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Increased sensitization to morphine-induced oral stereotypy in aged rats

Clifford M. Knapp, Shivkumar H. Jha, Conan Kornetsky*

Departments of Psychiatry and Pharmacology, Boston University School of Medicine ,715 Albany Street, R-620, Boston, MA 02118, USA

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Abstract

Sensitization develops to the stereotypic biting behavior that appears with the repeated administration of high dose morphine to rats. Because there is evidence that this behavior is dopamine-mediated and that there are age-related changes in dopamine systems, we compared the development and expression of morphine-induced biting behavior in aged (24 months) and young rats (5 months). Animals were treated with four sensitizing 10 mg/kg doses of morphine or saline, followed by three weekly challenges with 4 mg/kg doses of morphine or saline. By the fourth sensitizing morphine dose and after the administration of each low dose challenge, the biting time was significantly greater for aged than for young morphine pre-treated rats. After the first weekly low dose challenge, the aged but not young animals expressed more biting than when they did after the last 10 mg/kg dose. These results indicate that sensitization to morphine-induced oral stereotypy is significantly greater in aged as compared to young rats. Age-related enhanced sensitivity to morphine-induced oral stereotypy might be related to age-induced increases in vulnerability to opioid-induced insults to the basal ganglia, and may be a model for certain diseases of this pathway.

Keywords: Sensitization; Stereotypy; Opiates; Morphine; Aging; Rats

1. Introduction

The repeated administration of morphine and related opioids to rats leads to the appearance of stereotyped biting behavior in response to challenges with these compounds (Fog, 1970; Pollock and Kornetsky, 1989). This behavior involves stereotyped repetitive biting of either inanimate objects (cage bars, etc.) or self-biting (of the digits or tail) for prolonged periods of time (Pollock and Kornetsky, 1989). Following the development of opioid-induced oral stereotypy, this behavior will be re-expressed after the administration of low doses of morphine. This form of sensitization to the effects of morphine has been shown to persist for months (Pollock and Kornetsky, 1996). The expression of morphine-induced oral stereotypic behavior may be mediated by dopaminergic systems. This is suggested by the finding that morphine-induced biting behavior is blocked by the administration of the dopamine D_1 receptor antagonist SCH 23390, although it should be noted that this behavior is not altered by the administration of the D_2 selective antagonist raclopride (Pollock and Kornetsky, 1989). Also, oral stereotypy is expressed in morphine-exposed animals by the administration of dopamine agonists including the dopamine D_2 receptor agonist bromocriptine and the indirect dopamine agonists amphetamine and amfonelic acid (Pollock, 1992), but not by lower doses of the non-selective dopamine agonist apomorphine.

Oral stereotypic behavior also can be produced by the administration of dopamine agonists including amphetamine, apomorphine, and amfonelic acid in animals after discontinuation of chronic treatment with the dopamine antagonist haloperidol (Pollock and Kornetsky, 1991; Tarsy and Baldessarini, 1974). The administration of the opioid antagonist naloxone concurrently with these dopamine agonists blocks the appearance of oral stereotypy in animals pre-treated with haloperidol (Pollock and Kornetsky, 1991). These findings suggest that the presentation of agonist-induced oral stereotypy may involve the dysregulation of

* Corresponding author. Tel.: +1 617 638 4320; fax: +1 617 638 5254.
E-mail address: ckornets@bu.edu (C. Kornetsky).

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dopaminergic control of behavior and that endogenous opioid systems are somehow implicated in this process.

In young drug naïve animals, oral stereotypy is not produced by the acute administration of either amphetamine (Crawford and Levine, 1997; Pollock, 1992) or low dose apomorphine (Pollock, 1992). In contrast, in aged rats, repetitive gnawing behavior is produced by the acute administration of amphetamine (Crawford and Levine, 1997). Also, the administration of either the dopamine D_1 agonist SKF 38393 or the D_2 agonist quinpirole results in significantly greater gnawing behavior in aged as compared to young rats, although the effects of SKF 38393 may not be pronounced. Stereotyped behavioral effects (e.g., yawning, stereotyped sniffing, licking or gnawing) induced by the administration of the mixed D_1/D_2 agonist apomorphine are potentiated with increasing age (Stoessl et al., 1989), although this effect of age, at least in part, may be due to age-related changes in the clearance and distribution of apomorphine (Campbell et al., 1984). When considered together, these results suggest the possibility that aging may alter the regulation of behavior by dopaminergic systems in a way that involves changes in neuronal function that are closely related to those that result from with either repeated opioid agonist or dopamine antagonist administration. In the present investigation, the question of whether aging alters the development of sensitization to morphine-induced biting behavior is addressed by an examination of the effects of repeated morphine administration on the expression of oral stereotypy in young and aged rats.

2. Methods

2.1. Animals

Male Brown Norway/Fischer 344 F_1 ($F344/BNF_1$) hybrid rats (Harlan Sprague Dawley/NIA, Indianapolis, IN) were individually housed, provided water and food ad libitum, and maintained on a 12–12 h light–dark cycle. All experiments were carried out during the light portion of the cycle. Young adult rats were 5 months old and weighed between 250 and 325 g, while aged rats were 24 months old and weighed between 375 and 525 g. All of the animals were gentled daily for 1 week to habituate them to handling before any experimental treatment.

These experiments were conducted with the approval of Institutional Animal Care and Use Committee (IACUC) at Boston University School of Medicine.

2.2. Drugs and dosing

The animals were placed into one of six treatment groups (see Table 1). These groups consisted of paired groups, each pair consisting of a young or aged group of animals. The first pair of groups, the morphine-pretreated (sensitized) groups, were treated with four doses of morphine (10 mg/kg SC) administered over a 36-h period. This treatment regimen has been shown in previous studies to sensitize young rats to morphine-induced stereotypic aggressive gnawing and chewing of self or inanimate objects (Pollock and Kornetsky, 1989). The second pair of groups, the saline-pretreated (un-sensitized) groups, was treated with saline during the "sensitization" phase of this experiment. The third pair of groups, the saline control groups, received saline injections throughout the study.

Starting 1 week after the last morphine or saline treatment in the sensitization phase of this experiment, animals received 'challenge' doses of either morphine or saline administered three times at weekly intervals. Morphine (4 mg/kg SC) was administered to the pair of groups that had been previously treated with morphine and to a second pair of animals that had been pretreated with saline. Saline was administered to the control animals during the challenge phase.

Morphine sulfate (NIDA, Rockville, MD), 10 or 4 mg/kg was dissolved in normal saline. Doses were made in 5 and 2 mg/ml volumes and injected subcutaneously.

2.3. Procedure

Immediately following all morphine or saline injections, the rats were placed in individual acrylic chambers (21×21×35 cm high) with a grid floor composed of horizontal steel bars (2.0 mm in diameter and separated by 10 mm). They were then observed continuously for 120 min during both the acquisition (sensitization) phase and the challenge phase. The time spent self- or bar-biting was recorded using the OBSERVER® software. In every experiment, the observer remained blind with respect to the specific drug treatment and

Table 1
Experimental treatment of each of the six experimental groups during the sensitization and challenge phases of the experiment

Experimental groups	(*N*)	Dose 1 (mg/kg)	Dose 2 (mg/kg)	Dose 3 (mg/kg)	Dose 4 (mg/kg)	Challenge 1 (mg/kg)	Challenge 2 (mg/kg)	Challenge 3 (mg/kg)
Group 1 (sensitized young)	(10)	10.0	10.0	10.0	10.0	4.0	4.0	4.0
Group 2 (sensitized aged)	(10)	10.0	10.0	10.0	10.0	4.0	4.0	4.0
Group 3 (un-sensitized young)	(6)	Saline	Saline	Saline	Saline	4.0	4.0	4.0
Group 4 (un-sensitized aged)	(6)	Saline	Saline	Saline	Saline	4.0	4.0	4.0
Group 5 (young saline controls)	(4)	Saline	Saline	Saline	Saline	Saline	Saline	Saline
Group 6 (aged saline controls)	(4)	Saline	Saline	Saline	Saline	Saline	Saline	Saline

treatment history of the animal. Animals were run in cohorts of six, randomly selected from each experimental group. Animals' behaviors were rated using a modification of the methods described by Kelley et al. (1988). These behaviors were categorized as self-biting (biting of tail or digits), bar-biting (biting of cage floor, bar or acrylic sides of chamber), rearing, grooming, locomotion, catalepsy, sleep, inactivity, urination and defecation. The investigator recorded the time that animals spent engaged in these behaviors by signaling the OBSERVER® program at the start of the session and then immediately after the transition from one type of behavior to another. The percent time over the 2-h observation period that animals spent engaged in biting behavior (either self- or bar-biting) was used as the dependent variable in this study.

2.4. Data analysis

To compare the duration of biting at each of the four sequential sensitization treatments, a two-way repeated-measures analysis of variance (ANOVA) was employed. When appropriate, post hoc comparisons were made using the Student–Newman–Keuls method. A one-way ANOVA was used for between-groups comparisons of the effects for each challenge of 4.0 mg/kg dose of morphine. A one-way repeated-measures ANOVA was used for within-group comparisons of the data obtained for the last sensitizing dose of morphine with the data obtained for the challenge doses. Post hoc comparisons using Dunnett's *t*-test were then made between data for the last sensitizing dose with values obtained for each challenge dose. All analyses were performed using Sigmastat® (Sigmastat versions 2.0, SPSS).

3. Results

The progressive increase in the percentage of time engaged in stereotypic biting behavior during the administration of the four sensitizing doses of morphine is illustrated in Fig. 1. Both aged and young rats showed increases in stereotypy with each subsequent dose. A statistically significant Age×Dose interaction between young and aged groups [$F(3,54)=3.63$; $p=0.018$] was observed for sensitizing doses. At the fourth sensitizing dose, there was a statistically significant difference between the young and aged animals in biting behavior ($p<0.001$). At the third dose, young animals showed significant biting behavior as compared to the first dose ($p=0.008$) while biting was significantly increased in the aged rats by the second dose ($p=0.007$).

Fig. 2 illustrates the extent of stereotypy in all groups after each of the three weekly 4.0 mg/kg challenge doses. All animals in both sensitized groups exhibited stereotyped biting after the administration of challenge doses. The biting began 5–15 min after administration of the challenge dose with onset of biting being more rapid for the aged as compared to the young animals. Compared to the last sensitizing 10 mg/kg dose, the mean percent time spent biting was greater in aged rats after the administration of the first 4 mg/kg challenge dose ($p<0.05$). The mean percent biting time was not significantly different between the last sensitizing dose and the second or third challenge in the aged morphine sensitized animals or any of the three challenges in young sensitized rats. Following the administration of the first morphine challenge dose, the percentage of total biting behavior time was significantly greater in aged rats as compared to young rats in the sensitized group,

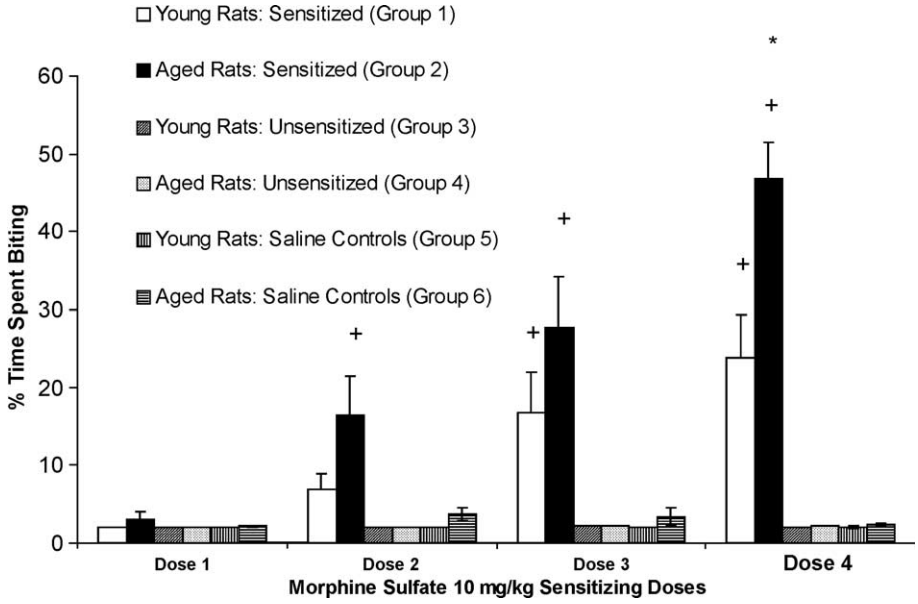

Fig. 1. Percentage time spent biting during the four 10 mg/kg dose or saline treatment at 12-h intervals for all six experimental groups. (Two percent of time spent biting was added to the data for all the groups to allow visualization of the results for saline-treated animals.) *Indicates a significant difference ($p<0.05$) between young and aged animals. $^{+}$Indicates for within-group comparisons a significant difference ($p<0.05$) between the first and subsequent morphine doses.

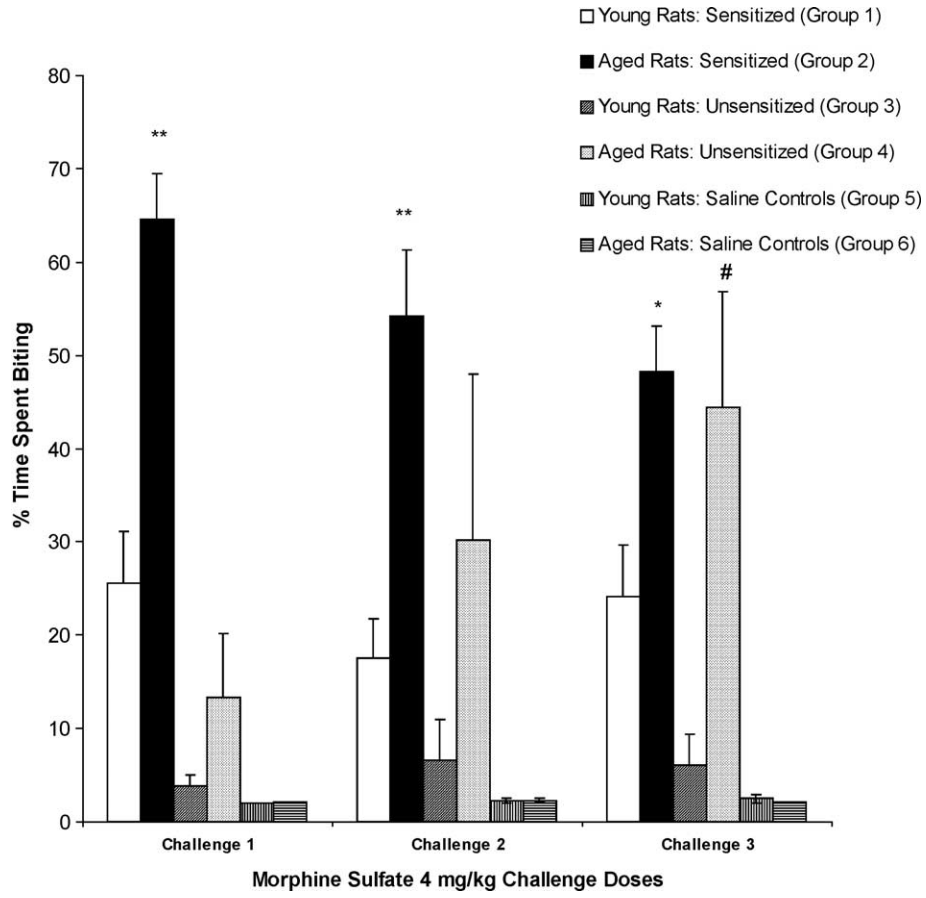

Fig. 2. Percentage time spent biting at each challenge dose of 4 mg/kg or saline. The first challenge dose was administered 1 week after the last sensitizing dose. Each subsequent challenge was one week thereafter. (Two percent of time spent biting was added to the data for all the groups to allow visualization of the results for saline-treated animals.) *Indicates a significant difference ($p<0.05$) between values obtained aged and young sensitized rats. **Indicates a significant difference ($p<0.001$) between values obtained for aged and young sensitized rats. $^{\#}$Indicates a significant difference ($p<0.05$) between values obtained for aged and young un-sensitized rats.

while no significant difference between aged and young animals existed in the saline-pretreated group. The second challenge dose had similar significant effects. By the third challenge, the sensitized groups of aged and young rats were dissimilar as earlier. However, by the third challenge dose, the percent of time spent biting was significantly greater for the un-sensitized group of aged rats than it was for the un-sensitized young rats. Saline controls never showed any significant increase in biting behavior.

4. Discussion

This study demonstrates that the acute administration of a moderately high single dose of morphine, in contrast to injection of amphetamine or of direct dopamine agonists, does not produce oral stereotypy in aged animals. The rate of increase in the time spent biting following the repeated administration of morphine, however, is significantly greater in aged than in young rats. Also, oral stereotypy develops in older animals following the administration of a few weekly injections of morphine at a dose that is too low to produce a similar effect in young animals. As with young animals, sensitization to morphine induced biting behavior persists for weeks after the development of the expression of this behavior.

The finding that the time spent in stereotyped biting becomes significantly greater following the repeated morphine administration in aged rats as compared to young animals being treated with the same dose of this drug suggests that aging somehow "sensitizes" rats to morphine's effects on at least some behaviors. At present, the mechanisms that are responsible for age-related changes in the sensitivity of rats to the oral stereotypy-inducing effects of morphine remain to be determined. Chronic morphine administration has been found to produce a variety of changes in young animals including increases in brain glucose utilization (Kraus et al., 1997) and μ-opioid receptor density (Vigano et al., 2003), desensitization of dopamine D_2 receptors (Nestby et al., 1995) and enhanced induction by morphine of guanylyl 5′-[γ-3^5S]thio]triphosphate G protein binding (Vigano et al., 2003). The effects of aging on these changes have yet to be examined.

One possible mechanism that must be considered as being responsible for age-related differences in sensitivities to the effects of morphine is the influence of the aging processes on the pharmacokinetics of this drug. The available evidence, however, suggests that the pharmacokinetics of morphine itself are unaltered by aging in rodents. In the mouse, similar percentages of a dose of labeled morphine entered the brains of mature and aged animals (Hoskins et al., 1986). Areas under the concentration–time curves for this drug for both plasma and brain tissue have not been found to differ significantly between aged and young animals after morphine injection (Van Crugten et al., 1997).

Morphine is metabolized by the rat into morphine-3-glucuronide and to a lesser extent into normorphine-3-glucuronide. These compounds, when administered into the brain, produce excitatory effects that range from chewing, increased grooming, and exploratory activity to seizure activity (Bartlett et al., 1994; Halliday et al., 1999; LaBella et al., 1979; Smith and Smith, 1998). These effects are blocked by the administration of the *N*-methyl-D-aspartate NMDA receptor antagonist LY274614, suggesting the possibility that morphine-3-glucuronide excitatory actions are mediated by NMDA receptor activity (Bartlett et al., 1994). Thus, activation of NMDA receptors by morphine-3-glucuronide might contribute to the development of morphine-induced oral stereotypy in rats. However, while maximal plasma concentrations of morphine-3-glucuronide are several folds higher in the plasma of aged as compared to young rats this metabolite was not detected in the brains of young or aged rats after the injection of morphine (Van Crugten et al., 1997) nor does it appear to enter the brain when it is exogenously administered (Bickel et al., 1996). Thus, at present, there is no evidence indicating that higher plasma concentrations of morphine-3-glucuonide seen after morphine administration in aged as compared to young rats might lead to an increased effect of this metabolite in the brain in older animals.

Only limited evidence is available as to how aging influences either opioid receptor systems or the effects of chronic morphine administration on these systems. The density of putative receptor binding sites for the opioid dihydromorphine significantly decline in several brain areas with age including the frontal poles, anterior cortex and the striatum (Messing et al., 1981). Findings concerning the influence of aging on the analgesic effects of morphine have not been consistent. When compared to young animals, aged animals have been found to be less (Jourdan et al., 2002), more (Smith and Gray, 2001) or equally (Van Crugten et al., 1997) sensitive to the analgesic effects of morphine. Aged animals showed enhanced hyperthermic response to a 5 mg/kg dose of morphine, but an attenuated response to the hypothermic effects of high dose morphine (McDougal et al., 1980). In comparison to young morphine-dependent rats, aged rats dependent on this drug exhibit less intense symptoms of withdrawal including rhinorhea, lacrimation and salivation (Simpkins, 1994). Finally, morphine-induced lowerings of brain stimulation reward thresholds, which reflect the actions of this drug on reward pathways, is produced by a similar range of doses of morphine in young and aged rats (Jha et al., 2004). Taken together, these findings are too inconsistent to allow a conclusion to be drawn as to whether aging increases or decreases the functioning of opioid systems.

The difference in morphine-induced sensitization between aged and young rats may be due to changes in the brain dopaminergic systems of aged rats. The expression of morphine-induced oral stereotypy is blocked by the administration of the dopamine D_1 receptor antagonist SCH23390 (Pollock and Kornetsky, 1989) and it occurs when dopamine agonists are administered to morphine sensitized rats. At 15 days after the completion of the administration of a series of morphine injections, challenge with a low dose of morphine resulted in both stereotyped behavior and an increase over control values in dopamine release in the core of the nucleus accumbens and in the caudate–putamen (Cadoni and Di Chiara, 1999). This evidence implicates the dopaminergic system in the expression of oral stereotypy in morphine-sensitized rats. Much of the evidence suggests that brain–dopamine systems are compromised with age. Dopamine-induced increases in striatal adenylate cyclase activity, mediated by dopamine D_1 receptors, are reduced in aged as compared to young rats (May and Sugawa, 1993; Schmidt and Thornberry, 1978). Binding sites labeled by the D_1 receptor ligand SCH23390 may be reduced in many brain regions (Nabeshima et al., 1994), although this has not been consistently reported (Araki et al., 1997). The number of striatal neurons containing dopamine D_1 receptor mRNA may be reduced in aged as compared to young rats (Zhang and Roth, 1997). Putative D_2 receptor binding sites in the rat striatum may decline with age (Donzanti et al., 1993; Han et al., 1989). However, some investigators have not found aged-related changes in D_2 receptor ligand binding in the striatum and other brain regions (Araki et al., 1997). In striatal tissue, the number of D_2 receptor mRNA containing neurons and the amounts of D_2 receptor mRNA are significantly lower in aged as compared to young rats (Della et al., 1992; Mesco et al., 1991; Valerio et al., 1994; Zhang et al., 1995). The administration of either the indirect dopamine agonist D-amphetamine or of the dopamine D_2 receptor agonist quinpirole results in the appearance of fewer Fos-positive nuclei in the nucleus accumbens of aged as compared to young rats (Crawford and Levine, 1997). These results suggest that the influence of dopamine and other dopamine agonists on neuronal activity may be diminished with aging, although this may not be case for the D_1 receptor agonist SKF 38393 (Crawford and Levine, 1997).

The evidence discussed above suggests that many of the effects of dopamine agonists may diminish with age.

However, some of the actions of these agents may increase with age. The induction of stereotyped behaviors produced by these agents is more pronounced in aged rats than it is in young rats (Crawford and Levine, 1997; Stoessl et al., 1989). Also, the administration of either D-amphetamine or apomorphine produces an increase in the proportion of striatal neurons exhibiting an increase in firing rates to those showing an inhibition of firing in aged as compared to young rats (Stanford et al., 2002). This age-related change in the ratio of neurons in the striatum that are excited, as opposed to those that are inhibited by dopamine agonists, may reflect a decrease in the dopamine D_2 receptor-mediated inhibition of glutamate release (Donzanti et al., 1993). It may also be related to age-related decreases in the inhibitory effects of dopamine on glutamate induced cell firing in the striatum (Cepeda et al., 1996). Elevations in basal concentrations of glutamate in the striatum that are associated with aging may also contribute to a reduction in the influence of dopamine on glutamatergic system functioning in aged rats (Donzanti et al., 1993; Massieu and Tapia, 1997). Aging, then, may lead to a disruption in the modulation by dopamine of the effects of cortico-striatal glutamatergic input to systems that regulate behavior. This may be of significance given the evidence that the glutamate and NMDA receptors, in particular, may play a role in the development of morphine-induced oral stereotypy (Livezey et al., 1995 and unpublished observations). It is therefore possible that alterations in dopamine regulation of glutamate activity in aged rats might play a role in the altered development of sensitization to morphine-induced oral stereotypy in these animals.

The apparent increased sensitivity of aged rats to the induction of oral stereotypy by morphine may reflect a particular vulnerability in dopaminergic or perhaps other systems that might be related to the development neurological disease in elderly individuals. It may also reflect a susceptibility to damage in cortico-striatal–thalamic circuits. Infusion of dopamine into the caudate of animals treated with reserpine leads to the appearance of stereotypic gnawing behavior (Fog and Pakkenberg, 1971) implicating the striatum in the production of such behavior. Insult to cortico-striatal–thalamic circuits in humans has been linked to the appearance of obsessive compulsive symptoms in certain neurological disorders including Parkinson's disease (Alegret et al., 2001). It has been suggested that these symptoms may be related to stereotyped behaviors in rats (Stein, 2003). In addition to stereotyped biting in the rat, stereotyped behaviors in other species may model features of obsessive compulsive behavior in humans (Stein et al., 1992). There is evidence that suggests that such behaviors in the horse are regulated by endogenous opioid peptides (Dodman et al., 1987; Dodman et al., 1988). Elucidation of the age-related changes in neuronal function that lead to the increased sensitivity of animals to morphine-induced stereotypy should help to establish the connection between this behavior in aged rats and human neurologic disorders.

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