AD_____

Award Number: W81XWH-10-1-0333

TITLE: Self-Injurious Behavior: An Animal Model of an Autism Endophenotype

PRINCIPAL INVESTIGATOR: Darragh Devine

CONTRACTING ORGANIZATION: University of Florida
Gainesville, FL 32611

REPORT DATE: January 2012

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-01-2012	Final	15 MAY 2010 - 14 DEC 2011

4. TITLE AND SUBTITLE

Self-Injurious Behavior: An Animal Model of an Autism Endophenotype

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0333

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Darragh Devine

E-Mail: dpdevine@ufl.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Florida
Gainesville, FL 32611

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Abstract on next page.

15. SUBJECT TERMS

Autism, self-injurious behavior, neuroscience, dopamine, DARPP-32, stress, anxiety

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	75	19b. TELEPHONE NUMBER *(include area code)*

14. ABSTRACT Self-injurious behavior (SIB) is a debilitating characteristic that is prevalent in autism spectrum disorders (ASD). Although the prevalence suggests that these children are highly vulnerable, the neurological mechanisms that confer vulnerability are unexplored. We have used an animal model to examine the expression and phosphorylation of DARPP-32, a signaling molecule that integrates dopaminergic and glutamatergic signaling, since these neurotransmitter systems are thought to play an important role in the etiology of SIB. In our model, SIB is brought about by repeated administration of the psychostimulant pemoline. We titrated the dose to a level that provokes robust SIB in about 50% of the rats, and identified an innate characteristic that confers vulnerability. When we pre-screened outbred Long-Evans rats for behavioral responsiveness to mild stress, the rats that were hyper-responsive (HR) self-injured more severely than less stress-responsive (LR) rats did. This finding has important implications for emotional and neurochemical factors that may contribute to vulnerability for SIB in ASD, and suggest that these children should be tested for stress-related vulnerability to develop SIB. In addition, we investigated DARPP-32 expression and activation (phosphorylation) in pemoline-treated HR and LR rats. The HR rats exhibited the most self-injury, and they had the highest levels of DARPP-32 expression. However, the pemoline-treatment did not differentially alter expression or phosphorylation of DARPP-32 in the HR vs. the LR rats. These findings suggest that a higher innate level of DARPP-32 in striatal medium spiny neurons may enhance vulnerability for pemoline-induced SIB, but the pemoline treatment does not induce self-injury through alterations in specific DARPP-32-mediated signaling mechanisms.

Table of Contents

Page

Introduction

Autism spectrum disorders (ASD) are characterized by complex constellations of symptoms, including social, cognitive, and behavioral pathology. Perhaps the most debilitating of these is self-injurious behavior (SIB) (*1-4*). Afflicted children generally hit or bite themselves, and strike their heads or other body parts against surfaces. These behaviors carry the risk of severe physical harm, and they interfere with all educational and socializing activities (*5-7*). In addition, SIB is extremely detrimental to families and care-givers of self-injurers (*7-9*), and the national cost of specialized services is over $3 billion annually (*10*). Interestingly, autistic children exhibit individual differences in vulnerability to develop SIB. Prevalence is generally estimated at about 18-20% (*1*), and the severity of SIB is highly variable (*1, 11*). Most research focuses on assessment and intervention for factors that maintain SIB (i.e. applied behavior analysis (*6, 12-14*). But the prevalence of SIB in autism (*1*) suggests that these children are particularly vulnerable to develop the behavior disorder. Nevertheless, little attention has been paid to the neurobiological basis of SIB, and little is known about biochemical factors that confer vulnerability.

We are studying the neurobiological basis of vulnerability for SIB using a pharmacological model in rodents (*15-24*). After repeated administration of the indirect monoamine agonist pemoline (*25, 26*), about 50% of the rats self-injure (*15, 21*). This allows us to examine behavioral and biochemical differences between self-injurious and non-injurious rats that are given identical manipulations. The focus of our current research is *dopamine- and cyclic AMP-regulated phosphoprotein of 32kDa* (DARPP-32). DARPP-32 is a postsynaptic integrator of monoaminergic and glutamatergic actions, and it exerts bidirectional regulation of intracellular mediators of plasticity. When phosphorylated at threonine-34 (i.e. pThr34DARPP-32) it is a potent inhibitor of protein phosphatase-1 (PP-1), so it increases neuronal excitability. Conversely, phosphorylation of DARPP-32 at Thr75 (i.e. pThr75DARPP-32) activates it to become a potent inhibitor of protein kinase A (PKA), so it opposes neuronal sensitization (*27*). It is highly expressed in at least 96% of striatal medium spiny neurons, including striatonigral and striatopallidal projections (*28*). Thus, it is ideally situated to mediate the behavioral consequences of repeated pemoline administration, and we believe that differences in its activity may function as the linchpin of vulnerability for SIB in our model.

Body

For more than 20 years, the foremost (arguably the only) neurobiological explanation for SIB was that it is driven by D1 receptor-mediated postsynaptic supersensitivity to dopaminergic striatal inputs (*29, 30*). Although this is a widely accepted theory, the evidence is based exclusively upon behavioral pharmacology studies (SCH-23390, LY-171555, etc.), using the neonatal 6-hydroxydopamine (6-OHDA) lesion model of SIB in rats. Moreover, there are actually no changes in D1 receptor gene expression or binding in the lesioned rats (*31, 32*), and evidence from studies with MK-801 suggest the additional involvement of glutamatergic neurotransmission in both the 6-OHDA and pemoline models (*19, 33, 34*). These data suggest that the neurobiological mechanisms are more complex than originally suspected. In fact, alterations in postsynaptic sensitivity are probably mediated by signaling mechanisms that are downstream from the receptors (*35*), but these mechanisms have not been defined. Overall, progress in our understanding of the neurobiology of SIB has lagged far behind progress in other pathological behaviors (e.g. dyskinesia's, stereotypy), largely because of a paucity of research investment in this field.

In order to investigate the neurobiological basis of vulnerability for SIB, we have characterized and refined the pemoline model of SIB in rats and mice. In our model, the animals receive daily injections of a moderately high dose of pemoline for 5-6 consecutive days. A principal advantage of this approach is that the induction of SIB is gradual, so we can identify subtle

changes in behavior during the etiology of SIB. In fact, we have now reported evidence indicating that the model has of face, construct, predictive, and etiological validity (*15, 16, 18, 20, 21, 24*). Furthermore, the induction of self-injury is dose-orderly (Fig. 1) (*15*), and we have now established a threshold dose (150 mg/kg/day), at which some animals do not self-injure throughout the treatment regimen, some exhibit only mild self-injury (denuding the fur and causing slight erythema/edema), and others exhibit severe self-biting that can occasionally even produce open lesions (an IACUC-approved termination criterion is then applied). In our rats, we assayed plasma and striatal pemoline concentrations at specific times throughout the treatment regimen, and these concentrations did not differ between non-injurious and severely self-injurious animals (Fig. 2) (*16*). Thus, we found that individual animals differ in vulnerability for pemoline-induced SIB, and this vulnerability is not based upon innate differences in drug metabolism.

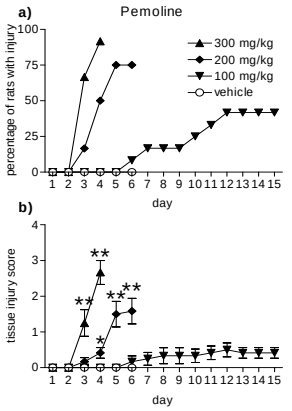

Fig. 1. Pemoline-induced self-injury was dose-orderly for incidence and severity. Values are daily a) percent of rats that had any tissue injury, and b) mean scores.

We also found that self-injurious pemoline-treated rats exhibit approximately 30% depletion in striatal dopamine content, compared with non-injurious controls (Fig. 4) (*20, 21*). This finding is redolent of postmortem findings in ASD (*36-39*) and other disorders, and raises the possibility that pemoline's actions eventually produce post-synaptic supersensitivity in our model.

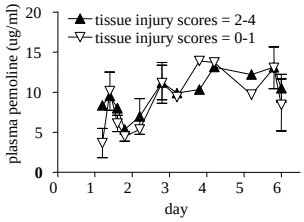

Fig. 2. Self-injurious and non-injurious rats did not differ in pemoline metabolism.

Since some rats self-injure and some are resistant when we use a moderately high dose of pemoline, we are investigating individual differences in vulnerability for pemoline-induced SIB. In one study, we adopted a well-established rodent model of individual differences in stress responsiveness (*40-43*). In this model, outbred rats are screened for responses to mild stress. Hyper-responsive (HR) rats exhibit exaggerated locomotor activation and very large increases in circulating corticosterone when compared with less-responsive (LR) conspecifics (*40, 41*). We have now discovered that individual differences in stress-responsiveness are highly correlated with vulnerability for pemoline-induced SIB. The HR rats reliably self-injure, and the LR rats are very resistant to the SIB-inducing actions of pemoline (Fig 3) (*44*). These findings are redolent of findings from clinical studies (*45-47*), and from studies with self-injurious non-human primates (*48-50*), where heightened stress responsiveness is associated with the etiology and expression of SIB.

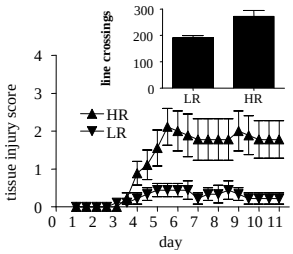

Fig. 3. HR rats exhibited more SIB than LR rats did. Values are novelty stress response (inset), and mean injury scores (main graph).

Interestingly, HR rats exhibit greater elevations in extracellular striatal dopamine concentrations than LR rats do after tail pinch stress (*51*). One possibility is that exaggerated dopaminergic activity accounts for greater striatal dopamine depletion during pemoline administration in the HR rats. This will have to be investigated further. However, the convergent evidence between our model, and the studies with human and non-human primate self-injurers suggest that stress-responsiveness is a critical feature in etiology and expression of SIB, and we now include assays of stress responsiveness in our ongoing studies.

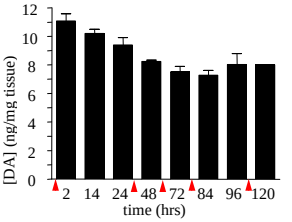

Fig. 4. Striatal [DA] declined in self-injurious pemoline-treated rats. Arrows indicate pemoline iniections.

In the current study, 36 male Long-Evans (LE) rats were screened

for individual differences in innate stress responsiveness. Each rat was placed under dim illumination into a novel environment consisting of a circular corridor (7 cm wide, 44 cm outer diameter). This constitutes a mild stressor for rats, and has been used to screen individual differences in locomotor responsiveness to the stress (i.e. crossings between equally-sized quadrants of the circular corridor during 60 minutes) (*40, 44*). The rats were then assigned to groups that were treated daily with pemoline (150 mg/kg/day) or peanut oil vehicle. The group assignments were counterbalanced by rank order of the locomotor scores (range = 113 to 363 counts, median = 225). In this study, we then analyzed the self-injury data using a median split of the pemoline-treated rats (none of the vehicle-treated rats self-injured, as expected). This analysis confirmed the previous finding. A greater percentage of the HR rats exhibited tissue injury, the tissue injury scores were greater ($F=2.599$, $p<0.01$), and the numbers of injured sites were greater ($F=2.523$, $p<0.01$) than in the LR rats (Fig. 5).

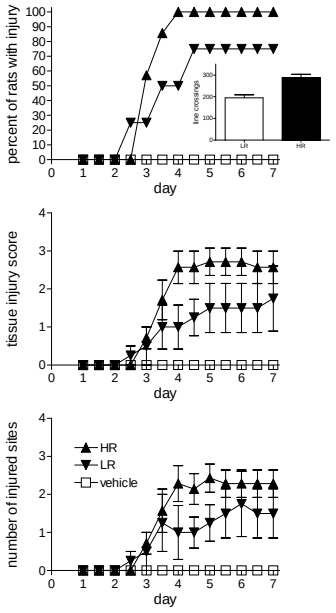

Fig. 5. A greater proportion of the HR rats self-injured, and the tissue injury scores and numbers of injured sites were greater than they were in the LR rats. Values are novelty stress response (inset), and daily percent of rats that had any tissue injury, and mean daily injury scores and numbers of injured sites

In our investigation of DARPP-32 gene expression, administration of pemoline did not specifically alter striatal DARPP-32 mRNA levels, despite the fact that it dramatically reduced concentrations of dopamine in this structure. Overall, the values in the pemoline-treated rats did not differ from those of the vehicle-treated controls. However, the levels of DARPP-32 mRNA were moderately higher in the stress hyper-responsive HR rats than they were in the less stress responsive LR rats (Fig. 6). These data concur with our finding that high levels of innate stress responsiveness confer vulnerability to develop self-injury, and implicate DARPP-32 signaling in this pathological predisposition.

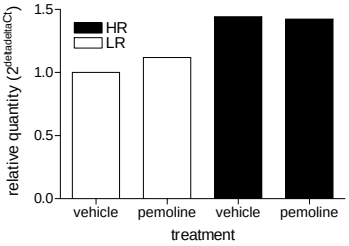

Fig. 6. DARPP-32 mRNA expression was higher in the HR rats, regardless of treatment condition.

On the other hand, the overall expression of pThr^{34}DARPP-32 and the overall expression of pThr^{75}DARPP-32 did not differ between groups, regardless of innate stress responsiveness or drug treatment (Fig. 7). This could be due to the fact that the striatum contains a heterogeneous population of cells that express D_1 and D_2 dopamine receptors. When stimulated by dopamine, the D_1 receptors signal an increase in phosphorylation at Thr^{34} (i.e. pThr^{34}DARPP-32). We had expected this effect to predominate, signaling sensitization of striatal neurons. However, during dopamine stimulation, the D_2 receptors signal a decrease in phosphorylation at Thr^{34}, and an increase in phosphorylation at Thr^{75} (i.e. pThr^{75}DARPP-32). Since the D_1 and D_2 receptors are localized on different cell populations, but those cell populations are highly intermixed, the cellular impacts of D_1 and D_2 stimulation are distinct, but the actions on D_2-expressing medium spiny neurons could obscure changes in the phosphorylation status of DARPP-32 in the D_1-expressing postsynaptic cells.

Key Research Accomplishments

- We identified that individual differences in innate characteristics of rats confer individual differences in vulnerability for pemoline-induced SIB, and we confirmed this finding by replicating it throughout the cohorts in the current studies. When we pre-screened outbred rats for responsiveness to the mild stress of a novel environment, individual rats that were

highly stress-responsive (HR) were also highly vulnerable for pemoline-induced self-injury. Less stress-responsive (LR) rats were less prone to self-injure when treated with pemoline.

- Our analyses of DARPP-32 gene expression revealed that the stress hyper-responsive HR rats had higher innate expression of DARPP-32 than did the less stress-responsive LR rats. This finding supports our hypothesis that pre-existing neurochemical characteristics of the HR rats render them particularly vulnerable to develop self-injurious behavior. The stress hyper-responsive HR rats appear to be predisposed to exhibit a higher level of postsynaptic signaling through this mechanism.
- Our analyses of DARPP-32 phosphorylation did not confirm our hypothesis that the self-injurious HR rats would have higher overall expression of pThr^{34}DARPP-32 and lower overall expression of pThr^{75}DARPP-32 during pemoline treatment. In fact, the overall phosphorylation status of DARPP-32 did not differentiate the HR from the LR rats in either control or pemoline-treated conditions. It remains possible that the outcome derives from the fact that there are two intermixed populations of medium spiny neurons in the striatum that could respond differently to alterations in dopaminergic signaling. One population expresses D_1 dopamine receptors that enhance pThr^{34}DARPP-32. The other population expresses D_2 dopamine receptors that oppose Thr^{34}DARPP-32 phosphorylation, and favor pThr^{75}DARPP-32. Potential treatment-induced increases in pThr^{34}DARPP-32 in D_1-receptor expressing medium spiny neurons could be masked by counterbalancing decreases in pThr^{34}DARPP-32 in D_2-expressing cells. Likewise, treatment-induced decreases in pThr^{75}DARPP-32 in D_1-expressing cells could be masked by counterbalancing increases in pThr75DARPP-32 in D_2-expressing cells
- Potential methods to address this unresolved question could include:
  1) use of flow cytometry to separate the striatal cells, but this approach could be compromised by difficulties in cell separation
  2) use of transgenic mice to alter the balance of D_1 and D_2 signaling
  3) use of transgenic mice or viral vectors to evaluate vulnerability in animals that express constitutively active or dominant negative isoforms of DARPP-32

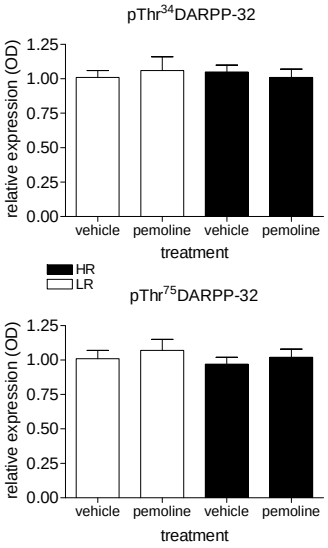

Fig. 7. DARPP-32 phosphorylation status did not differ between groups, regardless of stress responsiveness or drug treatment condition.

Reportable Outcomes

manuscripts

1. Muehlmann, A.M., Kies, S.D., Turner, C.A., Wolfman, S., Lewis, M.H., and Devine, D.P. (in press). Self-injurious behavior: Limbic disregulation and stress effects in an animal model, *Journal of Intellectual Disabilities Research.* (Special issue on self-injurious behavior)

2. Reynolds, S., Millette, A., and Devine, D.P. (in press). Sensory and motor characterization in the post-natal valproate rat model of autism, *Developmental Neuroscience*

3. Muehlmann, A.M., Wilkinson, J.A., and Devine, D.P. (2010). Individual differences in vulnerability for self-injurious behavior: Studies using an animal model, *Behavioural Brain Research*, **217:** 148-154.

abstracts

1. Weinstock, N.J. and Devine, D.P. (2011) A mouse model of pemoline-induced self-injurious behavior. *Society for Neuroscience Abstracts,* **37:** 776.11

2. Van Matre, A.M., Wolfman, S., and Devine, D.P. (2010). Neurotensin plays a modulatory role in self-injurious behavior: biochemical analyses using an animal model, *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** 29.

invited presentations

1. Devine, D.P. (2011). Disregulation of dopamine and glutamate neurotransmission in animal models of self-injurious behavior, *Experimental Biology 2011,* S369.

2. Devine, D.P. (2010). The role of stress-responsiveness in self-injurious behaviors: Biochemical studies in an animal model, *First International Congress on Borderline Personality Disorder*, **1:** S-031.

3. Devine, D.P. (2010). Biochemical factors that confer individual differences in vulnerability for self-injurious behaviour, in symposium on “Behavioral and biological frontiers in the analyses of self-injurious behavior”, *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** Symposium 13.

funding

Recent attempts to extend funding for this project include an R03 application for a study using mice with mutant forms of Pp1r1b gene, and a related project submitted to the Autism Speaks Foundation. Neither has been successful to date.

I have obtained a small grant from the Woodruff Foundation (September 2011-Sept 2012) to investigate the potential contribution of glucocorticoid receptors in conferring vulnerability for self-injury in the HR rats. The title of that grant is *Self-injurious behavior: a biomarker at the interface of stress and dopamine function*.

Conclusions

The findings from the current studies reveal a clear association between innate stress responsiveness and vulnerability for self-injurious behavior. (*44*). This adds to our previous characterizations of the animal model, and further supports its validation, since elevated emotionality (particularly anxiety is strongly implicated in *expression* of SIB among autistic children (*52-54*). However, specific associations between stress responsiveness and *vulnerability* for self-injury have not been investigated adequately in autistic populations. If at-risk children could be identified early, interventions may be possible to prevent, rather than simply respond to established patterns of self-injurious behaviors.

Innate differences in DARPP-32 expression may contribute to the heightened vulnerability of stress hyper-responsive animals, but the current data do not indicate a clear role for DARPP-32 phosphorylation in sensitized behavioral responses of the animals during pemoline treatment. Additional targets should be investigated. One clear priority is expression of glucocorticoid receptors on the dopaminergic neurons. Dopaminergic neurons are regulated by glucocorticoid inputs (for review see *55*), so it will be interesting to see if individual differences in expression of these receptors contribute to the differences in vulnerability between HR and LR rats. One possibility is that greater expression of GR on dopaminergic neurons mediate an interaction with the corticosteroids to enhance pemoline-induced depletion of dopamine stores. This and other possibilities will need to be investigated further.

References

1. J. L. Matson *et al.*, *Res. Dev. Disabil.* **17**, 135 (1996).
2. F. Poustka, S. Lisch, *Acta Paedopsychiatr.* **56**, 69 (1993).
3. S. R. Schroeder, C. S. Schroeder, B. Smith, J. Dalldorf, *J. Autism. Child Schizophr.* **8**, 261 (1978).
4. L. Bartak, M. Rutter, *J. Autism. Child Schizophr.* **6**, 109 (1976).
5. J. L. Matson, N. F. Minshawi, M. L. Gonzalez, S. B. Mayville, *Behav. Modif.* **30**, 496 (2006).
6. J. L. Matson, M. Nebel-Schwalm, *Res. Dev. Disabil.* **28**, 567 (2007).
7. R. L. Findling, *J Clin. Psychiatry* **66** Suppl 10, 26 (2005).
8. K. Sarimski, *J. Intellect. Disabil. Res.* **41** (Pt 1), 70 (1997).
9. J. Bromley, E. Emerson, *J. Intellect. Disabil. Res.* **39** (Pt 4), 341 (1995).
10. National Institutes of Health Consensus Statement, *J. Autism Dev. Disord.* **20**, 403 (1989).
11. J. Rojahn, A. J. Esbensen, in *Self-Injurious Behavior: Gene-Brain-Behavior Relationships*, S. R. Schroeder, M. L. Oster-Granite, T. Thompson, Eds. (American Psychological Association, Washington, D.C., 2002) ,ch. 3, pp. 41-77.
12. J. E. Favell *et al.*, *Behav. Ther.* **13**, 529 (1982).
13. S. Kahng, B. A. Iwata, A. B. Lewin, *Am. J. Ment. Retard.* **107**, 212 (2002).
14. G. P. Hanley, B. A. Iwata, B. E. McCord, *J. Appl. Behav. Anal.* **36**, 147 (2003).
15. S. D. Kies, D. P. Devine, *Pharmacol. Biochem. Behav.* **79**, 587 (2004).
16. A. M. Muehlmann, D. P. Devine. *Soc. Neurosci. Abstr.* **33**, 799.20. (2007).
17. B. L. Blake *et al.*, *Dev. Neurosci.* **29**, 241 (2007).
18. A. M. Muehlmann, B. D. Brown, D. P. Devine, *J. Pharmacol. Exp. Ther.* **324**, 214 (2008).
19. A. M. Muehlmann, D. P. Devine, *Behav Brain Res* **189**, 32 (2008).
20. D. P. Devine, A. M. Muehlmann, in *Selbstverletzendes Verhalten bei Stressassoziierten Erkrankungen (Self-Injurious Behaviour in Stress-Associated Disorders)*, C. Schmahl, C. Stiglmayr, Eds. (Verlag W. Kohlhammer, Stuttgart, Germany, 2009) ,ch. 3, pp. 39-60.
21. A. M. Muehlmann, D. P. Devine. Keystone Symp. **C2**, 206. (2008).
22. A. M. Muehlmann, S. Wolfman, D. P. Devine. *Soc. Neurosci. Abstr.* **34**, 446.29. (2008).
23. A. M. Muehlmann, J. A. Wilkinson, D. P. Devine. *Soc. Neurosci. Abstr.* **32**, 487.17. (2006).
24. A. M. Muehlmann, S. D. Kies, C. A. Turner, M. H. Lewis, D. P. Devine, *J Intellect. Disabil. Res.*, (in press).
25. G. M. Everett, *Fed. Proc.* **35**, 405 (1976).
26. H. C. Cromwell, B. H. King, M. S. Levine, *Dev. Neurosci.* **19**, 497 (1997).
27. P. Svenningsson *et al.*, *Annu. Rev. Pharmacol Toxicol.* **44**, 269 (2004).
28. S. I. Walaas, P. Greengard, *J. Neurosci.* **4**, 84 (1984).
29. G. R. Breese, A. A. Baumeister, T. C. Napier, G. D. Frye, R. A. Mueller, *J. Pharmacol. & Exp. Ther.* **235**, 287 (1985).
30. G. R. Breese *et al.*, *Brain Res. Brain Res. Rev.* **48**, 57 (2005).
31. K. M. Dewar, J.-J. Soghomonian, J. P. Bruno, L. Descarries, T. A. Reader, *Brain Res.* **536**, 287 (1990).
32. G. E. Duncan *et al.*, *J. Neurochem.* **61**, 1255 (1993).
33. H. E. Criswell, K. B. Johnson, R. A. Mueller, G. R. Breese, *J. Pharmacol. Exp. Ther.* **265**, 1001 (1993).
34. B. H. King, D. Au, R. E. Poland, *Dev. Neurosci.* **17**, 47 (1995).
35. S. P. Sivam, S. Pugazhenthi, V. Pugazhenthi, H. Brown, *J. Neurosci. Res.* **86**, 339 (2008).
36. T. Brucke, E. Sofic, W. Killian, A. Rett, P. Riederer, *J Neural Transm.* **68**, 315 (1987).
37. M. Ernst, A. J. Zametkin, J. A. Matochik, D. Pascualvaca, R. M. Cohen, *Lancet* **350**, 638 (1997).

38. K. Jellinger, D. Armstrong, H. Y. Zoghbi, A. K. Percy, *Acta Neuropathol. (Berl)* **76**, 142 (1988).
39. P. Riederer *et al.*, *Brain Dev.* **7**, 351 (1985).
40. P. V. Piazza, J.-M. Deminière, M. Le Moal, H. Simon, *Science* **245**, 1511 (1989).
41. M. Kabbaj, D. P. Devine, V. R. Savage, H. Akil, *J. Neurosci.* **20**, 6983 (2000).
42. J. D. H. Stead *et al.*, *Behav. Genet.* **36**, 697 (2006).
43. M. M. Blanchard, D. Mendelsohn, J. A. Stamp, *Neurosci. Biobehav. Rev.* **33**, 1145 (2009).
44. A. M. Muehlmann, J. A. Wilkinson, D. P. Devine, *Behav. Brain Res.* **217**, 148 (2011).
45. C. A. Sandman, J. L. Barron, A. Chicz-DeMet, E. M. DeMet, *Am. J. Ment. Retard.* **95**, 84 (1990).
46. F. J. Symons, K. A. Sutton, C. Walker, J. W. Bodfish, *Am. J Ment. Retard.* **108**, 13 (2003).
47. A. S. Kemp *et al.*, *Psychiatry Res.* **157**, 181 (2008).
48. M. A. Novak, *Am. J. Primatol.* **59**, 3 (2003).
49. L. A. Dellinger-Ness, L. Handler, *Clin. Psychol. Rev.* **26**, 503 (2006).
50. M. D. Davenport, C. K. Lutz, S. Tiefenbacher, M. A. Novak, J. S. Meyer, *Biol. Psychiatry* **63**, 990 (2008).
51. F. Dellu, P. V. Piazza, W. Mayo, M. Le Moal, H. Simon, *Neuropsychobiol.* **34**, 136 (1996).
52. R. Sovner, S. Fogelman, *Semin. Clin. Neuropsychiatry* **1**, 105 (1996).
53. S. E. Lindauer, I. G. DeLeon, W. W. Fisher, *J. Appl. Behav. Anal.* **32**, 103 (1999).
54. W. M. A. Verhoeven *et al.*, *Pharmacopsychiatry.* **32**, 13 (1999).
55. *Ann. N. Y. Acad. Sci.* **1018**, 192 (2011).

Appendices

paper in Journal of Disabilities Research (in press); paper in Developmental Neuroscience (in press); and paper in Behavioral Brain Research (2011), 5 Abstracts, and Curriculum Vitae

JIDR Journal of Intellectual Disability Research
Published on behalf of mencap and in association with IASSID

Journal of Intellectual Disability Research doi: 10.1111/j.1365-2788.2011.01485.x

1

Self-injurious behaviour: limbic dysregulation and stress effects in an animal model

A. M. Muehlmann,[1,2] S. D. Kies,[1] C. A. Turner,[3] S. Wolfman,[1] M. H. Lewis[2] & D. P. Devine[1]

1 *Behavioral and Cognitive Neuroscience Program, Department of Psychology, University of Florida, Gainesville, Florida, USA*
2 *Department of Psychiatry, University of Florida, Gainesville, Florida, USA*
3 *Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, Michigan, USA*

Abstract

Background Self-injurious behaviour (SIB) is prevalent in neurodevelopmental disorders, but its expression is highly variable within, and between diagnostic categories. This raises questions about the factors that contribute to aetiology and expression of SIB. Expression of SIB is generally described in relation to social reinforcement. However, variables that predispose vulnerability have not been as clearly characterised. This study reports the aetiology and expression of self-injury in an animal model of pemoline-induced SIB. It describes changes in gross neuronal activity in selected brain regions after chronic treatment with pemoline, and it describes the impact that a history of social defeat stress has on the subsequent expression of SIB during pemoline treatment.

Methods *Experiment 1* – Male Long-Evans rats were injected on each of five consecutive days with pemoline or vehicle, and the expression of SIB was evaluated using a rating scale. The brains were harvested on the morning of the sixth day, and were assayed for expression of cytochrome oxidase, an index of sustained neuronal metabolic activity. *Experiment 2* – Male Long-Evans rats were exposed to a regimen of 12 daily sessions of social defeat stress or 12 daily sessions of handling (i.e. controls). Starting on the day after completion of the social defeat or handling regimen, each rat was given five daily injections of pemoline. The durations of self-injurious oral contact and other stereotyped behaviours were monitored, and the areas of tissue injury were quantified.

Results *Experiment 1* – Neuronal metabolic activity was significantly lower in a variety of limbic and limbic-associated brain structures in the pemoline-treated rats, when compared with activity in the same regions of vehicle-treated controls. In addition, neuronal activity was low in the caudate–putamen, and in subfields of the hypothalamus, but did not differ between groups for a variety of other brain regions, including nucleus accumbens, substantia nigra, ventral tegmentum, thalamus, amygdala, and cortical regions. *Experiment 2* – All the pemoline-treated rats exhibited SIB, and whereas the social defeat regimen did not alter the total amount of self-injurious oral contact or other stereotyped behaviours, it significantly increased the severity of tissue injury.

Conclusions A broad sampling of regional metabolic activity indicates that the pemoline regimen produces enduring changes that are localised to specific limbic, hypothalamic and striatal structures. The potential role of limbic function in aetiology of SIB is further supported by the finding that pemoline-induced self-injury is exacerbated by prior exposure to social defeat stress. Overall, the results suggest brain targets that should be investigated further, and increase our understanding of the putative role that stress plays in the pathophysiology of SIB.

Keywords animal model, Lesch–Nyhan syndrome, pemoline, self-injury, social defeat, stress

Correspondence: Dr Darragh P. Devine, Behavioral and Cognitive Neuroscience Program, Department of Psychology, University of Florida, PO Box 112250, Center Drive, Gainesville, FL 32611-2250, USA (e-mail: dpdevine@ufl.edu).

Introduction

Self-injurious behaviour (SIB) is a debilitating characteristic that is exhibited by individuals with a broad variety of neurodevelopmental disorders (Rojahn & Esbensen 2002). It is seen in virtually all people with Lesch–Nyhan syndrome (Lesch & Nyhan 1964; Schretlen *et al.* 2005), most people with Prader–Willi syndrome (Symons *et al.* 1999) and approximately one-third of children with autism (Matson *et al.* 1996). Interestingly, it is not a definitive trait of most diagnostic categories. Rather, expression is heterogeneous within most of these groups, but shared across diagnoses. This suggests that characteristics that are symptomatic of multiple forms of intellectual disabilities may tend to be co-morbid with self-injury, or may even predispose vulnerability for the behavioural pathology.

Clinical studies reveal that SIB is particularly prevalent in disorders where ongoing distress and pathological irritability are prominent features (Anderson & Ernst 1994; Sovner & Fogelman 1996), and emotional stress may be a key trigger for SIB (Anderson & Ernst 1994; Lindauer *et al.* 1999). Furthermore, abnormal activity of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis is a common characteristic of intellectually disabled self-injurers (Verhoeven *et al.* 1999; Sandman *et al.* 2003, 2008; Symons *et al.* 2003; Kemp *et al.* 2008). However, it is not clear if LHPA axis dysregulation is a predisposing factor, or a consequence of SIB in these studies.

The relationship between emotional stress and self-injury is also seen in captive non-human primates. Self-injurious rhesus macaques display greater emotional responsiveness than their non-injurious counterparts do (Novak 2003), and the stress of relocation to novel housing produced long-lasting increases in self-biting behaviour in these animals (Davenport *et al.* 2008). In addition, we recently reported that individual differences in innate stress responsiveness predict vulnerability for induction of self-injury in the pemoline model of SIB (Muehlmann *et al.* 2011). Despite these intriguing implications for the role of stress in aetiology and expression of SIB, remarkably little study has been conducted on the manner in which stress and self-injury interact in intellectually disabled populations.

We have conducted an extensive characterisation of the pemoline model in rats, and our studies reveal several features that converge with observations from intellectually disabled self-injurers. For example, each pemoline-treated rat targets a specific tissue site (e.g. one forepaw, but not the other), and repeatedly injures that site (Kies & Devine 2004), in a manner that resembles the stereotypic body site preferences that are seen in human self-injurers (Symons & Thompson 1997). In addition, we found that pemoline-induced SIB is diminished by risperidone, valproate and topiramate (Muehlmann *et al.* 2008), drugs that are partially effective in human self-injurers (Ruedrich *et al.* 1999; McCracken *et al.* 2002; Shapira *et al.* 2002; Accardo 2003).

We now report two studies using the pemoline model of SIB in rats. In the first study, we used cytochrome oxidase (CO) histochemistry to identify brain regions that are impacted by chronic pemoline treatment in our model of SIB. The mitochondrial CO holoenzyme catalyses the final step in a process of electron transport that is tightly coupled to oxidative phosphorylation of adenosine diphosphate (yielding the highly energetic adenosine triphosphate) (Hatefi 1985). In brain tissue, this activity is required mostly for neuronal (as opposed to glial) activity, especially membrane repolarisation (Wong-Riley 1989). In contrast to more transient markers of neuronal activity (e.g. *c-fos*), the overall level of CO provides a reliable index of *sustained* neuronal function. Therefore, it has been described as a useful marker of neuroplasticity (Konkle & Bielajew 2004).

In the second study, we explored the impact of a prior history of stress exposure on the aetiology and expression of SIB, using the *social defeat* model. In this model, an experimentally naïve male rat (the *intruder*) is placed into the home cage of a larger male conspecific (the *resident*). The territorially dominant resident characteristically pins the intruder in a supine position (Miczek *et al.* 2004; Green & Devine 2009; Marcinkiewcz *et al.* 2009). This social defeat procedure activates limbic, hypothalamic and brainstem structures that are implicated in processing of stressful stimuli in the intruder rat (Martinez *et al.* 2002), and it is thought to emulate social stressors that rats may be exposed to in their natural habitats (Huhman 2006).

Methods

Experiment 1 – cytochrome oxidase

Animals

Sixteen male Long-Evans (LE) rats weighing 150–175 g (Charles River Laboratories, Raleigh, NC, USA) were housed in a climate-controlled vivarium with a 12 h/12 h light/dark schedule (lights on at 6:00 h). 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. Starting on the first day of pemoline treatment, each rat was individually housed, in order to ascertain that any injuries were self-inflicted. All the experimental procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida, and all the procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2011).

Drugs

The indirect monoamine agonist pemoline (Fuller *et al*. 1978; Gilbert *et al*. 1978) (2-amino-5-phenyl-1,3-oxazol-4-one; Spectrum Chemicals, New Brunswick, NJ, USA) was suspended at a concentration of 50 mg/ml in warm peanut oil (held at approximately 36°C), with constant stirring.

Pemoline treatment and assays of self-injury

On each of five consecutive mornings, approximately 2 h after the lights were turned on, each rat was examined for injuries, weighed and injected. Independent groups were treated with pemoline (200 mg/kg/day, s.c.; $n = 10$) or vehicle (4 ml/kg/day, s.c.; $n = 6$). The dose of pemoline was selected on the basis of our previous studies (Kies & Devine 2004), in which we found that reliable self-injury is produced by administration of 200 mg/kg/day, whereas a higher dose (300 mg/kg/day) produced very rapid onset of more severe self-injury, and a lower dose (100 mg/kg/day) did not produce reliable SIB.

The injections were delivered to the nape of the neck or either flank on a rotating basis. The examinations consisted of visual inspection of each rat's head, forepaws, ventrum, hindpaws and tail. Each rat was assigned a tissue damage score according to the presence and extent of injuries (see the rating scale in Table 1). The placement of each self-inflicted injury and the number of sites of tissue damage were also recorded. In addition, the length of each injury was measured. Injuries on the ventrum were consistently oval, and injury on the tail (only one rat) encompassed the circumference of the tail along a portion of its length. Accordingly, the length of each injury at these sites provided an approximation of the relative sizes of injuries. Injuries on the paws were less regular in shape, so the length of the injury along the paw and up the limb was taken as an overall approximation of the extent of the injury.

Table 1 Tissue injury rating scale (adapted from Kies & Devine 2004)

Score	Severity	Description
0	No injury	No tissue damage
1	Very mild injury	Slight oedema, pink moist skin, involves small area
2	Mild injury	Moderate oedema, slight erythema, slightly denuded skin, involves medium area and/or involves multiple sites
3	Moderate injury	Substantially denuded skin, substantial oedema and erythema, large area and/or involves multiple sites
4	Severe injury	Open lesion, requires immediate euthanasia

The rats were checked again for injuries every evening, but these scores were not included in the data analysis (i.e. one score was counted per day from each rat). The evening scores (which resembled the morning scores quite closely) were used to make certain that no animal was allowed to severely injure itself overnight without intervention. In any case where an open lesion was identified (score = 4 on the rating scale), the rat expressing the open lesion was immediately euthanised.

On the final morning of the experiment (day 6, 8:00 h–10:00 h), each rat was checked again for injuries, and then rapidly decapitated. Each brain

was rapidly removed, frozen in 2-methylbutane at –40°C, and stored at –80°C.

Cytochrome oxidase histochemistry

Each brain was cryosectioned at 20 μm in the coronal plane. Sections were mounted onto microscope slides on ice, and frozen at –20°C overnight. The slides were then stained for CO histochemistry according to methods that were modified slightly from those of Gonzalez-Lima & Jones (1994). Briefly, the frozen slides were fixed with 0.5% glutaraldehyde and 10% sucrose in 0.1 M PO_4 buffer (5 min), rinsed with 10% sucrose: 0.1 M PO_4 buffer (15 min), and incubated with stirring for 60 min in the dark in pre-oxygenated 0.1 M PO_4 buffer containing 5% sucrose, 0.05% diaminobenzidine, 0.0075% cytochrome c, 0.002% catalase and 0.25% dimethyl sulfoxide. The slides were then fixed in 10% buffered formalin with 10% sucrose for 30 min, dehydrated in graded EtOH (30, 50, 70, 90, 95, 100%) and immersed in xylene (20 min). The slides were coverslipped, and optical density (OD) was quantified for all regions of interest.

Quantification of cytochrome oxidase

Digital images of the sections were captured using a *Northern Light* R95 white trans-illuminator (Imaging Research Inc.) and a Photometrics *CoolSnap* CCD camera (Roper Scientific) connected to a personal computer. Semi-quantitative densitometry was performed using MCID Basic software (Imaging Research Inc.). Regions of interest were identified with reference to a standard rat brain atlas (Paxinos & Watson 1998), and a standard sampling box was used in the data collection window for each of these regions. Bilateral OD measures were sampled from three sections per region. External background measures were taken from outside each section, used to correct each sample OD, and the measures from the six samples per region were averaged. During this procedure, the experimenter was unaware of the experimental conditions associated with any section.

Data analyses

Two of the pemoline-treated rats were euthanised on day 4, because of injury. In these cases, the missing self-injury data were replaced by repeating the final score that was attained for each dependent measure through the end of the experiment. The brains were harvested and used for the CO assays, in the same manner as the brains of the rats that completed the experiment. This strategy was used to avoid the potential that the data would over- or underestimate the outcomes if the most severe self-injurers were eliminated. One additional rat was found dead on day 4. The cause was unknown, and it was removed from the experiment.

The percentage of rats that self-injured, mean tissue injury scores, numbers of injured sites and total length of injuries were plotted across days. Between-groups differences in tissue injury scores, number of injured sites and size of injuries were each evaluated using repeated-measures analyses of variance (RM-ANOVA). Effects were treated as statistically reliable when the *P*-values were less than 0.05. All significant effects were further analysed with Bonferroni post-tests.

The corrected OD measures were compared between groups using unpaired *t*-tests for each selected brain region. Between-groups differences were treated as statistically reliable when the *P*-values were less than 0.05. In order to present the outcomes of all the 38 anatomical regions that were assayed, the averaged OD scores for the pemoline-treated rats were subtracted from the scores for the vehicle-treated rats, and the data were then graphed as difference scores.

Experiment 2 – social defeat stress

Animals

Twenty-three male LE rats weighing 150–175 g (Charles River Laboratories) were pair-housed in the same manner as the rats in Experiment 1, with a 12 h/12 h light/dark schedule (lights on at 6:00 h) and *ad libitum* food and water. These were the experimental rats and were used as the 'intruders'. An additional six vasectomised male LE rats weighing 400–450 g were pair-housed with six female LE rats weighing 200–225 g in a separate housing room with an opposite 12 h/12 h light/dark schedule (lights off at 6:00 h). These rats were used as the 'residents'.

Social defeat

Each male resident was trained to exhibit dominance behaviour by repeatedly removing the female and introducing a smaller male rat into the cage. All the residents used in this study consistently demonstrated dominance behaviour in pre tests of social dominance. Social defeat sessions with the experimental intruders were then run during the dark phase of the resident rats' light cycle, starting at approximately 7:00 h. At the beginning of each social defeat session, the female resident was removed from the home cage. Ten minutes later, an intruder rat was placed into the home cage with the resident rat. The rats were then allowed to interact for 5 min or until the intruder displayed a submissive behaviour three times. Submissive behaviour was defined as supine posture, with the resident male rat on top, for at least 2 s. After this direct interaction phase, each intruder was removed, placed into a 10 cm × 10 cm × 15 cm double-layered wire mesh cage, and returned to the home cage of the resident male. This indirect interaction phase allowed the intruder rat to be out of physical contact but still experience stressful sensory stimuli. The intruder was maintained in the wire mesh cage until 10 min had elapsed from the start of the direct interaction phase, equalising the total duration of the stress session across rats. After the 10-min social defeat session concluded, both the female resident and the intruder were returned to their respective home cages. The intruder rats were subjected to social defeat once daily for 12 consecutive days, seeing each resident twice in that period, 6 days apart. Control rats were handled for 2 min each day for 12 days to ascertain that group differences could not be attributed to additional handling stress in the defeated rats.

Drugs

Pemoline was suspended at a concentration of 50 mg/ml in peanut oil. In order to get the pemoline into suspension, the solution was stirred overnight.

Pemoline treatment and assays of self-injury

Following the 12-day social defeat regimen, the rats were inspected, weighed and injected with pemoline at 150 mg/kg (s.c.) each morning for five consecutive days. In this experiment, a slightly lower dose was used than in the CO experiment, because the dose in that experiment (200 mg/kg/day) produced substantial SIB, and we anticipated that the stress exposure would exacerbate the expression of pemoline-induced SIB. These injections were administered at the nape of the neck and either flank on a rotating basis in the same manner as in Experiment 1. The inspections were conducted in the same manner as in Experiment 1, and like in Experiment 1, they were repeated in the evening.

In this experiment, the inspections were video-recorded, still images of the injured tissue were captured, and the MCID Basic software was used to outline the injured tissue, and to calculate the area of injury in mm^2. In addition, night-vision cameras were focused on the cages of the rats (one camera per cage), and 5-min time samples were recorded once every 3 h over the entire day. The duration of self-injurious oral contact and stereotypy were quantified during each videotaped interval by a trained observer. Self-injurious oral contact was defined as oral contact that stayed fixed on any specific site (e.g. forepaw) for longer than 2 s. This was differentiated from grooming, which is oral contact that continues to move along a body part or from one body part to another. The stereotypy measure is a compilation of the duration of stereotyped bobbing and licking, wherein the rat either bobbed its head or licked the side or floor of the cage repeatedly, and the duration of stereotyped digging, sniffing, or burrowing through the bedding. The duration (in seconds) of self-injurious oral contact and stereotypy were each summed over the entire day (i.e. from the eight video samples) and divided by the total number of seconds recorded.

Data analyses

Six rats were euthanised before the end of the experiment (four stressed rats and two control rats) because of injury. In these cases, the missing data were replaced in the same manner as in Experiment 1.

Between-groups differences in size of injuries, duration of self-injurious oral contact and duration of stereotypy were each evaluated using RM-ANOVA. Effects were treated as statistically reliable

when the *P*-values were less than 0.05. All significant effects were further analysed with pre-planned Fisher's least significant difference post-tests.

Results

Experiment 1 – cytochrome oxidase

Tissue injury was observed by day 4 in all the pemoline-treated rats (Fig. 1A). In the latter half of the experiment, the tissue injury scores ($F_{5,65} = 22.48$, $P < 0.0001$), number of injured sites ($F_{5,65} = 18.97$, $P < 0.0001$) and total size of injuries ($F_{5,65} = 15.63$, $P < 0.0001$) were greater than the corresponding measures in the control group (Fig. 1B–D).

Cytochrome oxidase expression was significantly lower in the pemoline-treated rats in the caudate–putamen (CPu), ventral pallidum (Fig. 2A), subregions of the septum and bed nucleus of stria terminalis (BNST) (Fig. 2B), specific hippocampal fields and hypothalamic nuclei (Fig. 2C), and the periaqueductal grey (PAG) (Fig. 2D). CO expression did not differ between the pemoline- and vehicle-treated groups in the substantia nigra, ventral tegmentum, core or shell of the nucleus accumbens septi (NAS), globus pallidus, limbic cortex, amygdaloid nuclei, subiculum, motor cortex, thalamic nuclei, or dorsal raphe (Fig. 2).

Experiment 2 – social defeat stress

All the pemoline-treated rats self-injured (Fig. 3A), and so the history of social defeat stress did not significantly affect the incidence of pemoline-

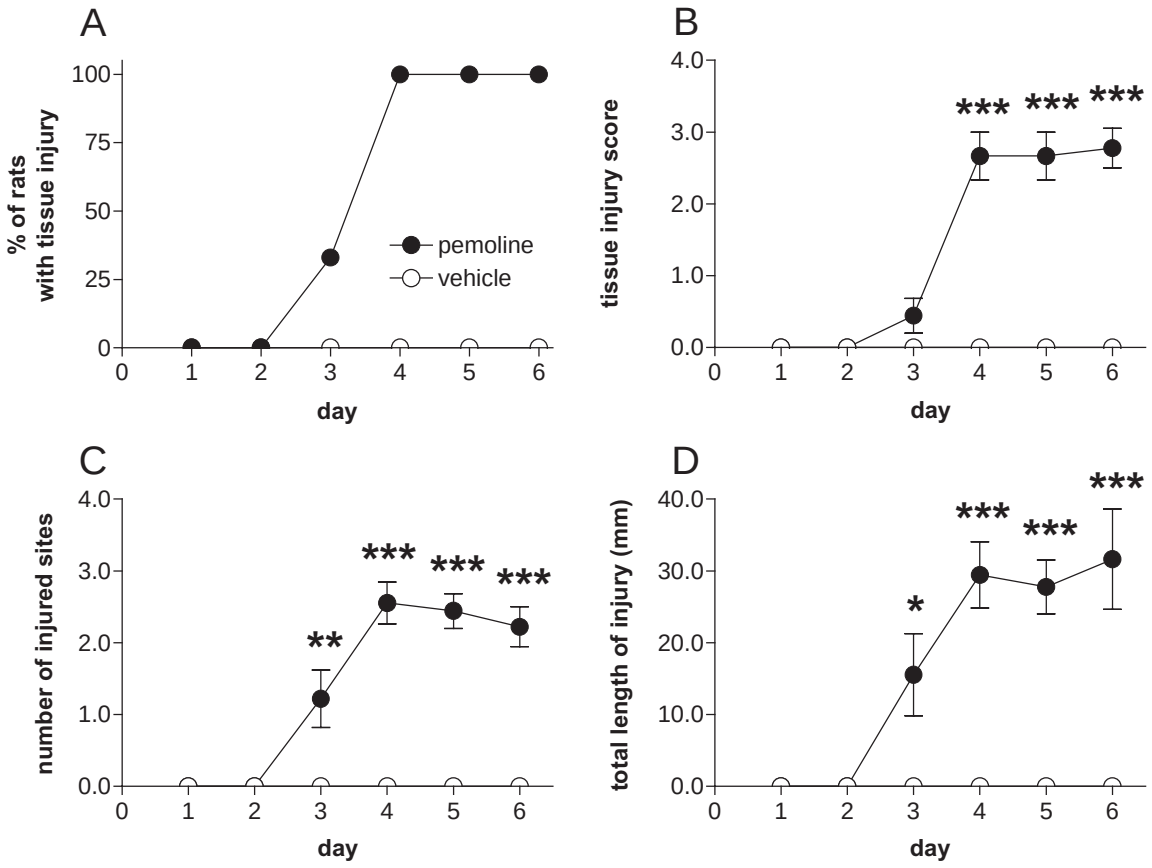

Figure 1 Incidence of pemoline-induced self-injury, tissue injury scores, number of injured sites and total length of injuries. Each measured increased across days. Self-injurious behaviour was observed in all the pemoline-treated rats, and in none of the vehicle-treated rats (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

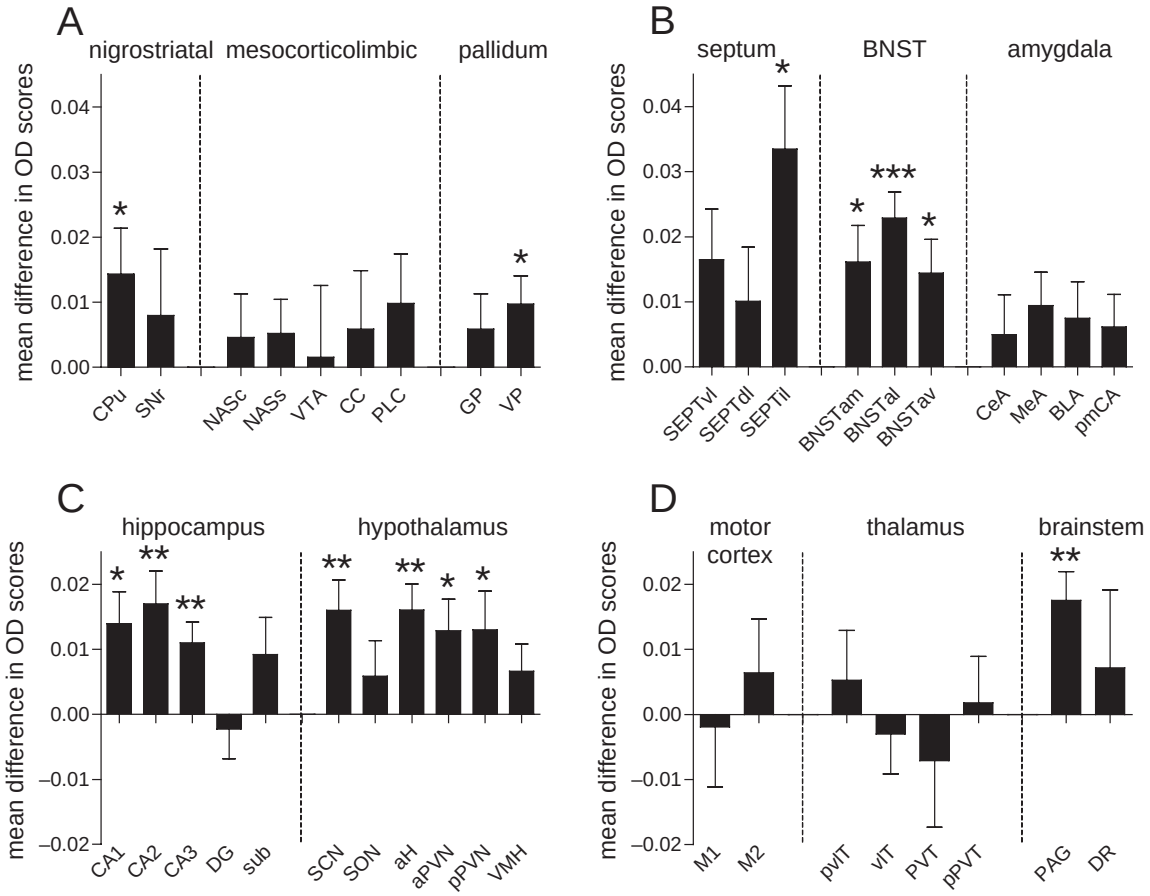

Figure 2 Cytochrome oxidase activity expressed as mean difference between the pemoline-treated and vehicle-treated rats. OD, optical density; CPu, caudate–putamen; SNr, substantia nigra (pars reticulata); NASc, nucleus accumbens septi (core); NASs, nucleus accumbens septi (shell); VTA, ventral tegmental area; CC, cingulate cortex; PLC, prelimbic cortex; GP, globus pallidus; VP, ventral pallidum; SEPTvl, ventral lateral septum; SEPTdl, dorsal lateral septum; SEPTil, interior lateral septum; BNST, bed nucleus of stria terminalis; BNSTam, anteromedial BNST; BNSTal, anterolateral BNST; BNSTav, anteroventral BNST; CeA, central amygdala; MeA, medial amygdala; BLA, basolateral amygdala; pmCA, post-medial cortical amygdala; CA1–CA3, cornu ammonis 1–3; DG, dentate gyrus; sub, subiculum; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; aH, anterior hypothalamus; aPVN, anterior paraventricular nucleus; pPVN, posterior paraventricular nucleus; VMH, ventromedial hypothalamus; M1 and M2, M1 and M2 motor cortex; pvlT, posterior ventrolateral thalamus; vlT, ventrolateral thalamus; PVT, paraventricular thalamus; pPVT, posterior paraventricular thalamus; PAG, periaqueductal grey; DR, dorsal raphe. All values are expressed as group means ± SEM (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

induced SIB. However, the sizes of the injuries were significantly greater in the rats that had a history of social defeat stress (Fig. 3B). The RM-ANOVA revealed a significant main effect of time ($F_{10,210} = 39.91$, $P < 0.01$) and a significant stress × time interaction ($F_{10,210} = 2.095$, $P < 0.05$), wherein rats first displayed injured tissue around day 2 or 3, and the size of injured tissue reached asymptote on day 4 or 5. There were no significant differences in the amount of time spent in self-injurious oral contact between stressed and control rats (Fig. 3C). There was a significant main effect of time ($F_{4,84} = 46.47$, $P < 0.01$) as all the rats began to show self-injurious oral contact on day 2, which peaked on day 3, and continued throughout the experiment; however, there was no significant main effect of stress nor a stress × time interaction effect. There were also no differences in the duration of other pemoline-induced stereotypies (Fig. 3D). The RM-ANOVA revealed a significant main effect of time

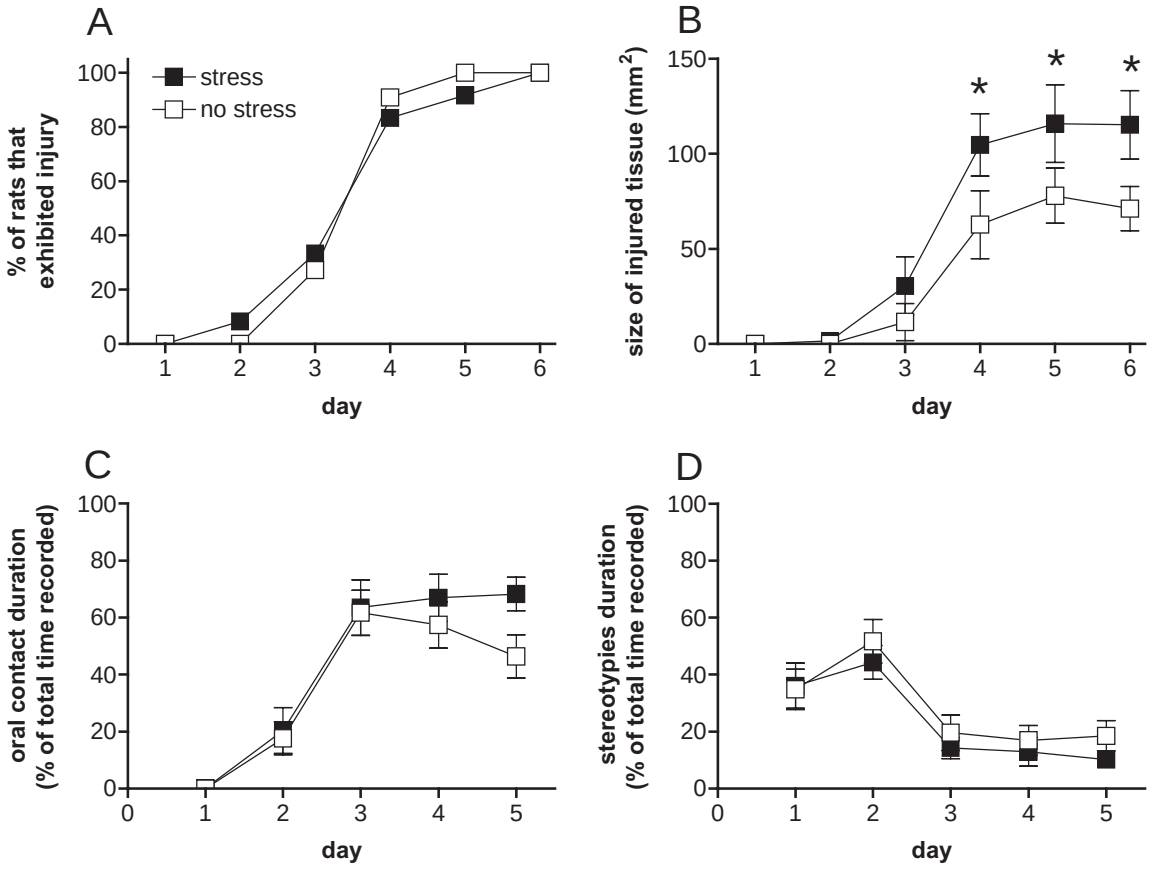

Figure 3 Effects of repeated social defeat stress on pemoline-induced self-injury and stereotypy. All the rats exhibited pemoline-induced self-injury (A); however, the rats with a history of repeated social defeat stress had larger areas of tissue damage (B). Repeated social defeat stress did not have any significant effect on pemoline-induced self-injurious oral contact (C) or whole body stereotypies (D). All values are expressed as group means ± SEM (*$P < 0.05$).

($F_{4,84} = 14.35$, $P < 0.01$), as all the rats showed moderate amounts of stereotypy on days 1 and 2, which lessened on days 3–5 as self-injurious oral contact duration increased. No significant main effect of stress or a stress × time interaction effect was found.

Discussion

In the CO histochemistry study, all the pemoline-treated rats exhibited self-injury, whereas none of the vehicle-treated rats injured (as expected). The anatomical assays revealed that sustained metabolic activity was suppressed in many of the 38 structures that were sampled from the self-injurious pemoline-treated rats, when compared with measures from the vehicle-treated rats. This suppression of neuronal activity was significant in one structure of the basal ganglia, and in a variety of limbic and limbic-associated structures. Although CO histochemistry provides only a gross characterisation of the regional effects of pemoline, it raises the possibility that direct or indirect impacts of the pemoline regimen in these regions may be associated with the induction of SIB.

Chronic pemoline treatment produced a statistically significant, but modest reduction in neuronal activity in the CPu, and the effect was not significant in the NAS or frontal cortex. The paucity of effects in the striatum and frontal cortex may seem surprising, as pemoline is an indirect monoamine agonist (Fuller *et al.* 1978; Gilbert *et al.* 1978), and

these regions are targets of extensive dopaminergic innervation (Graybiel & Ragsdale 1979). Moreover, all measures of dopamine function were decreased in samples of CPu and NAS taken from individuals with Lesch–Nyhan syndrome (Lloyd *et al.* 1981; Ernst *et al.* 1996), and these biomarkers are generally ascribed a critical role in the aetiology of SIB in both Lesch–Nyhan syndrome (Schretlen *et al.* 2005) and animal models of the disorder (Breese *et al.* 1984). However, postsynaptic neurons expressing excitatory D_1 and inhibitory D_2 dopamine receptors are intermingled in the striatum and frontal cortex (Gaspar *et al.* 1995; Le Moine & Bloch 1995), so pemoline-stimulated dopamine efflux would have opposing actions on these cells. As CO is most responsive to increases in membrane repolarisation (Wong-Riley 1989), the opposing actions of dopamine on D_1- and D_2-expressing cells could obscure the physiological effects of pemoline in these parts of the rat brain. CO histochemistry may be better suited to examine indirect impacts of pemoline on neurons that are downstream from these initial effects.

The other sites that exhibited significant suppression of neuronal metabolic activity were the ventral pallidum, interior lateral septum, BNST, subfields of the hippocampus, hypothalamic nuclei, and the PAG. On the other hand, other septal nuclei, dentate gyrus and subiculum, and the amygdala were not significantly impacted by chronic pemoline treatment. Thus, it seems that neuronal activity was suppressed only in a select subset of limbic-associated structures. Neuronal activity was not systematically altered in motor cortex or thalamus, suggesting a lack of involvement of these structures. Overall, the limbic/hypothalamic structures that exhibited significant pemoline-induced changes in neuronal metabolic activity, and the nigrostriatal and mesocorticolimbic systems, should each be examined more closely. The potential that these structures participate in the induction of SIB, and the specific biochemical alterations that occur in these structures require further analysis.

The suggestion that limbic structures may participate in pemoline-induced SIB is supported by evidence that emotional stress contributes to the *expression* of SIB in clinical populations (Favell *et al.* 1982; Anderson & Ernst 1994; Lindauer *et al.* 1999) and animal models (Stodgell *et al.* 1998; Davenport *et al.* 2008). For example, escape from or avoidance of aversive situations (i.e. stressors) is recognised as a basic communicative function that drives SIB in intellectually disabled self-injurers (Favell *et al.* 1982), and footshock stress increases the expression of SIB in the neonatal 6-hydroxydopamine (6-OHDA) lesion model in rats (Stodgell *et al.* 1998). These observations led us to examine whether a background of emotional distress might influence the *aetiology* of SIB in the pemoline model. We selected the social defeat model of emotional stress because it activates the aforementioned limbic, hypothalamic and brainstem mechanisms (Martinez *et al.* 2002). Additionally, repeated social defeat increases basal plasma corticosterone concentrations (Covington & Miczek 2001), and this is consistent with reports that LHPA axis function is dysregulated in intellectually disabled self-injurers (Verhoeven *et al.* 1999; Sandman *et al.* 2003, 2008; Symons *et al.* 2003; Kemp *et al.* 2008) [although it should be noted that both elevated and suppressed basal corticosterone concentrations have been found (Verhoeven *et al.* 1999; Symons *et al.* 2003)].

In our study of the impact of social defeat on subsequent acquisition of pemoline-induced SIB, all the pemoline-treated rats self-injured, regardless of whether they were pre-exposed to social defeat stress or not. Therefore, it was not possible to see any stress-induced increase in the incidence of self-injury. Likewise, there were no significant differences in the daily durations of self-injurious oral contact – perhaps also owing to a ceiling effect. However, the severity of tissue injury was significantly greater in the previously stressed rats, as indicated by the overall sizes of injured tissue in these rats. Thus, a history of psychosocial stress appears to contribute to the aetiology of SIB. When considered along with our recent finding that stress-hyper-responsive rats are particularly vulnerable for pemoline-induced SIB, the results indicate that stress reduction may be an important intervention in populations that are at risk for development of SIB. These findings may also have implications for the role that impoverished institutional environments have played in the aetiology of SIB among the intellectually disabled.

Studies of the brain structures that are impacted by social defeat may also help to focus the search for neuroanatomical substrates that contribute to the aetiology of SIB. Interestingly, *c-fos* expression is

activated in many limbic, hypothalamic and brainstem structures after acute defeat. The response habituates in some of these structures (e.g. septum, lateral hypothalamus, central amygdala), but enduring effects are found in others (BNST, paraventricular nucleus, medial amygdala, PAG, raphe) (Martinez *et al.* 1998). As social defeat stress enhanced the severity of self-injury in rats that were subsequently treated with pemoline, the brain regions in which there is overlap between the persistent actions of social defeat, and the brain regions where pemoline produces reductions in sustained neuronal activity may be particularly important in mediating the contribution of stress to the aetiology of pemoline-induced SIB. These areas (BNST, paraventricular nucleus and PAG) may be particularly interesting targets for additional investigation.

References

Accardo P. (2003) Risperidone in children with autism and serious behavioral problems. *Journal of Pediatrics* **142,** 86–7.

Anderson L. T. & Ernst M. (1994) Self-injury in Lesch-Nyhan disease. *Journal of Autism and Developmental Disorders* **24,** 67–81.

Breese G. R., Baumeister A. A., McCown T. J., Emerick S. G., Frye G. D. & Mueller R. A. (1984) Neonatal-6-hydroxydopamine treatment: model of susceptibility for self-mutilation in the Lesch-Nyhan syndrome. *Pharmacology, Biochemistry, and Behavior* **21,** 459–61.

Covington H. E. III & Miczek K. A. (2001) Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration 'binges'. *Psychopharmacology* **158,** 388–98.

Davenport M. D., Lutz C. K., Tiefenbacher S., Novak M. A. & Meyer J. S. (2008) A rhesus monkey model of self-injury: effects of relocation stress on behavior and neuroendocrine function. *Biological Psychiatry* **63,** 990–6.

Ernst M., Zametkin A. J., Matochik J. A., Pascualvaca D., Jons P. H., Hardy K. *et al.* (1996) Presynaptic dopaminergic deficits in Lesch-Nyhan disease. *New England Journal of Medicine* **334,** 1568–72.

Favell J. E., Azrin N. A., Baumeister A. A., Carr E. G., Dorsey M. F., Forehand R. *et al.* (1982) The treatment of self-injurious behavior. *Behavior Therapy* **13,** 529–54.

Fuller R. W., Perry K. W., Bymaster F. P. & Wong D. T. (1978) 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. *Journal of Pharmacy and Pharmacology* **30,** 197–8.

Gaspar P., Bloch B. & Le M. C. (1995) D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. *European Journal of Neuroscience* **7,** 1050–63.

Gilbert J. C., Allen J. M., Townsend B. G. & Wyllie M. G. (1978) Drugs affecting the central nervous system: effects of pemoline and tricyclic antidepressants on nerve terminal adenosine triphosphatase activities and neurotransmitter release. *Neuropharmacology* **17,** 419–21.

Gonzalez-Lima F. & Jones D. (1994) Quantitative mapping of cytochrome oxidase activity in the central auditory system of the gerbil: a study with calibrated activity standards and metal-intensified histochemistry. *Brain Research* **660,** 34–49.

Graybiel A. M. & Ragsdale C. W. Jr (1979) Fiber connections of the basal ganglia. *Progress in Brain Research* **51,** 239–83.

Green M. K. & Devine D. P. (2009) Nociceptin/Orphanin FQ and NOP receptor gene regulation after single or repeated social defeat exposure. *Neuropeptides* **43,** 507–14.

Hatefi Y. (1985) The mitochondrial electron transport and oxidative phosphorylation system. *Annual Review of Biochemistry* **54,** 1015–69.

Huhman K. L. (2006) Social conflict models: can they inform us about human psychopathology? *Hormones and Behavior* **50,** 640–6.

Kemp A. S., Fillmore P. T., Lenjavi M. R., Lyon M., Chicz-DeMet A., Touchette P. E. *et al.* (2008) Temporal patterns of self-injurious behavior correlate with stress hormone levels in the developmentally disabled. *Psychiatry Research* **157,** 181–9.

Kies S. D. & Devine D. P. (2004) Self-injurious behaviour: a comparison of caffeine and pemoline models in rats. *Pharmacology, Biochemistry, and Behavior* **79,** 587–98.

Konkle A. T. & Bielajew C. (2004) Tracing the neuroanatomical profiles of reward pathways with markers of neuronal activation. *Reviews in the Neurosciences* **15,** 383–414.

Le Moine C. & Bloch B. (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *Journal of Comparative Neurology* **355,** 418–26.

Lesch M. & Nyhan W. L. (1964) A familial disorder of uric acid metabolism and central nervous system function. *American Journal of Medicine* **36,** 561–70.

Lindauer S. E., DeLeon I. G. & Fisher W. W. (1999) Decreasing signs of negative affect and correlated

self-injury in an individual with mental retardation and mood disturbances. *Journal of Applied Behavior Analysis* **32,** 103–6.

Lloyd K. G., Hornykiewicz O., Davidson L., Shannak K., Farley I., Goldstein M. *et al.* (1981) Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. *New England Journal of Medicine* **305,** 1106–11.

Marcinkiewcz C. A., Green M. K., Devine D. P., Duarte P., Vierck C. J. & Yezierski R. P. (2009) Social defeat stress potentiates thermal sensitivity in operant models of pain processing. *Brain Research* **1251,** 112–20.

Martinez M., Phillips P. J. & Herbert J. (1998) Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *European Journal of Neuroscience* **10,** 20–33.

Martinez M., Calvo-Torrent A. & Herbert J. (2002) Mapping brain response to social stress in rodents with c-fos expression: a review. *Stress* **5,** 3–13.

Matson J. L., Baglio C. S., Smiroldo B. B., Hamilton M., Paclawskyj T. R., Williams D. *et al.* (1996) Characteristics of autism as assessed by the Diagnostic Assessment for the Severely Handicapped-II (DASH-II). *Research in Developmental Disabilities* **17,** 135–43.

McCracken J. T., McGough J., Shah B., Cronin P., Hong D., Aman M. G. *et al.* (2002) Risperidone in children with autism and serious behavioral problems. *New England Journal of Medicine* **347,** 314–21.

Miczek K. A., Covington H. E. III, Nikulina E. M. Jr & Hammer R. P. (2004) Aggression and defeat: persistent effects on cocaine self-administration and gene expression in peptidergic and aminergic mesocorticolimbic circuits. *Neuroscience and Biobehavioral Reviews* **27,** 787–802.

Muehlmann A. M., Brown B. D. & Devine D. P. (2008) Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy. *Journal of Pharmacology and Experimental Therapeutics* **324,** 214–23.

Muehlmann A. M., Wilkinson J. A. & Devine D. P. (2011) Individual differences in vulnerability for self-injurious behavior: studies using an animal model. *Behavioural Brain Research* **217,** 148–54.

National Research Council (2011) *Guide for the Care and Use of Laboratory Animals*, National Academies Press, Washington, D.C.

Novak M. A. (2003) Self-injurious behavior in rhesus monkeys: new insights into its etiology, physiology, and treatment. *American Journal of Primatology* **59,** 3–19.

Paxinos G. & Watson C. (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th edn. Academic Press, San Diego, CA.

Rojahn J. & Esbensen A. J. (2002) Epidemiology of self-injurious behavior in mental retardation: a review. In: *Self-Injurious Behavior: Gene-Brain-Behavior Relationships* (eds S. R. Schroeder, M. L. Oster-Granite & T. Thompson), pp. 41–77. American Psychological Association, Washington, D.C.

Ruedrich S., Swales T. P., Fossaceca C., Toliver J. & Rutkowski A. (1999) Effect of divalproex sodium on aggression and self-injurious behaviour in adults with intellectual disability: a retrospective review. *Journal of Intellectual Disability Research* **43**(Part 2), 105–11.

Sandman C. A., Touchette P., Lenjavi M., Marion S. & Chicz-DeMet A. (2003) Beta-endorphin and ACTH are dissociated after self-injury in adults with developmental disabilities. *American Journal on Mental Retardation* **108,** 414–24.

Sandman C. A., Touchette P. E., Marion S. D. & Chicz-DeMet A. (2008) The role of proopiomelanocortin (POMC) in sequentially dependent self-injurious behavior. *Developmental Psychobiology* **50,** 680–9.

Schretlen D. J., Ward J., Meyer S. M., Yun J., Puig J. G., Nyhan W. L. *et al.* (2005) Behavioral aspects of Lesch–Nyhan disease and its variants. *Developmental Medicine and Child Neurology* **47,** 673–7.

Shapira N. A., Lessig M. C., Murphy T. K., Driscoll D. J. & Goodman W. K. (2002) Topiramate attenuates self-injurious behaviour in Prader-Willi syndrome. *International Journal of Neuropsychopharmacology* **5,** 141–5.

Sovner R. & Fogelman S. (1996) Irritability and mental retardation. *Seminars in Clinical Neuropsychiatry* **1,** 105–14.

Stodgell C. J., Loupe P. S., Schroeder S. R. & Tessel R. E. (1998) Cross-sensitization between footshock stress and apomorphine on self-injurious behavior and neostriatal catecholamines in a rat model of Lesch-Nyhan syndrome. *Brain Research* **783,** 10–18.

Symons F. J. & Thompson T. (1997) Self-injurious behaviour and body site preference. *Journal of Intellectual Disability Research* **41,** 456–68.

Symons F. J., Butler M. G., Sanders M. D., Feurer I. D. & Thompson T. (1999) Self-injurious behavior and Prader-Willi syndrome: behavioral forms and body locations. *American Journal on Mental Retardation* **104,** 260–9.

Symons F. J., Sutton K. A., Walker C. & Bodfish J. W. (2003) Altered diurnal pattern of salivary substance P in adults with developmental disabilities and chronic self-injury. *American Journal on Mental Retardation* **108,** 13–18.

Verhoeven W. M. A., Tuinier S., van den Berg Y. W. M. M., Coppus A. M. W., Fekkes D., Pepplinkhuizen L. *et al.* (1999) Stress and self-injurious behavior; hormonal and serotonergic parameters in mentally retarded subjects. *Pharmacopsychiatry* **32,** 13–20.

Wong-Riley M. T. (1989) Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *Trends in Neurosciences* **12,** 94–101.

Accepted 16 August 2011

Sensory and Motor Characterization in the Post-natal Valproate Rat Model of Autism

Stacey Reynolds PhD, OTR/L[1], Alexandre Millette[2] Darragh P. Devine PhD[3]

[1] Department of Occupational Therapy, Virginia Commonwealth University, Box 980008 Richmond, VA 23298; reynoldsse3@vcu.edu

[2] Department of Department of Applied Physiology and Kinesiology, University of Florida, Box 118205, Gainesville, FL 32611-8205 millette1991@hotmail.com

[3] Department of Psychology, Behavioral and Cognitive Neuroscience Program, University of Florida, Box 112250, Gainesville, FL 32611-2250; dpdevine@ufl.edu

Corresponding Author: Stacey Reynolds
Box 980008 Richmond, VA 23298
Phone: (352)273-6126
Fax: (352) 273-6042
reynoldsse3@vcu.edu

Abstract

Although autism is diagnosed according to three core features of social deficits, communication impairments, and repetitive or stereotyped behaviors, other behavioral features such as sensory and motor impairments are present in more than 70% of individuals with autism spectrum disorders. Exposure of rat pups to the teratogen valproate during sensitive periods of brain development has been shown to elicit behavioral features associated with autism diagnosis and has been proposed as a valid animal model of the disorder. The purpose of this study was to characterize sensory and motor performance in rats post-natally treated with valproate. Thirty four rat pups were injected with either valproate (150 mg/kg) or saline on post-natal days 6-12. Auditory and tactile startle as well as auditory sensory gating was assessed during both the juvenile and adolescent stages of development; motor testing was conducted during late adolescence and included a sunflower seed eating task and a vermicelli-handling task. Valproate-treated rats were under-responsive to auditory stimuli, showed deficits in auditory sensory gating, and demonstrated impairments in motor speed and performance. These findings suggest that post-natal valproate treatment elicits sensory and motor features often seen in individuals with ASD. Further, the hypo-sensitivity seen in post-natally valproate-treated rats contrasted with hyper-sensitivity previously reported in pre-natally valproate-exposed rats. This suggests that timing of teratogenic exposure during early brain development may be important to consider when investigating the neurobiological basis of sensory-motor impairments in ASD.

Sensory and Motor Characterization in the Post-natal Valproate Rat Model of Autism

1. Introduction

Autism is a neurodevelopmental disorder characterized by three core diagnostic features: social deficits, communication impairments, and repetitive or stereotyped behaviors. Associated autistic characteristics such as sensory processing impairments and deficits in motor coordination are highly prevalent, yet have received less attention from the research community. As scientists continue to study patterns of symptom manifestation and the developmental course of the disorder, sensory and motor features may be particularly important to consider in both human and animal paradigms.

1.1 Sensory and Motor features of Autism Spectrum Disorders

Atypical responses to sensory stimulation have been reported in approximately 70-90% of individuals with autism spectrum disorders (ASD) [1, 2]. Behavioral manifestations of atypical sensory processing have been most commonly classified as over-responding (hypersensitivity) and under-responding (hyposensitivity) [3] with both patterns being identified in the ASD population [1, 4]. Further, these patterns of over or under responding appear to be strongly associated with other autism related behaviors and functional performance in this population.

Tactile over-responsivity in particular has been associated with rigid and inflexible behavior, repetitive verbalizations, and visual stereotypies [5]. Over-responsivity in or across other sensory systems (e.g. visual & auditory) have also been found to predict high levels of stereotypies, compulsions, and rituals/sameness behaviors in children with autism; suggesting that over-responsivity and repetitive behaviors may share a common underlying neurobiology [6]. Similarly, Liss and colleagues [7] found that over-responsive children with ASD tended to have high over-focusing scores and high rates of perseverative behavior. These authors suggest that over-responsivity and repetitive behaviors may both be related to deficits in arousal regulation (over-arousal) which may lead to over-selective attention to sensory stimuli, perseverative preoccupations and repetitive movements. While the arousal theory has not been fully explored, sensory over-responsivity has been strongly associated with anxiety in children and adolescents with Asperger's syndrome [8], adults with autism [9], and in children with other neurodevelopmental disorders [10]. Elevated sympathetic arousal (electrodermal reactivity) in response to normal levels of sensory stimulation has also been shown to predict sleep deficits in children with autism, and behavioral patterns of over-responding have been strongly correlated with sleep problems in this population [11].

Whereas sensory under-responsivity has been commonly reported in ASD [12], it has been less well studied than over-responsivity. Under-responsive behaviors demonstrated by children with autism include not responding to their name being called, lacking attention to novel objects, ignoring loud sounds, and not responding to touch/pain stimuli [1]. Patterns of sensory under-responsivity have been associated with impaired academic performance, poor attention to cognitive tasks and social skill deficits in children with ASD [13, 8]. Liss and colleagues [7] found under-responsivity clustered with low adaptive functioning in children with autism, and suggested that under-responsivity may be related to low intellectual functioning in this

population. Interestingly, academic performance and social interactions are areas that can be affected by depression [14]. In children and adolescents with ASD under-responsiveness has been correlated with depressive symptoms, with the strength of the relationship between under-responsivity and depression increasing with age [8].

Similar to sensory processing impairments, motor impairments are highly pronounced in individuals with ASD, with multiple studies indicating that 100% of their sample population show impairments in at least one area of motor development [15-17]. Gross motor clumsiness and impairments in gait and balance have been commonly reported in both children and adults with ASD [18]. In addition to a delayed onset of walking, toddlers with autism have also been shown to lack a mature heel-toe pattern and have a more waddling gait compared to age-matched controls [19]. These delays may be related to the high prevalence of hypotonia in ASD, which is present in approximately 50% of children with the disorder [20]. Fine motor deficits and impairments in upper limb coordination have also been commonly reported in the ASD population. Gernsbacher and colleagues [21] showed that in the first two years of life, fine motor skills such as pointing, clapping, reaching and constructional play were delayed in children later diagnosed with ASD. Older children and adolescents with autism have been shown to have difficulty with modulation of grip and force and with accuracy in reach-to-grasp movements that are needed for tasks such as shoe tying and buttoning [22, 23]. Though a fair degree of variability in performance has been reported, gross and fine motor coordination problems have been associated with reduced performance in daily life skills, play (e.g. running, throwing, jumping) and academic tasks such as handwriting in children with ASD, making these important behaviors to consider for this population [24, 25].

Interestingly, atypical sensory and motor features have been found to be salient early markers for ASD, identifiable as early as four months of age [26, 27]. While the cause of atypical sensory and motor features in autism is not known, their manifestation early in life suggests that exploration of factors inducing neurological changes during early brain development is warranted.

1.2 Teratogenic Effects of Valproate

Exposure of the developing brain to teratogens is known to produce a myriad of symptoms and disorders that influence sensory and motor function. Valproate use during pregnancy (used by mothers as an anti-epileptic, mood-stabilizing drug or migraine medication) has been associated with a higher prevalence of autism and autistic-linked behaviors in offspring [28, 29]. Whereas autism prevalence in the typical population has been estimated around 1% [30], Rasalam and colleagues [28] found that 8.9% of children exposed to valproate met the criteria for either autistic disorder or Asperger syndrome; this suggests that exposure to valproate leads to approximately a 10 fold increase in the risk for developing an autism spectrum disorder. While sensory and motor behaviors in children exposed to valproate have not been fully characterized, case study reports lend insight into features of that may be of particular relevance to this population. Williams and Hersh [31] described a 5 ½ year old boy exposed to valproate in utero and later diagnosed with autism. In addition to meeting core diagnostic criteria for autism, the child presented with muscular hypotonia, awkward gait, hyper-reflexia, and was reported to

cover his ears in response to hearing certain sounds. He also had insistence on routines connected with bathing and eating; features common in children with sensory over-responsivity. In a larger case report, Williams and colleagues [32] presented five children with both fetal valproate exposure and autism. In each of these cases children presented with some form of motor impairment including decreased muscle tone, gross and fine motor incoordination, and atypical gait; sensory responsivity was not described in this group.

The strong association between valproate exposure in utero and the development of autism led Rodier and colleagues [33] to explore the effects of prenatal valproate treatment in rats. Since this publication, multiple studies have been published using pre- or post-natal valproate treatment to elicit neurological and behavioral features of autism in rodent models. The use of this valproate rodent model has potential utility for understanding the development and neurobiological basis of sensory and motor features seen in ASD.

1.3 The Valproate Model of Teratogenicity in Rodents

A single pre-natal injection of valproate on embryonic day 12.5 (E12.5; corresponding to human 1st trimester following neural tube closure) has been shown most consistently to elicit autistic-like features such as repetitive behaviors and decreased social interaction in rodents [34]. Neurological features observed in rats treated with valproate on E12.5 are similar to those seen in post-mortem ASD human brains; these features include loss of cerebellar neurons [35] and altered dendritic arborization in pyramidal cells suggestive of impaired pruning [36]. These valproate-exposed rats also show signs of tactile hypersensitivity during pre-pubertal adolescence on post-natal days (PND) 30-50, and impaired auditory sensory gating during adolescence and adulthood (PND 90-120) [37, 38]. Impairments in sensory gating have been proposed to lead to sensory overload and strong reactions to environmental stimuli [37].

Evaluation of motor skills in this model is limited. Schneider and Przewłocki [38] found impaired swimming performance in pre-natally valproate-exposed rats on PND8 and PND12; however, no delays were noted on PND10 or PND11. Wagner and colleagues [39] found that mice pre-natally exposed to valproate had impaired righting reactions in the early juvenile stage but found no differences in vestibular function (negative geotaxis test) or motor development. Tests of fine motor function have not been assessed in this model.

Valproate has also been administered to rodents during the early post-natal period (PND 0-14). Since rodents are born in an altricial state, the status of their developing neurological sensory systems roughly correspond with features associated with human brain development in the third trimester. For example, in the rodent auditory system, it has been shown that the cochlea matures and hearing onset occurs around PND11, and that bone conduction-related events can be measured as early as PND7 [40]. In humans, auditory responses can be evoked in utero as early as the 27th prenatal week, around the beginning of the third trimester [41, 42]. Further, general patterns of brain development, such as the brain growth spurt that occurs near the end of the third trimester in humans, occurs between PND1-10 in rats [43, 44] supporting the notion that brain development in the final human trimester corresponds roughly to the first 1-2 weeks of rodent post-natal development [45, 46].

During this early rodent post-natal period there is also a high degree of synaptogenesis and refinement of neurological connections, which is dependent on a balance between cellular excitation and inhibition [47]. Since valproate enhances inhibitory GABAergic activity [48, 49], it has the potential to disrupt brain development during this period. Similar to pre-natal injections, post-natal valproate injections in rats have been shown to elicit autistic-like behaviors. Chomiak and colleagues [50] administered single doses of valproate (150 mg/kg) to rat pups for a maximum of two weeks starting at PND 6; these rats showed a significant reduction in social play compared to litter-matched controls. Further, the post-natally valproate-treated rats showed an enlarged temporal association cortex and a temporarily accelerated pattern of neuronal development in the temporal lobe; these findings correspond to research indicating enlargement of temporal lobe regions in individuals with autism [51, 52]. In mice, post-natal injection of valproate has been shown to impair vestibular function and mid-air righting reactions [39]. To our knowledge, no tests of sensory responsivity or skilled motor coordination have been conducted in the rodent post-natal valproate model. The purpose of this study, therefore, was to characterize sensory responsivity, sensory gating, and skilled motor performance in rats post-natally treated with valproate.

2. Methods

2.1 Animals

Thirty-four Long-Evans rats (24 male, 10 female) were bred in our lab using four experimentally-naïve females. On PND3, four litters were cross fostered so there were 8-9 pups per litter. The same day, the gender of each animal was established and each pup was randomly assigned to either the valproate or control group. To the best of our ability, each group had the same number of males (11 control, 13 valproate-treated) and females (5 control, 5 valproate-treated), and the same number of pups from each litter. Every day from PND6 - PND12 the offspring received an i.p. injection of saline (control) or sodium valproate dissolved in 0.9% saline at 150 mg/kg/day [50]. The weight of the pups was monitored daily. Eye opening was recorded on PND13-PND16 and scored as either 0=Both Eyes Closed, 1=One Eye Open, or 2=Both Eyes Open. Pups were weaned on PND23 and pair-housed with animals matched by group and gender.

2.2 Measures

2.2.1 Sensory Responsivity and Gating

Sensory testing was conducted for each rat (n=34) on PND23 (juvenile stage, prior to weaning) and PND45 (adolescence) during the first five hours of the light part of the daily light cycle. Auditory startle responses, tactile startle responses, and auditory sensory gating were measured using SR-LAB equipment (San Diego Instruments, San Diego, CA). The background sound level in the chamber was 70dB. Prior to the testing, each rat was acclimated to the test chamber for five minutes. Each rat was then exposed to 20 consecutive trials of the auditory startle stimuli which were bursts of white noise (10 KHz, 120 dB/40 milliseconds) presented at an average inter-trial interval of 10 seconds (variable 5-15 second range). At the end of the 20 trials a no-stimulus trial was presented. The auditory startle session was followed by a two

minute break with background level sound (70dB) only. Next, each rat was exposed to 20 consecutive trials of an auditory prepulse (80dB/20 milliseconds) paired with the startle stimulus (120 dB/40 milliseconds). Frequency for the prepulse and stimulus was set at 10KHz and there was a 100ms interval between the prepulse and the stimulus. Each rat was exposed to 20 trials followed by a no-stimulus trial. The average inter-trial interval was 10 seconds. Following another 2 minute break, each rat was exposed to 20 consecutive trials of the tactile startle stimulus which was a gentle air puff (PSI=20; lasting ~40 milliseconds) delivered to the mid-dorsal surface. Again, the inter-trial interval was 10 seconds and the 20 trials were followed by a single no-stimulus trial. The primary variable of interest for each session was the peak response magnitude (V-Max) which was defined as the highest voltage during the response window.

2.2.3 Sunflower Seed Task

Grasp and bilateral manual coordination was assessed using a sunflower seed eating task [53] on PND49. During the week prior to testing, all rats were exposed to sunflower seeds in their home cages (5 days of exposure, 3 seeds each time) in order to overcome neophobic responses and establish skill in handling [54]. Each rat was then acclimated to a clear plastic arena (14” x 11” x 11”) for five minutes one day prior to testing. Each rat was partially food restricted (8-10g/day/rat) for one night prior to testing to increase motivation for task performance. On the morning of the test, three sunflower seeds were positioned in the front right corner of the testing chamber. The time needed to open all three seeds, as well as the number of pieces of shell produced, was measured. All testing was done within the first four hours of the light part of the daily light cycle in a dimly illuminated room.

2.2.2 Vermicelli Handling Test

The vermicelli handling test has been used as a measure of forepaw dexterity in rodent models of stroke and neurodegenerative disease [54, 55]. We chose this task to look at fine motor dexterity, reaching accuracy, postural control, and motor planning in the valproate- and vehicle-treated rats. During the week prior to testing, all rats were exposed to 3” strands of Mueller’s Brand vermicelli in their home cage (5 days of exposure, 3 strands each time) [54]. For two nights prior to testing the rats were partially food restricted (8-10g/day/rat) to encourage task performance. On PND50, each rat was placed individually into a clear observation cage (same as above). Three vermicelli strands were placed one at a time in the observation cage and the rats’ eating behavior was recorded. A digital video camera was mounted on a tripod, which was repositioned during the test session so as to best record the rat’s paw movements and posture during eating. All testing occurred in a dimly lit room during the first five hours of the light part of the daily light cycle. After all testing was completed the video recordings were reviewed for quality. Trials were removed if less than 90% of the recorded eating session showed a clear view of the paws/digits, head and body posture. Eight trials did not meet these criteria and therefore a total of 94 trials were included for analysis.

Videos were scored by personnel blinded to the experimental conditions. Inter-observer reliability for all coded behaviors was greater than 80% with an average agreement rate of 89.9% (± 6.4%). During coding the videos were played at 6% normal speed. The primary variable of interest was the total number of paw adjustments per trial. As defined by other

groups, an adjustment was scored each time there was a visible release of the pasta (not a drop) or a reformation of the digits holding the pasta via motor patterns of flexion/extension, or abduction/adduction [55].

A code sheet was developed for specific and relevant atypical sensory motor behaviors based on an initial blinded review of 18 pasta-trials, nine trials randomly selected from each experimental group. Behaviors included on the code sheet were number of drops, failure to contact reaches, angling with head tilt, abnormal posture, use of a unilateral paw technique, and twirling of the pasta. Specific descriptions of the first four behaviors are defined elsewhere [54, 55]. Unilateral contact was defined as any time the rat held the pasta in one paw for five seconds or more during eating without visible contact with the other paw. This was distinct from a Failure to Contact code which was indicated each time the rat reached for the pasta but failed to make contact with its target. Twirling was defined as any time the pasta was rotated 180 degrees during positioning of the pasta for eating. This was seen as a potential indicator of impaired motor planning. The total time the rat spent actively eating (biting, chewing) the three pasta strands was recorded. All rats completed the task by consuming all three pasta pieces.

2.3 Statistical Analysis

For all analyses an alpha ≤ 0.05 significance level was considered to be significant. The assumption of normality was assessed for all outcome variables and when the assumption was rejected the appropriate non-parametric tests were utilized. Statistical analyses were conducted using PSAW Statistical Package 18.0. When examining group differences for multiple dependent variables, MANOVA models were used. A repeated measures ANOVA was used for analysis of weight data which were taken over several days during early development. For sensory and motor variables all the rats were initially analyzed in one model, followed by a separate analysis for males and females.

3. Results

3.1 Health and Development

All rats gained weight from approximately 14g on PND 6 (14.4g ± 1.2g) up to approximately 60g on PND 23 (60.4 ± 4.8). A two-factor repeated measures ANOVA was used to analyze body weight between control and valproate-treated groups from PND6 (pre-treatment) to PND23 (weaning). The analysis revealed a significant increase in weight over time ($F(17, 544)=2130.89$; $p<.0001$). There was no significant time*group interaction ($F(17, 544)=1.38$; $p=.218$).

There were also no significant differences found between groups for eye opening from PND13-PND16. By PND15, 81% of the controls and 78% of the valproate-treated rats had their eyes open. All pups had both eyes open by PND16.

3.2 Startle Responsivity

Valproate-treated rats exhibited significantly smaller auditory startle responses compared to responses of control rats ($F(1, 33)=4.72$; $p<.05$). These differences were present in male rats on PND23 ($F(1, 23)=5.36$; $p<.05$) and PND45 ($F(1, 23)=11.65$; $p<.01$). Female rats treated with valproate showed significantly smaller responses on PND45 ($F(1, 9)=8.03$; $p<.05$) but not on PND23 ($F(1, 9)=1.62$; $p=.240$). Tactile startle response magnitude did not differ significantly between groups ($F(1, 33)=.689$; $p=.509$) (Figure 1).

3.3 Sensorimotor Gating

In the control group, the startle response after a prepulse was inhibited by 47% on PND23 and by 57% on PND45. In the valproate-treated group the percent change was less apparent, with only a 36% reduction on PND23 and a 30% change on PND45. Group differences in prepulse inhibition were significant overall ($F(1, 33)=8.68$; $p<.01$) and when examined separately at PND23 ($F(1, 33)= 4.31$; $p<.05$) and PND45 ($F(1, 33)=11.69$; $p=<.01$). This trend remained when males were examined separately on PND45 ($F(1, 23)=5.76$; $p<.05$) and approached significance on PND23 ($F(1, 23)=3.98$; $p=.059$). In females, group differences in percent change were not seen at PND23 ($F(1, 9)=.360$; $p=.565$) but were found to be significant on PND45 ($F(1, 9)=8.84$; $p<.05$) with valproate-treated females showing very little change in response magnitude when presented with a pre-pulse stimulus (Figure 2).

3.4 Motor Performance

The Sunflower Seed Eating task was completed significantly faster by the control group compared to the valproate-treated group ($F(1,33)=4.19$; $p<.05$) but no differences were found in the number of shell pieces left after task completion, with the group means being nearly identical ($F(1, 33)=0$;. $p=1.00$). When group differences between males and females were examined separately, no significant differences emerged for time or number of shell pieces.

During the Vermicelli Handling Test, valproate-treated rats made significantly more paw adjustments ($F(1, 30)=11.85$; $p<.01$) and dropped the pasta significantly more often ($F(1, 30)=15.82$, $p<.001$) than the control rats did. These findings remained significant when males ($F(1, 20)=5.01$; $p<.05$) and females ($F(1, 9)=7.00$; $p<.05$) were examined separately. Valproate-treated rats also engaged in more atypical sensory-motor behaviors. Due to the non-normal distribution of the majority of the count variables, we used a Mann-Whitney U Test (1-tailed Sig.) to examine group differences. Based on this analysis, valproate-treated rats were found to use a unilateral technique significantly more often than control rats ($p<.001$) and had more observed twirls ($p<.05$), failure to contact reaches ($p<.05$) and atypical postures ($p<.05$) than the control group. When males were examined separately, use of the unilateral technique, twirls, and failure to contact reaches continued to be significantly different between the control and valproate-treated groups ($p<.01$- $p<.05$). For female rats, only use of a unilateral technique significantly differed between the groups ($p<.05$) (Figure 3).

A time score was recorded for each vermicelli trial (3 trials per subject). When all three time points were entered into a MANOVA model, the model was not found to be significant ($F(1, 30)=1.78$; $p=.174$). However, when the trials were examined separately, the third trial was found to be significantly different between groups ($F(1, 30)=5.38$; $p=<.05$) with the valproate-treated

rats taking significantly longer to complete the final trial (Figure 5). No differences were found between groups when males and females were examined separately.

Based upon visual examination of the vermicelli time trial data (Figure 4), there appeared to be a learning effect, with control rats (and not valproate-treated rats) showing improved speed with each trial. A repeated measures ANOVA was therefore used to examine the effects of time and group*time interaction. While there was a significant effect of time ($F(2, 58)=4.77$; $p<.05$) no time*group interaction was found ($F(2, 58)=.803$; $p=.453$).

4. Discussion

Here we have shown that early post-natal exposure to valproate leads to altered sensory responsivity, sensory gating, and motor performance in rats. Post-natally valproate-treated rats were under-responsive to auditory stimuli and showed deficits in auditory sensory gating. Sensory under-responsivity has been identified as the more prevalent form of sensory dysfunction in ASD, and it is the sensory characteristic that best distinguishes children with ASD from children with other neurodevelopmental disorders [1, 12]. Further, deficits in prepulse inhibition have been shown in adults with autism [56] and in children with autism and Fragile-X syndrome [57]. Therefore, an animal model that exhibits auditory under-responsivity and sensory gating deficits may be particularly relevant for this diagnostic group.

Interestingly, *pre*-natal valproate exposure has been shown to lead to sensory over-responsivity, particularly in the tactile domain [38] and this characteristic is redolent of another subset of autistic children who are overly sensitive to sensory stimuli. When considered alongside our data, this suggests that valproate exposure during salient pre- and post-natal periods of brain development in rodents may elicit the spectrum of over and under-responsivity that is seen in individuals with ASD. No other animal model of autism has been shown to exhibit this range of behaviors. The data further suggests that the timing of teratogenic insult may invoke differing deficits in neural development and, therefore, a comparison of the biochemical impacts of the pre- and post-natal valproate models may help us to understand the nature of toxic insults that lead to specific behavioral abnormalities on the autism spectrum.

The finding that under-responsivity was only found in the auditory domain, is relevant in the context of the rats' early post-natal brain development and the timing of the valproate injection. Sensitive periods for development of the auditory system occur over the first few weeks of post-natal development in rats. The injection time (PND6-12) corresponds to the sensitive period of development of the primary auditory cortex (A1) and the sub-cortical auditory system, which has been shown to range from PND4-14 [58,59] with refinement of receptive fields continuing throughout the first month of life . In contrast, rapid cortical map plasticity occurs in Layer 4 of the somatosensory cortex during the first week of post-natal rodent development [60] with distribution of thalamic afferents in the primary somatosensory cortex (S1) starting around PND0 and showing mature topographic orientation in relation to cortical barrels around PND4-PND7[61, 62]. While development of the somatosensory cortex continues to be refined over the first few weeks of life, particularly in Layer 2/3[63, 64], it is possible that PND0 to ~PND5 would be the most salient for disruption of tactile responsivity. This is one possible explanation for why there were no significant differences in tactile responsivity between our control and valproate-

treated rats, since our injections did not begin until PND6. Future studies are warranted which examine effects on tactile startle when injections are done in the first five days of post-natal development; this has yet to be examined in the rat or mouse model.

The effects of post-natal valproate exposure on sensory gating were also interesting to consider. Change scores (reported in percent change from auditory startle to auditory startle + prepulse) were significantly different between groups, with the valproate-treated rats showing a much smaller change in response magnitude. This may be due to the fact that their response to the auditory stimulus alone was significantly lower than that of controls and therefore the amount of gating required was much less; it is possible that initial startle responses in the valproate treated rats were so low that a floor effect was seen when the pre-pulse stimulus was presented. This would suggest that in this particular animal model, the problem may be one of initial orientation rather than inhibition. Using electroencephalography (EEG), children with autism have been shown to have smaller amplitude responses to novel auditory stimuli, particularly in the P300 wave, compared to typical children and children with learning disorders [65, 66]. Whitehouse & Bishop [67] also showed that children with autism showed reduced orienting to novel tones presented in a sequence of speech sounds, suggesting potential links between auditory orienting responses and speech/language impairments in this population. While sensory gating deficits remain important to consider in the autism population, and in animal models of the disorder, additional testing paradigms are needed to understand and characterize these sensory processing characteristics more fully.

Gender-specific differences in sensory processing were also interesting to consider in this model. Female rats treated with valproate did not differ significantly from control rats during the juvenile stage but were found to have significantly diminished auditory startle responses and altered sensory motor gating during adolescence. It is possible that significant differences at PND23 were not found due to the small number of subjects in the female group. However, while effect sizes for both auditory startle and auditory gating were moderate in adolescence ($p\eta^2$ = .501, $p\eta^2$ = .525) they were relatively small at the juvenile stage ($p\eta^2$ = .168, $p\eta^2$ = .043). This suggests that even with a larger n, effects of valproate treatment would likely not be found in females at PND23 for these dependent variables. Human studies have shown that girls are often identified as autistic at a later age than boys [68] and this may suggest differences in the timing of symptom manifestation. To the best of our knowledge, no human studies have explored changes in sensory responsivity or sensory gating in children with ASD over time so it is unclear if this sensory processing shift in our adolescent rats translates to any human phenomena. Certainly, this is an area for further exploration.

Finally, group differences in motor performance were apparent between the valproate-treated and the control rats. Valproate-treated rats performed both the sunflower-seed eating test and the final trial of the vermicelli handling test slower than control animals did. Therefore, while both groups were capable of performing the task (i.e. eating the item), the valproate-treated rats performed the task less efficiently. Since the valproate-treated rats required more paw adjustments and engaged in more atypical sensory motor behaviors (e.g. twirling, use of unilateral technique) it appears that these rats had more difficulties in finer aspects of motor control and/or difficulty coordinating the functions of the forepaws bilaterally. Since optimal

motor output is guided by accurate and efficient intake and processing of sensory input, impaired somatosensory processing is one explanation for the observed motor deficits. Despite the fact that we did not see differences in tactile startle responsivity between our valproate and non-valproate treated groups, the fine motor/motor-learning deficits observed in the motor tasks suggest that sensory-motor cortical development was affected by valproate-treatment. Future studies may consider the use of discrimination-based tests for assessing somatosensory function in these animals, rather than a tactile startle paradigm which assesses the modulation of stimulus response. Regardless of the etiology, the observed deficits in motor behaviors such as forepaw dexterity and coordinated bilateral paw movements suggest that the post-natal valproate model does in fact mimic many of the atypical motor behaviors seen in children with ASD.

5. Conclusions

Valproate use in humans during pregnancy has been shown to lead to a significantly higher incidence of autism and autism-related behaviors in offspring. Valproate treatment in rodents has, therefore, been suggested as a valid model for eliciting autistic-like behaviors in developmental neuroscience research. The goal of the present study was to characterize sensory responsivity, sensory gating, and skilled motor performance in rats post-natally treated with valproate. We conclude that this model does mimic many of the features seen in individuals with ASD including auditory under-responsivity which has not been documented in other animal models of autism. While results clearly indicate specific effects of valproate, future studies should consider use of an untreated and unhandled control group to establish a developmental time course of normal sensory and motor behavior. Use of pre and post-natal valproate injections during sensitive periods of brain development may also prove useful for eliciting both under and over responsivity in specific sensory systems and provide a valid model for studying the range of sensory and motor deficits observed individuals with ASD.

Figure Captions

Figure 1. Auditory (top) and tactile (bottom) startle response magnitude data for male and female rats in the control and valproate-treated groups on post-natal day (PND) 23 and 45 (* $p < 0.05$; ** $p < 0.01$).

Figure 2. Acoustic pre-pulse inhibition in male and female rats in the control and valproate-treated groups on post-natal day (PND) 23 and 45 (* $p < 0.05$).

Figure 3. Sensory-motor behaviors observed in male and female rats in the control and valproate-treated groups during the vermicelli-handling task. Each session included three pasta trials (* $p < 0.05$; ** $p < 0.01$).

Note: Uni = Unilateral Technique; Twirl = rotation of the pasta ≥ 180°; FTC= Failure to Contact reach; Posture= observation of atypical posture; Head= Angling of pasta with head tilt.

Figure 4. Time required to complete each trial of the vermicelli-handling task in control and valproate-treated rats (* $p < 0.05$).

Acknowledgements

This project was funded by the National Center for Medical Rehabilitation Research and the National Institute of Neurological Disorders and Stroke (K12 HD055929) Rehabilitation Research Career Development Program. Thanks to Dr. Jeffrey Kleim and Nagheme Thomas for assistance and training in tests of rodent motor performance.

References

1. Baranek GT, David FJ, Poe M D, Stone WL, Watson LR: Sensory experiences questionnaire: Discriminating sensory features in young children with autism, developmental delays, and typical development. J Child Psychol Psyc 2006; 47:591-601.

2. Lane AE, Young RL, Baker AE, Angley MT: Sensory processing subtypes in autism: association with adaptive behavior. J Autism Dev Disord 2010; 40: 112-122.

3. Miller LJ, Anzalone ME, Lane SJ, Cermak SA, Osten ET: Concept evolution in sensory integration: A proposed nosology for diagnosis. Am J Occup Ther 2007; 61:135-140.

4. Leekam SR, Nieto C, Libby SJ, Wing L, Gould J: Describing the sensory abnormalities of children and adults with autism. J Autism Dev Disord 2007; 37: 894-910.

5. Baranek GT, Foster LG, Berkson G: Tactile defensiveness and stereotyped behavior. Am J Occup Ther 1996; 51: 91-95.

6. Boyd BA, Baranek GT, Sideris J, Poe MD, Watson LR, Patten E, Miller H:Sensory features and repetitive behaviors in children with autism and developmental delays. Autism Res 2010; 3: 78-87.

7. Liss M, Saulnier C, Fein D, Kinsbourne M: Sensory and attention abnormalities in autistic spectrum disorders. Autism 2006; 10: 155-172.

8. Pfeiffer B, Kinnealey M, Reed C, Herzberg G: Sensory modulation and affective disorders in children and adolescents with Asperger's disorder. Am J Occup Ther 2005; 59: 335-345.

9. Gillott A, Standen PJ: Levels of anxiety and sources of stress in adults with autism. J Intellect Dis 2007; 11: 359-370.

10. Reynolds S, Lane SJ: Sensory over-responsivity and anxiety in children with ADHD. Am J Occup Ther 2009; 63: 433-440.

11. Reynolds S, Thacker L, Lane SJ: Sensory processing, physiological stress, and sleep behaviors in children with and without autism spectrum disorders. Occup Ther J Res; DOI: 10.3928/15394492-20110513-02

12. Rogers SJ, Ozonoff S: Annotation: What do we know about sensory dysfunction in autism? A critical review of the empirical evidence. J Child Psychol Psyc 2005; 46: 1255-1268.

13. Ashburner J, Ziviani J, Rodger S: Sensory processing and classroom emotional, behavioral, and educational outcomes in children with autism spectrum disorder. Am J Occup Ther 2008; 62: 564-573.

14. Field T, Diego M, Sanders C: Adolescent depression and risk factors. Adolescence 2001; 36: 491-498.

15. Ghaziuddin M, Butler E. Clumsiness in autism and Asperger syndrome: A further report. J Intell Disabil Res 1998; 42: 43-48.

16. Green D, Baird G, Barnett AL, Henderson L, Huber J, Henderson SE: The severity and nature of motor impairment in Asperger's syndrome: A comparison with specific developmental disorder of motor function. J Child Psychol Psyc 2002; 43: 655-658.

17. Provost B, Lopez BR, Heimerl S: A comparison of motor delays in young children: Autism spectrum disorder, developmental delay, and developmental concerns. J Autism Dev Disord 2007; 37: 321-328.

18. Fournier KA, Hass CJ, Naik SK, Lodha N, Cauraugh JH: Motor coordination in autism spectrum disorders: A synthesis and meta-analysis. J Autism Dev Disord 2010; 40: 1227-1240.

19. Esposito G, Venuti P: Analysis of toddlers' gait after six months of independent walking to identify autism: A preliminary study. Percept Motor Skill 2008; 106: 259-269.

20. Ming X, Brimacombe M, Wagner GC: Prevalence of motor impairment in autism spectrum disorders. Brain Dev 2007; 29: 565-570.

21. Gernsbacher MA, Sauer EA, Geye HM, Schweigert EK, Goldsmith HH: Infant and toddler oral- and manual-motor skills predict later speech fluency in autism. J Child Psychol Psyc 2008; 49: 43-50.

22. David FJ, Baranek GT, Giuliani CA, Mercer VS, Poe MD, Thorpe DE: A pilot study: Coordination of precision grip in children and adolescents with high functioning autism. Pediatr Phys Ther 2009; 21: 205-211.

23. Mari M, Castiello U, Marks D, Marraffa C, Prior M: The reach-to-grasp movement in children with autism spectrum disorder. Philos Trans R Soc Lond B Biol Sci 2003; 358: 393-403.

24. Kopp S, Beckung E, Gillberg C: Developmental coordination disorder and other motor control problems in girls with autism spectrum disorder and/or attention-deficit/hyperactivity disorder. Res Dev Disabil 2010; 31: 350-361.

25. Kushki A, Chau T, Anagnostou E: Handwriting difficulties in children with autism spectrum disorders: A scoping review. J Autism Dev Disord; DOI 10.1007/s10803-011-1206-0.

26. Baranek GT: Autism during infancy: A retrospective video analysis of sensory-motor and social behaviors at 9-12 months of age. J Autism Dev Disord 1999; 29: 213-224.

27. Teitelbaum P, Teitelbaum O, Nye J, Fryman J, Maurer RG: Movement analysis in infancy may be useful for early diagnosis of autism. Proc Natl Acad Sci U S A. 1998; 95: 13982-13987.

28. Rasalam AD, Hailey H, Williams JH, Moore SJ, Turnpenny PD, Lloyd DJ, Dean JC: Characteristics of fetal anticonvulsant syndrome associated with autistic disorder. Dev Med Child Neurol 2005; 47: 551-555.

29. Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T, Dean JC: A clinical study of 57 children with fetal anticonvulsant syndromes. J Med Genet 2000; 37: 489-497.

30. Fombonne E: Epidemiology of pervasive developmental disorders. Pediatr Res 2009; 65: 591-598.

31. Williams PG, Hersh JH: A male with fetal valproate syndrome and autism. Dev Med Child Neurol 1997; 39: 362-634.

32. Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH: Fetal valproate syndrome and autism: additional evidence of an association. Dev Med Child Neurol 2001; 43: 202-206.

33. Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J: Embryological origin for autism: Developmental anomalies of the cranial nerve motor nuclei. J Comp Neurol 1996; 370: 247-261.

34. Kim KC, Kim P, Go HS, Choi CS, Yang S, Cheong JH, Shin CY, Ko KH:The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. Toxicol Lett 2011; 201: 137-142.

35. Ingram JL, Peckham SM, Tisdale B, Rodier PM: Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. Neurotoxicol Teratol 2000; 22: 319-324.

36. Snow WM, Hartle K, Ivanco TL: Altered morphology of motor cortex neurons in the VPA rat model of autism. Dev Psychobiol 2008; 50: 633-639.

37. Markram K, Rinaldi T, La Mendola D, Sandi C, Markram H: Abnormal fear conditioning and amygdala processing in an animal model of autism. Neuropsychopharmacology 2008; 33, 901-912.

38. Schneider T, Przewlocki R: Behavioral alterations in rats prenatally exposed to valproic acid: Animal model of autism. Neuropsychopharmacol 2005; 30, 80-89.

39. Wagner GC, Reuhl KR, Cheh M, McRae P, Halladay AK: A new neurobehavioral model of autism in mice: Pre- and postnatal exposure to sodium valproate. J Autism Dev Disord 2006; 36: 779-793.

40. Geal-Dor M, Freeman S, Li G, Sohmer H: Development of hearing in neonatal rats: air bone conducted ABR thresholds. Hear Res 1993; 69: 236-242.

41. Froemke RC, Jones BJ: Development of auditory cortical synaptic receptive fields. Neurosci Biobehav Rev 2011; 35: 2105-2113

42. Moore JK, Linthicum FH: The human auditory system: A timeline of development. Int J Audiol 2007; 46: 460-478.

43. Dobbing J: Under-nutrition and the developing brain. The relevance of animal models to the human problem. Am J Dis Child 1970; 120:411-415.

44. Dobbing J. The later development of the brain and its vulnerability. In: Davis JA, Dobbing J, editors. Scientific foundations of paediatrics. Baltimore: University Park Press; 1981. P. 744-748.

45. Clancy B, Finlay BL, Darlington RB, Anand KJS: extrapolating brain development from experimental species to humans. Neurotoxicology 2007; 28: 931-937.

46. Clancy B, Darlington RB, Finlay BL: Translating developmental time across mammalian species. Neuroscience 2001; 105: 7-17.

47. Pohl-Guimaraes F, Krahe TE, Medina AE: Early valproic acid exposure alters functional organization in the primary visual cortex. Exp Neurol 2011; 228: 138-148.

48. Loscher W: Basic pharmacology of valproate: A review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs 2002; 16: 669-694.

49. Gobbi G, Janiri L: Sodium-and magnesium-valproate in vivo modulate glutamatergic and GABAergic synapses in the medial prefrontal cortex. Psychopharmacol 2006; 185: 255-262.

50. Chomiak T, Karnik V, Block E, Hu B: Altering the trajectory of early postnatal cortical development can lead to structural and behavioural features of autism. BMC Neurosci 2010; 11: 1-10.

51. Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P: A clinicopathological study of autism. Brain 1998; 121: 889-905.

52. Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, Gilmore J, Piven J: Magnetic resonance imaging and head circumference study of brain size in autism: Birth through age 2 years. Arch Gen Psychiat 2005; 62: 1366-1376.

53. Wishaw IQ, Coles BLK: Varieties of paw and digit movement during spontaneous food handling in rats: Postures, bimanual coordination, preferences, and the effect of forelimb cortex lesions. Behav Brain Res 1996; 77: 135-148.

54. Tennant KA, Asay AL, Allred RP, Ozburn AR, Kleim JA, Jones TA: The vermicelli and capellini handling tests: Simple quantitative measures of dexterous forepaw function in rats and mice. J Visual Exp 2010; 41: pii2076.

55. Allred RP, Adkins DL, Woodlee M, Husbands LC, Maldonado MA, Kane JR, Schallert T, Jones TA: The vermicelli handling test: A simple quantitative measure of dexterous forepaw function in rats. Journal of Neurosci Meth 2008; 170: 229-244.

56. Perry W, Minassian A, Lopez B, Maron L, Lincoln A: Sensorimotor gating deficits in adults with autism. Biol Psychiat 2007; 61: 482-486.

57. Yuhas J, Cordeiro L, Tassone F, Ballinger E, Schneider A, Long JM, Ornitz EM, Hessl D: Brief report: Sensorimotor gating in idiopathic autism and autism associated with fragile X syndrome. J Autism Dev Disord 2011; 41: 248-253.

58. de Villers-Sidani E, Simpson KL, Lu Y, Lin RCS, Merzenich MM: Manipulating critical period closure across different sectors of the primary auditory cortex. Nat Neurosci 2008; 11: 957-965.

59. Fathke RL, Gabriele ML: Patterning of multiple layered projections to the auditory midbrain prior to experience. Hear Res 2009; 249: 36-43.

60. Feldman DE, Brecht M: Map plasticity in the somatosensory cortex. Science 2005; 310:810-815.

61. Inan M, Crair MC: Development of cortical maps: Perspectives from the barrel cortex. Neuroscientist 2007; 13: 49-61.

62. Schlaggar BL, O'Leary DDM: Early development of the somatotopic map and barrel patterning in rat somatosensory cortex. J Comp Neurol 1994; 346: 80-96.

63. Stern EA, Maravall M, Svoboda K: Rapid development and plasticity of layer 2/3 maps in rat barrel cortex *in vivo*. Neuron 2001; 31: 305-315.

64. Lendvai B, Stern E, Chen B, Svoboda K: Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*. Nature 2000; 404: 876-881.

65. Courchesne E, Kilman BA, Galambos R, Lincoln AJ: Autism: Processing of novel auditory information assessed by event-related brain potentials. Electroencephalogr Clin Neurophysiol 1984; 59: 238-248.

66. Kemner C, Verbaten MN, Cuperus JM, Camfferman G, van Engeland H: Auditory event-related brain potentials in autistic children and three different control groups. Biol Psychiat 1995; 38: 150-165.

67. Whitehouse AJO, Bishop DVM: Do children with autism 'switch off' to speech sounds? An investigation using event-related potentials. Developmental Sci 2008 11: 516-524.

68. Giarelli E, Wiggins LD, Rice CE, Levy SE, Kirby RS, Pinto-Martin J, Mandell D: Sex differences in the evaluation and diagnosis of autism spectrum disorders among children. Disabil Health J 2010; 3: 107-116.

Behavioural Brain Research 217 (2011) 148–154

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Individual differences in vulnerability for self-injurious behavior: Studies using an animal model

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

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

ARTICLE INFO

Article history:
Received 11 March 2010
Received in revised form 5 October 2010
Accepted 12 October 2010
Available online 23 October 2010

Keywords:
Self-injurious behavior
Individual differences
Stress
Autism
Lesch-Nyhan syndrome
Animal model

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.

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

* Corresponding author. Tel.: +1 352 273 2174; fax: +1 352 392 7985.
E-mail address: dpdevine@ufl.edu (D.P. Devine).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.10.012

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 disregulation 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 adrenocorticotropic 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 D_1 receptor binding in the caudate, and lower levels of dopamine D_2 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 150–175 g upon delivery (Charles River Laboratories, Raleigh, NC) were housed in a climate controlled vivarium with a 12 h/12 h light/dark schedule (lights on at 7:00 a.m.). Standard laboratory rat chow (Lab Diet 5001) and tap water were available *ad libitum* The rats were pair-housed in standard polycarbonate cages (43 cm × 21.5 cm × 25.5 cm) during 6 days of acclimation to the housing facility and for 8 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 1–2 h 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 min. 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 10 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 mm^2.

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 min time samples were recorded once per hour for 8 h 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 s. 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 s.

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 s 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 s. A subsequent rear was only counted after the rat resumed and maintained contact with the cage floor for at least 1 s. 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 analysed 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 analysed 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 mm^2.

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 (Fig. 2B–D). 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

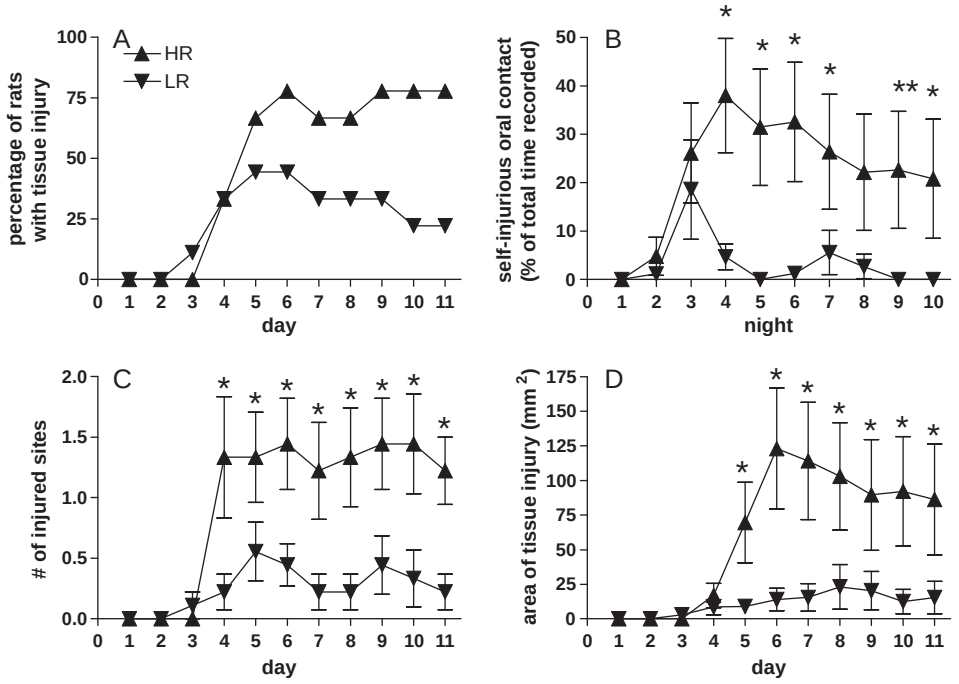

Fig. 1. Individual differences in innate stress-responsiveness predict vulnerability for pemoline-induced self-injury. (A) Most of the HR rats exhibited pemoline-induced SIB, whereas only a small proportion of the LR rats did. The HR rats also (B) spent more time injuring, (C) injured more body sites, and (D) had larger total areas of tissue injury than the LR rats did. All values expressed are group means ± 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 *rho*	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.

Significant correlations are depicted as follows: $^{*}p < 0.05$; $^{**}p < 0.01$.

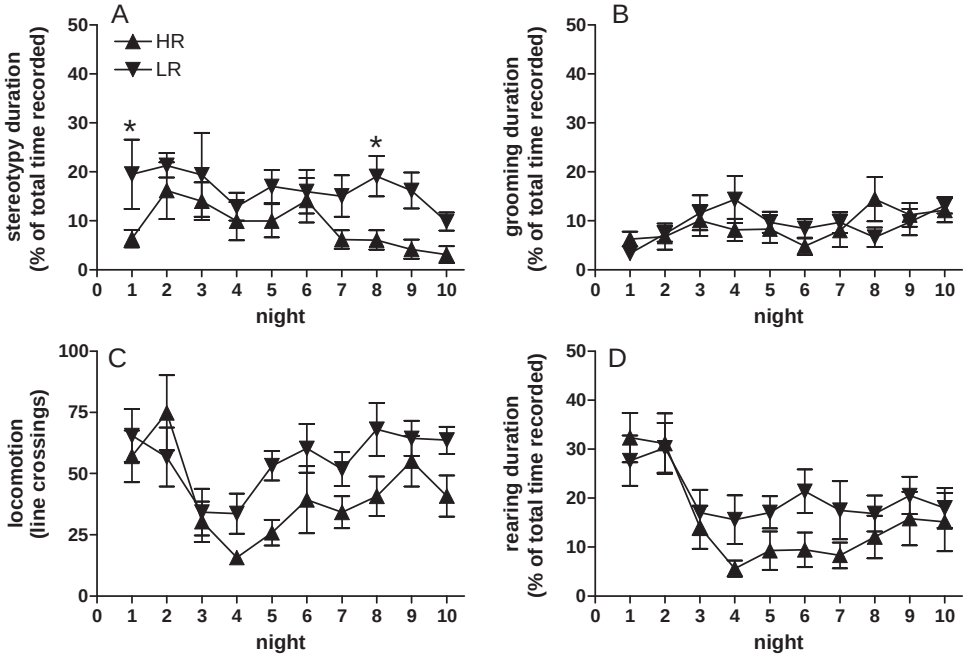

Fig. 2. Individual differences in stress-responsiveness do not predict the overall expression of ancillary behaviors during pemoline treatment. (A) The LR rats exhibited significantly more pemoline-induced stereotypy than the HR rats did, but this effect was small. The (B) duration of grooming, (C) frequency 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 ± S.E.M. Significant differences between HR and LR rats are depicted as follows: $^{*}p < 0.05$.

($F_{(9,144)} = 5.048$, $p < 0.01$). 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 behavioral 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-injure [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 do not 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.

Acknowledgements

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. 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.

References

[1] Anderson LT, Ernst M. Self-injury in Lesch-Nyhan disease. J Autism Dev Disord 1994;24:67–81.
[2] Anderson LT, Ernst M, Davis SV. Cognitive abilities of patients with Lesch-Nyhan disease. J Autism Dev Disord 1992;22:189–203.
[3] Baghdadli A, Pascal C, Grisi S, Aussilloux C. Risk factors for self-injurious behaviors among 222 young children with autistic disorders. J Intellect Disabil Res 2003;47:622–7.
[4] Blake BL, Muehlmann AM, Egami K, Breese GR, Devine DP, Jinnah HA. Nifedipine suppresses self-injurious behaviors in animals. Dev Neurosci 2007;29:241–50.
[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.
[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;68:315–24.
[7] Clarke DJ, Waters J, Corbett JA. Adults with Prader-Willi syndrome: abnormalities of sleep and behavior. J R Soc Med 1989;82:21–4.
[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 Retard 2004;29:119–58.
[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.
[10] Cromwell HC, Levine MS, King BH. Cortical damage enhances pemoline-induced self-injurious behavior in prepubertal rats. Pharmacol Biochem Behav 1999;62:223–7.
[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;63:990–6.
[12] Dellinger-Ness LA, Handler L. Self-injurious behavior in human and non-human primates. Clin Psychol Rev 2006;26:503–14.
[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. Neuropsychobiology 1996;34:136–45.
[14] Devine DP, Muehlmann AM. Tiermodelle für selbstverletzendes Verhalten (Animal models of self-injurious behavior). In: Schmahl C, Stiglmayr C, editors. Selbstverletzendes verhalten bei stressassoziierten erkrankungen (Self-injurious behavior 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;86:347–55.
[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.
[17] Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM. Low medial prefrontal dopaminergic activity in autistic children. Lancet 1997;350:638.
[18] Findling RL. Pharmacologic treatment of behavioral symptoms in autism and pervasive developmental disorders. J Clin Psychiatry 2005;66(Suppl 10):26–31.
[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;30:197–8.
[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. Neuropharmacology 1978;17:419–21.
[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.
[22] Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, et al. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc Natl Acad Sci U S A 1986;83:9779–83.
[23] Hartley SL, Maclean Jr WE, 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;136:140–5.
[24] Hooks MS, Colvin AC, Juncos JL, Justice Jr JB. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Res 1992;587:306–12.
[25] Hooks MS, Jones GH, Neill DB, Justice Jr JB. Individual differences in amphetamine sensitization: dose-dependent effects. Pharmacol Biochem Behav 1992;41:203–10.
[26] Hooks MS, Jones GH, Smith AD, Neill DB, Justice Jr JB. Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 1991;38:467–70.
[27] Hooks MS, Jones GH, Smith AD, Neill DB, Justice Jr JB. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 1991;9:121–8.
[28] Hooks MS, Juncos JL, Justice Jr JB, Meiergerd SM, Povlock SL, Schenk JO, et al. Individual locomotor response to novelty predicts selective alterations in D_1 and D_2 receptors and mRNAs. J Neurosci 1994;14:6144–52.
[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, et al. The functions of self-injurious behavior: an experimental-epidemiological analysis. J Appl Behav Anal 1994;27:215–40.
[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;198:333–40.
[32] Joyce PR, McHugh PC, McKenzie JM, Sullivan PF, Mulder RT, Luty SE, et al. A dopamine transporter polymorphism is a risk factor for borderline personality disorder in depressed patients. Psychol Med 2006;36:807–13.
[33] Kabbaj M. Individual differences in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol Disord Drug Targets 2006;5:513–20.
[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;20:6983–6.
[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. Neuropharmacology 2004;47(Suppl 1):111–22.
[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. Psychopharmacology 2001;158:382–7.
[37] Kemp AS, Fillmore PT, Lenjavi MR, Lyon M, Chicz-DeMet A, Touchette PE, et al. Temporal patterns of self-injurious behavior correlate with stress

hormone levels in the developmentally disabled. Psychiatry Res 2008;157: 181–9.
[38] Kies SD, Devine DP. Self-injurious behavior: a comparison of caffeine and pemoline models in rats. Pharmacol Biochem Behav 2004;79:587–98.
[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.
[40] Li Y, Hu XT, Berney TG, Vartanian AJ, Stine CD, Wolf ME, et al. Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. Synapse 1999;34:169–80.
[41] Lloyd KG, Hornykiewicz O, Davidson L, Shannak K, Farley I, Goldstein M, et al. Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. N Eng J Med 1981;305:1106–11.
[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.
[43] Matson JL, Nebel-Schwalm M. Assessing challenging behaviors in children with autism spectrum disorders: a review. Res Dev Disabil 2007;28:567–79.
[44] Muehlmann AM, Brown BD, Devine DP. Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy. J Pharmacol Exp Ther 2008;324:214–23.
[45] Muehlmann AM, Devine DP. Glutamate-mediated neuroplasticity in an animal model of self-injurious behavior. Behav Brain Res 2008;189:32–40.
[46] Muehlmann AM, Wolfman S, Devine DP. Examining the effects of chronic stress on self-injurious behavior in an animal model. Soc Neurosci 2008;34:446–529. Abstracts.
[47] Novak MA. Self-injurious behavior in rhesus monkeys: new insights into its etiology, physiology, and treatment. Am J Primatol 2003;59:3–19.
[48] Piazza PV, Deminière J-M, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science 1989;245:1511–3.
[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;10:3903–7.
[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;602:169–74.
[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.
[53] Sandman CA, Barron JL, Chicz-DeMet A, DeMet EM. Plasma β-endorphin levels in patients with self-injurious behavior and stereotypy. Am J Ment Retard 1990;95:84–92.
[54] Stead JDH, Clinton S, Neal C, Schneider J, Jama A, Miller S, et al. Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. Behav Genet 2006;36: 697–712.
[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;783:10–8.
[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;104:260–9.
[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;108:13–8.
[58] Tordjman S, Anderson GM, McBride PA, Hertzig ME, Snow ME, Hall LM, et al. Plasma beta-endorphin, adrenocorticotropin hormone, and cortisol in autism. J Child Psychol Psychiatry 1997;38:705–15.
[59] Xiao-Mian S, Jing Y, Chongxuna Z, Min L, Hui-Xing D. Study of 99mTc-TRODAT-1 imaging on human brain with children autism by single photon emission computed tomography. Conf Proc IEEE Eng Med Biol Soc 2005;5:5328–30.

Weinstock, N.J. and Devine, D.P. (2011) A mouse model of pemoline-induced self-injurious behavior. *Society for Neuroscience Abstracts,* **37:** 776.11

Self-injurious behavior (SIB) is a debilitating disorder in which patients bite, punch and hit themselves, often resulting in serious bodily harm. This behavior is most common in disorders with intellectual handicaps such as Autism, L-N syndrome, and Prader-Willi Syndrome. Self-injury is also common in the intellectually handicapped population in general, as well as in disorders in which the patients display normal intelligence such as schizophrenia, Tourette's and Borderline Personality Disorder. Currently, behavioral therapy is the best tool we have to help treat self-injury, but many afflicted individuals are extremely refractory. We have been working with a model of pharmacologically-induced self-injury in order to increase understanding of the neurochemical mechanisms that underlie vulnerability for the behavior disorder in these populations. In this model, rats are injected daily with pemoline until they display tissue damage, and we have reported evidence of face, construct, etiological, and predictive validity. In an effort to utilize cutting-edge molecular and genetic techniques, we have now developed a mouse model of pemoline-induced SIB. We have found that C57BL/6 mice injected twice daily with pemoline display self-injurious behavior in a dose-orderly manner. This finding will provide the basis for extending our findings into a new species and allow us to explore the biochemical mechanisms that underlie pemoline-induced self-injury in genetically-vulnerable animals.

Van Matre, A.M., Wolfman, S., and Devine, D.P. (2010). Neurotensin plays a modulatory role in self-injurious behavior: biochemical analyses using an animal model, *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** 29.

Introduction: The most debilitating of all the maladaptive behaviors in developmental disabilities is self-injurious behavior (SIB). SIB consists of stereotyped behaviors that can produce physical injury (e.g. head-banging, face-punching, self-biting). Pemoline, an indirect monoamine agonist, produces stereotyped self-biting in rats and is used as an animal model of human SIB. Previously we found that changes in dopaminergic and glutamatergic neurotransmission are involved in initiation of pemoline-induced self-injury. We've also shown that circulating corticosterone (a stress hormone) is elevated in pemoline-treated rats and that chronic stress potentiates the self-injury. We are now focusing on modulators of dopamine, glutamate, and stress systems as potential mechanisms that underlie the expression of self-injury, and as targets for pharmacotherapy. Neurotensin is a neuropeptide that increases dopamine and glutamate release in the striatum and stimulates the hypothalamic-pituitary-adrenal axis to increase circulating corticosterone levels.

Methods: In experiment 1, male Long Evans rats were administered either pemoline (150 mg/kg) or peanut oil vehicle each day for five days. On the sixth day the rats were killed, their brains removed, and the striatum and ventral tegmentum were rapidly dissected. A neurotensin radioimmunoassay (RIA) was used to measure neurotensin content within these structures. In experiment 2, rats received daily injections of pemoline (150 mg/kg/day; s.c.) and either 0 or 0.01 mg/kg of the neurotensin 1 receptor agonist, PD149163 (s.c.), twice daily for five days. In experiment 3, rats received daily injections of pemoline (150 mg/kg/day) and either 0 or 1.0 mg/kg of the neurotensin 1 receptor antagonist, SR48692, twice daily for five days. SIB was measured by quantifying the duration of self-injurious oral contact with the skin and by measuring the size of injured tissue. To determine whether the effects of the neurotensin targeted drugs were presynaptic or postsynaptic, we analyzed neuronal content of dopamine and serotonin and their metabolites. We also evaluated the phosphorylation status of a postsynaptic protein, DARPP-32, to determine the effects of the neurotensin targeted drugs on this critical integrator of dopamine, glutamate, and neurotensin signaling in postsynaptic neurons of the striatum.

Results: In experiment 1 we found that neuronal neurotensin content in the striatum was higher in pemoline-treated rats (i.e. the self-injurers) than in vehicle-treated controls. No differences in ventral tegmental neurotensin content were found between pemoline- and vehicle-treated rats. In experiments 2 & 3 we found that the neurotensin 1 receptor agonist and antagonist had opposite effects on pemoline-induced SIB. The agonist significantly increased the time spent injuring and the severity of the injury, whereas the antagonist significantly decreased the time spent injuring and the severity of the injury. On measures of presynaptic monoamine content, both drugs (when administered twice daily during concurrent repeated pemoline injections) increased neuronal concentrations of dopamine and serotonin and their metabolites (HVA, DOPAC, and 5HIAA). Since these drugs had opposite effects on behavior, we conclude that the relationship between presynaptic monoamine concentrations and the expression of pemoline-induced SIB is not straightforward. We are continuing to evaluate the effects of the neurotensin 1 receptor agonist and antagonist on DARPP-32 expression and phosphorylation in pemoline- and vehicle-treated rats.

Conclusions: Although our investigations into the role of neurotensin in pemoline-induced SIB are still on-going, we suggest that targeting neurotensin neurotransmission may be a clinically relevant pharmacotherapeutic strategy to reduce human SIB.

Devine, D.P. (2011). Disregulation of dopamine and glutamate neurotransmission in animal models of self-injurious behavior, *Experimental Biology 2011*, S369.

Symposium Title: ***Autism and PDD: Neuropathology, pharmacotherapies, and new directions***
Division of Behavioral Pharmacology, ASPET

Chair: Ellen Walker, PhD
Department of Pharmaceutical Sciences
Temple University School of Pharmacy

Synopsis: Autism is a heritable neurodevelopmental disorder classified within a broader domain of pervasive developmental disorders (PDD). Autism is characterized by impairments in three core domains: social interaction, language development, and patterns of behavior (restricted and stereotyped). The clinical pattern and severity of impairments vary and the level of cognitive functioning of individuals with autism spans the range from extreme mental retardation to advanced intellect. This symposium will address the status of the neuropathology, the genetic and environmental risk factors, and the pharmacology of autism. At the present time, models and drug treatment strategies are directed toward treating particular target symptom domains such as: motor hyperactivity and inattention; aggression and self-injurious behavioral; stereotypical and repetitive behavior; and, core social and language impairments. The applications of new pharmacological strategies and model development will be stressed in this symposium.

Titles:

Robert Schultz, Ph.D., ***The Brain in Autism: Perspectives from Neuropsychology and Neuroimaging.***
An overview of the current understanding of this complex disorder and its biological foundations. The key features of the clinical syndrome can be introduced through neuropsychology, genetic, and neuroimaging studies.

Michael G. Aman, Ph.D., ***Neurochemistry in the pathophysiology of autism.***
The challenge for the development of drugs for the core symptoms of autism can be addressed in this presentation. The current literature and pharmacological treatments strategies for inattention, overactivity, impulsiveness, communication/social impairment, irritability, self-injurious behavior, and aggression will be discussed.

Diane C. Chugani, Ph.D., ***Serotonin dysregulation in autism spectrum disorders***.
Serotonin has been implicated in many behaviors observed in individuals who have autism. The serotonin system is also of interest because of its essential role in brain development and impact on critical areas of the brain that may be dysfunctional in individuals who have autism. This presentation would review the various roles of serotonin in autism research.

Darragh Devine, PhD., ***Dopamine and glutamatergic neurotransmission in animal models of self-injurious behavior.***
The pharmacology of both dopamine and glutamate in animal models of self-injurious behaviors is proposed in addition to the validity of these models for predicting new treatments.

Craig Powell, M.D., Ph.D., ***Behavioral Genetics and Mouse Behavioral Tasks Relevant to Autism.***
Research focusing on genes that mediate social behavior in mice may help identify neural circuitry and pharmacology essential for normal social interaction, and lead to novel animal models of the autism behavioral phenotype.

Devine, D.P. (2010). The role of stress-responsiveness in self-injurious behaviors: Biochemical studies in an animal model, *First International Congress on Borderline Personality Disorder*, **1:** S-031.

The Role of Stress-Responsiveness in Self-Injurious Behaviors: Biochemical Studies in an Animal Model

We are investigating a rodent model of self-injury, a feature that is prevalent in Borderline Personality Disorder (BPD). Long-Evans rats were injected daily with the monoamine agonist pemoline, and self-biting was monitored. Individual rats differed in vulnerability for self-injury, and the outcomes were predicted *a priori*, by evaluating innate stress-responsiveness. Stress hyper-responsive (HR) rats exhibited self-biting behaviour, whereas less stress-responsive (LR) rats did not. Self-biting was increased by a history of emotional stress exposure. The HR and LR rats did not differ in basal measures of nociceptive (thermal) sensory sensitivity, and although pemoline treatment decreased sensitivity, the effects did not differ between the HR and LR rats. We propose that over-responsive neuroendocrine mechanisms alter monoamine function in pemoline-treated HR rats, and that the resulting neurochemical changes contribute to expression of self-injury. Environmental stress further promotes monoaminergic dysfunction, and overall these stress-responsive characteristics resemble abnormalities found in BPD. Pemoline-induced subsensitivity to nociceptive stimulation is also compatible with reports from BPD patients. However, since pain sensitivity did not differ between the vulnerable HR and resistant LR rats during pemoline treatment, the data suggest that diminished pain sensitivity may contribute to, but is not a definitive feature of vulnerability for etiology of SIB.

Devine, D.P. (2010). Biochemical factors that confer individual differences in vulnerability for self-injurious behaviour, in symposium on "Behavioral and biological frontiers in the analyses of self-injurious behavior", *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** Symposium 13.

TITLE: Biochemical Factors that Confer Individual Differences in Vulnerability for Self-Injury
AUTHORS: Darragh P. Devine, Ph.D.
AFFLIATIONS: Department of Psychology, Behavioral Neuroscience Program, University of Florida

Introduction: Self-injurious behavior (SIB) is highly prevalent in developmental disabilities. Interestingly, it is not a definitive trait of most diagnostic categories. Rather, some children within each diagnostic group develop self-injury, whereas others do not. Accordingly, there seems to be a shared vulnerability that underlies its etiology across disorders, and there is abundant evidence that the vulnerability for SIB derives (at least in part) from a neuropathological basis. One important clue is that SIB is particularly prevalent in disorders where ongoing distress, pathological irritability, and disregulation of stress hormones are prominent features [1-3]. Additional clues come from clinical trials of pharmacological interventions, from post-mortem neurochemical analyses, and from studies in animal models.
Methods: We are examining the neurobiological basis of vulnerability for SIB in an animal model. After repeated treatment with pemoline (a monoamine uptake blocker), rats exhibit self-injurious biting. We have refined and validated this model, improved behavioral measures of SIB, and identified multiple lines of convergence between clinical SIB and the pemoline model [4]. In one experiment, 18 Long-Evans rats were screened for individual differences in behavioral and hormonal responsiveness to the mild stress of a novel environment. The rats were then categorized as high stress-responders (HR) or low stress-responders (LR) by median split. All the rats were then given daily injections of pemoline (150 mg/kg/day) for 10 days, and self-injurious biting was evaluated.
Results: Seven of the 9 HR rats and 1 of the 9 LR rats exhibited substantial self-biting behavior. Furthermore, 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 stress-responsiveness scores were significantly correlated with each of these measures of self-injury. The HR rats did not exhibit enhanced responses on other measures of psychostimulant action (stereotypy, grooming, locomotion, rearing), and these measures were not correlated with stress-responsiveness.
Discussion: Overall, we found that individual rats differ in vulnerability for pemoline-induced SIB, and pre-existing stress-responsive phenotypes contribute to this vulnerability. An important feature of our model is that SIB onsets gradually in the vulnerable rats during treatment with pemoline. This allows us to examine neuroplastic modifications that occur during the induction of SIB, to identify whether specific neurochemical alterations coincide with the development of SIB, and to characterize neurochemical differences between vulnerable and invulnerable rats. This may provide insight into biochemical mechanisms that confer innate vulnerability for the self-injurious phenotype, reveal convergent mechanisms where heightened stress responsiveness contributes to altered neurotransmission, and identify potential targets for further biochemical and pharmacological investigation.

Key References:
1. Anderson, L.T., Ernst, M. (1994). Self-injury in Lesch-Nyhan disease, *J. Autism. Dev. Disord.*, **24**: 67-81.
2. Sovner, R., Fogelman, S. (1996). Irritability and Mental Retardation, *Semin. Clin. Neuropsych.*, **1**: 105-114.
3. Kemp, A.S., Fillmore, P.T., Lenjavi, M.R., Lyon, M., Chicz-DeMet, A., Touchette, P.E., Sandman, C.A. (2008). Temporal patterns of self-injurious behavior correlate with stress hormone levels in the developmentally disabled, *Psychiatry Res.*, **157**: 181-189.
4. Muehlmann, A.M., Brown, B.D., and Devine, D.P. (2008). Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy, *J. Pharmacol. Exp. Ther.*, **324**: 214-223.

Darragh Patrick Devine, Ph.D.
Associate Professor
Director, Behavioral and Cognitive Neuroscience Program
University of Florida
Department of Psychology
P.O. Box 112250
Gainesville, FL, 32611-2250

office: (352) 273-2174
lab: (352) 273-2192
fax: (352) 392-7985
e-mail: dpdevine@ufl.edu
website: http://www.psych.ufl.edu/~dpdevine/home/

January 2012

Curriculum Vitae

Languages English and French
Citizenships Ireland and Canada (permanent resident USA)

Current Position

Associate Professor and Director
Behavioral and Cognitive Neuroscience Program
University of Florida
Departments of Psychology and Neuroscience

Education

Post-Doctoral Research Fellowship
University of Michigan, School of Medicine, Department of Psychiatry
Molecular and Behavioral Neuroscience Institute
(formerly Mental Health Research Institute)
Ann Arbor, Michigan,
supervisors: Huda Akil, Ph.D. & Stanley J. Watson, M.D., Ph.D.
1993 – 1998

Doctor of Philosophy (Ph.D.), Psychology
Concordia University
Center for Studies in Behavioral Neurobiology
Montréal, Québec, Canada
dissertation advisor: Roy A. Wise, Ph.D.
graduated 1993

Master of Arts (M.A.), Psychology
Carleton University
Department of Psychology
Ottawa, Ontario, Canada
thesis advisor: Nicholas P. Spanos, Ph.D.
graduated 1988

Bachelor of Science (B.Sc.), Psychobiology
Concordia University
Department of Psychology
Montréal, Québec, Canada
graduated 1982

Diplôme d'études collégiales (D.E.C.), Social Sciences
Dawson College C.E.G.E.P.
Montréal, Québec, Canada
graduated 1975

Honors and Awards

Colonel Allen R. and Margaret G. Crow Term Professor
University of Florida, 2010-2011

Feature story in recognition of undergraduate mentoring
University of Florida CLAS annual report, 2006-2007

Recognition for Mentoring Undergraduate "Anderson Scholars"
University of Florida, College of Liberal Arts and Sciences
fall 2001, fall 2005, fall 2008

"You Made a Difference" Faculty Honoree for Undergraduate Mentoring
University of Florida, College of Liberal Arts and Sciences
spring 2004

"**Teacher of the Year" Award**
University of Florida, College of Liberal Arts and Sciences
Awarded spring 2003

Society for Neuroscience Annual Press Book
Featured research on self-injury from my laboratory
fall 2002

Spokesperson, Concordia University Campaign to promote Graduate Studies
1995 and 1996

Governor General of Canada's Gold Medal for Research
awarded October 1994

Governor General of Canada's Gold Medal

James McKeen Cattell Award
Section of Psychology, New York Academy of Sciences
Finalist, June 1995

Prix d'Excellence de l'Académie des Grands Montréalais
Finalist, June 1994

Research Experience

Associate Professor (2005 – present)
Assistant Professor (1998 – 2005)
University of Florida
Behavioral Neuroscience Program
Departments of Psychology and Neuroscience
Gainesville, Florida

Post-Doctoral Research Associate (1993 – 1998)
University of Michigan, School of Medicine, Department of Psychiatry
Mental Health Research Institute
Ann Arbor, Michigan,
supervisors: Huda Akil, Ph.D. & Stanley J. Watson, M.D., Ph.D.

Research Assistant (1988 – 1993)
Concordia University
Center for Studies in Behavioral Neurobiology and Department of Psychology
Montréal, Québec, Canada
supervisor: Roy A. Wise, Ph.D.

Research Assistant (1986 – 1988)
Carleton University
Department of Psychology
Ottawa, Ontario, Canada
supervisor: Nicholas P. Spanos, Ph.D.

Clinical Experience

Behaviour Consultant and Assistant Coordinator (1986 – 1988)
Ottawa-Carleton Behaviour Management &
Children's Aid Society of Ottawa-Carleton
1370 Bank St.
Ottawa, Ontario, Canada K1H 7Y3

Pavilion Head (Clinical Manager) (1980 – 1986)
Les Promotions Sociales Taylor-Thibodeau
Garry Taylor Center
231 Elm Ave.
Beaconsfield, Québec, Canada H9W 2E2

Educator (1976 – 1980)
Child Care and Child Development Centers Inc.
Garry Taylor Center
231 Elm Ave.
Beaconsfield, Québec, Canada H9W 2E2

In these three clinical positions, I used a model of applied behaviour analysis to treat maladaptive behaviour disorders and skill deficits in autistic, intellectually-handicapped and brain-injured individuals. In all three positions, I specialized in assessment and treatment of severe self-injurious behavior, a debilitating characteristic that is highly prevalent in autism and other neurodevelopmental disorders. I supervised a team of Behaviour Consultants and a few teams of Educators and Child Care Specialists. I also provided training courses for parents, teachers, staff, and other professionals who worked with the affected individuals. In addition, I lectured at a variety of Colleges in the Montréal and Ottawa areas, speaking to diverse populations (e.g. Early Childhood Education Association of Ottawa-Vanier, Canadian Association for Head Injury) about the management of behaviour disorders in developmentally-disabled and brain-injured individuals.

My clinical interests in neurodevelopmental disorders and self-injury contributed significantly to my research interests in Neuroscience. Hence, my laboratory is committed to a comprehensive research program that investigates the neurobiological basis of vulnerability for self-injury using animal models.

Teaching Experience

Associate Professor, 2005 – present
Assistant Professor, 1998 – 2005
University of Florida, Department of Psychology
Behavioral Neuroscience Program
Gainesville, Florida

undergraduate courses:
PSB 3004, Physiological Psychology
PSB 3912, Introduction to Research in Physiological Psychology
PSB 3054/3340, Behavioral Neuroscience
PSB 4810, Neurobiology of Learning and Memory
PSB 4934, Behavioral Neuroendocrinology
PSB 4934, Neurobiology of Developmental Disorders
PSB 4905, Individual Research
PSY 4970, Senior Thesis Research
IDS 4906, Interdisciplinary Senior Thesis Research in Neurosciences

graduate courses:
PSB 5935, Foundations of Molecular Neurobiology
PSB 6099, Graduate Proseminar in Physiological and Comparative Psychology
PSB 6930, Molecular Neurobiology
PSB 7248, Neurobiology of Stress and Stress-Related Psychopathology
PSB 7249, Mechanisms of Neuroplasticity

Course Instructor, 1995 – 1998
University of Michigan, School of Medicine, Department of Psychiatry
Ann Arbor, MI, 48109-0720
undergraduate courses:
UC 280, Undergraduate Research Opportunities

Teaching Exp. (cont'd…)

Teaching Assistant, 1988 – 1989
Concordia University
Center for Studies in Behavioral Neurobiology and Department of Psychology
Montréal, Québec, Canada H3G 1M8
undergraduate courses:
PSYC 310, Research Methods I
PSYC 311, Research Methods II
PSYC 315, Statistical Analysis I
PSYC 316, Statistical Analysis II

Graduate Students and Post-Docs Supervised (as primary mentor/chair of thesis committee):

name	dates	program	UF degree earned	UF degree pending	current placement
Martin Repetto, M.D., Ph.D.	1999-2000	Post-doctoral fellow	post doc		Psychiatrist, University Illinois, Champaign IL
Jaime Tartar (Simpkiss), Ph.D	2000-2004	Psych – Beh Neurosci	Ph.D.		Assistant Professor Nova Southeastern U
Staci Kies, B.A., M.S.	2000-2003	Psych – Beh Neurosci	M.S.		Private Practice, Atlanta GA
Michael A. Misilmeri, M.S.	1999-2003	Psych – Beh Neurosci	M.S.		Pharma Rep, CA
Kristen Stone, M.S.	2004-2007	Psych – Beh Neurosci	M.S.		Advanced Learning Centers Altamonte Springs, FL
Megan K. Green, Ph.D.*	2003-2007	Psych – Beh Neurosci	M.S., Ph.D		Post-doctoral fellow, University of Texas
Amber Muehlmann, Ph.D.*, **	2003-2011	Psych – Beh Neurosci	M.S., Ph.D.		U. Florida, Psychiatry
Nathan Weinstock, B.S.	2005-2009	Psych – Beh Neurosci	B.S., M.S.	Ph.D.	U. Florida, Psychology
Catherine Marcinkiewcz Ph.D	2007-2010	IDP - Neuroscience	M.S., Ph.D.		U. North Carolina
Ryan Keith M.S. (co-chair)	2010-present	Psych – Beh Neurosci	M.S.	Ph.D.	U. Florida, Psychology
Stacey Reynolds, Ph.D.	2010-present	K-12 Post-doctoral Research Associate			U. Florida, Psychology (Assistant Professor)
William Lin, B.S.	2010-present	Psych – Beh Neurosci		M.S., Ph.D.	U. Florida, Psychology
Xiaomeng Yuan, B.S.	2010-present	Psych – Beh Neurosci		M.S., Ph.D.	U. Florida, Psychology

* indicates winners of the Robert A. and Phyllis Levitt Award
** indicates winner of the Schumacher Award

Undergraduate Students Supervised (as primary mentor/chair of thesis committee):

name	dates	program	UF degree earned	UF degree pending	current placement
Jennifer Felger, B.S.	2000-2001	Psychology	B.S.		Emory U. Biol/Biomed S
Fabian Fernandez, B.S. *,**	2001-2002	IDS Behav Neurosci	B.S.		Stanford U. Neuroscienc
Martina C. Bauer, B.S. *	2002-2004	IDS Behav Neurosci	B.S.	M.D.	U. Florida Sch of Medicir
Carrie A. Hersh *	2002-2005	IDS Behav Neurosci	B.S.		Nova Southeastern U.
Kir-Wei Chen	2004-2006	Psychology	B.S.		Boston U Sch of Medicin
Allessa Duren	2004-2006	IDS Behav Neurosci	B.S.		FSU School of Medicine
Andrea Naccarato *, **	2004-2007	IDS Behav Neurosci	B.S.		Mote Marine laboratory
Emily Barbieri *	2004-2007	IDS Behav Neurosci	B.S.		Washington State Univ
Jenny Wilkinson *, **	2004-2007	IDS Behav Neurosci	B.S.	M.D.	U. Florida Sch of Medicir
Christopher McDonald	2007-2008	Psychology	B.S.		FSU School of Medicine
Peter Duarte	2004-2008	IDS Behav Neurosci	B.S.		
Evan Loewy ***	2007-2009	IDS Behav Neurosci	B.S.		U Miami Sch of Medicine
Shannon Wolfman ***	2006-2010	IDS Behav Neurosci	B.S.		U. of Chicago
Kanita Beba ***	2007-2010	IDS Behav Neurosci	B.S.		U. Florida Sch of Medicir
Gabriella Fernandez	2009 - 2010	Psychology		B.S.	U. Florida
Karly Lorbeer*	2009 - preser	Psychology		B.S.	U. Florida

* indicates members of University Scholars program
** indicates Anderson Scholars
*** indicates Howard Hughes Medical Institute "Science for Life" Scholar

Graduate Students Supervised as secondary mentor/member of thesis committee:

name	dates	program	UF degree earned	UF degree pendir	current placement
Laurie Geran, Ph.D.	1998-2003	Psych – Beh Neurosc	Ph.D.		Post-doctoral fellow Ohio State University
Cortney Turner, Ph.D.	1999-2003	Neuroscience	Ph.D.		Post-doctoral fellow University of Michigan
Michelle Miller, M.S.	2000-2002	Psych – Beh Analysis	M.S.		
Steven Rowell, M.S.	2004-2005	Psychology	M.S.		
Cheryl Vaughan, Ph.D.	2000-2006	Psych – Beh Neurosc	M.S., Ph.D.		Post-doctoral fellow Georgia State University
Chris King, Ph.D.	2000-2006	Neuroscience	Ph.D.	Post-do	Research Assistant Professor University of Florida
Irene Glenn, M.S.	2003-2006	Psych – Beh Analysis	M.S.		
David Dietz, Ph.D.	2002-2007	Neuroscience	M.S. Ph.D. (FSU		Post-doctoral fellow Mt. Sinai Medical Center
Anaya Mitra, Ph.D.	2006-2008	Psych – Beh Neurosc	M.S., Ph.D.		Post-doctoral fellow University of Minnesota
Linda Wen-Hua Lee, Ph.D.	2003-2009	Neuroscience	M.S., Ph.D.		Post-doctoral fellow U. Florida
Yoko Tanimura, Ph.D.	2004-2010	Psych – Beh Neurosc	M.S., Ph.D.		Post-doctoral fellow U. Rochester Med Center
Jolene Sy	2008 - 2011	Psych – Beh. Analysi	M.S., Ph.D.		St. Louis University
Michael La Sala	2010 - present	Neuroscience		Ph.D.	U. Florida
Sofia Beas	2011-present	Neuroscience		Ph.D.	U. Florida

Undergraduate Students Supervised as secondary mentor/member of thesis committee:

name	dates	program	UF degree earned	UF degree pending	current placement
Sean Kearns, B.S.	1999-2000	IDS Behav Neurosci	B.S.	.	Grad student, U. Florida
Sharon Karackattu, B.S.	1999-2000	IDS Behav Neurosci	B.S.		Grad student, MIT
Jolie Haun, B.S.	1999-2000	Psychology	B.S.		Grad student, FSU.
Leslie Guttman, B.S.	2000-2002	IDS Behav Neurosci	B.S.		
Jesse Cushman, B.S.	2000-2002	IDS Behav Neurosci	B.S.		Grad student, UCLA
Alexander Bayevsky, B.S.	2003-2004	IDS Behav Neurosci	B.S.		
Sarah Williams, B.S.	2003-2005	IDS Behav Neurosci	B.S.		
Courtney Whitehurst, B.S.	2004-2005	IDS Behav Neurosci	B.S.		
Jessica Saul, B.S.	2004-2005	Psychology	B.S.		
Althea Bardin, B.S.	2005-2006	Psychology	B.S.		
Randy Colvin	2004-2007	IDS Behav Neurosci	B.S.		
Tana Bleser	2006-2007	Psychology	B.S.		Grad student, U Florida
Rebecca Wright	2006-2007	IDS Behav Neurosci	B.S.		
Ellen Espenschied	2006-2008	IDS Behav Neurosci	B.S.		
Parin Chheda	2006-2007	IDS Behav Neurosci	B.S.		
Samantha Baer *	2007-2008	IDS Behav Neurosci	B.S.		
Sara El-Sherbini	2007-2009	Psychology	B.S.		
Nicholas Maling	2008-2009	IDS Behav Neurosci	B.S.		
Emily Korszen	2010-2010	IDS Behav Neurosci	B.S.		
Jessica McElroy	2009-2010	IDS Behav Neurosci	B.S.		
Christopher Herring	2009-2010	Psychology	B.S.		
Elon Richman	2009-present	IDS Behav Neurosci		B.S.	
Gabrielle Hall	2010-present	Psychology		B.S.	

* indicates member of University Scholars program

Research Funding

Current Funding

The Woodruff Foundation
Self-injurious behavior: a biomarker at the interface of stress and dopamine function
role: Principal Investigator
September 2011 – September 2012

NIH - National Institute on Child Health and Human Development (N.I.C.H.D.)
Rehabilitation Research Career Development Program
An Investigation into the Neurobiological Basis of Sensory Processing Disorder
#K12 HD055929
role: Mentor (Krista Van Den Borne and Kenneth Ottenbacher are coPIs)
February 2010 – January 2013

Pending Funding

Research Opportunity Fund
Neurochemical basis of vulnerability for self-injurious behavior in autism spectrum disorders
role: Principal Investigator
selected as finalist in two-stage review process – currently under review

Past Funding

Congressionally Directed Medical Research Programs
Department of Defense
Autism Research Program Concept Award
Self-Injurious Behavior: An Animal Model of an Autism Endophenotype
role: Principal Investigator
May 2010 – December 2011

NIH – National Institute on mental Health (N.I.M.H.)
A Novel Class of Orally-Applied Inhibitors of Aggressive Behaviour
#K18 OD008065-01
role: co-Principal Investigator and Mentor
September 2010 – September 2011

NIH - National Institute on Drug Abuse (N.I.D.A.)
A Home-Based Behavioral Treatment for Cigarette Smoking
#R01 DA019580
role: co-Investigator (Jesse Dallery is PI)
April 2007 – March 2011

NIH - National Institute on Mental Health (N.I.M.H.)
Vulnerability for Self-Injurious Behavior: Neurobiological Mechanisms
#F31 MH079675
role: Mentor
May 2008 – April 2010

NIH - National Institute of Neurological Disease and Stroke (N.I.N.D.S.)
Integrative and Translational Training in Pain Research
#T-32 training grant
role: Mentor

NIH - National Institute on Aging (N.I.A.)
Neurobiological Mechanisms of Cognitive Impairment in Aging
#T-32 training grant
role: Mentor
August 2008 – August 2010

Research Funding (cont'd…)

UF – CLAS Preliminary Study Grant (P.S.G.)
Self-Injurious Behavior: Biochemical Basis for Individual Differences in Vulnerability
role: Principal Investigator
May 2009 – May 2010

National Science Foundation (N.S.F.)
Stress-Modulating Actions of Orphanin FQ (N/OFQ)
grant #0515136
role: Principal Investigator
August 2005 – August 2009

Cure Autism Now Foundation (C.A.N.)
Self-Injurious Behavior: Pharmacological Studies in a Rat Model
role: Principal Investigator
February 2006 – October 2008

National Alliance for Autism Research (N.A.A.R.)
Self-Injurious Behavior: Pharmacotherapy in a Rat Model
role: Principal Investigator
August 2005 – August 2007

Evelyn F. McKnight Brain Research Foundation
Chronic Stress-Induced Neuroadaptations Cause Age-Associated Memory Impairment
role: Principal Investigator
December 2002 – August 2006

National Institutes of Health (N.I.C.H.D.)
grant #1R03 HD38239-01
Self-Injurious Behavior: Identification of Molecular Markers
role: Principal Investigator
April 2001 – October 2003

National Institutes of Health (N.I.D.A.)
grant #R01 DA08920-06A1
The Orphanin System: Role in Stress and Addiction
role: co-Investigator (Huda Akil was P.I.)
December 1999 – November 2005

National Institutes of Health (N.I.M.H.) R21
grant #1 R21 MH65678-01
Repetitive and Stereotyped Behavior Disorders in Autism
Development of the Florida Autism Center of Excellence
role: co-Investigator and Director of the Behavioral Neuroscience Core
(Mark H. Lewis was P.I.)
October 2001 – September 2003

Research Opportunities Fund Award
Self-Injurious Behavior: Identification of Environmental Determinants
role: Principal Investigator (Mark H. Lewis was co-P.I.)
April 2001 – March 2002

CLAS Research Award (UF)
Stress-Modulating Actions of Orphanin FQ
role: Principal Investigator
February 2001 – January 2002

Research Funding (cont'd…)

CLAS Research Award (UF)
Self-Injurious Behavior: Molecular Markers for Initiation & Recovery
role: Principal Investigator
January 1999 – December 1999

Fellowships

Fonds de la Recherche en Santé du Québec (F.R.S.Q.)
Dopaminergic Regulation of Opioid Receptor Gene Expression
Post-doctoral Fellowship
1993-1996

Medical Research Council of Canada (M.R.C.)
Behaviourally-Reinforcing Actions of Opioids in the Ventral Tegmentum
Post-graduate Fellowship
1991-1992

Natural Sciences and Engineering Research Council of Canada
Opioid Receptor Regulation of Mesolimbic Dopamine Neurotransmission
Post-graduate Fellowship
1989-1991

Fonds pour la Formation de Chercheurs et l'Aide à la Recherche
D_1 and D_2 Dopamine Receptor Functions in the Substantia Nigra
Post-graduate Fellowship
1989-1990

Publications:

Thesis and Dissertation:

1. Devine, D. P. (1993). The involvement of ventral tegmental opioid receptors in mediation of opiate-reward and in modulation of mesolimbic dopamine: Behavioural and neurochemical analyses, ***Doctoral Dissertation,*** Concordia University, Montréal, Canada.
2. Devine, D. P. (1988). An empirical evaluation of the latent structure of cognitive analgesia strategies**,** ***Master's thesis,*** Carleton University, Ottawa, Canada.

Papers in Preparation:

1. Devine, D.P. (in preparation). Self-injurious behavior: Biochemical factors that confer vulnerability in intellectually-handicapped populations, *Journal of Intellectual Disabilities Research.*
   * This paper is an invited *Annotation*. The journal publishes a maximum of 4 of these reviews per year.
2. Edge, C., Rice, K. G., and Devine, D.P. (in preparation). Physiological stress reactivity of perfectionists, *Psychoneuroenmdocrinology.*
3. Green, M. K., Devine, D. P., Vierck, C. J., and Yezierski, R. P. (in preparation). Effects of duloxetine on nociceptive reflexes and operant measures of pain, *Pain*.

Published Papers and Chapters:

1. Reynolds, S., Millette, A., and Devine, D.P. (in press). Sensory and motor characterization in the post-natal valproate rat model of autism, *Developmental Neuroscience*.
2. Marcinkiewcz, C. A. and Devine, D.P. (submitted). The organic cation transporter 3, Gene expression and behavioral characterization in an animal model, *Genes Brain and Behavior.*
3. Muehlmann, A.M., Kies, S.D., Turner, C.A., Wolfman, S., Lewis, M.H., and Devine, D.P. (in press). Self-injurious behavior: Limbic disregulation and stress effects in an animal model, *Journal of Intellectual Disabilities Research.* (Special issue on self-injurious behavior)
4. Wolfman, S. and Devine, D. P. (2011). The Effects of Chronic Social Stress on a Rodent Model of Self-Injurious Behavior, *J. Undergraduate Research*, **12 (3):** 1-4.
5. McDonald, C. J., Murphy, T. K., and Devine, D. P. (in press). Relationship between cortisol concentration and tic severity in children with Tourette's syndrome, *J. Undergraduate Research*, **12 (3):** 1-4.

Published Papers and Chapters (cont'd…):

6. Devine, D.P. (2011). Animal Models of Self-Injurious behaviour: An Overview, ch. 4, In: *Psychiatric Disorders: Methods and Protocols*, Vol. 829, F. H. Kobeissy (Ed.), Humana Press, New York, NY. *pp. 68-85.*
7. Devine, D.P. (2011). The Pemoline Model of Self-Injurious Behaviour, ch. 9, In: *Psychiatric Disorders: Methods and Protocols*, Vol. 829, F. H. Kobeissy (Ed.), Humana Press, New York, NY. *pp. 153-170.*
8. Muehlmann, A.M., Wilkinson, J.A., and Devine, D.P. (2011). Individual differences in vulnerability for self-injurious behavior: Studies using an animal model, *Behavioural Brain Research*, **217:** 148-154.
9. Green, M. K. and Devine, D. P. (2009). Nociceptin/Orphanin FQ and NOP receptor gene regulation after single or repeated social defeat exposure, *Neuropeptides*, **43:** 507-514.
10. Marcinkiewcz, C.A., Green, M.K., Devine, D. P., Duarte, P., Vierck, C. J., and Yezierski, R. P. (2009). Social defeat stress potentiates thermal sensitivity in operant models of pain processing, *Brain Research*, **1251:** 112-120. **** recommended by Faculty of 1000 Medicine, March 4 2009.**
11. Devine, D. P. and Muehlmann, A. M. (2009). Tiermodelle für selbstverletzendes Verhalten (Animal models of self-injurious behaviour), In: *Selbstverletzendes Verhalten bei stressassoziierten Erkrankungen* (*Self-Injurious Behaviour in Stress-Associated Disorders*), C. Schmahl and C. Stiglmayr (Eds.), pp 39-60, Verlag W. Kohlhammer, Stuttgart.
12. Muehlmann, A.M. and Devine, D. P. (2008). Glutamate-mediated neuroplasticity in an animal model of self-injurious behaviour, *Behavioural Brain Research*, **189:** 32-40.
13. Muehlmann, A.M., Brown, B.D. and Devine, D. P. (2008). Pemoline-induced self-injurious behavior: a rodent model of pharmacotherapeutic efficacy, *Journal of Pharmacology and Experimental Therapeutics*. **324:** 214-223.
14. Green, M.K., Barbieri, E.V., Brown, B.D., Chen, K.-W., and Devine, D. P. (2007). Roles of the bed nucleus of stria terminalis and of the amygdala in N/OFQ-mediated anxiety and HPA axis activation, *Neuropeptides*, **41:**.399-410.
15. King, C.D., Devine, D. P., Vierck, C. J., Mauderli, A., and Yezierski, R. P. (2007). Opioid modulation of reflex versus operant responses following stress in the rat, *Neuroscience*, **147:** 174-182.
16. Blake, B. L., Muehlmann, A. M., Egami, K., Breese, G. R., Devine, D. P., and Jinnah, H. A. (2007). Nifedipine suppresses self-injurious behaviors in animals, *Developmental Neuroscience*, **29:** 241-250.
17. Devine, D. P. (2007). Foreword: Effects of stress on behaviour and biology. *J. Undergraduate Research,* **8 (5)**.
18. Barbieri, E. V. and Devine, D. P. (2007). N/OFQ-mediated anxiety: Role of the bed nucleus of stria terminalis, *J. Undergraduate Research,* **8 (5).**
19. Naccarato, A. M. and Devine, D. P. (2007). Rats exhibit behavioral despair after repeated social defeat stress, *J. Undergraduate Research,* **8 (5).**
20. Wilkinson, J.A. and Devine, D. P. (2007). Self-injurious behavior: Investigating individual differences in vulnerability and sensory thresholds in an animal model, *J. Undergraduate Research,* **8 (5).**
21. Tartar, J. L., King, M. A., and Devine, D. P. (2006). Glutamate-mediated neuroplasticity in a limbic input to the hypothalamus, *Stress*, **9:** 13-19.
22. Hersh, C. M. and Devine, D. P. (2005). The effects of environmental challenges on nociceptin/orphanin FQ-induced anxiety, *Journal of Undergraduate Research.* **6 (7)**.
23. Kies, S. D. and Devine, D. P. (2004). Self-injurious behaviour: a comparison of caffeine and pemoline models in rats, *Pharmacology Biochemistry & Behavior*, **79:** 587-598.
24. Fernandez, F., Misilmeri, M. A., Felger, J. C., and Devine, D. P. (2004). Nociceptin/Orphanin FQ increases anxiety-related behaviour and circulating levels of corticosterone during neophobic tests of anxiety, *Neuropsychopharmacology*, **29:** 59-71.
25. Bauer M. C. and Devine, D. P. (2004). The effects of J113397, an orphanin/nociceptin FQ receptor antagonist, on the limbic-hypothalamic-pituitary-adrenal axis, *Journal of Undergraduate Research.* **6 (2)**.
26. King, C. D., Devine, D. P., Rodgers, J., Vierck, C. J., and Yezierski, R. P. (2003). Differential effects of stress on escape and reflexive responses to noxious thermal stimuli in the rat, *Brain Research*, **987:** 214-222.

Published Papers and Chapters (cont'd…):

27. Kabbaj, M., Yoshida, S., Numachi, Y., Matsuoka, H., Sato, M., Devine, D. P., and Ueda, T. (2003). Methamphetamine differentially regulates hippocampal glucocorticoid and mineralocorticoid receptor mRNAs in Fisher and Lewis rats, *Molecular Brain Research*. **117:** 8-14.
28. Simpkiss, J. L. and Devine, D. P. (2003). Responses of the HPA axis after chronic variable stress: effects of novel and familiar stressors, *Neuroendocrinology Letters*. **24:** 75-81.
29. Devine, D. P., Hoversten, M. T., Ueda, Y., and Akil, H. (2003). Nociceptin/orphanin FQ content is decreased in forebrain neurones during acute stress, *Journal of Neuroendocrinology*, **15:** 69-74.
30. Fernandez, F. and Devine, D. P. (2002). N/OFQ exerts anxiogenic actions in an open field, *Journal of Undergraduate Research.* **3 (10)**.
31. Devine, D. P., Watson, S. J., Jr. and Akil, H. (2001). Nociceptin/orphanin FQ regulates neuroendocrine function of the limbic-hypothalamic-pituitary-adrenal axis, *Neuroscience*. **102:** 541-553.
32. Kabbaj, M., Devine, D. P., Savage, V., and Akil, H. (2000). Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules, *Journal of Neuroscience*. **20:** 6983-6988.
33. Akil, H., Meng, F., Devine, D. P. and Watson, S. J. (1997). Molecular and neuroanatomical properties of the endogenous opioid system: implications for treatment of opiate addiction, In**:** Seminars in Neuroscience - Strategies for the Treatment of Opiate Abuse, L. L. Iversen and B. H. Herman (Eds.), vol. 9, pp. 70-83 Academic Press, New York.
34. Devine, D. P., Taylor, L., Reinscheid, R. K., Monsma, F. J., Jr., Civelli, O. and Akil, H. (1996). Rats rapidly develop tolerance to the locomotor-inhibiting effects of the novel neuropeptide orphanin FQ, *Neurochemical Research.* **21:** 1387-1396.
35. Devine, D. P., Reinscheid, R. K., Monsma, F. J., Jr., Civelli, O. and Akil, H. (1996). The novel neuropeptide orphanin FQ fails to produce conditioned place preference or aversion, *Brain Research.* **727:** 225-229.
36. Carlezon, W. A. Jr., Devine, D. P. and Wise, R. A. (1995). Habit-forming actions of nomifensine in nucleus accumbens, *Psychopharmacology.* **122:** 194-197.
37. Devine, D. P. and Wise, R. A. (1994). Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats, *Journal of Neuroscience*. **14:** 1978-1984.
38. Devine, D. P., Leone, P. and Wise, R. A. (1993). Mesolimbic dopamine neurotransmission is increased by administration of μ-opioid receptor antagonists, *European Journal of Pharmacology*. **243:** 55-64.
39. Devine, D. P., Leone, P., Pocock, D. and Wise, R. A. (1993). Differential involvement of ventral tegmental area *mu*, *delta* and *kappa* opioid receptors in modulation of basal mesolimbic dopamine release: *In vivo* microdialysis studies, *Journal of Pharmacology & Experimental Therapeutics.* **266:** 1236-1246.
40. Devine, D. P., Leone, P. and Wise, R. A. (1993). Striatal tissue preparation facilitates early sampling in microdialysis and reveals an index of neuronal damage, *Journal of Neurochemistry.* **61:** 1246-1254. (A commentary on this paper was published in *Current Separations*, (1994). 12: 200.)
41. Devine, D. P., Leone, P. and Wise, R. A. (1993). Surgical preparation of striatal tissue facilitates early sampling in microdialysis and reveals an index of neuronal damage, *Current Separations.* **12:** 68.
42. Devine, D. P., Leone, P., Carlezon, W. A. Jr. and Wise, R. A. (1993). Ventral mesencephalic opioid receptors are involved in modulation of basal mesolimbic dopamine neurotransmission: An anatomical localization study, *Brain Research.* **622:** 348-352.
43. Devine, D. P. and Spanos, N. P. (1990). Effectiveness of maximally different cognitive strategies and expectancy in attenuation of reported pain, *Journal of Personality & Social Psychology.* **58:** 672-678.
44. Devine, D. P. and Spanos, N. P. (1988). Distraction, imagery, and expectancy factors in cognitive analgesia, *Canadian Psychology.* **29:** 187.

Abstracts / Conference Proceedings:

1. Reynolds, S., Millette, A., and Devine, D.P. (2011) Sensory and motor characterization in the post-natal valproate model of autism. *Society for Neuroscience Abstracts,* **37:** 57.18.
2. Weinstock, N.J. and Devine, D.P. (2011) A mouse model of pemoline-induced self-injurious behavior. *Society for Neuroscience Abstracts,* **37:** 776.11
3. LaSala, M., Devine, D.P. and Zolotukhin, S. (in press). Long-term salivary PYY(3-36) treatment modulates aggressive behavior, *Obesity Society*.
4. Devine, D.P. (2011). Disregulation of dopamine and glutamate neurotransmission in animal models of self-injurious behavior, *Experimental Biology 2011,* S369.
5. Marcinkiewcz, C.A. and Devine, D.P. (2010). Stress-modulated polyspecific cation transporters in the brain: Role in individual vulnerability for psychopathology, *American College of Neuropharmacology*, **49:** 225.
6. Devine, D.P. (2010). The role of stress-responsiveness in self-injurious behaviors: Biochemical studies in an animal model, *First International Congress on Borderline Personality Disorder*, **1:** S-031.
7. Edge, C. Rice, K. G. and Devine, D.P. (2010). Perfectionism, emotion regulation, and physiological stress reactivity, *World Conference on Stress & Anxiety Research*, **31:** 63.
8. Marcinkiewcz, C.A. and Devine, D.P. (2010). Blockade of the organic cation transporter-3 (OCT3) attenuates depressive-like behavior in Wistar-Kyoto rats: Implications for treatment-resistant depression, *Society for Neuroscience Abstracts,* **36:** 885.12
9. Marcinkiewcz, C.A. and Devine, D.P. (2010). Social defeat stress differentially modulates hippocampal expression of the organic cation transporter-3 in rats exhibiting behavioral depression, *International Behavioral Neuroscience Society* **19:** 115.
10. Devine, D.P. (2010). Biochemical factors that confer individual differences in vulnerability for self-injurious behaviour, in symposium on "Behavioral and biological frontiers in the analyses of self-injurious behavior", *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** Symposium 13.
11. Van Matre, A.M., Wolfman, S., and Devine, D.P. (2010). Neurotensin plays a modulatory role in self-injurious behavior: biochemical analyses using an animal model, *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, **43:** 29.
12. Devine, D.P. (2009). The physiological basis of self-injurious behavior: Studies in an animal model, *Association for Behavioral and Cognitive Therapies.* **43:** 109.
13. Van Matre, A.M., Wolfman, S., and Devine, D.P. (2009). Neurotensin plays a modulatory role in pharmacologically-induced self-injurious behavior, *Society for Neuroscience Abstracts,* **35:** 734.10
14. Marcinkiewcz, C. and Devine, D.P. (2009). Stress-induced changes in neural expression of the organic cation transporter: Insights from an animal model of depression, *Society for Neuroscience Abstracts,* **35:** 866.6
15. Green, M.K., Devine, D.P., Vierck, C.J., and Yezierski, R.P. (2009). Effects of duloxetine (Cymbalta) on escape and lick/guard behaviors in rats, *American Pain Society,* **28:** 195.
16. Acosta, A., Aslanidi, G., Geguchadze, R., Wright, A., Campbell-Thompson, A., Voutetakis, A,. Baum, B.J., Devine, D.P., and Zolotukhin, S. (2009). Modulating Long-Term Feeding and Social Behavior Using Non-Invasive Gene Therapy, *American Society of Gene Therapy,* **12:** 169.
17. Van Matre, A.M., Wolfman, S., and Devine, D.P. (2009). The Role of Neurotensin in an Animal Model of Self-Injurious Behavior, *International Meeting for Autism Research*. **8:** 120.82
18. Weinstock, N.J., Marcinkiewcz, C., and Devine, D.P. (2008). Chronic variable social stress: behavioral and biochemical studies in an animal model, *Society for Neuroscience Abstracts.* **34:** 282.13.
19. Marcinkiewcz, C., Sand, S., Stoll, M.L., Reep, R.L., and Devine, D.P. (2008). Journey to the center of the brain: An inquiry-based approach to teaching neuroscience in a K-12 classroom, *Society for Neuroscience Abstracts.* **34:** 222.13.
20. Green, M.K., Devine, D.P., Vierck, C.J., and Yezierski, R.P. (2008). Effects of duloxetine (Cymbalta) on escape and lick/guard behaviors in normal rats, *Society for Neuroscience Abstracts.* **34:** 267.17

Abstracts / Conference Proceedings (cont'd…):

21. Marcinkiewcz, C., Duarte, P., Vierck, C.J., Yezierski, R.P., and Devine, D.P. (2008). Stress and pain: Effects of psychosocial stress on thermal nociception in an operant model of pain processing, *Society for Neuroscience Abstracts.* **34:** 364.18.
22. Muehlmann, A.M., Wolfman, S., and Devine, D.P. (2008). Examining the effects of chronic stress on self-injurious behavior in an animal model, *Society for Neuroscience Abstracts.* **34:** 446.29.
23. Devine, D.P. and Muehlmann, A.M. (2008). Self-injurious behavior: Strategies for investigating behavioral pathology in an animal model, *Keystone Symposium: Towards Identifying the Pathophysiology of Autistic Syndromes*. **C2:** 104.
24. Muehlmann, A.M. and Devine, D.P. (2008). Self-injurious behavior: Individual differences in neurotransmitter concentrations using an animal model, *Keystone Symposium: Towards Identifying the Pathophysiology of Autistic Syndromes*. **C2:** 206.
25. Weinstock, N.J., Kabbaj, M., and Devine, D.P. (2007). Effects of social defeat stress on limbic expression of connexins, *Society for Neuroscience Abstracts.* **33:** 298.23.
26. Muehlmann, A.M. and Devine, D.P. (2007). Characterization of drug titers and neurotransmitter concentrations during induction and maintenance of pharmacologically-induced self-injurious behavior, *Society for Neuroscience Abstracts.* **33:** 799.20.
27. Devine, D.P., Wilkinson, J.A., and Muehlmann, A.M. (2007). Sensory thresholds in an animal model of self-injurious behavior, *Society for Neuroscience Abstracts.* **33:** 799.21.
28. Green, M.K. and Devine, D.P. (2007). Effects of acute and chronic social defeat stress on the regulation of the NOP receptor, *Society for Neuroscience Abstracts.* **33** 734.20.
29. Muehlmann, A.M., Wilkinson, J.A., and Devine, D.P. (2007). Sensory thresholds and drug metabolism in an animal model of self-injurious behavior, *International Meeting for Autism Research*. **6:** PS6.6.
30. Stone, K.L., Naccarato, A.M., and Devine, D.P. (2006). Rats exhibit behavioral despair after social defeat stress, *Society for Neuroscience Abstracts.* **32:** 287.17.
31. Muehlmann, A.M., Wilkinson, J.A., and Devine, D.P. (2006). Individual differences in etiology of self-injurious behavior, using an animal model, *Society for Neuroscience Abstracts.* **32:** 487.17.
32. Green, M.K. and Devine, D.P. (2006). Effects of acute and chronic social defeat stress on the regulation of N/OFQ and the NOP receptor, *Society for Neuroscience Abstracts.* **32:** 808.13.
33. Devine, D.P., Kies, S.D., Wilkinson, J.A., and Muehlmann, A.M. (2006). Individual differences in expression of self-injurious behavior, using an animal model, *International Meeting for Autism Research*. **5:** PS1.65.
34. Muehlmann, A.M. and Devine, D.P. (2006). Self-injurious behavior: Pharmacological studies in an animal model, *International Meeting for Autism Research*. **5:** PS1.69
35. Green, M.K. Barbieri, E. V., Brown, B. and Devine, D.P. (2005). N/OFQ-mediated anxiety: Role of the bed nucleus of stria terminalis, *Society for Neuroscience Abstracts.* **31:** 184.2.
36. Muehlmann, A.M. and Devine, D.P. (2005). Pemoline-induced self-injury: Investigating the neurobiological basis in an animal model, *Society for Neuroscience Abstracts.* **31:** 448.11.
37. Devine, D.P. and Muehlmann, A.M. (2005). Pemoline-induced self-injury: Pharmacological validation of an animal model, *Society for Neuroscience Abstracts.* **31:** 448.12.
38. Stone, K. L. and Devine, D.P. (2005). Analysis of the resident-intruder interaction during social defeat stress, *Society for Neuroscience Abstracts.* **31:** 873.15.
39. King, C.D., Devine, D.P., Vierck, C.J., and Yezierski, R.P. (2004) Effects of acute stress on two different behavioral measures of thermal nociception, *American Pain Society*. **24:** 5227.
40. Muehlmann, A.M. and Devine, D.P. (2004). A pharmacological challenge of pemoline-induced self-injury using tramadol, *Society for Neuroscience Abstracts.* **30**: 116.4.
41. Kies, S. D., Kabbaj, M. and Devine, D.P. (2004). A comparison of caffeine- and pemoline-induced self-injury in rats, *Society for Neuroscience Abstracts.* **30**: 116.5.
42. Lopes, K. and Devine, D.P. (2004). Elevation of HPA axis activity in response to repeated social defeat stress in rats, *Society for Neuroscience Abstracts.* **30**: 426.12.

Abstracts / Conference Proceedings (cont'd…):

43. Green, M.K. and Devine, D.P. (2004). N/OFQ-mediated anxiety: Roles of the bed nucleus of stria terminalis and the amygdala, *Society for Neuroscience Abstracts.* **30**: 762.5
44. Devine, D.P., Bauer, M.C., Kolesov, S. Hersh, C. and Tartar, J.L. (2004). N/OFQ-induced anxiogenesis: Pharmacological and environmental challenges, *Society for Neuroscience Abstracts.* **30**: 762.6
45. Devine, D.P. (2004). Chronic Stress-Induced Neuroadaptations Cause Age-Associated Memory Impairment, *Evelyn F. McKnight Brain Research Foundation, Board of Trustees Meeting,* **2**.
46. Kies, S. D. and Devine, D.P. (2003). The effects of pemoline on neuronal metabolic activity in a rodent model of self-injurious behavior, *Society for Neuroscience Abstracts.* **29:** 318.17.
47. King, C.D., Devine, D.P., Vierck, C. J., and Yezierski, R. P. (2003). Differential effects of naloxone on acute stress-induced changes in somatosensory processing, *Society for Neuroscience Abstracts.* **29:** 908.3.
48. Misilmeri, M.A. and Devine, D.P. (2003). The NOP receptor antagonist J-113397 activates HPA neuroendocrine activity in unstressed rats, *Society for Neuroscience Abstracts.* **29:** 396.13.
49. Simpkiss, J.L, King, M.A., and Devine, D.P. (2003). Electrophysiologically-induced changes in HPA-axis functioning, *Society for Neuroscience Abstracts.* **29:** 192.8.
50. Kabbaj, M. and Devine, D.P. (2003). Interactions between N/OFQ and the limbic HPA axis: biochemical markers, *Society for Neuroscience Abstracts.* **29:** 392.10.
51. Simpkiss, J.L, Divine, J., Bauer, M.C., and Devine, D.P. (2003). Amygdaloid mediation of N/OFQ-induced anxiety, *Society for Neuroscience Abstracts.* **29:** 396.14.
52. Kabbaj, M. and Devine, D.P. (2003). Interactions between N/OFQ and the limbic HPA axis: biochemical markers, *International Narcotics Research Conference Abstracts.* **34:** O VII-1.
53. Simpkiss, J.L, Divine, J., Bauer, M.C., and Devine, D.P. (2003). Amygdaloid mediation of N/OFQ-induced anxiety, *International Narcotics Research Conference Abstracts.* **34:** P 124.
54. Devine, D.P. (2003). Chronic Stress-Induced Neuroadaptations Cause Age-Associated Memory Impairment, *Evelyn F. McKnight Brain Research Foundation, Board of Trustees Meeting,* **1:** 3-4.
55. Kies, S. D., Turner, C. A., Lewis, M. H., and Devine, D.P. (2002). Effects of environmental complexity in an animal model of self-injury, *Society for Neuroscience Abstracts.* **28:** 207.8.
56. Misilmeri, M.A., Fernandez F., Felger, J.C., and Devine, D.P. (2002). Intracerebroventricular microinjections of N/OFQ produce anxiogenic effects in neophobic tests of anxiety, *Society for Neuroscience Abstracts.* **28:** 571.11.
57. Fernandez, F., Misilmeri, M.A., and Devine, D.P. (2002). Validation of a modified open field: testing anxiety in the rat, *Society for Neuroscience Abstracts.* **28:** 571.10.
58. Simpkiss, J. L. and Devine, D.P. (2002). Electrophysiological changes in central stress circuitry, *Society for Neuroscience Abstracts.* **28:** 865.10.
59. King, C.D., Rodgers, J.C., Devine, D.P., Vierck, C. J., and Yezierski, R. P. (2002). The effects of chronic stress on thermal sensitivity in rats, *Society for Neuroscience Abstracts.* **28:** 156.8.
60. Misilmeri, M. A., Fernandez F., Felger, J. C., and Devine, D.P. (2002). Limbic actions of nociceptin/orphanin FQ (N/OFQ): hormones and anxiety, *Frontiers in Addiction Research, NIDA Director's Symposium.* 1: 44.
61. Kies, S. D. and Devine, D.P. (2002). Quantification of self-injurious behavior in an animal model of pemoline-induced self-injury, *International Meeting for Autism Research*. 2: P1.2.4
62. Fernandez, F., Felger, J. C., Misilmeri, M. A., and Devine, D.P. (2002). Nociceptin/orphanin FQ (N/OFQ) is anxiogenic in tests of rat neophobia, *International Narcotics Research Conference Abstracts.* 33: S46.
63. Whiting, S. K. and Devine, D.P. (2001). Individual differences in caffeine- and pemoline-induced self-injury, *Society for Neuroscience Abstracts.* 27: 775.14.
64. Misilmeri, M. A., Gregory, G. W. and Devine, D.P. (2001). Microinjections of N/OFQ into the VTA and NAcc produce opposite effects in conditioned place preference, *Society for Neuroscience Abstracts.* 27: 320.9.

Abstracts / Conference Proceedings (cont'd…):

65. Simpkiss, J. and Devine, D.P. (2001). Altered regulation of the HPA axis after exposure to chronic unpredictable stress: Effects of novel and familiar stressors, *Society for Neuroscience Abstracts.* 27: 412.5.
66. Whiting, S. K. and Devine, D.P. (2001). Factors that predicate self-injury in an animal model of self-injurious behavior, *International Meeting for Autism Research.* 1: P1.2.4
67. Misilmeri, M. A., Gregory, G. W., Akil, H. and Devine, D.P. (2001). Motivational effects of N/OFQ administered into the nucleus accumbens and ventral tegmental area, *International Narcotics Research Conference Abstracts.* **32:** 46.
68. Whiting, S. K., Repetto, M.J., and Devine, D.P. (2000). Evaluation of an Animal Model of Caffeine-Induced Self-Injury. *Society for Neuroscience Abstracts.* **26:** 538.1.
69. Misilmeri, M. A., and Devine, D.P. (2000) Neuroanatomical sites mediating orphanin FQ-induced modulation of LHPA axis activity. *Society for Neuroscience Abstracts.* **26:** 539.3.
70. Misilmeri, M. A., Akil, H. and Devine, D.P. (2000). OFQ-induced modulation of LHPA axis activity: a neuroanatomical study. *International Narcotics Research Conference Abstracts.* **31:** 79.
71. Repetto, M. J., Padron, A. M., McWilliams, H. J. and Devine, D.P. (1999). Amygdaloid injections of orphanin FQ (OFQ) increase activity of the hypothalamic-pituitary-adrenal axis. *Society for Neuroscience Abstracts.* **25:** 590.10.
72. Devine, D.P., McWilliams, H. J., Padron, A. M. and Repetto, M. J. (1999). Intra-amygdala injection of orphanin FQ increases plasma corticosterone concentration. *International Narcotics Research Conference Abstracts.* **30:** 18.
73. Devine, D.P., Hoversten, M. and Akil, H. (1998). Acute stress reduces striatal orphanin FQ content**.** *Society for Neuroscience Abstracts.* **24:** 536.19.
74. Kabbaj, M., Devine, D.P., Watson, S. J. and Akil, H. (1998). Individual differences in physiological responses to an anxiogenic stressor. *Society for Neuroscience Abstracts.* **24:** 564.12.
75. Devine, D.P., Hoversten, M. and Akil, H. (1998). Alterations in neuronal orphanin FQ content following exposure to an acute stressor. *International Narcotics Research Conference Abstracts.* **29:** 79.
76. Devine, D.P., Watson, S. J., Civelli, O. and Akil, H. (1997). Intracerebroventricular administration of the neuropeptide orphanin FQ alters responses of the HPA axis to stress *Society for Neuroscience Abstracts.* **23:** 480.9.
77. Devine, D.P., Watson, S. J., Civelli, O. and Akil, H. (1997). Orphanin FQ increases plasma corticosterone responses to stress, *International Narcotics Research Conference Abstracts.* **28:** 36.
78. Devine, D.P., Reinscheid, R., Monsma, F., Civelli, O. and Akil, H. (1996). Motivational and motoric effects of intracerebroventricular administration of the novel neuropeptide orphanin FQ. *Society for Neuroscience Abstracts.* **22:** 521.4.
79. Devine, D.P., Reinscheid, R., Monsma, F., Civelli, O. and Akil, H. (1996). A behavioural characterization of the effects of intracerebroventricular administration of the novel neuropeptide orphanin FQ. *International Narcotics Research Conference Abstracts.* **27:** 84.
80. Watson, S. J., Mansour, A., Meng, F., Devine, D.P., Civelli, O. and Akil, H. (1996). Orphanin FQ peptide and receptor: structure-function, anatomical and behavioral analysis. *International Narcotics Research Conference Abstracts.* **27:** 30.
81. Devine, D.P. and Akil, H. (1996). A behavioural and anatomical characterization of the motivational and motor-impairing effects of the novel neuropeptide orphanin. *Albert J. Silverman Research Conference Abstracts.* **7:** 19.
82. Devine, D.P. and Akil, H. (1995). Ventral tegmental area microinjections of a mu-opioid receptor antagonist produce conditioned place aversion. *Society for Neuroscience Abstracts.* **21:** 292.11.
83. Devine, D.P. and Akil, H. (1995). Motivational effects of a mu-opioid receptor antagonist microinjected into the ventral tegmentum. *Albert J. Silverman Research Conference Abstracts.* **6:** 27.
84. Devine, D.P., Watson, S. J. and Akil, H. (1994). The relative distributions of mRNAs encoding for mu and delta opioid receptors and for glutamic acid decarboxylase (GAD) in the ventral mesencephalon, *Society for Neuroscience Abstracts.* **20:** 705.5.

Abstracts / Conference Proceedings (cont'd…):

85. Devine, D.P., Watson, S. J. and Akil, H. (1994). Distributions of mRNAs encoding mu and delta opioid receptors, and glutamic acid decarboxylase (GAD) in the ventral mesencephalon, *Albert J. Silverman Research Conference Abstracts.* **5:** 13.
86. Devine, D.P., Leone, P. and Wise, R. A. (1993). Knife-cut lesions of the ventral mesencephalon differentially affect ventral striatal dopamine and dopamine metabolite concentrations, *Society for Neuroscience Abstracts.* **19:** 40.11.
87. Devine, D.P., Leone, P. and Wise, R. A. (1993). Striatal tissue preparation facilitates early sampling in microdialysis and reveals an index of neuronal damage, *Albert J. Silverman Research Conference Abstracts.* **4:** 13.
88. Devine, D.P. Leone, P. and Wise, R. A. (1992). Extracellular nucleus accumbens dopamine is increased by microinjections of selective μ opioid antagonists into the ventral tegmental area, *Society for Neuroscience Abstracts.* **18:** 417.6.
89. Devine, D.P. Leone, P. and Wise, R. A. (1992). Involvement of ventral tegmental μ, δ, and κ opioid receptors in reward and in modulation of mesolimbic dopamine: Intracranial self-administration and microdialysis studies, *International Narcotics Research Conference Abstracts.* **25:** 125.
90. Devine, D.P., Leone, P. and Wise, R. A. (1992). Modulation of mesolimbic dopamine by ventral tegmental area μ, δ, and κ opioid receptors: *in vivo* microdialysis studies, *Dopamine '92: From Neurobiology to Neuropathology Abstracts,* 109.
91. Devine, D.P., Leone, P., Pocock, D. and Wise, R. A. (1991). Microinjections of selective μ and δ agonists into the ventral tegmentum increase extracellular nucleus accumbens dopamine: An *in vivo* microdialysis study, *Society for Neuroscience Abstracts.* **17:** 132.6.
92. Devine, D.P., Leone, P., Pocock, D. and Wise, R. A. (1991). The effects of selective opiates in the ventral tegmentum on extracellular dopamine in the nucleus accumbens: An *in vivo* microdialysis study, *International Brain Research Organization Abstracts.* **3:** P60.14.
93. Devine, D.P. and Wise, R. A. (1990). Self-administration of morphine, [D-Ala^2,N-Me-Phe^4-Gly^5-ol]-enkephalin (DAGO), and [D-Pen^2,D-Pen^5]-enkephalin (DPDPE) into the ventral tegmentum of the rat, *Society for Neuroscience Abstracts.* **16**: 382.19.
94. Devine, D.P. and Spanos, N.P. (1988), Cognitive strategies, expectancy, and the attenuation of experimental pain, *Canadian Psychological Association / Societe Canadienne de Psychologie*. **49:** 8.

Invited Talks:

1. The biochemical basis of self-injurious behavior: focus on dopaminergic and glutamatergic signaling. *Symposium on "Autism and Pervasive Developmental Disorders: Neuropathology, Pharmacotherapies, and New Directions",* at the *Experimental Biology* conference. Washington DC (April 9, 2011).
2. The role of stress responsiveness in an animal model of self-injury. *Symposium on "Interaction of Disturbed Pain Processing and Self-Injurious Behavior",* at the *First International Congress on Borderline Personality Disorder.* Berlin Germany (July 3, 2010). co-Chair of the symposium, w/ Dr. C. Schmahl.
3. Biochemical factors that confer individual differences in vulnerability for self-injurious behavior. *Symposium on "Behavioral and biological frontiers in the analyses of self-injurious behavior"*, at the *Gatlinburg Conference on Research & Theory in Intellectual & Developmental Disabilities*, Annapolis VA (March 19, 2010). co-Chair of the symposium, w/ Dr. F. Symons.
4. The physiological basis of self-injurious behavior: Studies in an animal model, *Symposium on "The Role of Universal Cognitive and Neurobiological Factors in Deliberate Self-Harm", Association for Behavioral and Cognitive Therapies*, New York, NY (November 21 2009).
5. Limbic Neuroplasticity: Insights into Stress-Induced Psychopathology, Opening address for *Eric Kandel Symposium, Nova Southeastern University*, Ft. Lauderdale, FL (February 2008).
6. Stress and anxiety responses: Regulation by the peptide neurotransmitter N/OFQ, *Department of Medicinal Chemistry, University of Mississippi*, Oxford, MS (April 2004).
7. Neurobiology of stress and anxiety responses: role of the neuropeptide N/OFQ, *Department of Neuroscience, Florida State University*, Tallahassee, FL (November 2003).
8. Interactions between N/OFQ and the limbic HPA axis: biochemical markers. *International Narcotics Research Conference,* Perpignan, France (July 2003).
9. Nociceptin/orphanin FQ (N/OFQ) is anxiogenic in tests of rat neophobia, *International Narcotics Research Conference*, Monterey, CA (July 2002).
10. The neurobiology of stress and drug abuse: Involvement of the endogenous opioid and opioid-orphan systems*, University of Florida, Department of Psychology,* Gainesville, FL (January 1998).
11. Behavioural and physiological properties modulated by nociceptin/orphanin FQ, *International Narcotics Research Conference*, Hong Kong, China (August 1997).
12. Neurobiology of opioid and opioid-like orphan systems: Implications for drug abuse and stress responses, *Maryland Psychiatric Research Center, University of Maryland, School of Medicine, Department of Psychiatry,* Baltimore MD (March 1997).
13. Neurobiology of opioid and opioid-like orphan systems: Implications for drug abuse and stress responses, *William Paterson University, Department of Biology,* Pattersonville, NJ (February 1997).
14. The involvement of opioid and opioid-like orphan systems in the neurobiology of stress and drug abuse, *University of California at Irvine, School of Medicine, Department of Pharmacology,* Irvine CA (November 1996).
15. The involvement of opioid and opioid-like orphan systems in the neurobiology of stress and drug abuse, *Albert Einstein College of Medicine, Department of Psychiatry,* New York, NY (September 1996).
16. A behavioral characterization of the effects of intracerebroventricular administration of the novel neuropeptide orphanin FQ, *International Narcotics Research Conference,* Long Beach, CA (July 1996).
17. Involvement of ventral tegmental μ, δ, and κ opioid receptors in reward and in modulation of mesolimbic dopamine: Intracranial self-administration and microdialysis studies, *International Narcotics Research Conference, Keystone Resort,* Colorado, CO (July 1992).
18. Behaviour management of the survivor of traumatic brain injury, *"New Beginnings Conference", Head Injury Association of Canada, Ottawa, Ontario Canada* (1987).
19. Self-injury as communicative behaviour: Implications for assessment and treatment, *Southeastern Ontario Community Support Programs, Ottawa, Ontario, Canada* (1987).

Talks at the University of Florida:

1. Self-Injurious Behaviour: an Animal Model of an Autism Endophenotype, *Howard Hughes Medical Institute (HHMI) "Science for Life" program,* (November 17, 2009).
2. Neurobiological Mechanisms that Underlie the Self-Injurious Endophenotype, *University of Florida Neuroscience Club* (October 22, 2009)
3. The Biochemical Basis of Self-Injury, *Seminar in Psychological Science* (October 1, 2009).
4. The Neurobiological Basis of Vulnerability for Self-Injurious Behaviour, *McKnight Brain Institute* (April 15, 2009)
5. Individual Differences in Vulnerability for Self-Injurious Behaviour, *Center for NeuroPsychological Studies, Veteran's Administration Hospital* (April 3, 2009)
6. A Rodent Model of Self-Injurious Behaviour, *University of Florida Neuroscience Club* (March 16, 2009)
7. Self-Injurious Behaviour: Findings from an Animal Model, *Howard Hughes Medical Institute (HHMI) "Science for Life" program, Turlington* (October 7, 2008).
8. Stress-Induced Limbic Neuroplasticity, *Seminar in Psychological Science* (February 29, 2008).
9. Self-Injurious Behaviour: Findings from an Animal Model, *Howard Hughes Medical Institute (HHMI) "Science for Life" program, Cancer Genetics Institute* (March 6, 2007).
10. Rodent Models of Psychopathology: Stress and Self-injury, *UF College of Veterinary Medicine and Animal Care Services* (March 16, 2004).
11. Chronic stress-induced neuroadaptations in health and disease, *Comprehensive Center for Pain Research* (December 7, 2004).
12. Chronic Stress-Induced Neuroadaptations Cause Age-Associated Memory Impairment, *Evelyn F. McKnight Brain Research Foundation, Board of Trustees Meeting* (February 2004).
13. Self-injurious behaviour: findings from an animal model, *Fifth Annual Autism Conference* (January 2004).
14. Stress responses and anxiety: studies on N/OFQ, individual differences, and neuroadaptations, *NIMH Center for the Study of Emotion and Attention* (March 2003).
15. Stress responses and anxiety: studies on N/OFQ, individual differences, and neuroadaptations, *McKnight Brain Institute* (October 2002).

Grant Reviews:

Qatar National Research Fund
National Priorities Research Program
grant reviewer, March 2011

State of Pennsylvania Depart of Health
Oak Ridge Associated Universities program
ad hoc grant reviewer, November 2010

Wellcome Trust (United Kingdom)
WT2 grant reviewer, August 2010

National Science Foundation
Neuroscience and Physiology section
Member, Graduate Research Fellowship Panel, Arlington, VA., March 5-7, 2010

National Institutes of Health – National Institute on Mental Health
ZMH1 ERB-L-02, R01/R21 – Neurodevelopmental Studies of Mental Illness
Member, Special Emphasis Panel, Washington D.C., February 22-23, 2010

Indo-US Science and Technology Forum – Smithsonian Institute
Birth Defects and Disabilities in the Developing World
ad hoc grant reviewer, September 2009

National Institutes of Health – Recovery Act Limited Competition
RC1 Challenge grants
stage 1 grant reviewer, June 2009

Wellcome Trust (United Kingdom)
WT2 grant reviewer, March 2009

Autism Speaks Foundation
Member, Scientific Review Panel, Philadelphia, PA, February 25-27, 2008

National Institutes of Health – National Cancer Institute
NCI-E GRB-F (Q1) – Stress and Cancer
Member, P01 Review Panel, Bethesda, MD, June 10-11, 2004

National Institutes of Health – National Institute on Drug Abuse
NIDA ZDA1RXL-E (14) - Chronic Stress and Its Relation to Drug Abuse
Member, Special Emphasis Panel, Washington, DC, April 22-23, 2003

Cornerstone Program Enhancement Grant (PEG) competition
Florida State University
ad hoc reviewer, January 2004

South Carolina Exp. Program to Stimulate Competitive Research (EPSCoR)
March, 2002

National Institutes of Health – Center for Scientific Review
ZRG1 BBBP-2, (BDCN-2) - Brain Disorders and Clinical Neuroscience
ad hoc reviewer, 2001

National Science Foundation
Integrative Biology & Neuroscience, Behavioral Neuroscience Division
ad hoc reviewer, 2000-2001

National Science Foundation
Integrative Biology & Neuroscience, Sensory Systems Division
ad hoc reviewer, 2000 and 2001

Department of Veteran's Affairs
ad hoc reviewer, VA Medical Center, Gainesville, FL 1999

Allegheny University of the Health Sciences (intramural grant program)
ad hoc reviewer, 1996

Editorial Service:

Associate Editor and Guest Editor, Special Issue of *Journal of Intellectual Disabilities Research,* Special Issue on "Self-Injurious Behavior"
anticipated publication in late 2011

Editorial Advisory Board, *The Open Neuropsychopharmacology Journal*
Bentham Science Publishers, 2008 – present

Editorial Advisory Board, *The Open Neuroendocrinology Journal*
Bentham Science Publishers, 2008 – present

Guest Editor, *Journal of Undergraduate Research*, 8 (5): 2007.

Journal Reviews:

Behavioural Brain Research	Behavioural Processes
Biological Psychiatry	Biochemical Pharmacology
Brain Research	British Journal of Pharmacology
Current Drug Targets	European Journal of Neuroscience
Int Journal of Neuropsychopharmacology	International Journal of Psychology
Journal of Intellectual Disabilities Research	Journal of Neurochemistry
Journal of Neurodevelopmental Disorders	Journal of Neuroendocrinology
Journal of Neuroscience	Journal of Neuropsychopharmacology
Journal of Pharmacology and Exp Therapeutics	Journal of Psychopharmacology
Neurobiology of Learning and Memory	Neuropharmacology
Neuropsychopharmacology	Neuroscience
Neuroscience Letters	Open J of Neuropsychopharm Res
Peptides	Pharmacol Biochemistry and Behavior
Pharmacological Research	Physiology and Behavior
Proc of the National Academy of Sciences	Psychopharmacology
Psychoneuroendocrinology	Stress, Int J on the Biology of Stress

Book Reviews:

Oxford University Press
Behavioral Neuroscience

Cengage/Wadsworth Publishing Company
Behavioral Neuroscience: Dynamics of Brain Body Function (book proposal)

Cengage/Wadsworth Publishing Company
Behavioral Neuroscience (book proposal)

Sanders Publishing Company
Biology of Psychology (book proposal)

Allyn & Bacon Publishing Company
Physiology of Behavior 9th Edition, Neil R. Carlson

Allyn & Bacon Publishing Company
CD accompanying *Physiology of Behavior* 9th Edition

Sage Publishing Company
Brain and Behavior 2nd Edition, Bob Garrett

Wadsworth Publishing Company
Biological Psychology 7th Edition, James W. Kalat

Worth Publishing Company
An Introduction to Brain and Behavior 1st Edition, Bryan Kolb & Ian Q. Whishaw

Worth Publishing Company
An Introduction to Brain and Behavior 2nd Edition, Bryan Kolb & Ian Q. Whishaw

Scientific Outreach:

Founding member, and member of the Executive Committee, North-Central Florida Chapter of the Society for Neuroscience

Junior Science Engineering, and Humanities Symposium (JSEHS) 2007 - 2011
laboratory host and judge for this national competition of high school students

Society for Neuroscience Annual Meeting, New Orleans, LA 2003
Short Course Guide for high school students

Published by the Society for Neuroscience in the Annual Press Release Book
Interviewed on National Public Radio
Interviewed on WUFL
Interview published in "Child Protection Report", Baltimore MD.
Interview published in "The Link" newsletter, University of Florida

Promotion Committees:

External referee for promotion of a Faculty Scientist in the Department of Pharmacology at the University of California at Irvine (UCI) (2001)

External referee for promotion of Associate Professor to Full Professor at the University of Mississippi (2004)

External referee for promotion of Assistant Professor to Associate Professor at Nova Southeastern University (2009)

External referee for the promotion of an Associate Professor at the University of California at Los Angeles (UCLA) (2010)

Service at U. of Florida:

Member of Institutional Animal Care and Use Committee (2000-2002)
Alternate Member of Institutional Animal Care and Use Committee (2002-2005)
Search committee member – Behavioral Neuroscience faculty position, 2002
Search committee member – Center for Smell and Taste faculty position, 2003
Search committee member – Learning and Memory/Aging faculty position, 2004
Search committee member – Behavioral Genetics faculty position, 2004
Judge for "Doris Thames Staff Excellence Award" (2003)
Judge for Undergraduate Scholars Program writing competition (2003-2004)
Interviewed for internet course on Careers in Psychology (2003)
Interviewed for internet course in Counseling Psychology (2004)
Merit committee member (2004-2005)
T.A./Fellowship/Admissions committee rep. for Beh Neurosci program (2006-ongoing)
Website design/support for Behavioral Neuroscience Program (1999-ongoing)
Lab host and Judge for Junior Science, Engineering and Humanities Symp. (2007-2011)
Director, Behavioral and Cognitive Neuroscience Program, (2009-ongoing)
Member, Policy and Planning Committee, UF Dept. of Psychology
Member, CLAS Sabbatical Committee (2010)
Member, CLAS Faculty Enhancement Opportunity (FEO) Committee (2010-2011)

Technical Skills:

Small Animal Surgery:
stereotaxic intracranial and intravenous (jugular) cannulation
electrolytic / chemical lesions
vasectomy, adrenalectomy, etc.
perfusion, brain removal and dissection, sectioning and staining

Behavioral Assays:
intravenous / intracranial models of drug self-administration
intracranial electrical self-stimulation
conditioned place preference
locomotor activity / sensitization / tolerance
motor skill / motor impairment battery ("Rat Olympics")
drug dependence / withdrawal
models of stress / non-habituating stress
models of anxiety (elevated plus maze, open field, etc.)
models of learning / memory (Morris water maze, T-maze, etc.)

Neurochemical Assays:
intracranial microdialysis
high-pressure liquid chromatography / U-V and electrochemical detection

Molecular Assays:
preparation of competent cells / DNA transformation / plasmid preps
cell culture /cell transfection / cell passage
mRNA transcription Rxn / labeling of riboprobes
DNA / RNA isolation / purification / quantification
polyacrylamide / agarose gel electrophoresis
radioactive / digoxigenin mRNA *in situ* hybridization
immunohistochemistry (glyoxylic acid, TH, etc.)
radioimmunoassay, ELISA
Western blots (incl. phospho-specific)
PCR / RT-PCR / RT-PCR arrays
autoradiography
densitometry and image analysis

Additional Training:

Protein Chemistry and Molecular Cloning
Molecular Biology Laboratory Techniques Course (GMS6004), 2010

Society for Neuroscience Short Course: DNA Microarrays: The New Frontier in Gene Discovery and Gene Expression Analysis, *Society for Neuroscience*, 2000

NIH: National Institute of Child Health and Human Development
Workshop on Self-Injurious Behavior, 1999

Society for Neuroscience Short Course: Neurobiology of Developmental Disorders, *Society for Neuroscience*, 1997

Society for Neuroscience Short Course: Basics of Molecular Biology for Neurobiologists, *Society for Neuroscience*, 1992

Society for Neuroscience Short Course: Microdialysis and Voltammetry
Society for Neuroscience, 1991

National Institute on Drug Abuse: Technical Review Meeting on Neurobiological Models for Evaluating Mechanisms Underlying Cocaine Addiction and Potential Pharmacotherapies for Treating Cocaine Abuse, *Society for Neuroscience*, 1991

Current Professional Associations:

Society for Neuroscience (SfN)
American Association for the Advancement of Science (AAAS)
American Society for Pharmacology and Experimental Therapeutics (ASPET)
Cure Autism Now Foundation, Orlando Chapter (CAN)
International Society for Autism Research (INSAR)
Interactive Autism Network (IAN)
Evelyn F. and William L. McKnight Brain Institute
University of Florida Comprehensive Center for Pain Research
University of Florida Genetics Institute