Published in final edited form as:

Behav Brain Res. 2011 February 2; 217(1): 148–154. doi:10.1016/j.bbr.2010.10.012.

Individual Differences in Vulnerability for Self-Injurious Behavior: Studies Using an Animal Model

Amber M. Muehlmann, Jennifer A. Wilkinson, and Darragh P. Devine

University of Florida, Department of Psychology, Behavioral Neuroscience Program, P.O. Box 112250, Gainesville, FL 32611-2250, U.S.A

Abstract

Self-injurious behavior (SIB) is a debilitating characteristic that is prevalent across a broad spectrum of neurodevelopmental disorders. In most of these disorders, some individuals exhibit SIB, whereas others do not. However, the neurobiological mechanisms that confer vulnerability are virtually unexplored. We examined innate characteristics that contribute to vulnerability or resistance for SIB in an animal model of the behavioral pathology. Eighteen outbred Long-Evans rats were screened for behavioral responsiveness to the mild stress of a novel environment. The rats were then categorized as high responders (HR; those rats that had the highest locomotor counts) or low responders (LR; those rats that had lower locomotor counts) by median split. All the rats were then given daily injections of the indirect monoamine agonist pemoline (150 mg/kg/day) for 10 days, and self-injury was evaluated. All 9 HR rats and 5 of the 9 LR rats exhibited self-injury. The HR rats spent more time self-injuring, injured more body sites, and caused larger areas of tissue damage than the LR rats did. Furthermore, the behavioral responsiveness to novelty stress was significantly correlated with each of these measures of self-injury. The HR rats did not exhibit substantially enhanced responses on other measures of psychostimulant action (stereotypy, grooming, locomotion, rearing). Accordingly, vulnerability to develop pemoline-induced SIB is positively correlated with, and can be predicted based upon, a behavioral measure of innate stress responsiveness. These findings suggest that characteristics that are common in developmental disorders may help predispose afflicted individuals to self-injure. The findings also extend the variety of behavioral pathologies (e.g. drug addiction) for which the HR/LR model predicts vulnerability.

Keywords

Self-injurious behavior; individual differences; stress; autism; Lesch-Nyhan syndrome; animal model

© 2010 Elsevier B.V. All rights reserved.

Corresponding author: Darragh P. Devine, Ph.D., University of Florida, Department of Psychology, Behavioral Neuroscience Program, P.O. Box 112250, Gainesville, FL 32611-2250, U.S.A. dpdevine@ufl.edu, Office phone: 352-273-2174, Fax: 352-392-7985.

Views and opinions of the authors do not reflect those of the US Army or the Department of Defense, and none of the authors have any conflict of interest to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Self-injurious behavior (SIB) is arguably the most devastating of all the behavioral problems that are commonly seen in individuals with neurodevelopmental disorders. These disorders include Lesch-Nyhan syndrome, Prader-Willi syndrome, autism, and intellectual disabilities in general. The prevalence and forms of SIB are highly variable when comparing these diagnostic groups, and within most of them, there are substantial individual differences in incidence and severity of SIB. Virtually all individuals with Lesch-Nyhan syndrome exhibit self-biting [1], whereas the incidence of skin-picking in Prader-Willi syndrome is estimated around 80–90% [7,56]. Approximately 50% of children with autism bang their heads and punch or slap themselves [3], and estimates of the rate of self-injury across other intellectually-disabled groups is highly variable [49]. The reasons for these individual differences are not well characterized, but these behaviors can produce severe physical harm, and they interfere with all educational and socializing activities [18,42,43].

The fact that SIB is prevalent in so many neurodevelopmental disorders suggests that common underlying variables may contribute to the etiology and expression of SIB in these diverse groups. Indeed, there is abundant evidence that social reinforcers (both positive and negative) contribute to the ongoing expression of SIB, and that manipulations that target these reinforcing contingencies can often produce dramatic improvements in behavior (for review see [29]). However, social explanations of SIB cannot fully explain the apparent vulnerability that underlies etiology of SIB in these populations, and biological factors appear to play an important role. For example, individuals with the paternal deletion subtype of Prader-Willi syndrome typically exhibit higher SIB scores than do Prader-Willi patients with the uniparental disomy subtype of the disorder [23]. Furthermore, SIB is not shaped by social consequences in about 30% of intellectually-disabled self-injurers [30], and it is not responsive to standard operant interventions in some neurodevelopmental disorders (e.g. Lesch-Nyhan syndrome [1]). Overall, it appears that social conditioning variables contribute to the etiology and maintenance of SIB in most intellectually-disabled self-injurers, but that biological variables associated with neurodevelopmental disorders confer critical vulnerability to develop the self-injurious phenotype.

Although various genetic and/or environmental insults may be the proximate causes of neurodevelopmental disorders, the functional similarity that contributes to vulnerability for SIB may include dysregulation of cortico-basal ganglia circuitry. This circuitry participates in motor behavior as well as habit learning and is regulated by neurotransmitter inputs that originate from several brain regions. As such, different genetic defects or environmental insults may lead to similar dysfunction in relation to basal ganglia output and behavior (for a review see [8]).

Disregulation of limbic and hormonal stress responses may also contribute. SIB is associated with heightened arousal and feelings of distress in a variety of neurodevelopmental disorders [1,2], and “stress reduction” is reported to be the most often-used form of intervention in boys with Lesch-Nyhan syndrome [2]. Furthermore, there is a correlation between SIB and circadian regulation of stress-related hormone secretion in clinical populations with intellectual disabilities. Abnormally elevated concentrations of circulating adrenocorticotrophic hormone (ACTH) and cortisol were found in plasma and salivary samples collected from self-injurers [37,53,57]. However, it is unclear if dysfunction of the limbic hypothalamic pituitary (LHPA) axis might be a predisposing factor, or a consequence of this devastating behavior in these self-injurious patients. The potential that stress-reactivity contributes to vulnerability for SIB has not been explored beyond these descriptive observations in human samples.

Heightened emotional responsiveness has also been reported in self-injurious rhesus monkeys, which display more vocalizations and threat behaviors than do their non-injurious counterparts [47]. In addition, stress exposure has been shown to exacerbate SIB in a variety of animal models, including neonatal 6-hydroxydopamine (6-OHDA) lesioned rats [5], environmentally-deprived non-human primates [12] and chronic treatment with psychostimulants [14,46]. For example, self-injurious biting is enhanced by acute footshock stress in the neonatal 6-hydroxydopamine (6-OHDA) lesion model of SIB [55], and relocation stress increased the expression of SIB in captive macaques with a prior history of self-injuring [11]. However, the potential role of innate stress-responsiveness in etiology of SIB is not revealed in these studies where the effects of acute stress exposure were examined in animals that already had well-established patterns of SIB.

Innate stress-responsiveness has been studied in a rodent model, examining individual differences in behavioral and hormonal activation during exposure to mild stress in outbred rats. In this model, the rats are screened for reactivity in a novel (circular corridor) environment. Those rats that exhibit high rates of stress-induced locomotor activation are known as high stress-responders or HR rats, and those rats that exhibit lower rates of locomotor activation are known as low responders or LR rats [48]. The HR/LR model is widely recognized as a model of vulnerability for drug addiction, because the HR rats self-administer stimulant drugs when they are made available at doses that are too low to support self-administration in the LR rats [35,36,48]. The HR rats also exhibit greater novelty-seeking, and less anxiety-related behavior in neophobic tests than the LR rats do (i.e. preferential exploration of a novel arm in a maze, greater exploration of the light compartment in a dark/light shuttlebox test, and greater exploration of the open arms of an elevated plus maze [13,34,54]). The HR rats are also extremely responsive to social stress exposure. Their expression of anxiety-related behavior is greatly enhanced after one week of social isolation [34], and their propensity to self-administer drugs is diminished by exposure to social defeat [36].

The HR and LR rats also differ in important biochemical characteristics. HR rats exhibit greater and more prolonged elevations in circulating corticosterone concentrations during novelty stress [34,48]. HR rats also have higher basal concentrations of extracellular dopamine in the nucleus accumbens (NAcc) than the LR rats do [24], and they exhibit greater and more prolonged increases in NAcc dopamine concentrations after tail pinch stress [13,50,51] or cocaine administration [24,27] than the LR rats do. The HR rats also express higher basal levels of D₁ receptor binding in the caudate, and lower levels of dopamine D₂ receptor binding in the caudate and nucleus accumbens than the LR rats do [28].

Although the HR/LR model has been studied primarily as a model of drug addiction, the characteristics of these animals suggests that they may have relevance for additional forms of psychopathology [13,31,34,54]. Accordingly, we investigated the potential that these innate individual differences in behavioral-responsiveness to mild stress predict individual differences in vulnerability to develop SIB in an animal model.

2. Materials and Methods

2.1 Animals

Eighteen male Long-Evans rats weighing 150–175g upon delivery (Charles River Laboratories, Raleigh, NC) were housed in a climate controlled vivarium with a 12h/12h light/dark schedule (lights on at 7:00a.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 cm × 21.5 cm × 25.5 cm) during 6 days of acclimation to the

housing facility and for eight days following screening of stress-responsiveness. This pair-housing was implemented to avoid the potential impact of prolonged isolation stress [34] and to allow recovery from any effect of the stress exposure that could have arisen during the novel environment screening. The rats were then singly-housed in identical polycarbonate cages upon initiation of pemoline treatment, in order to ascertain that any injuries were self-inflicted. All the experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2003), and were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida.

2.2 Drugs

The indirect monoamine agonist pemoline [19,20] (2-amino-5-phenyl-1,3-oxazol-4-one; 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.

2.3 Stress-Responsiveness Screening

Innate individual differences in locomotor-responsiveness to mild stress were screened by testing the rats' behavior in novel circular corridor environments. Each circular corridor was constructed by placing a small plastic cylinder inside a larger plastic cylinder (forming the inner and outer walls of the corridor, respectively). Both cylinders were washed with 4% bleach and standard bedding was placed on the floor (7 cm wide, 44 cm outer diameter) of the corridor. The lights in the testing room were turned off and dim illumination was provided by small lamps placed at the floor beside each circular corridor (less than 10 lux within the circular corridor). Each rat was placed individually into this novel environment between one to two hours after lights on (i.e. between 8 and 9 a.m.). Video cameras secured to the ceiling were used to record locomotor behavior for 60 minutes. A trained observer quantified locomotion by dividing the video image of the corridor into equal 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. The rats were classified as HRs or LRs by median split, and this classification was verified by comparison with a large sample of rats we have run in our laboratory in related studies of stress-responsiveness (n=336; median = 223 line crossings; unpublished data). Additional biochemical measures (e.g. LHPA axis function) were not collected during the screening for stress responsiveness, as the association of these measures with the behavioral activation is very well-established and robust (e.g.[34,48]), and collection of these samples would require intrusive procedures that could have affected the subsequent expression of SIB.

2.4 Drug treatment

Starting on the 9th day after the stress-responsiveness screening, an experimenter blind to the novelty stress responses of the rats, weighed and injected each rat with pemoline at 150 mg/kg (s.c.) each day (between 9:00 and 10:00 a.m.) for ten days. This dose regimen produces self-biting in approximately 50% of rats [38]. The injections were administered at the nape of the neck and either flank on a rotating basis. Note that a vehicle-treated control group is not included in this experiment because we have previously reported that vehicle-treated rats never exhibit SIB in our experimental protocol [38].

2.5 Assays of Self-Injury and Related Behaviors

Each rat was visually inspected every morning at the time of the pemoline injection and then again every evening (5:00 – 6:00 p.m.), and the inspections were videotaped. During these inspections, each rat was held in front of a video camera and the head, forepaws, hindpaws, ventrum and tail were displayed. The total numbers of tissue injuries (denuded skin,

erythema, edema, or open lesion) was noted for each rat. Any rat with an open lesion was immediately euthanized (2 of the HR rats and none of the LR rats). Still images of the injured tissue were captured from the videotapes, and MCID software (Imaging Research Inc., St. Catharines, ON, Canada) was used to draw outlines around the injured tissue, and to calculate the total area of injury for each rat in mm².

In addition to the scoring of injury sites, night-vision video-cameras were focused on the cages of the rats (one camera per cage), and 5-minute time samples were recorded once per hour for 8 hours each night (total 400 min per rat). The duration of self-injurious oral contact, stereotypy, grooming, rearing and the amount of locomotion were quantified in the home cage during each time sample, by a trained observer who was blind to the HR or LR designation. Self-injurious oral contact was defined as all oral contact that stayed fixed at any specific site on the body for longer than 2 seconds. This was differentiated from grooming, which is oral contact with any part of the body that moves in a predictable manner along the length of a body part (e.g. licking along a length of a forelimb or flank), or from site to site on the body (e.g. oral contact with the forepaws, that then moves up each forelimb and continues to the ventrum). In all our observations of grooming behavior, oral contact is not sustained at any spot on the body for longer than 2 seconds.

A second trained but experimentally-blind observer re-scored a subset of the video-recordings to evaluate inter-observer reliability for the duration of self-injurious oral contact (one randomly-selected night of recordings for each rat).

The duration of stereotyped behaviors was also quantified from the video time samples. These behaviors included episodes of stereotyped head-bobbing, cage-licking, and digging/sniffing/burrowing through the bedding, that exceeded 3 sec duration [10]. Since individual rats exhibited substantial differences in the expression of stereotypy (e.g. some primarily exhibited head-bobbing or cage-licking, whereas others exhibited digging/sniffing/burrowing) the stereotypy scores are reported as aggregate scores that compile the total duration of all these stereotyped behaviors.

Home-cage rearing and locomotion were also scored from the video recordings. Rearing was counted each time a rat lifted both forepaws off the cage floor for at least 3 sec. A subsequent rear was only counted after the rat resumed and maintained contact with the cage floor for at least 1 sec. Locomotion was counted by sectioning the video image of the cage into thirds (i.e. drawing lines on the television monitor dividing the cage into three equal parts along its length) and tallying the number of times the rat's forepaws crossed these lines. However, a subsequent crossing of that line was only counted after the rat crossed the other line. Thus, each locomotor count represents a minimum of 14.3 cm traversing of the cage.

2.6 Statistical analyses

Differences in the percentage of rats that exhibited pemoline-induced self-injury in the HR and LR rat groups were analyzed by a chi-square test. Between-groups differences in novelty stress-induced locomotion (circular corridor line crossings), duration of self-injurious oral contact, number of injured sites, area of the injured tissue, duration of stereotypy, grooming, home-cage rearing, and the amount of home-cage locomotion were each evaluated using mixed model repeated measures analyses of variance (RM-ANOVA). Outcomes were treated as statistically reliable when the p-values were less than 0.05. All significant between-groups effects were further analyzed with pre-planned Fisher's least significant difference (LSD) post-hoc comparisons.

The relationship between each rat's stress-responsiveness (total novelty-induced locomotion score) and each of the measures of self-injury were also evaluated with correlational analyses. Pearson correlations were calculated to determine the relationship between stress-responsiveness and mean nightly self-injurious oral contact duration, maximal area of tissue injury, and mean nightly stereotypy scores. In addition, the novelty stress-induced locomotion scores were converted to rank order (from low to high) and a Spearman correlation was calculated comparing these rank orders with the rats' numbers of injured sites. Similar Pearson correlation analyses were also conducted to evaluate the relationship between stress-responsiveness and each of the measures of ancillary behaviors (stereotypy, grooming, home-cage locomotion, and home-cage rearing).

Two HR rats were euthanized before the end of the experiment (both on day 5) because they had open lesions. 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 under-estimate the area of tissue damage and self-injurious oral contact scores, and to avoid the potential that the group means would over- or under-estimate the scores for the other behaviors when the most severe self-injurers were terminated.

3. Results

The total number of locomotor counts during 60 min exposure to the novel environment (circular corridor) stress ranged from 156 to 448. The rats that exhibited greater than the median amount of locomotion were classified as HR (mean = 271.67 ± 22.86) and the rats that exhibited less than the median amount of locomotion were classified as LR (mean = 191.33 ± 7.96).

A greater percentage of the HR rats than LR rats exhibited pemoline-induced self-injury (defined as the presence of any denuded skin, erythema, edema, or open lesion) during the 11 days of the experiment; ($\chi^2 = 30.55$, $p < 0.0001$). Overall, each of the nine HR rats self-injured, whereas only 5 of the 9 LR rats exhibited any evidence of self-injury. Furthermore, the self-induced tissue injury was more transient in the LR rats (mean = 2.5 days) than it was in the HR rats (mean = 6.1 days). Two of the 5 self-injurious LR rats had mild tissue injury that was only visible on days 5 or 6, and these injuries healed before the next inspection. On the other hand, 8 of the 9 HR rats had visible injury that persisted for 3 or more days, usually through the end of the experiment. The proportion of rats that self-injured on each day of the experiment is depicted in Fig. 1A. The HR rats also spent more time self-injuring (Fig. 1B), had more sites of tissue injury (Fig. 1C), and had larger total areas of tissue injury (Fig. 1D) than the LR rats did. For the self-injurious oral contact measure (Fig. 1B), there were significant main effects of time ($F_{(9,144)} = 3.106$, $p < 0.01$) and stress-responsiveness ($F_{(1,144)} = 5.354$, $p < 0.05$), and there was a significant time by stress-responsiveness interaction ($F_{(9,144)} = 2.091$, $p < 0.05$), indicating that the HR rats exhibited self-injurious oral contact throughout nights 3 through 10, whereas the LR rats typically did not. For the number of injury sites (Fig. 1C), the RM-ANOVA revealed significant main effects of time ($F_{(10,160)} = 8.905$, $p < 0.01$) and stress-responsiveness ($F_{(1,160)} = 6.805$, $p < 0.05$), and a significant time by stress-responsiveness interaction ($F_{(10,160)} = 3.604$, $p < 0.01$). These outcomes derive from the fact that 6 of the HR rats, and only 2 of the LR rats had self-inflicted tissue injuries at more than one site. For the measures of areas of tissue injury (Fig. 1D), there were significant main effects of time ($F_{(10,160)} = 5.440$, $p < 0.01$), and stress-responsiveness ($F_{(10,160)} = 4.924$, $p < 0.05$), and a significant time by stress-responsiveness interaction ($F_{(10,160)} = 3.147$, $p < 0.01$). Four of the HR rats, and only one of the LR rats had tissue injury areas greater than 100 mm².

In addition, the Pearson correlations between total line crossings in the stress-responsive screening with the mean oral contact duration scores, and with the area of tissue injury scores were each significant. The Spearman correlation between the rank-ordered stress-screening scores and the number of injured sites was also statistically significant. The correlations between these measures are depicted in Table 1.

Overall, the HR and LR rats did not differ on most measures of ancillary behaviors (i.e. grooming, home-cage locomotion, and home-cage rearing) during the regimen of pemoline treatment (Figs. 2B, 2C, and 2D). However, the LR rats expressed slightly longer daily total durations of stereotypy than the HR rats did. The RM-ANOVA revealed a significant main effect of stress-responsiveness ($F_{(1,144)} = 7.730$, $p < 0.05$) for the aggregate stereotypy scores (Fig. 2A). On the other hand, the Pearson correlation comparing total novelty stress-induced locomotion with mean stereotypy was not statistically significant (Table 1). Grooming was a very low rate behavior for which a significant main effect of time was found ($F_{(9,144)} = 2.107$, $p < 0.05$), but the daily durations did not differ between the HR and LR rats (Fig. 2B), and the Pearson correlation comparing novelty stress-induced locomotion and grooming was not statistically significant (Table 1). Similarly, the rates of home-cage locomotion did not differ between the HR and LR rats (Fig. 2C), although they changed across the experimental days ($F_{(9,144)} = 5.048$, $p < 0.01$). In addition, the daily durations of home-cage rearing behavior did not differ between the HR and LR rats (Fig. 2D), although they did change across days ($F_{(9,144)} = 6.373$, $p < 0.01$). The Pearson correlations between novelty stress-induced locomotion and these measures of home-cage activities also failed to reach statistical significance (Table 1).

4. Discussion

The HR/LR model of stress-responsiveness has been studied as a rodent model of individual differences in vulnerability for drug abuse [35,48], trait anxiety [13,34,54], and responsiveness to treatment for major depressive disorder [31]. Since the clinical expression of SIB may also be related to behavioral and hormonal responses to stress [1,14,37,53,57], we hypothesized that individual differences in innate stress-responsiveness might predict which rats are vulnerable for pemoline-induced SIB. Our data support this hypothesis. The HR rats are more susceptible to develop pemoline-induced SIB and in doing so exhibit longer durations of self-injurious oral contact, more sites of injury, and larger areas of tissue damage than the LR rats do. Of course, the HR and LR phenotypes are complex (as we have described), and so additional co-varying characteristics of these animals (e.g. differences in sensation-seeking or trait anxiety) may also contribute to the behavioural outcomes. However, these possibilities have not been explored.

In this study, the HR and LR rats were classified by median split of the locomotor responses to mild stress using the data from the current set of 18 rats. The group assignments were verified by comparison with a large set of rats that we have screened in other studies, using identical experimental parameters (unpublished data). Regardless of the database used for comparison, the assignment of the rats was identical. This suggests that the trait is generally replicable across cohorts (although we recommend that HR/LR assignments of small cohorts should be checked against a larger database for consistency in studies of this type). However, even though the HR/LR model is widely used to study individual differences in innate stress-responsiveness, the procedure to classify animals into HR and LR groups is statistically questionable, since the underlying locomotor response to novelty is a continuous variable. Therefore, we further verified the relationship between innate stress-responsiveness and the behavioral measures during pemoline treatment using analyses of the underlying correlations. The positive Pearson correlations between stress-responsive locomotor activation in the novel environment and each of the measures of self-injury (duration of self-

injurious oral contact, number of injured sites, and area of tissue damage, see Table 1) provides additional support for the conclusion that individual differences in innate stress-responsiveness contribute to the expression of pemoline-induced SIB.

In fact, the individual differences in innate stress-responsiveness appeared to predict vulnerability for self-injury quite specifically, in contrast with the lack of association between stress-responsiveness and each of the other behavioral measures in this study. The stress-responsiveness of the rats did not predict the amount of pemoline-induced grooming, home-cage locomotion, or home-cage rearing. There were also no significant between-groups differences in body weights (data not shown), and although there was a marginal between-groups difference in the aggregate stereotypy scores, this was not confirmed by the analysis of the correlation between stress-responsiveness and stereotypy.

The lack of association between stress-responsiveness and psychostimulant-induced locomotor activation might appear puzzling upon first glance. Indeed, HR rats exhibit transiently greater locomotion after acute administration of amphetamine (0.5–1.0 mg/kg) [25], and there are conflicting reports about individual differences in locomotor responses when a higher (1.5 mg/kg) dose of amphetamine was used [25,48]. The HR rats exhibit greater locomotor sensitization when they are treated intermittently with low doses of amphetamine (0.5–1.0 mg/kg/day), [15,25,26]. However, HR and LR rats do not differ in locomotor sensitization when injected repeatedly with a higher (1.5 mg/kg) dose of amphetamine [25,33]. Although pemoline doses cannot be compared directly with amphetamine doses, the doses of pemoline used in the current study were certainly in a high range – as this is necessary to produce self-injury. Accordingly, the lack of association between stress-responsiveness and each of the measures of home cage activity during pemoline treatment is compatible with other published reports in which HR and LR rats were treated with amphetamine.

In our experiments, we have consistently observed that repeated injections of pemoline are required to produce SIB when a moderately high dose of pemoline is used [4,14,38,44,45]. Interestingly, co-administration of MK-801 blocked the induction of SIB in the pemoline model [39,45]. This finding implicates glutamatergic neurotransmission in etiology of pemoline, which is not surprising, since dopaminergic and glutamatergic neurotransmission are highly interactive. However, glutamatergic signaling is also strongly implicated in sensitization of dopaminergic neurotransmission [40]. Thus, although we cannot rule out the possibility that pemoline accumulates over the days of treatment, the data suggest that sensitization may play an important role in the etiology of pemoline-induced SIB [45].

Most importantly, the data suggest that the pemoline model may be useful for investigations of the neurochemical basis of individual differences in vulnerability to self-injury [9,14]. One fundamental problem with pharmacological models is that the drug may have multiple effects, some of which are relevant, and some of which may be irrelevant for the pathological condition that is being modeled. Since individual rats differ in expression of SIB, we are currently investigating the relevant and irrelevant actions of the drug by comparing biochemical differences between the brains of rats that self-injure and those that don't when these rats are given identical drug treatments.

Moreover, the fact that the stress-responsive HR rats are more highly vulnerable for SIB suggests some interesting targets for these investigations. One important candidate is the aforementioned pre-existing individual differences in dopaminergic signaling. This includes the findings that HR rats have higher basal concentrations of extracellular dopamine [24], larger stress-induced and cocaine-stimulated elevations in these concentrations [24,27], and the differences in D1 and D2 receptor binding in the HR and LR rats [28]. In fact, the

innately elevated D1 receptor binding levels in the HR rats may prove to be particularly interesting, because data from the 6-OHDA model of SIB suggest that D1 receptor-mediated supersensitivity may play a particularly important role in the etiology of SIB in the lesioned rats (see [5]).

Since there are important interactions between the LHPA axis and the mesencephalic dopamine projections, the highly responsive LHPA axis of the HR rats may play an important role in mediating these differences between HR and LR rats. GR immunoreactivity is extensively co-localized with tyrosine hydroxylase immunoreactivity in the nigrostriatal and mesocorticolimbic dopamine neurons [22], and this appears to have functional significance for dopaminergic responses to stress. Stress-induced increases in accumbens dopamine concentrations are blocked in adrenalectomized rats, and these glucocorticoid-dependent elevations in extracellular dopamine concentrations are greater and more sustained in intact HR rats than they are in intact LR rats [13,50,51]. Overall, these differences in basal dopaminergic tone, acute drug-responsiveness, and glucocorticoid actions between the HR and LR rats could contribute to their differential vulnerability for pemoline-induced SIB.

In conclusion, innate stress-responsiveness predicts vulnerability or resistance for self-injury in the pemoline model of SIB. The heightened susceptibility of the HR rats likely results from heightened sensitivity or plasticity of the mesencephalic dopamine projections, and/or exaggerated interactions between the LHPA axis and these systems. Interestingly, these implications are redolent of current knowledge of the biochemical basis of SIB in clinical populations. Most studies have focused on Lesch-Nyhan syndrome, in which post-mortem analyses reveal diminished dopamine content in the basal ganglia that is thought to be associated with post-synaptic striatal supersensitivity to dopamine [41,52]. Dopaminergic abnormalities have also been reported in autism spectrum disorders [17,21,59], including Rett syndrome [6,16], and a polymorphism in the dopamine transporter gene is linked to borderline disorder [32] – a condition in which SIB is extremely prevalent. Furthermore, abnormal emotional and hormonal-responsiveness to stress has been associated with the expression of self-injury in neurodevelopmental disorders [1,2,14,37,57]. Hyper-responsiveness of the LHPA axis during stress has also been reported in autism, and this stress hyper-responsiveness was particularly pronounced in those with the most severe core symptoms [58]. Since this subgroup of autistic patients is particularly vulnerable for self-injury [3], it is possible that heightened stress-responsiveness plays an important role in their vulnerability for the behavior disorder. Overall little attention has been given to interactions between stress-responsiveness and dopaminergic function in clinical populations of self-injury. The convergence between our findings in the pemoline model and the observations in Lesch-Nyhan syndrome, autism, and other disorders suggests that this may be a promising area of investigation.

Acknowledgments

The authors would like to thank Brandon Brown for his assistance scoring the video-recorded images in this project.

This research was supported by grants from the Cure Autism Now Foundation (Autism Speaks) and the Department of Defense Autism Research Program under award number AR093546, as well as an F31 fellowship (#MH079675) from the National Institute of Mental Health.

References

1. Anderson LT, Ernst M. Self-injury in Lesch-Nyhan disease. *J Autism Dev Disord.* 1994; 24:67–81. [PubMed: 8188575]

2. Anderson LT, Ernst M, Davis SV. Cognitive abilities of patients with Lesch-Nyhan disease. *J Autism Dev Disord.* 1992; 22:189–203. [PubMed: 1624404]
3. Baghdadli A, Pascal C, Grisi S, Aussilloux C. Risk factors for self-injurious behaviours among 222 young children with autistic disorders. *J Intellect Disabil Res.* 2003; 47:622–627. [PubMed: 14641810]
4. Blake BL, Muehlmann AM, Egami K, Breese GR, Devine DP, Jinnah HA. Nifedipine suppresses self-injurious behaviors in animals. *Dev Neurosci.* 2007; 29:241–250. [PubMed: 17047321]
5. Breese GR, Knapp DJ, Criswell HE, Moy SS, Papadeas ST, Blake BL. The neonate-6-hydroxydopamine-lesioned rat: a model for clinical neuroscience and neurobiological principles. *Brain Res Brain Res Rev.* 2005; 48:57–73. [PubMed: 15708628]
6. Brucke T, Sofic E, Killian W, Rett A, Riederer P. Reduced concentrations and increased metabolism of biogenic amines in a single case of Rett-syndrome: a postmortem brain study. *J Neural Transm.* 1987; 68:315–324. [PubMed: 3559543]
7. Clarke DJ, Waters J, Corbett JA. Adults with Prader-Willi syndrome: abnormalities of sleep and behaviour. *J R Soc Med.* 1989; 82:21–24. [PubMed: 2629712]
8. Cromwell HC, King BH. The role of the basal ganglia in the expression of stereotyped, self-injurious behaviors in developmental disorders. *Int Rev Res Ment Ret.* 2004; 29:119–158.
9. Cromwell HC, King BH, Levine MS. Pemoline alters dopamine modulation of synaptic responses of neostriatal neurons in vitro. *Dev Neurosci.* 1997; 19:497–504. [PubMed: 9445087]
10. Cromwell HC, Levine MS, King BH. Cortical damage enhances pemoline-induced self-injurious behavior in prepubertal rats. *Pharmacol Biochem Behav.* 1999; 62:223–227. [PubMed: 9972687]
11. Davenport MD, Lutz CK, Tiefenbacher S, Novak MA, Meyer JS. A rhesus monkey model of self-injury: effects of relocation stress on behavior and neuroendocrine function. *Biol Psychiatry.* 2008; 63:990–996. [PubMed: 18164279]
12. Dellinger-Ness LA, Handler L. Self-injurious behavior in human and non-human primates. *Clin Psychol Rev.* 2006; 26:503–514. [PubMed: 16713051]
13. Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H. Novelty-seeking in rats - Biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. *Neuropsychobiol.* 1996; 34:136–145.
14. Devine, DP.; Muehlmann, AM. Tiermodelle für selbstverletzendes Verhalten (Animal models of self-injurious behavior). In: Schmah, C.; Stiglmayr, C., editors. *Selbstverletzendes Verhalten bei Stressassoziierten Erkrankungen (Self-Injurious Behaviour in Stress-Associated Disorders)*. Stuttgart, Germany: Verlag W. Kohlhammer; 2009. p. 39-60.
15. Dietz DM, Tapocik J, Gaval-Cruz M, Kabbaj M. Dopamine transporter, but not tyrosine hydroxylase, may be implicated in determining individual differences in behavioral sensitization to amphetamine. *Physiol Behav.* 2005; 86:347–355. [PubMed: 16126238]
16. Dunn AJ, Swiergiel AH, Palamarchouk V. Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. *Ann N Y Acad Sci.* 2004; 1018:25–34. [PubMed: 15240349]
17. Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM. Low medial prefrontal dopaminergic activity in autistic children. *Lancet.* 1997; 350:638. [PubMed: 9288051]
18. Findling RL. Pharmacologic treatment of behavioral symptoms in autism and pervasive developmental disorders. *J Clin Psychiatry.* 2005; 66 (Suppl 10):26–31. [PubMed: 16401147]
19. Fuller RW, Perry KW, Bymaster FP, Wong DT. Comparative effects of pemoline, amfonelic acid and amphetamine on dopamine uptake and release in vitro and on brain 3,4-dihydroxyphenylacetic acid concentration in spiperone-treated rats. *J Pharm Pharmacol.* 1978; 30:197–198. [PubMed: 24701]
20. Gilbert JC, Allen JM, Townsend BG, Wyllie MG. Drugs affecting the central nervous system: effects of pemoline and tricyclic antidepressants on nerve terminal adenosine triphosphatase activities and neurotransmitter release. *Neuropharmacol.* 1978; 17:419–421.
21. Gillberg C, Svennerholm L. CSF monoamines in autistic syndromes and other pervasive developmental disorders of early childhood. *Br J Psychiatry.* 1987; 151:89–94. [PubMed: 2445409]

22. Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, Okret S, Yu ZY, Goldstein M, Steinbusch H. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. *Proc Natl Acad Sci U S A*. 1986; 83:9779–9783. [PubMed: 2879285]
23. Hartley SL, Maclean WE Jr, Butler MG, Zarcone J, Thompson T. Maladaptive behaviors and risk factors among the genetic subtypes of Prader-Willi syndrome. *Am J Med Genet A*. 2005; 136:140–145. [PubMed: 15940679]
24. Hooks MS, Colvin AC, Juncos JL, Justice JB Jr. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. *Brain Res*. 1992; 587:306–312. [PubMed: 1525663]
25. Hooks MS, Jones GH, Neill DB, Justice JB Jr. Individual differences in amphetamine sensitization: dose- dependent effects. *Pharmacol Biochem Behav*. 1992; 41:203–210. [PubMed: 1539070]
26. Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Individual differences in locomotor activity and sensitization. *Pharmacol Biochem Behav*. 1991; 38:467–470. [PubMed: 2057515]
27. Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse*. 1991; 9:121–128. [PubMed: 1821483]
28. Hooks MS, Juncos JL, Justice JB Jr, Meiergerd SM, Povlock SL, Schenk JO, Kalivas PW. Individual locomotor response to novelty predicts selective alterations in D₁ and D₂ receptors and mRNAs. *J Neurosci*. 1994; 14:6144–6152. [PubMed: 7931568]
29. Iwata, B.; Roscoe, EM.; Zarcone, J.; Richman, DM. Environmental determinants of self-injurious behavior. In: Schroeder, SR.; Oster-Granite, ML.; Thompson, T., editors. *Self-Injurious Behavior: Gene-Brain-Behavior Relationships*. Washington, D.C: American Psychological Association; 2002. p. 99-103.
30. Iwata BA, Pace GM, Dorsey MF, Zarcone JR, Vollmer TR, Smith RG, Rodgers TA, Lerman DC, Shore BA, Mazalesk JL. The functions of self-injurious behavior: an experimental-epidemiological analysis. *J Appl Behav Anal*. 1994; 27:215–240. [PubMed: 8063623]
31. Jama A, Cecchi M, Calvo N, Watson SJ, Akil H. Inter-individual differences in novelty-seeking behavior in rats predict differential responses to desipramine in the forced swim test. *Psychopharmacology (Berl)*. 2008; 198:333–340. [PubMed: 18438645]
32. Joyce PR, McHugh PC, McKenzie JM, Sullivan PF, Mulder RT, Luty SE, Carter JD, Frampton CM, Robert CC, Miller AM, Kennedy MA. A dopamine transporter polymorphism is a risk factor for borderline personality disorder in depressed patients. *Psychol Med*. 2006; 36:807–813. [PubMed: 16623961]
33. Kabbaj M. Individual differences in vulnerability to drug abuse: the high responders/low responders model. *CNS Neurol Disord Drug Targets*. 2006; 5:513–520. [PubMed: 17073654]
34. Kabbaj M, Devine DP, Savage VR, Akil H. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. *J Neurosci*. 2000; 20:6983–6986. [PubMed: 10995843]
35. Kabbaj M, Evans S, Watson SJ, Akil H. The search for the neurobiological basis of vulnerability to drug abuse: using microarrays to investigate the role of stress and individual differences. *Neuropharmacol*. 2004; 47 (Suppl 1):111–122.
36. Kabbaj M, Norton CS, Kollack-Walker S, Robinson TE, Watson SJ, Akil H. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine taking-behavior. *Psychopharmacol*. 2001; 158:382–387.
37. Kemp AS, Fillmore PT, Lenjavi MR, Lyon M, Chiciz-DeMet A, Touchette PE, Sandman CA. Temporal patterns of self-injurious behavior correlate with stress hormone levels in the developmentally disabled. *Psychiatry Res*. 2008; 157:181–189. [PubMed: 17913241]
38. Kies SD, Devine DP. Self-injurious behaviour: A comparison of caffeine and pemoline models in rats. *Pharmacol Biochem Behav*. 2004; 79:587–598. [PubMed: 15582667]
39. King BH, Au D, Poland RE. Pretreatment with MK-801 inhibits pemoline-induced self-biting behavior in prepubertal rats. *Dev Neurosci*. 1995; 17:47–52. [PubMed: 7621748]

40. Li Y, Hu XT, Berney TG, Vartanian AJ, Stine CD, Wolf ME, White FJ. Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. *Synapse*. 1999; 34:169–180. [PubMed: 10523754]
41. Lloyd KG, Hornykiewicz O, Davidson L, Shannak K, Farley I, Goldstein M, Shibuya M, Kelley WN, Fox IH. Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. *N Eng J Med*. 1981; 305:1106–1111.
42. Matson JL, Minshawi NF, Gonzalez ML, Mayville SB. The relationship of comorbid problem behaviors to social skills in persons with profound mental retardation. *Behav Modif*. 2006; 30:496–506. [PubMed: 16723427]
43. Matson JL, Nebel-Schwalm M. Assessing challenging behaviors in children with autism spectrum disorders: A review. *Res Dev Disabil*. 2007; 28:567–579. [PubMed: 16973329]
44. Muehlmann AM, Brown BD, Devine DP. Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy. *J Pharmacol Exp Ther*. 2008; 324:214–223. [PubMed: 17925479]
45. Muehlmann AM, Devine DP. Glutamate-mediated neuroplasticity in an animal model of self-injurious behaviour. *Behav Brain Res*. 2008; 189:32–40. [PubMed: 18243356]
46. Muehlmann AM, Wolfman S, Devine DP. Examining the effects of chronic stress on self-injurious behavior in an animal model. *Society for Neuroscience Abstracts*. 2008; 34:446.29.
47. Novak MA. Self-injurious behavior in rhesus monkeys: New insights into its etiology, physiology, and treatment. *Am J Primatol*. 2003; 59:3–19. [PubMed: 12526035]
48. Piazza PV, Deminière J-M, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. *Science*. 1989; 245:1511–1513. [PubMed: 2781295]
49. Rojahn, J.; Esbensen, AJ. Epidemiology of self-injurious behavior in mental retardation: A review. In: Schroeder, SR.; Oster-Granite, ML.; Thompson, T., editors. *Self-Injurious Behavior: Gene-Brain-Behavior Relationships*. Washington, D.C: American Psychological Association; 2002. p. 41-77.
50. Rougé-Pont F, Deroche V, Le Moal M, Piazza PV. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *Eur J Neurosci*. 1998; 10:3903–3907. [PubMed: 9875367]
51. Rougé-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H. Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. *Brain Res*. 1993; 602:169–174. [PubMed: 8448654]
52. Saito Y, Ito M, Hanaoka S, Ohama E, Akaboshi S, Takashima S. Dopamine receptor upregulation in Lesch-Nyhan syndrome: a postmortem study. *Neuropediatrics*. 1999; 30:66–71. [PubMed: 10401687]
53. Sandman CA, Barron JL, Chiczy-DeMet A, DeMet EM. Plasma β -endorphin levels in patients with self-injurious behavior and stereotypy. *Am J Ment Retard*. 1990; 95:84–92. [PubMed: 2167106]
54. Stead JDH, Clinton S, Neal C, Schneider J, Jama A, Miller S, Vazquez DM, Watson SJ, Akil H. Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. *Behav Genet*. 2006; 36:697–712. [PubMed: 16502134]
55. Stodgell CJ, Loupe PS, Schroeder SR, Tessel RE. Cross-sensitization between footshock stress and apomorphine on self-injurious behavior and neostriatal catecholamines in a rat model of Lesch-Nyhan syndrome. *Brain Res*. 1998; 783:10–18. [PubMed: 9479035]
56. Symons FJ, Butler MG, Sanders MD, Feurer ID, Thompson T. Self-injurious behavior and Prader-Willi syndrome: behavioral forms and body locations. *Am J Ment Retard*. 1999; 104:260–269. [PubMed: 10349467]
57. Symons FJ, Sutton KA, Walker C, Bodfish JW. Altered diurnal pattern of salivary substance P in adults with developmental disabilities and chronic self-injury. *Am J Ment Retard*. 2003; 108:13–18. [PubMed: 12475363]
58. Tordjman S, Anderson GM, McBride PA, Hertzog ME, Snow ME, Hall LM, Thompson SM, Ferrari P, Cohen DJ. Plasma beta-endorphin, adrenocorticotropin hormone, and cortisol in autism. *J Child Psychol Psychiatry*. 1997; 38:705–715. [PubMed: 9315980]

59. Xiao-Mian S, Jing Y, Chongxuna Z, Min L, Hui-Xing D. Study of ^{99m}Tc -TRODAT-1 imaging on human brain with children autism by single photon emission computed tomography. *Conf Proc IEEE Eng Med Biol Soc.* 2005; 5:5328–5330. [PubMed: 17281454]

Fig. 1.

Individual differences in innate stress-responsiveness predict vulnerability for pemoline-induced self-injury. (A) Most of the HR rats exhibited pemoline-induced SIB, whereas only a small proportion of the LR rats did. The HR rats also (B) spent more time injuring, (C) injured more body sites, and (D) had larger total areas of tissue injury than the LR rats did. All values expressed are group means $\pm$ S.E.M. Significant differences between HR and LR rats are depicted as follows: *, $p < 0.05$.

Fig. 2.

Individual differences in stress-responsiveness do not predict the overall expression of ancillary behaviors during pemoline treatment. (A) The LR rats exhibited significantly more pemoline-induced stereotypy than the HR rats did, but this effect was small. The (B) duration of grooming, (C) frequency of home-cage locomotor counts, and (D) duration of home-cage rearing did not differ between the HR and LR rats. All values expressed are group means $\pm$ S.E.M. Significant differences between HR and LR rats are depicted as follows: *, $p < 0.05$.

Table 1

Correlations between scores for novelty stress-induced locomotion and the measures of self-injury and ancillary behaviors.

stress responsiveness	SIB/ancillary behaviors	test	correlation	significance
line crossings	mean self-injurious oral contact	Pearson r	0.498	0.018, *
line crossings (rank order)	number of injured sites	Spearman ρ	0.419	0.042, *
line crossings	largest area of tissue damage	Pearson r	0.608	0.004, **
line crossings	mean stereotypy	Pearson r	-0.204	0.418, n.s.
line crossings	mean grooming	Pearson r	-0.145	0.567, n.s.
line crossings	mean home-cage locomotion	Pearson r	-0.219	0.383, n.s.
line crossings	mean home-cage rearing	Pearson r	-0.308	0.214, n.s.