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Title page

Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy

Amber M. Muehlmann, Brandon D. Brown, Darragh P. Devine

University of Florida, Department of Psychology, Behavioral Neuroscience Program, P.O. Box

112250, Gainesville, Florida, 32611-2250   (AMM, BDB, and DPD)

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Running Title Page

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Corresponding Author: Darragh P. Devine
University of Florida
Department of Psychology
P.O. Box 112250
Gainesville, Florida
32611-2250

phone: 352-392-0601 ext. 294
fax: 352-392-7985
e-mail: dpdevine@ufl.edu

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693 words in Introduction
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Nonstandard Abbreviations:
Self-injurious behavior (SIB)
Obsessive-compulsive disorder (OCD)
Selective serotonin reuptake inhibitor (SSRI)
Aspartate aminotransferase (AST)
Gamma-aminobutyric acid (GABA)
N-methyl-D-aspartate (NMDA)
α amino 3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)
Analysis of variance (ANOVA)
Repeated measures analysis of variance (RM-ANOVA)
Least Significant Difference (LSD)
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Abstract

Self-injury is a devastating, maladaptive behavior disorder that is common in developmental disabilities, and is comorbid with numerous psychiatric disorders. Examples of self-injurious behavior (SIB) include head-banging, self-biting, and self-punching. The neurochemical basis of SIB is unknown, however many different classes of drugs are prescribed (e.g. neuroleptics, atypical neuroleptics, anti-epileptics, opioid antagonists) to reduce these behaviors. These drugs have all shown clinically significant but limited efficacy in patient populations, and no class of drug is effective for all patients. The development and characterization of a valid animal model could provide important information regarding the neurochemical basis of SIB and could be used to screen potential new pharmacotherapies. In one model of SIB, high doses of pemoline are administered to rats. Using this model, we evaluated the effectiveness of three drugs (risperidone, valproate and topiramate) that reduce SIB in humans. We also screened the potential effectiveness of tramadol, a drug that decreases stereotyped and compulsive behaviors, but has not been assessed in human self-injurers. We found that risperidone, valproate and topiramate each significantly attenuate pemoline-induced SIB, whereas tramadol does not. These findings suggest that the pemoline model of SIB has predictive validity across a range of drug classes, and implicate important potential neurochemical mechanisms that may contribute to the behavior disorder. The findings also indicate that tramadol may not be an effective pharmacotherapy for SIB.

Introduction

Self-injurious behavior (SIB) is a devastating, maladaptive behavior disorder that is highly prevalent in autistic and intellectually handicapped populations, as well as a variety of well-defined genetic syndromes (e.g. Lesch-Nyhan and Prader-Willi syndromes) (Rojahn and Esbensen, 2002). It is also common in schizophrenia, borderline personality disorder, obsessive-compulsive disorder (OCD) and Tourette's syndrome (Burgess, 1991; Primeau and Fontaine, 1987; Robertson et al., 1989). In all these groups, the self-injurious actions include head-banging, self-biting, skin-picking, self-punching, and many other forms (Rojahn and Esbensen, 2002). These behaviors can interfere with normal educational and socializing activities, and they can produce severe medical complications. Furthermore, the expression of SIB is extremely destructive for families and caretakers of the self-injurer, and the economic burden is staggering.

Although little is known about the neurochemical basis of SIB, a variety of studies have implicated disregulation of dopaminergic, serotonergic, GABAergic, and opioid neurotransmission (for review see Winchel and Stanley, 1991). Accordingly, clinical trials have been conducted with classical neuroleptics (e.g. haloperidol), selective serotonin reuptake inhibitors (SSRIs), and the opioid antagonist naltrexone (Janowsky et al., 2005a; Janowsky et al., 2005b; Sandman et al., 1990). Although beneficial effects have been reported in many of these trials, non-responders have been identified for each of these drug classes, and there are contradictory reports that these drugs did not have positive therapeutic effects (e.g. Willemsen-Swinkels et al., 1995).

More recent reports have described relatively consistent reductions in SIB during treatment with the atypical neuroleptic risperidone (Risperdol) (Masi, 2004). Positive outcomes have also been

described during treatment with the anti-epileptic and mood-stabilizing drug valproate

(Depakote) (Ruedrich et al., 1999) and preliminary trials with the anti-epileptic topiramate

(Topamax) have been promising (Shapira et al., 2002). However, it should be noted that these

three compounds have not been studied as extensively as the older drug classes have.

Furthermore, interpretation of these clinical trials (and those of the older drugs) is hampered by

the fact that most trials are conducted with uncontrolled open label designs, owing to the severe

nature of the disorder. In addition, the effects of concomitant behavior therapy are generally not

reported, and interactions with additional ongoing drug interventions may obscure the treatment

outcomes. Precise monitoring of specific behaviors may also be difficult throughout the daily

lives of patients, so many trials have relied upon more global impressions of the patients'

progress, rather than specific counts of SIB (e.g. Caicedo and Williams, 2002).

In light of these difficulties in the design and interpretation of clinical drug trials for SIB, it

would be useful to have an animal model with good predictive validity for pharmacotherapeutic

effects. The availability of such a model could benefit patient populations by helping to pre-

screen potential pharmacotherapies under controlled laboratory conditions, with precisely

defined and quantifiable dependent measures.

In one animal model of SIB, rodents exhibit self-biting behavior after administration of high

doses of the indirect monoamine agonist pemoline (2-amino-5-phenyl-1,3-oxazol-4-one)

(Mueller and Hsiao, 1980; Kies and Devine, 2004). The pemoline-induced SIB occurs in a dose-

orderly and stereotyped manner after 3-5 daily injections (Kies and Devine, 2004), and it can be

blocked by co-administration of haloperidol, pimozide, or naltrexone (Mueller and Nyhan, 1982;

King et al., 1993). Accordingly, the model seems to be responsive to classical interventions that

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have been at least partially successful in treatment of human self-injurers. However, the efficacy

of atypical neuroleptics and anti-epileptics has not been evaluated in the pemoline model of SIB.

Since risperidone and valproate each have established records of beneficial effects in human self-

injurers, we examined the predictive validity of the pemoline model by investigating the

effectiveness of these two drugs against pemoline-induced SIB. We also evaluated the

pharmacotherapeutic potential of topiramate, since there is preliminary evidence that it is

effective in treatment of SIB in autism and Prader-Willi syndrome, and we have challenged the

pemoline-induced SIB with tramadol (Ultram), an atypical opiate agonist that is thought to be

beneficial in OCD and Tourette's syndrome (Goldsmith et al., 1999; Shapira et al., 1997), but

has not previously been assessed for treatment of clinical SIB. In addition, we evaluated the

impact of the experimental manipulations on regulation of the hypothalamic-pituitary-adrenal

axis, and we investigated potential organotoxicity during pemoline administration.

Methods

Animals

One hundred and seventy two male Long Evans (LE) rats weighing 225-275 grams were purchased from Charles River Laboratories (Raleigh, NC; risperidone, valproate and topiramate experiments) or from Harlan Inc. (Indianapolis, IN; tramadol experiment). The rats were housed in a climate controlled vivarium with a 12hr/12hr light/dark schedule (lights on at 7 a.m.). Standard laboratory rat chow (Lab Diet 5001) and tap water were available *ad libitum*. The rats were pair-housed in standard polycarbonate cages (43 x 21.5 x 25.5 cm) during 5-7 days of acclimation to the housing facility. After the acclimation period the rats were singly-housed in identical polycarbonate cages. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and all experiments were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida.

Drugs

Pemoline (Spectrum Chemicals, New Brunswick, NJ) was suspended at a concentration of 50 mg/ml in warm peanut oil (held at approximately 36° C), with constant stirring. Risperidone (Sigma-Aldrich Co., St. Louis, MO) was dissolved in 45% (w/v) hydroxypropyl-β-cyclodextrin at concentrations of 0, 0.1, 0.5 and 1.0 mg/ml. Sodium valproate (Sigma-Aldrich Co.) was dissolved in 0.04% (w/v) Na_2EDTA at concentrations of 0, 50, 100 and 200 mg/ml and was adjusted to a neutral pH of 7.4. Topiramate was provided by Johnson & Johnson (Raritan, NJ), and was dissolved in sterile saline at concentrations of 0, 1, 3 and 10 mg/ml. Tramadol hydrochloride (Sigma-Aldrich Co.) was dissolved in sterile saline at concentrations of 0, 0.1, 1.0, and 10 mg/ml.

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Experimental Procedures

Drug Treatments:

Twenty-nine of the rats were weighed and injected with pemoline (200 mg/kg s.c.) at approximately 8:00 a.m. on each of five consecutive days. These injections were administered at the nape of the neck or either flank on a rotating basis. The rats were also injected twice daily with risperidone (0, 0.1, 0.5 or 1.0 mg/kg, i.p.; n = 6-8 per group) on each of the five days. The risperidone injections were administered at approximately 8:00 am (immediately after the pemoline injection) and again at approximately 6:00 pm. The rest of the rats received daily pemoline injections at 200 mg/kg, and twice-daily injections of either valproate (0, 50, 100 or 200 mg/kg, i.p.; n = 9 rats per group), topiramate (0, 1, 3 or 10 mg/kg, s.c.; n = 8-9 rats per group), or tramadol (0, 0.1, 1.0 or 10 mg/kg, s.c.; n = 18 rats per group) for five days, following the same procedures as in the risperidone experiment.

These doses of risperidone, valproate, topiramate, and tramadol were selected because they do not produce sedative effects in rats. Risperidone did not diminish any measure of activity after acute or chronic (once daily for 20 days) administration of doses ranging from 0.01- 10 mg/kg (Drago et al., 1997). Valproate did not diminish locomotion or rearing when administered acutely or chronically (21 days) at doses ranging from 50-400 mg/kg (Fernandez et al., 1988). Topiramate did not diminish locomotion, and it did not affect any of a variety of measures of motor abilities when tested at 80 and 160 mg/kg (Alaverdashvili et al., 2005). Tramadol did not affect motor abilities at doses up to 30 mg/kg (Guneli et al., 2007).

Assays of Self-Injury

The rats were visually inspected each time they were injected (i.e. twice per day for five days), and the inspections were videotaped. For these inspections, each rat was held in front of a video camera and the head, forepaws, hindpaws, ventrum and tail were manually turned toward the camera by the experimenter. The presence or absence of tissue injury (denuded skin, erythema, edema or open lesion) was noted for each rat. Any rat with an open lesion was immediately euthanized. In addition, still photographs of the injured tissue were taken from the videotapes, outlines were drawn around the injured tissue, and MCID software was used to calculate the area of tissue damage in mm^2.

Night-vision cameras were focused on the cages of the rats (one camera per cage) each night, and five-minute time samples were recorded once per hour for eight hours. These recordings were scored for duration of self-injurious oral contact, duration of grooming, duration of inactivity and the amount of locomotion. Self-injurious oral contact was defined as all oral contact that stayed fixed on any one body part for longer than two seconds. Grooming was defined as oral contact with any part of the body that continued to move from site to site on the body (e.g. oral contact with the forepaws, then moving up each forelimb and continuing to the ventrum, in which the contact was not sustained on any spot on the body for longer than two seconds). Inactivity was defined as complete lack of movement except respiratory movements. Locomotion was counted by sectioning the cage into three equal parts (along the length of the cage) and tallying the number of times the rat's forepaws entered into a different section without returning to the section that it occupied immediately prior to that movement

On the morning of the sixth day each rat was visually inspected. Immediately after this inspection the rat was terminated, and the thymus and adrenal glands were removed.

Health Status of the Rats

Thymus involution and adrenal hyperplasia are well known consequences of chronic stress exposure in rats. Accordingly, the thymus and adrenal glands were weighed in order to determine whether the stress of the experiment was substantial enough to affect the endocrine or immune status of the pemoline-treated rats.

An additional 30 rats (100-125 g; Harlan Inc.) were used to evaluate the potential that the pemoline regimen could induce organotoxicity in the rats. Rats received either no treatment (n = 6), peanut oil injections (n = 6), or pemoline injections at 100 mg/kg/day for 14 days (n = 6), 200 mg/kg/day for five days (n = 6) or 300 mg/kg/day for four days (n = 6). The rats were rapidly decapitated the morning after the last injection. The trunk blood was collected and plasma was isolated by centrifugation at 1000xg for five minutes at 4 C. The plasma was frozen on dry ice and stored at -80 C. Organic toxicity was examined by assaying extinction of NADH by aspartate aminotransferase (AST), according to the procedure of Kus et al. (2004).

Statistical Analyses

Between-groups differences in self-injurious oral contact, area of the tissue damage, grooming, inactivity, and locomotion were each evaluated using repeated measures analyses of variance (RM-ANOVA). The area under the curve (AUC) was then determined for the self-injurious oral contact scores and for the area of tissue damage for each rat in each of the pharmacological challenge experiments. The correlations between these AUCs were then analyzed using a Pearson correlation to examine the relationship between the behavioral (oral contact) and tissue (area of damage) measures in each of the experiments.

The body weights were also analyzed by RM-ANOVA, and the glandular weights were each compared using a one-way ANOVA for each experiment.

All significant effects were further analyzed with Least Significant Difference (LSD) post-tests for all the ANOVAs and RM-ANOVAs. Between-groups differences in all dependent measures were treated as statistically reliable when the p-values were less than 0.05.

Some rats were euthanized before the end of the experiment because they had an open lesion. In these cases, the missing data were replaced by repeating the final score that was attained for each dependent measure through the end of the experiment. This strategy was used to avoid the potential that the group means would underestimate the self-injurious oral contact and the area of tissue damage scores, and to avoid the potential that the group means would over- or under-estimate the locomotor, inactivity, and grooming scores when the most severe self-injurers had to be removed from any group. The numbers of rats that were terminated early in each group are summarized in Table 1.

Inter-observer reliability

Inter-observer reliability for the overnight behavioral scoring was assessed by two observers scoring the same 300 minutes of videotaped samples (60 clips lasting five minutes each with all concentrations of all drugs represented in this sampling each night). A quantitative measure of reliability was determined by taking the total number of seconds that each observer scored the same behavior in each time sample and dividing by the total number of seconds scored (i.e. 300 seconds).

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Results

Risperidone experiment

Fewer rats exhibited pemoline-induced self-injury when co-treated with risperidone, in comparison with the group that did not receive risperidone injections (Fig. 1a). The duration of self-injurious oral contact was significantly less in the risperidone-treated rats than it was in the vehicle-treated rats ($F_{(3,100)} = 3.175$, $p < 0.05$; Fig. 1b), and the risperidone-treated rats displayed smaller areas of tissue damage than the vehicle-treated rats did ($F_{(30,250)} = 2.444$ $p < 0.05$; Fig. 1c). Additionally, the AUC for self-injurious oral contact and the AUC for area of tissue damage were significantly correlated ($r = 0.5416$, $p < 0.05$; Fig. 1d) for all the vehicle- and risperidone-treated rats.

Risperidone did not significantly affect the duration of time spent grooming ($F_{(12,100)} = 1.253$, $p > 0.05$; Fig. 2a). On the other hand, it did significantly increase the duration of inactivity overnight ($F_{(3,76)} = 3.482$, $p < 0.05$; Fig. 2b), although there were no significant time or group by time interaction effects. The amount of locomotion was initially high, and it decreased significantly across the days treatment in all the groups ($F_{(4,100)} = 8.709$, $p < 0.05$; Fig. 4c). Furthermore, the rats that were treated with the highest dose of risperidone exhibited higher rates of locomotion compared to the vehicle-treated rats ($F_{(3,100)} = 3.874$, $p < 0.05$; Fig. 2c), but there were no group by time interaction effects.

Valproate experiment

Fewer rats exhibited pemoline-induced self-injury in the groups that were treated with the two highest doses of valproate than in the other two groups (Fig. 3a). In agreement with this

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observation, the area of tissue damage was significantly less in the group treated with the highest

dose of valproate ($F_{(30,320)} = 1.953$, $p < 0.05$; Fig. 3c). However, the rats that received the lowest

dose of valproate actually displayed significantly larger areas of tissue damage than the vehicle-

treated rats did. Furthermore, valproate administration did not significantly affect the duration of

self-injurious oral contact ($F_{(12,128)} = 0.6282$, $p > 0.05$; Fig. 3b). When analyzed together, the

AUC for self-injurious oral contact and the AUC for area of tissue damage for the vehicle- and

valproate-treated rats were significantly correlated ($r = 0.6253$, $p < 0.05$; Fig 3d).

Valproate administration did not significantly affect any of the other behaviors that were

measured during the experiment. Although the time spent grooming ($F_{(4,128)} = 5.286$, $p < 0.05$;

Fig. 4a), inactive ($F_{(4,128)} = 4.753$, $p < 0.05$; Fig. 4b), and the amount of locomotion recorded on

videotapes overnight ($F_{(4,128)} = 20.19$, $p < 0.05$; Fig. 4c) each decreased significantly across the

days of the experiment, there were no significant between-groups differences and there were no

group by time interaction effects.

Topiramate experiment

All the rats that were treated with either vehicle or the low dose of topiramate, and nearly all the

rats that were treated with the two higher doses of topiramate, exhibited pemoline-induced self-

injury (Fig. 5a). Overall, the incidence and expression of SIB was greater in this experiment

than it was in the other experiments, and more rats were euthanized because of severe SIB than

in the other experiments (see Table 1). This is attributed to the fact that we used a new batch of

pemoline, which was more potent than the batch that we used in the other experiments. This

difference in potency was confirmed by a small difference in the drug purity (according to the

drug supplier's certificate of analysis) and we have continued to observe differences in potency

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in additional experiments we recently conducted. Accordingly, we have reduced our working dose to 150 mg/kg in those subsequent experiments (manuscripts in preparation). The rats that received the two higher doses of topiramate, however, displayed less self-injurious oral contact ($F_{(12,128)} = 2.002$, $p < 0.05$; Fig. 5b) and smaller areas of tissue damage (($F_{(30,320)} = 1.569$, $p < 0.05$; Fig. 5c) despite the high potency of the pemoline. The AUCs for self-injurious oral contact and area of tissue damage were significantly correlated ($r = 0.5513$, $p < 0.05$; Fig. 5d) for all the vehicle- and topiramate-treated rats.

Topiramate did not significantly affect the amount of time spent grooming. Topiramate-treated rats did exhibit more inactivity than vehicle-treated rats did ($F_{(12,120)} = 1.9$, $p < 0.05$; Fig. 6b), and this effect was most pronounced during the last two experimental nights. Locomotion decreased significantly throughout days of the experiment ($F_{(4, 120)} = 62.88$, $p < 0.05$; Fig. 6c) but there were no between-groups differences.

Tramadol experiment

More rats exhibited pemoline-induced self-injury in each of the tramadol-treated groups than in the vehicle-treated group (Fig. 7a). However, the duration of self-injurious oral contact ($F_{(12,164)} = 1.193$, $p > 0.05$; Fig. 7b) and the area of tissue damage ($F_{(30,680)} = 1.108$ $p > 0.05$; Fig. 7c) were not significantly altered in the tramadol-treated rats, compared to those of the vehicle-treated rats. Additionally, the AUC for self-injurious oral contact and the AUC for area of tissue damage were significantly correlated in all the vehicle- and tramadol-treated rats ($r = 0.4782$, $p < 0.05$; Fig. 7d).

Tramadol did not significantly affect the other behaviors that were measured during the experiment. The time spent grooming ($F_{(4,164)} = 9.595$, $p < 0.05$; Fig. 14a), inactive ($F_{(4,164)} =$

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4.823, $p < 0.05$; Fig. 14b) and the amount of locomotion ($F_{(4,164)} = 7.454$, $p < 0.05$; Fig. 14c) changed significantly across the days of the experiment, however, there were no significant between-groups differences or group by time interaction effects for any of these behaviors.

Health Status of the Rats

Body weight of all the rats described herein declined throughout the first three days of pemoline treatment, but then rebounded back to baseline by day 6 of the experiment (data not shown). No significant differences in thymus or adrenal gland mass were found, replicating our previous findings (Kies and Devine, 2004), except for a slight thymus involution in the group of rats treated with the highest dose of valproate (data not shown).

No sign of organic toxicity was found in the pemoline-treated rats. AST activity was consistently below 300 U/L (Table 2). In comparison, Kus et al. (2004) reported AST levels as high as 844 ± 125 U/L in rats with toxic liver damage, and 295 ± 35 U/L in controls.

Inter-observer reliability

The two observers' scores of the duration of overnight behaviors matched exactly 98.3% of the time.

Discussion

The control rats in each of these experiments spent a very high percentage of time engaged in self-injurious oral contact. Accordingly, the rats appear to be very focused on this behavior, but despite this focus, risperidone, valproate, and topiramate were each effective in reducing the amount and/or severity of self-injury. These drugs did not consistently alter the expression of grooming, inactivity, or locomotion in any of the experiments. Furthermore, the body weights, the glandular masses, the AST assays, and the overall appearance of the rats indicated that they were healthy. Accordingly, the effectiveness of risperidone, valproate and topiramate each appeared to result from specific attenuation of the target behaviors, rather than a more general effect, such as sedation or malaise.

The neurochemical mechanisms whereby pemoline causes SIB are not known. Pemoline is a long-lasting indirect monoamine agonist that blocks neuronal uptake of dopamine and norepinephrine after acute administration (Molina and Orsingher, 1981). Its effects on serotonergic uptake have been less well studied, and the impacts of the chronic high dose pemoline regimen on neurochemical regulation have not been systematically characterized. The involvement of dopaminergic mechanisms in pemoline-induced SIB is suggested by the fact that the SIB is eliminated by co-administration of haloperidol or pimozide (Mueller and Nyhan, 1982). Furthermore, dopaminergic neurotransmission is often disregulated in clinical populations that exhibit SIB (Turner and Lewis, 2002). Noradrenergic mechanisms are indirectly implicated in the pemoline model by the fact that isolated mice self-injure after clonidine administration (Razzak et al., 1977). Serotonergic mechanisms are implicated by the fact that co-administration of the SSRI paroxetine increases the expression of SIB in the pemoline model (Turner et al., 1999).

Risperidone Experiment

The dose-orderly decreases that were observed in all the measures of self-injury are congruent with reports of risperidone's efficacy in clinical trials (Masi, 2004). Thus, the results of this study suggest convergence in the neurochemical mechanisms that contribute to the pathology of SIB in clinical subjects and in the pemoline model, and generate support for the predictive validity of the pemoline model.

The robust effect of risperidone in the pemoline model also concurs with reports that risperidone attenuates SIB in the neonatal 6-OHDA model in rats (Allen et al., 1998) and in the amphetamine model in mice (Wagner et al., 2004). Risperidone is a benzisoxazole derivative that functions as an antagonist with high affinity at 5-HT_{2A} receptors, moderate affinity at dopamine D_2 receptors, and lower affinity at α_1 and α_2 adrenoceptors (Leysen et al., 1988). Since risperidone blocks 5-HT_{2A} receptors, and is effective in these animal models and in clinical SIB, it appears that serotonergic neurotransmission may play an important role in the etiology of SIB. At the very least, therapies targeted at this system may have the potential to correct neurotransmitter disregulation that contributes to the disorder. This also concurs with the negative effects of paroxetine in the pemoline model of SIB (Turner et al., 1999), and suggests that SSRIs may be contraindicated in SIB. Since risperidone also blocks D_2 receptors, dopaminergic actions are also implicated in SIB. This suggestion is further supported by clinical observations (Turner and Lewis, 2002), by the effect of classical neuroleptics in the pemoline model (Mueller and Nyhan, 1982; King et al., 1993), and by the fact that the D_1 receptor antagonist SCH 23390 blocks SIB in the neonatal 6-OHDA model (Allen et al., 1998).

Valproate Experiment

In the valproate challenge of pemoline-induced SIB, the beneficial effects were again consistent with the effectiveness in clinical self-injury (Ruedrich et al., 1999). However, valproate was less effective than was risperidone in the dose ranges that were evaluated. In fact, the lowest dose of valproate actually seemed to slightly increase the appearance of tissue injury, and valproate did not significantly reduce the amount of oral contact at any of the doses that were tested. Reductions in the incidence of self-injury and the amount of tissue damage were most apparent when the highest dose (200 mg/kg) was used. The discrepancy between the effectiveness of valproate in reducing the incidence and expression of tissue damage and its lack of effect on duration of self-injurious oral contact suggests that valproate did not reduce the overall expression of stereotyped oral behaviors, but did reduce the severity of those behaviors.

Valproate inhibits neuronal excitability by increasing extracellular GABA concentrations (Nau and Loscher, 1982). This raises the possibility that valproate attenuates pemoline-induced SIB by inhibiting monoaminergic neurotransmission through GABAergic actions in the substantia nigra, ventral tegmental area, cortex, and /or striatum. This is consistent with a report that the GABA agonist baclofen reduced SIB in intellectually handicapped self-injurers (Primrose, 1979).

On the other hand, valproate also blocks voltage-gated Na^+ and Ca^{2+} channels (McLean and MacDonald, 1986), and N-methyl-D-aspartate (NMDA)-mediated cellular depolarizations (Zeise et al., 1991). Accordingly, although the effects of valproate suggest interesting targets for investigation of the neurobiological basis of SIB, it is not clear at this time whether it reduces self-injury through GABAergic mechanisms, cation channel actions, or even alterations in intracellular signaling.

Topiramate Experiment

The effects of topiramate on pemoline-induced SIB were also consistent with the clinical observations (Shapira et al., 2002), providing further support for the predictive validity of the pemoline model of SIB. These data are particularly interesting in relation to a study in which topiramate reduced the sizes and severity of wounds in subjects with Prader-Willi syndrome who exhibited severe skin-picking (Shapira et al., 2002). Although the measures of wound expression in this open-label study were more precise than is typical of clinical studies of SIB, the findings are complicated by the fact that topiramate has been found to promote dermal healing (Shapira et al., 2003). In the current analysis with pemoline-induced SIB, topiramate not only decreased tissue damage – it also decreased the duration of self-injurious oral contact, suggesting that the clinical report accurately revealed a decrease in actual skin-picking behavior, rather than an unrelated dermal action of the drug.

Topiramate, like valproate exerts inhibitory effects on neuronal excitability. It enhances GABAergic neurotransmission by increasing GABA-mediated Cl^- influx through $GABA_A$ receptors (White et al., 1997). It also inhibits Na^+ and/or Ca^{2+} channels (DeLorenzo et al., 2000), and it decreases kainate-evoked current through α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Gibbs et al., 2000). Accordingly, topiramate has the potential to interact with neurotransmission in multiple systems, and the specific mechanism(s) whereby it decreases SIB are unknown.

Tramadol Experiment

Although tramadol decreases compulsive behaviors in individuals with OCD and Tourette's syndrome (Goldsmith et al., 1999; Shapira et al., 1997), it did not significantly affect any

dependent measure of SIB in the pemoline model. One possible interpretation of these results is that the pemoline-induced SIB is not a compulsive behavior. This interpretation contrasts with our observations that the rats focus on specific tissue sites (Kies and Devine, 2004) and are often difficult to distract when they are engaged in self-biting behavior. However, the clinical efficacy of tramadol as an anti-compulsive agent is not yet well established since the reports are based on open label trials with small sample sizes. Moreover, it is possible that the dosage or dosing regimen (b.i.d.) of tramadol that we used in this study may not have been aggressive enough. The potential that compulsions play an important role in pemoline-induced SIB merits further evaluation.

On the other hand, the pharmacological actions of tramadol may explain the outcome of this experiment. Tramadol is an opioid receptor agonist that exhibits moderate affinity to mu receptors (Raffa et al., 1992), an action that is consistent with its efficacy in clinical trials and animal models of compulsions. However, tramadol also blocks reuptake of norepinephrine and serotonin (Raffa et al., 1992). These actions (especially blockade of serotonin reuptake may account for the lack of efficacy of tramadol in the pemoline model of SIB.

Summary and Conclusions

Although there is no universally effective pharmacological treatment for SIB, the results of the experiments with risperidone, valproate, and topiramate provide evidence of the predictive validity of the pemoline model of SIB. Thus, the pemoline model of SIB may be a valuable tool for pre-screening potential pharmacotherapeutic interventions for SIB in a controlled laboratory setting with quantifiable dependent measures. The tramadol experiment suggests that this treatment does not hold therapeutic promise. It is not clear if the failure of tramadol to diminish

pemoline-induced SIB might be due to its blockade of norepinephrine or serotonin uptake, but the results of this and other studies (Allen et al., 1998; Turner et al., 1999) suggest that drugs such as SSRIs, that block monoamine uptake should be considered with caution.

Additional examinations of the therapeutic effects of pharmacological manipulations in the pemoline model may be useful to identify neurobiological factors that contribute to the etiology and expression of SIB. Although the specific pharmacological actions that underlie the efficacy of these drug challenges are currently unknown, comparisons of drug-induced neurochemical alterations that differentiate self-injurious pemoline-treated rats from non-injurious rats that are treated with these pharmacological challenges may help to reveal important neurochemical variables that underlie SIB.

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Footnotes

Address correspondence to:
Dr. Darragh P. Devine
University of Florida
Department of Psychology
Behavioral Neuroscience Program
P. O. Box 112250
Gainesville, FL 32611-2250

e-mail dpdevine@ufl.edu

Legends for figures

Figure 1. Effects of risperidone on pemoline-induced self-injury: Risperidone dose-dependently decreased the incidence of pemoline-induced SIB (A), the time spent injuring (B), and the area of tissue damage (C). The amount of time spent injuring and the area of tissue damage across days were highly correlated (D). All values expressed are group means ± S.E.M. Significant differences between vehicle- and risperidone-treated rats are depicted as follows: * $p < 0.05$ for comparisons between risperidone at 1.0 mg/kg and vehicle; + $p < 0.05$ for comparisons between risperidone at 0.5 mg/kg and vehicle; # $p < 0.05$ for comparisons between risperidone at 0.1 mg/kg and vehicle.

Figure 2. Effects of risperidone on grooming, inactivity, and locomotion: Risperidone did not significantly affect time spent grooming (A). Risperidone-treated rats did exhibit more inactivity as compared to vehicle-treated rats (B). Risperidone-treated rats did locomote significantly more overnight as compared to vehicle-treated rats. All values expressed are group means ± S.E.M. Significant differences between vehicle- and risperidone-treated rats are depicted as follows: * $p < 0.05$ for comparisons between risperidone at 1.0 mg/kg and vehicle; # $p < 0.05$ for comparisons between risperidone at 0.1 mg/kg and vehicle.

Figure 3. Effects of valproate on pemoline-induced self-injury: The two highest doses of valproate lessened the incidence of pemoline-induced SIB (A). However, no dose of valproate tested reduced the overall percent duration of self-injurious oral contact (B). The rats treated with the lowest dose of valproate (50 mg/kg) had significantly larger areas of tissue damage; the rats treated with the highest dose of valproate (200 mg/kg) had significantly smaller areas of tissue damage (C). Despite this, the amount of time spent injuring and the area of tissue damage

across days remained correlated (D). All values expressed are group means ± S.E.M.

Significant differences between vehicle- and valproate-treated rats are depicted as follows: * p <

0.05 for comparisons between valproate at 200 mg/kg and vehicle; # p < 0.05 for comparisons

between valproate at 50 mg/kg and vehicle.

Figure 4. Effects of valproate on grooming, inactivity and locomotion: Valproate did not

significantly affect time spent grooming (A), time spent inactive (B), or the amount of

locomotion (C). All values expressed are group means ± S.E.M.

Figure 5. Effects of topiramate on pemoline-induced self-injury: All, or nearly all the rats in each

treatment group exhibited pemoline-induced SIB (A). However, topiramate lessened the time

spent injuring (B), and the area of tissue damage (C). The amount of time spent injuring and the

area of tissue damage across days were also correlated (D). All values expressed are group

means ± S.E.M. Significant differences between vehicle- and topiramate-treated rats are

depicted as follows: * p < 0.05 for comparisons between topiramate at 10 mg/kg and vehicle; + p

< 0.05 for comparisons between topiramate at 3.0 mg/kg and vehicle; # p < 0.05 for comparisons

between topiramate at 1.0 mg/kg and vehicle.

Figure 6. Effects of topiramate on grooming, inactivity and locomotion: Topiramate did not

significantly affect time spent grooming (A). Topiramate-treated rats did exhibit more inactivity

as compared to vehicle-treated rats (B). Topiramate had no significant effect on the amount of

locomotion (C). All values expressed are group means ± S.E.M. Significant differences

between vehicle- and topiramate-treated rats (LSD) are depicted as follows: * p < 0.05 for

comparisons between topiramate at 10 mg/kg and vehicle; + p < 0.05 for comparisons between

topiramate at 3.0 mg/kg and vehicle; # $p < 0.05$ for comparisons between topiramate at 1.0

mg/kg and vehicle.

Figure 7. Effects of tramadol on pemoline-induced self-injury: The percentage of rats that

exhibited injury was high in all tramadol-treated groups (A). Tramadol did not significantly

affect the amount of time spent injuring (B) or the area of tissue damage (C). The total amount

of self-injurious oral contact was positively correlated with the area of injured tissue (D). All

values expressed are group means ± S.E.M.

Figure 8. Effects of tramadol on grooming, inactivity and locomotion: (a) Tramadol did not

significantly affect (a) time spent grooming, (b) time spent inactive, or (c) the amount of

locomotion. All values expressed are group means ± S.E.M.

Table 1. Numbers of rats euthanized due to severe self-injury in each treatment group		
Treatment		**euthanized/total**
risperidone	0 mg/kg	1/8
	0.1 mg/kg	0/6
	0.5 mg/kg	0/7
	1.0 mg/kg	0/8
valproate	0 mg/kg	2/9
	50 mg/kg	2/9
	100 mg/kg	1/9
	200 mg/kg	0/9
topiramate	0 mg/kg	6/8
	1.0 mg/kg	7/9
	3.0 mg/kg	4/9
	10.0 mg/kg	5/9
tramadol	0 mg/kg	2/18
	0.1 mg/kg	1/18
	1.0 mg/kg	1/18
	10.0 mg/kg	1/18

Table 2. Aspartate aminotransferase (AST) activity in control and pemoline-treated rats

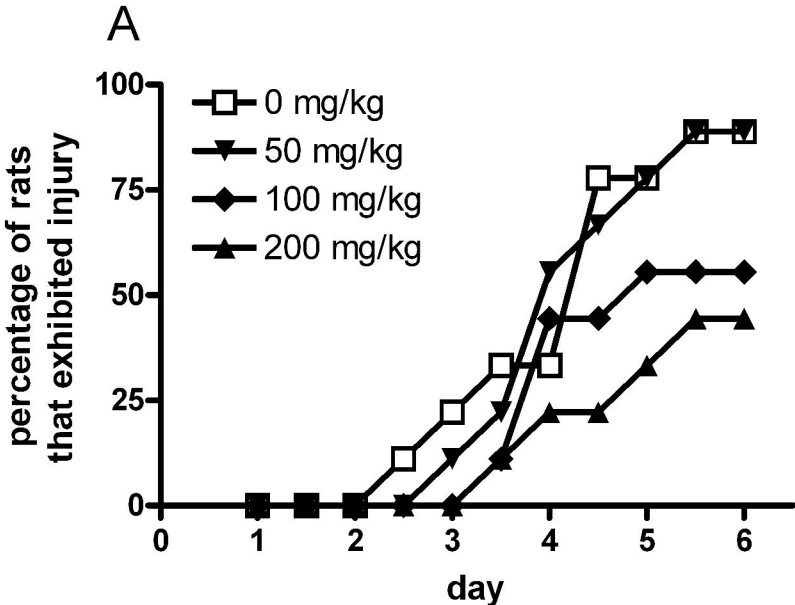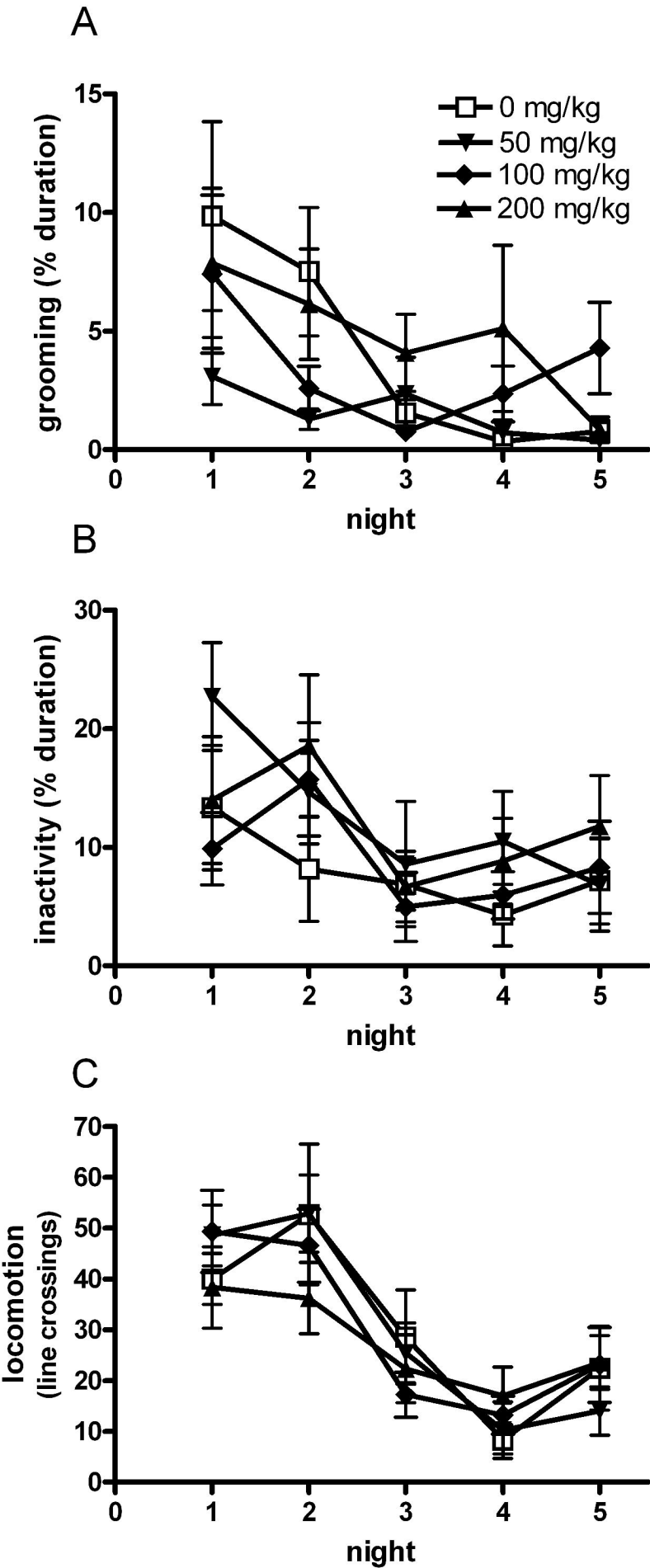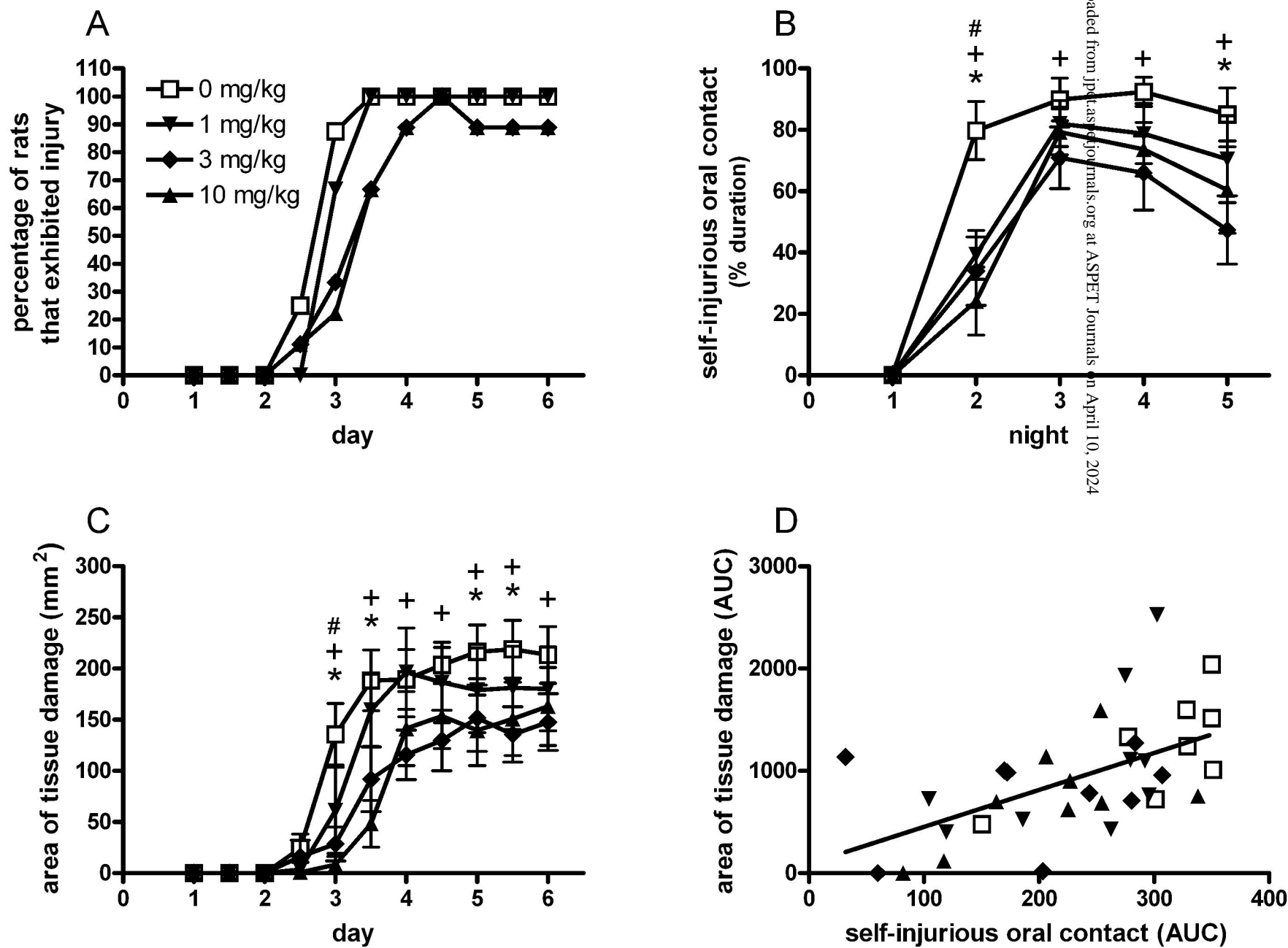| Treatment	AST (units/liter ± SEM)
no treatment	268.9 ± 21.37
0 mg/kg pemoline	193.6 ± 14.16
100 mg/kg pemoline	237.5 ± 15.59
200 mg/kg pemoline	293.7 ± 12.88
300 mg/kg pemoline	252.4 ± 10.31

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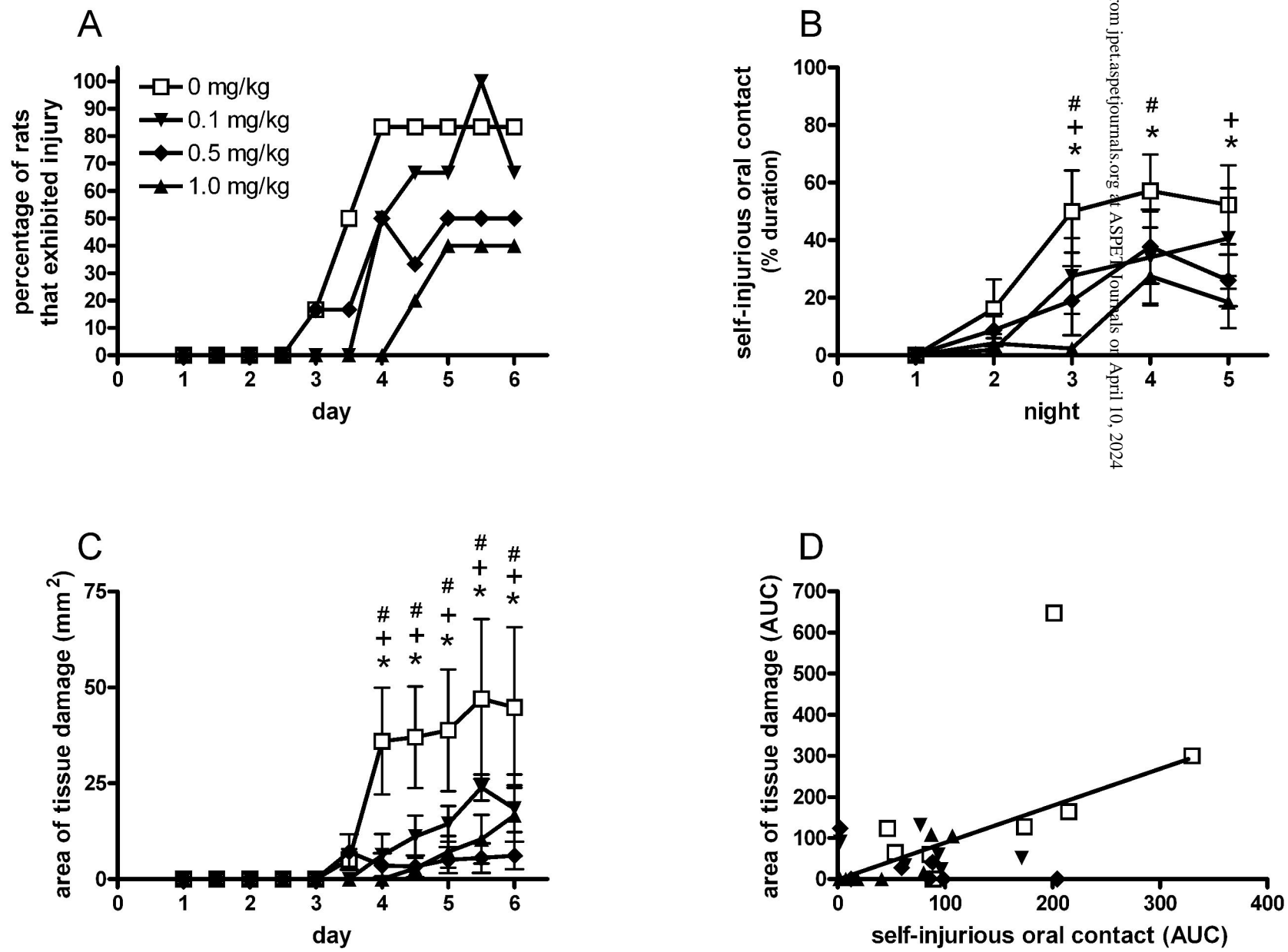
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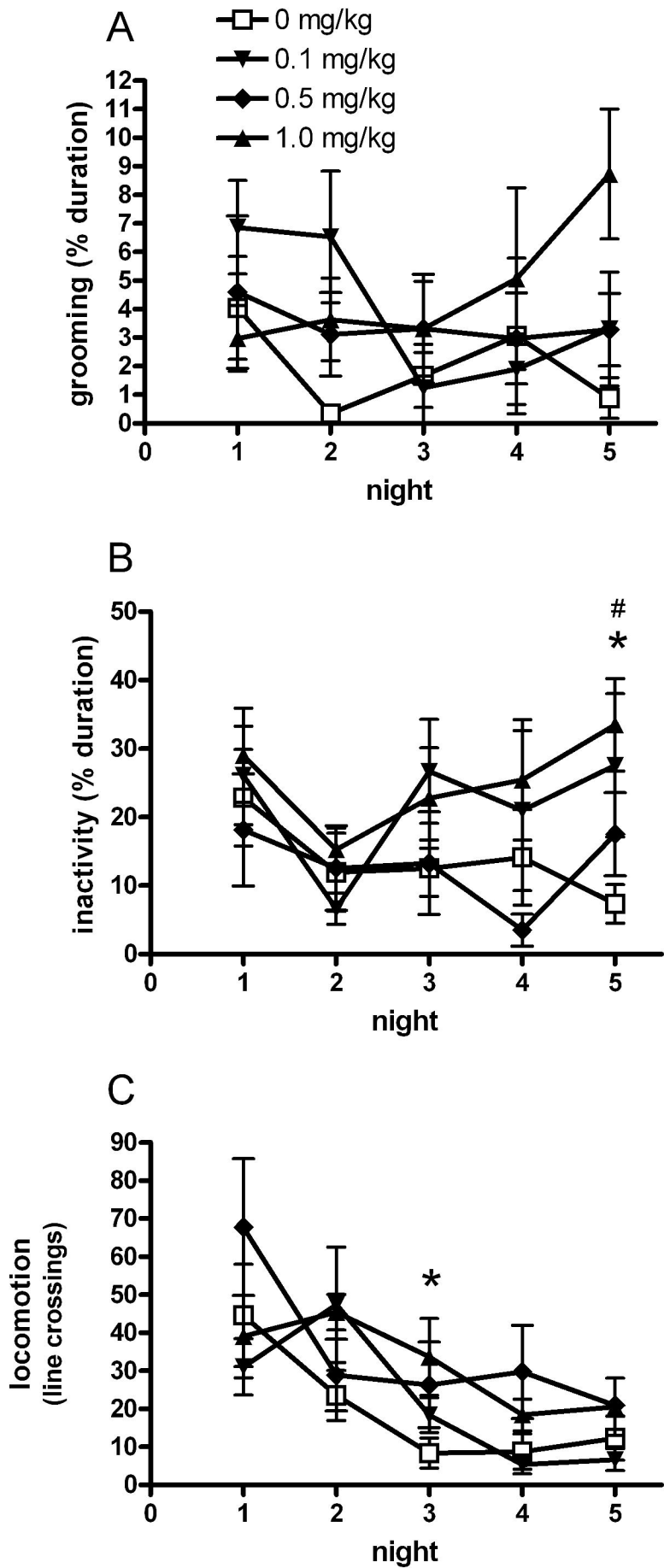
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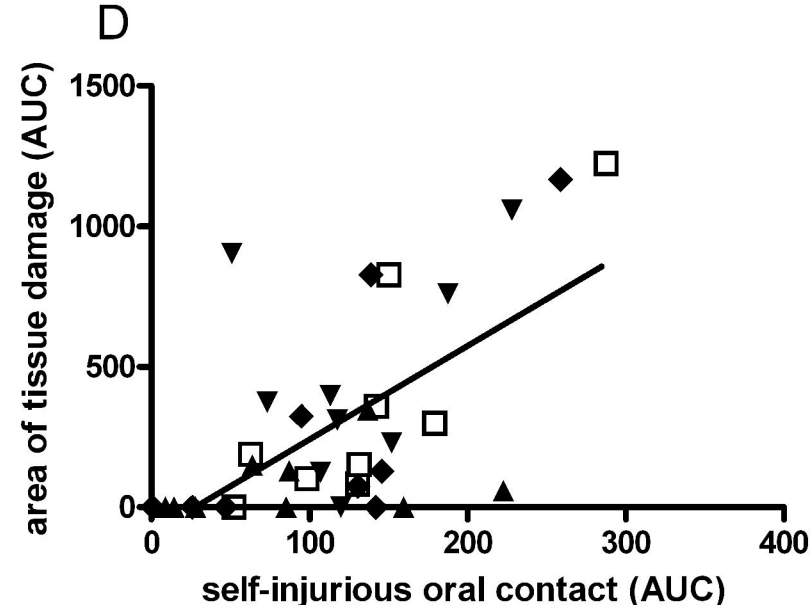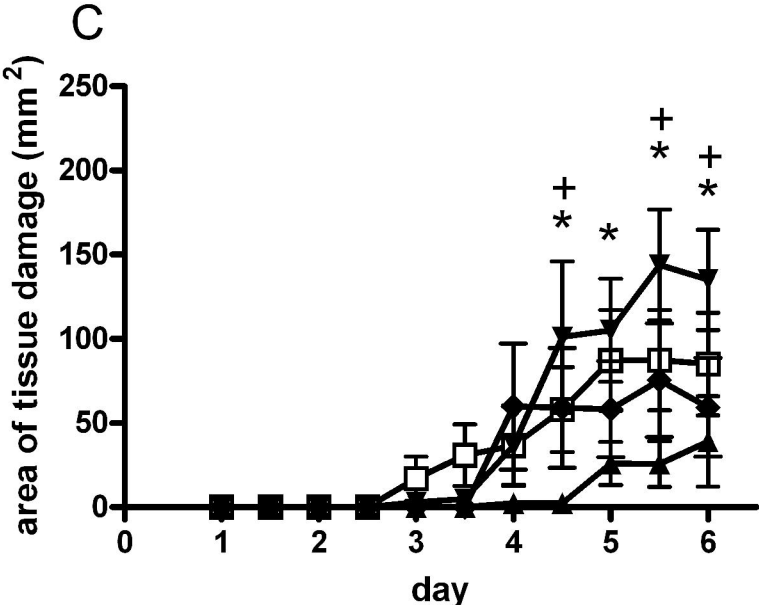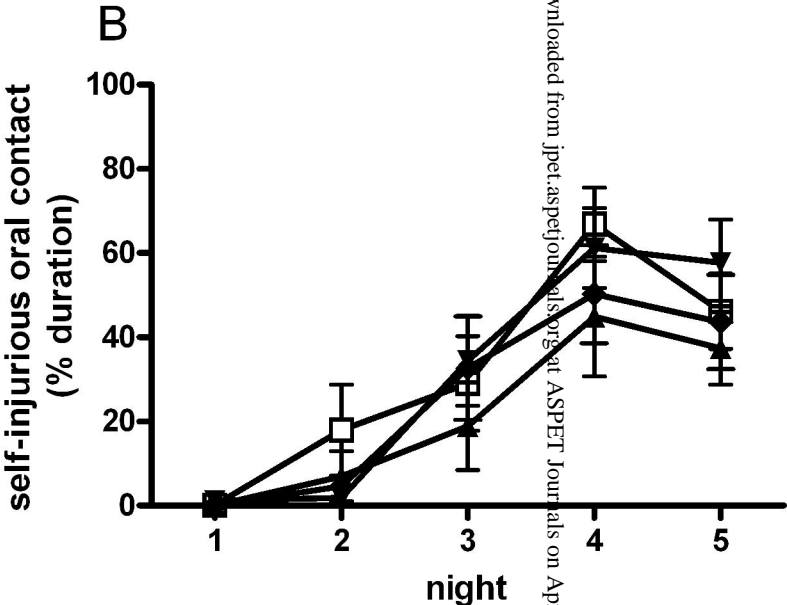
Figure 4

Figure 5

Figure 6

Figure 7

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