A COMPARISON OF CAFFEINE AND PEMOLINE MODELS OF SELF-INJURY IN RATS

By

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This document is dedicated to my parents, Ken and Debbie Kies, for their emotional support during the writing of this thesis.

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Chronic and stereotyped self-injurious behavior (SIB) is a maladaptive and debilitating behavior disorder, which can often have life-threatening consequences. It is exhibited predominantly by autistic and intellectually handicapped individuals, including those with a variety of specific genetic disorders. Disregulation of dopamine neurotransmission appears to be an important neurochemical feature of a variety of disorders in which SIB is observed, and several animal models have been developed in which dopamine function is altered. We have investigated the etiology of SIB in two of these models, using caffeine, an adenosine antagonist, and pemoline, an indirect dopamine agonist, in rats. In these investigations, we identified that caffeine produces only mild self-injury, and the effective doses are highly toxic. Pemoline was effective across a range of doses, and the expression of pemoline-induced self-injury occurred in a dose-orderly manner. Furthermore, effective pemoline doses were identified at which self-injury was only seen in a subset of the rats. This suggests that there may be

individual differences in vulnerability to self-injure in the pemoline model of SIB. These individual differences are reminiscent of the fact that individuals with specific clinical disorders (e.g., autism) differ in their vulnerability to self-injure. Accordingly, research with the pemoline model of self-injury may help to uncover the biological factors that underlie individual differences in vulnerability to exhibit self-injury.

INTRODUCTION

Self-injurious behavior (SIB) is a devastating, chronic and usually stereotyped behavior disorder in which tissue damage is self-inflicted. This maladaptive behavior disorder is commonly seen in intellectually handicapped populations, wherein the severity of the SIB can range from mild to life-threatening. The expression of self-injury differs between clinical groups of intellectually handicapped individuals. Self-injury to the head and/or hands is often seen in autistic and other intellectually handicapped populations (Symons & Thompson 1997), skin-picking is commonly seen in Prader-Willi syndrome (Hellings & Warnock, 1994; Schepis et al., 1994), and lip-, tongue-, and digit-biting is seen in Lesch-Nyhan syndrome (Anderson & Ernst, 1994). In these groups, the behavior disorder is often highly resistant to treatment (Anderson & Ernst, 1994).

In some instances, SIB is co-expressed with other behavior disorders, especially stereotypy. In fact, it has been proposed that SIB occurs on a continuum with stereotypy, wherein SIB is a severe or sensitized expression of stereotypy (Barron & Sandman, 1984; Guess & Carr, 1991) and the basal ganglia have been specifically implicated in this co-morbidity because of the apparently overlapping neural mechanisms involved in the expression of both of these behavioral abnormalities (Turner & Lewis, 2002).

It has been estimated that 8-20% of a general population of intellectually handicapped individuals exhibit some form of SIB (Schroeder et al., 1978; Oliver et al., 1987) and the incidence of SIB is higher in institutionalized populations than it is in community-based groups (Oliver et al., 1987). Furthermore, the incidence of SIB varies

between groups of individuals that differ in the nature of their intellectual handicaps. In Lesch-Nyhan patients, all (Nyhan, 1968a; Nyhan, 1968b; Partington & Hennen, 1967) or nearly all individuals (Mitchell & McInnes, 1984) exhibit self-biting behavior and the severity of the SIB varies from individual to individual. These individual differences in expression of SIB in Lesch-Nyhan patients appear to be related to the age of onset of the SIB (Anderson & 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% exhibit some form of self-injury (Berney et al., 1999), and 34 % of autistic individuals exhibit SIB (Matson et al., 1996). The high incidence of SIB in these various disorders suggests that there is something about intellectual handicaps in general that predisposes individuals to exhibit SIB. Furthermore, even within these disorders, some but not all exhibit SIB, and the severity of SIB may vary between afflicted individuals. Accordingly, there appear to be individual differences in vulnerability to acquire this devastating behavior disorder both between groups with different disorders, and within specific types of disorder.

Dysregulation of dopamine neurotransmission appears to be an important neurochemical feature of a variety of 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 (Ernst et al., 1996; Lloyd et al., 1981). Saito et al. (1999) further identified that the reduced dopamine content in the caudate and putamen was accompanied by an increase in D1 and D2 receptors. Taken together, these data suggest that dopamine receptor supersensitivity may be involved in the expression of SIB. Additional neurochemical

disregulation has been found in adenosine (Page & Coleman, 1998; Rosenberger-Debiesse & Coleman, 1986; Sweetman & Nyhan, 1970) opioid (Coid et al., 1983; Gillberg et al., 1985; Saito et al., 1999; Sandman, 1988; Sandman et al., 1990; Willemsen-Swinkels et al., 1996) and serotonin (Castells et al., 1979; Jankovic et al., 1988) systems in Lesch-Nyhan syndrome, autism, and other disorders in which SIB is expressed.

Investigation of the neurobiological mechanisms that participate in the development and expression of SIB has been facilitated by the identification of a variety of animal models of this behavior disorder. These models include social isolation in early development (Harlow & Harlow, 1962; Harlow et al., 1965; Seay & Harlow, 1965), neonatal 6-hydroxydopamine (6-OHDA) lesions followed by dopamine agonist administration in adulthood (Breese et al., 1984), and administration of pharmacological agents that block adenosine receptors, (Hoefnagel, 1968; Mardikar et al., 1969; Sakata & Fuchimoto, 1973) or augment dopamine function (Genovese et al., 1969; Sivam, 1995).

We have investigated the etiology of SIB in caffeine- and pemoline-treated rats. Caffeine is a non-selective adenosine receptor antagonist and chronic caffeine administration has been reported to induce self-injury in rats (Kasim & Jinnah, 2002; Mueller et al., 1982; Mueller & Nyhan, 1983; Minana et al., 1984; Minana & Grisolia 1986; Peters, 1967) if extremely high doses are administered repeatedly. Pemoline is an indirect dopamine agonist that acts by blocking the reuptake of dopamine. Administration of pemoline at a very high dose is known to produce a rapid onset of stereotypy and SIB (Cromwell et al., 1997; Cromwell et al., 1999; Mueller & Hsiao, 1980) whereas moderately high doses of pemoline are known to produce SIB after

repeated administration across several days (Mueller & Hsiao, 1980; Mueller & Nyhan, 1982; Mueller et al., 1986; Turner et al., 1999). In these investigations of the caffeine and pemoline models, we identified that caffeine produces self-injury only when administered repeatedly at doses that are highly toxic. Pemoline was effective across a range of doses, and self-induced tissue trauma was only seen in a subpopulation of the rats. This suggests that there may be individual differences in vulnerability to self-injure in this animal model of SIB.

METHODS

Animals

Male Long Evan rats weighing 100-125g were housed in a climatically-controlled vivarium with a 12 hr light: 12 hr dark cycle (lights on at 3:00 p.m. for the caffeine experiment, and 7:00 a.m. for the pemoline experiment). All the rats had free access to food and water. The rats were pair-housed for 1 week in standard polyethylene cages (43 x 21.5 x 25.5 cm) prior to the repeated caffeine or pemoline administration. Starting on the first day of caffeine or pemoline treatment, each rat was individually housed in standard caging (to ascertain that any recorded injuries were self-inflicted). All the procedures in these experiments were pre-approved 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.

Drugs

Caffeine was obtained from Sigma-Aldrich Co. and pemoline was obtained from Spectrum Chemicals. The caffeine was suspended in warm saline at a concentration of 20mg/ml, and the pemoline was suspended in warm peanut oil at a concentration of 50mg/ml. Both suspensions were kept warm and stirred constantly right up to the injection time. Independent groups of rats were given daily subcutaneous (s.c.) injections of caffeine (140 or 185 mg/kg/day for 15 days; n = 6 or 12 rats per group) or pemoline (100 mg/kg/day for 15 days, or 200mg/kg/day for 5 days, or 300 mg/kg/day for 4 days; n

= 12 rats per group). Additional groups of rats were injected daily with saline for 15 days or peanut oil for 5 days (1.0 ml/kg; n = 6 rats per group). All injections (caffeine, pemoline, and both vehicles) were administered daily between 8:30 and 9:30 a.m.

Experimental Procedure

Each morning, the rats were checked for injuries, weighed, and injected with caffeine, pemoline, or vehicle. The rats were checked for injuries again every evening. A self-injury score was recorded each morning and evening, according to the presence and extent of injuries (see table 1 for the scale that was used to evaluate self-inflicted tissue damage). The placement of each self-inflicted injury was also recorded, and the size of each injury was measured with a ruler. In any case where an open lesion was identified, the rat expressing the open lesion was immediately euthanized.

On the final day (day 16 for 140 mg/kg caffeine, 185 mg/kg caffeine, 100 mg/kg pemoline, and saline groups; day 6 for 200 mg/kg pemoline and peanut-oil groups; day 4 for 300 mg/kg pemoline group) of the experiment (unless the rat had to be euthanized early), each rat was rapidly decapitated one hour after "lights on". The trunk blood was collected, and plasma was isolated by centrifugation at 2800 rpm / 1,000 rcf for 5 minutes 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.

Histology

The adrenal and thymus glands were weighed. Plasma adrenocorticotrophic hormone (ACTH) concentrations were quantified by immunoradiometric assay (IRMA), using a kit from Nichols Institute Diagnostics. Plasma corticosterone (CORT) concentrations were quantified by radioimmunoassay (RIA) using a kit from Diagnostic Products Corporation.

Statistical Analyses

The saline and peanut oil vehicle control groups were compared using 2 x 15 repeated measures analyses of variance (ANOVA) for measurements of self-injury (SIB score, size of tissue damage, and number of sites) and body weight. These vehicle-treated groups were also compared using t-tests for the adrenal and thymus weights, and for the plasma ACTH and CORT concentrations. Neither group of vehicle-treated rats exhibited any self-injury, and there were no significant between-groups differences in any of the other measures (results not shown), and so the groups were combined and used as the common control group for the caffeine and pemoline experiments (vehicle group n = 12).

The self-injury scores, number of self-injury sites and total size of self-injuries were each analyzed using 2-way repeated measures ANOVAs. These scores were analyzed with a 3 x 15 (group x day) ANOVA to compare the 185, 140, and control groups for the caffeine experiment. In light of the fact that the pemoline experiments had to be terminated on differing days for each dose group, three ANOVA procedures were conducted. The first ANOVA was a 4 x 4 (group x day) procedure comparing the 300 mg/kg, 200 mg/kg, 100 mg/kg and vehicle control groups across the four days when all groups were run. A 3 x 6 ANOVA was used to compare the 200 mg/kg, 100 mg/kg, and vehicle control groups across the six days that these three groups were all run, and a 2 x 15 ANOVA was used to compare the 100 mg/kg and vehicle control groups across the 15 days when these remaining two groups were run. The vehicle-treated group was used as the control group in both experiments. Data for tissue damages were utilized from the morning recordings only - the evening scorings (which generally resembled the morning scorings quite closely) were simply used to make certain that no animal was allowed to

seriously injure itself overnight without intervention. All significant effects were further analyzed with Newman-Keul's post tests, 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 various doses for each drug treatment.

Between-groups differences in adrenal and thymus weights, and in plasma ACTH and CORT concentrations were each analyzed using one-way ANOVAs for each experiment (caffeine and pemoline), followed by Newman-Keul's post tests for all significant between groups differences. Between-groups differences in the rats' body weights were analyzed with 2-way repeated measures ANOVAs in the same manner as were the self-injury scores.

Table 1. Qualitative SIB scale: The rats were scored using these rankings (0-4), based on the severity of self-inflicted tissue damage.

SCORE	SEVERITY	DESCRIPTION
0	no SIB	None
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 minor tissue loss
4	severe SIB	amputation of digits, and/or clear open lesions requires euthanasia

RESULTS

Tissue Trauma

Some, but not all, of the caffeine-treated rats exhibited self-induced tissue damage (see Figure 1a). The number of rats in each group that exhibited tissue damage increased across days of treatment, reaching a peak around day 8 in the group that was treated with 185 mg/kg/day, and around day 12 in the rats that were treated with 140 mg/kg/day.

The experiment was terminated on day 16 because of the health conditions of the caffeine-treated rats – In fact, one rat in the 185 mg/kg group died on day 14, apparently due to the toxic effects of chronic caffeine administration. Furthermore, when we used a higher dose of caffeine, the dose was lethal early in the course of treatment, and the experiment had to be discontinued before any self-injury was observed (data not shown).

Administration of pemoline also produced self-inflicted tissue trauma, and in contrast to the caffeine-induced self-injury, these effects were dose-orderly (see Figure 1b). The self-induced tissue trauma occurred in a greater number of the rats, and onset earlier in the rats that were treated with the higher doses of pemoline. In this experiment, the group that was treated with 300 mg/kg/day was terminated on day 4 because a significant number of the rats exhibited one or more open lesions. The experiment was terminated on day 6 for the group that was treated with 200 mg/kg/day because of the tissue trauma, and the experiment was terminated on day 16 for the group that was treated with 100 mg/kg/day, although these rats did not exhibit open lesions.

The daily scoring of caffeine-induced self-inflicted tissue damage revealed that there were significant between-groups differences in the severity of tissue damage across the 15 days of the experiment (interaction effect: $F(28, 35) = 1.73$, $p < 0.05$; see Table 1 for the ranked scale of tissue damage scores). Furthermore, the group that was treated with 185 mg/kg/day exhibited significantly higher tissue trauma scores than did the vehicle-treated controls near the end of the experiment (see Fig. 2a). There were no significant differences in tissue trauma scores between the group that was treated with 140 mg/kg/day, and the group that was treated with 185 mg/kg/day, and so the severity of the caffeine-induced self-injury did not occur in a dose-orderly manner.

The severity of tissue-damage in the pemoline-treated rats was dose-orderly, with the higher doses producing significantly higher tissue trauma scores than did the lower doses across the days that each group of rats was tested (see Fig. 2d). The rats that were tested with 300 mg/kg/day of pemoline exhibited significantly higher trauma scores than did the other groups of rats during the 4 days (interaction effect: $F(9,47) = 10.88$, $p < 0.01$). The group of rats that was treated with 200 mg/kg/day of pemoline exhibited significantly higher trauma scores than did the 100 mg/kg and vehicle group during the 6 days that they were tested (interaction effect: $F(10,35) = 11.97$, $p < 0.01$). In addition, the group of rats that was treated with 100 mg/kg/day of pemoline exhibited significantly higher trauma scores than did the vehicle-treated group of rats during the 15 days that they were tested (interaction effect: $F(14,23) = 2.87$, $p < 0.01$).

The measures of the total size of tissue damage and the number of tissue damage sites revealed a pattern of results that resembled the results using the ranked scores of tissue trauma. The caffeine-treated rats exhibited greater sizes of tissue damage

(interaction effect: $F(28, 35) = 1.58$, $p < 0.05$), and greater numbers of sites of tissue damage (interaction effect: $F(28,35) = 1.93$, $p < 0.01$) than did the vehicle-treated controls (which did not exhibit tissue trauma), but the effects did not differ between the two groups of caffeine-treated rats, and hence were not dose orderly (see Fig 2b and 2c). On the other hand, the pemoline-treated rats exhibited dose-orderly between-groups differences in size and numbers of self-induced tissue damages (see Fig. 2e and 2f). The rats that were tested with 300 mg/kg/day of pemoline exhibited significantly larger (interaction effect: $F(9, 47) = 8.39$, $p < 0.01$) and more numerous (interaction effect: $F(9,47) = 8.22$, p 0<.01) damages than did the other groups of rats across the 4 days that they were tested. The group of rats that was treated with 200 mg/kg/day of pemoline exhibited significantly larger (interaction effect: $F(10,35) = 4.93$, $p<.01$) and more numerous (interaction effect: $F(10,35) = 5.40$, $p <0.01$) damages than did the 100 mg/kg and vehicle groups of rats across the 6 days that they were tested. The group of rats that was treated with 100 mg/kg/day of pemoline exhibited significantly larger (interaction effect: $F(14,23) = 3.17$, $p < 0.01$) and more numerous (interaction effect: $F(14,23) = 2.97$, $p < 0.01$) damages than did the vehicle-treated group of rats across the 15 days that they were tested.

Interestingly, there was a tendency that the rats that were treated with caffeine exhibited injury sites on their tails, and did not injure other body sites. The rats that were treated with 200 and 300 mg/kg/day of pemoline primarily injured their forepaws and ventrum (both thorax and abdomen), whereas the rats that were treated with 100 mg/kg/day primarily injured their tails (see table 2).

Hypothalamic-Pituitary-Adrenal Axis Functioning

Repeated caffeine administration produced substantial alterations in HPA axis activity, in that basal ACTH levels were significantly increased ($F(2,35) = 88.92$, $p < 0.01$) in both doses of caffeine (see Figure 3a), however these effects were not dose-orderly since the 185 and 140 mg/kg groups did not differ significantly. In addition, basal CORT levels were significantly increased in the higher dose of caffeine ($F(2,35) = 8.40$, $p < 0.01$) compared to the vehicle group (see Figure 3b).

Repeated pemoline administration produced substantial alterations in HPA axis activity, so that 200 mg/kg/day produced significantly higher elevations in circulating ACTH ($F(2,33) = 15.80$, $p<.01$) and CORT ($F(2,33) = 7.14$, $p<.01$) concentrations (see Figure 3c and 3d). Hormonal data are not available for the rats that were treated with 300 mg/kg/day because they were euthanized at a different time of the day (early evening) than were the other groups (early morning) owing to the severity of injury that had developed at that time.

Repeated caffeine administration also produced substantial alterations in adrenal and thymus gland masses (see Fig. 4a and 4b), producing significant hypertrophy of the adrenal glands ($F(2,35) = 4.15$, $p < 0.05$) in the 185 mg/kg group and significant atrophy of the thymus glands ($F(2,35) = 41.85$, $p < 0.01$) in both 140 mg/kg and 185 mg/kg groups, when these glandular weights were adjusted for between-groups differences in body weights (see description of body weight differences, below). The repeated administration of pemoline produced adrenal hypertrophy only at the highest (300 mg/kg/day) dose ($F(3, 44) = 11.66$, $p < 0.01$), and did not significantly alter thymus weights in any of the groups of rats ($F(3,44) = 1.74$, $p > 0.05$) (see Figure 4c and 4d).

In the experiment with repeated caffeine administration, the caffeine-treated rats did not gain weight as rapidly as did the rats that were treated with vehicle (interaction effect: $F(28,35) = 11.44$, $p < 0.01$), and there were differences between weight gain of the rats in the groups that were treated with the 140 and 185 mg/kg/day doses (see Fig. 4c). In the pemoline experiment, there were also significant between group differences in the weight gains in the four treatment groups (see Fig. 4f). In fact, the rats that were treated with the highest dose (300 mg/kg/day, $F(9,47) = 27.26$, $p<.01$) exhibited weight loss, and the rats in the 200 mg/kg/day group ($F(10,35) = 14.34$, $p<.01$) exhibited suppressed weight gain, but the rats that were treated with 100 mg/kg/day only differed in their body weights from the vehicle group during the early days of the experiment ($F(14,23) = 2.86$, $p<.05$)

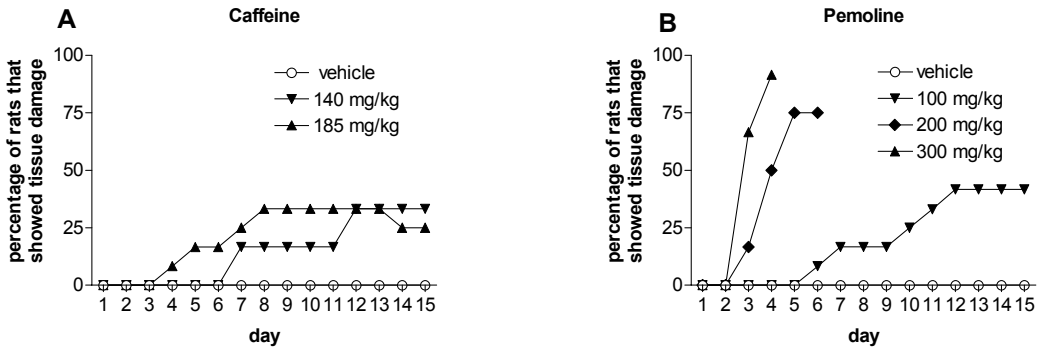

Figure 1. Incidence of SIB: Pemoline, but not caffeine, administration produced self-injury in a dose-orderly manner. a) Some, but not all, of the caffeine-treated rats self-injured. b) In pemoline-treated rats, the onset of SIB occurred earlier and the total number of rats that self-injured was greater in rats treated with the higher doses of pemoline. None of the rats treated with saline or peanut oil exhibited any signs of tissue damage.

Table 2. Topography of SIB: Rats that self-injured in the caffeine-treated group predominantly exhibited raw skin on their tails, with little damage on the paws and no damage on the ventrum. The rats that self-injured in the pemoline-treated group, exhibited tissue damage on the tails, paws or ventrum, depending upon the dose administered. The number of rats exhibiting tissue damage on the tails, paws or ventrum are listed for each of the pharmacologically-treated groups. All the groups have 12 rats, except for the 140 mg/kg caffeine group, which has 6 rats.

GROUP	forepaws	hindpaws	ventrum	tail
vehicle	0	0	0	0
185 caffeine	0	2	0	3
140 caffeine	1	0	0	2
300 pemoline	9	1	5	2
200 pemoline	4	2	7	1
100 pemoline	1	0	0	4

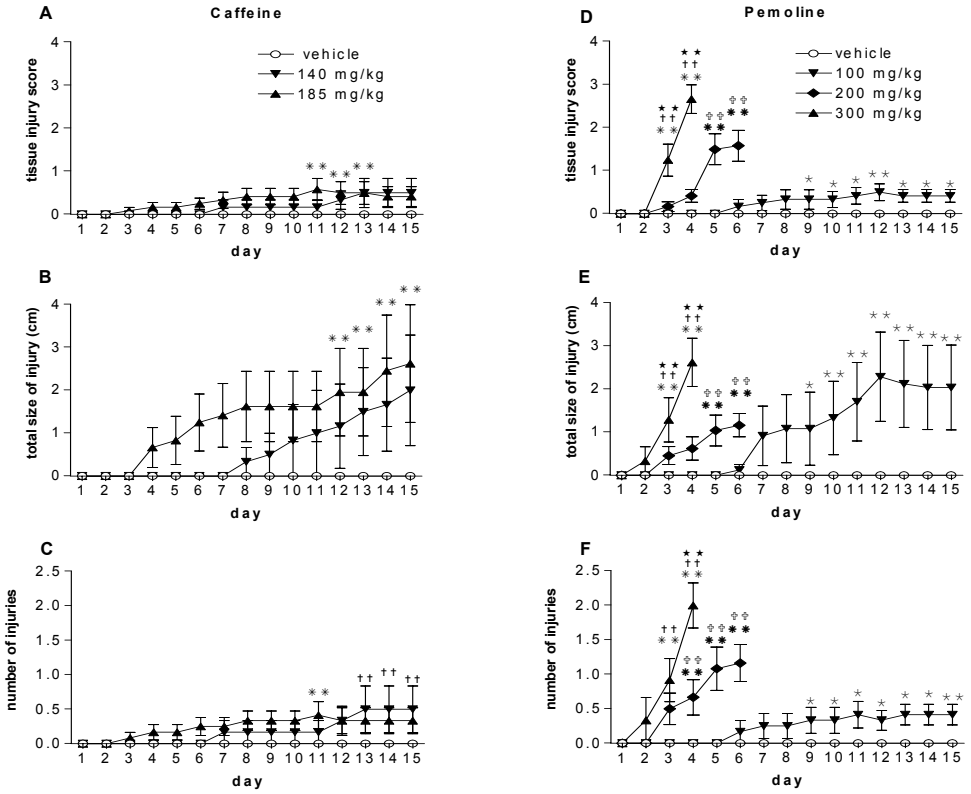

Fig. 2. Caffeine- and Pemoline-Induced Self-Injury: The rats that were treated with caffeine exhibited significant self-inflicted tissue trauma, as indicated by a) tissue trauma scores, b) overall measures of tissue trauma size, and c) total number of tissue damages across 15 days of treatment. The rats that were treated with pemoline also exhibited self-inflicted tissue trauma, as indicated by d) tissue trauma scores, e) overall measures of tissue trauma size, and f) total number of tissue damages across 4, 6, or 15 days of treatment. V6alues expressed are group means ± the standard error of the mean (SEM). Significant between-groups differences are depicted as follows: ✳✳ $p < 0.01$ comparing 185 mg/kg caffeine with vehicle; ✝✝ $p < 0.01$ comparing 140 mg/kg caffeine with vehicle; ✳✳ $p < 0.01$ comparing 300 mg/kg pemoline with vehicle; ✝✝ $p < 0.01$ comparing 300 mg/kg pemoline with 100 mg/kg pemoline; ★★ $p < 0.01$ comparing 300 mg/kg pemoline with 200 mg/kg pemoline; ❋❋ $p < 0.01$ comparing 200 mg/kg pemoline with vehicle; ✞✞ $p < 0.01$ comparing 200 mg/kg pemoline with 100 mg/kg pemoline; ✶ $p < 0.05$, ✶✶ $p < 0.01$ comparing 100 mg/kg pemoline with vehicle.

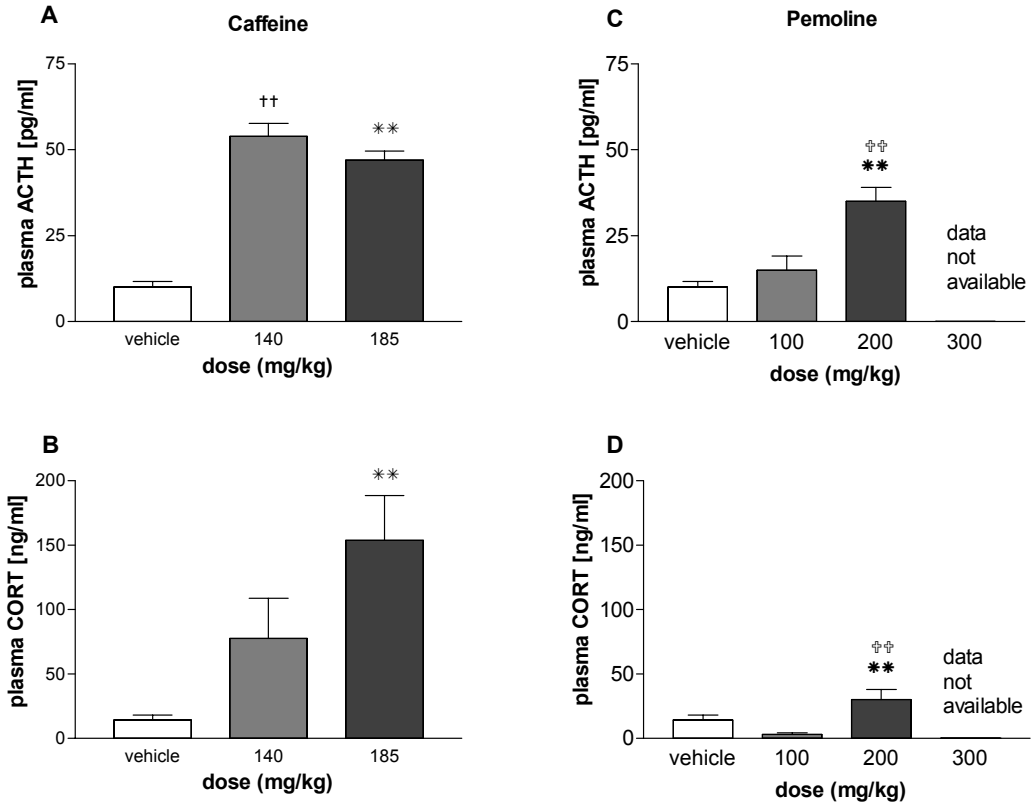

Figure 3. Basal Levels of Stress Hormones: The rats that were treated with caffeine exhibited significant increases in a) basal ACTH levels, however, only the higher dose of caffeine significantly altered b) basal CORT levels. The rats that were treated with 200 mg/kg/day pemoline also exhibited significant alterations in basal stress hormones, as indicated by c) ACTH levels and d) CORT levels. Values expressed are group means ± SEM. Significant between-groups differences are depicted as follows: ✳✳ $p < 0.01$ comparing 185 mg/kg caffeine with vehicle; †† $p < 0.01$ comparing 140 mg/kg caffeine with vehicle; ✱✱ $p < 0.01$ comparing 200 mg/kg pemoline with vehicle; ✞✞ $p < 0.01$ comparing 200 mg/kg pemoline with 100 mg/kg pemoline.

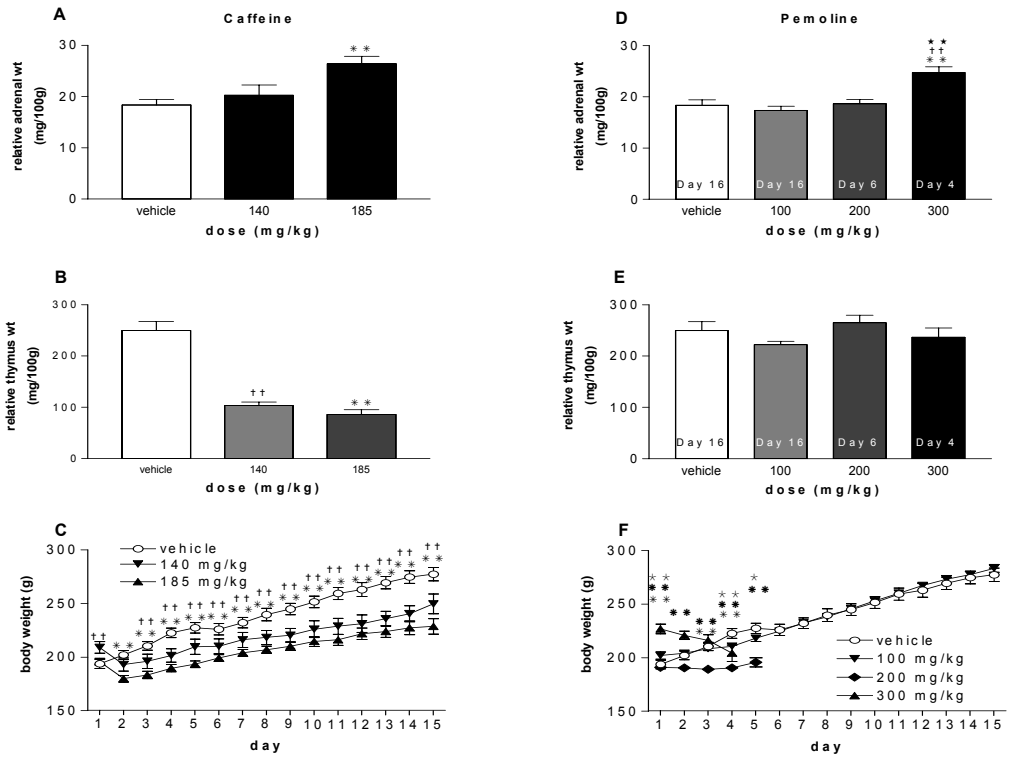

Figure 4. Alterations in Glandular and Body Weight: The rats that were treated with caffeine exhibited alterations in glandular weight, as indicated by a) adrenal hypertrophy in the 185 mg/kg group and b) thymus atrophy in both groups. c) The caffeine-treated rats did not gain weight as rapidly as did the rats that were treated with vehicle. The rats that were treated with the highest dose of pemoline showed d) adrenal hypertrophy, but repeated pemoline administration did not alter e) thymus weights. Administration of pemoline affected f) body weight, in that the 300 mg/kg group lost weight, while the 200 mg/kg group did not gain weight; however, the 100 mg/kg group did not significantly differ from the vehicle towards the end of the experiment. Values expressed are group means ± SEM. Significant between-groups differences are depicted as follows: ✳✳ $p < 0.01$ comparing 185 mg/kg caffeine with vehicle; ✝✝ $p < 0.01$ comparing 140 mg/kg caffeine with vehicle;✳✳ $p < 0.01$ comparing 300 mg/kg pemoline with vehicle; ✝✝ $p < 0.01$ comparing 300 mg/kg pemoline with 100 mg/kg pemoline; ★★ $p < 0.01$ comparing 300 mg/kg pemoline with 200 mg/kg pemoline; ❋❋ $p < 0.01$ comparing 200 mg/kg pemoline with vehicle, ✶✶ $p < 0.01$ comparing 100 mg/kg pemoline vehicle. For body weight, (graphs c and f), significance symbols are shown for treatment groups vs. vehicle group only.

DISCUSSION

Previous reports of caffeine- and pemoline-induced self-injury have described the effects of caffeine or pemoline treatments either by reporting the numbers of rats that exhibited tissue damage (Kasim & Jinnah, 2002; Minana et al., 1984; Minana & Grisolia, 1986; Mueller & Nyhan, 1982; Mueller et al., 1982; Mueller & Nyhan, 1983; Mueller et al., 1986; Peters, 1967), or by reporting the severity of the tissue damage using a rating scale (King et al., 1993; King et al., 1995; Mueller & Hsiao, 1980; Turner et al., 1999). In the present experiments, we directly compared the effectiveness of caffeine and pemoline treatments. We measured the numbers of rats that exhibited tissue trauma daily during treatment with each dose of each drug, and we measured the severity of tissue trauma with a 5-point scale of tissue damage (King et al., 1993; King et al., 1995; Turner et al., 1999). In addition, we assessed the number of trauma sites, and the size of tissue trauma each day during treatment with each of the pharmacological manipulations.

This phenomenological evaluation of these two pharmacological models across days of treatment revealed important differences between caffeine- and pemoline-induced SIB. In fact, the caffeine-induced SIB was mild (never exceeding "2" on the rating scale), and this mild self-injury occurred only in a small number of the rats, even though the doses that were required to produce these self-injurious outcomes were in the range that produced extreme toxicity. The caffeine-treated rats exhibited severe signs of malaise at all doses tested, including behavioral lethargy, reduced weight gain, porphyrhin secretions around the eyes and snout, alterations in HPA axis function, and

even death. One of the twelve rats died during treatment with 185 mg/kg of caffeine, a finding that is consistent with a previous report (Peters, 1967) that this dose administered intragastrically produced approximately 10% mortality. In our preliminary studies, a higher dose produced immediate mortality in more than 50% of the rats – so that experiment was immediately discontinued, and we were unable to examine whether doses higher than 185 mg/kg would induce greater self-injury due to these toxic actions.

Repeated administration of pemoline produced substantially greater incidence and severity of self-induced tissue trauma than did caffeine, and in contrast to the effects of caffeine, these effects of pemoline were dose-orderly. Furthermore, the pemoline-treated rats exhibited significantly fewer and less severe signs of drug-induced toxicity, especially at the 100 and 200 mg/kg doses. Porphyrhin secretions were not observed in the rats that were treated with the 100 and 200 mg doses of pemoline, all the pemoline-treated rats exhibited hyperactivity rather than lethargy or malaise, 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. Accordingly, the 100-300 mg/kg dose range effectively produced self-injury that was accompanied by minimal impact upon the health status of the rats. However, it should be noted that higher doses of pemoline do appear to produce substantial toxicity, and 500 mg/kg/day has been shown to produce approximately 50% mortality (Genovese et al., 1969).

The caffeine and pemoline models also differed in terms of the topographical expression of SIB. In the caffeine-treated rats, tissue damage was generally restricted to the tail; there was very little tissue trauma on the forepaws and no tissue damage on the ventrum (Table 2). The mildness of the caffeine-induced self-injury, coupled with the

fact that it was focused on the tail, contrasts with previous reports that described severe self-inflicted injuries on the paws (Mueller et al., 1982; Mueller & Nyhan, 1983) or on the paws and tails (Peters, 1967) of caffeine-treated rats. The reason for this apparent contradiction is unclear. However, we did observe that the caffeine-treated rats had extensive amounts of dark red porphyrhin secretions on their forepaws, where they spread these secretions from their snouts onto their paws during grooming. These secretions closely resembled blood, and the encrusted secretions on the forepaws of our rats looked like severe injury, until we washed the paws, and found no injury underneath. Furthermore, self-biting behavior was never observed in casual observations in the caffeine-treated rats. In the pemoline-treated rats, the extent of tissue damage was much greater, and was more commonly exhibited on the forepaws and ventrum (thorax and abdomen). In contrast to injuries in the caffeine-treated rats, the tail was the least common area of injury. This is consistent with previous reports of pemoline-induced SIB (Mueller & Hsiao, 1980; Mueller et al., 1986). In the pemoline-treated rats, the self-biting behavior was highly stereotyped, with rats often showing biting that started at the forepaws, and moved on to the ventral thorax and abdomen, and this self-biting behavior was consistently observed in casual observations.

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. In all three doses of chronic pemoline administration, some of the rats self-injured, whereas some of the rats 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 behaviors. 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, in that this biological vulnerability may have a significant impact upon our understanding of the etiology of clinical SIB in human populations. Individual differences in vulnerability to self-injure also occurred in the caffeine model, but the toxicity of this treatment is problematic, and therefore, the pemoline model appears to be a better model for the study of factors that determine individual differences in this vulnerability. In fact, the 200 mg/kg dose of pemoline appears to be close to the ED50 for induction of SIB and seems to be a reasonable dose to use when investigating individual differences in brain functioning, drug sensitivity, and hormonal responses that may shed light on individual differences in the clinical populations. These investigations could be coupled with studies in genetic models of SIB (Kasim & Jinnah, 2002), in effects of cortical (Cromwell et al., 1999) and other brain lesions, and in pharmacological manipulations (e.g. antagonist challenges) that could alter vulnerability (King et al., 1993; King et al., 1995) to exhibit 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 behavior.

LIST OF REFERENCES

Anderson L.T., Ernst, M. (1994) Self-injury in Lesch-Nyhan disease. Journal of Autism.and Developmental.Disorders 24: 67-81.

Barron J.L., Sandman, C.A. (1984) Self-injurious behavior and stereotypy in an institutionalized mentally retarded population. Applied Research in Mental Retardation 5: 499-511.

Berney T.P., Ireland M., Burn J. (1999) Behavioural phenotype of Cornelia de Lange syndrome. Archives of the Disabled Child 81(4): 289-304

Breese G.R., Baumeister A.A., McCown T.J., Emerick S.G., Frye G.D., Mueller R.A. (1984) Neonatal-6-hydroxydopamine treatment: Model of susceptibility for self-mutilation in the Lesch-Nyhan syndrome. Pharmacology Biochemistry and Behavior 21: 459-461.

Castells S., Chakrabarti C., Winsberg B.G., Hurwic M., Perel J.M., Nyhan W.L. (1979) Effects of L-5-hydroxytryptophan on monoamine and amino acids turnover in the Lesch-Nyhan syndrome. Journal of Autism and Developmental Disorders 9: 95-103.

Coid J., Allolio B., Rees L.H. (1983) Raised plasma metenkephalin in patients who habitually mutilate themselves. Lancet 2: 545-546

Cromwell H.C., King B.H., Levine M.S. (1997) Pemoline alters dopamine modulation of synaptic response of neostriatal neurons. Developmental Neuroscience 19: 497-504.

Cromwell H.C., Levine M.S., King B.H. (1999) Cortical damage enhances pemoline-induced self-injurious behavior in prepubertal rats. Pharmacology Biochemistry and Behavior 62(2): 223-227

Edelson S.M. (1984) Implications of sensory stimulation in self-destructive behavior. American Journal of Mental Deficits 89: 140-145.

Ernst M., Zametkin A.J., Matochik J.A., Pascualvaca D., Jons P.H., Hardy K., Hankerson, J.G., Doudet D.J., Cohen R.M. (1996) Presynaptic dopaminergic deficits in Lesch-Nyhan disease. New England Journal of Medicine 334: 1568-1572.

Genovese E., Napoli P.A., Bolega-Zonta N. (1969) Self-aggressiveness: a new type of behavioral change induced by pemoline. Life Sciences 8(1): 513-515

Gilbert S., Spellacy E., Watts R.W.E. (1979) Problems in the behavioural treatment of self-injury in the Lesch-Nyhan syndrome. Developmental Medicine in Child Neurology 21: 795-800.

Gillberg C., Terenius L., Lonnerholm G. (1985) Endorphin activity in childhood psychosis. Spinal fluid levels in 24 cases. Archives of General Psychiatry 42: 780-783.

Guess D., Carr E. (1991) Emergence and maintenance of stereotypy and self-injury. American Journal of Mental Retardation 96(3): 299-319

Harlow H.F., Dodsworth R.O., Harlow M.K. (1965) Total social isolation in monkeys. Proceedings of the National.Academy of Sciences U.S.A 54: 90-97.

Harlow H.F., Harlow M.K. (1962) Social deprivation in monkeys. Scientific American 207: 136-146.

Hellings J.A., Warnock J.K. (1994) Self-injurious behavior and serotonin in Prader-Willi Syndrome. Psychopharmacological Bulletin 30(2): 245-50.

Hoefnagel D. (1968) Seminars on the Lesch-Nyhan syndrome: Summary. Federal Proceedings 27: 1042-1046.

Jankovic J., Caskey T.C., Stout J.T., Butler I.J. (1988) Lesch-Nyhan syndrome: A study of motor behavior and cerebrospinal fluid neurotransmitters. Annals of Neurology 23: 466-469.

Kasim S., Jinnah H. (2002) Pharmacologic thresholds for self-injurious behavior in a genetic mouse model of Lesch-Nyhan disease. Pharmacology Biochemistry and Behavior 73: 583-592

King B.H., Au D., Poland R.E. (1993) Low-dose naltrexone inhibits pemoline-induced self-biting behavior in prepubertal rats. Journal of Child and Adolescent Psychopharmacology 3(2): 71-79.

King B.H., Au D., Poland R.E. (1995) Pretreatment with MK-801 inhibits pemoline-induced self-biting behavior in prepubertal rats. Developmental Neuroscience 17: 47-52

Lloyd K.G., Hornykiewicz O., Davidson L., Farley I., Goldstein M., Shibuya M., Kelley W.N., Fox I.H. (1981) Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. New England Journal of Medicine 305: 1106-1111.

Mardikar B.R., Srinivasan S., Balwani J.H. (1969) On the phenomenom of caffeine induced automutilation. Indian Journal of Physiological Pharmacology 13(4): 261-263

Matson J.L., Baglio C.S., Smiroldo B.B., Hamilton M., Packlowskyj T., Williams D., Kirk-patrick-Sanchez S. (1996) Characteristics of autism as assessed by Diagnostic Assessment for Severely Handicapped-II DASH-II. Research of Developmental Disabilities 17: 135-43.

Minana M.D., Portoles M., Jorda A., Grisolia S. (1984) Lesch-Nyhan Syndrome, Caffeine model: increae of purine and pyrimidine enzymes in rat brain. Journal of Neurochemistry 43: 1556-1560.

Minana M.D., Grisolia S. (1986) Caffeine Ingestion by rats increases noradrenaline turnover and results in self-biting. Journal of Neurochemistry 47: 728-732.

Mitchell G., McInnes R.R. (1984) Differential diagnosis of cerebral palsy: Lesch-Nyhan syndrome without the self-mutilation. Canadian Medical Association Journal 130(10): 1323-4.

Mueller K., Hollingsworth E., Pettit H. (1986) Repeated pemoline produces self-injuroius behavior in adult and weanling rats. Pharmacology Biochemistry and Behavior 25: 933-938.

Mueller K., Hsiao S. (1980) Pemoline-induced self-biting in rats and self-mutilation in the deLange syndrome. Pharmacology Biochemistry and Behavior 13(5): 627-631.

Mueller K., Nyhan W.L .(1982) Pharmacologic control of pemoline induced self-injurious behavior in rats. Pharmacology Biochemistry Behavior 16: 957-963.

Mueller K., Nyhan W.L. (1983) Clonidine potentiates drug-induced self-injurious behavior in rats. Pharmacology Biochemistry Behavior 18: 891-894.

Mueller K., Saboda S., Palmour R., Nyhan W.L. (1982) Self-injurious behavior produced in rats by daily caffeine and continuous amphetamine. Pharmacology Biochemistry and Behavior 17: 613-617.

Nyhan W.L. (1968a) Clinical features of the Lesch-Nyhan syndrome. Introduction--clinical and genetic features. Federal Proceedings 27(4): 1027-33.

Nyhan W.L. Lesch-Nyhan syndrome. (1968b) Summary of clinical features. Federal Proceedings 27(4): 1034-41.

Oliver C., Murphy G.H., Corbett J.A. (1987) Self-injurious behavior in people with mental handicap: a total population study. Journal of Mental Deficit Research 31: 147-62.

Page T. Coleman, M. (1998) De novo purine synthesis is increased in the fibroblasts of purine autism patients. Advanced Experimental and Medical Biology 431: 793-796.

Partington M., Hennen B.K. (1967) Lesch-Nyhan Syndrome: Self-destructive behavior, mental retardation, neurodisease, and hyperuricaemia. Developmental and Medical Child Neurology 9(5): 563-72.

Peters J.M. (1967) Caffeine-induced hemorrhagic automutilation. Archives of International Pharmacodynamic Therapy 169(1): 139-46.

Rosenberger-Debiesse J., Coleman M. (1986) Preliminary evidence for multiple etiologies in autism. Journal of Autism Developmental Disorders 16: 385-392.

Saito Y., Ito M., Hanaoka S., Ohama E., Akaboshi S., Takashima S. (1999) Dopamine receptor upregulation in Lesch-Nyhan syndrome: a postmortem study. Neuropediatrics 30: 66-71.

Sakata T., Fuchimoto H. (1973) Stereotyped and aggressive behavior induced by sustained high doses of theophylline in rats. Japan Journal of Pharmacology 23(6): 781-5.

Sandman, C.A. (1988) b-endorphin disregulation in autistic and self-injurious behavior: a neurodevelopmental hypothesis. Synapse 2: 193-199.

Sandman C.A., Barron J.L., Chicz-DeMet A., DeMet E.M. (1990) Plasma b-endorphin levels in patients with self-injurious behavior and stereotypy. American Journal of Mental Retardation 95: 84-92.

Schepis C., Failla P., Siragusa M., Romano C. (1994) Skin-picking: best cutaneous feature in recognization of Prader-Willi Syndrome. International Journal of Dermatology 33(12): 866-7.

Schoeder S.R., Schroeder C.S., Smith B., Dalldorf J. (1978) Prevalence of self-injurious behaviors in a large state facility for the retarded: a three year follow-up study. Journal of Autism and Childhood Schizophrenia 8(3): 261-269.

Seay B., Harlow H.F. (1965) Maternal separation in the rhesus monkey. Journal of Nervous Mental Disorders 140: 434-441.

Sivam S.P. (1989) D1 dopamine receptor-mediated substance P depletion in the striatonigral neurons of rats subjected to neonatal dopaminergic denervation: implications for self-injurious behavior. Brain Research 500(1-2): 119-30.

Sweetman L., Nyhan W.L. (1970) Detailed comparison of the urinary excretion of purines in a patient with the Lesch-Nyhan syndrome and a control subject. Biochemical Medicine 4: 121-134.

Symons F.J., Butler M.G., Sanders M.D., Feurer I.D., Thompson T. (1999) Self-injurious behavior and Prader-Willi syndrome: behavioral forms and body locations. American Journal of Mental Retardation 104(3): 260-9.

Symons F.J., Thompson T. (1997) Self-injurious behavior and body site preference. Journal of Intellectual Disabilities Research 41(6): 456-68.

Turner C.A., Lewis M.H. (2002) Dopaminergic mechanisms in self-injurious behavior and related disorders. American Psychological Association, Washington, D.C. In *Self-injurious behavior: gene-brain-behavior relationships* (Editors: Schroeder S.R., Oster-Granite M.L., Thompson T.) 165-179.

Turner C.A., Panksepp J., Bekkedal M., Borkowski, Burgdorf (1999) Paradoxial effects of serotonin and opiods in pemoline-induced self-injurious behavior. Pharmacology Biochemistry and Behavior 63(3): 361-366.

Willemsen-Swinkels S.H.N., Buitelaar J.K., Weijnen F.G., Thijssen J.H.H., Van Engeland H. (1996) Plasma beta-endorphin concentrations in people with learning disability and self-injurious and/or autistic behaviour. British Journal of Psychiatry 168: 105-109.

BIOGRAPHICAL SKETCH

Staci Kies was born on February 5th, 1978, in Joliet, IL. In 1986, she moved to Melbourne, FL. After graduating high school in 1996, she attended Brevard Community College, where she received her Associate of Arts in May 1997. Staci then went on to attend the University of Central Florida, where she obtained her Bachelor of Science in psychology in May 1999. From August 1999 to the present, she has been pursuing a graduate degree in behavioral neuroscience in the Psychology Department of the University of Florida.