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Dopamine receptor modulation of repetitive grooming actions in the rat: potential relevance for Tourette syndrome

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Abstract

Studies of rodent grooming can provide valuable insight for dopamine contributions to the initiation, organization, and repetition of motor patterns. This information is useful for understanding how brain dysfunctions contribute to movement disorders such as Tourette syndrome and obsessive compulsive disorder, in which patients are driven to reiterate particular movement patterns. In rodents, dopamine D1 receptor stimulation causes a complex behavioral super-stereotypy in the form of excessive production and rigid execution of whole sequences of movements known as syntactic grooming chains. Sequential super-stereotypy of grooming chains may be particularly advantageous for modeling movement sequences and treatments in Tourette syndrome and related disorders. Here, we report that co-administration of haloperidol, one available treatment for Tourette syndrome and primarily a D2 receptor antagonist, prevented D1 stimulation with SKF38393 from inducing sequential super-stereotypy, which manifests as an exaggeration of the tendency to complete all four phases of a syntactic chain in rigid serial order once the first phase has begun. In a separate experiment, we showed that in contrast to acute D1 agonist administration, 39 hour withdrawal from chronic (3 weeks) administration of the D1 antagonist SCH23390 (which has been suggested to increase D1 receptor expression in the basal ganglia) did not elicit sequential super-stereotypy after drug cessation. Instead, rats suddenly removed from repeated SCH23390 spent more time performing simple stereotypies that included intense scratching and biting behaviors. Together, these results have implications for understanding how dopamine receptors facilitate particular stereotypies manifest in animal models of Tourette syndrome and obsessive compulsive disorder.

Keywords

Dopamine D1 receptor; Dopamine D2 receptor; Tourette syndrome; Stereotypy; Grooming; Haloperidol

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Introduction

Dysfunction of the basal ganglia involving alteration in dopamine neurotransmission is proposed to contribute to a range of movement disorders (Albin et al., 1989;Albin, 2006). Patients with Parkinson's disease, which is caused by destruction of nigrostriatal dopamine neurons, show deficiencies in performing movement sequences (Agostino et al., 1992;Benecke et al., 1987;Harrington et al., 1991). In contrast, patients with Tourette syndrome experience repetitive undesired movements interjected into ongoing behavior known as tics (Berardelli et al., 2003). Simple tics are “repetitive stereotyped jerks” while complex motor tics “consist of a wide variety of muscle jerks and contractions in different muscle groups organized in sequence and coordinated movements resembling normal motor gestures” (Berardelli et al., 2003). The efficacy of anti-dopaminergic agents such as haloperidol in treating Tourette symptoms, along with other clinical and basic science findings, have contributed to the concept that abnormal dopamine signaling and aberrations in basal ganglia processing are important factors contributing to the pathophysiology of Tourette syndrome (Albin et al., 2006;Frey et al., 2006;Jimenez-Jimenez et al., 2001;Mink, 2006;Segawa, 2003;Singer et al., 2002).

The biological basis of Tourette syndrome is thought to overlap that of obsessive compulsive disorder (OCD), a condition which is characterized by intrusive thoughts (obsessions) and urges to repeat rigid behavioral patterns (compulsions) (Goodman et al., 2006). Although the serotonin system is most often implicated in OCD, the dopamine system may be disrupted as well (Kim et al., 2003). Dopamine antagonists may need to be added to the treatment regimen for some OCD patients, especially when OCD is co-morbid with Tourette syndrome (McDougle et al., 1994). As dopamine and basal ganglia dysfunction likely contribute to Tourette syndrome and OCD, it is reasonable to use rodent models to elucidate whether basic dopamine receptor actions, such as interactions between receptors and changes in receptor number, can modulate abnormal repetition of specific movements or movement patterns. Grooming sequences and simple stereotypies are among the motor behaviors that can be used in animals to answer such queries.

Grooming involves an innate set of movements used by many mammalian species to care for the body (Berridge, 1990;Richmond et al., 1978;Young et al., 1991). During grooming bouts, rodents perform facial strokes, lick and scratch the body, and gnaw at the extremities (Bolles, 1960;Richmond et al., 1978). These movements are usually executed in a fairly flexible arrangement, but on regular occasions rats perform a series of grooming movements in a highly predictable order (13,000 times greater than chance)(Berridge et al., 1987). This rigid sequential pattern is known as a “syntactic grooming chain” (Berridge et al., 1987). The basal ganglia are important for the regulation of syntactic grooming chains(Aldridge, 2005). For example, an intact striatum is necessary for the correct implementation of grooming chains, as lesions of the dorsolateral striatum impair the completion of syntactic grooming chains (Berridge, 1989a;Berridge et al., 1992;Cromwell et al., 1996). Extracellular recordings of neural activity in the same region of dorsolateral striatum in rats have demonstrated that neurons in those regions code the entire grooming sequence pattern as a whole, especially firing in terminal phases. Those neurons also discriminate between the sequential pattern and those same grooming movements produced in different orders outside of the syntactic chain (Aldridge et al., 1993;Aldridge et al., 1998;Meyer-Luehmann et al., 2002). By comparison, neural activity in the pars reticulata region of the substantia nigra appears to code the initiation of the pattern, responding especially to the onset of chains (Meyer-Luehmann et al., 2002). This suggests that the basal ganglia play coordinated roles both in the initiation and organization of sequential patterns, rather than in just the elementary component movements within a pattern.

In rodents, dopamine is a crucial neurotransmitter for implementation of the sequential grooming pattern (Berridge, 1989b). Rodent studies have revealed that 6-hydroxydopamine lesions of dopaminergic neurons impair the correct completion of grooming chain patterns, similar to neostriatal lesions (Berridge, 1989b). In contrast, excessive dopamine neurotransmission caused by knockdown of the dopamine transporter expression in transgenic mice triggers sequential super-stereotypy in the form of exceedingly rigid execution of syntactic grooming chains (Berridge et al., 2005). There seems to be an especially important function for the dopamine D1 receptor in this dopamine contribution to grooming chain implementation in rodents. First, mutant mice lacking D1A receptors are less likely to complete grooming chains than normal mice (Cromwell et al., 1998). In contrast, peripheral or central administration of D1 receptor-specific agonists increase grooming chain initiation and the probability that grooming chains will be completed (ie, “sequential super-stereotypy”), whereas a D2 agonist does not (Berridge et al., 2000a;Berridge et al., 2000b). The apparently excessive repetition of grooming sequences observed in the D1 agonist-treated rat has been speculated to be a potential model for complex tics observed in Tourette syndrome and for OCD (Albin et al., 2006).

Dopamine and its receptors are clearly implicated in super-stereotypy, but some of the details of that involvement and how they may lend insights into Tourette syndrome and OCD remain unclear. The current set of studies was undertaken to further investigate the idea that sequential super-stereotypy is induced by excessive activation of dopamine D1 receptors, and that it could be modulated by co-treatment with a D2 receptor antagonist. In doing so, we aim to better understand how dopamine influences the extent to which organized movements are carried out and reiterated. This is crucial for developing hypotheses about the brain pathologies that lead to disorders involving compulsive movements and for developing animal models of those aberrant behaviors.

For the first experiment, we were interested in clarifying the potential remaining role of endogenous stimulation of dopamine D2 receptors in sequential super-stereotypy. That is, normal co-activation levels of D2 receptors by endogenous dopamine might be important in supporting the production of super-stereotypy by D1 receptor over-stimulation, even if D2 over-stimulation by a D2 agonist is not sufficient by itself to cause the stereotypy (Berridge et al., 2000a). This role for endogenous D2 receptor participation is suggested by the clinical efficacy of neuroleptics in reducing tics and related symptoms in human Tourette patients (Jimenez-Jimenez et al., 2001). Since the mechanism of action for neuroleptics is likely in part due to D2 receptor blockade, examination of this issue would provide insight into potential mechanisms underlying the motor tics of Tourette syndrome and its treatment (Arnt et al., 1998;Robertson et al., 2000). Such information could also be advantageous in assessing whether the rodent syntactic chain pattern of repetitive movement sequences is truly relevant to modeling sequential tics or stereotypies observed in OCD. The initiation and completion of grooming sequences was compared for conditions in which the rats were given only the D1 agonist SKF38393 to elicit super-stereotypy versus the D1 agonist plus one of several different doses of the primarily D2 antagonist haloperidol. Control conditions in which the rats were given only vehicle injections or only haloperidol were also included. We confirmed the hypothesis that D2 receptors facilitate D1-induction of overly rigid behavioral sequences by demonstrating that haloperidol pre-treatment prevented sequential super-stereotypy. Since haloperidol also reduces tics in Tourette syndrome, this also provides support for incorporating super-stereotypy into modeling complex tics and compulsions.

Another issue regarding Tourette syndrome and its models is the possibility that long-term changes in receptor number and/or sensitivity may lead to motor dysfunction (Segawa, 2003), so may also be used in chronic treatment animal models to mimic the induction of

super-stereotypy by acute administration of a direct agonist. A drug regimen of the chronic D1 antagonist SCH23390 followed by a wash-out period has been suggested to simultaneously increase D1 receptor expression and D1 agonist-induced grooming (Creese et al., 1985;Hess et al., 1986;Parashos et al., 1990;Schwartz et al., 2003). If the over-expression of D1 receptors after cessation of D1 antagonist is equivalent to the over-stimulation of the same receptors by an acute D1 agonist, then chronic SCH23390 might also be expected to facilitate super-stereotypy. On the other hand, that prediction would not be true if there are important differences between the two treatments. We report that cessation of D1 receptor blockade did not lead to super-stereotypy, but instead elicited only a simple motor stereotypy. Simple stereotypy is the same single movement is repeated again and again, and here was manifest as repetitive scratching and biting movements after cessation of chronic SCH23390 administration. Simple stereotypies, as well as sequential super-stereotypies that model more complex repetitive behavior, may both be relevant for animal investigations of tics and compulsions (Korff et al., 2008;Swerdlow et al., 2006). Thus, our results indicate that different dopaminergic mechanisms may contribute to simple stereotypies versus sequential super-stereotypy, suggesting that it is useful to investigate both simple and sequential stereotypies when modeling Tourette syndrome and OCD.

Results

Haloperidol modulation of sequential super-stereotypy induced by D1 agonist administration

Total grooming—To determine haloperidol and SKF38393 effects upon the general quantity of grooming behavior, we estimated the total time spent grooming by the rats, which included all types of grooming: syntactic chains, non-chain grooming, and scratching/nail biting. In line with previous studies, D1 receptor stimulation with SKF38393 more than doubled the amount of time spent grooming (Fig 1A; main effect of drug treatment: $F=20.356$, $p<0.05$; vehicle + SKF vs. vehicle + vehicle, $p<0.05$). When haloperidol was administered prior to SKF38393, the middle and highest doses (0.1 and 1.0 mg/kg) of haloperidol prevented SKF38393 from inducing an increase in grooming duration (haloperidol (0.1 or 1.0 mg/kg) + SKF vs. vehicle + SKF, $p<0.05$). The combination of the 1.0 mg/kg dose of haloperidol with SKF38393 lowered total grooming to below baseline levels (haloperidol (1.0 mg/kg) + SKF vs. vehicle + vehicle, $p<0.05$). When administered alone prior to vehicle (in the absence of SKF38393), this highest dose of haloperidol was the only dose to significantly reduce grooming time compared to baseline (haloperidol (1.0 mg/kg) + vehicle vs. vehicle + vehicle, $p<0.05$). Thus, both normal levels of grooming (sequentially flexible + rigid syntactic chains), and enhanced levels caused by D1 receptor stimulation, depend upon endogenous D2 receptor stimulation.

Syntactic grooming chain initiation—To identify the effects of haloperidol on the quantity of grooming chains produced by rats, with or without co-administration of SKF38393, we tallied the number of grooming chains produced by rats in vehicle baseline and drug conditions. The absolute number of grooming chains initiated by the rats rose after administration of the D1 agonist SKF38393 (Fig 1B; main effect of drug treatment: $F=71.646$, $p<0.05$; vehicle + SKF vs. vehicle + vehicle, $p<0.05$). Pre-treatment with the middle dose of haloperidol blunted the stimulatory effect of SKF38393 on grooming chain number by approximately 50% (haloperidol (0.1 mg/kg) + SKF vs. vehicle + SKF, $p<0.05$), and pre-treatment with the highest haloperidol dose completely blocked the SKF38393 increase (haloperidol (1.0 mg/kg) + SKF vs. vehicle + SKF, haloperidol (1.0 mg/kg) + SKF vs. vehicle + vehicle, $p<0.05$). None of the haloperidol + vehicle conditions differed significantly from the baseline vehicle + vehicle condition. These data indicate that

haloperidol not only diminishes total time spent grooming, but also blocks the number of syntactic grooming chains initiated from being increased by treatment with SKF 38393.

The absolute number of grooming chains initiated was divided by the total amount of time spent grooming in order to determine whether syntactic grooming chains were more or less likely to be initiated relative to the total amount of grooming. Intriguingly, co-administration of haloperidol at the highest dose tested (1.0 mg/kg) with SKF38393 nearly tripled the number of grooming chains initiated per minute of grooming compared to SKF38393 alone (Fig 1C; main effect of drug treatment: $F=20.22$, $p<0.05$; haloperidol (1.0 mg/kg) + SKF vs. vehicle + SKF, $p<0.05$). Rats displayed a relatively higher likelihood to initiate grooming chains when given SKF38393 combined with either 0.1 or 1.0 mg/kg haloperidol pre-treatments compared to when the same doses of haloperidol were administered in combination with vehicle or when compared to the vehicle + vehicle baseline (haloperidol (0.1 mg/kg) + SKF vs. haloperidol (0.1 mg/kg) + vehicle or vs. vehicle + vehicle, $p<0.05$; haloperidol (1.0 mg/kg) + SKF vs. haloperidol (1.0 mg/kg) + vehicle or vs. vehicle + vehicle, $p<0.05$). SKF38393 alone did not significantly affect the relative rate of syntactic grooming chain initiation compared to vehicle + vehicle baseline (vehicle + SKF vs. vehicle + vehicle, NS). Thus, dopamine D1 stimulation combined with D2 blockade may be especially effective at promoting the initiation of sequentially-stereotyped sequences of grooming, even though D2 blockade tended to reduce the amount of grooming and number of grooming chains.

Syntactic grooming chain completion—D1 receptor activation also increased the probability that grooming chains would be completed syntactically through terminal phases, once they were begun (Fig 1D; Main effect of drug treatment: $F=5.757$, $p < 0.05$; vehicle + SKF vs. vehicle + vehicle, $p<0.05$), thus confirming previous reports that D1 stimulation induces sequential super-stereotypy. The percentage of grooming chains that were completed syntactically from Phase 1 to Phase 4 rose from 46% during baseline to 77% after SKF38393. Pre-treatment with the 0.01 and 0.1 mg/kg doses of haloperidol did not alter the effects of SKF38393 on chain completion. The highest dose of haloperidol completely blocked the sequential-super-stereotypy effect of SKF38393, preventing syntactic chains from becoming more likely to be completed through Phase IV (haloperidol (1.0 mg/kg) + SKF vs. vehicle + SKF, $p<0.05$), and returning grooming chain completion to baseline levels (haloperidol (1.0 mg/kg) + SKF vs. vehicle + vehicle, NS). This is a central finding-that D1 agonist-induced super-stereotypy is reduced by D2 receptor blockade, and is in concordance with the use of haloperidol as a treatment for Tourette syndrome.

We also examined the effects of haloperidol on grooming chain completion in the absence of SKF38393. Some rats in the haloperidol + vehicle conditions did not initiate any grooming chains (1 of 7 rats for 0.1 mg/kg haloperidol + vehicle, and 4 of 8 rats for 1.0 mg/kg haloperidol + vehicle), and thus were not included in the computation of chain completion. Even so, haloperidol alone significantly reduced grooming chain completion (haloperidol (1.0 mg/kg) + vehicle vs. vehicle + vehicle, $p<0.05$). Only half of the rats even initiated a single grooming chain after the highest dose of haloperidol alone, and none of the rats completed that one grooming chain with this treatment. In every case of incomplete grooming chains, chest licking took the place of phase 4 flank licking. In 3 of the 4 cases, rats also skipped phase 3 bilateral strokes. Thus, endogenous D2 receptor stimulation seems to be necessary for grooming chain organization in the normal brain.

A separate experiment examined whether effects similar to acute D1 stimulation could be produced by the sudden cessation of chronic D1 blockade: Chronic dopamine D1 blockade followed by a drug-free period may induce a transient up-regulation of D1 receptors (Creese et al., 1985;Hess et al., 1986;Parashos et al., 1990;Schwartz et al., 2003).

Cessation of a D1 antagonist might therefore conceivably produce an excessive D1 signal with effects that overlap with D1 stimulation, even though there are many differences between the two manipulations. This experiment assessed the overlap and differences in effects by measuring changes in grooming behavior produced by release from chronic D1 blockade.

Total grooming—Rats that had been treated chronically with SCH23390 groomed more than the control, chronic vehicle-treated rats during the vehicle challenge test (Fig. 2A; $t = -2.437$, $p<0.05$). This increase was anticipated to occur based on previous reports of D1 receptor up-regulation following cessation of SCH23390 that corresponds increased D1 agonist-induced grooming (Creese et al., 1985;Hess et al., 1986;Parashos et al., 1990;Schwartz et al., 2003).

Syntactic grooming chains—Cessation of three weeks of chronic SCH23390 administration, followed by a 39 hour drug-free period [intended to up-regulate D1 receptors (Creese et al., 1985;Hess et al., 1986;Parashos et al., 1990;Schwartz et al., 2003)], did not alter the initiation of grooming chains in rats. During the vehicle challenge, there was no difference in the absolute number of grooming chains between the rats that had been given daily SCH23390 and those that had been given daily vehicle (Fig 2B; $t = -1.019$, NS). The level of grooming chain initiation relative to the total amount of grooming also was unaltered by chronic SCH23390 (Fig 2C; $t = -2.0129$, NS). Finally, withdrawal from chronic SCH23390 treatment also did not significantly alter grooming chain completion (Fig 2D; $t = 1.098$, NS). These results show that cessation from SCH23390 does not elicit sequential super-stereotypy, nor does it have any effect on other measures of grooming chains.

Scratching and nail, tail, or body biting—Withdrawal from previous exposure to chronic SCH23390 more than doubled the amount of scratching and biting that occurred during the vehicle challenge, compared to chronic vehicle treatment (Fig 2E; $t = -3.024$, $p<0.05$). These simple motor stereotypies were simple repetitions of individual movements, rather than changes in more complex syntactic grooming chains. The simple motor stereotypies accounted for most of the increase in total grooming time caused by cessation from chronic exposure to the D1 antagonist. Thus, it is possible that simple stereotypies are modulated by elevations in D1 receptor density whereas sequential super-stereotypy is more likely related to excess stimulation of normal numbers of D1 receptors.

Discussion

The first results from these experiments show that sequential super-stereotypy was prevented by co-administration of a D2 antagonist. This supports the notion that endogenous D2 receptor activation indeed influences sequential super-stereotypy (an elevated tendency to complete an entire rigid sequence of actions) induced by a D1 agonist, as measured via this complex serial pattern (i.e., enhanced syntactic grooming chain completion) (Figure 1D). These data support the hypothesis that the D2 class of dopamine receptors at endogenous levels of activation could contribute to D1 agonist-stimulation of sequential super-stereotypy of an instinctive pattern of behavior. Consistent with previous reports, the D1 agonist SKF38393 by itself increased grooming time and the absolute number of syntactic grooming chains (Figures 1A, B). Haloperidol abolished both of these effects of SKF38393, suggesting that haloperidol possesses a general ability to suppress grooming (Figures 1A, B). D1 stimulation with SKF38393 also increased the likelihood that once started a syntactic chain would be completed all the way to its terminal phase (that is, the D1 agonist caused super-stereotypy when administered alone) (Figure 1D). As predicted, co-administration of haloperidol completely blocked the sequential super-stereotypy elicited by SKF38393, as well as prevented syntactic chain completion in the absence of D1 stimulation

(Figure 1D), which indicates that endogenous D2 receptor activation may be needed for sequential movement organization to be implemented.

We also report that sudden cessation of chronic D1 blockade, which may allow the expression of a potentiated D1 signal generated by up-regulated D1 receptors, produced a simple motor stereotypy in the form of repeated scratching and biting. This regimen (ie, withdrawal from SCH23390 followed by a 39-hour drug-free period for the purpose of increasing D1 receptor expression) caused rats to spend more than double the amount of time spent by control rats scratching the body with hind paws and performing biting actions directed towards the nails, tail, or body. Some evidence exists that scratching elicited by peptides is dependent on D1 receptor stimulation (Van Wimersma Greidanus et al., 1989). This is distinct from sequential “super-stereotypy”, or tendency to organize multiple movements into complex yet rigid serial patterns such as syntactic grooming chains (Berridge et al., 2000a;Berridge et al., 2000b). As a caveat, we note that although we did not analyze dopamine receptor expression in the current study, increases in D1 receptor expression and grooming have been reported to occur in the striatum and/or substantia nigra pars reticulata using similar pharmacological paradigms (Creese et al., 1985;Hess et al., 1986;Parashos et al., 1990;Schwartz et al., 2003). Thus, release of D1 receptors from chronic blockade may be sufficient to generate simple motor stereotypies, but sequential super-stereotypy requires D1 receptor activation of the type provided by an actual agonist such as SKF38393.

The control of sequential super-stereotypy by interactions between D1 and D2 receptors involved some unexpected complexities. D2 receptors are often thought to provide a permissive or facilitating role for D1-mediated behaviors (Molloy et al., 1986;Waddington et al., 1995a). For example, haloperidol has been reported to reduce grooming elicited by SKF38393 in the current study and in a previous report by Molloy and colleagues (Molloy et al., 1987). In many instances D2 activation may foster D1-driven behaviors, but occasionally tonic D2 stimulation is reported to suppress D1-actions (Rosengarten et al., 1986;Waddington et al., 1995a). Haloperidol combined with SKF38393, but not either alone, fostered an increase in the chain initiation rate (Figure 1C). That is, blockade of D2 receptors enhanced the likelihood that D1 stimulation would increase the relative rate of syntactic grooming chain initiation. In other words, endogenous D2 receptor stimulation may dampen the shift to initiate syntactic grooming that is produced by D1 stimulation (Berridge et al., 2000a). The opposite effects of the haloperidol + SKF39393 treatment on syntactic grooming chain initiation rate and completion supports the idea that the brain mechanisms needed to begin a series of organized movements may be differentially regulated from those needed to syntactically arrange those movements into a finished pattern.

Haloperidol is a neuroleptic that predominately blocks dopamine D2 receptors, and possesses some affinity for dopamine D1, serotonin 2A, and α2-adrenergic receptors, among others (Arnt et al., 1998). The most plausible interpretation of our current results therefore is that blockade of D2 receptors played the predominant role in preventing super-stereotypy, though further work would be useful to confirm the receptor specificity of this interpretation. If so, it is not surprising that D2 receptors could have a role in movement sequencing, given their high expression in the striatum, suspected aberrant functioning in motor disorders, and known ability to regulate other D1-mediated behaviors (Parashos et al., 1990;Strange, 1993;Wachtel et al., 1992;Waddington et al., 1995b).

It should be noted that the highest dose (1.0 mg/kg) of haloperidol significantly reduced both grooming time and chain completion when administered in the absence of SKF38393 (Figure 1A,D). At high doses, haloperidol has been reported to induce catalepsy in rats and

parkinsonism and sedation in patients (Janssen, 1965;Robertson et al., 2000). Thus, particularly with the highest dose, we cannot determine from this study whether haloperidol specifically blocked the effects of D1 stimulation or if the haloperidol effect was due to a more general suppression of movement. The middle dose of haloperidol (0.1 mg/kg) diminished the stimulatory effect of SKF38393 on grooming time and number of grooming chains without significantly reducing those grooming measures when administered alone (Figure 1A,D). Also, at this dose the animals did not appear akinetic. This could suggest that the suppressive effects of the middle dose of haloperidol on grooming sequences were not necessarily a consequence of generalized movement inhibition, but was likely related to a decrease in all forms of grooming.

A defining characteristic of Tourette syndrome is the production of diverse stereotypies: motor tics (both simple or complex) involving single or repetitive movements, as well as some more complex sequentially-organized stereotypies, such as those involving vocalization (Mink, 2001). In addition, OCD is an overlapping and apparently related disorder that can involve highly complex stereotypies of action sequences and thought sequences. Haloperidol and related dopamine antagonists are among the pharmacological treatments available for Tourette syndrome and OCD (McDougle et al., 1994;Robertson et al., 2000). The ability of haloperidol to improve tics contributed to the idea that there may be excessive dopamine in the basal ganglia in Tourette syndrome patients, which has more recently gained support from neuroimaging studies (Albin et al., 2006;Frey et al., 2006;Singer et al., 2002). There is evidence for enhanced stimulated dopamine release in the putamen (Singer et al., 2002) and dopamine receptor supersensitivity in Tourette patients (Segawa, 2003). Tics in Tourette syndrome and related stereotypies have been proposed to arise in part from dysfunction of the direct striatonigral output pathway, preventing the correct suppression of unwanted movements (Mink, 2006). D1 receptors are highly expressed in striatonigral direct output pathway neurons (Harrison et al., 1990). While the role of D1 receptors specifically in Tourette syndrome and OCD remains unclear, overly sensitive D1 receptors are thought to contribute to excessive movements observed in other L-DOPA induced dyskinesias and in the extreme self-directed biting behaviors of Lesch-Nyhan syndrome (Aubert et al., 2005;Breese et al., 2005;Corvol et al., 2004;Grondin et al., 1999;Rascol et al., 2001;Taylor et al., 2005). Therefore, enhanced D1 receptor sensitivity, acute over-stimulation of D1 receptors, or up-regulation of D1 receptors, all of which promote D1-mediated signaling, may be important factors in disorders like Tourette syndrome.

Tourette syndrome and related disorders involve excess movements that are often sequentially organized and are difficult to control. The intense, repetitive nature of the grooming movements observed during super-stereotypy, along with the relationship of super-stereotypy to basal ganglia activation and over-stimulation of dopamine D1 receptors, led to the notion that sequential super-stereotypy could potentially be used as a model to study the brain mechanisms that underlie serially-patterned tics and related behavioral pathologies (Albin et al., 2006;Berridge et al., 2000a;Berridge et al., 2005). The suppression of sequential super-stereotypy by haloperidol demonstrated here supports the use of syntactic chain completion as part of a model for Tourette syndrome.

In the second experiment, a potential D1 receptor up-regulation (caused by chronic receptor blockade) induced only a simple repetitive action, namely scratching and nail/tail biting, but did not induce sequential super-stereotypy of grooming patterns. Mutant mice with enhanced D1 receptor signaling are known to display tic-like stereotypies, including perseverative gnawing and non-aggressive biting of littermates (Campbell et al., 1999). D1 receptor stimulation is needed for apomorphine to elicit stereotypies that include sniffing, biting, gnawing, licking, and other mouth movements (Delfs et al., 1990;Iorio et al., 1983).

The observation that acute pharmacological activation of D1 receptors seems to enhance the repetition and stereotyped pattern of complex movement sequences, whereas chronically-induced increases in D1 receptor expression enhanced only the repetition of simpler scratching actions could be relevant to the balance of stereotypies that emerge in Tourette syndrome. The details of chronic versus acute mechanisms of dopamine receptor activation in the basal ganglia thus may be important to determine the nature of motor difficulties, manifested either as simple movement repeats or as sequentially rigid patterns of actions, in disorders like Tourette syndrome and OCD.

If problems of D1 versus D2 receptor function are indeed contributing factors in Tourette syndrome, then it is plausible to think of excessively repetitive forms of rodent grooming behavior, such as syntactic chains and simple scratching, as models for particular features the disorder. These models may clarify how alterations of dopamine D1 and D2 receptors can change information flow through the basal ganglia and lead to aberrant behavior. Rodent models have provided valuable insight to neurological disorders like stroke and Parkinson's disease, but developing animal models of Tourette syndrome has proven to be more difficult (Cenci et al., 2002;Swerdlow et al., 2006). The rodent grooming behaviors described here may complement current rodent models of Tourette syndrome and related disorders, such as those based on sensorimotor gating deficits, to help gain a better understanding of what happens in the brain in Tourette syndrome (Swerdlow et al., 2006),

Experimental procedure

Animals

Adult male Sprague-Dawley rats (Harlan) were housed as pairs in acrylic cages and maintained on a reverse phase light/dark cycle (lights off at 8:00 am, lights on at 8:00 pm). Rats were provided with standard rodent chow and water ad libitum. Experiments were approved by the University Committee on Use and Care of Animals at the University of Michigan.

Observation chamber

On the mornings of the experimental procedures, rats were transported in their home cages from a University-run vivarium to the lab, where they were kept in a dark, quiet room until experiments commenced. For all acclimation and test sessions, rats were individually transferred to an acrylic cylinder (27 cm tall, 25 cm in diameter) within a larger, enclosed behavioral observation chamber with a glass floor. A video camera was aimed toward a mirror angled beneath the cylinder to allow for video recordings of the rat from below. Red house lights were used to illuminate the chamber and to avoid disruption of circadian rhythms. Rats were handled and acclimated to the observation chamber on at least 3 occasions prior to testing.

Pharmacological treatments & behavioral recording

Experiment #1—In separate test sessions, rats (n=8) were injected with haloperidol (0.01, 0.1, or 1.0 mg/kg; ip; Sigma-Aldrich) or its vehicle in a within-subject design (1:1 ethanol:sterile water, 1 ml/kg, ip) and placed into the observation chamber. Fifteen minutes later, rats were removed from the chamber, injected with the dopamine D1 receptor agonist SKF38393 (8 mg/kg, ip; Sigma-Aldrich) or its vehicle (sterile water, 1 ml/kg, ip) and returned to the chamber. Thus, treatment groups were haloperidol-SKF38393; vehicle-SKF38393; vehicle-haloperidol; and vehicle-vehicle. Further, all haloperidol groups contained the 3 different doses. Rats received 7 or 8 of the drug combinations in various orders arranged so that drug order would not determine the group outcome. Digital video recordings of free range behavior were taken for one hour following the second injection. At

least 3 days elapsed between each test session, and test sessions took place between 8:00 am and 3:00 pm.

Experiment #2—A separate set of rats were given injections of vehicle (n=4; sterile water; 1 ml/kg, ip) or the D1 dopamine receptor antagonist SCH23390 (n=6; 0.5 mg/kg, ip; Sigma-Aldrich) twice daily (8:00 am and 5:00 pm) for 20 or 21 days. Following at least one of each of the daily chronic treatment injections, rats were placed in the behavioral chamber for 30 minutes before being returned to their home cages. Following the remaining chronic injections, rats were returned directly to their home cages. Thirty-nine hours following the final evening chronic SCH23390 injection, rats were injected with vehicle (sterile water; 1 ml/kg, ip) and placed in the observation chamber, where digital video recordings of free range behavior were taken for one hour. Rats were then given an injection of the D1 agonist SKF38393 (1 mg/kg, ip), and video-recorded for an additional hour (See Supplementary Data, Figure S1).

Behavioral analysis

For the purposes of the current study, grooming was defined as any time in which the rat's paws or mouth contacted the body. Grooming behaviors were composed of a variety of movements, including head and face washing, paw licking, body licking, biting directed towards the nails, tail, or body, and scratching with the hind paws. Each test session was scored offline for grooming behaviors as described below, at speeds ranging from single frame-by-frame analysis (each frame=1/30th of 1 second) to real-time. The observers (JLT, AKR, LG, MB, and LJ) received extensive training to perform the grooming analyses. Observers agreed on 98% of the time spent grooming samples and on 96% of the syntactic grooming chain scores.

Time spent grooming—To estimate total time spent grooming, at every 1-minute interval on the video the observer(s) identified whether or not the rat was grooming. This scoring procedure yielded 60 samples for each 1-hour session. The number of samples in which grooming occurred were tabulated to estimate the number of minutes spent grooming, divided by the total number of observations (60), and multiplied by 100, to yield an estimate of the percent of time spent grooming in a recording session. For experiment #2, each sample of grooming in which “scratching and biting” occurred was also noted, and the amount of time spent scratching and biting was estimated in the same manner.

Syntactic grooming chains—For a detailed analysis of grooming sequences, the entire continuous recording session was inspected in order to identify and evaluate syntactic grooming chains. As stated by Kalueff and colleagues, since syntactic grooming chains are short (less than 5 sec), many would have been missed with the sampling method described above (Kalueff et al., 2007). Syntactic grooming chains are highly predictable sequences of 15-25 individual paw strokes and licking movements that occasionally occur within grooming bouts (Berridge et al., 1987). Syntactic grooming chains are organized into 4 consecutive phases. Phase 1 of the grooming chain consists of 5-9 rapid elliptical forepaw strokes around the nose at a rate of at least 6 Hz. Phase 2 is composed of 1-5 unilateral, alternating paw strokes that may extend up toward the level of the eye. Phase 3 strokes are bilateral and symmetric, usually extending past the ear. Phase 4 is a rapid turn of the head followed by movement of the snout towards the side of the body to commence licking of the flank. Grooming chains are initiated when Phase 1 is immediately followed by Phases 2 or 3. Grooming chains that are considered to be syntactically perfect are those in which Phase 1 is followed consecutively by Phases 2, then 3, then 4. No other movements may be inserted and no omission of grooming phases may occur. A trained observer examined the continuous video from each one-hour session and identified every time that syntactic

grooming chains were initiated, which is reported as the absolute number of grooming chains. The absolute number of grooming chains was divided by the total amount of time spent grooming (min) as a measure of the relative number of grooming chains initiated per minute of total grooming. Each grooming chain was also evaluated for syntactic completion, and is expressed as percent of grooming chains completed.

Statistical analysis

For the SKF38393/haloperidol experiment, a one-way mixed model analysis of variance was conducted to test for main effects of drug treatment. Post-hoc testing using a Bonferroni adjustment was used to determine if significant differences existed between the 8 different treatments ($p<0.05$). For the chronic SCH23390 experiment, an independent samples t-test was used to determine whether significant differences existed between the treatment groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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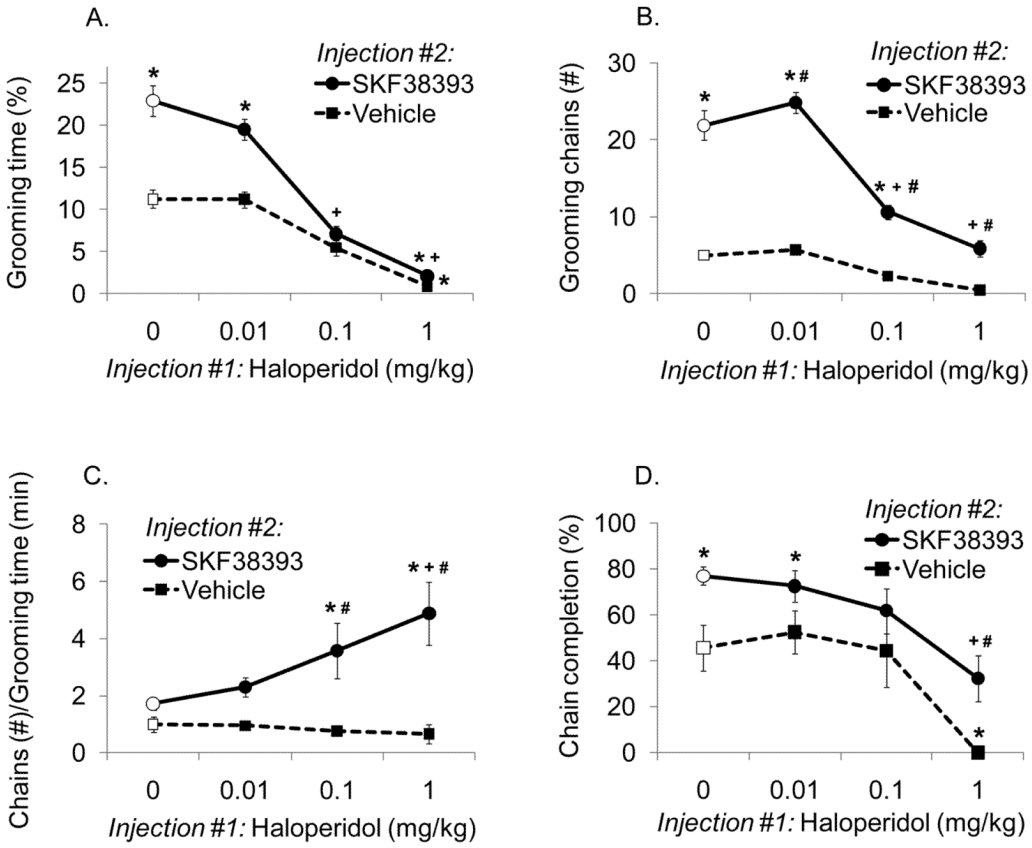

Figure 1. Haloperidol modifies the influence of SKF38393 on syntactic chain grooming

The combined effects of vehicle (0 mg/kg) or haloperidol (0.01, 0.1, and 1.0 mg/kg, ip) with vehicle or SKF39393 (8 mg/kg, ip) on A) time spent grooming (percent of observation time), B) absolute number of syntactic grooming chains initiated, C) initiation of syntactic grooming chains relative to total grooming (number of grooming chains initiated/time spent grooming) and D) percentage of syntactic grooming chains completed perfectly during the 60 min long observation period. Open square denotes vehicle + vehicle (baseline activity), closed squares denote haloperidol + vehicle (haloperidol effect), open circle denotes vehicle + SKF38393 (D1 agonist effect), closed circles denote haloperidol + SKF38393. *p<0.05 vs. vehicle + vehicle; +p<0.05 vs. vehicle + SKF38393; #p<0.05 vs. the same dose of haloperidol + vehicle.

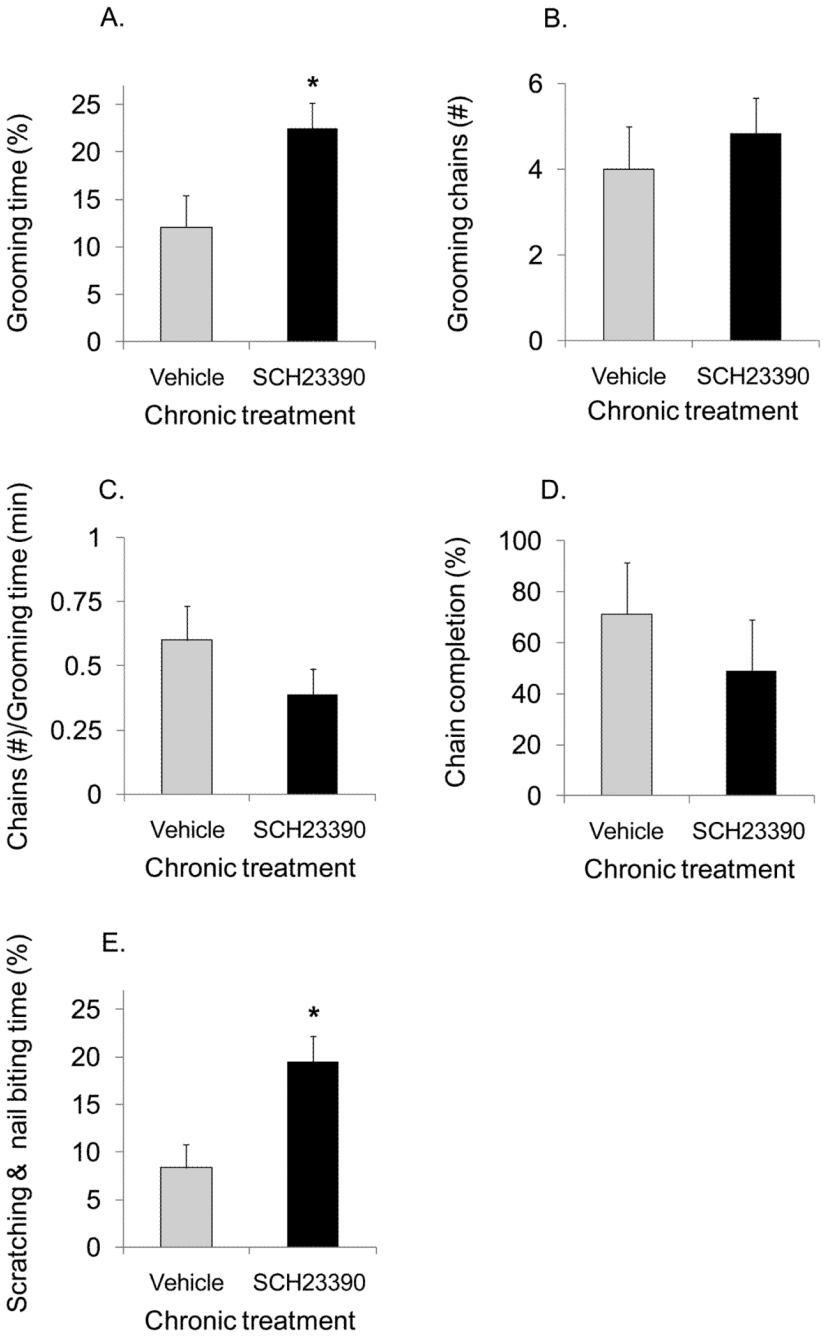

Figure 2. Previous exposure to SCH23390 increases scratching and biting without modifying syntactic chain grooming

The effects of a history of chronic SCH23390 (0.5 mg/kg, ip, bid) and chronic vehicle on A) time spent grooming (percent of observation time), B) absolute number of syntactic grooming chains, C) initiation of syntactic grooming chains relative to total grooming (number of grooming chains initiated /time spent grooming), D) percentage of syntactic grooming chains completed perfectly, and E) time spent scratching and nail or tail biting (percent of observation time), during the 60 min long vehicle-challenge observation period. *p<0.05 chronic SCH23390 vs. chronic vehicle.