The role of anxiety in vulnerability for self-injurious behaviour: studies in a rodent model

X. Yuan and D. P. Devine

University of Florida, Department of Psychology, Behavioral and Cognitive Neuroscience

Program, Gainesville, FL 32611-2250, USA

Corresponding Author:

Darragh P. Devine, Ph.D.
University of Florida
Department of Psychology
945 Center Drive
Gainesville, FL 32611-2250
Tel: (352) 273-2174
Fax: (352) 392-7985
e-mail: dpdevine@ufl.edu

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Abstract

Self-injurious behaviour (SIB) is a debilitating characteristic that is highly prevalent in autism and other neurodevelopmental disorders. Pathological anxiety is also common, and there are reports of comorbid anxiety and self-injury in some children. We have investigated potential interactions between anxiety and self-injury, using a rat model of pemoline-induced self-biting. In one experiment, rats were pre-screened for trait anxiety by measuring expression of anxiety-related behaviour on the elevated plus maze and open field emergence test. The rats were then treated with pemoline once daily for ten days, and vulnerability for pemoline-induced self-injury was evaluated. This revealed modest correlations between innate levels of anxiety-related behaviour in the open field test (time in the start box, and latency to enter the open field), and vulnerability for pemoline-induced self-biting (total duration of self-injurious oral contact, and total size of tissue injury). Measures in the elevated plus maze were not significantly correlated with vulnerability for pemoline-induced self-injury. In a second experiment, rats were treated with the beta-carboline FG 7142 twice daily, during 5 days of treatment with pemoline. The rats that were treated with this anxiogenic drug exhibited greater duration of self-injurious oral contact, and larger injuries than vehicle-treated controls did. Overall, these results suggest that anxiety may contribute to the etiology and/or expression of self-injurious behaviour, and indicate that further research is warranted.

1. Introduction

Self-injurious behaviour (SIB) is a clinically challenging problem in which an individual inflicts deliberate, and sometimes even severe self-harm. This irrational behaviour is difficult to comprehend, and its developmental origins are elusive [1]. Nevertheless, SIB is prevalent among people with a variety of neurodevelopmental disorders including autism spectrum disorder (ASD) [2], Fragile X syndrome [2,3], Lesch-Nyhan syndrome [4,5], Prader-Willi syndrome [6], and others [7].

One interesting lead in the quest to understand SIB is that pathological anxiety is also common in neurodevelopmental disorders where SIB is prevalent [8-14]. For example, Sukhodolsky and colleagues [15] reported that 43% of children with pervasive developmental disorders (PDD) meet clinical criteria for at least one anxiety disorder. Other reports have estimated that the prevalence of anxiety disorders is as high as 55-84% of children in this population [16,17], and there is evidence that pathological anxiety is associated with greater expression of disruptive, aggressive, and oppositional behaviours [18]. Significant correlations have also been reported between the incidence of anxiety disorders and the presence of SIB among children with ASD [19] and Fragile X syndrome [3]. However, these studies do not identify whether anxiety disorders might contribute to the etiology of self-injury, or if they might occur as a secondary consequence (e.g. due to the child's apprehension regarding self-inflicted injury), or if they might even just co-vary with SIB under the influence of other factors, such as level of cognitive function or expressive language skills.

Clinical trials with buspirone (an azapirone drug with anxiolytic effects) have provided evidence supporting a more direct interaction between anxiety and SIB. These trials concluded that treatment with buspirone attenuated self-injury in adults with intellectual handicaps [20], or ASD [21]. However, these open label and uncontrolled trials should be interpreted with caution.

Some recent studies in animal models lend additional support to the idea that anxiety and self-injury are associated. For example, high levels of innate anxiety-related behaviours have been found in SAPAP3 [22], Slitrk5 [23], and Shank3 [24] mutant mice, all of which exhibit stereotypic over-grooming that results in tissue injury. Moreover, SIB appears to be common in captive animals outside of laboratory settings. Domestic dogs and cats with separation anxiety syndrome typically exhibit self-injurious denuding of the fur and mutilation of the skin [25,26], and exotic birds pluck out their own feathers when emotionally distressed [27]. Furthermore, buspirone and fluoxetine (an antidepressant drug with anxiolytic actions) each attenuated SIB in non-human primates [28,29]. Overall, these observations suggest that SIB is a response that can occur in captive animals under conditions of emotional distress, and pharmacological intervention with anxiolytic drugs may be beneficial.

In a rodent model of SIB, administration of pemoline produces self-injurious gnawing and biting of the limbs and ventrum in rats and mice [30]. These effects are observed after a single administration of a high dose, or after repeated administration of lower doses [31-34]. This model has been used to identify neurobiological mechanisms that underlie expression of SIB [35-39], and factors that can exacerbate the ability of pemoline to induce SIB [39,40]. The model has also been used to evaluate potential pharmacotherapy for SIB [31,41-43]. In addition, we have used the pemoline model to tease apart individual differences in vulnerability for pemoline-induced SIB. In these studies, we found that behavioural responsiveness to mild stress is positively correlated with vulnerability for pemoline-induced SIB [44], and that prior exposure to chronic emotional stress increases severity of tissue injury when the rats are subsequently treated with pemoline [39].

In the studies reported herein, we examined the potential role of anxiety as a factor that can exacerbate pemoline-induced SIB. In the first experiment, we prescreened outbred rats using the elevated plus maze (EPM) and open field emergence test (OFET) prior to treatment with pemoline. We then assayed associations between innate expression of anxiety-related behaviours, and vulnerability for pemoline-induced SIB. In the second experiment, we repeatedly administered the anxiogenic drug FG 7142 [45] during pemoline treatment, and evaluated the impact on SIB.

2. Materials and Methods

2.1. Animals

Fifty one male Wistar rats weighing 176~200g were purchased from Charles River Laboratories (Raleigh, NC). Fifteen rats were used in Experiment 1, and 36 rats were used in Experiment 2. All the rats were initially paired-housed in standard polycarbonate cages (43 cm x 21.5 cm x 25.5 cm) with a 12 hr: 12 hr light/dark schedule (lights on at 7:00 a.m.). Standard rat chow (Lab Diet 5001) and tap water were available *ad libitum*, and the room temperature and humidity were controlled. Prior to the start of behavioural testing, the rats were allowed to acclimate to the housing conditions for one week. Then, after the behavioural prescreening was completed (see below), the rats were transferred to individual housing in the standard polycarbonate cages. All the experimental procedures were pre-approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Pemoline an (indirect monoamine agonist) [46,47] was purchased from Spectrum Chemicals (New Brunswick, New Jersey), and was suspended in peanut oil at 50 mg/ml. FG 7142 (a beta-carboline, partial inverse agonist at the benzodiazepine allosteric site of the GABA-A receptor)

[45] was purchased from Sigma-Aldrich Inc. (St. Louis, MO), and was suspended in 1.0% (w/v) carboxymethylcellulose (CMC) at concentrations of 0, 1, 3, or 10 mg/ml.

2.3. Experiment 1

Each rat was prescreened sequentially on the EPM and OFET. Since we have already identified that individual differences in innate stress responsiveness predict vulnerability for pemoline-induced SIB [44], we also prescreened the rats by exposing them to the mild stress of a novel environment in the circular corridor test (CCT) [48]. In order to avoid any influence of the CCT on anxiety-related behaviours in the EPM and OFET, stress responsiveness in the CCT was assayed last. These three behavioural tests were all conducted between 8:00 a.m. and 1:00 p.m., with 48 hrs between tests.

2.3.1. Elevated Plus Maze (EPM)

Individual differences in anxiety-related behaviour were assayed using the EPM according to methods we have previously described [49]. The EPM was constructed of black acrylic with four 45 cm x 12 cm arms and a 12 cm x 12 cm central platform. The opposing closed arms were surrounded by 45 cm high walls, and the open arms had 0.6 cm high ledges. The maze was elevated 90 cm above the floor, and illuminated by a light bulb above the central platform. The light intensity was approximately 46-60 lux in the open arms, and 19-30 lux in the closed arms. A video camera suspended above the maze was used to record each rat's behaviour during the test.

At the beginning of the 5-min test, each rat was individually placed on the central platform facing one of the closed arms. Then it was allowed to freely explore the maze. Latency to enter an open arm, and time spent in the open and closed arms was scored by a trained experimenter using "Noldus Observer" software (Noldus Information Technology). Crossings into the open

arms were only counted when the rat crossed the threshold with all four paws. Accordingly, time spent on the central platform, or hesitating to enter an open arm is included in the scores for time in closed arms.

2.3.2. Open Field Emergence Test (OFET)

Individual differences in anxiety-related behaviour were also screened using the OFET as we have previously described [49]. The OFET consisted of a 90 cm x 90 cm black acrylic arena connected to a 20 cm x 30 cm start box. The open arena and start box had 60 cm high walls and open tops, and were separated by a 20 cm x 20 cm acrylic door. The light intensity was approximately 15 lux in the start box and 30 lux in the open arena. A video camera suspended above the apparatus was used to record each rat's behaviour during the test.

At the beginning of the test, each rat was individually placed into the start box. After 60 sec, the door was opened remotely, and it was left open for the remainder of the session. Thus, the rat was allowed to freely explore the open arena and the start box for five min. Latency to enter the open arena, and time spent in the open arena / start box was scored by a trained experimenter using the Observer software. Crossings from the start box into or out of the open arena was only counted when the rat crossed the threshold with all four paws.

2.3.3. Circular Corridor Test (CCT)

The circular corridor was constructed by placing a small plastic cylinder inside a larger plastic cylinder, forming a 7 cm wide corridor with an outer diameter of 44 cm. The light intensity was approximately 10 lux throughout the circular corridor. A video camera suspended above the apparatus was used to record each rat's behaviour during the test.

At the beginning of the test, each rat was individually placed into the circular corridor and the rat was allowed to freely explore it for 60 min. The video image of the session was divided into four equal quadrants, and line crossings were counted each time the rat's snout crossed into a new quadrant. Crossing a boundary between any two quadrants was not counted again until the rat crossed into a different quadrant, thereby assuring that each line crossing counted only when at least a full quarter of the circular corridor was traversed.

2.3.4. Pemoline Treatment

The rats were assigned to vehicle or pemoline treatment groups (0 or 75 mg/kg/day, s.c.) in a counterbalanced manner according to the line crossing scores in the CCT, thereby distributing those that may be more or less vulnerable for pemoline-induced SIB in equal proportions across the groups [44]. Then, starting on the seventh to ninth day after the CCT, each rat was weighed, inspected for injuries (see below), and injected with vehicle or pemoline once daily on each of 10 consecutive days (between 9:00 and 10:00 a.m.).

2.3.5. Assays of Self-Injury and other Behaviours

At 9:00 a.m. and 5:00 p.m. of each day of the vehicle or pemoline treatments, each rat was visually inspected for physical evidence of injury. All inspections were recorded with digital cameras, and images were captured of each rat's head, forepaws, hindpaws, ventrum, and tail. In addition, four 5-minute time samples of rats' behaviours were recorded in their home cages each day during the treatments (6:00 a.m., 12:00 p.m., 6:00 p.m., and 12:00 a.m.).

The duration of self-injurious oral contact (defined as oral contact with the torso or limbs that stayed fixed at any body site for 2 seconds or longer) was quantified from the 5-min time sample recordings using the Observer software. Sizes of injuries (cm²) were measured from the digital images with MCID image analysis software (Imaging Research Inc.). Stereotypy, grooming,

and rearing were also quantified from the time sample recordings using the Observer software. Stereotypy was defined as episodes of cage licking, head bobbing and/or digging/burrowing/sniffing through the bedding that continued for more than 3 sec. Since the expression of the specific forms of stereotypy was different for each individual rat, the stereotypy scores are reported as aggregate scores that compile the total duration of all these stereotyped behaviours. Grooming was defined as oral contact along the torso or limbs, staying fixed for less than 2 seconds. Rearing was defined as whenever a rat raised both of its forepaws off the bedding for at least 3 seconds. All the behavioural measures and the evaluations of injury size were performed by a trained experimenter who was blind to the treatment conditions.

In all our experiments on pemoline-induced SIB, any rat that has an open lesion is immediately euthanized. No rats were euthanized early in this experiment, using this relatively low dose of pemoline. All the rats were terminated 24 hours after the last vehicle or pemoline injection.

2.3.6. Statistical Analyses

In order to evaluate the counterbalanced assignment of the rats to the vehicle and pemoline treatment groups, potential between-groups differences in each of the prescreened behavioural variables (latency to an open arm and time in the closed arms in the EPM; latency to the open arena and time in the start box in the OFET, and line crossings in the CCT) were each analyzed with T-tests. In addition, correlations between latency to enter an open arm (EPM), and latency to enter the open arena were evaluated using Pearson's correlation. Correlations between time in the closed arms (EPM), and time in the start box were also evaluated using Pearson's correlation.

The percentage of rats that self-injured during vehicle/pemoline treatment (defined as the presence of denuded skin, erythema, edema, or open lesion) was plotted across the days of the experiment for the vehicle and pemoline-treated groups. Between-groups differences in total duration of self-injurious oral contact, and in average daily measures of tissue injury size were each analyzed with repeated measures analyses of variance (RM-ANOVA). In addition, between-groups differences in stereotypy, grooming, and rearing were each analyzed by RM-ANOVA. All significant between-groups differences ($p < 0.05$) were further analyzed with t-tests.

The average duration of oral contact, stereotypy, grooming, and rearing were each calculated from all 40 time samples for each rat. The average injury size was calculated from the 21 inspections. Pearson's correlations were calculated between the average duration of oral contact and each of the measures of anxiety-related behaviour (latency to enter the open arms and time spent in the closed arms of the EPM, and latency to enter the open arena and time spent in the start box of the OFET). In addition, Pearson correlations were calculated between the average injury sizes and each of the measures of anxiety-related behaviour.

2.4. Experiment 2

2.4.1. Open Field Emergence Test (OFET)

The rats were acclimated to the housing conditions, as in Experiment 1. Then, as the results of Experiment 1 revealed a correlation between anxiety-related behaviour in the OFET and vulnerability for pemoline-induced SIB, each rat was prescreened in the OFET according to the methods described for Experiment 1.

2.4.2. Drug Treatment

The rats were assigned to dose groups of FG 7142 (0, 1, 3, 10 mg/kg/b.i.d., i.p.) in a counterbalanced manner according to their performance in the OFET (time spent in the start box). The doses of FG7142 were selected on the basis of our previous research, in which we reported that 10 mg/kg FG7142 increased latency to enter and reduced time spent in the open arena of the OFET [49]. Since we were interested in subtle individual differences in anxiety states, we included lower doses in addition to the 10 mg/kg dose.

Each rat was weighed and injected with FG 7142 twice per day at approximately 8:00 a.m. and 5:00 p.m. on each of 5 consecutive days. Immediately after the morning administration of FG 7142 each day, pemoline (75 mg/kg/day, s.c.) was administered to each rat.

2.4.3. Assays of Self-Injury and other Behaviours

Each rat was visually inspected twice daily. All afternoon inspections were recorded, images were captured of each rat, and four 5-minute time samples of rats' behaviours were recorded, as in Experiment 1. The percentage of rats with injury, injury sizes, stereotypy, grooming, and rearing were each quantified as in Experiment 1. In this experiment, duration of self-injurious oral contact was scored whenever oral contact remained fixed on a body site for 5 sec or longer, and grooming was scored when oral contact remained fixed for less than 5 sec. As in Experiment 1, all the behavioural measures and the evaluations of injury size were performed by a trained experimenter who was blind to the treatment conditions.

Any rat with an open lesion was immediately euthanized. Accordingly, 1 rat treated with pemoline plus vehicle, 3 rats treated with pemoline plus the low dose of FG 7142, 4 rats treated with pemoline plus the medium dose of FG 7142, and 1 rat treated with pemoline plus the high dose of FG 7142 were euthanized before the end of the treatment. In these cases, the missing data were replaced by repeating the final score that was attained for each dependent measure

through to the end of the experiment. This was done to prevent the possibility that the data for the most severe self-injurers would be selectively affected by attrition. The remaining rats were euthanized 24 hours after the last pemoline injection.

2.4.4. Statistical Analyses

Between-groups differences in each of the measures of self-injury, grooming, stereotypy, and rearing were analyzed using RM-ANOVAs. Significant effects ($p < 0.05$) were further analyzed with t-tests comparing scores for each FG 7142 dose group with the corresponding scores for the vehicle-treated group.

3. Results

3.1. Experiment 1

The counterbalanced assignment of the rats to the vehicle and pemoline treatment groups resulted in groups that did not differ in any prescreened measure of anxiety-related behaviour (EPM or OFET) or stress responsiveness (CCT: see Table 1). This helps to assure that the rats with higher or lower vulnerability for pemoline-induced SIB were equally distributed between the groups.

The pemoline treatment regimen produced self-inflicted tissue injury in 56% of the rats by the last day of the experiment (Fig 1A), and as expected, there were no injuries in the vehicle-treated controls (Fig. 1A and 1C). The 2-way RM-ANOVA of the duration of oral contact (Fig. 1B) revealed a significant main effect of group ($F_{(1, 13)} = 5.298$, $p < 0.05$), and analyses of the injury size data (Fig. 1C) revealed a significant group x time interaction ($F_{(10, 130)} = 2.317$, $p < 0.05$). There were also significant between-groups differences in the expression of stereotypy and rearing during the pemoline treatment. The 2-way RM-ANOVA for duration of stereotypy (Fig. 2A) revealed significant main effects of group ($F_{(1, 13)} = 30.54$, $p < 0.01$), time ($F_{(9, 117)} =$

5.126, $p < 0.01$), and a group x time interaction ($F_{(9, 117)} = 3.319$, $p < 0.01$). The 2-way RM-ANOVA for duration of rearing (Fig. 2C) revealed significant main effects of time ($F_{(9, 117)} = 2.706$, $p < 0.01$), and a group x time interaction ($F_{(9, 117)} = 2.184$, $p < 0.05$). There were no significant differences in the expression of grooming behaviours between the vehicle and pemoline-treated groups (Fig. 2B).

Correlations between self-injury measures (average oral contact duration and average injury size) with the prescreened measures of anxiety-related behaviour are summarized for the pemoline-treated rats in Table 2. The OFET reliably predicted individual differences in vulnerability for pemoline-induced SIB (duration of self-injurious oral contact and tissue injury size), whereas the behavioural measures on the elevated plus maze did not correlate with individual differences in vulnerability for pemoline-induced SIB. In addition, the correlations between the duration of grooming, stereotypy and rearing, and the measures of anxiety-related behaviour are summarized for the pemoline-treated rats in Table 2. There were no significant correlations between the prescreened measures of anxiety-related behaviour and the measures of stereotypy, grooming, or rearing.

Since scores on the OFET reliably predicted individual differences in vulnerability for pemoline-induced SIB, whereas scores on the EPM did not, we evaluated the correlations between the scores in these two tests. Latency to enter the open arms (EPM) and latency to enter the open arena (OFET) were not significantly correlated ($r = 0.2260$, $p = 0.2072$). Similarly, time spent in the closed arms (EPM) and time spent in the start box were not significantly correlated ($r = 0.1664$, $p = 0.2767$).

3.1.2. Experiment 2

In Experiment 2, we prescreened the rats using the OFET, because the results of Experiment 1 revealed that prescreening on the OFET predicted individual differences in vulnerability for pemoline-induced SIB. Accordingly, we used the total time in the start box to counterbalance the assignment of the rats to the groups that were treated with the vehicle and the three doses of FG 7142. The counterbalancing resulted in groups that did not differ in either of the prescreened measures of anxiety-related behaviour (time in start box and latency to open arena) in the OFET (see Table 3).

During the middle days of the experiment, a greater percentage of the rats that were treated with combined pemoline and FG 7142 exhibited tissue injury, in comparison with those that were treated only with pemoline. However, on the last day of the experiment, all the rats in all the groups had some sign of injury (Fig. 3A). Nevertheless, the duration of self-injurious oral contact, and the sizes of injury differed significantly between the groups. The 2-way RM-ANOVA of the duration of oral contact (Fig. 3B) revealed a significant main effect of group ($F_{(3, 32)} = 3.265$, $p < 0.05$), and a significant main effect of time ($F_{(4, 128)} = 20.42$, $p < 0.01$). Analyses of the injury size data (Fig. 3C) revealed a significant main effect of time ($F_{(5, 160)} = 18.23$, $p < 0.01$), and the group x time interaction approached significance ($F_{(15, 160)} = 1.70$, $p = 0.06$).

There were also significant between-groups differences in the expression of stereotypy during the treatments with pemoline and the doses of FG 7142. The 2-way RM-ANOVA for duration of stereotypy (Fig. 4A) revealed significant main effects of group ($F_{(3, 32)} = 3.273$, $p < 0.05$) and time ($F_{(4, 128)} = 19.10$, $p < 0.01$). The 2-way RM-ANOVAs for duration of grooming and rearing (Fig. 4B and 4C) revealed only significant main effects of time ($F_{(4, 128)} = 4.008$, $p < 0.01$ for grooming), ($F_{(4, 128)} = 31.10$, $p < 0.01$ for rearing). On these measures, there were no significant group differences, and no group x time interactions.

4. Discussion

In the first of the two experiments reported herein, outbred male Wistar rats were prescreened on two neophobic tests of anxiety-related behaviours. The rats were acclimated to the housing conditions, housing and testing were in adjacent spaces, and care was exercised to avoid additional stress exposure prior to testing. Thus we sought to measure innate (or trait) levels of anxiety-related behaviours (see reference [50]), and to evaluate the potential relationship between trait anxiety and SIB. Furthermore, we used a somewhat low dose of pemoline (75 mg/kg) that was sufficient to induce significant levels of self-biting in only a subset of the rats, revealing those that are more vulnerable, and those that are more resistant. Accordingly, only 56% of the pemoline-treated rats exhibited tissue injury, and the injury was not severe in any of the rats in Experiment 1. We have previously found that low-dose regimens are well-suited to identify subtle individual differences in vulnerability [39,44].

In this experiment, the rats exhibited individual differences in the measures of anxiety-related behaviours in both the EPM and OFET tests. This was especially true in the case of the OFET, and expression of anxiety-related behaviours in the OFET proved to be a modest predictor of vulnerability for pemoline-induced SIB. On the other hand, individual differences in anxiety-related behaviours in the EPM did not correlate with the rats' vulnerability for pemoline-induced SIB. The reason for the discrepancy between the results using the OFET and EPM is not clear, but the lack of significant correlations between measures on the EPM and suggest that there are real differences between these tests. One possibility is that the two tests may assess different aspects of emotionality [51], which could differ in their relevance for etiology of SIB. However, both tests are thought to assess approach/avoidance toward novel stimuli [52]. A more likely possibility is based upon differences in the procedures of the two tests. In the EPM test, the rat is placed directly onto the apparatus, and immediately makes a choice between entering an open arm, or retreating into a closed arm. Thus, the handling of the animal is

contiguous with the behavioural demands of the test, which could potentially affect the outcomes. The OFET we employed is a modification of the emergence test that was first reported by Paré and colleagues [53]. In our version, the rat is placed into a start box, and there is a delay prior to opening of the gate, allowing the behavioral task (latency to enter the open arena) to be uncoupled from the handling [49]. Furthermore, by leaving the relatively small and secure start box available throughout the testing session, we have added a highly reliable measure to the OFET, and we have found that overall, the OFET is somewhat more sensitive than the EPM to the effects of a variety of anxiety-modulating drugs [49].

Nevertheless, the ability of innate anxiety-related behaviour to predict SIB was not very powerful in the present experiment. This may be because the experiment was conducted with outbred Wistar rats. These rats exhibit individual differences in anxiety-related behavior, but there is no reason to suspect that they experience the pathological levels of anxiety that are found in some children with autism and other neurodevelopmental disorders [3,8-19]. It remains possible that more extreme basal levels of anxiety could have a more robust influence on the outcomes. In fact, we have previously reported that a prior history of chronic exposure to emotional stress increases rats' vulnerability for pemoline-induced SIB [39]. Of course, emotional stress and anxiety are distinct phenomena, and we did not specifically measure anxiety-related behaviour in our stress study. However, exposure to emotionally stressful stimuli can increase the expression of anxiety-related behaviours in animal models (for review see reference [54]).

The findings of Experiment 1 are also compatible with a previous report, which indicated that diazepam reduced self-biting during oral administration of a single dose of pemoline, but that the effects were inconsistent [31]. The small role of individual differences in innate levels of anxiety (Experiment 1), and the small effects of diazepam [31] both suggest that trait anxiety in the absence of pathological conditions makes only a small contribution to the etiology of SIB.

In Experiment 1, pemoline treatment also elevated the expression of stereotypy and rearing. These findings are in accord with the stimulant action of pemoline [55]. Interestingly, the elevated stereotypy and rearing decreased over days, suggesting either that the rats habituated to the stimulant actions, or that the behavioural repertoire shifted and became more focused on SIB, effectively replacing the other motor activities. It is also interesting to note that the vehicle and pemoline-treated groups did not differ in the amount of grooming during the experiment, and there were no apparent alterations in grooming behaviour as the pemoline-treated rats gradually exhibited more SIB. Overall, the lack of correlation between any of these behaviours and the measures in the OFT and EPM suggests that stereotypy, grooming, and rearing were not associated with the individual differences in trait anxiety.

The modest relationship between individual differences in innate levels of anxiety-related behaviours and vulnerability for pemoline-induced SIB led us to conduct the second experiment, in which we pharmacologically elevated the anxiety state in independent groups of rats to levels that could be considered pathological. In this experiment, the rats were prescreened for anxiety-related behaviour in the OFET, and group assignments were balanced so that the groups did not differ in basal expression of these behavioural characteristics. Then FG 7142 or vehicle was co-administered twice daily during the pemoline treatment regimen. FG 7142 increases the expression of anxiety-related behaviors in a variety of behavioral tests [45,56-59], and induces *c-fos* expression in brain regions that are implicated in anxiety. This includes prefrontal cortex, amygdala, and striatum [60,61]. Accordingly, the elevated expression of SIB in the groups that were treated with pemoline plus FG 7142 support the hypothesis that pathological levels of anxiety do promote vulnerability for SIB in the pemoline model.

One obvious caveat is that the effects of FG 7142 were not dose-orderly. This could indicate

that there was heterogeneity in the level of response of individual rats to the drug. In fact, a previous study indicated that FG 7142 increased self-biting in rhesus macaques that had a prior history of spontaneous SIB, but that the effect was limited to a subset of the self-injurious monkeys. This led to the conclusion that SIB was related to anxiety in some, but not all the monkeys [62]. Another explanation is that there was a ceiling effect in the current study. The lowest dose of FG 7142 produced a robust enhancement of the pemoline-induced SIB in the rats, and therefore, higher doses did not add significantly to that effect.

Another issue is that 100 percent of the rats in the control group exhibited some amount of tissue injury during treatment with pemoline at 75 mg/kg/day. This contrasts with the 56 percent that had tissue injury in Experiment 1, when treated with the same dose of drug. The only difference between these groups is that the control group in Experiment 2 had additional injections of CMC vehicle, twice daily. The addition of this stressor could explain the effect. However, the amount of tissue injury in this control group was minor, and the measures of oral contact and average injury size were similar in the two experiments.

In Experiment 2, there were significant between-groups differences in stereotypy, but these differences did not appear to reveal any systematic effects of FG 7142, and the post tests revealed only sporadic differences. There were no significant impacts of the addition of FG 7142 to the pemoline regimen in the expression of grooming or rearing behaviours, confirming the lack of association between anxiety and the expression of these behaviours during pemoline treatment.

In summary, the findings that are reported herein suggest that normal individual differences in trait anxiety may play a small role in vulnerability for SIB in our animal model, and that severe anxiety significantly enhances vulnerability for SIB when a threshold dose of pemoline is

administered.

The clinical implications should be interpreted cautiously, and in relation to known characteristics of children with autism and other neurodevelopmental disorders. These children exhibit heterogeneous levels and types of anxiety. The problem can range from moderate anxiety that interferes with daily activities, to severity that falls in the range of clinical pathology and reaches diagnostic criteria for generalized anxiety, panic, phobia, separation anxiety, and/or social anxiety disorders [8,10,15,18]. Attempts have been made to identify animal models of anxiety that relate more or less to specific forms, such as generalized anxiety, social anxiety, and phobias [63-66]. However, the manipulations reported herein do not discriminate between specific forms of anxiety. Further research is needed to provide a more detailed characterization of the potential contribution of anxiety to SIB. This endeavor may be aided by the use of inbred animal models of pathological trait anxiety, such as the BALB/C mouse [50], by animal models that may relate more specifically to particular forms of anxiety [63-66], and of course by controlled clinical trials of anxiolytic pharmacotherapy in children at risk for SIB.

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Figure Captions

Figure 1. In Experiment 1, treatment with pemoline produced self-injury that was dose-orderly in terms of A) the percentage of rats that incurred tissue injury, B) the duration of self-injurious oral contact, and C) the sizes of injuries on the forepaws and ventrum. Significant differences between groups are depicted as follows: * $p < 0.05$ for comparisons between the pemoline-treated and vehicle-treated rats. Values expressed are group means $\pm$ SEM.

Figure 2. In Experiment 1, treatment with pemoline produced significantly elevated duration of A) stereotypy, and C) rearing, but did not produce significant between-groups differences in duration of grooming. Significant differences between groups are depicted as follows: * $p < 0.05$, ** $p < 0.01$ for comparisons between the pemoline-treated and vehicle-treated rats. Values expressed are group means $\pm$ SEM.

Figure 3. In Experiment 2, FG 7142-produced elevations in the A) percentage of rats with tissue injury, B) duration of oral contact, and C) injury size. Significant differences between the groups treated with the different FG 7142 doses are presented as follows: * $p < 0.05$ for comparisons between the rats treated with FG 7142 at 1 mg/kg/day and the rats treated with the vehicle, # $p < 0.05$ for comparisons between the rats treated with FG 7142 at 3 mg/kg/day and the rats treated with the vehicle, and + $p < 0.05$ for comparisons between the rats treated with FG 7142 at 10 mg/kg/day and the rats treated with the vehicle.

Figure 4. In Experiment 2, the low dose of FG 7142-produced a transient suppression of A) stereotypy. There was no effect on B) grooming and C) rearing. The significant differences are presented as follows: * $p < 0.05$ for comparisons between the rats treated with FG 7142 at 1

mg/kg/day and the rats treated with the vehicle, and $\dagger p < 0.05$ for comparisons between the rats treated with FG 7142 at 10 mg/kg/day and the rats treated with the vehicle

Figure 1

Figure 2

A

B

C

Figure 3

Figure 4

Table 1. Counterbalancing of the group assignments resulted in groups that were equivalent in all the prescreened behavioural measures.

behavioural test	dependent measure	0 mg/kg (mean $\pm$ SEM)	75 mg/kg (mean $\pm$ SEM)	t (df)	p
EPM	latency to open arm (sec)	15.39 $\pm$ 4.77	47.87 $\pm$ 28.54	0.8652 (14)	> 0.05
	time in closed arms (sec)	212.65 $\pm$ 22,41	227.66 $\pm$ 12.66	0.6335 (14)	> 0.05
OFET	latency to open arena (sec)	149.94 $\pm$ 41.81	100.98 $\pm$ 39.25	0.1985 (14)	> 0.05
	time in start box (sec)	279.08 $\pm$ 11.13	225.26 $\pm$ 17.99	0.8115 (14)	> 0.05
CCT	line crossings	143.33 $\pm$ 28.08	163.50 $\pm$ 16.87	0.6583 (14)	> 0.05

Table 2. Correlations between the measures of self-injury (oral contact and injury size), stereotypy, grooming, rearing, and the prescreened measures of anxiety-related behaviours for all the pemoline-treated rats. Pearson's *r* was calculated for each pair of correlations between the behavioural measures in the OFET or EPM and the behavioural measures during pemoline treatment. All significant correlations are depicted in bold font. The significance of each correlation is depicted as **p* < 0.05 and ***p* < 0.01 (1-tailed).

dependent measure	EPM				OFET			
	latency to open arm		time in closed arm		latency to open arena		time in start box	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
oral contact	-0.0690	0.4300	0.0370	0.4624	0.8309	0.0028**	0.7796	0.0066**
injury size	-0.2135	0.2906	-0.0912	0.4078	0.6497	0.0291*	0.6165	0.0385*
stereotypy	-0.2983	0.2178	-0.0126	0.4871	-0.0225	0.4770	-0.1527	0.3474
grooming	-0.2928	0.2222	-0.0498	0.4494	0.1786	0.3229	0.2735	0.2382
rearing	0.2367	0.2699	0.3271	0.1951	-0.267	0.2437	-0.3139	0.2054

Table 3. Counterbalancing of the group assignments resulted in groups that were all equivalent in measures of anxiety-related behaviours in the OFET.

OFET dependent measure	0 mg/kg (mean $\pm$ SEM)	1.0 mg/kg (mean $\pm$ SEM)	3.0 mg/kg (mean $\pm$ SEM)	10 mg/kg (mean $\pm$ SEM)	F (df)	p
latency to open arena (sec)	93.29 $\pm$ 42.75	81.56 $\pm$ 41.75	41.15 $\pm$ 32.39	80.16 $\pm$ 41.76	0.32 (3,32)	> 0.05
time in start box (sec)	188.76 $\pm$ 24.89	190.81 $\pm$ 25.01	188.07 $\pm$ 20.44	192.50 $\pm$ 24.60	0.01 (3,32)	> 0.05