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Pharmacologic thresholds for self-injurious behavior in a genetic mouse model of Lesch–Nyhan disease

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Abstract

Congenital deficiency of hypoxanthine-guanine phosphoribosyl transferase (HPRT) causes Lesch–Nyhan disease in humans, which is associated with severe and recurrent self-injurious behavior (SIB). The HPRT-deficient knockout mouse model, however, does not display this unusual behavior. The present studies tested whether these mice might be more vulnerable to pharmacologic agents known to cause SIB in normal rodents, including clonidine, Bay K 8644, GBR 12909, methamphetamine, pemoline and caffeine. The results provided three conclusions. First, normal mice did not display SIB using some drugs known to provoke the behavior in rats (GBR 12909, caffeine), indicating important species differences in the expression of the behavior. Second, the C57BL/6J mice did not display SIB using drugs effective for other strains of mice (methamphetamine, pemoline), indicating important strain differences in expression of the behavior. Finally, there was no evidence that the HPRT-deficient mice were more susceptible to SIB when it occurred (clonidine, Bay K 8644).

Keywords: Mouse model; Stereotypic behavior; Review

1. Introduction

Self-injurious behavior (SIB) encompasses an array of abnormal behaviors including self-biting, self-hitting, head banging and others (Schroeder et al., 2001; Winchel and Stanley, 1991). It is most commonly seen in profound mental retardation and autism, but it also occurs in several neurodevelopmental disorders including Tourette syndrome, Rett syndrome, Cornelia de Lange syndrome, Smith–Magenis syndrome and neuroacanthocytosis. In adults, SIB also occasionally appears in thought, mood, or personality disorders. SIB is most frequent and severe in Lesch–Nyhan disease, which is caused by mutation of the gene encoding hypoxanthine-guanine phosphoribosyl transferase (HPRT). This condition has therefore served as a prototype for the study of the behavior (Jinnah and Friedmann, 2000).

The pathogenesis of SIB is very poorly understood, and several pharmacologic models have been developed in animals to facilitate research on its neurobiological basis (Jinnah et al., 1990; Moy et al., 1997). Several models have employed methylxanthines (caffeine and theophylline), clonidine, neonatal 6-hydroxydopamine, psychostimulants (methamphetamine, amphetamine and pemoline), GBR 12909 or ± Bay K 8644. These pharmacologic models have been valuable for providing insight into the neurochemical systems involved in the expression of SIB, for screening drugs that might have efficacy against SIB, and for designing potential treatment strategies in humans.

In addition to these pharmacologic models, a genetic HPRT-deficient ($HPRT^-$) mouse model for Lesch–Nyhan disease has been produced (Hooper et al., 1987; Kuehn et al., 1987). These mutants do not display SIB, but they show abnormal behavioral responses to a variety of drugs (Jinnah et al., 1991, 1992). One study suggested that SIB could be induced in these mutants with 9-ethyladenine (Wu and Melton, 1993), but this finding could not be reproduced in a second study (Edamura and Sasai, 1998). The purpose of the current studies was to determine if the $HPRT^-$ mice might be particularly vulnerable to other pharmacological agents known to cause SIB.

Abbreviations: SIB, self-injurious behavior; HPRT, hypoxanthine-phosphoribosyl transferase.

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2. Methods

2.1. Animals

These studies were conducted with C57BL/6J mice and congenic HPRT$^-$ mutants (C57BL/6J$^{HPRT.BM3}$) obtained from Jackson Laboratories (Bar Harbor, ME). They were maintained in groups of 6–12 on a 14:10-h light–dark cycle in the Johns Hopkins animal facilities for at least 2 weeks prior to behavioral testing. All had free access to food and water, except those for clonidine testing, which were housed individually for 4 weeks and had food withdrawn the night prior to testing. All mice were 6–8 weeks of age at the time of testing, except for those treated with ±Bay K 8644, which were 3–4 weeks of age. All animal procedures were conducted with approval from the local Animal Care and Use Committee in accordance with guidelines described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

±Bay K 8644, caffeine, clonidine, methamphetamine, GBR 12909, haloperidol and pemoline were obtained from Sigma-RBI (St. Louis, MO). Clonidine, methamphetamine and GBR 12909 were dissolved in distilled water and injected subcutaneously at a volume of 10 ml/kg. Caffeine was dissolved in 0.9% saline and injected subcutaneously at a volume of 10 ml/kg. ±Bay K 8644 was first dissolved in a 50:50 mixture of ethanol and Tween-80 to provide a stock solution of 10 mg/ml. The stock was diluted with distilled water before use and injected at a volume of 10 ml/kg. Pemoline was delivered by gastric lavage after suspending it in 5% gum arabic.

Dosing regimens for each drug were based on those previously reported to cause SIB. A single large dose was used in the case of ±Bay K 8644, clonidine and pemoline. Repeated treatments were used for GBR-12909 (once daily for 3–4 days), methamphetamine (every 3 h for 12 h) and caffeine (twice daily for 4 days).

2.3. Behavioral evaluation

After receiving a drug, mice were housed individually in clear plastic cages similar to their home cage and the presence or absence of SIB was recorded. Clonidine and ±Bay K 8644 provoke SIB acutely (Jinnah et al., 1999a,b; Morpurgo, 1968; Razzak et al., 1975), so animals were monitored for 1 min every 10 min for up to 2 h. Caffeine, GBR 12909, methamphetamine and pemoline provoke SIB after a long delay (Mueller and Nyhan, 1982; Shishido et al., 2000; Sivam, 1995), so animals were monitored for longer periods. GBR 12909 treated animals were examined at 2, 4, 6 and 8 h following each of three daily doses. Methamphetamine treated mice were examined every hour for 12 h. Animals treated with pemoline were examined at 2, 4, 6, 8, 24 and 36 h following a single dose. Animals treated with caffeine were examined at 1, 2, 4 and 8 h following each dose.

To prevent unnecessary pain or suffering, SIB was terminated with gaseous methoxyflurane as soon as it was observed. Clonidine-treated mice were then given an intraperitoneal injection of 2 mg/kg haloperidol, causing sedation until they recovered from the influence of the drugs. The ±Bay K 8644 treated mice were given an intraperitoneal injection of 10 mg/kg nifedipine, which rapidly terminates SIB. The immediate termination of SIB precluded measurements related to the persistence or severity of SIB. As a result, subtle differences in these parameters would not have been detected in these studies.

In addition to SIB, each of the test drugs caused other abnormal behaviors, which were also recorded. The severity of dystonic motor dysfunction (Jinnah et al., 2000) associated with ±Bay K 8644 was recorded on a four-point scale (Table 1). GBR 12909, methamphetamine and pemoline caused hyperactivity and stereotypical behaviors. Behavioral changes associated with these drugs were recorded using a behavioral inventory method (Fray et al., 1980) and a five-point rating scale originally developed for amphetamine (Table 2). With the behavioral inventory method, each animal was observed for exactly 1 min at specified intervals for the duration of the recording period and each minute divided into four 15-s bins. The occurrence of specific behaviors (ambulation, hyperactive running, sniffing, rearing, gnawing, grooming and tongue protrusion) during the interval was recorded.

2.4. Data analysis

Data derived from the ±Bay K motor disability scale were averaged over the entire 60-min recording interval to provide a single score for each mouse. Individual scores were then combined to provide a composite average for each experimental group, and the group averages were compared by two-way ANOVA with drug dose and HPRT status as the main variables. Scores derived from the stimulant scale were also combined for each time interval

Table 1
Motor disability scale

Behavior	Score
Normal motor behavior	0
Slightly slowed or abnormal motor behavior, but not bad enough to impair any activities	1
Mild impairment; limited ambulation unless disturbed, transient abnormal postures or infrequent falls	2
Moderate impairment; limited ambulation evenwhen disturbed, frequent abnormal postures, frequent falls but still able to get upright most of the time	3
Severe impairment; almost no ambulation, sustained abnormal postures, not upright most of the time	4

Table 2
Stimulant scale

Behavior	Score
Sleeping	0
Awake inactive	1
Active, exploring or grooming	2
Hyperactive	3
Hyperactive ambulation with intermittent stereotypy (gnawing, licking, sniffing in one spot)	4
Persistent stereotypy with little ambulation	5
Persistent stereotypy with no ambulation	6

to provide composite averages for each group that was also compared by two-way ANOVA. For behavioral inventory data, the average percent time for each behavior at each time interval was analyzed by MANOVA, with HPRT status and interval as the main variables.

3. Results

3.1. Clonidine

Preliminary experiments in separate groups of 10 normal C57BL/6J mice were conducted to determine the doses of clonidine required to provoke SIB. At doses of 25 mg/kg, the mice demonstrated a reduction in spontaneous activity, severe tremors when walking, Straub tail and piloerection. At 50 mg/kg, these phenomena were more severe. The mice also developed intermittent fisting (flexion of the forepaws or hindpaws), exophthalmos and intermittent compulsive licking or gnawing of the cage walls. At 75–100 mg/kg, the mice had difficulty walking, experienced tremors and spent most of their time lying motionless. Three mice did not survive treatment at 100 mg/kg. SIB was seen in half the animals treated with 50 mg/kg or above. The behavior was characterized exclusively by subtle persistent biting of one forepaw without vocalization.

Table 3
Clonidine-induced SIB

Variable	SIB
Grouped	
C57BL/6J male	0/10
C57BL/6J female	0/10
Isolated	
C57BL/6J male	4/10
C57BL/6J female	0/10
Grouped	
Male HPRT$^+$	0/10
Male HPRT$^-$	0/10
Isolated	
Male HPRT$^+$	4/10
Male HPRT$^-$	4/10

A dose of 50 mg/kg was used for assessment of the influence of sex and housing conditions on the expression of SIB in normal mice. One group of 10 male and 10 female mice was housed together in groups of 8–12, while another group was housed individually for at least 4 weeks. The group-housed mice did not display SIB. None of the isolation-housed females displayed SIB, but 4/10 of isolation-housed males displayed SIB (Table 3).

The ability of 50 mg/kg clonidine to provoke SIB was next evaluated in 10 HPRT$^+$ and 10 HPRT$^-$ male mice,

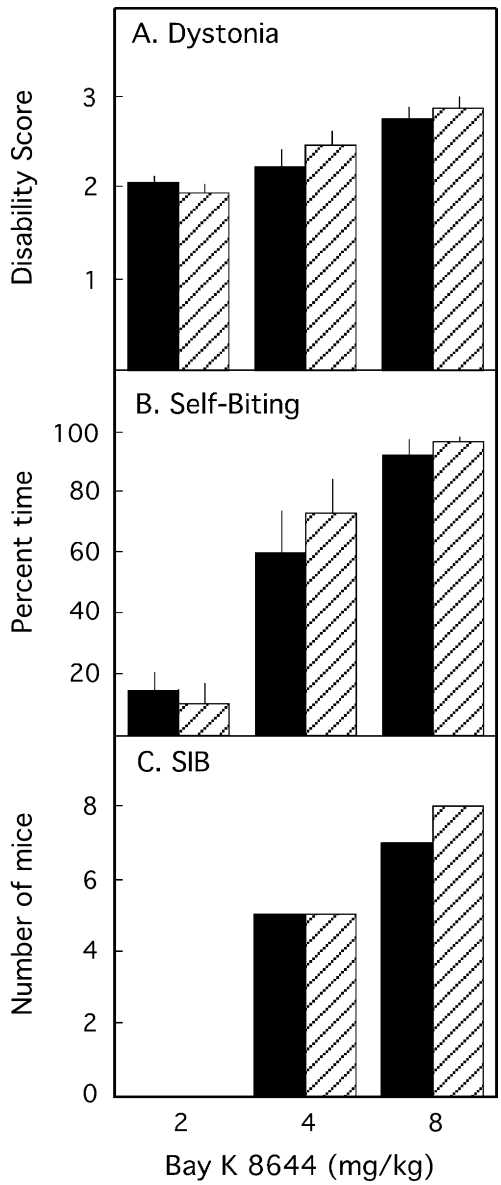

Fig. 1. Behaviors induced by ±Bay K 8644. Results represent average values ±S.E.M. for 10 normal mice (solid bars) and 10 HPRT$^-$ mice (open bars) for motor disability (A), average percentage of time self-biting (B) and number of animals progressing to SIB (C). Data for motor disability and self-biting were analyzed by two-way ANOVA with HPRT status and drug dose as the main variables, and no significant differences were detected between the groups ($P > .10$).

before and after isolation. None of the group-housed HPRT$^+$ or HPRT$^-$ mice displayed SIB. After the same mice were housed individually for 4 weeks, 4/10 HPRT$^+$ and 4/10 HPRT$^-$ mice displayed SIB (Table 3).

3.2. Bay K 8644

The behavioral effects of 2, 4 and 8 mg/kg ±Bay K 8644 were compared in separate groups of 10 HPRT$^+$ and 10 HPRT$^-$ weanling mice, as previously described (Jinnah et al., 1999a,b). In both groups of mice, there were dose-dependent increases in motor disability, self-biting and SIB (Fig. 1). However, normal and mutant mice displayed quantitatively similar responses for all three behaviors.

A second experiment was conducted with additional groups of 10 HPRT$^+$ and 10 HPRT$^-$ weanling mice, using a repeated dosing paradigm where the mice received escalating doses of ±Bay K 8644 separated by 2 days. Once again, there were no significant differences between the normal and mutant mice (results not shown). In a third experiment, 10 HPRT$^+$ and 10 HPRT$^-$ mice were tested at 6–8 weeks of age with the repeated dosing schedule. Again there was no evidence for increased SIB in the mutant mice (results not shown).

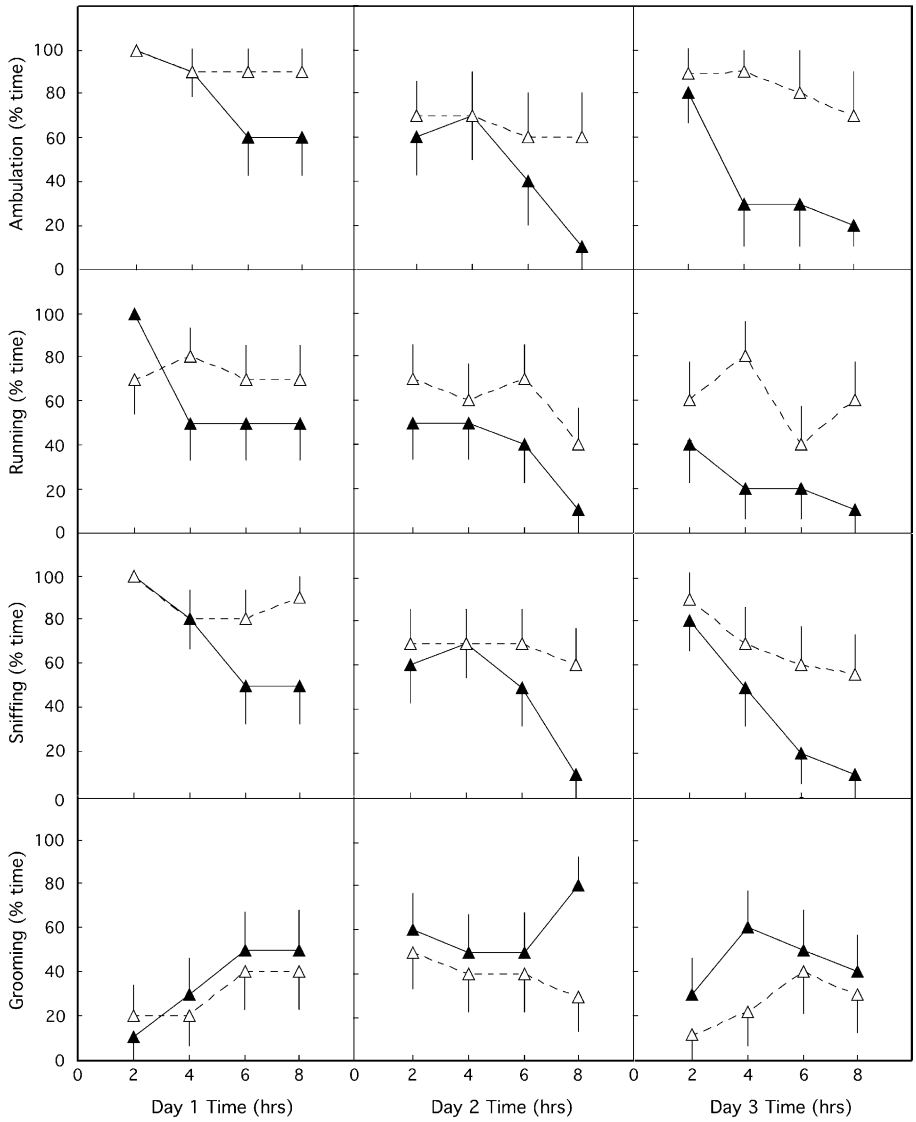

Fig. 2. Profile of stereotypic behaviors induced by GBR 12909. Behaviors were monitored every 2 h for 8 h after the administration of 20 mg/kg GBR 12909 on 3 consecutive days. Results represent average values ±S.E.M. for five normal mice (solid triangles) and five HPRT$^-$ mice (open triangles). Data were analyzed by multivariate ANOVA with HPRT status, treatment day and recording time as the main variables. The overall analysis revealed highly significant effects for all three main variables (P<.01). Statistically significant differences between normal and HPRT$^-$ mice were observed for ambulation (P<.001), running (P<.001), sniffing (P<.001) and grooming (P<.03). There were no significant differences between the two groups of mice for gnawing (P>.2) or rearing (P>.2).

3.3. GBR 12909

To determine if GBR 12909 would cause SIB in normal C57BL/6J mice, preliminary experiments were conducted with separate groups of 10 mice treated at daily intervals for 4 days with 10, 20 and 40 mg/kg, as previously described for rats (Sivam, 1995). All doses caused locomotor hyperactivity and stereotypical behaviors analogous to those reported for rats. However, SIB was never observed, despite using doses twice that reported to be effective in rats (Sivam, 1995).

To determine if $HPRT^+$ and $HPRT^-$ male mice would display different patterns of behavior with GBR 12909, groups of five normal and five mutant mice were treated at daily intervals for 3 days with 20 mg/kg. Normal and mutant mice could be distinguished by quantitative differences in locomotor behavior and certain stereotypic behaviors (Figs. 2 and 3). In comparison with normal mice, $HPRT^-$ mice had lower scores for grooming but higher scores for ambulation, running and sniffing. There was no difference in gnawing or rearing behaviors between $HPRT^+$ and $HPRT^-$ mice, and none of the mice displayed SIB.

3.4. Methamphetamine

Groups of 4 $HPRT^+$ and 4 $HPRT^-$ mice were given repeated doses of 5 mg/kg methamphetamine at 3-h intervals for 12 h, as previously described (Kita et al., 2000; Shishido et al., 2000). Additional groups of four $HPRT^+$ and four $HPRT^-$ mice were treated with repeated doses of 10 or 15 mg/kg methamphetamine. Pronounced locomotor hyperactivity was observed after the first dose of methamphetamine. Subsequent doses produced exaggerated stereotypical behaviors that persisted through the entire observation period. These behaviors included grooming, sniffing, taffy-pulling and gnawing of the bedding material. However, there were no obvious differences between normal and mutant mice, and SIB was not observed in any of the animals (results not shown).

3.5. Pemoline

To determine if pemoline would cause SIB in normal C57BL/6J mice, preliminary experiments were conducted with separate groups of eight males and eight females with doses of 100, 200, 300 or 500 mg/kg. As previously described for rats, progressively increasing doses were associated with hyperactivity, gnawing, sniffing and tongue protrusion (results not shown). There was no SIB at any dose and no difference between male and female mice. At the 500 mg/kg dose, 5/8 male and 5/8 female mice died.

The susceptibility of five normal and five $HPRT^-$ mice was evaluated using a dose of 250 mg/kg. $HPRT^+$ and $HPRT^-$ mice displayed similar stereotypy scores and similar patterns of stereotypical behaviors. There was a slight increase in tongue protrusion in the $HPRT^-$ mice, which was not significant (results not shown). None of the mice displayed SIB.

3.6. Caffeine

In pilot studies, 10 normal C57BL/6J mice were injected with incremental doses of caffeine given twice daily over 4 days to determine the dose required to provoke SIB. Each mouse received a total of 100 mg/kg on Day 1, 150 mg/kg on Day 2, 200 mg/kg on Day 3 and 300 mg/kg on Day 4. The mice became hypoactive at these high doses, without excessive grooming behavior, as described for rats. Unlike previous reports using these doses in rats (Ferrer et al., 1982; Minana and Grisolia, 1986; Mueller et al., 1982; Peters, 1967), none of the mice showed SIB. At the 300-mg/kg dose, all mice died.

The susceptibility of five normal and five $HPRT^-$ mice was evaluated the same way. SIB was again absent, and none survived treatment with 300 mg/kg.

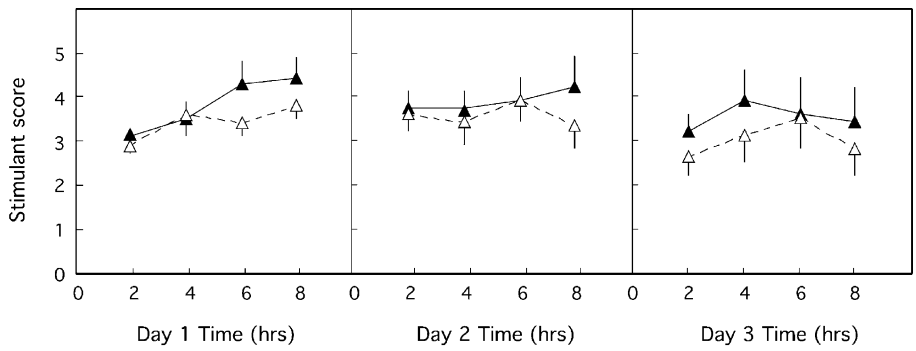

Fig. 3. Stereotypy severity scores induced by GBR 12909. Results represent average values ±S.E.M. for five normal mice (solid triangles) and five $HPRT^-$ mice (open triangles). Data were analyzed by multivariate ANOVA with HPRT status, treatment day and recording time as the main variables. The overall analysis revealed significant effects for HPRT status only ($P<.05$).

4. Discussion

4.1. HPRT$^-$ mouse

The purpose of the current studies was to assess the sensitivity of HPRT$^-$ mice to pharmacologic agents known to be capable of provoking SIB in normal animals. The results provide no evidence that the HPRT$^-$ mice have increased susceptibility to any of the six agents used. Only clonidine and ±Bay K 8644 provoked SIB in normal or HPRT$^-$ mice. Though caffeine, GBR 12909 and pemoline reliably cause SIB in rats, they apparently do not do so in mice, at least with the dosing paradigms employed. However, each of these drugs caused behavioral changes, confirming their neuropharmacologic activity. These results point to important species differences in the neuropharmacology of SIB.

Despite the absence of an increased susceptibility to SIB, the results confirm that HPRT$^-$ mice are not behaviorally normal. Their stereotypical behaviors in response to GBR 12909 were quantitatively higher than those observed in normal mice. This phenomenon is consistent with prior studies demonstrating that the HPRT$^-$ mice display enhanced behavioral sensitivity to drugs that promote dopamine release such as amphetamine, methylphenidate and amfonelic acid (Jinnah et al., 1991, 1992). On the other hand, other studies have shown HPRT$^-$ mice to have significantly reduced striatal dopamine levels (Finger et al., 1988; Jinnah et al., 1994, 1999a,b). The mechanisms responsible for the low dopamine levels and the paradoxical enhanced behavioral sensitivity to agents promoting dopamine release in the HPRT$^-$ mice remain unknown. The paradox is not the result of dopamine receptor supersensitivity, since the HPRT$^-$ mice have normal behavioral responses to direct dopamine receptor agonists (Jinnah et al., 1992).

4.2. Clonidine

At doses of 5–20 mg/kg, clonidine typically causes aggressive behavior in normal mice (De Feo et al., 1983; Morpurgo, 1968; Nikulina and Klimek, 1993; Ushijima et al., 1984). At 20–100 mg/kg, it causes the mice to gnaw compulsively on any available objects, such as food pellets, bedding or cage bars. If there is nothing to bite, the animals bite their own paws (Bhattacharya et al., 1988; Katsuragi et al., 1984; Razzak et al., 1975, 1977). SIB typically emerges within 10–30 min of drug administration and lasts less than 2 h. In this model, SIB is increased after fasting, isolation housing and male sex (Katsuragi et al., 1984; Razzak et al., 1975).

Our studies confirm that high dose of clonidine can provoke SIB in mice, particularly male mice after isolation. However, the results provide no evidence that HPRT$^-$ mutants are more susceptible to this phenomenon.

The pharmacological properties of clonidine are complex, and the mechanisms by which it provokes SIB remain unclear. It is most commonly used as a $\alpha 2$ adrenergic receptor agonist, though it also binds to adenosine and imidazoline receptors (Egelen et al., 1998; Kulkarni and Mehta, 1984; Stone and Taylor, 1978). Clonidine-induced SIB does not appear to be mediated by activation of adrenergic receptors, since adrenergic receptor antagonists do not consistently block SIB (Katsuragi et al., 1984; Razzak et al., 1977). Several investigators have proposed the involvement of dopaminergic (Bhattacharya et al., 1988), purinergic (Katsuragi et al., 1984; Razzak et al., 1977) or serotonergic mechanisms (Bhattacharya et al., 1988).

4.3. Bay K 8644

±Bay K 8644 causes SIB in mice in a dose and age dependent manner (Jinnah et al., 1999a,b). Low doses typically cause self-biting without injury while higher doses cause persistent self-biting leading to tissue injury. This behavior is much worse in younger weanling mice compared to older adults. The mice typically bite their forepaws, shoulders or abdomen. The biting begins 10–20 min after injection and lasts 60–90 min. ±Bay K 8644 does not cause exaggerated stereotypy, but is associated with severe motor dysfunction best characterized as generalized dystonia (Jinnah et al., 2000).

±Bay K 8644 caused severe dystonia, self-biting and SIB in both HPRT$^+$ and HPRT$^-$ mice, but there was no evidence to suggest that any of these behaviors were more frequent or severe in the HPRT$^-$ mice.

±Bay K 8644 functions as an L-type calcium channel activator that increases calcium channel fluxes in response to depolarizing stimuli (Triggle and Rampe, 1988). The SIB provoked by ±Bay K 8644 appears to be caused by activation of these channels since pretreating mice with L-type calcium channel blockers can block it (Jinnah et al., 1999a,b).

4.4. GBR 12909

In rats, acute administration of 2–20 mg/kg GBR 12909 causes locomotor hyperactivity and stereotypical behaviors (Heikkila and Manzino, 1984), while repeated daily administration of 20 mg/kg over 2–4 days causes the emergence of SIB, which consists of gnawing or licking the trunk, paws or tail (Sivam, 1995). Acute administration of GBR 12909 causes hyperactivity and stereotypy in mice, just as it does in rats. However, chronic administration of high doses of GBR 12909 to normal and HPRT$^-$ mice did not cause SIB. Though there was no SIB, HPRT$^-$ mice showed altered stereotypical behaviors. These results are consistent with prior studies showing that dopaminergic agents produce quantitatively different behavioral responses in normal and HPRT$^-$ mice (Jinnah et al., 1991, 1992), but the results provide no evidence that HPRT$^-$ are more susceptible to the ability of GBR 12909 to provoke SIB.

GBR 12909 acts as a reuptake inhibitor of dopamine at the presynaptic dopaminergic sites, leading to increased availability of dopamine at postsynaptic dopamine receptors (Heikkila and Manzino, 1984). SIB is therefore thought to result from overstimulation of dopamine receptors (Sivam, 1995).

4.5. Methamphetamine

Amphetamine and methamphetamine typically cause locomotor hyperactivity at low doses and exaggerated stereotypical behavior with higher doses in rodents. Very high doses provoke SIB in both rodents and nonhuman primates (Ellison and Eison, 1983; Hohn and Lasagna, 1960; Shishido et al., 2000), usually biting of the paws or abdomen. Amphetamine has even been reported to cause self-injurious nail and finger biting in susceptible children (Sokol et al., 1991). SIB provoked by these psychostimulants does not require extreme doses, but can also be seen when lower doses are given chronically by constant infusion (Huberman et al., 1977; Mueller and Nyhan, 1983; Mueller et al., 1982) or by repeated dosing (Brien et al., 1977; Kita et al., 2000; Lara-Lemus et al., 1997). SIB also occurs when the drugs are microinjected directly into the striatum of rats (Dickson et al., 1994; Kelley et al., 1989; Kelley et al., 1988).

Although a prior study documented SIB with high doses of amphetamine in normal BALB/c mice (Hohn and Lasagna, 1960), SIB was not observed in normal or HPRT$^-$ mice on a C57BL/6J or 129/J background in a previous study using doses up to 32 mg/kg (Jinnah et al., 1991). Repeated administration of lower doses of amphetamine or methamphetamine has also been reported to cause SIB in BALB/c mice (Brien et al., 1977; Kita et al., 2000; Shishido et al., 2000), but was not observed in the present study with C57BL/6J mice. These results suggest important mouse strain differences in the susceptibility to psychostimulant-induced SIB.

4.6. Pemoline

Pemoline has behavioral effects similar to amphetamine or methamphetamine, except for a much longer duration of action (McMillen, 1983). High doses of pemoline also cause SIB in rats (Cromwell et al., 1999; King et al., 1995, 1998; Mueller et al., 1986; Mueller and Hsiao, 1980; Mueller and Nyhan, 1982; Turner et al., 1999) and a single report described SIB in Swiss albino mice (Genovese et al., 1969). It consists of gnawing of the digits, paws, thorax, abdomen or root of the tail. The self-biting is accompanied by hyperactivity and stereotypical behaviors such as gnawing, sniffing and tongue protrusion (Mueller and Nyhan, 1982). The HPRT$^+$ and HPRT$^-$ C57BL/6J mice used in these experiments did not exhibit SIB at any dose. These results again suggest important mouse strain difference in susceptibility to psychostimulant induced SIB.

The mechanism by which amphetamine, methamphetamine or pemoline causes SIB appears to be due to the ability of these drugs to release dopamine from the brain (Jinnah et al., 1990).

4.7. Methylxanthines

Low doses of caffeine stimulate motor activity while high doses cause hypoactivity. In rats, daily administration of 100–200 mg/kg caffeine causes an initial phase of motor hyperactivity with stereotypical behaviors followed by hypoactivity (Mueller et al., 1982). Self-biting of the paws and root of the tail occurs in 10–80% of animals within a few days. The effect is not limited to caffeine but can also be seen with other methylxanthines such as theophylline (Morgan et al., 1970; Sakata and Fuchimoto, 1973) or aminophylline (Lloyd and Stone, 1981). The expression of SIB is influenced by nutritional status and strain of rat used, and the mortality rate is significant (Lloyd and Stone, 1981; Mueller and Nyhan, 1983; Peters, 1967; Sakata and Fuchimoto, 1973). The majority of studies have employed rats, though one study employed rabbits (Morgan et al., 1970). There are no published reports on methylxanthine-induced SIB in mice. Our studies suggest caffeine may not have the same influence in mice, or at least the C57BL/6J strain.

The mechanism of methylxanthine-induced SIB remains unclear. These drugs have multiple effects in the brain, including antagonism of adenosine receptors, inhibition of phosphodiesterase, and mobilization of intracellular calcium stores (Daly et al., 1981; Krizaj et al., 1999). Low doses are thought to be selective for adenosine receptors, but the doses required to provoke SIB are likely to influence other processes.

4.8. Summary

Our comparison reveals a number of important differences among the pharmacologic models for SIB. From a purely technical perspective, clonidine and ±Bay K 8644 are the simplest models to evaluate behaviorally because responses occur within 1–2 h. In addition, exaggerated grooming and licking responses are not prominent after treatment with clonidine or ±Bay K 8644 but are seen with GBR 12909, amphetamine, methamphetamine and pemoline. Some have argued that SIB in response to psychostimulants is not a specific behavioral response, but rather an indirect consequence of over-grooming (Braun and Chase, 1986; Hartgraves and Randall, 1986). The absence of significant stereotypy with clonidine or GBR 12909 facilitates the discrimination of SIB from similar repetitive behaviors.

Though clonidine and ±Bay K 8644 do not cause exaggerated stereotypies, both drugs cause other abnormal behaviors. The doses of clonidine required to provoke SIB are associated with severe tremor and prostration, both of which may interfere with the expression of SIB. In addition,

Table 4
Animal models for SIB

Model	Neurochemical mediator	Exaggerated stereotypy	Aggression	Other behaviors	Dosing	Species tested
Clonidine	unknown	no	yes	tremor	acute	mouse
Methylxanthine	unknown	transient	no	hyperactivity, tremor	chronic	rat or rabbit
Pemoline	dopamine	yes	no	hyperactivity	chronic	mouse or rat
Methamphetamine	dopamine	yes	yes	hyperactivity	chronic	mouse, rat or primate
GBR-12909	dopamine	yes	no	hyperactivity	chronic	rat
6-Hydroxydopamine	dopamine	yes	yes	hyperactivity	delayed	rat
±Bay K 8644	calcium channels	no	yes	dystonia	acute	mouse or rat

these doses are near lethal in mice. ±Bay K 8644 causes severe dystonia, which can also interfere with the expression of SIB. Therefore, any studies involving SIB provoked by clonidine or ±Bay K 8644 must account for these potentially confounding behaviors.

Another important difference among the pharmacologic models is drug specificity (Table 4). The pharmacologic mechanisms responsible for caffeine and clonidine-induced SIB are unknown, because the high doses required likely affect many neurochemical systems. GBR 12909, amphetamine, methamphetamine and pemoline are thought to involve dopamine systems, but the requirement for chronic delivery or very high doses is likely to directly or indirectly influence other neurochemical pathways. ±Bay K 8644 is a calcium channel activator, and the neurochemical systems influenced by it remain to be determined.

Finally, it is important to consider the relevance of these models to their human counterparts. The HPRT$^-$ mouse provides a true genetic model for Lesch–Nyhan disease by virtue of mutations in the same gene. It has proved to be a valuable genetic, biochemical and neurochemical model for the disease (Jinnah and Breese, 1997). It has not provided a useful behavioral model, since it does not display any analogous neurobehavioral abnormalities. The absence of behavioral correlates is not unique to the HPRT$^-$ mouse model of Lesch–Nyhan disease, as there are now a large number of transgenic and knockout mouse models that lack behavioral correlates to their human counterparts (Erickson, 1989). The pharmacologic models, in contrast, reproduce a key behavioral element of Lesch–Nyhan disease, SIB. However, they have no direct influence on the HPRT$^-$ gene, the gene product or relevant biochemical pathways. These models are therefore more accurately considered tools to study the behavioral phenomenon of SIB rather than specific models for Lesch–Nyhan disease. While the pharmacologic and genetic models provide incomplete reproductions of Lesch–Nyhan disease as a whole, they are nonetheless valuable for studying specific aspects of the disease (Jinnah and Breese, 1997).

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