Self-Injurious Behavior: Investigating Individual Differences in an Animal Model

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ABSTRACT

Self-injurious behavior (SIB) is a devastating disorder that consists of deliberate acts of self-inflicted tissue damage commonly exhibited by individuals with developmental disabilities, autism, and certain genetic syndromes. SIB is exhibited by only a portion of individuals with these disorders and the severity of SIB varies widely among them, suggesting that there are specific individual differences in vulnerability to express SIB within these groups. This SIB is modeled pharmacologically in rats. Repeated moderately high doses of pemoline produce highly compulsive and stereotyped SIB in some rats. We have evaluated the potential that individual differences in stress responsiveness correspond to individual differences in vulnerability for SIB in this pemoline model. Individual differences in stress-responsiveness were screened by exposing rats to a novel environment, in which high responders (HRs) exhibit larger locomotor and hormonal responses to the stressor, whereas less responsive rats are known as low responders (LRs). We found that HRs were more vulnerable to exhibit pemoline-induced SIB than LRs were. However, HRs and LRs did not significantly differ in most other behaviors including grooming and inactivity. These results suggest that individual differences in stress responsiveness play a major role in mediating individual differences in the vulnerability to exhibit pemoline-induced SIB. These results will allow future research to focus on the neurobiological and neurochemical characteristics that underlie vulnerability for SIB in the self-injurious HR rats.

INTRODUCTION

Self-injurious behavior (SIB) is a devastating disorder that consists of deliberate acts of self-inflicted tissue damage including head-banging, skin-picking, and self-biting. Individuals with intellectual and developmental disabilities, autism, and certain genetic syndromes commonly exhibit SIB. The behavior is usually stereotyped and repetitive and ranges in severity from mild to life threatening (Thompson and Caruso, 2002). Furthermore, the disorder often persists through adulthood and is difficult to treat (Matson et al., 2007).

The prevalence, form, and expression of SIB are highly variable. Prevalence rates among individuals with intellectual disabilities range from 1.7% to 65.9% (Rojahn and Esbensen, 2002), depending on the extent of the intellectual disability, the residential setting, and the etiology of the disorder (Thompson and Caruso, 2002). In addition, particular groups of self-injurers exhibit particular forms of SIB. For example, biting of the lips,
tongue, and digits is seen in Lesch-Nyhan syndrome (Anderson and Ernst, 1994), skin-picking is seen in Prader-Willi Syndrome (Symons et al., 1999) and face-hitting and head-banging are common in autism and other developmentally disabled populations (Symons and Thompson, 1997). SIB is exhibited by only a portion of individuals with these disorders and the severity of SIB varies widely among them (Thompson and Caruso, 2002). This suggests that there are specific individual differences in vulnerability to express SIB within these groups.

The neurobiological mechanisms underlying the vulnerability to self-injure are largely unknown, but evidence from human clinical pathology and animal models has implicated neurochemical disregulation in SIB, predominately in monoaminergic systems. An autopsy study of three Lesch-Nyhan patients revealed much lower dopamine concentrations in the caudate-putamen (Lloyd et al., 1981) and a recent positron emission tomography study of this population showed significantly reduced dopamine transporters compared to controls (Wong et al., 1996). Additional evidence for disregulation can be seen in animal models of SIB. When the dopaminergic system of neonatal rats is lesioned by 6-hydroxydopamine (6-OHDA) and dopamine agonists are subsequently administered to them as adults, rats exhibit severe self-injury (Breese et al., 1984). Another animal model of SIB involves repeated administration of moderately high doses of pemoline (2-imino-5-phenyl-4-oxazolidione), an indirect monoamine agonist, which produces highly compulsive and stereotyped SIB in some rats (King, 2002; Kies and Devine, 2004).

We have chosen to use the pemoline model of SIB, because it is both effective and predictable. As the dosage of pemoline increases, the percentage of self-injurious rats increases; this dose-orderly effect indicates that the treatment has a reliable outcome (Kies and Devine, 2004). Additionally, some of the characteristics of SIB in pemoline-treated rats resemble characteristics of SIB in humans. For example, stress exposure has been shown to enhance pemoline-induced SIB (Kies et al., 2004) and children with Lesch-Nyhan syndrome exhibit higher rates of self-injury associated with highly stressful activities (Anderson and Ernst, 1994). Moreover, children raised in environmentally deprived institutional settings display high rates of SIB (Beckett et al., 2002), while increased environmental complexity reduces pemoline-induced SIB (Kies et al., 2004). Furthermore, drugs that are used to lessen SIB in clinical populations (eg. valproate and risperidone; Ruedrich et al., 1999; McCracken et al., 2002) are also effective in reducing pemoline-induced self-injury (Devine and Muehlmann, 2005). Finally, moderately high doses of pemoline induce only a subset of rats to self-injure, which is similar to the fact that only a subset of individuals with developmental disabilities self-injure (Kies and Devine, 2004).

In light of the fact that some, but not all rats self-injure when treated with pemoline, we are studying individual differences in vulnerability to acquire SIB using this pemoline model. The fact that pemoline-induced SIB can be modified by chronic stress suggests that inherent stress-responsiveness may contribute to vulnerability for SIB. Therefore, we are investigating individual differences in behavioral responses to mild stress as potential predictors of vulnerability to develop pemoline-induced SIB. In this paradigm, rats are exposed to the mild stress of a novel, circular environment. Some rats exhibit large locomotor and hormonal responses to the stressor, and are known as high responders (HRs), whereas less responsive rats are known as low responders (LRs). HRs have also been described as "novelty-seeking," as they seek out new environments,
despite the fact that this stressful event elicits higher plasma concentrations of corticosterone and greater increases in mesolimbic dopamine transmission than in LRs (Piazza et al., 1989). Furthermore, it is possible to selectively breed for divergence in novelty-seeking traits, indicating that these differences in stress-responsiveness are heritable (Stead et al., 2006).

We have also investigated an alternative way of assessing individual differences in a neophobic test of anxiety by using an open-field. Hughes (1972) studied reactions to novelty in an open field environment, and Fernandez et al. (2004) modified this apparatus by adding a start box to one wall. This addition improved the sensitivity of the open-field by permitting the measurement of numerous anxiety-related behaviors such as latency to enter, number of entries, and time spent in the open-field. In the open-field, the rats can choose to stay within the safety of a small start box or investigate the open field. This test allowed us to examine anxiety-related behavior in another novel environment and compare this behavior with locomotor behavior in the circular corridor to determine which is a better predictor of vulnerability to exhibit pemoline-induced SIB.

This study provides evidence that a simple behavioral test can be used to screen rats for their vulnerability to exhibit pemoline-induced SIB, allowing future studies to examine how neurochemical mechanisms differ between vulnerable and resistant rats. This information may have important implications for our understanding of the biological basis of SIB in human self-injurers.

**METHODS**

**Animals**

Male Long-Evans rats (Charles River) were housed in an AAALAC-approved vivarium. The temperature, humidity, and light schedule were automatically controlled and the rats were maintained on a 12-hour light cycle (lights on at 7:00am). Food and water were available *ad-libitum*. The rats were pair-housed for 6-7 days of acclimation to the facility in standard polycarbonate cages (43 x 21.5 x 25.5 cm). The rats remained pair-housed until the first day of pemoline treatment, when they were individually-housed to insure that any injuries were self-inflicted. All procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

**Drugs**

Pemoline (Spectrum Chemical Co., New Brunswick, NJ) was suspended in warm peanut oil at a concentration of 50mg/ml by constant stirring at 36ºC. At approximately 9:00am, the rats were weighed and injected subcutaneously. The injections were administered using 21 gauge needles at three alternating sites (the nape of the neck, the right flank, and the left flank).

**Apparatus**
Each circular corridor (Fig. 1) consisted of a small clean wastebasket placed inside of a large clean garbage can (forming the inner and outer walls of the corridor, respectively). Standard bedding was placed on the path (7 cm wide, 44 cm outer diameter).

Figure 1. Circular Corridor. Each rat is placed on the circular path and allowed to freely explore for 60 minutes.

The open-field (Fig. 2) was a black acrylic 90 cm square. A small start box was attached to the open-field by a guillotine door that was lifted by means of a pulley system from outside the testing room.

Figure 2. Open-Field. Each rat is placed into the small start box for one minute of acclimation and then allowed to freely explore the open-field for five minutes.

**Experimental Procedures**
Eighteen of the rats weighing 275-350 grams were acclimated to the housing facility and screened for individual differences in stress-responsiveness in the circular corridor. The rats were screened for 60 minutes and recorded using a ceiling mounted video camera. A trained observer quantified locomotion by dividing the video of the corridor into quadrants. One count was recorded each time the rat crossed a line, but no further counts from that line were made until the rat crossed another line. Therefore, small movements back and forth were not counted as locomotion.

Starting one day after screening, the rats were handled on each of three consecutive days for five minutes in the morning, to habituate them to handling. One day after the last handling day, the rats were tested in the open-field at approximately 9:00am. Each rat was first allowed one minute of habituation to the start box. Then the guillotine door was lifted to allow the rat five minutes to explore the open-field and attached start box. The testing was recorded using a ceiling mounted video camera. An observer who was blind to the circular corridor behavior scored the videotapes for the latency to first enter the open-field, number of entries into the open-field, and time spent in the open-field. One day after open-field testing, all of the rats began pemoline treatment for ten days at 150 mg/kg.

**Assays of Self-Injury and other behaviors**

During pemoline (or vehicle) treatment, the rats were visually inspected at the time of the injection and at approximately 5:00pm, and the inspections were videotaped. The rats were physically manipulated, and the head, forepaws, hindpaws, ventrum, and tail of each rat were displayed to the video camera and an injury score was assigned (see Table 1). Still photographs were taken from the video and the area of tissue damage was measured using MCID Image Analysis software. An observer who was blind to the drug treatment later rescoring the injuries.

<table>
<thead>
<tr>
<th>Score</th>
<th>Classification</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>no SIB</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>very mild SIB</td>
<td>denuded skin, edema or erythema; involves small area</td>
</tr>
<tr>
<td>2</td>
<td>mild SIB</td>
<td>denuded skin, edema or erythema; involves medium area or multiple small sites</td>
</tr>
<tr>
<td>3</td>
<td>moderate SIB</td>
<td>denuded skin, edema or erythema; involves large area or multiple medium sites</td>
</tr>
<tr>
<td>4</td>
<td>severe SIB</td>
<td>open lesion. Requires euthanasia.</td>
</tr>
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Night vision cameras focused on the home cage of each rat recorded self-injurious oral contact, grooming, locomotion, and inactivity. Five-minute samples were recorded once per hour for eight hours each night, beginning at 8pm. The videotapes were scored to assay the duration of self-injurious oral contact, grooming, inactivity, and locomotion. Self-injurious oral contact was defined as any oral contact that remained fixed on a body part for more than two seconds. Grooming was defined as oral contact that continued to move from body part to body part and did not remain localized for more than two seconds. Inactivity was defined as total lack of movement except for respiration. Locomotion was quantified by dividing the cage into three equal sections length-wise and counting the number of times the rat entered a new section, excluding entrances back into the previously occupied section.

On the morning after the last day of pemoline administration (day 11), each rat was inspected, assigned an injury score, and then rapidly decapitated. The brain was removed from each rat and rapidly frozen in 2-methylbutane at -40°C and later stored at -80°C. The brains will be used in future histochemical analyses not included in this study. The thymus and adrenal glands were also removed and weighed to assess the health status of each rat.

**Statistical Analyses**

Differences between HRs and LRs in circular corridor and open-field behavior were compared using t-tests. Pemoline-induced self-injury between HRs and LRs, including tissue damage scores, size of the injured tissue, and self-injurious oral contact were compared using repeated measures analyses of variance (RM-ANOVA). Body weight, grooming, inactivity, and locomotion were also compared using RM-ANOVA. All significant effects (p<0.05) were further analyzed with Fisher’s Least Significant Difference (LSD) post-tests. Glandular weights were first converted to relative weights (mg gland/100g bodyweight) and then compared using t-tests.

Rats that received an injury score of 4, indicating an open lesion, were euthanized before the end of the experiment (this affected 2 rats). For these rats, 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 potentially underestimating the group means of injury scores, when the most severe injurers were removed from the group. The same rationale was also used to prevent under- or over-estimating locomotion, inactivity, and grooming scores.

**RESULTS**

A database of circular corridor locomotor scores was used to classify the stress responsiveness status (i.e. HR or LR) of each rat. This normative database derived from the scores of 172 rats that were previously tested in the Devine laboratory under the same conditions as the rats in the current experiment. Its use minimizes the potential misclassification of stress-responsiveness status that could occur if the rats’ circular corridor scores were compared to only those of other rats in their respective experiment. Rats with circular corridor scores
Results show that the HRs had significantly higher locomoter counts than did the LRs in the first three ten-minute intervals \( [F(1,80) = 10.39, p<0.05] \) (Fig. 3a) and significantly higher total line crossings \( [t(16) = 3.223, p<0.05] \) (Fig 3b).

In the open-field, there were no significant differences between the HRs (n= 10) and the LRs (n= 8) in anxiety-related behaviors, including latency to enter \( [t(16) = 0.976, p>0.05] \) (Fig. 4a), number of entries \( [t(16) = 1.082, p>0.05] \) (Fig. 4b), and total time spent in the open field \( [t(16) = 0.308, p>0.05] \) (Fig. 4c). However, the HRs did show a consistent trend towards fewer anxiety-related behaviors than the LRs did.

The HRs were more vulnerable to the self-injury inducing effects of pemoline. A greater percentage of the HRs self-injured in comparison with the percentage of LRs that self-injured (Fig. 5a), spent significantly more time in self-injurious oral contact \( [F(1,144) = 5.010, p<0.05] \) (Fig. 5b), and the SIB exhibited by the HRs was significantly more severe, compared to that of the LRs \( [F(1,320) = 8.171, p<0.05] \) (Fig. 5c).
Figure 5. Pemoline-induced SIB. (a) HRs were more likely to exhibit pemoline-induced SIB, (b) spent significantly more time in self-injurious oral contact, and (c) exhibited injury that was more severe as compared to LRs. Values in b and c are group means ± S.E.M. Significant differences between HRs and LRs (LSD) are depicted as: * p<0.05.

Individual differences in stress-responsiveness (i.e.: HR/LR status) did not correlate with individual differences in most of the other behaviors that were measured in this experiment. The HRs were not significantly different than LRs in percentage of time spent grooming \([F(1,135) = 0.1436, p>0.05]\) (Fig. 6a) or inactive \([F(1,144) = 0.5089, p>0.05]\) (Fig. 6b). The LRs did show significantly higher overnight locomotion scores than did HRs \([F(1,135) = 5.536, p<0.05]\) (Fig. 6c). The HRs and LRs were not significantly different in measures of health status. There was a significant time effect for body weight \([F_{(10,160)} = 17.53, p<0.05]\), wherein all rats exhibited weight loss during the first four days of the experiment, followed by six days of weight gain, and this did not differ between the HR and LR groups (Fig. 7a). Glandular weights at the end of the experiment were also not significantly different, including thymus \([t(16) = 2.120, p=0.05]\) (Fig. 7b), and adrenal weight \([t(16) = 1.523, p>0.05]\) (Fig. 7c)

Figure 6. Other Behaviors. There were no significant differences between HRs and LRs in time spent (a) grooming or (b) inactive. (c) LRs exhibited overnight locomotion significantly more than HRs did on nights 5 and 6. All values expressed are group means ± S.E.M. Significant differences between HRs and LRs (LSD) are depicted as: * p<0.05.
There were no significant differences between HRs and LRs in (a) body weight, (b) thymus weight, and (c) adrenal weight. However, there was a significant time effect in (a) body weight, wherein all rats exhibited weight loss during the first four days of the experiment, followed by six days of weight gain. All values expressed are group means ± S.E.M.

DISCUSSION

In this study, we investigated the potential that individual differences in stress-responsiveness could predict vulnerability to exhibit pemoline-induced SIB. The HR/LR model of individual differences in responses to the mild stress of a novel environment has been well established (Piazza et al., 1989) using Sprague-Dawley rats. We have shown that this model is effective in differentiating these individual differences in Long-Evans rats. Furthermore, screening a large number of rats in the circular corridor has revealed that the stress-responsiveness scores follow a normal distribution.

Following circular corridor screening, we examined the anxiety-related behaviors of the rats, in order to compare performance in the circular corridor and in our modified open field (Fernandez et al., 2004). While the group differences in exploratory behaviors in the open field were not statistically significant, the HRs did show trends toward lower anxiety-related behaviors as compared to the LRs in all three measures. Previous studies have shown that HRs exhibit fewer anxiety-related behaviors than LRs do in the elevated plus maze and light/dark boxes (Kabbaj et al., 2000), and also in a standard open-field, using a larger number of selectively bred rats (Stead et al., 2006). Our results are consistent with these findings and the outcomes might have reached statistical significance if a larger cohort of rats had been tested.

Individual differences in behavioral responses to mild stress reliably predicted the individual rats' vulnerability to develop pemoline-induced SIB. The HRs were more likely to exhibit pemoline-induced self-injury, spent significantly more time in self-injurious oral contact, and the tissue damage exhibited by the HR rats was significantly more severe, compared to that of the LR rats. The dose (150mg/kg/day) of pemoline used and the 10-day treatment regimen created excellent conditions for revealing these differences between HRs and LRs. Furthermore, the HRs and LRs did not differ in most other behaviors, including time spent grooming or inactive. Differences in overnight locomotion were seen on nights 5 and 6, but the HR rats that are exhibiting compulsive behavior might be expected to locomote less. There were also no differences in measures
of health status (i.e. body, thymus and adrenal weights) between the vulnerable HRs and the relatively resistant LRs. Overall, these results suggest that individual differences in stress-responsiveness contribute to individual differences in vulnerability to exhibit pemoline-induced SIB. This will allow future research to focus on the neurobiological and neurochemical differences between self-injurious HRs and non-self-injurious LRs that underlie the differences in vulnerability for pemoline-induced SIB.

REFERENCES


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