The Effects of Chronic Social Stress on a Rat Model of Self-Injurious Behavior

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Self injurious behavior (SIB) is a debilitating characteristic of many developmental and genetic disorders. Common forms of SIB include self-biting, head-banging, self-punching, and skin-picking. Rats repeatedly treated with the psychostimulant drug pemoline exhibit self-biting behavior, which closely resembles the SIB seen in human self-injurers. In these clinical populations, evidence suggests that emotional stress exacerbates SIB. Therefore, the effects of chronic social stress were examined in this rat model of SIB. The type of stress used, social defeat stress (SD), is a potent processive stressor which models the type of emotional stress that a human might experience. In this study, the chronically stressed rats exhibited larger areas of tissue damage due to SIB than did non-stressed rats. There were no significant differences between the groups for self-injurious oral contact or for incidence of SIB. Thus, stress increases the severity of self-biting behavior but appears to have no effect on the time spent injuring or on whether or not SIB is initiated.

INTRODUCTION

Self-injurious behavior (SIB) is a debilitating characteristic of many developmental and genetic disorders, such as autism and Lesch-Nyhan syndrome. Common forms of SIB include self-biting, head-banging, self-punching, and skin-picking (Thompson and Caruso, 2002). The effects of SIB are devastating. For many patients, education becomes nearly impossible, as does social interaction. Patients could also potentially cause themselves severe injury, and the emotional and financial burden on caretakers is significant (Matson et al., 2006; Matson and Nebel-Schwalm, 2006). The neurochemical basis of SIB is unknown. Although some treatments exist, many of those who self-injure do not respond to any of them (Anderson and Ernst, 1994; Underwood et al., 1989).

Rats repeatedly treated with the psychostimulant drug pemoline begin to self-injure (Kies and Devine, 2004). This self-biting behavior is caused by biochemical changes in the brain, such as alterations in neurotransmitter levels and receptors induced by pemoline, which is an indirect dopamine (DA) agonist. The injury that these rats express closely resembles SIB in human populations in that it seems compulsive and is confined to specific areas (Kies and Devine, 2004). Validity for this animal model of SIB is also evidenced by individual differences in the vulnerability to self-injure that exists in both the rat and human populations (e.g., 30% of autistic children self-injure) (Kies and Devine, 2004). Additionally, the drugs that have shown therapeutic benefits in human populations also protect rats from self injuring (Muehlmann et al., 2008). In human populations, stress has been reported to exacerbate SIB (Anderson and Ernst, 1994). Also, stress cross-sensitizes with psychostimulants like amphetamine (Dietz et al., 2008), so stress likely also cross-sensitizes with pemoline. Since it seems that SIB may be a sensitization effect of pemoline (Muehlmann and Devine, 2008), we would expect an increase in SIB after repeated stress. Therefore, we explored the effects of chronic social defeat stress on SIB in the rat model. All aspects of the experiment were pre-approved by the Institutional Animal Care and Use Committee.

To produce chronic social stress, each rat was placed in an emotionally stressful environment in which a dominant male rat pins him. This type of stress, called social defeat (SD) stress, is a potent processive stressor that more closely parallels the type of emotional stress that a human would experience than would a tail shock or some other systemic stressor (Huhman, 2006). Additionally, SD stress causes a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Covington and Miczek, 2001). Self-injurious human populations show a dysregulation of stress hormones (Hessl et al., 2002; Curin et al., 2003). Another advantage of using SD stress is that rats do not habituate to its stressful effects, so it maintains its potency over the entire period, allowing for chronic stress (Dietz et al., 2008). Understanding the role that stress plays in SIB may help to develop more effective behavioral therapies and may also reveal underlying neurochemical mechanisms that can lead to improved phamacotherapies.

METHODS

Animals

Experimental Animals (Intruders). Twenty-three male Long Evans rats weighing between 150g and 175g when they arrived were pair housed during acclimation to the
facility and the stress regimen. They were given free access to standard rat chow and tap water and lived in a climate-controlled environment. Their light dark cycle provided 12 hours of light and 12 hours of dark, and the lights were turned on at 6:00 AM. The rats were housed in standard polycarbonate cages (43 cm x 21.5 cm x 25.5 cm).

**Resident Animals.** Six male Long Evans rats, after vasectomies, were each housed with a cycling female Long Evans rat in a room separate from the experimental animals. The male rats weighed 200–225g upon arrival. They were given the same access to food and water that the intruder rats were given and housed in the same types of cages. Their light/dark cycle was reversed, however, with 12 hours of dark starting at 6:00 AM. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

**Social Defeat**

The residents were housed with their females for at least two weeks before social defeat began. The residents were trained to exhibit dominant behavior. At 7:00 AM, during the residents’ dark schedule, a female was removed from her cage and the male left alone for ten minutes. Then, an intruder rat was placed inside the resident’s cage. The intruder was considered defeated if he displayed a supine posture with the resident rat on top of him for at least three seconds. After either three defeats or five minutes, whichever came first, the intruder was removed from the resident’s cage and placed into a small, double-layered wire mesh cage (10 cm x 10 cm x 15 cm). The wire cage with the intruder inside was then placed inside the resident’s cage for the remainder of the ten minute period. The wire cage allowed for continued indirect contact with the resident. Each intruder was exposed to social defeat for 12 days in a row. The schedules were made so that each intruder saw each resident only twice and only after six days. The rats that were part of the non-stressed group were handled for two minutes each for every day of social defeat.

**Drug Treatment**

Pemoline (2-amino-5-phenyl-1,3-oxazol-4-one; Spectrum Chemicals, New Brunswick, New Jersey) was suspended at a concentration of 50 mg/ml in peanut oil. The pemoline and peanut oil were left stirring overnight to make sure the pemoline was in suspension.

The day following the last day of social defeat, the rats were each weighed and injected with 150 mg/kg of pemoline each morning for five days. The injections were administered subcutaneously on either flank, changing sides each day.

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**Assessing Self Injury and Stereotopy**

Each rat was shown to a camera every morning before injections and every afternoon to record any injury (denuded skin, erythema, edema, or open lesion). Any rat with an open lesion was immediately euthanized. Images from the video allowed the computer program MCID software (Imaging Research Inc., St. Catherines, ON, Canada) to evaluate the size of the injury in mm².

The rats were videotaped in five-minute segments every three hours throughout the duration of pemoline treatment to get a random, representative sample of their behaviors throughout the day and night. A trained observer then quantified the behaviors. Any oral contact that stayed on the same part of the body for more than two seconds counted as self-injurious oral contact. Grooming was considered to be sustained oral contact that did not focus on the same part of the body for more than two seconds. Stereotyped behavior was considered to be head bobbing or licking of the cage floor. The duration of each behavior was summed over the day and divided by 2400 seconds, the total number of seconds recorded in a day.

**Statistics**

Repeated measures analyses of variance (RM-ANOVA) was used to determine between group differences in self-injurious oral contact, size of tissue damage, and stereotypy, which were treated as statistically reliable when the p-values were less than 0.05. All significant effects were further analyzed with pre-planned Fisher’s least significant difference (LSD) post-tests. Missing data from the four rats that were euthanized before the conclusion of the experiment were replaced by repeating the final datum obtained from each rat for each measure.

**RESULTS**

Rats that are repeatedly injected with pemoline exhibit self-biting behavior (Figure 1A). All rats, regardless of past SD stress exposure, exhibited SIB in response to this dose of pemoline (150 mg/kg) (Figure 1A), indicating that stress history does not affect incidence or onset of self-injury. However, rats that were exposed SD stress exhibited significantly larger areas of tissue damage due to SIB than did rats that were not exposed to SD stress [F(10,210) = 2.095, p < 0.05] (Figure 1B). This suggests that stress exposure increases the severity of SIB. Stress history also did not affect self-injurious oral contact (i.e. time spent injuring) (Figure 1C) or other pemoline-induced stereotopies (Figure 1D). Inter-observer reliability was r = 0.9647 for the duration of oral contact and r = 0.9306 for the duration of stereotypy and was determined a Pearson correlation. These results suggest that stress does not affect the onset or duration of pemoline-induced behaviors, including SIB.
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Discussion

Since all rats regardless of stress history exhibited SIB, it can be concluded that the dose of pemoline used and not the effects of stress account for the incidence of SIB. Since the stressed group of rats displayed significantly larger areas of tissue damage due to self-injury than did non-stressed rats (B), stress history did not affect the duration of self-injurious oral contact (C). Stress history did not affect other pemoline-induced stereotopies (D). All values are expressed as group means ± S.E.M. (* p < 0.05).

Evidence indicates that glutamate plays a role in pemoline-induced SIB (Muehlmann et al., 2008). The glutamatergic system is responsible for the sensitizing effects of cocaine and amphetamine (Wolf, 1998), suggesting that SIB might be a sensitized response to the effects of pemoline. The effect of stress on the severity of the self-biting behavior supports this idea, since SD stress cross sensitizes with psychostimulants (Dietz et al., 2008). Since this study indicates that sensitization and stress play an important role in SIB, future studies will focus on the role of glutamate in pemoline-induced behavioral sensitization and how it relates to the interactions between SIB and dysregulation of the HPA axis. Learning more about the neurobiological mechanisms that mediate pemoline-induced SIB can help us to develop more effective treatment strategies for human populations of self-injurers.
REFERENCES


