PHARMACOLOGICAL CHALLENGES OF AN ANIMAL MODEL OF SELF-INJURIOUS BEHAVIOR

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2005
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by

Amber M. Muehlmann
This document is dedicated to my parents, Richard and Susan Muehlmann, my brother, Aaron Muehlmann, and my loving family in Gainesville, Florida, Nicholas, Shelby and Maximus Van Matre. Your love and support has allowed me to complete this work in only two years. Thank you.
ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Mark Lewis, Dr. Andy Shapira and Dr. Timothy Vollmer, for their time, as well as Dr. George Casella for all of his help with the statistical analyses. I would especially like to thank my advisor, Dr. Darragh Devine, for his guidance throughout the development and completion of these projects. I also wish to thank my labmates, both past and present, who have helped tremendously with these experiments.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGMENTS</th>
<th>iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>x</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION .................................................................1

2 METHODS ........................................................................12

   Animals........................................................................12
   Drugs..........................................................................12
   Experimental Procedures...............................................13
       Drug Treatments-Experiment 1: Risperidone .....................13
       Drug Treatments-Experiment 2: Valproate .......................13
       Drug Treatments-Experiment 3: Nifedipine .....................14
       Drug Treatments-Experiment 4: Tramadol .......................14
       Drug Treatments-Experiment 5: Memantine .....................14
   Behavioral and Histological Asssays-All experiments.........14
   Statistical Analyses.....................................................16

3 RESULTS ........................................................................21

   Experiment 1: Risperidone ............................................21
   Experiment 2: Valproate ..............................................28
   Experiment 3: Nifedipine .............................................35
   Experiment 4: Tramadol ..............................................42
   Experiment 5: Memantine .............................................49
   Inter-observer reliability ............................................56

4 DISCUSSION ....................................................................57

LIST OF REFERENCES .......................................................69
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Injury score rating scale</td>
</tr>
</tbody>
</table>

TABLE
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Effects of risperidone on pemoline-induced self-injury</td>
<td>23</td>
</tr>
<tr>
<td>3.2</td>
<td>Effects of risperidone on the incidence of self-injury</td>
<td>24</td>
</tr>
<tr>
<td>3.3</td>
<td>Effects of risperidone on the induction and maintenance of pemoline-induced self-injury</td>
<td>24</td>
</tr>
<tr>
<td>3.4</td>
<td>Effects of risperidone on the duration of pemoline-induced self-injurious oral contact</td>
<td>25</td>
</tr>
<tr>
<td>3.5</td>
<td>Effects of risperidone on the induction and maintenance of pemoline-induced self-injurious oral contact</td>
<td>25</td>
</tr>
<tr>
<td>3.6</td>
<td>Effects of risperidone on the grooming, inactivity and locomotion</td>
<td>26</td>
</tr>
<tr>
<td>3.7</td>
<td>Effects of risperidone on the health status of the rats</td>
<td>27</td>
</tr>
<tr>
<td>3.8</td>
<td>Effects of valproate on pemoline-induced self-injury</td>
<td>30</td>
</tr>
<tr>
<td>3.9</td>
<td>Effects of valproate on the incidence of self-injury</td>
<td>31</td>
</tr>
<tr>
<td>3.10</td>
<td>Effects of valproate on the induction and maintenance of pemoline-induced self-injury</td>
<td>31</td>
</tr>
<tr>
<td>3.11</td>
<td>Effects of valproate on the duration of pemoline-induced self-injurious oral contact</td>
<td>32</td>
</tr>
<tr>
<td>3.12</td>
<td>Effects of valproate on the induction and maintenance of pemoline-induced self-injurious oral contact</td>
<td>32</td>
</tr>
<tr>
<td>3.13</td>
<td>Effects of valproate on the grooming, inactivity and locomotion</td>
<td>33</td>
</tr>
<tr>
<td>3.14</td>
<td>Effects of valproate on the health status of the rats</td>
<td>34</td>
</tr>
<tr>
<td>3.15</td>
<td>Effects of nifedipine on pemoline-induced self-injury</td>
<td>37</td>
</tr>
<tr>
<td>3.16</td>
<td>Effects of nifedipine on the incidence of self-injury</td>
<td>38</td>
</tr>
</tbody>
</table>
3.17 Effects of nifedipine on the induction and maintenance of pemoline-induced self-injury .................................................................38
3.18 Effects of nifedipine on the duration of pemoline-induced self-injurious oral contact .................................................................39
3.19 Effects of nifedipine on the induction and maintenance of pemoline-induced self-injurious oral contact ..................................................39
3.20 Effects of nifedipine on the grooming, inactivity and locomotion .................40
3.21 Effects of nifedipine on the health status of the rats .....................................41
3.22 Effects of tramadol on pemoline-induced self-injury .....................................44
3.23 Effects of tramadol on the incidence of self-injury .......................................44
3.24 Effects of tramadol on the induction and maintenance of pemoline-induced self-injury .................................................................45
3.25 Effects of tramadol on the duration of pemoline-induced self-injurious oral contact .................................................................45
3.26 Effects of tramadol on the induction and maintenance of pemoline-induced self-injurious oral contact ..................................................46
3.27 Effects of tramadol on the grooming, inactivity and locomotion .....................47
3.28 Effects of tramadol on the health status of the rats ........................................48
3.29 Effects of memantine on pemoline-induced self-injury ...................................51
3.30 Effects of memantine on the incidence of self-injury .....................................51
3.31 Effects of memantine on the induction and maintenance of pemoline-induced self-injury .................................................................52
3.32 Effects of memantine on the duration of pemoline-induced self-injurious oral contact .................................................................52
3.33 Effects of memantine on the induction and maintenance of pemoline-induced self-injurious oral contact ..................................................53
3.34 Effects of memantine on the grooming, inactivity and locomotion ..................54
3.35 Effects of memantine on the health status of the rats .....................................55
Self-injurious behavior (SIB) is a devastating behavior disorder that involves acts directed at a person’s own body and causes damage to skin and underlying tissues. These actions are often expressed in a stereotyped manner and include, but are not limited to, self-biting, head banging and self-punching. SIB is exhibited by people with intellectual handicaps, particularly people with severe and profound intellectual impairment, and by people with several different congenital developmental disorders (e.g., Lesch-Nyhan syndrome, autism and Prader-Willi syndrome). Animal models of SIB have been developed using environmental restriction, neurotoxins, and pharmacological manipulations. These models, in combination with post-mortem and in vivo clinical data, have provided evidence that monoaminergic disregulation is an important factor in the development and expression of SIB. Pemoline, an indirect monoamine agonist, produces stereotyped SIB in rats when administered at high doses. We have investigated the potential therapeutic effectiveness of five drugs (risperidone, valproate, nifedipine,
tramadol and memantine) in this model of pemoline-induced SIB. These pharmacological challenges of the pemoline model were chosen in order to achieve four specific objectives. These objectives were to evaluate the predictive validity of the pemoline model, to test the generalizability of pharmacological interventions between several animal models of SIB, to investigate the pharmacotherapeutic potential of two of the drugs, and to further investigate the neurobiological mechanisms that contribute to pemoline-induced SIB. Risperidone and valproate effectively decreased the occurrence of SIB in clinical trials, and they each decreased the occurrence of pemoline-induced SIB. Nifedipine blocked SIB in the 6-hydroxydopamine and Bay-K 8644 models, and it decreased the pemoline-induced SIB. We also investigated the pharmacotherapeutic potential of tramadol (a drug that attenuates compulsive behaviors in obsessive-compulsive disorder and Tourette’s syndrome) and memantine (a glutamate receptor antagonist that has shown promise in treatment of Alzheimer’s disease and other clinical disorders). These drugs did not significantly lessen the occurrence of pemoline-induced SIB. Each of these experiments also reveals important new information regarding the neuronal changes that occur during chronic pemoline administration. These new findings will lead to future experiments on neurobiological changes that produce SIB, and they may help to identify potential neurobiological targets for new pharmacotherapies.
CHAPTER 1
INTRODUCTION

Self-injurious behavior (SIB) is a devastating, maladaptive behavior disorder that is common in intellectually handicapped populations. The self-injurious actions are usually highly stereotypic (Symons & Thompson, 1997), and they result in immediate or delayed damage to the skin or underlying tissues. Self-injurers exhibit many different forms of SIB, including head banging (Thompson & Caruso, 2002), self-biting (Nyhan, 1968), skin-picking (State et al., 1999), and self-punching (Oliver et al., 1987); and although individual self-injurers usually exhibit stereotyped patterns of behavior that are directed at specific and generally invariant body sites (Bodfish et al., 1995), there is great diversity in the forms of self-injury within and across clinical groups. These behaviors vary from mild self-injury producing bruises or calluses to severe self-injury leading to permanent tissue damage or tissue loss.

In addition to the dire physical consequences that self-injurers inflict upon themselves, these behaviors also limit social and cognitive development. SIB often results in exclusion from educational and socializing activities, and it interferes with all normal activities of daily living. SIB is highly destructive for families and caregivers who live and work with self-injurers, leading to increased stress (Sarimski, 1997) and feelings of despair (Bromley & Emerson, 1995). There are also significant costs to society (estimated at $3 billion in 1989), as self-injurers require additional resources in terms of specialized care and professional interventions (NIH Consensus Development Conference Statement, 1989).
SIB is positively correlated with the occurrence of non-injurious stereotypies and compulsions (Bodfish et al., 1995) and has even been hypothesized as being a compulsive behavior in itself (King, 1993). In fact, Powell and colleagues (1996) found that 46% of their self-injurious sample engaged in self-restraint in an apparent attempt to interrupt their self-injury – suggesting that these individuals were resisting a compulsive need to self-injure. Unfortunately, these self-restraining behaviors did not produce any decrease in the occurrence of SIB.

SIB is also associated with stress, wherein SIB increases in stressful situations (e.g., being around new people, being sick or when having restraints removed) (Anderson & Ernst, 1994), and there is a disproportionately high prevalence of SIB in disorders that involve abnormal amounts of distress (Sovner & Fogelman, 1996; Lindauer et al., 1999). Estimates of the population prevalence of SIB range from 1.7% to 65.9% of the intellectually handicapped in general. These estimates vary considerably because definitions of SIB are inconsistent; some studies include mild SIB whereas others only report the incidence of moderate to severe SIB (for review see Rojahn & Esbensen, 2002). Estimates also differ because individuals with severe or profound intellectual disabilities are more likely to self-injure than individuals with mild or moderate intellectual disabilities (McClintock et al., 2003), and because individuals in institutions are more likely to self-injure than those who are not in institutions (Eyman & Call, 1977). It is unclear, however, if the greater severity of SIB in institutionalized populations is actually caused by the institutional environment, if it is because the more severely intellectually handicapped are more likely to live in an institution (Eyman & Call, 1977), or if it is because the SIB is the reason for institutionalization (Eyman et al., 1972).
SIB also presents as a phenotypic trait of many specific congenital developmental disorders. Approximately 44% of individuals with Cornelia de Lange syndrome self-injure by head banging, self-scratching and finger biting (Berney et al., 1999). Among girls with Rett syndrome, 50% compulsively wring or bite their hands until there are lesions on the skin (Sansom et al., 1993). Estimates of SIB in autism are more variable, as are all the characteristics of autism. One study found mild SIB in 21.5%, moderate SIB in 17.1% and severe SIB in 14.6% of their autistic sample (Baghdadli et al., 2003).

Additionally, 80% of individuals with Prader-Willi syndrome will compulsively pick at their skin, leading to sores and infection (Symons et al., 1999). SIB is almost always observed in Lesch-Nyhan syndrome, but there have been rare cases where the expression of SIB has been delayed or non-existent (Mitchell & McInnes 1984; Singh et al., 1986; Hatanaka et al., 1990; Adler & Wrabetz, 1996). The severity of their SIB is usually extreme and many individuals with Lesch-Nyhan syndrome exhibit self-injury that causes tissue loss and deformity of the hands and face. (Nyhan, 1968; Anderson & Ernst, 1994).

The biological basis of Lesch-Nyhan syndrome is any single point mutation in the HPRT enzyme, which renders the enzyme completely inactive. The biological consequences of that deficiency that lead to SIB are not understood. There is disregulation in a variety of neurotransmitter systems, and most studies have indicated a prominent disregulation of dopamine. Studies of post-mortem brain tissue have shown a significant loss of dopamine functioning in the nigrostriatal and mesolimbic dopamine terminals, as measured by decreases in dopamine content and functional activity of tyrosine hydroxylase and dopa decarboxylase (Lloyd et al., 1981). Significant reductions in dopa decarboxylase activity and dopamine storage have also been found in the caudate,
putamen, frontal cortex and ventral tegmental complex in an *in vivo* investigation using positron emission tomography (PET) imaging with a fluorodopa F 18 tracer (Ernst et al., 1996). Using PET, Wong and colleagues (1996) found decreased binding of the radiolabeled dopamine transporter ligand, WIN-35,428, (50-63% reduction in the caudate and 64-75% in the putamen) in individuals with Lesch-Nyhan syndrome. This indicates a significant loss of dopaminergic nerve terminals. There is also an upregulation of D₁ and D₂ receptors in the striatum (Saito et al., 1999), suggesting post-synaptic supersensitivity for dopamine in the striata of individuals with Lesch-Nyhan syndrome. Reduced concentrations of the dopamine metabolite, homovanillic acid (HVA), have also been seen in cerebrospinal fluid (Jankovic et al., 1988). Increased concentrations of serotonin and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), have also been found in the putamen (Lloyd et al., 1981). There is evidence that decreased dopamine functioning may cause increases in serotonin levels in the brain (Mrini et al., 1995). Marked decreases in caudate, putamen and cerebral volume in individuals with Lesch-Nyhan syndrome have also been seen using magnetic resonance imaging (Harris et al., 1998). Overall, these data indicate that there is profound disregulation of monoamine systems in the brains of Lesch-Nyhan syndrome, and that nigrostriatal and mesocorticolimbic dopamine neurotransmission may play a particularly important role in the etiology and expression of self-injury.

Many drugs have been prescribed to help reduce the incidence of SIB in clinical populations. Unfortunately, no one medication, or class of medications, has proven effective for all patients. This suggests that these medications may not be able to correct the behavioral overlay (e.g., escape from a demanding task) that is associated with the
SIB. Clinical trials with typical neuroleptics such as haloperidol have produced inconsistent results. Some studies report successful attenuation of SIB (Janowsky et al., 2005) and some studies report failures to decrease SIB (Mace et al., 2001). Interestingly, Durand (1982) found that neither haloperidol nor mild punishment reduced a case of severe SIB, but a combination of haloperidol and behavioral intervention did significantly decrease the occurrence of SIB. This suggests that the effectiveness of pharmacological treatment in human self-injurers may be complicated by environmental conditions that influence the expression of the behavior disorder. Clinical trials with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), have also produced inconsistent results. In a blind, placebo-controlled experiment using fluoxetine in children with Obsessive Compulsive Disorder, King and colleagues (1991) reported an emergence of both SIB and obsessive self-injurious ideations in six children, four of whom had to be hospitalized. Decreased SIB with fluoxetine treatment has also been reported (e.g., Ricketts et al., 1993), but many of those trials have not included placebo control or have not used blind observers to measure the dependent outcomes. In addition, naltrexone, an opioid antagonist, has produced conflicting results in its effectiveness to reduce SIB in clinical trials. There are reports that naltrexone produced increases in SIB (Benjamin et al., 1995), decreases in SIB (Symons et al., 2001) and no effects on SIB (Willemsen-Swinkels et al., 1995). Valproate, an indirect GABA agonist (that also has actions in other systems), has also reduced the incidence of SIB in small clinical trials with autistic (Hollander et al., 2001) and intellectually handicapped individuals (Kastner et al., 1993). Risperidone, an atypical neuroleptic that affects multiple monoaminergic systems, has had the most consistent effects across different patient groups in reducing the amount of
aggression, directed at both the self and others, in children and adults with Lesch-Nyhan syndrome (Allen & Rice, 1996), autism (McCracken et al., 2002; Caicedo & Williams, 2002) and mental retardation (Cohen et al., 1998). In summary, the results of these clinical trials have provided evidence of the involvement of dopaminergic, serotonergic, opioid and GABAergic systems in clinical SIB, and these data further suggest that there are sub-groups of self-injurers, who may respond differently to different kinds of pharmacotherapy.

Animal models of SIB have also provided important information regarding the neurobiological basis of SIB. These animal models include neonatal lesions, environmental deprivation and pharmacological manipulations. In one model, 6-hydroxydopamine (6-OHDA) is used to lesion striatal dopamine neurons in neonatal rat pups. When these lesioned rats become adults they begin to self-injure after administration of either direct or indirect dopamine agonists (e.g., apomorphine, l-dopa), which affect multiple dopamine receptors (Breese et al., 1984b; Breese et al., 1984a). Furthermore, agonists that are effective only at the D1 class of dopamine receptors (e.g., SKF 38393) will effectively induce SIB, whereas D2-selective agonists do not induce SIB in the 6-OHDA model (Breese et al., 1985). Additionally, D1 antagonists block the SIB (Breese et al., 1985). It has been hypothesized, therefore, that SIB in this model is due to a supersensitivity at the D1 receptors. This is consistent with evidence of a dopamine supersensitivity in individuals with Lesch-Nyhan syndrome (Saito et al., 1999). Risperidone and nifedipine (an L-type calcium channel blocker, which decreases the amount of dopamine released in the caudate (Okita et al., 2000) have lowered the incidence of SIB in the 6-OHDA model (Blake et al., 2004). In summary, the 6-OHDA
model provides further evidence of dopamine’s important role in SIB. It is unclear if altered functioning of other neurotransmitter systems may also contribute to the induction of SIB in the 6-OHDA model.

Early environmental and maternal deprivation of non-human primates has been found to produce abnormal behaviors including stereotyped locomotion, abnormal socialization and SIB (Harlow & Harlow, 1962). The occurrence of whole-body stereotypies and the severity of SIB increase with an apomorphine challenge (Lewis et al., 1990), which suggests dopamine supersensitivity. These changes in dopaminergic functioning are similar to those found in individuals with Lesch-Nyhan syndrome and provide further evidence that dopamine disregulation is an important contributor to SIB. There is also a significant decrease in the density of immunoreactivity for tyrosine hydroxylase, substance P and leucine-enkephalin in the striatum and related basal ganglia regions in rhesus monkeys with a history of social isolation (Martin et al., 1991). This suggests that early environmental deprivation directly affects the development of the dopaminergic and peptidergic systems in the striatum. Once again, this resembles the striatal disorganization seen in Lesch-Nyhan syndrome (Wong et al., 1996; Harris et al., 1998).

A variety of different classes of pharmacological manipulations have been used to induce self-injury in animals. Bay-K 8644, an L-type calcium channel agonist, causes dose-orderly expression of self-biting in mice (Jinnah et al., 1999; Jinnah et al., 2003). Moreover, Bay-K 8644- induced SIB is eliminated by administration of nifedipine (Jinnah et al., 1999), which demonstrates that Bay-K 8644-induced SIB is specifically due to actions on the L-type calcium channels. Injections of Bay-K 8644 directly into the
striatum produce significant increases in dopamine release in a dose dependent fashion (Jinnah et al., 1999). Additionally, administration of fluoxetine (Kasim et al., 2002) and amphetamine (Kasim & Jinnah, 2003) (serotonin and dopamine agonists, respectively) each increase Bay-K 8644-induced SIB and administration of drugs that antagonize serotonin (Kasim et al., 2002) or dopamine decrease Bay-K 8644-induced SIB (Kasim & Jinnah, 2003). These results demonstrate that Bay-K 8644-induced SIB, like SIB seen in clinical populations, is associated with changes in dopaminergic and serotonergic neurotransmission.

Caffeine, a non-selective adenosine receptor antagonist, has also been reported to induce SIB when administered repeatedly at very high doses (Miñana et al., 1984). However, it was recently reported that the caffeine-induced SIB is not dose orderly, that the self-injury is extremely mild and only seen in a small percentage of the animals, and the required doses are highly toxic (Kies & Devine, 2004).

GBR-12909, an indirect dopamine agonist that blocks the dopamine transporter and the uptake of dopamine into synaptic vesicles, produces dose- and time-orderly induction of SIB in rats. GBR-12909-induced SIB is blocked by 6-OHDA lesions of nigrostriatal dopaminergic neurons, which suggests GBR-12909 produces SIB by altering presynaptic dopamine (for review see Sivam, 1996). This is consistent with evidence of altered presynaptic dopamine functioning in individuals with Lesch-Nyhan syndrome (Lloyd et al., 1981).

Pemoline, an indirect monoamine agonist, has also been used as a pharmacological model of SIB (Genovese et al., 1969; Mueller & Hsiao, 1980). Chronic administration of moderately high doses of pemoline produces dose-orderly self-injury in
a large majority of rats in a few days time (Kies & Devine, 2004). SIB in this model is usually directed towards the forepaws and abdomen, but is occasionally directed at the hindpaws and tail (Mueller & Hsiao, 1980; Kies & Devine, 2004). Pemoline-induced SIB is potentiated by impoverished environmental conditions during development (Kies et al., 2002) and by stress exposure (Kies et al., 2004). This is consistent with characteristics of clinical SIB, which is more prevalent in environmentally impoverished conditions, such as institutions than it is in community-based populations (Eyman et al., 1972; Eyman & Call, 1977), and is commonly expressed in stressful situations (Anderson & Ernst, 1994). An examination of brain structures affected by pemoline administration, using an assay for cytochrome oxidase (the end product of the mitochondrial electron transport chain and a marker of on-going neuronal activity) indicated that there is a significant pemoline-induced down-regulation of neuronal activity in the caudate nucleus, septum, bed nucleus of the stria terminalis, hippocampus, periaqueductal grey and some hypothalamic nuclei (Kies & Devine, 2003). These results suggest that the pemoline acts upon the dopaminergic nigrostriatal and mesolimbic pathways, and that there is significant indirect impact on a variety of limbic structures that are known to participate in processing of emotionally-relevant stimuli (Herman et al., 1996, Herman & Cullinan 1997). Disregulation of the nigrostriatal dopamine pathway is strongly implicated in clinical populations in which SIB is manifested and negative affect and SIB are highly correlated in some individuals with intellectual handicaps (as previously discussed) (Lindauer et al., 1999). In addition to dopaminergic actions, there is evidence that other neurotransmitter systems are involved in pemoline-induced SIB. Specifically, King and colleagues (1995) have found that pemoline-induced SIB is attenuated by MK-801
administration, which suggests glutamatergic involvement. Additionally, paroxetine (an SSRI) significantly potentiated pemoline-induced SIB, suggesting a role of serotonin in the pemoline model (Turner et al., 1999).

Based on the results from these investigations of SIB using the pemoline model, we have begun to further characterize the model in rats by pharmacologically challenging the induction of SIB with five specific drugs. Those drugs are risperidone (Risperdal), valproate (Depakote), nifedipine, tramadol (Ultram) and memantine (Namenda). We have examined the predictive validity of the pemoline model by investigating the effectiveness of two drugs that have been clinically useful in reducing SIB, risperidone and valproate.

Additionally, we have examined the effectiveness of nifedipine, an L-type calcium channel antagonist, to lessen pemoline-induced SIB. Nifedipine has been used to lower the incidence of self-injury in the 6-OHDA model and to decrease Bay-K 8644-induced SIB (as previously discussed). The purpose of this investigation is to evaluate the generalizability between animal models as this may help to reveal whether common neurobiological mechanisms contribute to the induction and expression of SIB in the various animal models. Commonality in these models may be useful in further investigating the neurobiological basis of SIB. In all of these drug challenges, we are also considering the specific biological mechanisms that are acted upon by the drug challenges in order to further evaluate the neurobiological mechanisms that underlie pemoline-induced SIB.

We have also evaluated the pharmacotherapeutic potential of two drugs that have not previously been assessed in clinical populations, using the pemoline model. Since
SIB is a behavior disorder that appears highly stereotypic and compulsive in clinical populations (Bodfish et al., 1995; King, 1993) and in the pemoline model, we investigated the effectiveness of tramadol to reduce the incidence of pemoline-induced SIB. Tramadol is a low affinity mu-opioid receptor agonist (Dhasmana et al., 1989), which also blocks reuptake of serotonin (Driessen & Reimann, 1992) and norepinephrine (Driessen et al., 1993). It has been shown to reduce the amount of compulsive behaviors in individuals with Obsessive Compulsive Disorder (OCD) (Goldsmith et al., 1999) and to reduce the amount of motor tics in individuals with Tourette’s syndrome (Shapira et al., 1997).

Additionally, we evaluated the effectiveness of memantine, a non-competitive NMDA receptor antagonist, to lessen SIB in the pemoline model. MK-801, another non-competitive NMDA receptor antagonist with high affinity, blocks SIB in the pemoline model (King et al., 1995). MK-801, however, cannot be used as a clinical pharmacotherapy because of its psychotomimetic side effects (Koek et al., 1988). Memantine lacks these side effects because it has a lower affinity for the NMDA receptor, and was recently approved by the FDA for use in Alzheimer’s patients (Molineuvo et al., 2005). In light of the effects of MK-801, we hypothesized that memantine could be clinically effective for treatment of clinical SIB.
CHAPTER 2
METHODS

Animals

Male Long Evans (LE) rats weighing 225-275 grams were housed in an
AAALAC-approved, climate controlled vivarium. The rats were maintained on a 12-
hour light/dark schedule with lights on at 7 am. Standard laboratory rat chow (Lab Diet
5001) and tap water were available ad libitum. The rats were pair-housed in standard
polycarbonate cages (43 x 21.5 x 25.5 cm) during 5-7 days of acclimation to the housing
facility. After the acclimation period the rats were singly-housed in similar
polycarbonate cages. All procedures were conducted in accordance with the Guide for
the Care and Use of Laboratory Animals published by the National Institutes of Health
and all procedures were pre-approved by the Institutional Animal Care and Use
Committee at the University of Florida.

Drugs

Pemoline (Spectrum Chemicals, New Brunswick, New Jersey) was suspended at a
concentration of 50 mg/ml in warm peanut oil (held at approximately 36° Celsius), with
constant stirring. Risperidone was purchased from Sigma-Aldrich Co. (St. Louis,
Missouri) and was suspended in a solution of 0.45% (w/v) hydroxypropyl-beta-
cyclodextrin. The risperidone was suspended at concentrations of 0, 0.1, 0.5 and 1.0
mg/ml. Sodium valproate was purchased from Sigma-Aldrich Co. and was suspended in
a solution of 0.04% (w/v) Na2EDTA. The valproate was suspended at concentrations of
0, 50, 100 and 200 mg/ml and was adjusted to a neutral pH of 7.4. Nifedipine was
purchased from Sigma-Aldrich Co. and was suspended in a solution consisting of 40% propylene glycol (v/v), 10% ethanol (v/v), 15% benzyl alcohol (v/v), 5% sodium benzoate (w/v) and approximately 35% distilled water (v/v). Nifedipine was suspended at concentrations of 0, 1.5, 5 and 15 mg/ml. Tramadol hydrochloride was purchased from Sigma-Aldrich Co. and was dissolved in sterile saline at concentrations of 0, 1.0, 10 and 100 mg/ml. Memantine was purchased from Sigma-Aldrich Co. and was dissolved in sterile saline at concentrations of 0, 3, 10 and 30 mg/ml.

**Experimental Procedures**

**Drug Treatments-Experiment 1: Risperidone**

Twenty-three male LE rats (Charles River Laboratories, Raleigh, NC) were weighed and injected with pemoline (200 mg/kg s.c.) at approximately 8:00 a.m. on each of five consecutive days. These injections were administered at the nape of the neck and either flank on a rotating basis, using 21 gauge needles. The rats were also injected twice daily with risperidone (0, 0.1, 0.5 or 1.0 mg/kg, i.p.) on each of the five days (n = 5-6 per group), using 26-gauge needles. The risperidone injections were administered at approximately 8:00 am (immediately after the pemoline injection) and approximately 6:00 pm.

**Drug Treatments-Experiment 2: Valproate**

Thirty-six male LE rats (Charles River Labs) received daily pemoline injections at 200 mg/kg and twice-daily injections of valproate (0, 50, 100 or 200 mg/kg, i.p.) for five days (n = 9 rats per group), following the same procedures as in Experiment 1.
Drug Treatments-Experiment 3: Nifedipine

Twenty-three male LE rats (Charles River Labs) received daily pemoline injections at 200 mg/kg and twice-daily injections of nifedipine (0, 3, 10 or 30 mg/kg, s.c.) for five days (n = 6 rats per group), following the same procedures as in Experiment 1.

Drug Treatments-Experiment 4: Tramadol

Seventy-two male LE rats (Harlan Inc., Indianapolis, Indiana) received daily pemoline injections at 200 mg/kg and twice-daily injections of tramadol (0, 0.1, 1.0 or 10 mg/kg, s.c.) for five days (n = 18 rats per group), following the same procedures as in Experiment 1.

Drug Treatments-Experiment 5: Memantine

Twenty-two male LE rats (Charles River Labs) received daily pemoline injections at 200 mg/kg and twice-daily injections of memantine (0, 3, 10 or 30 mg/kg, i.p.) for five days (n = 5-6 rats per group). The injection procedures were similar to the procedures in Experiment 1 except that memantine was administered 30 minutes before pemoline each day, and then again at approximately 6:00 p.m.

Behavioral and Histological Assays - All Experiments

The rats were visually inspected each time they were injected (i.e. twice per day for five days), and the inspections were videotaped. These inspections were also performed on the morning of the sixth day, but no injections were administered on the sixth day. The rats were held in front of a video camera and the head, forepaws, hindpaws, ventrum and tail were displayed. An injury score (see Table 1) was assigned to describe the presence (or absence) and severity of all injuries. An observer blind to the
drug treatment independently re-scored the injuries from the videotapes, and inter-
observer reliability was assessed.

After the morning injections of pemoline and challenge drug (i.e. risperidone,
valproate, nifedipine, tramadol or memantine) each rat was placed back into its home
cage. A locomotor monitor (San Diego Instruments, San Diego, CA) was then raised
around each cage in order to measure the locomotor activating (or inhibiting) effects of
the pemoline and challenge drug. Each locomotor monitor had four LED sensors spaced
along the length of the cage. A locomotor count was recorded each time the rat
interrupted a photocell sensor, and then no further counts were recorded from that sensor
until the rat interrupted another photocell sensor. Accordingly, each locomotor count
represented actual movement across the cage (approximately 3.5 inches) rather than
repetitive interruption of any single photocell sensor. The rats’ locomotion was
monitored for two hours after the injections each morning.

A variety of behaviors were recorded using night-vision cameras, where a camera
was focused on the cage of each rat each night. Five-minute time samples were recorded
once per hour for eight hours each night. These recordings were scored for duration of
self-injurious oral contact, duration of grooming, duration of inactivity and the amount of
locomotion. Self-injurious oral contact was defined as all oral contact that stayed fixed
on any one body part for longer than two seconds. Grooming was defined as oral contact
with any part of the body that continued to move from site to site on the body (e.g., oral
contact with the paws, then moving up each arm and continuing to the ventrum, in which
the contact was not sustained in any spot on the body for longer than two seconds).
Inactivity was defined as complete lack of movement except respiratory movements.
Locomotion was counted by sectioning the cage into three equal parts (along the length of the cage) and tallying the number of times the rat entered into a different section without returning to the section that he occupied immediately prior to that movement.

On the morning of the sixth day each rat was inspected and assigned an injury score. Immediately after this inspection the rat was rapidly decapitated. Each rat’s brain was removed and rapidly frozen in 2-methylbutane at -40°C and later stored at -80°C. These brains are being retained for histochemical analyses to compare a variety of potential neurochemical differences between self-injurious and non-injurious rats that were treated with the pemoline and challenge drugs. These analyses are not included in this thesis. The thymus and adrenal glands were also removed, frozen on dry ice and stored at -80°C. These glands were later weighed in order to determine the health status of the rats.

**Statistical Analyses**

The induction of self-injury was determined by graphing the injury scores of each rat across the six days of the experiment. The onset of SIB was defined for each experiment at the time the first rat in that experiment exhibited self-injury (i.e. received an injury score of 1 or more). A linear regression line was then plotted from the time of onset to the time when injury scores began to asymptote, and the slope of that line was determined for each rat. Between groups differences in the slopes of the regression lines were then compared using a one-way analysis of variance (ANOVA). All significant effects were further analyzed with Fisher’s Least Significant Difference (LSD) post-tests for each experiment.
The maintenance of the self-injury was compared between the groups by taking the mean of the injury scores that were recorded for each rat during the final days of the experiment (i.e. all days after the asymptote was reached). On those days, the injury scores leveled off or began to decline in most groups. A one-way ANOVA was used to compare the mean injury scores for the rats in each treatment group in each experiment. All significant effects were further analyzed with Fisher’s LSD post-tests.

The percentage of time that the rats exhibited self-injurious oral contact (data scored from the 40 minutes of videotaped recordings for each night) was used to quantify the behavioral expression of self-injury. The induction of self-injurious oral contact was assessed from the first night until the night that the most self-injurious group reached the peak mean duration of self-injurious oral contact. The duration of self-injurious oral contact data were then transformed by calculating the square root of the percentage of time spent with self-injurious oral contact to equalize the variability between the treatment groups (see below). A linear regression line was then plotted from the first night to the night when the duration of self-injurious oral contact peaked, using the square root transformed scores, and the slope of that line was determined for each rat. Between groups differences in the slopes of the regression lines were then compared using an ANOVA for each experiment. All significant effects were further analyzed with LSD post-tests.

The maintenance of the self-injurious oral contact was compared by taking the mean of the transformed percentage of oral contact data for the final nights of the experiment, when the percent of self-injurious oral contact duration started to decline in the groups that were treated with pemoline and vehicle. The mean of the transformed
duration of self-injurious oral contact was determined for each rat. These data were
compared for each treatment group using a one-way ANOVA. All significant effects
were further analyzed with LSD post-tests.

The dose of pemoline (200 mg/kg/day) that was used in these experiments
induces self-injury in approximately 75% of the rats. Consequently, there was substantial
variability of the duration of self-injurious oral contact within each treatment group. In
fact, in each experiment we observed some rats with self-injurious oral contact for 100%
of the time sampled and other rats, in the same treatment group, with no self-injurious
oral contact whatsoever (see Results). Accordingly, square root transformations of the
duration of self-injurious oral contact were used to control for this variability (Ott &
Longnecker, 2001). The power of the statistical analyses of injury scores and oral contact
duration was also diminished by the fact that the range of doses for the challenge drugs
included doses that were ineffective at reducing the different measures of pemoline-
induced SIB. In these cases, the statistical outcome was confounded by the ineffective
dose(s). We therefore set the acceptable alpha error level at 0.1 for all analyses of injury
scores and oral contact durations.

The duration of grooming behavior, inactivity, and the amounts of overnight
locomotion and post-injection locomotion (measured by the locomotor monitors) were
compared for each group using 4x5 (group x day) repeated measures ANOVAs. All
significant effects were further analyzed with LSD post-tests.

Body weights were analyzed by two-way (4 groups x 5 days) repeated measures
ANOVA. Glandular weights were compared using a one-way ANOVA for each
experiment. All significant effects were further analyzed with LSD post-tests.
Some rats were euthanized before the end of the experiment because they had an injury score of 4 (open lesion or amputated digit). 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 underestimate the injury scores and self-injurious oral contact scores, and to avoid the potential that the group means would over- or under-estimate the locomotor, inactivity, and grooming scores when the most severe self-injurers were removed from any group.

The prevalence of pemoline-induced SIB is dose-dependent (Kies and Devine, 2004), and we chose to work with a dose (200 mg/kg/day) that would induce SIB in some but not all of the rats. Accordingly, the variability in expression of SIB is high in all the groups of rats, including the control (pemoline + vehicle) groups. Furthermore, the doses of challenge drugs included ineffective doses in most of the experiments. These factors decreased the power of our ANOVAs to identify effective treatments. In light of these statistical issues, treatment-associated decreases in injury scores and self-injurious oral contact scores were treated as statistically reliable when the p-values were less than 0.10. Between-groups differences in all other dependent measures were treated as statistically reliable when the p-values were less than 0.05.
Table 2.1. Injury score rating scale (adapted from Turner et al., 1999).

<table>
<thead>
<tr>
<th>Score</th>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no self-injury</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>very mild self-injury</td>
<td>denuded skin, edema or erythema; involves small area</td>
</tr>
<tr>
<td>2</td>
<td>mild self-injury</td>
<td>denuded skin, edema or erythema; involves medium area or multiple small sites</td>
</tr>
<tr>
<td>3</td>
<td>moderate self-injury</td>
<td>denuded skin, edema or erythema; involves large area or multiple medium sites</td>
</tr>
<tr>
<td>4</td>
<td>severe self-injury</td>
<td>open lesion, amputated digit. Requires euthanasia.</td>
</tr>
</tbody>
</table>
CHAPTER 3
RESULTS

Experiment 1: Risperidone

In the experiment with risperidone, there were no signs of self-injury until the second night during the pemoline treatment. On the morning of day 3, mild injury was observed in one rat in the group that was treated with pemoline plus vehicle, and in one rat that was treated with pemoline plus the medium dose (0.5 mg/kg) of risperidone. The injury scores of the vehicle-treated rats reached an asymptote around the morning of day 4 (see Fig. 3.1). During the experiment, two rats were assigned an injury score of 4, and both were in the vehicle-treated group. One was put down on day 4; the other reached an injury score of 4 on day 6 and was terminated with the other rats.

By the final morning of the experiment, 5 of the 6 vehicle-treated rats exhibited injury scores of 1 or higher (i.e. had self-induced tissue damage). Fewer rats in each of the risperidone-treated groups exhibited injury scores of 1 or higher, and this effect was dose-orderly. The prevalence of positive injury scores throughout the experiment is depicted in Fig. 3.2.

The rats that were treated with risperidone exhibited lower injury scores than the vehicle-treated rats did, and this effect was dose-orderly (Fig. 3.1). The induction of self-injury (i.e. the slope of the injury scores starting with the onset on day 3 to the asymptote on day 4) occurred at a significantly lower rate in all the risperidone-treated groups, than in the vehicle-treated group ($F_{(3, 22)} = 7.340, p< 0.01$; Fig. 3.1 and Fig. 3.3a). The mean injury scores during the maintenance phase (i.e. the asymptotic expression from day 4 to
day 6) were significantly lower in the risperidone-treated rats than they were in the vehicle–treated rats, and this effect was roughly dose-orderly \( (F_{(3,22)} = 5.276, p< 0.01; \) Fig. 3.1 and Fig. 3.3b).

The duration of self-injurious oral contact was also significantly less in the risperidone-treated rats than it was in the vehicle-treated rats (Fig. 3.4). The induction of self-injurious oral contact (taken from night 1 until the peak on night 3) was significantly lower in the risperidone-treated rats than in the vehicle-treated rats \( (F_{(3,22)} = 4.380, p< 0.10; \) Fig. 3.4 and Fig. 3.5a). The maintenance of the self-injurious oral contact (taken from night 3 until night 5) appeared to be lower in the risperidone-treated rats, but this effect did not reach statistical significance \( (F_{(3,22)} = 2.336, p> 0.10; \) Fig. 3.4 and Fig. 3.5b).

Risperidone did not significantly affect the duration of time spent grooming \( (F_{(12,76)} = 0.7929, p> 0.05; \) Fig. 3.6a). Although risperidone did significantly increase the duration of inactivity overnight \( (F_{(3,76)} =3.393, p< 0.05; \) Fig. 3.6b), there were no significant time or group by time interaction effects. The amount of locomotion recorded on videotapes overnight decreased significantly across the days of the experiment \( (F_{(4,76)} =10.67, p< 0.05; \) Fig. 3.6c), but there were no significant between-groups differences, and there were no group by time interaction effects. The amount of post-injection locomotion (counts taken from the photocell monitors each morning) was not significantly affected by risperidone treatment \( (F_{(12,76)} = 0.9274, p> 0.05; \) Fig. 3.6d).

All the groups exhibited weight loss for the first four days, followed by a slight weight gain. There was a significant interaction between group and time for body weight \( (F_{(15,95)} =1.947, p< 0.05; \) Fig 3.7a), wherein the risperidone-treated rats exhibited less
weight loss than the vehicle-treated rats did. Risperidone treatment, however, did not appear to impact the health of the rats. There were no significant between-groups differences in thymus ($F_{(3,22)} = 2.630, p > 0.05$; Fig. 3.7b), or adrenal weights (left adrenal: $F_{(3,22)} = .4862, p > 0.05$; right adrenal: $F_{(3,22)} = .5737, p > 0.05$; Fig. 3.7 c,d).

Fig. 3.1. Effects of risperidone on pemoline-induced self-injury: Risperidone dose-dependently delayed the onset of self-injury, with the highest dose of risperidone blocking the pemoline-induced self-injury until day 4. Risperidone also dose-dependently attenuated the severity of pemoline-induced self-injury, as measured by the injury scores. All values expressed are group means ± S.E.M.
Fig. 3.2. Effects of risperidone on the incidence of self-injury: Risperidone dose-dependently lowered the incidence of self-injury, as measured by the percentage of rats that exhibited injury on day 6 of the experiment.

Fig. 3.3. Effects of risperidone on the induction and maintenance of pemoline-induced self-injury: (a) Risperidone dose-dependently reduced the induction of pemoline-induced self-injury. (b) Risperidone also dose-dependently reduced the maintenance of pemoline-induced self-injury. All values expressed are group means ± S.E.M. Significant differences between the pemoline plus vehicle and pemoline plus risperidone treated groups (LSD) are depicted with asterisks, where * indicates p < 0.10.
Fig. 3.4. Effects of risperidone on the duration of pemoline-induced self-injurious oral contact: Risperidone reduced the overall percent duration of self-injurious oral contact. Self-injurious oral contact peaked on day 4 for most groups. All values expressed are group means ± S.E.M.

Fig. 3.5. Effects of risperidone on the induction and maintenance of pemoline-induced self-injurious oral contact: (a) Risperidone dose-dependently decreased the induction of pemoline-induced self-injurious oral contact. (b) Although it appeared that risperidone also reduced the maintenance of pemoline-induced self-injurious oral contact in a dose-orderly manner, this effect was not statistically significant. All values expressed are group means ± S.E.M. Significant differences between pemoline plus vehicle and pemoline plus risperidone treated groups (LSD) are depicted with asterisks, where * indicates p < 0.10.
Fig. 3.6. Effects of risperidone on grooming, inactivity and locomotion: (a) Risperidone did not significantly affect time spent grooming. (b) Risperidone-treated rats did exhibit more inactivity as compared to vehicle-treated rats. Significant differences between vehicle- and risperidone-treated rats (LSD) are depicted as follows: * p< 0.05 for comparisons between risperidone at 1.0 mg/kg and vehicle. Risperidone had no significant effect on locomotion, either (c) overnight or (d) after injections. All values expressed are group means ± S.E.M.
Fig. 3.7. Effects of risperidone on the health status of the rats: (a) there was a significant interaction between time and group for body weight. Pemoline plus vehicle treated rats lost more weight than pemoline plus risperidone treated rats did. Significant differences between vehicle- and risperidone-treated rats (LSD) are depicted as follows: * p< 0.05 for comparisons between risperidone at 1.0 mg/kg and vehicle; ☆ p< 0.05 for comparisons between risperidone at 0.5 mg/kg and vehicle; # p< 0.05 for comparisons between risperidone at 0.1 mg/kg and vehicle. Risperidone did not significantly affect the weights of the (b) thymus glands or (c,d) adrenal glands. All values expressed are group means ± S.E.M.
Experiment 2: Valproate

In the experiment with valproate, there were no signs of self-injury during the first day or night of pemoline treatment. The onset of self-injury occurred on day 2, with a rat in the pemoline plus vehicle group exhibiting mild injury. The onset of self-injury was further delayed in all the valproate treated groups. The injury scores of the vehicle-treated rats reached an asymptote around the morning of day 5 (see Fig. 3.8). Five rats were assigned an injury score of 4, two in the vehicle-treated group (one euthanized on day 4, the other on day 5), two rats in the lowest dose (50 mg/kg) of valproate group (one euthanized on day 4, the other on day 5) and one in the medium dose (100 mg/kg) of valproate group (euthanized on day 4).

By the final morning of the experiment, 8 of the 9 vehicle-treated rats exhibited injury scores of 1 or higher (i.e. had self-induced tissue damage). Fewer rats in the groups treated with the middle and high doses of valproate exhibited injury scores of 1 or higher, and this effect was dose-orderly. The prevalence of positive injury scores throughout the experiment is depicted in Fig. 3.9.

The rats that were treated with valproate exhibited lower injury scores than the vehicle-treated rats did, and this effect was primarily observed at the highest dose of valproate (Fig. 3.8). The induction of self-injury (i.e. the slope of the injury scores starting with the onset on day 2 to the asymptote on day 5) occurred at a significantly lower rate in the valproate-treated groups, than in the vehicle-treated groups \(\text{F}(3,35) = 2.593, p< 0.10\; \text{Fig. 3.8 and 3.10a}\), and this effect was primarily observed at the highest dose of valproate. The mean injury scores during the maintenance phase (i.e. the asymptotic expression from day 5 to day 6) were significantly lower in the valproate-
treated rats than they were in the vehicle-treated rats ($F_{(3,35)} =2.420, p< 0.10$; Fig. 3.10b), and this effect was seen primarily in the highest dose of valproate.

In contrast to the effects of valproate on actual tissue damage (i.e. injury scores), valproate administration did not significantly affect the duration of self-injurious oral contact (Fig. 3.11). The induction of self-injurious oral contact (taken from night 1 until the peak on night 4) was not significantly affected by valproate treatment ($F_{(3,35)} =.8013, p> 0.10$; Fig. 3.12a). The maintenance of self-injurious oral contact (taken from night 4 until night 5) was also not significantly reduced by valproate ($F_{(3,35)} =.3864, p> 0.10$; Fig. 3.12b).

Valproate administration did not significantly affect any of the other behaviors that were measured during the experiment. Although the time spent grooming ($F_{(4,128)} =5.378, p> 0.05$; Fig. 3.13a), or inactive ($F_{(4,128)} =3.784, p> 0.05$; Fig. 3.13b), and the amount of locomotion recorded on videotapes overnight ($F_{(4,128)} =20.19, p> 0.05$; Fig. 3.13c) decreased significantly across the days of the experiment, there were no significant between-groups differences and there were no group by time interaction effects. Post-injection locomotion, recorded with the photocell monitors immediately after pemoline and valproate injections each morning, was not significantly affected by valproate treatment ($F_{(3,35)} =1.991, p> 0.05$; Fig. 3.13d).

All groups exhibited weight loss for the first four days, followed by a slight weight gain. This weight loss was significant across days of the experiment ($F_{(5,160)} =10.79, p< 0.05$; Fig. 3.14a). There were no significant between-groups differences or group by time interaction effects. Thymus weights were found to be significantly different between groups ($F_{(3,35)} =5.841, p< 0.05$; Fig. 3.14b), but no significant
differences were found between the valproate-treated groups and the vehicle-treated group. There were also no significant between-groups differences on adrenal weights (left: $F_{(3,35)} = .6223, p > 0.05$; right: $F_{(3,35)} = .8762, p > 0.05$; Fig. 3.14c,d).

![Graph showing effects of valproate on pemoline-induced self-injury](image)

**Fig. 3.8.** Effects of valproate on pemoline-induced self-injury: Valproate delayed the onset of self-injury. The two highest doses of valproate (100 and 200 mg/kg) attenuated the severity of pemoline-induced self-injury, as measured by the injury scores. All values expressed are group means ± S.E.M.
Fig. 3.9. Effects of valproate on the incidence of self-injury: The middle and high doses of valproate lowered the incidence of self-injury, as measured by the percentage of rats that exhibited injury on day 6 of the experiment.

Fig. 3.10. Effects of valproate on the induction and maintenance of pemoline-induced self-injury: (a) Valproate reduced the induction of pemoline-induced self-injury. (b) Valproate also reduced the maintenance of pemoline-induced self-injury. All values expressed are group means ± S.E.M. Significant differences between the pemoline plus vehicle and pemoline plus valproate treated groups (LSD) are depicted with asterisks, where * indicates p< 0.10.
Fig. 3.11. Effects of valproate on the duration of pemoline-induced self-injurious oral contact: Valproate did not significantly reduce the overall percent duration of self-injurious oral contact. Self-injurious oral contact peaked on night 4 for all groups. All values expressed are group means ± S.E.M.

Fig. 3.12. Effects of valproate on the induction and maintenance of pemoline-induced self-injurious oral contact: (a) Valproate did not significantly affect induction of pemoline-induced self-injurious oral contact. (b) Valproate also did not significantly affect the maintenance of pemoline-induced self-injurious oral contact. All values expressed are group means ± S.E.M.
Fig. 3.13. Effects of valproate on grooming, inactivity and locomotion: Valproate did not significantly affect (a) time spent grooming or (b) time spent inactive. Valproate also had no significant effect on locomotion, either (c) overnight or (d) after injections. All values expressed are group means ± S.E.M.
Fig. 3.14. Effects of valproate on the health status of the rats: (a) There was a significant effect of time on body weight, wherein all groups lost weight during the first four days of the experiment and then exhibited a slight weight gain. (b) Valproate did significantly affect the weights of the thymus glands, however, no significant differences were found between the valproate-treated groups and the vehicle-treated group. (c,d) Valproate did not significantly affect the weights of the adrenal glands. All values expressed are group means ± S.E.M.
Experiment 3: Nifedipine

In the experiment with nifedipine, there were no signs of self-injury until the second day during the pemoline treatment. On the afternoon of day 2, mild injury was observed in one rat that was treated with pemoline plus the medium dose (10 mg/kg) of nifedipine. The injury scores of the vehicle-treated rats reached an asymptote around the morning of day 5 (Fig. 3.15). During the experiment, thirteen rats were assigned an injury score of 4 and were euthanized before the end of the experiment. The rats in the vehicle-treated group exhibited extremely severe self-injury. In fact, all the rats in the vehicle-treated group received an injury score of 4 and were terminated early (one on day 3, four on day 4 and one on day 5). Three rats in the group treated with pemoline plus the low dose (3 mg/kg) of nifedipine reached an injury score of 4 (one on day 4 and two on day 6). One rat from the group treated with pemoline plus the middle dose (10 mg/kg) of nifedipine reached an injury score of 4 on day 4 and three rats in the group treated with pemoline plus the high dose (30 mg/kg) of nifedipine group were euthanized early (one on day 3 and two on day 4).

By the final morning of the experiment, all rats exhibited injury scores of 1 or higher (i.e. had self-induced tissue damage) in the group treated with pemoline plus vehicle and the group treated with pemoline plus the low dose (3 mg/kg) of nifedipine. All but one rat in each of the groups that were treated with pemoline plus the middle (10 mg/kg) and high (30 mg/kg) doses of nifedipine also exhibited injury scores of 1 or higher. The prevalence of positive injury scores throughout the experiment is depicted in Fig. 3.16.

The rats that were treated with nifedipine exhibited lower injury scores than the vehicle-treated rats did (Fig. 3.15). The induction of self-injury (i.e. the slope of the
injury scores starting with the onset on day 2 to the asymptote on day 4) occurred at a significantly lower rate with all the nifedipine-treated groups, than in the vehicle-treated group \( (F(3,22) =2.626, p< 0.10; \text{Fig. 3.15 and Fig. 3.17a}) \). The mean injury scores during the maintenance phase (i.e. the asymptotic expression from day 4 to day 6) were significantly lower in the nifedipine-treated rats than they were in the vehicle-treated rats \( (F(3,22) =3.041, p< 0.10; \text{Fig. 3.15 and Fig. 3.17b}) \).

In contrast to the effects of nifedipine on actual tissue damage (i.e. injury scores), nifedipine administration did not significantly affect the duration of self-injurious oral contact (\text{Fig. 3.18}). The induction of self-injurious oral contact (taken from night 1 until the peak on night 3) was not significantly affected by nifedipine treatment \( (F(3,21) =0.4573, p> 0.10; \text{Fig. 3.18 and Fig. 3.19a}) \). The maintenance of self-injurious oral contact (taken from night 3 until night 5) was also not significantly reduced by nifedipine \( (F(3,22) =0.7444, p> 0.10; \text{Fig. 3.18 and Fig. 19b}) \).

Nifedipine did not significantly affect any of the other behaviors that were measured during the experiment. Time spent grooming \( (F(4,72) =4.676, p< 0.05; \text{Fig. 3.20a}) \), inactive \( (F(4,72) =6.579, p< 0.05; \text{Fig. 3. 20b}) \) and the amount of locomotion recorded on videotapes overnight \( (F(4,72) =23.94, p< 0.05; \text{Fig. 3.20c}) \) or recorded with the photocell monitors immediately after pemoline and nifedipine injections each morning \( (F(4,76) =10.79, p< 0.05; \text{Fig. 3. 20d}) \) all changed significantly across the days of the experiment, but no significant group by time interaction effects were found.

All groups exhibited weight loss during the experiment. This weight loss was significant across days of the experiment \( (F(5,95) =86.06, p< 0.05; \text{Fig. 3.21a}) \). There were no significant between-groups differences or group by time interaction effects.
Nifedipine treatment significantly affected thymus weights ($F_{(3,21)} = 6.917$, $p < 0.05$; Fig. 3.21b), with the thymus glands of nifedipine-treated animals weighing significantly less than the glands of the vehicle-treated animals did. Adrenal weights, however, were not different between groups (left: $F_{(3,21)} = .4816$, $p > 0.05$; right: $F_{(3,21)} = 1.095$, $p > 0.05$; Fig. 3.21c,d).

Fig. 3.15. Effects of nifedipine on pemoline-induced self-injury: Nifedipine dose dependently attenuated the severity of pemoline-induced self-injury, as measured by the injury scores. Nifedipine did not delay the onset of pemoline-induced self-injury. All values expressed are group means ± S.E.M.
Fig. 3.16. Effects of nifedipine on the incidence of self-injury: The highest doses of nifedipine lowered the incidence of self-injury, as measured by the percentage of rats that exhibited injury on day 6 of the experiment.

Fig. 3.17. Effects of nifedipine on the induction and maintenance of pemoline-induced self-injury: (a) Nifedipine reduced the induction of pemoline-induced self-injury. (b) Nifedipine also decreased the maintenance of pemoline-induced self-injury. All values expressed are group means ± S.E.M. (Significant differences between pemoline plus vehicle and pemoline plus nifedipine treated groups (LSD) are depicted with asterisks, where * indicates p< 0.10).
Fig. 3.18. Effects of nifedipine on the duration of pemoline-induced self-injurious oral contact: Nifedipine did not significantly reduce the overall percent duration of self-injurious oral contact. Vehicle-treated rats exhibited self-injurious oral contact for 100% of the night, beginning on night 3. All values expressed are group means ± S.E.M.

Fig. 3.19. Effects of nifedipine on the induction and maintenance of pemoline-induced self-injurious oral contact: (a) Nifedipine did not significantly affect the induction of pemoline-induced self-injurious oral contact. (b) Nifedipine also did not significantly affect the maintenance of pemoline-induced self-injurious oral contact. All values expressed are group means ± S.E.M.
Fig. 3.20. Effects of nifedipine on grooming, inactivity and locomotion: Nifedipine did not significantly affect (a) time spent grooming or (b) time spent inactive. Nifedipine also had no significant effect on locomotion, either (c) overnight or (d) after injections. All values expressed are group means ± S.E.M.
Fig. 3. Effects of nifedipine on the health status of the rats: (a) There was a significant effect of time on body weight, wherein all groups lost weight during the six days of the experiment. Nifedipine significantly affected the (b) thymus gland weights. Significant thymus involution was seen in all rats in the nifedipine-treatment groups. Nifedipine, however, had no significant effect on (c,d) adrenal gland weights. All values expressed are group means ± S.E.M. (Significant differences between pemoline plus vehicle and pemoline plus nifedipine treated groups (LSD) are depicted with asterisks, where * indicates p< 0.05).
**Experiment 4: Tramadol**

In the experiment with tramadol, there were no signs of self-injury until the second day of pemoline treatment. On day 2, mild injury was observed in one rat in the group treated with pemoline plus vehicle, and one rat that was treated with pemoline plus the medium dose (1.0 mg/kg) of tramadol. The injury scores of the vehicle-treated rats reached an asymptote around the morning of day 4 (Fig. 3.22). During the experiment, five rats were assigned an injury score of 4. Two rats in the vehicle-treated group (one euthanized on day 2, the other on day 3), one rat in the group treated with the lowest dose (0.1 mg/kg) of tramadol (euthanized on day 6), one rat in the group treated with the medium dose (1.0 mg/kg) of tramadol (euthanized on day 4) and one rat in the group treated with the highest dose (10 mg/kg) of tramadol (euthanized on day 3) were assigned an injury score of 4.

By the final morning of the experiment, 12 of 18 vehicle-treated rats exhibited injury scores of 1 or higher (i.e. had self-induced tissue damage). More rats in each of the tramadol-treated groups exhibited positive injury scores than in the vehicle-treated group. The prevalence of positive injury scores throughout the experiment is depicted in Fig. 3.23.

The rats that were treated with tramadol did not exhibit lower injury scores than did the vehicle-treated rats (Fig. 3.22). The induction of self-injury (i.e. the slope of the injury scores starting with the onset on day 2 to the asymptote on day 4) was not significantly affected by tramadol treatment ($F_{(3,71)} = 1.532, p > 0.10$; Fig. 3.22 and Fig. 3.24a). The mean injury scores during the maintenance phase (i.e. the asymptotic expression from day 4 to day 6) was also not significantly affected by tramadol treatment ($F_{(3,71)} = .7915, p > 0.10$; Fig. 3.22 and Fig. 3.24b).
The duration of self-injurious oral contact was also not significantly altered in the tramadol-treated rats (Fig. 3.25). The induction of self-injurious oral contact (taken from night 1 until the peak on night 3) was not significantly affected by tramadol treatment ($F(3, 42) = 1.942, p > 0.10$; Fig. 3.25 and Fig. 3.26a). The maintenance of self-injurious oral contact (taken from night 3 until night 5) was also not significantly reduced by tramadol ($F(3,42) = 0.8608, p > 0.10$; Fig. 3.25 and Fig. 3.26b).

Tramadol did not significantly affect the other behaviors that were measured during the experiment. The time spent grooming ($F(4,164) = 9.596, p < 0.05$; Fig. 3.27a), inactive ($F(4,164) = 5.113, p < 0.05$; Fig. 3.27b) and the amount of locomotion recorded on the videotapes overnight ($F(4,164) = 7.454, p < 0.05$; Fig. 3.27c) or recorded with the photocell monitors immediately after pemoline and tramadol injections each morning ($F(4,272) = 3.050, p < 0.05$; Fig. 3.27d) were significantly changed across days of the experiment. However, there were no significant between-groups differences or group by time interaction effects for any of these behaviors.

All groups exhibited weight loss during the first four days of the experiment, followed by a slight weight gain. This weight loss was significant across days of the experiment ($F(5,340) = 22.90, p < 0.05$; Fig. 3.28a). No between-groups differences or interaction effects were significant. Tramadol treatment, however, did not appear to impact the health of the rats. There were no significant between-groups differences in thymus ($F(3, 71) = 0.7867, p > 0.05$; Fig. 3.28b), or adrenal weights (left: $F(3, 71) = 0.5434, p > 0.05$; right: $F(3, 71) = 1.016, p > 0.05$; Fig. 3.28c,d).
Fig. 3.22. Effects of tramadol on pemoline-induced self-injury: Tramadol did not affect pemoline-induced self-injury, as measured by the injury scores. All values expressed are group means ± S.E.M.

Fig. 3.23. Effects of tramadol on the incidence of self-injury: Tramadol did not significantly affect the incidence of self-injury, as measured by the percentage of rats that exhibited self-injury on day 6 of the experiment.
Fig. 3.24. Effects of tramadol on induction and maintenance of pemoline-induced self-injury: Tramadol did not significantly affect the induction of pemoline-induced self-injury. (b) Tramadol also did not significantly affect the maintenance of pemoline-induced self-injury. All values expressed are group means ± S.E.M.

Fig. 3.25. Effects of tramadol on the duration of pemoline-induced self-injurious oral contact: Tramadol did not significantly affect the overall percent duration of pemoline-induced self-injurious oral contact. Self-injurious oral contact peaked on day 3 for most groups. All values expressed are group means ± S.E.M.
Fig. 3.26. Effects of tramadol on the induction and maintenance of pemoline-induced self-injurious oral contact: (a) Tramadol did not significantly affect the induction of pemoline-induced self-injurious oral contact. (b) Tramadol also did not significantly affect the maintenance of pemoline-induced self-injurious oral contact. All values expressed are group means ± S.E.M.
Fig. 3.27. Effects of tramadol on grooming, inactivity and locomotion: (a) Tramadol did not significantly affect time spent grooming. (b) Tramadol did significantly alter the amount of time spent inactive. However, no tramadol-treated group differed significantly from the vehicle-treated group. Tramadol had no significant effect on locomotion, either (c) overnight or (d) after injections. All values expressed are group means ± S.E.M.
Fig. 3.28. Effects of tramadol on the health status of the rats: (a) There was a significant effect of time on body weight, wherein all groups lost weight during the first four days of the experiment. Tramadol did not significantly affect the weights of the (b) thymus glands or (c,d) adrenal glands. All values expressed are group means ± S.E.M.
Experiment 5: Memantine

In the experiment with memantine, there were no signs of self-injury until the second night during the pemoline treatment. On the morning of day 3, moderate injury was observed in one rat in the group treated with pemoline plus vehicle. The injury scores of the vehicle-treated rats reached asymptote around the afternoon of day 4 (Fig. 3.29). During the experiment, five rats were assigned an injury score of 4, one in the vehicle-treated group (euthanized on day 3), two rats in the group treated with the lowest dose (3 mg/kg) of memantine (one euthanized on day 3, the other on day 4) and two rats in the group treated with the medium dose (10 mg/kg) of memantine (one euthanized on day 4, the other on day 6).

By the final morning of the experiment, 5 of the 6 vehicle-treated rats exhibited injury scores of 1 or higher (i.e. had self-induced tissue damage). All rats in the groups treated with the lowest and medium doses (3 and 10 mg/kg) of memantine exhibited injury scores of 1 or higher, and 4 out of the 6 rats in the group treated with the highest dose (30 mg/kg) of memantine exhibited injury scores of 1 or higher. The prevalence of positive injury scores throughout the experiment is depicted in Fig. 3.30.

The rats that were treated with memantine did not exhibit lower injury scores than the vehicle-treated rats did. The induction of self-injury (i.e. the slope of the injury scores starting with the onset on day 3 to the asymptote on day 4) was not significantly affected by memantine treatment ($F_{(3,21)} = 1.042, p > 0.10$; Fig. 3.29 and Fig. 3.31a). The mean injury scores during the maintenance phase (i.e. the asymptotic expression from day 4 to day 6) was also not significantly affected by memantine treatment ($F_{(3,21)} = 0.4803, p > 0.10$; Fig. 3.29 and Fig. 3.31b).
The duration of self-injurious oral contact was also not significantly affected in the memantine-treated rats, as compared to the oral contact durations in the vehicle-treated rats (Fig. 3.32). The induction of self-injurious oral contact (taken from night 1 until the asymptote on night 4) was not significantly affected by memantine treatment ($F_{(3,21)} = 0.5208, p > 0.10$; Fig. 3.32 and Fig. 3.33a). The maintenance of the self-injurious oral contact (taken from night 4 until night 5) was also not significantly affected by memantine ($F_{(3,21)} = 0.1132, p > 0.10$; Fig. 3.32 and Fig. 3.33b).

Although there were significant changes in the duration of grooming ($F_{(4,72)} = 5.442, p < 0.05$; Fig. 3.34a) and inactivity ($F(4,72) = 4.869, p < 0.05$; Fig. 3.34b) across the days of the experiment, there were no significant between-groups differences or interaction effects with memantine treatment. There were significant group by day interactions for both overnight locomotion (taken from the overnight videotapes) ($F_{(12,72)} = 2.948, p < 0.05$; Fig. 3.34c) and post-injection locomotion (counts taken from the locomotor monitors) ($F_{(12,72)} = 7.753, p < 0.05$; Fig. 3.34d), wherein memantine-treated rats exhibited greater locomotion than did vehicle-treated rats.

All groups exhibited weight loss for the first four days, followed by a slight weight gain. There was a significant interaction between group and time for body weight ($F_{(30,180)} = 2.243, p < 0.05$; Fig. 3.35a), wherein the memantine-treated rats exhibited more weight loss than the vehicle-treated rats did. Thymus weights were found to be significantly different between groups ($F_{(3,21)} = 3.657, p < 0.05$; Fig. 3.35b), but no significant differences were found between the weights in the memantine-treated and vehicle-treated groups. Adrenal weights were not significantly affected by memantine.
administration (left adrenal: $F_{(3,21)} = 0.5132$, $p > 0.05$; right adrenal: $p > 0.05$, $F_{(3,21)} = 2.857$, $p > 0.05$; Fig. 3.35c,d).

Fig. 3.29. Effects of memantine on pemoline-induced self-injury: Memantine did not affect pemoline-induced self-injury, as measured by the injury scores. All values expressed are group means ± S.E.M.

Fig. 3.30. Effects of memantine on the incidence of self-injury: Memantine did not significantly affect the incidence of self-injury, as measured by the percentage of rats that exhibited self-injury on day 6 of the experiment.
Fig. 3.31. Effects of memantine on induction and maintenance of pemoline-induced self-injury: Memantine did not significantly alter either (a) induction or (b) maintenance of pemoline-induced self-injury. All values expressed are group means ± S.E.M.

Fig. 3.32. Effects of memantine on the duration of pemoline-induced self-injurious oral contact: Memantine did not significantly affect pemoline-induced self-injurious oral contact. Self-injurious oral contact peaked on day 4 for most groups. All values expressed are group means ± S.E.M.
Fig. 3.33. Effects of memantine on the induction and maintenance of pemoline-induced self-injurious oral contact: Memantine did not significantly affect either (a) induction or (b) maintenance of pemoline-induced self-injurious oral contact. All values expressed are group means ± S.E.M.
Fig. 3.34. Effects of memantine on grooming, inactivity and locomotion: Memantine did not significantly affect (a) time spent grooming or (b) time spent inactive. Memantine-treated rats exhibited significantly greater counts of locomotion, both (c) overnight and (d) post-injection. Significant differences between vehicle- and memantine-treated rats (LSD) are depicted as follows: * p< 0.05 for comparisons between memantine at 30 mg/kg and vehicle; ☆ p< 0.05 for comparisons between memantine at 10 mg/kg and vehicle; # p< 0.05 for comparisons between memantine at 3 mg/kg and vehicle. All values expressed are group means ± S.E.M.
Fig. 3.35. Effects of memantine on the health status of the rats: (a) There was a significant interaction between time and group for body weight. The rats in the pemoline plus memantine groups lost more weight than the rats in the pemoline plus vehicle group did. Significant differences between vehicle- and memantine-treated rats (LSD) are depicted as follows: * p< 0.05 for comparisons between memantine at 30 mg/kg and vehicle; ☆ p< 0.05 for comparisons between memantine at 10 mg/kg and vehicle; # p< 0.05 for comparisons between memantine at 3 mg/kg and vehicle. (b) There were significant between-groups differences in the weights of the thymus glands, however, no significant differences were found between the memantine-treated groups and the vehicle-treated group. Memantine had no affect adrenal gland weight (c,d). All values expressed are group means ± S.E.M.
Inter-observer reliability

Inter-observer reliability for injury scores across the five experiments was as follows. In 93% of cases, the two observers’ scores matched exactly. In 6% of cases, the scores were mismatched by one point on the 5-point scale (e.g., one observer assigned a score of 2, and the other observer assigned a score of 3). In less than 1% of cases, the scores were mismatched by 2 points, and the scores were never mismatched by 3 points or more.
CHAPTER 4  
DISCUSSION

The results of the current study replicate previous findings that approximately 75% of the rats exhibit SIB when treated with pemoline at 200 mg/kg/day, and most of the self-injury was targeted at the forepaws and ventrum (Kies and Devine, 2004). In four of the five current experiments, approximately 75-80% of the rats exhibited tissue injury when injected daily with 200 mg/kg pemoline (i.e. those that were injected with pemoline plus vehicle). The only exception was the nifedipine experiment in which 100% of the rats self-injured when treated with pemoline and the vehicle (see discussion below). The fact that some of the rats in the groups that were treated with pemoline and vehicle did not self-injure in four of the five experiments, suggests that there are individual differences in vulnerability to develop pemoline-induced SIB. Individual differences in vulnerability to develop pemoline-induced SIB resemble the expression of SIB in clinical populations. Even in disorders with a high prevalence of SIB, there are individuals who do not self-injure. Individual differences in the vulnerability to develop pemoline-induced SIB may provide a useful tool for investigating the neurobiological differences between rats that exhibit pemoline-induced SIB and those that do not.

In these experiments we used multiple measures to characterize pemoline-induced SIB. These included injury scores that detail the severity and the prevalence of injury in each treatment group. We also evaluated a measure of the behavioral expression of SIB, using the duration of self-injurious oral contact on the body, and we used the injury scores and the oral contact scores to evaluate the rate of onset and the ongoing
maintenance of the pemoline-induced self-injury. An analysis of the behavioral expression, or duration, of SIB has generally not been used (for an exception, see King et al., 1995). It has even been proposed that the behavioral expression of pemoline-induced SIB could not be quantified because of its resemblance to normal grooming (Mueller & Hsiao, 1980). In fact, we found that there was general concordance between the measures of self-injury, and that the measure of prolonged oral contact reliably discriminates between grooming and SIB. Additionally, we found that the quantification of the behavioral expression of SIB highlighted important information about the pemoline-induced SIB; information that the injury scores alone were not sensitive enough to decipher. The oral contact data from the overnight videos indicated that there was pemoline-induced self-biting behavior before there were any signs of injury. The quantification of SIB also indicated that the behavioral expression of SIB actually peaks and then declines during the five nights of the experiment in most of the groups of rats that were treated with pemoline plus vehicle. The injury scores remained at asymptotic levels around this time and so they did not accurately reflect this eventual decrease in self-biting behavior. The reason for the decline in behavioral expression of pemoline-induced SIB is not known. Perhaps the decline in self-injurious oral contact results from tolerance to the injury-inducing effect of pemoline. This decline could also be a response to the pain of injuring tissue that has been traumatized. This interesting finding will require further investigation. On the other hand, the analysis of the self-injurious oral contact was not very sensitive to the severity of biting. In two of the experiments (valproate and nifedipine), the rats that were treated with the drug challenges exhibited lowered injury scores, but no significant effect on oral contact scores. Apparently, these
rats engaged in prolonged oral contact, with less severe self-biting so that they exhibited lower amounts of tissue damage than the vehicle-treated rats did. Overall, these multiple dependent variables each measure different aspects of pemoline-induced SIB and allow for a more thorough characterization of the self-injury.

The drugs that were evaluated in these experiments were designed to provide specific information about the pemoline model of SIB, and about the potential effects of drug challenges in this model. In particular, the drug challenges were designed to assess the predictive validity of the model (i.e. risperidone and valproate), the generalizability of the pemoline model in relation to other animal models of SIB (i.e. risperidone and nifedipine), and pharmacotherapies that may have clinical potential (i.e. tramadol and memantine). The results of these pharmacological studies generated support for the predictive validity and generalizability of the pemoline model. They did not yield any promising leads for previously untested pharmacotherapy, but these studies revealed interesting information about the potential use of the model to uncover the neurobiological basis of SIB. The fact that the rats expressed lower amounts of SIB if they were treated with some of the challenge drugs (risperidone, valproate, and nifedipine) suggests that the neurochemical mechanisms that are directly or indirectly addressed by these drugs may be important mediators of the induction and expression of SIB, and these mechanisms may be important targets for future development of pharmacotherapies.

The fact that risperidone attenuates pemoline-induced SIB suggests that the model has predictive validity. Risperidone decreased SIB in both clinical samples (Allen & Rice, 1996; Cohen et al., 1998; McCracken et al., 2002; Caicedo & Williams, 2002) and
in this animal model. Additionally, risperidone has lessened the occurrence of SIB in another animal model (Allen et al., 1998). These results provide evidence for the generalizability between the different animal models of SIB. This generalizability will allow for neurobiological analyses that can highlight the common substrates that lead to SIB in these models. The results from the risperidone experiment also provide further evidence that disregulated monoaminergic neurotransmission is important for the etiology and maintenance of SIB. Pemoline is a monoamine agonist (Molina & Orsinghen, 1981) and risperidone is a monoamine antagonist (Leysen et al., 1988). Thus, the opposing actions of these drugs, as they induce SIB and block SIB, suggests that disregulated monoaminergic systems cause SIB in the pemoline model. This is consistent with the evidence of disregulated monoaminergic neurotransmission in clinical populations that exhibit SIB (Lloyd et al., 1981; Ernst et al., 1996; Wong et al., 1996).

The inconsistency between the effects of valproate on injury scores and its effects on duration of self-injurious oral contact suggests that the effects of valproate were more subtle than were the effects of risperidone. These results show that valproate lessened the severity of the pemoline-induced self-injury, as measured by the injury scores, but did not reduce the expression of stereotyped oral behaviors, as quantified from the overnight videotaped samples. The significant valproate-induced decrease in the severity of the tissue injury further indicates that the pemoline model has predictive validity since this finding is consistent with decreases in SIB in autistic and intellectually handicapped patients treated with valproate (Kastner et al., 1993; Hollander et al., 2001). Valproate increases extracellular GABA concentrations by blocking the degradation of GABA by GABA transaminase (Loscher, 1993). It also blocks voltage-gated sodium channels.
(McLean & McDonald, 1986), calcium channels (Kito et al., 1994) and protein kinase C (Chen et al., 1994). Accordingly, it is not clear whether valproate reduced pemoline-induced self-injury through GABAergic mechanisms, ion channels actions or through alterations in cell signaling pathways. But, it did not appear that the injury-reducing effects of valproate were due to general sedation because the duration of self-injurious oral contact, grooming behaviors, inactivity and amount of locomotion were not different between the groups.

The fact that nifedipine attenuated pemoline-induced self-injury, and appeared to produce an overall inhibition of the self-injurious oral contact (although this did not reach statistical significance), concurs with the findings that nifedipine lowers the SIB in the 6-OHDA (Blake et al., 2004) and Bay-K 8644 (Jinnah et al., 1999) models. This indicates that there is generalizability between these different animal models of SIB. This could indicate that there are commonalities in the neurobiological actions that initiate SIB in each of these animal models. Identification of these common mechanisms could help to characterize the neurobiological conditions that are necessary and sufficient to induce SIB. One obvious factor is dopamine disregulation, and this investigation using nifedipine contributes further evidence that dopamine is important in the expression of SIB. L-type calcium channels are found predominantly on the presynaptic neurons (Okita et al., 2000) in the striatum and cortex (Hirota and Lambert, 1997) and are associated with increased release of dopamine from the caudate when activated (Okita et al., 2000). Additionally, blocking these channels decreases the rate of action potentials of midbrain dopaminergic neurons (Mercuri et al., 1994).
One potential confounding variable in this study is that the vehicle for the nifedipine contained ethanol. Ethanol was not used in any of the other experiments in this or previous studies, and the effect of ethanol on pemoline-induced SIB is not known. The rats that just received pemoline plus vehicle exhibited more severe self-injury than the rats did in any other experiment, even though all the groups of rats were treated with pemoline at 200 mg/kg/day. All the rats in the pemoline plus vehicle group were euthanized before the end of the experiment because they reached an injury score of 4. Accordingly, it is possible that this anomalous outcome may have actually resulted from an interaction between the ethanol and the pemoline in this experiment. This possibility will require further study. Nevertheless, nifedipine significantly reduced the overall SIB, demonstrating the effectiveness of this drug challenge.

Significant thymus involution was observed in all the nifedipine-treated groups. This is consistent with reports that nifedipine administration causes thymic apoptosis (Balakumaran et al., 1996). Accordingly, it does not appear that nifedipine could have any clinical potential for treatment of SIB, but further studies with this drug may help to reveal neurobiological mechanisms that underlie the induction and maintenance of SIB in the various animal models in which it is effective.

Although tramadol has been reported to decrease compulsive behaviors in individuals with OCD and Tourette’s syndrome (Goldsmith et al., 1999; Shapira et al., 1997) it did not significantly affect any of our dependent measures of SIB in the pemoline model. One potential interpretation of these results is that pemoline-induced SIB is not a compulsive behavior. This is contradictory to our casual observations, where we have noticed that the rats are extremely difficult to distract after they have initiated
self-biting behavior and that even if the injury site is out of reach for the rat (i.e. when the paw is being shown to the camera during the visual inspections) the rat will begin to nibble on other items, such as the examiners glove or lab coat. Another possibility is that tramadol does not disrupt compulsive behavior patterns with enough strength to combat the compulsive nature of pemoline-induced SIB. The two studies that describe the effectiveness of tramadol to combat compulsive behaviors in OCD and Tourette’s syndrome are open-labeled and they included small sample sizes. Thus, the clinical efficacy of tramadol is not yet well established. Moreover, it is possible that the dosage (0, 0.1, 1.0, 10 mg/kg) or dosing regimen (b.i.d.) of tramadol that we used in this study may not have been aggressive enough. We chose doses below 25 mg/kg/day because higher doses have been shown to induce abnormal chewing movements, vigorous grooming and spasms (Matthiesen et al., 1998). Accordingly, it seems unlikely that higher doses of tramadol would be effective in the pemoline model of SIB. The potential that compulsions play an important role in pemoline-induced SIB merits further evaluation.

The fact that memantine did not affect any of our dependent measures of pemoline-induced SIB is disappointing from a clinical perspective. Although the pemoline model is not a definitive screening tool for the efficacy of therapeutic drugs, the results from the memantine study suggest that it may not be an effective drug for reducing SIB in clinical populations. This negative outcome is somewhat surprising since memantine and MK-801 are both non-competitive NMDA receptor antagonists (Wong et al., 1986; Bormann, 1989) and MK-801 blocked pemoline-induced SIB (King et al., 1995). This difference in findings could be explained by the difference in
experimental procedures. King and colleagues investigated interactions between MK-801 and pemoline only acutely, as MK-801 and pemoline were administered only once in their study. In the present study the effects of blocking the NMDA receptor were studied with repeated administration of memantine (b.i.d.) and pemoline (q.d.) for five days. Although there is no specific reason to suspect that these different dosing regimens would produce differing outcomes, it is possible that higher doses or more frequent administration of memantine may have had some effect. However, memantine has been found to produce some learning deficits and ataxia when administered at 20 mg/kg (Hesselink et al., 1999) and in our study we used doses up to 30 mg/kg b.i.d. Accordingly, higher doses of memantine may not be appropriate in this model.

Another potential reason for the effectiveness of MK-801 and the ineffectiveness of memantine is that the actions of these drugs at the NMDA receptor differ substantially from each other. MK-801 exhibits high affinity binding to activated NMDA receptors, and becomes trapped in the ionophore where it cannot be displaced. Accordingly, MK-801 blocks the physiological actions that result from transient release of glutamate (e.g., learning and memory) and it also blocks pathological actions that result from prolonged glutamate stimulation (e.g., apoptotic cascades in neurodegenerative diseases). Memantine, on the other hand, exhibits low affinity binding to the ionophore of the activated NMDA receptors. As such, the binding is transient. Accordingly, memantine is neuroprotective in conditions where there is prolonged glutamate stimulation (e.g., neurodegenerative disorders such as Alzheimer’s disease) because it decreases the calcium influx during the prolonged stimulation. However, under basal conditions, when the postsynaptic NMDA receptor is generally quiescent, memantine is not bound to the
receptors, which allows transient binding of glutamate and the opening of the ion channel to occur. If memantine does bind, the binding is rapidly reversible, and so the next time normal transient glutamate stimulation occurs, the process is repeated. Accordingly, memantine is only a very low potency antagonist against normal physiological actions of glutamate at NMDA receptors (for review see Sonkusare et al., 2005). In fact, a comparative analysis reveals that MK-801 exhibits very high potency blockade of NMDA receptor-dependent hippocampal long term potentiation (LTP), whereas memantine has only very low potency and does not affect LTP (Frankiewicz et al., 1996).

The discrepancy between the effects of MK-801 (King et al., 1995) and the lack of effects of memantine (present results) coupled with the observation that there is a lag between the onset of treatment and the onset of SIB when the rats are treated with a moderately high dose of pemoline (Kies and Devine, 2004; present results), raises the interesting possibility that glutamate-mediated neuroadaptations may play an important role in pemoline-induced SIB. In this case, the MK-801 was effective because it blocked the ability of pemoline to initiate these adaptations, and memantine was ineffective because it did not block the neuroadaptations. An analysis of the systems in which pemoline induces neuroadaptation through glutamate-mediated actions may reveal neuropathological disregulation in the pemoline-treated rats that exhibit SIB. Identification and characterization of deregulated systems that differentiate self-injurious from non-injurious rats may provide interesting leads to examine neuropathological substrates that underlie SIB in clinical populations.

In the valproate, nifedipine and tramadol experiments, the drug challenges had no significant impact on any of the measures of grooming, inactivity or locomotion
(overnight videotapes and photocell counts). In the risperidone experiment, the duration of inactivity on the last night of the experiment was significantly greater in rats treated with the highest dose of risperidone, as compared to vehicle-treated rats. It does not appear that risperidone was exerting its effect through sedative actions, however, because duration of grooming and amount of locomotion were not different between risperidone-and vehicle-treated groups. During the first two days of the memantine experiment, the memantine-treated rats exhibited greater locomotor counts, both overnight and after the morning injections, compared to that of the vehicle-treated rats. Beginning on day 3, the locomotor counts of the memantine-treated rats no longer differed from that of the vehicle-treated rats. The differences in locomotor counts between memantine- and vehicle-treated rats are mostly inconsequential because memantine had no significant effect on pemoline-induced self-injury.

The rats remained reasonably healthy throughout the experiments. Pemoline caused some weight loss, which began to level off or improve on day 3 or 4 in most experiments. This may be due to the psychomotor stimulating effects of pemoline or altered feeding behaviors during pemoline treatment. Chromodaccryorrhea (porphyrin containing secretions around the eyes; Payne, 1994) was noticed in some of the rats. These secretions have been associated with stress (Harkness & Ridgway, 1980; Ross, 1994; Chen et al., 1997) and with toxic actions of a variety of drugs (Moser, 1991; Sauer et al., 1995; Pegg et al., 1996; Graziano et al., 1996; Sauer et al., 1997). The presence of chromodaccryorrhea replicates a previous finding when rats were treated daily with 200 mg/kg pemoline (Kies & Devine, 2004), but as in the previous study, this was a minor
effect that occurred in a very small number of the rats, and did not appear to be associated
with the induction or maintenance of SIB.

In summary, these projects provide evidence of the predictive validity of the
pemoline model to mimic the SIB seen in clinical populations as risperidone and
valproate lessened pemoline-induced SIB. They also provide more evidence of
monoamine involvement in SIB because risperidone and nifedipine each reduced the self-
injury in the pemoline model. Results from these experiments also indicate that common
neurobiological substrates may underlie the induction of SIB in multiple animal models,
as evidenced by the fact that nifedipine consistently blocks SIB in these various animal
models. Also, the current evidence suggests that glutamate-mediated neuroadaptations
may be involved in producing pemoline-induced SIB since memantine did not affect
pemoline-induced SIB. This interesting possibility merits further attention.
Unfortunately, the tramadol experiment suggests (within the limits that the model may
predict clinical efficacy) that this treatment does not hold therapeutic promise.

In accordance with the observations of these experiments, the predictive validity
and pre-clinical screening potential of the pemoline model should be investigated further
using drugs that are effective in decreasing SIB in different clinical populations. In light
of the fact that the monoamine agonist pemoline only produced SIB after days of
treatment, and that a monoamine antagonist blocked the effect, the dynamic regulation of
neurotransmission in dopaminergic, serotonergic, and noradrenergic systems should be
examined in relation to the onset and expression of the SIB. Additionally, the role of
specific glutamate-mediated neuroadaptations should also be investigated. Challenging
the pemoline model of SIB with drugs that inhibit the protein kinases that mediate
neuroadaptations, such as a protein kinase B and protein kinase C, will begin to elucidate the changing mechanisms that produce pemoline-induced SIB. Additionally, challenging the pemoline model with a protein kinase C inhibitor and with another GABA agonist, topiramate, will also help decipher whether valproate reduced the pemoline-induced self-injury through its actions on GABA or its actions on the kinase. These future experiments will improve our knowledge about the neurobiological mechanisms that are producing and maintaining this devastating behavior disorder and will lead to the elucidation of potential targets for new pharmacotherapies.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Amber Marie Muehlmann graduated cum laude from San Diego State University with a Bachelor of Arts in May 2002. She began her graduate education in August 2003 working towards her Master of Science degree in the behavioral neuroscience program in the Psychology Department at the University of Florida.