ELSEVIER

European Journal of Pharmacology 371 (1999) 103–111

ejp

www.elsevier.nl/locate/ejphar

Differential response of cortical–limbic neuropotentiated compulsive mice to dopamine D_1 and D_2 receptor antagonists

Keith M. Campbell, Michael J. McGrath, Frank H. Burton *

Department of Pharmacology, University of Minnesota, 3-249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455, USA

Received 26 November 1998; received in revised form 8 March 1999; accepted 16 March 1999

Abstract

We previously created transgenic mice in which dopamine D_1 receptor-expressing (D1 +) neurons in regional subsets of the cortex and amygdala express a neuropotentiating cholera toxin (CT) transgene. These 'D1CT' mice engage in complex biting, locomotor and behavioral perseverance–repetition abnormalities that resemble symptoms of human compulsive disorders associated with cortical–limbic hyperactivity. Because excessive cortical–limbic stimulation of striatal motor pathways may play a critical role in causing compulsive disorders, we examined the responsiveness of D1CT mice to dopamine D_1 and D_2 receptor antagonists. D1CT mice were found to be largely resistant to the cataleptic action of the D_1 receptor antagonist SCH23390. The abnormal repetitive leaping of D1CT mice was similarly unaffected by SCH23390. In contrast, the D1CT mice displayed supersensitivity to cataleptic induction by the D_2 receptor antagonist sulpiride. These data are consistent with the hypothesis that complex compulsions are mediated by chronic excessive corticostriatal (and/or amygdalostriatal) glutamatergic stimulation of the striatal direct and indirect motor pathways.

Keywords: Compulsive behavior; Glutamate; Obsessive–Compulsive Disorder (OCD); Tourette's syndrome; Catalepsy; (Mouse)

1. Introduction

In humans, compulsive behavioral disorders such as Obsessive–Compulsive Disorder (OCD) are associated with cortical–limbic hyperactivity and excessive stimulation of striatal motor pathways (Kurlan et al., 1990; Breiter et al., 1996). Consequently, studying how different neuron subtypes within distinct cortical layers or limbic nuclei may selectively exert effects upon striatal motor output pathways and upon behavior may be useful in elucidating the role of cortical–limbic stimulation in the origin of compulsions. We have recently characterized a novel transgenic mouse model of cortical and limbic neurostimulation (Campbell et al., 1999). These 'D1CT' mice express a neuropotentiating transgene, which encodes an intracellular form of the G_s-activating and cyclic adenosine monophosphate (cAMP)-elevating enzyme cholera toxin (CT) (Burton et al., 1991, 1998; Zeiger et al., 1997), in a restricted subset of dopamine D_1 receptor-expressing (D1 +) neurons in piriform cortex layer II, somatosensory/insular cortex layers II–III, and the intercalated nucleus of the amygdala. Chronic potentiation of these cortical and limbic neurons in the D1CT mice causes them to engage in non-aggressive repeated biting of cagemates during grooming, repeated leaping, and episodes of perseverance or repetition of any and all normal behaviors. These complex behavioral abnormalities uniquely resemble the symptoms of human compulsive disorders such as OCD.

In OCD, compulsive episodes, which can exist with or without associated obsessions (Rapoport et al., 1992), are associated with hyperactivity in the same regions likely to be stimulated in D1CT mice, namely the amygdala and cortical regions including the orbitofrontal and somatosensory/insular cortex (Horwitz et al., 1991; Breiter et al., 1996), which in humans have been proposed to cause compulsions by excessively stimulating the striatum through excitatory corticostriatal inputs (Divac et al., 1977; Reubi and Cuenod, 1979; Fonnum et al., 1981). Supporting this hypothesis, severance of these corticostriatal inputs (which are predominantly glutamatergic) effectively suppresses the compulsive behaviors of patients with severe OCD (Bernstein et al., 1975; Kurlan et al., 1990) as well as suppresses dopamine-induced psychoactive behaviors in

* Corresponding author. Tel.: +1-612-626-0187; Fax: +1-612-625-8408; E-mail: burto006@tc.umn.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00184-3

rodents (Cenci and Björklund, 1993). In D1CT mice, the somatosensory/insular and piriform cortical D1+ neurons chronically potentiated by the D1CT transgene (Campbell et al., 1999) are glutamatergic projection neurons (Huang et al., 1992; Bergson et al., 1995; Gaspar et al., 1995; Grobin and Deutch, 1998) that excite deeper layer corticostriatal glutamatergic neurotransmission (Yamamoto et al., 1990; Kaneko et al., 1994), and laterally project to and excite the orbitofrontal cortex, an integrative cortical area that also projects to the striatum (Morecraft et al., 1992; Barbas, 1993). In the amygdala, the D1+ neurons in the intercalated nucleus that are chronically potentiated in D1CT mice are GABA(ergic) (gamma-aminobutyric acid releasing) neurons that regulate excitatory output to the cortex and striatum from other amygdaloid nuclei (McDonald, 1987; Scibilia et al., 1992; McDonald and Augustine, 1993; Gerfen and Wilson, 1996). Their chronic potentiation in D1CT mice, as well as electrical stimulation of the intercalated nucleus itself, triggers anxiogenic and startle behaviors consistent with increased overall limbic output (Rosen and Davis, 1988; McGrath et al., in preparation). Thus, D1CT mice not only exhibit behaviors resembling the biting, motor and perseverance–repetition compulsions of OCD, but also may share a similar neuroanatomical basis, in which topographically-restricted cortical–limbic hyperactivity may excessively stimulate striatal motor pathways.

Consequently, it would be worthwhile to examine the effect of the D1CT mice's chronic cortical–limbic neuron potentiation upon their efferent striatal motor pathways. In the striatum, dopamine D_1 receptor activation acts cooperatively with afferent glutamate in exciting neurons that stimulate motion via the direct striatonigral pathway of motor output, while dopamine D_2 receptor activation attenuates afferent glutamatergic excitation of neurons that suppress motion via the indirect striatopallidal pathway (Cepeda et al., 1993). Accordingly, agents that elevate or mimic dopamine stimulate motor activity and compulsions through these striatal pathways (Fog, 1975; Fung and Uretsky, 1980; Gold et al., 1989; Koshikawa et al., 1989; Karler et al., 1994), while D_1 and D_2 receptor antagonists suppress these behaviors, and at higher doses induce catalepsy, by blocking these striatal D_1 and D_2 receptor-regulated motor pathways (Fog, 1975; Koshikawa et al., 1989). Here, we have investigated the interaction of our D1CT mice with drugs known to inhibit the striatal direct or indirect motor pathways. This study indicates that D1CT mice exhibit changes in their striatal responsiveness to D_1 and D_2 receptor antagonists that suggest their compulsion-like behaviors are the consequence of chronic cortical–limbic glutamatergic excitation of striatal neurons, consistent with current models of afferent glutamatergic regulation of striatal output (Alexander and Crutcher, 1990; Carlsson, 1995; Carlsson and Carlsson, 1990; Gerfen and Wilson, 1996; Schmidt and Kretschmer, 1997). Moreover, these data suggest that the reported up-regulation of striatal D_2 receptors in human compulsive disorders (Wolf et al., 1996) could similarly be a consequence of chronic afferent glutamatergic stimulation of the striatum.

2. Materials and methods

2.1. Animals

Adult female Balb/c and Balb/c-inbred D1CT (D1CT-7 line) transgenic mice and non-transgenic littermates were housed in groups of two to five in a temperature-controlled room and were kept on a 12:12 light–dark cycle. Experiments were conducted during the light phase of the cycle. With the exception of testing times, the mice had ad lib access to food and water. Care was taken to ensure that the mice used in this study received no unnecessary discomfort. Animals were maintained and studies were carried out in accordance with the Animal Welfare Act and the N.I.H. Guide for the Care and Use of Laboratory Animals, under the approval of the University of Minnesota Institutional Animal Care and Use Committee. The University of Minnesota animal facility is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

2.2. Waveform display analysis of D1CT mouse behavior

Waveform display analysis (Campbell et al., 1998) was used to examine the behavior of D1CT and control non-transgenic mice. Briefly, videotapes of drug-naive D1CT or control non-transgenic mice were continuously observed for a 15-min period beginning 15 min after the mice were individually placed into a clean cage containing fresh bedding, food and water. The observations were performed blindly with respect to transgenic status. EthoMac (v1.10©, The University of Minnesota) software was used for behavioral state entry, and for calculation and tabulation of behavioral state event timing, number and duration. Switching from one behavior to another was recorded by depressing keys corresponding to each behavioral state in real time on the computer keyboard. EthoMac was also used to produce graphical coordinate output suitable for graphical waveform display of behavior. Graphical coordinate data was saved as a text file and then imported directly into CA-Cricket Graph III (Computer Associates International) to produce graphical waveform displays.

The behaviors scored included: (1) sniff; (2) still (remaining in one position with an occasional head movement); (3) rear; (4) gnaw (either gnawing against the side of the Plexiglas cage or gnawing of sawdust bedding); (5) locomotion; (6) dig (into the sawdust bedding); (7) groom; (8) hang (from the wire bar cage lid or stand/rear on the water bottle sipper spout); (9) leap (animal standing on its hind paws and repeatedly jumping up and down against the wall or corner of the cage); (10) drink; (11) eat (either

from the food container of the wire cage lid or from items picked from the bedding); and (12) other (any activity that does not fit into the previous categories).

2.3. Catalepsy assay (bar test)

Female D1CT or control non-transgenic littermates were injected i.p. with either the dopamine D_1 receptor antagonist, *R*(+)-SCH23390 (RBI, Nantic, MA) (0, 0.025, 0.1, 0.5, and 2 mg/kg), or the dopamine D_2 receptor antagonist, *S*(−)-sulpiride (RBI, Nantic, MA) (0, 20, and 100 mg/kg). *R*(+)-SCH23390 was dissolved in water, while *S*(−)-sulpiride was dissolved in one drop of acetic acid before being diluted with water, as previously described (Zarrindast and Haibibi-Moini, 1991). Each mouse served as its own within-group control, receiving both vehicle and drug. Separate groups of mice were used for SCH23390 and sulpiride. Based on reports showing no development of short-term tolerance to SCH23390 (Hess et al., 1988) or sulpiride (Scatton, 1977; Imperato et al., 1994), each mouse received drug doses in ascending order over consecutive days. Later confirmation that the D1CT mouse's marked resistance to the highest dose of SCH23390 was not actually due to increased development of tolerance to lower doses was obtained in a follow-up study in which drug-naive D1CT and control mice were given the highest dose of SCH23390. To perform the catalepsy assay, the forepaws of the mice were placed on a horizontal bar secured 5 cm above the floor. Duration of catalepsy was determined by counting the time until the mouse either removed both front paws from the bar or climbed onto the bar with one of its hindpaws, with a cutoff time of 5 min (Rowlett et al., 1991). For SCH23390, catalepsy was measured 15 min after injection, as previously described (Ushijima et al., 1995). For sulpiride, catalepsy was measured 3 and 4 h following injection, which represented the period of peak cataleptic effect in control mice (data not shown) and which is within the peak effect time period observed by others (Zarrindast and Haibibi-Moini, 1991). Because the time spent immobile on the bar by control non-transgenic mice given the highest dose (100 mg/kg) of sulpiride at 3-h post-injection was more equivalent than at 4-h post-injection to the time on the bar with the highest dose (2 mg/kg) of SCH23390, the 3-h post-injection sulpiride catalepsy data were evaluated in this study. During the D_1 receptor antagonist (SCH23390) catalepsy study, two pairs of mice (a pair consisted of a D1CT mouse and a control non-transgenic littermate) were eliminated to prevent unnecessary discomfort to the animals. One pair was eliminated on day four (at the 0.5 mg/kg dose), due to self- or cagemate-inflicted wounds. Another pair was eliminated on day 5 (at the 2 mg/kg dose) of this same study because the D1CT transgenic mouse had a seizure during the i.p. injection. Therefore, to prevent further trauma to these mice, they were immediately eliminated from the study. The observations for all catalepsy assays were performed blindly with respect to transgenic status and drug dose.

2.4. Time-sampling assay of immobility and repetitive leaping

For sulpiride, immediately after completion of the catalepsy assay, D1CT mice or control non-transgenic mice were individually placed into a clean Plexiglas cage with no food, water or bedding, and observed for 10 s windows every 5 min for 1 h. The behaviors selected for scoring were 'leaping' (animal standing on its hind paws and repeatedly jumping up and down against the wall or corner of the cage), which is an abnormal repetitive locomotor behavior present in D1CT mice and absent in control siblings (Campbell et al., 1999), and 'still' (remaining in one position with an occasional head movement), a separate indicator of cataleptic tendencies. A behavior was scored if it lasted at least 3 consecutive seconds (s) during the 10 s window of observation (Fray et al., 1980). For SCH23390, the assay was performed as above, except that a group of D1CT mice and control non-transgenic littermates different from those used in the catalepsy assay were observed. As with the catalepsy assay, each mouse received i.p. injections of SCH23390 in ascending doses over consecutive days, and time-sampling was performed 15 min after administration of SCH23390. The observations for all time-sampling assays were performed blindly with respect to transgenic status and drug dose.

2.5. Statistics

Data were analyzed by repeated measures analysis of variance (ANOVA) (within subjects for dose, between subjects for genotype) to establish overall significance of genotype effect on dose responses. If there was a significant dose × genotype interaction, then individual comparisons within subjects for each dose vs. vehicle were made by two-tailed paired *t*-test and between subjects (genotype) comparisons were made by two-tailed *t*-test. Significance was assumed at $P < 0.05$. Data are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. Illustration of D1CT mouse behavioral disorder

D1CT transgenic mice have been shown to engage in complex abnormal behaviors resembling those of human compulsive disorders such as OCD, including perseverance or repetition of any and all normal behaviors, repeated non-aggressive biting during grooming, and abnormal repeated leaping (Campbell et al., 1999). Fig. 1 shows an example 'behavioral waveform display' that illustrates some of these complex behavioral abnormalities of the

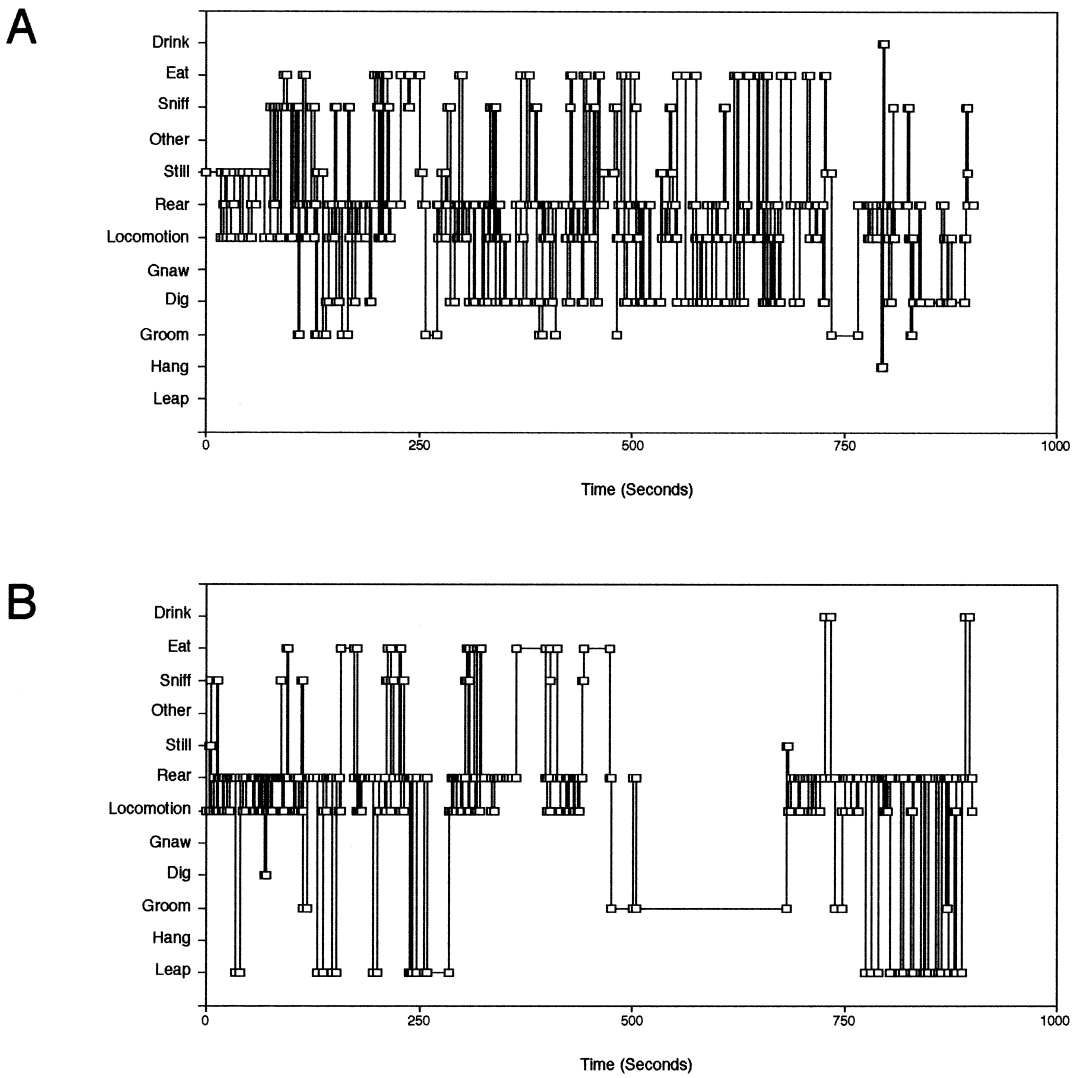

Fig. 1. Waveform display of locomotor and behavioral perseverance–repetition abnormalities of D1CT mice. (A) Control non-transgenic mouse. (B) D1CT mouse. Shown are waveform displays of mouse behavior (Campbell et al., 1998). The behavioral states in which the mice engaged are shown on the *y*-axis while time is shown on the *x*-axis. A horizontal line denotes the time that the mice engaged in a single behavioral state, while switching from one behavioral state to another is denoted by a vertical line. Areas of rapid behavioral switching appear as thickened vertical bars, which are due to the merging of many single vertical lines.

D1CT mice. Behavioral waveform display analysis provides both statistical analysis of the frequency and duration of total behaviors in a population of subjects, as well as graphical illustrations of examples of behavior as a 'waveform display' (Campbell et al., 1998). In this example, a typical D1CT mouse (Fig. 1B) is shown engaging in repetitive episodes of abnormal leaping behavior, which occurs frequently in the D1CT transgenic population (averaging one occurrence every 2 min per D1CT mouse) but which has never been observed in control non-transgenic mice (Fig. 1A) (Campbell et al., 1999). This leaping behavior consists of the animal standing on its hind paws and repeatedly jumping up and down against the corner or wall of the cage. The D1CT mouse in Fig. 1B is also shown engaging in another abnormal behavior typical of D1CT mice, abnormally perseverant episodes of any behavior (in this case, grooming), as well as reduced behavioral interspersion compared to control non-transgenic mice (Fig. 1A). Although only perseverant grooming is shown in Fig. 1B, D1CT mice can perseverantly engage in any behavior within their behavioral repertoire, which remains as full as that of non-transgenic mice (not shown). It is important to note that the D1CT mice's cortical–limbic induced leaping, grooming-associated biting and episodic perseverance of any and all behaviors is not the same as the general hyperactivity and specific stereotypic behaviors induced by broadly-acting dopaminergic drugs. This has been further confirmed by evidence that cocaine treatment masks these unique OCD-like behaviors of D1CT mice while inducing typical stereotypic and hyperactive behaviors identical to those in cocaine-treated control mice (Campbell et al., 1999).

Of the unique D1CT mouse behavioral abnormalities, repeated leaping is the simplest to detect and quantify using less labor intensive time-sampling observation methods (Fray et al., 1980). Consequently we chose to monitor the incidence of this perseverative–repetitive abnormality, along with the extent of catalepsy, in the D_1 and D_2 receptor antagonist experiments described below.

3.2. D1CT mice are resistant to the effects of a dopamine D_1 receptor antagonist

Because dopamine D_1 receptor antagonists suppress motion by blocking dopaminergic excitation of the motion-inducing 'direct' striatal pathway, we tested whether a D_1 receptor antagonist could suppress the chronic behavioral activation in our D1CT mouse model of cortical–limbic induced compulsions. The bar test revealed that D1CT mice were largely resistant to the cataleptic effects of the dopamine D_1 receptor antagonist SCH23390 (Fig. 2). Repeated measures ANOVA (within subjects for dose, between subjects for genotype) revealed a significant effect of genotype ($F[1,8] = 13.818$; $P = 0.0059$) and dose ($F[4,32] = 6.648$; $P = 0.0005$), as well as a significant dose × genotype interaction ($F[4,32] = 4.686$; $P = 0.0043$). Therefore, we made independent comparisons to determine from where the significant interaction arose. At all but the highest dose of SCH23390, D1CT mice exhibited no significant catalepsy, measured as an increase in the amount of time spent with their forepaws on the bar (Fig. 2). Only at the highest dose of 2 mg/kg SCH23390 did D1CT mice exhibit significant but modest catalepsy, compared to no drug (6.400 ± 1.965 vs. 0.800 ± 0.800 s, respectively; $P = 0.026$). Even so, D1CT mice were far less cataleptic than their control non-transgenic siblings at this highest dose (6.400 ± 1.965 vs. 146.200 ± 46.136 s, respectively; $P = 0.0164$) (Fig. 2). D1CT mice were also found to be far less cataleptic than their non-transgenic siblings at a lower dose of 0.5 mg/kg SCH23390 (20.200 ± 16.972 vs. 150.200 ± 57.448 s, respectively; $P = 0.0167$). Because prior studies indicated that significant tolerance does not develop to repeated doses of SCH23390 over a several-day injection regimen (Hess et al., 1988), and because each D1CT and control mouse received all drug doses in ascending order, the development of tolerance to SCH23390 as an explanation for the D1CT mice's marked resistance to the cataleptic effect of this drug was considered unlikely. Nevertheless, to confirm that increased tolerance to repeated drug doses did not underlie

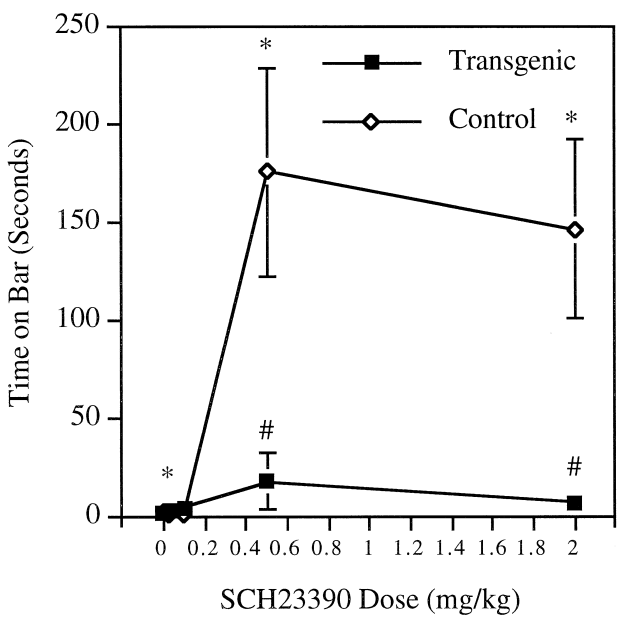

Fig. 2. D1CT mice are resistant to dopamine D_1 receptor antagonist-induced catalepsy. Shown is the mean time spent immobile in the horizontal bar forepaw placement test after administration of 0, 0.025, 0.1, 0.5, and 2 mg/kg SCH23390. Filled symbols, D1CT mice; Open symbols, control non-transgenic siblings; $n = 7$ per group, * = $P < 0.05$ compared to no drug using a two-tailed paired *t*-test; # = $P < 0.05$ compared to control non-transgenics at the given dose using a two-tailed unpaired *t*-test. Repeated measures ANOVA (within subjects for dose, between subjects for genotype) revealed a significant effect of genotype ($F[1,8] = 13.818$; $P = 0.0059$) and dose ($F[4,32] = 6.648$; $P = 0.0005$), as well as a significant dose × genotype interaction ($F[4,32] = 4.686$; $P = 0.0043$). Therefore, the above independent comparisons were justified.

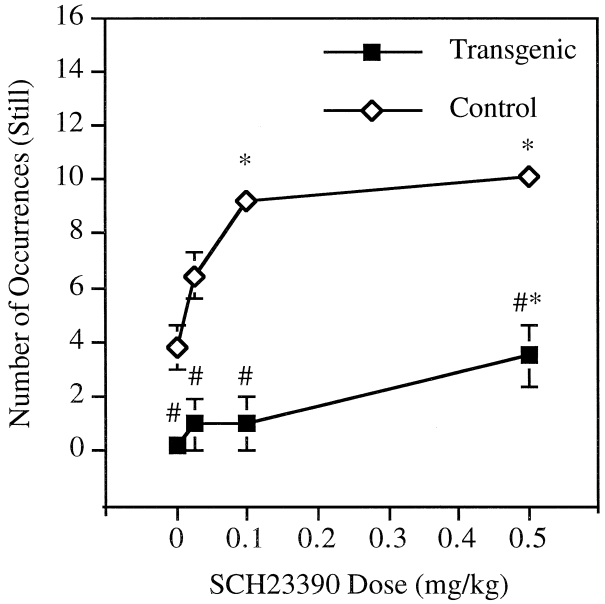

Fig. 3. D1CT mice are slightly behaviorally inhibited by a dopamine D_1 receptor antagonist. Shown is the mean number of occurrences of 'still' detected in a time-sampling assay after administration of 0, 0.025, 0.1, and 0.5 mg/kg SCH23390. Filled symbols, D1CT mice; Open symbols, control non-transgenic siblings; $n = 7$ per group, * = $P < 0.05$ compared to no drug using a two-tailed paired *t*-test; # = $P < 0.05$ compared to control non-transgenic siblings at the given dose using a two-tailed unpaired *t*-test. Repeated Measures ANOVA (within subjects for dose, between subjects for genotype) indicated a significant effect of genotype ($F[1,14] = 75.409$; $P < 0.0001$) and dose ($F[3,42] = 68.43$; $P < 0.0001$), as well as a significant dose × genotype interaction ($F[3,42] = 3.456$; $P = 0.0247$). Therefore, the above independent comparisons were justified.

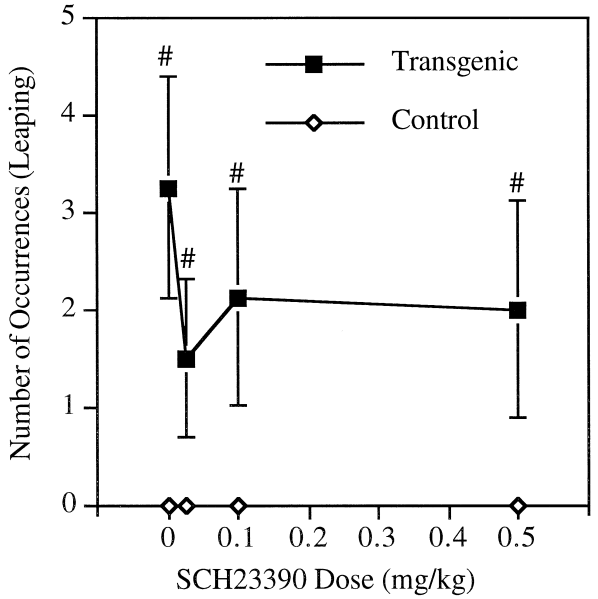

Fig. 4. Abnormal behavior of D1CT mice is resistant to a dopamine D_1 receptor antagonist. Shown is the mean number of occurrences of abnormal leaping behavior in D1CT mice administered 0, 0.025, 0.1, and 0.5 mg/kg SCH23390. Filled symbols, D1CT mice; Open symbols, control non-transgenic siblings; $n = 7$ per group, $\# = P < 0.05$ compared to control non-transgenic siblings at the given dose using a two-tailed unpaired *t*-test. Repeated Measures ANOVA (within subjects for dose, between subjects for genotype) indicated no significant effect of dose on leaping ($F[3,42] = 6.547$; $P = 0.2463$), nor a significant dose by genotype interaction ($F[3,42] = 6.547$; $P = 0.2463$), while confirming a significant effect of genotype on leaping ($F[1,14] = 78.766$; $P = 0.0383$).

the D1CT mice's drug resistance, we performed a follow-up study in which D1CT and control mice that were drug-naive were given the highest (2 mg/kg) dose of SCH23390. This confirmed that even drug-naive D1CT mice, in which tolerance could not have occurred, were markedly resistant to SCH23390-induced catalepsy. For example, there was no significant difference in the time spent on the bar at a dose of 2 mg/kg SCH23390 between drug-naive D1CT mice and D1CT mice that had previously received the drug (19.000 ± 15.853 vs. 6.400 ± 1.965 s, respectively; $P = 0.4926$); similarly, control non-transgenic mice exhibited no effect of prior drug exposure on their pronounced cataleptic response to 2 mg/kg SCH23390 (146.200 ± 46.136 vs. 177.000 ± 48.790 s for drug-naive; $P = 0.6620$) (not shown). These data cumulatively indicate that D1CT mice are resistant to the cataleptic effects of SCH23390 and that this resistance is not due to elevated tolerance in these mice.

However, the resistance of the D1CT mice to behavioral inhibition by SCH23390 was not total. Informal observation of D1CT mouse behavior after drug injection indicated that brief and intermittent episodes of akinesia did occur in response to the D_1 antagonist. This anecdotal observation was confirmed by the increase in the amount of 'still' behavior exhibited by D1CT mice in a time-sampling assay at a dose of 0.5 mg/kg SCH23390 (3.500 ± 1.150 vs. 0.188 ± 0.132 s with vehicle; $P = 0.0246$) (Fig. 3). D1CT mice nevertheless remained considerably less 'still' than their control non-transgenic siblings at all SCH23390 doses, including no drug (Fig. 3).

Although SCH23390 caused modestly increased 'stillness' in D1CT mice, no similar decrease in their abnormal repetitive leaping behavior was observed in the time-sampling assay (Fig. 4). In fact, there was no significant decrease in the number of leaping events in D1CT mice at any dose of the D_1 antagonist (Fig. 4). These data cumulatively indicate that, relative to their control non-transgenic siblings, D1CT mice's activity level (and in particular, their abnormal cortical–limbic induced behavior) are almost completely unaffected by a blockade of striatal D_1 receptors.

3.3. D1CT mice are supersensitive to the effects of a dopamine D_2 receptor antagonist

Because dopamine D_2 receptor antagonists suppress motion by blocking dopaminergic inhibition of the motion-suppressing 'indirect' striatal pathway, we simi-

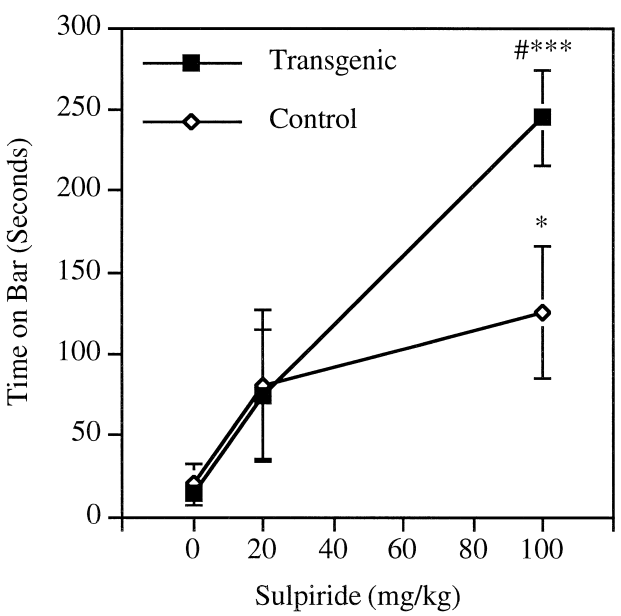

Fig. 5. D1CT mice are supersensitive to dopamine D_2 receptor antagonist-induced catalepsy. Shown is the mean time spent immobile in the bar test at 3 h after administration of 0, 20, and 100 mg/kg sulpiride. Filled symbols, D1CT mice; Open symbols, control non-transgenic siblings; $n = 6$ per group, $* = P < 0.05$ and $*** = P < 0.001$ compared to no drug using a two-tailed paired *t*-test; $\# = P < 0.05$ compared to control non-transgenics at the given dose using a two-tailed unpaired *t*-test. Repeated measures ANOVA (within subjects for dose, between subjects for genotype) at 3-h post-injection indicated a significant dose × genotype interaction ($F[2,22] = 3.665$; $P = 0.0423$). Therefore, the above independent comparisons were justified.

larly tested whether a D_2 receptor antagonist could suppress the chronic behavioral activation of D1CT mice. Interestingly, in contrast to their insensitivity to a D_1 receptor antagonist, D1CT mice exhibited supersensitivity to behavioral inhibition by the D_2 receptor antagonist sulpiride. As shown in the bar test of catalepsy, D1CT mice were significantly more cataleptic than control non-transgenic mice in response to the highest dose (100 mg/kg) of sulpiride at a typical 3-h post-injection period (244.571 ± 29.694 vs. 125.5 ± 40.027 s, respectively; $P = 0.0332$) (Fig. 5). Abnormal repetitive leaping was absent in D1CT mice when administered either 20 mg/kg or 100 mg/kg sulpiride (not shown), presumably because the mice were cataleptic (not shown) at both doses when the time-sampling assay for leaping was performed (4 h after drug administration).

Because the highest doses of the D_2 receptor antagonist sulpiride and the D_1 receptor antagonist SCH23390 caused equivalent extents of catalepsy in control non-transgenic mice (125.500 ± 40.027 vs. 158.000 ± 57.580 s, respectively; $P = 0.7412$) (Fig. 5 vs. Fig. 2), the sensitivity of D1CT mice to sulpiride but not to SCH23390 is not due to an arbitrarily high dose of sulpiride relative to SCH23390. Instead, it appears to reflect selective changes in the responsiveness of the D1CT mice to these two classes of cataleptic drugs.

4. Discussion

Our data reveal that D1CT mice (which exhibit a complex, cortical–limbic induced biting, movement and behavioral perseverance–repetition disorder reminiscent of the compulsions associated with cortical–limbic hyperactivity in human OCD and related psychomotor illnesses) are resistant to behavioral inhibition by the dopamine D_1 receptor antagonist SCH23390, but are supersensitive to behavioral inhibition by the dopamine D_2 receptor antagonist sulpiride. As discussed below, these results may shed light on the interaction of corticostriatal (and/or amygdalostriatal) glutamatergic and nigrostriatal dopaminergic regulation of the striatal motor pathways known to mediate both motor activation and catalepsy. Our findings are also consistent with the hypothesis that compulsive psychomotor disorders including OCD may involve excessive glutamatergic input into the striatum. Further supporting this hypothesis, it has recently been reported that LY354740, a novel presynaptic metabotropic glutamate receptor agonist that reduces excessive (but not basal) glutamate efflux, decreased phencyclidine (PCP)-induced prefrontal glutamate levels, hyperlocomotion and stereotypic head rolling in rats (Moghaddam and Adams, 1998), suggesting a causative role of excessive prefrontal cortical glutamate in PCP psychosis and possibly other forms of psychotic and associated compulsive behaviors.

4.1. D1CT mouse resistance to dopamine D_1 receptor antagonists is consistent with excessive glutamatergic stimulation of the striatum

The resistance of D1CT mice to doses of SCH23390 that can cause complete catalepsy in control mice (Figs. 2–4) shows that their chronic cortical–limbic neurostimulation is sufficient to cause continued behavioral activation even in the presence of a systemically administered (and thus globally acting) dopamine D_1 receptor antagonist. The current model of the direct (striatonigral) motor output pathway leads to the prediction that, under conditions of chronic glutamatergic input to the striatum, behavioral resistance to D_1 receptor antagonists would develop. This is because D_1 receptor activation facilitates glutamatergic excitation of the D1 + striatal neurons that induce motion by the direct pathway (Fig. 6A) (Cepeda et al., 1993). Hence, excessive glutamatergic excitation of these striatal D1 + neurons would reduce their dependence on D_1 receptors to provide cooperative excitation, making activation of these receptors less necessary (Fig. 6B). These D1 + striatal neurons, and their motor output, should thus become resistant to inhibition by D_1 receptor antagonists (Fig. 6C), which we observed in the D1CT mice (Figs. 2–4). In summary, these behavioral data are consistent with the hypothesis that chronic and excessive corticostriatal and/or amygdalostriatal glutamate will excite striatal D1 + neurons and behavior even when cooperative excitation by nigrostriatal dopamine stimulation of D_1 receptors is blocked (Fig. 6B–C).

4.2. D1CT mouse supersensitivity to dopamine D_2 receptor antagonists is consistent with excessive glutamatergic stimulation of the striatum

The cataleptic supersensitivity of D1CT mice to doses of sulpiride that did not cause as much catalepsy in control mice (Fig. 5) shows that their chronic cortical–limbic neurostimulation is sufficient to make them unusually susceptible to behavioral suppression by a systemically administered (and thus globally acting) dopamine D_2 receptor antagonist. The current model of the indirect (striatopallidal) motor output pathway leads to the prediction that, under conditions of chronic glutamatergic input to the striatum, behavioral supersensitivity to D_2 receptor antagonists would develop—a response opposite to that seen to D_1 receptor antagonists. This is because D_2 receptor activation attenuates, rather than facilitates, glutamatergic excitation of the D2 + striatal neurons that suppress motion by the indirect pathway (Fig. 6A) (Cepeda et al., 1993). Hence, excessive glutamatergic excitation of these striatal D2 + neurons would increase their dependence on D_2 receptors to provide an inhibitory counterbalance, making activation of these receptors even more necessary (and possibly leading to compensatory increases in D_2 receptor number or sensitivity) (Fig. 6B). Blocking this counterbalance using D_2 receptor antagonists would disinhibit the

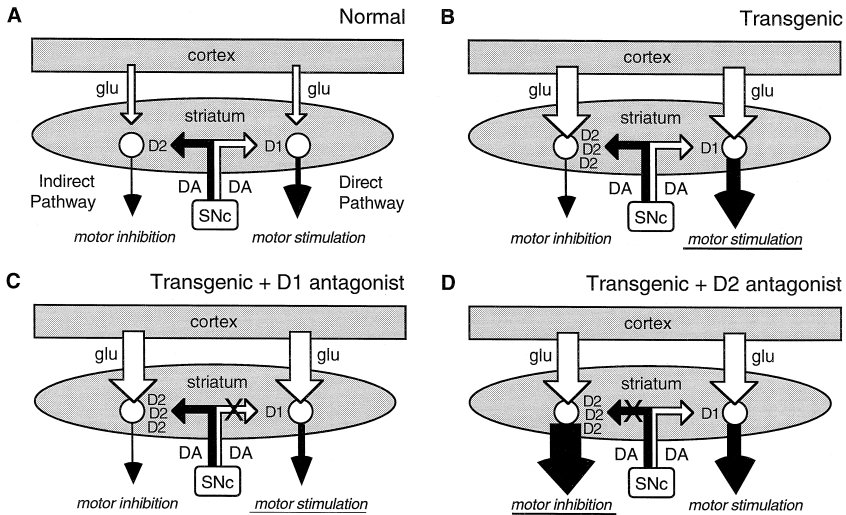

Fig. 6. Neuroanatomical model of cortical–limbic induced compulsions in D1CT mice. (A) Cortical–limbic glutamate and nigral dopamine effects on striatal output in normal mice. Dopamine and glutamate cooperatively excite the dopamine D_1 receptor-mediated 'direct' pathway that induces motion, while dopamine inhibits glutamatergic excitation of the dopamine D_2 receptor-mediated 'indirect' pathway that suppresses motion. (B) Cortical–limbic glutamate and nigral dopamine effects on striatal output in D1CT mice. Excessive glutamatergic excitation of the direct pathway alleviates the need for cooperative excitation by dopamine D_1 receptors and increases motor output, while excessive glutamatergic excitation of the indirect pathway is attenuated by inhibitory dopamine D_2 receptors (which would be predicted to increase in number or sensitivity to counterbalance excessive glutamatergic excitation). (C) Dopamine D_1 receptor antagonist effect on striatal motor output in D1CT mice. Blockade of co-excitatory dopamine D_1 receptors does not reverse the excessive glutamatergic excitation of the motion-inducing direct pathway. The net result is behavioral resistance to dopamine D_1 receptor antagonists. (D) Dopamine D_2 receptor antagonist effect on striatal motor output in D1CT mice. Blockade of inhibitory dopamine D_2 receptors allows excessive glutamatergic excitation of the motion-suppressing indirect pathway. The net result is increased sensitivity to the cataleptic effects of dopamine D_2 receptor antagonists. Key: Light arrows, excitatory input; dark arrows, inhibitory input; circles, D1 + or D2 + neurons; DA = dopamine; glu = glutamate; SNc = substantia nigra pars compacta; D_1 = dopamine D_1 receptor subtype; D_2 = dopamine D_2 receptor subtype; D_1 antagonist = dopamine D_1 receptor antagonist; D_2 antagonist = dopamine D_2 receptor antagonist.

glutamatergic hyperexcitation of these motion-suppressing striatal D2 + neurons, leading to super-inhibition of motor activity through the indirect pathway (Fig. 6D). Consistent with this prediction, D1CT mice demonstrated supersensitivity to D_2 receptor antagonist-induced catalepsy (Fig. 5). In this regard, it is intriguing to note that striatal D_2 receptors are abnormally increased in proportion to the severity of symptoms in Tourette's Syndrome (Wolf et al., 1996), a disorder typified not only by tics but by OCD-like compulsions, and which is responsive to treatment with D_2 receptor antagonists (Erenberg, 1992). Thus, chronic glutamatergic excitation of D2 + striatal neurons associated with compulsive behaviors may not only underlie our D1CT mice's supersensitivity to D_2 receptor antagonists but also suggests a potential mechanism for the elevation of striatal D_2 receptors in Tourette's Syndrome or related compulsive disorders. In the future, quantifying the changes in D1CT mouse cortical–limbic metabolic activity, behavioral responses to glutamate receptor-binding drugs, and striatal D_2 receptor density may further elucidate how cortical–limbic neurostimulation interacts with the striatum to generate complex compulsive disorders.

Acknowledgements

We thank Matt Veldman for technical assistance and Uyen Campbell for assistance in preparing the manuscript. This work was supported by NIH training grant T32DA07097 (K.M.C.), NIH research grant R03MH53553 (F.H.B.), a research grant from the Tourette's Syndrome Association (F.H.B.), and the Jeff Sutton Memorial Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression and Rochester Area Alliance for the Mentally Ill (F.H.B.)

References

Alexander, G.E., Crutcher, M.D., 1990. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci. 12, 266–271.

Barbas, H., 1993. Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. Neuroscience 56 (4), 841–864.

Bergson, C., Mrzlijak, L., Smiley, J.F., Pappy, M., Levenson, R., Goldman-Rakic, P.S., 1995. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptor in primate brain. J. Neurosci. 15, 7821–7836.

Bernstein, I.C., Callahan, W.A., Jaranson, J.M., 1975. Lobotomy in private practice. Arch. Gen. Psychiatry 32 (8), 1041–1047.

Breiter, H.C., Rauch, S.L., Kwong, K.K., Baker, J.R., Weisskoff, R.M., Kennedy, D.N., Kendrick, A.D., Davis, T.L., Jiang, A., Cohen, M.S., Stern, C.E., Belliveau, J.W., Baer, L., O'Sullivan, R.L., Savage, C.R., Jenike, M.A., Rosen, B.R., 1996. Functional magnetic resonance imaging of symptom provocation in obsessive–compulsive disorder. Arch. Gen. Psychiatry 53 (7), 595–606.

Burton, F.H., Hasel, K., Bloom, F.E., Sutcliffe, J.G., 1991. Pituitary hyperplasia and gigantism in mice caused by a cholera toxin transgene. Nature 350, 74–77.

Burton, F.H., Forss-Petter, S., Battenberg, E., Bloom, F.E., Sutcliffe, J.G., 1998. Lethal psychomotor abnormalities in mice caused by a neural cholera toxin transgene. Transgenics 2, 249–257.

Campbell, K.M., Rohland, R.M., McGrath, M.J., Satoskar, S.D., Burton, F.H., 1998. Detecting complex repetitive or stereotypic behaviors by graphing behavior as a multifrequency waveform. Physiol. Behav. 64 (1), 83–91.

Campbell, K.M., de Lecea, L., Severynse, D.M., Caron, M.G., McGrath, M.J., Sparber, S.B., Sun, L.-Y., Burton, F.H., 1999. OCD-like behaviors caused by a neuropotentiating transgene targeted to cortical and limbic D1+ neurons. J. Neurosci., in press.

Carlsson, A., 1995. Neurocircuitries and neurotransmitter interactions in schizophrenia. Intl. Clin. Psychopharmacol. 10, 21–28, Suppl. 3.

Carlsson, M., Carlsson, A., 1990. Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. Trends Neurosci. 13 (7), 272–277.

Cenci, M.A., Björklund, A., 1993. Transection of corticostriatal afferents reduces amphetamine- and apomorphine-induced striatal fos expression and turning behavior in 6-hydroxydopamine-lesioned rats. Eur. J. Neurosci. 5, 1062–1070.

Cepeda, C., Buchwald, N.A., Levine, M.S., 1993. Neuromodulatory actions of dopamine in the neostriatum are dependent on the excitatory amino acid receptor subtypes activated. Proc. Natl. Acad. Sci. USA 90, 9576–9580.

Divac, I., Fonnum, F., Storm-Mathisen, J., 1977. High affinity uptake of glutamate in terminals of corticostriatal axons. Nature 266, 377–378.

Erenberg, G., 1992. Treatment of Tourtette syndrome with neuroleptic drugs. Adv. Neurol. 58, 241–243.

Fog, R., 1975. Neuroleptic action of clozapine injected into various brain areas in rats. Intl. Pharmacopsychiatry 10 (2), 89–93.

Fonnum, F., Storm-Mathisen, J., Divac, I., 1981. Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neuroscience 6 (5), 863–873.

Fray, P.J., Sahakian, B.J., Robbins, T.W., Koob, G.F., Iversen, S.D., 1980. An observational method for quantifying the behavioral effects of dopamine agonists: contrasting effects of D-amphetamine and apomorphine. Psychopharmacology 69, 253–259.

Fung, Y.K., Uretsky, N.J., 1980. The effect of dopamine uptake blocking agents on the amphetamine-induced circling behavior in mice with unilateral nigro-striatal lesions. J. Pharmacol. Exp. Ther. 214 (3), 651–656.

Gaspar, P., Bloch, B., LeMoine, C., 1995. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. Eur. J. Neurosci. 7 (5), 1050–1063.

Gerfen, C.R., Wilson, J.W., 1996. The basal ganglia. Hndbk. Chem. Neuroanat. 12, 371–468.

Gold, L.H., Geyer, M.A., Koob, G.F., 1989. Neurochemical mechanisms involved in behavioral effects of amphetamines and related designer drugs. NIDA Re. Monogr. 94, 101–126.

Grobin, A.C., Deutch, A.Y., 1998. Dopaminergic regulation of extracellular gamma-aminobutyric acid levels in the prefrontal cortex of the rat. J. Comp. Neurol. 323 (3), 341–358.

Hess, E.J., Norman, A.B., Creese, I., 1988. Chronic treatment with dopamine receptor antagonists: behavioral and pharmacologic effects on D1 and D2 dopamine receptors. J. Neurosci. 8 (7), 2361–2370.

Horwitz, B., Swedo, S.E., Grady, C.L., Pietrini, P., Schapiro, M.B., Rapoport, J.L., Rapoport, S.I., 1991. Cerebral metabolic pattern in obsessive–compulsive disorder: altered intercorrelations between regional rates of glucose utilization. Psychiatry Res. 40 (4), 221–237.

Huang, Q., Zhou, D., Chase, K., Gusella, J.F., Aronia, N., DiFiglia, M., 1992. Immunocytochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system and thalamic reticular nucleus. Proc. Natl. Acad. Sci. USA 89, 11988–11992.

Imperato, A., Obinu, M.C., Carta, G., Mascia, M.S., Casu, M.A., Dazzi, L., Gessa, G.L., 1994. Neuroleptics cause stimulation of dopamine D1 receptors and their desensitization after chronic treatment. Eur. J. Pharmacol. 264, 55–60.

Kaneko, T., Caria, M.A., Asanuma, H., 1994. Information processing within the motor cortex: II. Intracortical connections between neurons receiving somatosensory cortical input and motor output neurons of the cortex. J. Comp. Neurol. 345 (2), 172–184.

Karler, R., Calder, L.D., Thai, L.H., Bedingfield, J.B., 1994. A dopaminergic–glutamatergic basis for the action of amphetamine and cocaine. Brain Res. 568 (1–2), 8–14.

Koshikawa, N., Aoki, S., Tomiyama, K., Kobayshi, M., Iwata, K., Sumino, R., Stephenson, J.D., 1989. Effects of intrastriatal injections of selective D-1 and D-2 agonists and antagonists on jaw movements of rats. Eur. J. Pharmacol. 163 (2–3), 227–236.

Kurlan, R., Kersun, J., Ballantine, H.T., Caine, E.D., 1990. Neurosurgical treatment of severe obsessive–compulsive disorder associated with Tourette's syndrome. Mov. Disord. 5 (2), 152–155.

McDonald, A.J., 1987. Organization of amygdaloid projections to the mediodosral thalamus and prefrontal cortex: a fluorescent retrograde transport study in the rat. J. Comp. Neurol. 261 (1), 46–58.

McDonald, A.J., Augustine, J.R., 1993. Localization of GABA-like immunoreactivity in the monkey amygdala. Neuroscience 52 (2), 281–294.

Moghaddam, B., Adams, B.W., 1998. Reversal of phencyclidine effects by a group II metabatropic glutamate receptor agonist in rats. Science 281, 1349–1352.

Morecraft, R.J., Geula, C., Mesulam, M.M., 1992. Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. J. Comp. Neurol. 323 (3), 341–358.

Rapoport, J.L., Swedo, S.E., Leonard, H.L., 1992. Childhood obsessive compulsive disorder. J. Clin. Psychiatry 53, 11–16, Suppl.

Reubi, J.C., Cuenod, M., 1979. Glutamate release in vitro from corticostriatal terminals. Brain Res. 176 (1), 185–188.

Rosen, J.B., Davis, M., 1988. Enhancement of acoustic startle by electrical stimulation of the amygdala, Behav. Neurosci., 102, 195–202, 324.

Rowlett, J.K., Pedigo, N.W. Jr., Bardo, M.T., 1991. Catalepsy produced by striatal microinjections of the D1 dopamine receptor antagonist SCH 23390 in neonatal rats. Pharmacol. Biochem. Behav. 40, 829–834.

Scatton, B., 1977. Differential regional development of tolerance to increase in dopamine turnover upon related neuroleptic administration. Eur. J. Neurosci. 46, 363–369.

Schmidt, W.J., Kretschmer, B.D., 1997. Behavioral pharmacology of glutamate receptors in the basal ganglia. Neurosci. Biobehav. Rev. 21 (4), 381–392.

Scibilia, R.J., Lachowicz, J.E., Kilts, C.D., 1992. Topographic nonoverlapping distribution of D_1 and D_2 dopamine receptors in the amygdaloid nuclear complex of the rat brain. Synapse 11 (2), 146–154.

Ushijima, I., Mizuki, Y., Yamada, M., 1995. Development of tolerance and reverse tolerance to haloperidol- and SCH23390-induced cataleptic effects during withdrawal periods after long-term treatment. Pharmacol. Biochem. Behav. 50 (2), 259–264.

Wolf, S.S., Jones, Q.W., Knable, M.B., Gorey, J.G., Lee, K.S., Hyde, T.M., Coppola, R., Weinberger, D.R., 1996. Tourette syndrome: prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. Science 273, 1225–1227.

Yamamoto, T., Samejima, A., Oka, H., 1990. The mode of synaptic activation of pyramidal neurons in the cat primary somatosensory cortex: an intracellular HRP study. Exp. Brain Res. 80 (1), 12–22.

Zarrindast, M.R., Haibibi-Moini, S., 1991. Blockade of both D-1 and D-2 dopamine receptors may induce catalepsy in mice. Gen. Pharmacol. 22 (6), 1023–1026.

Zeiger, M.A., Motoyasu, S., Gusev, Y., Westra, W.H., Takiyama, Y., Dooley, W.C., Kohn, L.D., Levine, M.A., 1997. Thyroid-specific expression of cholera toxin A1 subunit causes thyroid hyperplasia and hyperthyroidism in transgenic mice. Endocrinology 138, 3133–3140.