy is not clear. In some studies, the caffeine was administered in the rats' food or water (Hoefnagel, 1968; Ferrer et al., 1982; Mueller and Nyhan, 1983; Minana et al., 1984; Casas-Bruge et al., 1985; Minana and Grisolia, 1986). The intake was therefore under control of the rats, and so it is difficult to determine the doses that produced SIB. In some studies, moderate to severe food restriction (Pfeiffer and Gass, 1962; Peters, 1967; Hoefnagel, 1968; Casas-Bruge et al., 1985), additional pharmacological treatments (Mueller and Nyhan, 1983), or lesions of dopaminergic neurons (Casas-Bruge et al., 1985) were administered to potentiate the ability of caffeine to produce SIB. Some studies reported lethal actions of caffeine in some of the rats (Peters, 1967; Hoefnagel, 1968; Mueller et al., 1982; Ferrer et al., 1982) in agreement with our own observations when we used higher doses of caffeine, although the additional toxic actions that we report were not described.

In contrast to the effects of caffeine, repeated administration of pemoline induced dose-orderly expression of self-injury that ranged from mild to severe. Oral contact with injured body sites was consistently observed during observations of the rats, and the SIB appeared to be highly compulsive. In fact, the rats were observed to gnaw on the sleeves or lapels of the experimenter's lab coat during the daily examinations, when they could not reach their paws or ventrum due to the experimenter's handling (note that the rats were not aggressive toward the experimenter). This is reminiscent of the compulsive nature of SIB in a variety of disorders (Stinnett and Hollender, 1970; Warnock and Kestenbaum, 1992; Hellings and Warnock, 1994; Bodfish et al., 1995) including Lesch–Nyhan syndrome. The pemoline-treated rats also exhibited significantly fewer and less severe signs of drug-induced toxicity, especially at the 100- and 200-mg/kg doses. Harderian secretions were not

observed in the rats that were treated with these doses of pemoline. Circulating hormone concentrations were only slightly elevated, and glandular masses were unchanged. All the pemoline-treated rats exhibited hyperactivity rather than lethargy or malaise throughout the experiment, and the pemoline was never lethal even at a dose (300 mg/kg/day) that produced very rapid onset of severe self-injury in more than 90% of the rats (it should be noted that 500 mg/kg/day produces 50% mortality; Genovese et al., 1969). Accordingly, the 100–200 mg/kg dose range effectively produced self-injury that was accompanied by minimal impact upon the health status of the rats. Moderate physiological compromise was seen at the 300-mg/kg dose. Overall, these observations suggest that the pemoline model may be highly useful in exploring the neurobiological basis of SIB.

The caffeine and pemoline models also differed in terms of the topographical expression of SIB. In the caffeine-treated rats, and in the rats treated with the lowest dose of pemoline, tissue damage was restricted to the tail—there was only occasional and minor irritation on the forepaws, and no tissue damage on the ventrum (Table 2). In the rats treated with the higher pemoline doses, the extent of tissue damage was greater, and was predominantly exhibited on the forepaws and ventrum (consistent with previous reports; Mueller and Hsiao, 1980; Mueller et al., 1986). The hindpaws and tail were the least common areas of injury. In the pemoline-treated rats, the self-biting behaviour was highly stereotyped, and targeted at specific tissue sites (e.g., left forepaw and ventrum, but not right forepaw). This topographical specificity is reminiscent of observations in human clinical populations in which specific forms of self-injury are specifically expressed by groups with particular disorders (Nyhan, 1968a,b; Anderson and Ernst, 1994; Schepis et al., 1994; Symons and Thompson, 1997), and in which individual human self-injurers generally have favoured target tissue sites (Symons and Thompson, 1997; Symons et al., 1999; Thompson and Caruso, 2002).

Evaluation of pemoline doses that were effective in approximately 50–75% of the rats (100–200 mg/kg) revealed that there are individual differences in vulnerability to self-injure in this pharmacological model. Some of the rats self-injured, whereas others did not. This is reminiscent of the fact that individuals within clinical populations (e.g., autistic individuals) appear to differ in their vulnerability or predisposition to exhibit self-injury so that only a subset of afflicted individuals demonstrate self-injurious behaviours (Rojahn and Esbensen, 2002). Accordingly, we believe that the pemoline model of self-injury may provide a useful tool to examine the neurobiological basis of individual differences in vulnerability to self-injure. This model has already revealed that cortical damage enhances expression of SIB (Cromwell et al., 1999), and that administration of naltrexone (King et al., 1993) and MK801 (King et al., 1995) inhibit SIB. In addition, the impact of environmental factors (e.g., stress exposure, environmental enrichment, operant conditioning) that could alter the innate predisposition to self-injure could be studied in the pemoline model of SIB. Ultimately, these studies may help increase our understanding of pathologies that are associated with self-injury, and lead towards improved prevention or treatment of self-injurious behaviour.

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