Pharmacology, Biochemistry and Behavior 94 (2010) 346–351

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

D1 priming enhances both D1- and D2-mediated rotational behavior and striatal Fos expression in 6-hydroxydopamine lesioned rats

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ARTICLE INFO

Article history:
Received 17 July 2009
Received in revised form 11 September 2009
Accepted 24 September 2009
Available online 2 October 2009

Keywords:
D1
D2
Dopamine
Quinpirole
SKF38393
Striatum
Priming
Sensitization
Fos
Rotational behavior

ABSTRACT

Rats with unilateral 6-hydroxydopamine (6-OHDA) lesions exhibit behavioral sensitization upon repeated treatment with dopamine agonists, a phenomenon called 'priming'. We examined the effectiveness of priming with D1 or D2 agonists on rotational behavior and striatal Fos expression following challenge with D1 or D2 agonists. Twenty-one days post-lesion, rats received three priming injections, spaced 3–6 days apart, with water, D1 agonist SKF38393 (10 mg/kg) or D2 agonist quinpirole (1 mg/kg). One week later, 6-OHDA rats were challenged with water, SKF38393 (1 or 10 mg/kg) or quinpirole (0.25 mg/kg). 6-OHDA rats challenged with SKF38393 (1 mg/kg) showed no contralateral rotational behavior, but robust striatal Fos expression in D1-primed animals. Challenge with SKF38393 (10 mg/kg) led to pronounced contralateral rotational behavior and striatal Fos expression in all priming groups — with the largest behavioral response in D1- and D2-primed rats. Quinpirole challenge (0.25 mg/kg) led to robust contralateral rotational behavior and striatal Fos expression in D1-primed animals, but only mild rotational behavior and baseline levels of striatal Fos expression in D2-primed animals. These data suggest that D1- or D2-priming enhances rotational behavior following challenge with D1 or D2 agonist, but only D1-priming enhances D1- and D2-mediated striatal Fos expression in 6-OHDA rats.

1. Introduction

Rats that have been unilaterally lesioned with 6-hydroxydopamine (6-OHDA) to destroy the nigrostriatal dopamine pathway exhibit contralateral rotational behavior following systemic administration of levodopa (L-dopa) or dopamine agonists (Hudson et al., 1993; Ungerstedt, 1971). This response is thought to reflect the asymmetric action of dopamine agonist at supersensitive dopamine receptors in the denervated striatum (Hudson et al., 1993; Ungerstedt, 1971). This rotational response shows sensitization upon repeated treatment with dopamine agonists, a phenomenon called "priming" (Carey, 1991). Priming of rotational behavior in 6-OHDA rats is long lasting and dependent on the timing of the administration of the priming drugs (Morelli et al., 1989; Pollack and Strauss, 1999).

Previously, we have used a three-injection priming paradigm to examine the effect of pretreatment with D1, D2 or D1/D2 dopamine agonists on subsequent D2-mediated responses in 6-OHDA rats (Pollack et al., 1997; Pollack and Strauss, 1999; Pollack and Yates, 1999). Our results have shown that 6-OHDA rats primed with three injections, spaced 3–6 days apart, with the D1/D2 agonist apomorphine (0.5 mg/kg) or the D1 agonist SKF38393 (10 mg/kg) demonstrate robust contralateral rotational behavior and pronounced expression of the immediate early gene (IEG) product c-Fos in the ipsilateral striatum following a challenge one week later with a low dose of the D2 agonist quinpirole (0.25 mg/kg) (Pollack et al., 1997; Pollack and Yates, 1999). By contrast, 6-OHDA rats primed with water and challenged with the same dose of quinpirole (0.25 mg/kg) do not rotate and show only baseline levels of striatal Fos expression (Pollack et al., 1997; Pollack and Yates, 1999). In addition, 6-OHDA rats primed with quinpirole (1 mg/kg) and challenged with low dose quinpirole (0.25 mg/kg) display enhanced contralateral rotational behavior without induction of striatal Fos expression (Pollack and Yates, 1999). These observations suggest that priming with D1 and D2 subtype selective dopamine agonists alters subsequent D2-mediated responses in dopamine-depleted rats, with prior D1 receptor stimulation required for D2-mediated Fos expression in the striatum.

The importance of dopamine agonist pretreatment (priming) on the expression of D1-mediated contralateral rotational behavior in 6-OHDA rats is a bit unclear as the outcome depends on the priming paradigm used. Using a single-injection priming paradigm, prior treatment with D1 or D2 agonists have specific dose- and time-dependent effects on the sensitization of subsequent D1-mediated responses (Morelli et al., 1989; Morelli and Di Chiara, 1987). In contrast, several groups have reported that multiple-injection priming paradigms led to tolerance of D1-mediated responses rather than sensitization (Asin et al., 1995; Asin and Wirtshafter, 1993; Engber et al., 1993; Rouillard et al., 1988). In addition, while

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doi:10.1016/j.pbb.2009.09.016

administration of D1 agonists induces striatal Fos expression in 6-OHDA rats (Robertson et al., 1989; 1992; Wirtshafter, 2007), it is unclear whether sensitization of D1-mediated rotational behavior and striatal Fos expression occur in parallel, suggesting a similar/same mechanism(s), or in a disconnected manner, suggesting separate/distinct mechanisms. Therefore, we have used our three-injection priming paradigm, which leads to robust sensitization of D2-mediated responses (Pollack et al., 1997; Pollack and Strauss, 1999; Pollack and Yates, 1999), to examine the effects of D1 and D2 agonist priming on contralateral rotational behavior and striatal Fos expression when 6-OHDA rats are challenged with the D1 agonist SKF38393 (1 or 10 mg/kg). In addition, we have run, in parallel, separate groups of 6-OHDA rats primed with D1 or D2 agonists, and challenged with the D2 agonist quinpirole (0.25 mg/kg) in order to permit a direct comparison of the effects of priming on D1- and D2-mediated responses.

2. Materials and methods

Male, Sprague–Dawley rats (270–300 g) were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg), i.p., and pretreated with desmethylimipramine (25 mg/kg, i.p.) prior to a stereotaxic injection of 4 μl 6-OHDA (3 mg/ml) and ascorbic acid (2 mg/ml) into the left medial forebrain bundle (coordinates from bregma: −3.7 AP, +1.6 ML, −8.8 DV) as described previously (Paxinos and Watson, 1986; Pollack et al., 1997). All rats were treated in a manner conforming to the National Institute of Health Guide for Care and Use of Laboratory Animals; the Animal Care Committee at the University of Massachusetts–Boston approved of all procedures.

Three weeks after 6-OHDA injection, rats were given three priming injections, i.p., at 3–6 day intervals, with either: water ($N=23$), the D1 agonist SKF38393 (10 mg/kg) ($N=32$) or the D2 agonist quinpirole (1 mg/kg) ($N=24$). Contralateral rotational behavior was measured for 30 min after each priming injection using an automated rotometer (AccuScan Instruments, Columbus OH). Rotational behavior during priming was used to assess the degree of dopamine-depletion in order to assure that only sufficiently lesioned animals were administered a subsequent challenge injection. Therefore, only those 6-OHDA rats that demonstrated ≥100 contralateral rotations during at least one of the three priming injections with SKF38393 or quinpirole were challenged 7–12 days later with either: water, SKF38393 (1 or 10 mg/kg) or quinpirole (0.25 mg/kg). The twelve experimental groups were (primed/challenged): water/water ($N=6$), water/SKF38393 (1 mg/kg) ($N=3$), water/SKF38393 (10 mg/kg) ($N=6$), water/quinpirole ($N=8$), SKF38393/water ($N=5$), SKF38393/SKF38393 (1 mg/kg) ($N=8$), SKF38393/SKF38393 (10 mg/kg) ($N=5$), SKF38393/quinpirole ($N=14$), quinpirole/water ($N=5$), quinpirole/SKF38393 (1 mg/kg) ($N=5$), quinpirole/SKF38393 (10 mg/kg) ($N=5$), and quinpirole/quinpirole ($N=9$).

Seven–twelve days following the third priming injection, 6-OHDA rats were challenged with water, SKF38393 (1 or 10 mg/kg) or quinpirole (0.25 mg/kg) and 360° contralateral rotational behavior was measured for 110 min using an automated rotometer. Two hours following the challenge injection, 6-OHDA rats were deeply anesthetized with pentobarbital (100 mg/kg) and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffer. Sixty-micrometer coronal brain sections were processed by immunohistochemistry using a c-Fos polyclonal antibody (1:15,000; PC35 Oncogene Science) as described previously (Pollack et al., 1997; Pollack and Strauss, 1999; Pollack and Yates, 1999). Fos-like immunoreactivity was visualized using a biotinylated secondary antibody followed by incubation with avidin/biotin-peroxidase (Elite ABC kit; Vector Labs), and developed with diaminobenzidine using nickel enhancement. To quantify Fos immunostaining, sections from the rostral dorsal striatum were analyzed under 10× magnification using a Scion Image Analysis System by one observer (L.I.T.) who was blind to the treatment groups. Fos data are expressed as the number (mean ± SEM) of cells expressing Fos-like immunoreactivity in a 1.2 mm^2 rectangular box placed over the dorsal striatum.

In water-primed groups, which did not display significant contralateral rotational behavior during priming or following challenge with water, SKF38393 (1 mg/kg) or quinpirole, the extent of dopamine-denervation was determined by assessing tyrosine hydroxylase (TH) immunostaining in 60-micron coronal sections as described previously (Pollack and Yates, 1999). An individual blind to the identity of the groups (L.I.T.) assessed TH staining in a qualitative manner. Therefore, the number of rats (N) in each water-primed group reflects only those 6-OHDA rats with sufficient loss of TH staining in the ipsilateral striatum, which was equivalent to TH staining in SKF38393-primed 6-OHDA rats, which were used as a positive control.

Contralateral rotations across the three priming injections in each priming group were analyzed using a repeated measure ANOVA followed by Tukey–Kramer multiple comparisons test, with $p<0.05$ considered significant (Instat v. 3). A two-way ANOVA (SPSS, v. 12.0, GLM) tested for effects of pretreatment (priming), challenge and interaction (pretreatment × challenge) on contralateral rotational behavior and striatal Fos expression, with $p<0.05$ considered significant.

3. Results

Across the three priming injections, 6-OHDA rats pretreated with water did not show any contralateral rotational behavior ($F(2,22)=0.24$, $p=0.7863$; Table 1). In contrast, 6-OHDA rats that received three priming injections with the D1 agonist SKF38393 (10 mg/kg) or the D2 agonist quinpirole (1 mg/kg) displayed a progressively higher number of contralateral rotations following the second and the third priming injections compared to the first priming injection in their respective group (SKF38393: $F(2,31)=34.89$, $p<0.0001$; quinpirole: $F(2, 23)=91.78$, $p<0.0001$; Table 1).

The twelve treatment groups, which varied by priming drug and challenge drug, allowed for a direct comparison of water-, D1-, and D2-priming (pretreatment) on subsequent contralateral rotational behavior and striatal Fos expression following a challenge with water, D1 or D2 agonist. There was a significant effect of pretreatment (priming) ($F(2,78)=18.08$, $p<0.0001$), challenge ($F(3,78)=54.12$, $p<0.0001$), and interaction (pretreatment × challenge) ($F(6,78)=4.81$, $p<0.0001$) on contralateral rotational behavior. There was also a significant effect of pretreatment (priming) ($F(2,78)=12.33$, $p<0.0001$), challenge ($F(3,78)=145.12$, $p<0.0001$), and interaction (pretreatment × challenge) ($F(6,78)=7.2$, $p<0.0001$) on striatal Fos expression.

When 6-OHDA rats were challenged with water there was no effect of priming group on the subsequent behavioral and cellular responses. 6-OHDA rats challenged with water did not show any

Table 1
Contralateral rotational behavior after each priming injection.

Priming groups	(N)	Priming injection #1	Priming injection #2	Priming injection #3
Water	(23)	0 ± 0	0 ± 0	0 ± 0
SKF38393	(32)	59 ± 15	192 ± 28[a]	259 ± 27[b]
Quinpirole	(24)	20 ± 3	95 ± 13[a]	164 ± 10[b]

Three weeks after injection of 6-OHDA, rats were treated with three priming injections with water, SKF38393 (10 mg/kg) or quinpirole (1 mg/kg), spaced 3–6 days apart. Data represent the number (mean ± SEM) of 360° contralateral rotations recorded in 30 min after each priming injection. N = number of animals in each group.
[a] Different from priming injection #1 of the same priming group, $p<0.05$.
[b] Different from priming injection #1 and #2 of the same priming group, $p<0.05$.

Table 2
Effect of priming with D1 or D2 agonist on the expression of D1- or D2-mediated contralateral rotational behavior.

Challenge treatment	Water-primed	SKF38393-primed	Quinpirole-primed
Water	0 ± 0	5 ± 1	4 ± 2
SKF38393 (1)	0 ± 0	141 ± 90	137 ± 136
SKF38393 (10)	522 ± 67[c]	1338 ± 239[a]	1090 ± 109[a]
Quinpirole (0.25)	27 ± 15	746 ± 81[b]	369 ± 55[a]

Rats were lesioned with 6-OHDA and three weeks later were primed with three injections of water, SKF38393 (10 mg/kg) or quinpirole (1 mg/kg), spaced 3–6 days apart. Seven–twelve days after the third priming injection, 6-OHDA rats were challenged with water, SKF38393 (1 or 10 mg/kg) or quinpirole (0.25 mg/kg). Data represent the number (mean ± SEM) of 360° contralateral rotations recorded in 110 min after challenge injection.

[a] Different from water-primed of the same challenge group, $p<0.05$.

[b] Different from water-primed and quinpirole-primed of the same challenge group, $p<0.05$.

[c] Different from water-primed/water-challenged, $p<0.05$.

Table 3
Effect of priming with D1 or D2 agonist on D1- or D2-mediated striatal Fos expression.

Challenge treatment	Water-primed	SKF38393-primed	Quinpirole-primed
Water	45 ± 9	112 ± 26	169 ± 32
SKF38393 (1)	109 ± 22	1078 ± 157[a]	377 ± 190
SKF38393 (10)	1767 ± 130[b]	1552 ± 124	1705 ± 70
Quinpirole (0.25)	40 ± 7	557 ± 70[a]	45 ± 6

Rats were lesioned with 6-OHDA and three weeks later were primed with three injections of water, SKF38393 (10 mg/kg) or quinpirole (1 mg/kg), spaced 3–6 days apart. Seven–twelve days after the third priming injection, 6-OHDA rats were challenged with water, SKF38393 (1 or 10 mg/kg) or quinpirole (0.25 mg/kg). Data represent the number (mean ± SEM) of Fos-positive cells counted in 1.2 mm^2 of the ipsilateral dorsal striatum 2 h after challenge injection.

[a] Different from water-primed and quinpirole-primed of the same challenge group, $p<0.05$.

[b] Different from water-primed/water-challenged, $p<0.05$.

contralateral rotational behavior (Table 2) and expressed only baseline levels of striatal Fos (Table 3; Fig. 1).

The behavioral and cellular responses in 6-OHDA rats following a challenge with the D1 agonist SKF38393 were dose-dependent and related to the priming group. There was no effect of priming on contralateral rotational behavior when 6-OHDA rats were challenged with low dose SKF38393 (1 mg/kg); these animals did not display any significant contralateral rotational behavior (Table 2). However, while challenge with high dose SKF38393 (10 mg/kg) led to significant contralateral rotational behavior in water-primed 6-OHDA rats, there was also an effect of priming since rotational behavior following challenge with SKF38393 (10 mg/kg) was more robust in D1- and D2-primed animals compared to water-primed 6-OHDA rats (Table 2). For striatal Fos expression, there was an effect of priming group when 6-OHDA rats were challenged with low dose SKF38393 (1 mg/kg). Only D1-primed 6-OHDA rats exhibited pronounced ipsilateral striatal Fos expression following challenge with low dose SKF38393 (1 mg/kg), with baseline levels of striatal Fos in water- and D2-primed animals (Table 3; Fig. 1). In contrast, the magnitude of striatal Fos expression following challenge with high dose SKF38393 (10 mg/kg) was independent of priming group; there were equally high levels of striatal Fos expression in all priming groups (Table 3; Fig. 1).

Challenge with the D2 agonist quinpirole led to behavioral and cellular responses in 6-OHDA rats that were dependent on the priming group. Following challenge with quinpirole (0.25 mg/kg) there was no rotational behavior in the water-primed group, moderate rotational behavior in the D2-primed group, and robust rotational behavior in the D1-primed group (Table 2). In addition, challenge with quinpirole led to D2-mediated striatal Fos expression in D1-primed animals only (Table 3; Fig. 1), with baseline levels of striatal Fos expression in water- and D2-primed 6-OHDA rats.

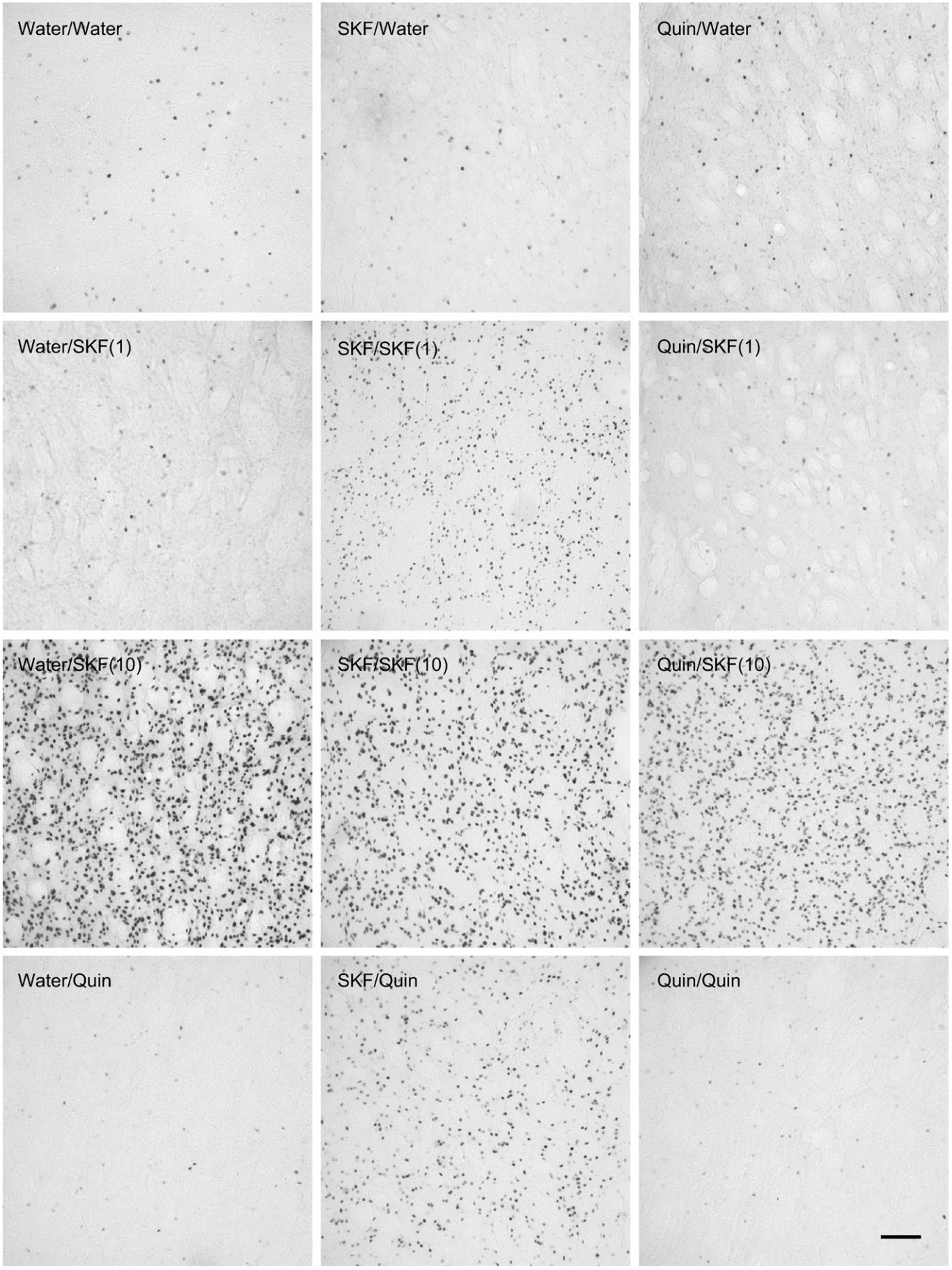
4. Discussion

The effects of priming on D1-mediated motor responses and striatal IEG expression varied according to the priming agent and the dose of SKF38393 used for the challenge injection. While 6-OHDA rats challenged with low dose SKF38393 (1 mg/kg) failed to display contralateral rotational behavior in any priming group, challenge with high dose SKF38393 (10 mg/kg) led to rotational behavior in all priming groups — with a moderate response in water-primed rats and a larger response, of equal magnitude, in D1- and D2-primed rats. Challenge with low dose SKF38393 (1 mg/kg) led to robust striatal Fos expression in D1-primed animals, but not in water- or D2-primed rats, while challenge with high dose SKF38393 (10 mg/kg) produced equally robust striatal Fos expression in all priming groups. Lastly, 6-OHDA rats challenged with the D2 agonist quinpirole (0.25 mg/kg) displayed enhanced contralateral rotational behavior and significant striatal Fos expression in D1-primed animals, while D2-primed rats showed only enhanced rotational behavior and baseline levels of striatal Fos expression — results that are consistent with our previous findings (Pollack and Yates, 1999).

The effects of the D1 agonist SKF38393 on rotational behavior were dose-dependent, and related to the time post-6-OHDA lesion when the drug was first administered. For example, 6-OHDA rats, which received the first priming injection with SKF38393 (10 mg/kg) at three weeks post-lesion, demonstrated only mild rotational behavior (~60 contralateral rotations/30 min; Table 1). In contrast, challenge of water-primed 6-OHDA rats with the same dose of SKF38393 (10 mg/kg), corresponding to six weeks post-lesion, produced significant contralateral rotational behavior (over 500 contralateral rotations/110 min; Table 2). While these rotational responses were measured for different durations of time (priming 30 min, challenge 110 min), another contributing factor is likely the duration of time post-6-OHDA lesion prior to the first administration SKF38393. Consistent with our results, Morelli et al. (1989) observed a significant role of time post-lesion on the behavioral effects of SKF38393 such that treatment of drug-naïve 6-OHDA rats with SKF38393 (2 mg/kg) led to rotational behavior at 60 and 90 days post-lesion, but not at 17 or 24 days post-lesion. These observations indicate that the time post-6-OHDA lesion plays a significant role on the development of dopamine receptor supersensitivity and its behavioral manifestations — an effect that is believed to be expedited by priming, but not dependent on it for its expression (Nadjar et al., 2009).

D1- and D2-priming had equivalent effects on enhancing D1-mediated rotational behavior when 6-OHDA rats were challenged with high dose SKF38393 (10 mg/kg), but had no effect on rotational behavior following challenge with low dose SKF38393 (1 mg/kg). These data are consistent with results using a single-injection priming paradigm in 6-OHDA rats, in which D1 and D2 agonists displayed both 'homologous' as well as 'heterologous' sensitization (Morelli et al., 1989; Morelli and Di Chiara, 1987). However, our data, together with past studies, create a fuller picture of this effect. Using a single-injection priming paradigm, challenge with SKF38393 (2 mg/kg) led to rotational behavior in D1- or D2-primed 6-OHDA rats (Morelli et al., 1989; Morelli and Di Chiara, 1987), whereas using

Fig. 1. Photomicrographs of Fos-like immunoreactivity in the ipsilateral striatum of 6-OHDA rats 2 h following challenge injection with water, SKF38393 or quinpirole. Each photomicrograph is labeled as 'priming treatment/challenge treatment'. Groups were primed with water (left column), SKF38393 (middle column) or quinpirole (right column), and challenged with water (first row), SKF38393 (1 mg/kg, second row), SKF38393 (10 mg/kg, third row) or quinpirole (0.25 mg/kg, fourth row). Scale bar = 0.1 mm.


Water/Water
SKF/Water
Quin/Water
Water/SKF(1)
SKF/SKF(1)
Quin/SKF(1)
Water/SKF(10)
SKF/SKF(10)
Quin/SKF(10)
Water/Quin
SKF/Quin
Quin/Quin

our three-injection priming paradigm, challenge with half this dose of SKF38393 (1 mg/kg) proved too low to elicit any rotational behavior in D1- or D2-primed 6-OHDA rats (Table 2). Taken together, these observations suggest that three priming injections do not give rise to any more pronounced degree of behavioral sensitization than a single priming injection. Therefore, a single injection with an efficacious dose of dopamine agonist is both adequate and sufficient to trigger the mechanism(s) responsible for D1-mediated behavioral sensitization in dopamine-depleted rats. In contrast, other groups have reported that longer, repeated treatments with D1 agonists can give rise to behavioral tolerance rather than sensitization (Asin et al., 1995; Asin and Wirtshafter, 1993; Engber et al., 1993). This effect could be due, in part, to the nature of the dopamine agonist used — the partial D1 agonist SKF38393 versus full D1 agonist A-77636 (Asin and Wirtshafter, 1993). However, behavioral tolerance was also related to the *timing* of the priming injections since daily, repeated treatment with the partial D1 agonist SKF38393 led to behavioral tolerance following prolonged treatment (Engber et al., 1993), even when behavioral sensitization was noted in the first few days of treatment (Rouillard et al., 1988).

The effects of SKF38393 on striatal Fos expression were dose-dependent, and sensitive to prior drug exposure (priming). While administration of SKF38393 (10 mg/kg) to water-primed 6-OHDA rats led to robust striatal Fos expression — an effect independent of D1- or D2-priming, this response was also dose-dependent since administration of low dose SKF38393 (1 mg/kg) to water-primed 6-OHDA rats did not induce striatal Fos expression (Table 3; Fig. 1). These data suggest that D1 receptor stimulation alone, administered at a sufficient dose, can induce striatal IEG expression in dopamine-depleted animals, an observation supported by others (Nadjar et al., 2009; Robertson et al., 1992; Wirtshafter, 2007). However, we also observed an effect of priming since D1-primed 6-OHDA rats challenged with low dose SKF38393 (1 mg/kg) displayed robust striatal Fos expression compared to rats primed with water or D2 agonist (Table 3, Fig. 1). These data suggest that prior D1 receptor stimulation can sensitize subsequent D1-mediated striatal IEG induction. A similar effect of D1-priming on subsequent striatal Fos expression was also observed in 6-OHDA rats challenged with the D2 agonist quinpirole (0.25 mg/kg) (Table 3; Fig. 1; Pollack and Yates, 1999). It is unclear whether the same mechanism(s) is involved in D1-priming of D1- and D2-mediated IEG responses. D1-mediated Fos induction occurs primarily in striatonigral neurons (Robertson et al., 1990), which comprise the 'direct pathway', which links the striatum to the basal ganglia output structures: substantia nigra pars reticulata (SNr) and globus pallidus pars interna (GPi) (termed entopeduncular nucleus in rodents) (Obeso et al., 2008). Interestingly, we reported that 'direct pathway' neurons also show robust Fos expression when 6-OHDA rats are primed with the D1/D2 agonist apomorphine (0.5 mg/kg) and challenged with quinpirole (Pollack et al., 1997), suggesting that similar priming mechanism(s) may be involved. However, this is probably not the case for several reasons. First, D1 and D2 receptors are localized to separate populations of striatal output neurons — with D1 receptors expressed primarily on 'direct pathway' striatonigral neurons, while D2 receptors are expressed primarily on striatopallidal neurons (Gerfen et al., 1990; Le Moine and Bloch, 1995); these neurons comprise the 'indirect pathway', which links the striatum to the SNr/GPi through synaptic connections in the globus pallidus pars externa and subthalamic nucleus (Obeso et al., 2008). Second, we found that the pattern of striatal Fos expression in D1-primed rats was slightly different following challenge with quinpirole and SKF38393 (data not shown); our results were consistent with prior studies, which reported that D2-mediated Fos expression was more concentrated in the dorsolateral striatum (Pollack and Yates, 1999), while D1-mediated Fos expression was more uniformly distributed in the striatum (Paul et al., 1995). Taken together, a more likely explanation is that D1-priming of D2-mediated striatal Fos expression occurs via a trans-synaptic mechanism, such as recurrent axon collaterals in the striatum (Taverna et al., 2008), rather than through direct effects within the same population of striatal output neurons (Gerfen et al., 1990; Le Moine and Bloch, 1995).

While the effects of the D1 agonist SKF38393 on behavioral and cellular responses in 6-OHDA rats are clearly dose-dependent, there appears to be a disconnection between the effective dose of SKF38393 required to stimulate significant rotational behavior and to induce striatal Fos expression. For example, while D1-priming could permit a challenge with low dose SKF38393 (1 mg/kg) to induce robust striatal Fos expression, the same dose of SKF3839 (1 mg/kg) did not produce any rotational behavior in D1-primed animals. A similar dosage disconnection was also observed in water-primed 6-OHDA rats challenged with high dose SKF38393 (10 mg/kg), which led to only moderate rotational behavior, but pronounced striatal Fos expression. And while D1- and D2-priming was able to potentiate SKF38393 (10 mg/kg)-mediated contralateral rotational behavior, D1- and D2-priming had no effect on SKF38393 (10 mg/kg)-mediated striatal Fos induction, which was already at maximal levels in water-primed 6-OHDA rats (Table 3; Fig. 1). Therefore, D1-mediated striatal Fos expression is able to occur independently of D1-mediated rotational behavior, and the level of striatal Fos expression is not necessarily related to the amount of rotational behavior. These data suggest that the priming of D1-mediated rotational behavioral and striatal Fos expression is likely mediated by separate mechanisms, and that Fos protein expression itself is not required for the expression of D1-mediated rotational behavior —observations consistent with other studies using single-injection priming paradigms (Paul et al., 1995; Robertson et al., 1989). However, other IEGs, most notably FosB and JunD proteins, appear to play a significant role in D1 receptor priming (Crocker et al., 1998; Vallone et al., 1997). In fact, dopamine-depletion combined with D1 receptor stimulation leads to numerous changes in striatal cell signaling including activation of ERK1/2/MAP kinase (Gerfen et al., 2002), DARPP-32 phosphorylation (Barone et al., 1994) as well as increased activator protein 1 (AP-1) binding (Huang and Walters, 1996; Kashihara et al., 1996). In addition, D1 receptor stimulation may underlie activation of ERK, which is thought to play a key role in cocaine-mediated behavioral sensitization (Lu et al., 2006). As such, these intracellular signals could ultimately affect the expression of genes, whose products may underlie the persistence of behavioral sensitization once it has been established (Pollack and Strauss, 1999). However, it is important to note that the number and timing of D1 agonist injections determines the nature of the subsequent response — sensitization versus tolerance in dopamine-depleted rats (Asin et al., 1995; Asin and Wirtshafter, 1993; Engber et al., 1993; Morelli et al., 1989; Morelli and Di Chiara, 1987; Rouillard et al., 1988). Recent studies suggest that intracellular trafficking and localization of D1 receptors (Berthet et al., 2009; Guigoni et al., 2007) and/or its heterodimer partner *N*-methyl-D-aspartate glutamate receptors (Fiorentini et al., 2006) may be related to subsequent behavioral responsiveness in animal models of L-dopa-induced dyskinesias. Therefore, future experiments should utilize treatment specific paradigms, run in parallel, which lead to sensitization or tolerance in dopamine-depleted rats, in order to determine which signaling molecules and intracellular processes underlie the opposing effects on D1-mediated plasticity.

Acknowledgements

We would like to thank Dr. Solange Brault for her assistance with the statistical analysis. This work was supported by the University of Massachusetts–Boston.

References

Asin KE, Bednarz L, Nikkel A, Perner R. Rotation and striatal c-fos expression after repeated, daily treatment with selective dopamine receptor agonists and levodopa. J Pharmacol Exp Ther 1995;273:1483–90.

Asin KE, Wirtshafter D. Effects of repeated D1 receptor stimulation on rotation and c-fos expression. Eur J Pharmacol 1993;235:167–8.

Barone P, Morelli M, Popolo M, Cicarelli G, Campanella G, Di Chiara G. Behavioural sensitization in 6-hydroxydopamine lesioned rats involves the dopamine signal transduction: changes in DARPP-32 phosphorylation. Neurosci 1994;61:867–73.

Berthet A, Porras G, Doudnikoff E, Stark H, Cador M, Bezard E, et al. Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesias. J Neurosci 2009;29: 4829–35.

Carey RJ. Chronic L-DOPA treatment in the unilateral 6-OHDA rat: evidence for behavioral sensitization and biochemical tolerance. Brain Res 1991;568:205–14.

Crocker SJ, Morelli M, Wigle N, Nakabeppu Y, Robertson GS. D1-receptor-related priming is attenuated by antisense-mediated 'knockdown' of fosB expression. Mol Brain Res 1998;53:69–77.

Engber TM, Marin C, Susel Z, Chase TN. Differential effects of chronic dopamine D1 and D2 receptor agonists on rotational behavior and dopamine receptor binding. Eur J Pharmacol 1993;236:285–93.

Fiorentini C, Rizzetti MC, Busi C, Bontempi S, Collo G, Spano P, et al. Loss of synaptic D1 dopamine/*N*-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesias in the rat. Mol Pharmacol 2006;69:805–12.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr FJ, et al. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 1990;250:1429–32.

Gerfen CR, Miyachi S, Paletzki R, Brown P. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 2002;22:5042–54.

Guigoni C, Doudnikoff E, Li Q, Bloch B, Bezard E. Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. Neurobiol Dis 2007;26:452–63.

Hudson JL, van Horne CG, Stromberg I, Brock S, Clayton J, Masserano J, et al. Correlation of apomorphine- and amphetamine-induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. Brain Res 1993;626: 167–74.

Huang K-X, Walters JR. Dopaminergic regulation of AP-1 transcription factor DNA binding activity in rat striatum. Neuroscience 1996;75:757–75.

Kashihara K, Akiyama K, Ishihara T, Shiro Y, Shohmori T. Levodopa but not bromocriptine induces AP-1 and CREB DNA-binding activity in the dopamine-depleted striatum of the rat. Life Sci 1996;58:159–70.

Le Moine C, Bloch B. D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 1995;355:418–26.

Lu L, Koya E, Zhai H, Hope B, Shaham Y. Role of ERK in cocaine addiction. Trends Neurosci 2006;29:695–703.

Morelli M, Di Chiara G. Agonist-induced homologous and heterologous sensitization to D-1- and D-2-dependent contraversive turning. Eur J Pharmacol 1987;141:101–7.

Morelli M, Fenu S, Garau L, Di Chiara G. Time and dose dependence of the 'priming' of the expression of dopamine receptor supersensitivity. Eur J Pharmacol 1989;162: 329–35.

Nadjar A, Gerfen CR, Bezard E. Priming for L-dopa-induced dyskinesias in Parkinson's disease: a feature inherent to the treatment or the disease? Prog Neurobiol 2009;87:1–9.

Obeso JA, Rodriguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, et al. Functional organization of the basal ganglia: therapeutic implications for Parkinson's Disease. Mov Disord 2008;23:S548–59.

Paul ML, Currie RW, Robertson HA. Priming of a D1 dopamine receptor behavioural response is dissociated from striatal immediate early gene activity. Neuroscience 1995;66:347–59.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 2nd ed. San Diego: Academic Press; 1986.

Pollack AE, Strauss JB. Time dependence and role of *N*-methyl-D-aspartate glutamate receptors in the priming of D2-mediated rotational behavior and striatal Fos expression in 6-hydroxydopamine lesioned rats. Brain Res 1999;827:160–8.

Pollack AE, Turgeon SM, Fink JS. Apomorphine priming alters the response of striatal outflow pathways to D2 agonist stimulation in 6-hydroxydopamine rats. Neuroscience 1997;79:79–93.

Pollack AE, Yates TM. Prior D1 dopamine receptor stimulation is required to prime D2-mediated striatal Fos expression in 6-hydroxydopamine lesioned rats. Neuroscience 1999;94:504–14.

Robertson GS, Vincent SR, Fibiger HC. Striatonigral projection neurons contain D1 dopamine receptor-activated c-fos. Brain Res 1990;523:288–90.

Robertson GS, Vincent SR, Fibiger HC. D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. Neuroscience 1992;49:285–96.

Robertson HA, Peterson MR, Murphy K, Robertson GS. D1-dopamine receptor agonists selectively activate striatal c-fos independent of rotational behavior. Brain Res 1989;503:346–9.

Rouillard C, Bedard P, Falardeau R, Di Paolo T. Repeated stimulation of D1 dopamine receptors increases the circling response to bromocriptine in rats with a 6-OHDA lesion. Eur J Pharmacol 1988;157:125–33.

Taverna S, Ilijic E, Surmeier DJ. Recurrent collateral connection of striatal medium spiny neurons are disrupted in models of Parkinson's disease. J Neurosci 2008;28: 5504–12.

Ungerstedt U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand Suppl 1971;367: 69–93.

Vallone D, Pellecchia MT, Morelli M, Verde P, DiChiara G, Barone P. Behavioural sensitization in 6-hydroxydopamine-lesioned rats is related to compositional changes in AP-1 transcription factor: evidence for induction of FosB- and JunD-related proteins. Mol Brain Res 1997;52:307–17.

Wirtshafter D. Rotation and immediate-early gene expression in rats treated with the atypical D1 dopamine agonist SKF 83822. Pharmacol Biochem Behav 2007;86:505–10.