BILATERAL CONTROL OF BRAIN ACTIVITY BY DOPAMINE D1 RECEPTORS: EVIDENCE FROM INDUCTION PATTERNS OF REGULATOR OF G PROTEIN SIGNALING 2 AND c-fos mRNA IN D1-CHALLENGED HEMIPARKINSONIAN RATS

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Abstract—Recent reports show that striatal dopamine D1-type receptors from one side of the normal rat brain can control brain activity (as measured by c-fos induction) on both sides of the brain. However, this phenomenon has not yet been studied in the presence of sensitized dopamine D1-type receptors. Here we address this issue by investigating the extent to which dopamine D1-type receptors control brain activation in rats with unilaterally sensitized dopamine D1-type receptors. Gene induction assays were used to identify activated regions from midbrain to forebrain in unilaterally 6-hydroxydopamine lesioned (hemiparkinsonian) rats challenged with the full dopamine D1-type agonist SKF82958 (3 mg/kg, 0.5 and 2 h). The genes used are c-fos, the proven neuronal activity marker, and Regulator of G protein Signaling 2, a gene we propose as a marker of signaling homeostasis. SKF82958-mediated induction of both genes is greatly enhanced in hemiparkinsonian rats compared with shams, in both the lesioned and the intact hemisphere. For example, in the denervated caudate-putamen at 2 h postinjection, this enhancement is more than 80-fold for c-fos and up to 20-fold for Regulator of G protein Signaling 2; for the intact side this is 35-fold for c-fos and 27-fold for Regulator of G protein Signaling 2. Cortical induction of c-fos and Regulator of G protein Signaling 2 was generalized to most neocortical regions and was essentially equivalent in both the denervated and intact hemispheres. Interestingly, hippocampal structures also showed strong bilateral induction of both genes. This overall pattern of brain activation can be accounted for by the basal ganglia thalamocortical and hippocampal circuits which both contain hemisphere-crossing connections and which can be initially activated in the lesioned hemisphere. Some regions, such as the intact striatum or the CA1 region, showed relatively low c-fos induction and relatively high Regulator of G protein Signaling 2 induction, possibly

indicating that these regions are engaged in unusually strong signaling regulation activities. Our results show that, besides basal ganglia-thalamocortical circuits, dopamine D1-type-mediated brain activation in hemiparkinsonian rats also involves hippocampal circuits. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: SKF82958, SKF38393, 6-hydroxydopamine, basal ganglia, hippocampus, Parkinson's disease.

Knowledge of the control of D1-type receptors (subtypes D1 and D5, further referred to as D1) over brain function is important to Parkinson's disease. Indeed, these receptors are among those influenced by the main drug used in the pharmacotherapy of Parkinson's disease, levodopa. Also, drugs acting specifically at D1 receptors may potentially be beneficial for the symptomatic treatment of Parkinson's disease (Wachtel, 1991; Chase, 1998; Mailman et al., 2001). D1 receptors of the striatum are known to exert control over the activity of basal ganglia-thalamocortical circuits via the so-called 'direct' striatal output pathway (Gerfen, 2004). However, recent reports suggest that the modulatory control of striatal D1 receptors over the activity of cortical and subcortical extrastriatal neurons may be more widespread than expected. For example, a unilateral intrastriatal injection of SKF38393 in the normal rat can induce c-fos in the ipsilateral cortex as well as in the contralateral nucleus accumbens shell (Blandini et al., 2003). In addition, a unilateral injection of the D1 antagonist SCH23390 into the normosensitive striatum is sufficient for a bilateral block of both c-fos and zif268 induction in the striatum and cerebral cortex by D1/D2 activation with systemic apomorphine (Steiner and Kitai, 2000). These notions that striatal D1 receptors from one side of the brain can control brain activity on both sides of the brain have yet to be examined in pathological conditions such as in experimental Parkinsonism. However, we recently observed that the expression of the Regulator of G protein Signaling type 2 (RGS2) gene in the striatum is dramatically upregulated by a D1 challenge with SKF82958 in unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats, both ipsilateral and contralateral to the lesion (Taymans et al., 2004). As sensitized dopamine receptors are found only in the lesioned hemisphere while RGS2 induction was seen in striata of both hemispheres, this result again indicates that the modulatory actions of D1 receptors cross the hemispheric divide.

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RGS2 is part of a protein family whose members share the common feature of regulating G protein coupled receptor signaling events via interactions with G proteins and effectors (for reviews see Kehrl and Sinnarajah, 2002; Hollinger and Hepler, 2002). The predominant regulatory interactions described specifically for RGS2 are the attenuation of Gq- and adenylyl cyclase- (AC) mediated signaling cascades (Kehrl and Sinnarajah, 2002). An additional feature of RGS2 is its rapid gene induction. For instance, we have previously shown that RGS2 can be rapidly regulated in the striatum by D1 receptors in a cAMP dependent manner (Taymans et al., 2003, 2004). RGS2 is also rapidly induced in the brain by a variety of other stimuli such as electroconvulsive seizures (Ingi et al., 1998; Gold et al., 2002) or precipitated morphine withdrawal (Gold et al., 2003). Because of its fast induction and its cellular role in signaling regulation, we and others have proposed that RGS2 gene expression changes may be indicative of a cellular response to changes in receptor signaling (Burcett et al., 1998; Taymans et al., 2003, 2004). In the present report, we would like to formally use RGS2 as a marker for signaling homeostasis. In this capacity, the use of RGS2 in activation studies is complementary to *c-fos*, with *c-fos* induction indicating neurons which have been activated and RGS2 induction indicating neurons which are actively responding to altered receptor signaling.

Although the D1-mediated induction patterns of RGS2 in hemiparkinsonian rats described above are most likely explained by the influence that D1 containing striatal neurons may have on extrastriatal regions via polysynaptic hemisphere-crossing connections, the exact role of different dopaminergic circuits remains unclear. Indeed, little information is available in D1 challenged hemiparkinsonian rats on marker gene induction in major structures of basal ganglia-thalamocortical circuitry other than the striatum such as globus pallidus, substantia nigra or thalamus. Also the contribution of other brain regions, such as the hippocampus or cortex which receive dopaminergic innervation and which contain D1 neurons, is unknown. Specifically, recent reports showing alterations in the hippocampus of Parkinson's disease patients make this structure interesting to monitor in experimental models of Parkinsonism (Camicioli et al., 2003; Bruck et al., 2004). The present report addresses these issues by monitoring D1-mediated brain activity in hemiparkinsonian rats using two genes: *c-fos*, a proven neuronal activity marker, and RGS2, a proposed marker for signaling homeostasis. Our results show that unilaterally sensitized D1 receptors influence neuronal activity bilaterally via circuits involving not only the basal ganglia, but also the hippocampus and cerebral cortex.

EXPERIMENTAL PROCEDURES

Animals

Animals were housed in a rat raising facility on a 12-h light/dark cycle with food and water *ad libitum*. All efforts were undertaken to minimize animal suffering and to minimize the number of the animals used in the course of the experiments. All procedures were conducted in strict accordance with the European Commu-

nities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the animal care and use committee of Johnson & Johnson Pharmaceutical Research and Development.

Stereotaxic lesions

For the 6-OHDA and sham lesions of the ascending medial forebrain bundle, male Wistar rats ($n=36$) weighing 190–240 g were anaesthetized with Nembutal (60 mg/kg i.p., Sanofi Santé Animale Benelux, Brussels, Belgium) and positioned in a Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA). Rats received a unilateral stereotaxic injection of 8 μ g 6-OHDA (dissolved in 4 μ l 0.9% NaCl containing 0.05% ascorbic acid, the lesion group) or solvent (4 μ l 0.9% NaCl containing 0.05% ascorbic acid, the sham group) into the right medial forebrain bundle. Coordinates used were 3.4 mm anterior and 1.6 mm lateral (right) from Bregma and 8.2 mm below the cortical surface according to the atlas of Paxinos and Watson (1986). After the infusion, the injection cannula was left in place for an additional 4 min to allow diffusion, and then carefully removed. Rats were allowed to recover for 4 weeks before drug treatment.

Drug dosing

The 6-OHDA lesioned rats were divided randomly into four groups of eight to 10. Sham lesioned rats were divided randomly into two groups of six. Rats from two lesion groups and one sham group were injected intraperitoneally with the full D1 agonist SKF82958 (3 mg/kg), the other groups received solvent (H_2O). The injection volumes were 1 ml/kg. Rats were killed by decapitation at 0.5 or 2 h post-injection for the lesion groups and at 2 h post-injection for the sham groups. The brains were dissected out and rapidly frozen in dry-ice-cooled 2-methylbutane ($-40^\circ C$). Brains were then conserved at $-70^\circ C$ until sectioning. Twenty-micron-thick frontal sections were cut out of the brains at the level of the caudate-putamen (Bregma +1.00 mm), globus pallidus (Bregma -0.92 mm), hippocampus/thalamus (Bregma -3.60 mm) and substantia nigra (Bregma -5.30) using a Leica C3050 cryostat microtome (Leica Microsystems, Wetzlar, Germany) and thaw-mounted on adhesive microscope slides (SuperFrost, Knittel Gläser, Germany). The sections were then kept at $-70^\circ C$ until use in *in situ* hybridization or radioligand autoradiography of dopamine transporter.

In a separate series of drug dosing, normal male Wistar rats weighing 180–220 g were randomly paired. Of each pair, one rat was dosed intraperitoneally with SKF82958 (3 mg/kg) and the second was administered vehicle (H_2O), as described in Taymans et al. (2003). The injection volumes were 1 ml/kg. The rats were killed in pairs by decapitation at 0.5, 1, 2, or 4 h after drug or vehicle injection. Three to four pairs of animals were used for each time point. The brains from each rat pair were dissected out in parallel and rapidly frozen in dry-ice cooled 2-methylbutane ($-40^\circ C$). Brains were then conserved at $-70^\circ C$ until sectioning. Twenty-micron-thick frontal hemisections, composed of one hemisphere of a drug dosed rat and one hemisphere of the corresponding vehicle dosed rat, as described in Taymans et al. (2003) were cut out of the brains at the level of the caudate-putamen using a Leica C3050 cryostat microtome (Leica Microsystems) and thaw-mounted on adhesive microscope slides (SuperFrost). The sections were then kept at $-70^\circ C$ until use in *in situ* hybridization.

Behavioral evaluation

On the day of injections (described above), animals were placed in polyvinyl chloride cylindrical boxes (diameter 50 cm) from 20 min prior to injection until kill. Animals were filmed, so as to evaluate the presence of rotational behavior and stereotypical behavior. Each animal was manually scored for 1 min every 10 min for number and direction of turns, as well as number of head rears.

Dopamine transporter (DAT) autoradiography

Because DAT is a marker for nigrostriatal fiber density, labeling of DAT with the dopamine transport inhibitor, [125 I]-RTI-55 (2000 Ci/mmol), and autoradiography was performed on the brain sections of the 6-OHDA and sham-lesioned animals in order to measure their degree of lesion. The experimental protocol applied is essentially described by Rocha et al. (1998). After thawing, the brain sections were dried under a cold air stream and pre-incubated (3×10 min) in 50 mM Tris–HCl (pH 7.4), 120 mM NaCl at room temperature. The slices were then incubated for 60 min at room temperature with the radioligand in buffer containing 50 mM Tris–HCl (pH 7.4), 120 mM NaCl and 100 nM citalopram to prevent labeling of the serotonin transporter. Non-specific binding was determined by addition to the incubation medium of 100 μ M cocaine. To stop the incubation, the slides were washed (1×1 min then 2×10 min) in 50 mM Tris–HCl (pH 7.4), 120 mM NaCl at 4 °C followed by a rapid dip in cold demineralized water and drying under a stream of cold air. Sections were exposed together with 125 I standard strips (Amersham, UK) to Kodak Ektascan NB film (Kodak, Chalon, France) for 4–7 days at room temperature.

In situ hybridization histochemistry (ISHH)

ISHH for RGS2 was performed as previously described (Taymans et al., 2002). Antisense 32 P-labeled riboprobes complementary to RGS2 mRNA were produced using T3 transcription systems (Promega, Madison, WI, USA), using a linearized RGS2 plasmid construct as a template (Taymans et al., 2002). After thawing, slides were dried under a cold air stream and subsequently fixed in paraformaldehyde. Sections were then deacetylated, dehydrated, delipidated and finally rehydrated and dried. Hybridization buffer containing 6.10^6 cpm of RGS2 probe per 100 μ l was applied to the sections. Sections were coverslipped and hybridized for 16 h at 60 °C in a humid chamber. Following hybridization, sections were rinsed in SSC 1 $\times$, washed in SSC 2 $\times$ –formamide, treated with RNase A and rinsed in SSC 2 $\times$. Finally, sections were rinsed in 70% ethanol and air dried. Sections were exposed to a Kodak Biomax MR film together with 14 C standard strips (ARC, St. Louis, MO, USA) for a period varying between 1 and 3 days.

For c-fos ISHH, a 29 mer oligonucleotide complementary to c-fos mRNA (nucleotides 7–36 of GenBank sequence X06769) was end labeled with 32 P-dATP (Amersham) using 12 units of terminal deoxynucleotidyl transferase (Roche, Mannheim, Germany) to a specific activity of $\sim 5.10^8$ cpm/ μ g. Sections were submitted to pre-hybridization treatment as described above. The end-labeled nucleotide was diluted in the hybridization buffer (same composition as above; Taymans et al., 2002) to 4.10^5 cpm per 100 μ l. The hybridization mixture was applied to the sections which were then coverslipped and hybridized for 16 h at 41 °C in a humid chamber. Following hybridization, sections were rinsed in SSC 1 $\times$ and submitted to wash steps in SSC 2 $\times$ –formamide (3×8 min) at 51 °C. Following rinsing in SSC 2 $\times$ (2×5 min), sections were rinsed in 70% ethanol and air dried. Sections were exposed to a Kodak Biomax MR film together with 14 C standard strips (ARC) for a period varying between 1 and 2 weeks.

Autoradiographic detection and quantification

Sections were exposed to films as described above. Following exposure, films were developed manually in Kodak D19 and fixed with Kodak Ready-matic. Autoradiograms were submitted to densitometric analysis using an MCID M4 digital analyzer (Imaging Research Inc., St. Catharines, Ontario, Canada). Polynomial calibration curves were fitted to relative density values from the known 14 C or 125 I radioactive standards that were co-exposed with the sections. It should be noted that the units of the radioactivity values given for ISHH sections labeled with 32 P are approximate

units as they are derived from 14 C standards. On each section, structures were delineated interactively (see Fig. 1 for diagrammatic representation of regional delineations) and pixel relative optical density values were measured in the outlined areas. For each pixel, these values were converted to radioactivity per milligram of tissue using the calibration curves and subsequently averaged over the measured area. Mean regional labeling densities were sampled from three to six sections per rat for RGS2 or c-fos mRNA. For DAT, the mean regional labeling density was given as the difference between the total binding and non-specific binding measured in adjacent sections and was sampled from two to four sections per rat.

For the time course, net c-fos induction is expressed as nCi/g tissue equivalent (teq) and is given by the mean regional labeling density of c-fos mRNA of the drug exposed caudate-putamen minus the mean regional labeling density of the corresponding control caudate-putamen.

For the lesion and sham groups, the degree of denervation for a given rat was given by 100% minus the percent DAT labeling in the caudate-putamen of the lesioned hemisphere versus the intact hemisphere. Only those lesion animals showing more than 95% reduction in nigrostriatal fiber density were included in the analysis (by using this cutoff, there was a minimum of six lesioned rats per drug or vehicle group). No sham animals were excluded from the analysis (sham group individuals shows less than 5% loss of DAT). In lesion rats, RGS2 or c-fos expression was measured for several brain regions (see Table 1 and Fig. 1) including regions of the cerebral cortex, striatum, hippocampus, thalamus and basal ganglia, identified according to the stereotaxic atlas by Paxinos and Watson (1986). These regions were chosen in relation to their relation to the direct activation of D1 receptors (i.e. striatal, neo-cortical or hippocampal structures) and eventual downstream circuit effects (i.e. structures of the basal ganglia–thalamocortical circuit). In sham rats, RGS2 or c-fos expression was measured in the caudate-putamen. For each region, density measurements were performed in both the lesioned (or sham) and intact hemispheres. Absolute values of c-fos and RGS2 expression levels are given for the caudate-putamen in Fig. 3. For all other figures and tables, RGS2 and c-fos gene expression changes for each region in the drug challenge groups are calculated by subtracting the average labeling density of the region quantified in the vehicle group from the average labeling density of the corresponding region in the drug challenge groups, i.e. the unilaterally 6-OHDA (or sham) -lesioned group killed 0.5 or 2 h following i.p. administration of H_2O .

Illustrations of film autoradiograms for the figures were digitally recorded with the MCID M4 digital analyzer as bright field photomicrographs. Illustrations were prepared with Adobe Photoshop version 7.0 (Adobe Systems Incorporated, San Jose, CA, USA) for contrast and brightness adjustments and cropping.

Statistics

For the time course, the log values of the mean c-fos values of each time point were compared using the analysis of variance (ANOVA), and the Tukey test was applied in post hoc analysis to determine which values were significantly different. As a control, the caudate-putamen expression values of the sham groups and the lesion 2 h groups were compared by two-way ANOVA using drug (D1 agonist or vehicle) and lesion (sham or lesion) as factors, followed by post hoc *t*-tests to describe differences between individual groups. Drug effects in the lesion groups were determined for each region with two-way ANOVA using time (0.5 h or 2 h) and drug (SKF82958 or vehicle) as factors, followed by post hoc *t*-tests to describe differences between individual groups. Interhemispheric differences were evaluated for those regions testing positive in the two-way ANOVA with a Student's *t*-test for paired observations. For related hippocampal structures (dentate gyrus (DG), CA1 and CA3 fields), RGS2 or c-fos were compared by

Fig. 1. Diagrammatic representation of analyzed brain regions. The delimitation of the regions submitted to densitometric analysis in hybridized sections is shown (see list of structures in Table 1). Measurements were performed in sections at the level of the striatum, globus pallidus, hippocampus and substantia nigra. AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; CA1; CA3; Cg, cingulate cortex; CPu,

Table 1. Anatomical regions surveyed

Cerebral cortex	Globus pallidus
Cingulate cortex	Hippocampal formation
Motor cortex	Dentate gyrus
Somatosensory cortex	CA1
Piriform cortex	CA3
EC	Thalamus
Medial septal nucleus	Medial geniculate nucleus
Striatum	Principal relay nuclei
Caudate/putamen rostral	Substantia nigra, pars compacta
Nucleus accumbens, shell	Substantia nigra, pars reticulata
Nucleus accumbens, core	
Olfactory tubercle	

These regions were chosen based on their relation to the direct activation of D1 receptors (i.e. striatal, neocortical or hippocampal structures) and eventual downstream circuit effects (i.e. structures of the basal ganglia–thalamocortical circuit).

ANOVA and the Bonferroni test for multiple comparisons was applied in post hoc analysis to determine which values were significantly different. For all tests, the required minimum levels for statistical significance were set at $P < 0.05$.

RESULTS

Transient upregulation of striatal c-fos by SKF82958 in normal rats

As shown in Fig. 2, systemic injection of the full D1 agonist SKF82958 (3 mg/kg i.p.) rapidly and transiently induced c-fos in the caudate-putamen. Maximum induction occurs at 0.5 h and c-fos levels return below detection limits at 4 h. Based on these observations, gene regulations in the lesioned animals given below were measured at 0.5 and 2 h following D1 challenge.

Induction of RGS2 and c-fos by SKF82958 in sham animals compared with lesioned animals

Caudate-putamen levels of c-fos and RGS2 mRNA were measured in brains of unilaterally 6-OHDA or sham lesioned rats at 2 h following injection of SKF82958 (3 mg/kg) or vehicle ($n=6$ for sham vehicle group 2 h, $n=6$ for sham drug group 2 h, $n=8$ for 6-OHDA vehicle group 2 h, $n=6$ for 6-OHDA drug group 2 h). For the 6-OHDA-lesioned animals, only those animals displaying a greater than 95% lesion as quantified by the reduction in DAT autoradiographic signal were included in the analysis. As revealed by two-way ANOVA using lesion and drug as factors, SKF82958 significantly increases c-fos and RGS2 in the caudate-putamen of both the sham and the lesion groups, and these inductions are significantly enhanced in the lesion group compared with the sham group by more than 80-fold for c-fos (for the lesioned hemisphere, net induction of 100 nCi/g teq in the lesion caudate-putamen vs. 1.2 nCi/g teq in the sham caudate putamen) and almost

caudate-putamen; GP, globus pallidus; LEnt, lateral entorhinal cortex; M, motor cortex; MG, medial geniculate thalamic nucleus; MS, medial septal nucleus; Pir, piriform cortex; S1, somatosensory cortex; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; Th, principal thalamic relay nuclei; Tu, olfactory tubercle.

Transient induction of c-fos in caudate-putamen by SKF82958 3 mg/kg ip

Fig. 2. Time course of SKF82958 induced c-fos upregulation in normal rat striatum. Animals were randomly paired and injected with the D1 agonist SKF82958 (3 mg/kg i.p.) or vehicle, as described in Experimental Procedures, and hemisections were collected by combining one hemisphere of the drug-injected rat (drug) with the opposite hemisphere of the corresponding vehicle-injected rat (ctrl). Graph points represent striatal c-fos (■) mRNA levels following drug injection relative to control at different time points (0.5–8 h, $n=3-4$ per time point, error bars represent the S.D.). Shown under the graph are representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of ^{33}P -labeled oligoprobes complementary to c-fos at 0.5 and 1 h. § $P<0.05$ relative to the 2 h graph point by Tukey post hoc test. Scale bar=5 mm.

20-fold for RGS2 (for the lesioned hemisphere, net induction of 1798 nCi/g teq in the lesion caudate-putamen vs. 91 nCi/g teq in the sham caudate-putamen) (Fig. 3). For the non-surgically manipulated hemisphere, these gene induction enhancements of 6-OHDA vs. sham in the caudate-putamen are 35-fold and 27-fold for c-fos and RGS2, respectively. A small proportion of the 6-OHDA-lesioned animals showed a degree of lesion of less than 95%. For these insufficiently lesioned animals which also received SKF82958, c-fos and RGS2 inductions were markedly lower than in the fully lesioned animals, further indicating that enhanced gene inductions were dependent on a full lesion.

c-fos Gene induction by SKF82958 in unilaterally 6-OHDA-denervated rats

Levels of c-fos induction were measured in brains of unilaterally 6-OHDA lesioned rats at 0.5 or 2 h following injection of SKF82958 (3 mg/kg i.p., $n=6$ 6-OHDA vehicle group 0.5 h, $n=7$ 6-OHDA drug group 0.5 h, n as above for 2 h groups). Only those animals displaying a greater than

95% lesion as quantified by the reduction in DAT autoradiographic signal were included in the analysis. The cerebral cortical, basal ganglia and other subcortical regions surveyed are listed in Table 1. At 0.5 h, the most significant changes are in striatal structures of both hemispheres as well as in the sensory and motor cortices of the lesioned hemisphere (Table 2, Fig. 3). Notable regions showing no significant induction are the globus pallidus and substantia nigra. There was a tendency for stronger c-fos induction in the lesioned hemisphere compared with the non-lesioned hemisphere in striatal and neocortical regions. This tendency was significant in the caudate-putamen, sensory cortex and olfactory tubercle. Within the hippocampal formation, c-fos appeared to be induced most strongly in the DG, although this was not statistically different from the vehicle group.

As can be seen from Table 2 and Figs. 3, 4 and 5, c-fos values were mostly higher at 2 h compared with 0.5 h. Of the regions without significant c-fos induction at 0.5 h, the cingulate cortex showed at 2 h significant c-fos levels; the globus pallidus and the substantia nigra nuclei did not.

Fig. 3. Expression of c-fos and RGS2 mRNA in unilaterally SHAM- or 6-OHDA-lesioned rats after D1 agonist treatment. (A) Illustrations of film autoradiograms show c-fos and RGS2 expression in frontal sections at the level of the striatum of rats having received a SHAM or 6-OHDA lesion of the right medial forebrain bundle and injected 4 weeks postsurgery with vehicle or the full D1 agonist SKF82958 (3 mg/kg s.c., 0.5 or 2 h). Exposure times for the autoradiograms in this figure are 10 days for all c-fos images and 36 h for all RGS2 images. Scale bar=5 mm. (B) Mean c-fos and RGS2 density values (mean $\pm$ S.E.M.) measured in the caudate putamen on the intact and lesioned side for the above groups ($n=6-7$ per group). Quantifications of c-fos and RGS2 induction per brain region are given in Tables 2 and 3. Note the striking difference in D1 induced c-fos or RGS2 induction between the SHAM and the 6-OHDA lesion groups, both in the lesioned and the intact hemisphere.

Significant interhemispheric differences were found only in striatal structures which all showed approximately two-fold higher c-fos levels in the lesioned hemisphere compared with the intact hemisphere (Table 2, Fig. 3). Intrahemispheric comparisons revealed that the DG showed significantly higher c-fos levels than other hippocampal structures (Fig. 8A).

RGS2 gene induction by SKF82958 in unilaterally 6-OHDA-denervated rats

The statistical tests used for c-fos were also applied to RGS2 values. With the exception of the piriform cortex of the lesioned hemisphere, only striatal regions showed significant RGS2 upregulation at 30 min following the SKF82958 injection in the hemiparkinsonian rats (Table 3,

Figs. 3 and 6). After 2 h, significant RGS2 upregulation is observed bilaterally in all neocortical, striatal and hippocampal structures measured as well as in the lateral septal nucleus (Table 3, Figs. 3, 6 and 7). In hippocampal structures, induction in the CA3 region is significantly less pronounced than in the DG or CA1 regions (Fig. 8B).

No significant interhemispheric differences were observed for RGS2 upregulation at either time point. No RGS2 expression changes were observed in the globus pallidus or in nigral regions.

Behavioral assessment of unilaterally 6-OHDA-denervated rats challenged with SKF82958

Contralateral rotations were observed within 10 min after the administration of SKF82958 in all rats showing com-

Table 2. c-fos Gene expression changes in rats lesioned in the right medial forebrain bundle with 6-OHDA and challenged with SKF82958 (3 mg/kg ip, 0.5 and 2 h)

Brain region	SKF82958 3 mg/kg ip 0.5 h				SKF82958 3 mg/kg ip 2 h			
	Intact		Lesion		Intact		Lesion	
	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.
Cingulate cortex	22.3	11.8	20.8	9.8	183.0** ^s	31.3	180.4*** ^s	32.9
Sensory cortex	15.6**	3.9	26.7*** ^x	3.0	111.0*** ^s	19.0	122.6*** ^s	25.1
Motor cortex	14.8*	4.8	26.7***	3.9	86.0*** ^s	13.8	96.8*** ^s	14.5
Piriform cortex	50.6*	18.3	96.4**	17.5	309.1* ^s	98.0	354.5*** ^s	61.7
EC	16.6	7.5	38.4**	7.9	148.8*** ^{ss}	29.7	209.7*** ^s	50.4
Caudate-putamen	17.8***	2.1	23.0*** ^x	0.7	45.4*	12.0	100.0*** ^{ss,xx}	9.6
Nucleus accumbens core	33.0***	5.2	37.6***	1.9	60.5*	15.8	130.4*** ^{ss,xx}	14.3
Nucleus accumbens shell	56.9***	2.4	72.3***	7.5	83.0*	24.1	201.1*** ^{ss,xx}	24.3
Olfactory tubercle	70.4***	11.4	173.1*** ^{xxx}	12.5	147.0**	35.5	266.9*** ^{s,x}	39.5
Lateral septum	28.6*	9.3	39.8**	10.6	193.6*** ^s	39.1	198.2*** ^s	38.1
Globus pallidus	0		0		0		0	
Medial geniculate nucleus	4.7**	1.0	9.7***	1.1	35.3*	8.0	65.8*** ^{ss}	13.3
Principal relay nuclei thalamus	3.2	2.1	5.6	2.4	29.7* ^s	7.7	48.3* ^s	14.1
Dentate gyrus	318.9*	120.6	321.2*	116.5	761.2**	176.6	858.7*** ^s	198.8
Hippocampus CA1	40.2*	11.9	33.1**	7.4	305.4*** ^s	64.6	292.5*** ^s	64.6
Hippocampus CA3	35.2*	9.7	45.9*	13.7	200.9*** ^s	29.2	257.6*** ^s	46.8
Substantia nigra pars compacta	0		0		0		0	
Substantia nigra pars reticulata	0		0		0		0	

Distribution of SKF82958 induced c-fos induction in hemiparkinsonian rats. Levels of c-fos mRNA following SKF82958 (3 mg/kg) or vehicle injection were measured in both hemispheres for several cortical and subcortical regions (listed in Table 1) by densitometric analysis of film autoradiograms of isotopic *in situ* hybridization histochemistry. Only those rats showing minimum 95% decrease in dopaminergic fibers were included in the analysis. Gene induction values were obtained for each structure by subtracting the mean densitometric values of the vehicle groups from the values of the corresponding drug groups. Shown are mean $\pm$ S.E.M. gene inductions at 0.5 h (left columns) and 2 h (right columns) expressed in nCi/g tissue equivalent. Values measured lower than 1 nCi/g tissue equivalent are given as 0. Representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of ³²P-labeled oligoprobes complementary to c-fos in the drug-challenged groups are displayed in Figs. 3, 4 and 5. *** Significant drug dependent gene expression change (drug vs. vehicle; $P < 0.05$; 0.01; 0.001 respectively). ^{s,ss} Significant time dependent gene expression change (2 h vs. 0.5 h; $P < 0.05$; 0.01; 0.001 respectively). ^{x,xx,xxx} Significant difference in gene expression changes for lesion hemisphere compared to intact hemisphere ($P < 0.05$; 0.01; 0.001 respectively).

plete denervation (>95%). In the 2 h group, rotations averaged five to seven contralateral rotations per minute from 10 to 70 min post-injection. Average contralateral rotations declined slowly after 70 min post-injection, reaching a value of 2.3 rotations per minute just prior to killing the rats at 2 h post-injection. Rotations were jerky and interspersed by bouts of head rearing. Head rearing was observed within 10 min after the administration of SKF82958. In the 2 h group, an average of 10–15 head rears per minute was observed from 10 min to 2 h post-injection.

DISCUSSION

This study was performed to investigate the extent to which D1 receptors control brain activation in rats with unilaterally sensitized D1 receptors. To this end, gene induction assays were used to identify activated regions from the midbrain to the forebrain in both the lesioned and non-lesioned hemispheres of hemiparkinsonian rats having received a D1 challenge. The genes used are the proven neuronal activity marker, c-fos, as well as the proposed marker of signaling homeostasis, RGS2. Our most significant findings are that brains of hemiparkinsonian rats

are activated bilaterally by a full D1 challenge and that this involves activation of basal ganglia-thalamocortical and hippocampal circuits.

Activation of structures of the basal ganglia-thalamocortical circuits in D1 challenged hemiparkinsonian rats

The D1 agonist used in the present study, SKF82958, is one of the most potent available (O'Boyle et al., 1989) and has previously been shown to induce c-fos and RGS2 in the normal rat striatum (Wang and McGinty, 1996; Svenningsson et al., 2000; Rushlow et al., 2000; Taymans et al., 2003, 2004). Again, in the present study, SKF82958 induces c-fos or RGS2 in all our experimental groups: normal rats, rats with a nonspecific lesion to the medial forebrain bundle (shams) and rats with a 6-OHDA lesion of the medial forebrain bundle (Figs. 2 and 3). However, it should be noted that the D1-mediated gene inductions observed here are heavily influenced by the 6-OHDA lesion. For instance, at 2 h postinjection, caudate-putamen c-fos levels induced by SKF82958 in the lesioned hemisphere are more than 80-fold higher in 6-OHDA-lesioned rats versus shams (sham, Fig. 3), while for RGS2 this difference is

Fig. 4. Regional pattern of c-fos expression 0.5 h following administration of SKF82958 to rats with deleted ascending dopamine pathways in the right hemisphere. As described in Experimental Procedures, animals were lesioned by stereotaxic injection of 6-OHDA into the medial forebrain bundle (right hemisphere) and injected with the D1 agonist SKF82958 (3 mg/kg i.p.) or vehicle at 4 weeks post-surgery and killed 0.5 h later. Shown are representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of 32 P-labeled oligoprobes complementary to c-fos in the drug-challenged group at the level of the globus pallidus, rostral hippocampus and caudal hippocampus. C-fos mRNA levels in the vehicle group were below the detection limit for most structures (not shown). Quantifications of c-fos induction per brain region are given in Table 2. Scale bar=5 mm.

almost 20-fold (Fig. 3). These important differences reproduce previous findings that D1 agonists much more readily induce genes in the denervated compared with the normal rat striatum (reviewed in Marshall et al., 1997; Gerfen, 2000) and can be attributed to the sensitized state of D1 receptors in the striatum of the 6-OHDA-lesioned hemisphere.

Intriguingly, enhanced gene activations are found also in the non-lesioned hemisphere. Indeed, for the caudate-putamen of the intact hemisphere, the difference in gene inductions at 2 h post-injection between 6-OHDA and sham groups is 35-fold and 27-fold for c-fos and RGS2, respectively (Fig. 3). As no sensitized D1 receptors are found in the intact hemisphere, these differences cannot be explained by the action of SKF82958 in the intact hemisphere. Rather, the D1 agonist is likely causing sensitized regions of the lesioned hemisphere to be hyperstimulated, and these regions in turn excite other regions transsynaptically. One initial focal point would logically be

Fig. 5. Regional pattern of c-fos expression 2 h following administration of SKF82958 to rats with deleted ascending dopamine pathways in the right hemisphere. As described in Experimental Procedures, animals were lesioned by stereotaxic injection of 6-OHDA into the medial forebrain bundle (right hemisphere) and injected with the D1 agonist SKF82958 (3 mg/kg i.p.) or vehicle at 4 weeks post-surgery and killed 2 h later. Shown are representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of 32 P-labeled oligoprobes complementary to c-fos in the drug challenged group at the level of the globus pallidus, rostral hippocampus and caudal hippocampus. C-fos mRNA levels in the vehicle group were below the detection limit for most structures (not shown). Quantifications of c-fos induction per brain region are given in Table 2. Scale bar=5 mm.

dopamine-denervated striatal regions. This hypothesis is consistent with our observation that neuronal activation revealed by c-fos induction at 0.5 h following D1 agonist injection is significantly higher in the olfactory tubercle and caudate-putamen of the lesioned hemisphere compared with the non-lesioned hemisphere. The fact that the intensity of striatal c-fos or RGS2 induction increases at 2 h compared with 0.5 h (Tables 2 and 3), contrarily to what is observed in the normal rat (Fig. 1, Taymans et al., 2004), suggests the activation of a closed, reinforcing circuit, as is the case for the basal-ganglia thalamocortical circuits. Finally, the contralateral rotations observed in these rats shortly after drug injection are also indicative of postsynaptic receptor supersensitivity in the denervated striatum (Ungerstedt, 1971; Gerfen, 2004).

From the focal point of the striatal structures of the lesioned hemisphere, it is possible for other structures, including structures in the contralateral hemisphere, to be stimulated through the basal ganglia–thalamocortical cir-

Table 3. RGS2 gene expression changes in rats lesioned in the right medial forebrain bundle with 6-OHDA and challenged with SKF82958 (3 mg/kg ip, 0.5 and 2 h)

Brain region	SKF82958 3 mg/kg ip 0.5 h				SKF82958 3 mg/kg ip 2 h			
	Intact		Lesion		Intact		Lesion	
	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.	Mean (nCi/g teq)	S.E.M.
Cingulate cortex	271.3	157.0	256.7	138.2	1954.3***§§	398.9	2002.8***§§	369.3
Sensory cortex	48.4	39.5	55.4	32.5	1138.0***§	251.6	1068.3***§§	196.6
Motor cortex	55.4	33.0	79.1	41.5	1070.6***§§	183.5	1174.2***§§	230.8
Piriform cortex	343.3	143.5	711.7*	227.3	4555.1***§§	727.6	6379.3***§	959.5
EC	0	12.9	0		156.6*§	44.4	260.9***§§	58.4
Caudate-putamen	469.2***	57.4	407.0***	50.4	2373.8*§	677.6	1798.9*§	602.9
Nucleus accumbens core	644.9*	190.3	517.1***	87.4	3292.9*§	836.7	2919.1***§	661.2
Nucleus accumbens shell	1001.3**	264.8	801.0***	78.9	4833.7***§	998.1	3424.8***§	699.4
Olfactory tubercle	585.2	280.3	1155.1***	180.2	4120.8*§	1196.8	4527.4***§§	939.7
Lateral septum	255.2	180.9	646.1	268.0	3082.5***§	697.3	3695.9***§	877.2
Globus pallidus	0		0		0		0	
Medial geniculate nucleus	0		8.5	4.1	8.2	7.0	21.3*	6.0
Principal relay nuclei thalamus	0		0		0		0	
Dentate gyrus	155.2	73.3	161.3	73.7	737.9***§§	104.9	962.0***§	188.2
Hippocampus CA1	22.4	19.2	28.5	19.8	539.2***§§§	66.9	626.8***§	92.1
Hippocampus CA3	33.5	21.0	39.7	21.7	119.4*§	41.1	177.3*§	47.7
Substantia nigra pars compacta	0		0		0		0	
Substantia nigra pars reticulata	0		0		0		0	

Distribution of SKF82958 induced RGS2 induction in hemiparkinsonian rats. Levels of RGS2 mRNA following SKF82958 or vehicle injection were measured in both hemispheres for several cortical and subcortical regions by densitometric analysis of film autoradiograms of isotopic *in situ* hybridization histochemistry. Only those rats showing minimum 95% decrease in dopaminergic fibers were included in the analysis. Gene induction values were obtained for each structure by subtracting the mean densitometric values of the corresponding vehicle groups from the values of the drug groups. Shown are mean±S.E.M. gene inductions at 0.5 h (left columns) and 2 h (right columns) expressed in nCi/g tissue equivalent. Values measured lower than 1 nCi/g tissue equivalent are given as 0. Representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of ³³P-labeled riboprobes complementary to RGS2 in both the drug and vehicle challenged groups are displayed in Figs. 3, 6 and 7. *** Significant drug dependent gene expression change (drug vs. vehicle; $P < 0.05$; 0.01; 0.001 respectively). § Significant time dependent gene expression change (2 h vs. 0.5 h; $P < 0.05$; 0.01; 0.001 respectively). §§§ No significant difference in gene expression changes for lesion hemisphere compared to intact hemisphere were found for RGS2 as measured by *t*-test.

cuits. Dopaminergic stimulation of striatofugal pathways decreases the activity of the basal ganglia output nuclei, i.e. the substantia nigra pars reticulata and the entopeduncular nucleus. Dopamine D1 receptors are classically associated with the direct striatonigral pathway, whereas D2 receptors are associated with the indirect striatopallidal pathway. The activation of the striatopallidal pathway by a D2 agonist in hemiparkinsonian rats leads to the induction of Fos protein expression in the globus pallidus (Paul et al., 1992; Marshall et al., 1993). Because the globus pallidus is free of c-fos expression in our experimental groups, it can be concluded that SKF82958 does not activate striatal D2 receptors in unilaterally dopamine depleted rats. The basal-ganglia output nuclei project predominantly and to some degree bilaterally to the motor (ventromedial and ventral anterior) and nonspecific (intralaminar and midline) thalamic nuclei (Gerfen et al., 1982; reviews Gerfen, 2004; Groenewegen and Witter, 2004). Therefore, striatal D1 receptor activation leads to reduced inhibitory tone of the basal ganglia output nuclei on their thalamic targets (MacLeod et al., 1990). Both groups of thalamic nuclei provide projections to the frontal cortex, and to a lesser degree, to the parietal cortex (Herkenham, 1979, 1980; Groenewegen and Berendse, 1994; Groenewegen and

Witter, 2004). These projections together with cortico-cortical and cortico-thalamo-cortical projections can account for the 'global' induction of both c-fos and RGS2 throughout the cortex. Although the effect of the selective D1/D5 agent in the cortex of the lesioned hemisphere is expected to be primarily mediated by dopaminergic pathways, the possible contribution of other pathways should be considered. Noradrenergic fibers innervating the cortex transit via the medial forebrain bundle (Morrison et al., 1978) where the lesion was performed and these may also have been damaged by the lesion, since desmethylinipramine was not given prior to 6-OHDA to minimize the impact on noradrenergic systems. Therefore, the possibility that a change in the noradrenergic innervation of the cortex may influence the activation of the cortex following systemic challenge with a D1 agonist cannot be excluded. Cortical activation achieved through these circuits is conveyed back to the striatum by the prominent crossed and uncrossed corticostriatal pathways (McGeorge and Faull, 1989; Berendse et al., 1992; Brog et al., 1993), thereby explaining the activation of the non-lesioned striatum.

It should be noted that some regions involved in these pathways, such as the substantia nigra pars reticulata and thalamic nuclei, show weak or no c-fos or RGS2 induction.

Fig. 6. Regional pattern of RGS2 expression 0.5 h following administration of SKF82958 or vehicle to rats with deleted ascending dopamine pathways in the right hemisphere. Rats were lesioned and dosed as described in the legend of Fig. 4 and in Experimental Procedures. Shown are representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of 32 P-labeled ribo-probes complementary to RGS2 in both the vehicle (left panels) and drug-challenged group (right panels) at the level of the globus pallidus, rostral hippocampus and caudal hippocampus. Quantifications of RGS2 induction per brain region are given in Table 3. Scale bar = 5 mm.

This is likely due to the predominant inhibitory innervation received by these structures. Both c-fos and RGS2 can be induced by increased levels of either cAMP or Ca^{2+} (Sassone-Corsi et al., 1988; Sheng et al., 1990; Heximer et al., 1997; Pepperl et al., 1998). Therefore, as a general rule, c-fos and RGS2 light up in regions expected to receive excitatory stimulation causing enhanced cAMP or Ca^{2+} signaling. Such is the case for the striatum where cAMP levels can be directly stimulated by the action of SKF82958 on dopamine D1 receptors. For its part, the cortex would receive enhanced glutamatergic innervation from thalamocortical neurons probably causing increased Ca^{2+} levels via activation of NMDA receptors.

Activation of hippocampal structures in D1 challenged hemiparkinsonian rats

Perhaps owing to the modest expression of D1-type receptors in the hippocampal formation (Savasta et al., 1986;

Fig. 7. Regional pattern of RGS2 expression 2 h following administration of SKF82958 or vehicle to rats with deleted ascending dopamine pathways in the right hemisphere. Rats were lesioned and dosed as described in the legend of Fig. 5 and in Experimental Procedures. Shown are representative bright field photomicrographs of autoradiograms illustrating *in situ* hybridization of 32 P-labeled ribo-probes complementary to RGS2 in both the vehicle (left panels) and drug-challenged group (right panels) at the level of the globus pallidus, rostral hippocampus and caudal hippocampus. Quantifications of RGS2 induction per brain region are given in Table 3. Scale bar = 5 mm.

Camps et al., 1990), the hippocampus is rarely included in studies of D1-mediated gene induction. Nevertheless, the study of hippocampal activity in models of Parkinsonism may be important in light of recent reports indicating that the hippocampus suffers alterations in Parkinson's disease patients (Camicioli et al., 2003; Bruck et al., 2004). D1 receptors are capable of influencing hippocampal gene expression since treatment of hippocampal slices with dopamine or SKF38393 leads to c-fos induction in the hippocampus (Kang et al., 2000), and systemic administration of SKF82958 induces c-fos in the hippocampus of normal rats (Crosio et al., 2003). Furthermore, hippocampal D1 receptors have the capacity to be sensitized as testified by findings that D1-mediated early long term potentiation at CA1 hippocampal synapses is enhanced by depletion of catecholamine stores (Otmakhova and Lisman, 1996).

Our results indicate that the deletion of ascending catecholaminergic fibers also results in sensitization of hippocampal structures and that therefore the hippocampus

Fig. 8. SKF82958-induced gene expression changes in hemiparkinsonian rats: Comparison of c-fos (A) and RGS2 (B) upregulation in hippocampal structures at 2 h. For both genes, induction levels are relatively high in the DG and relatively low in the CA3 region. Differences between c-fos and RGS2 induction are observed in the CA1 region which shows relatively low induction for c-fos and relatively high levels of upregulation for RGS2. ##, ###—for DG and CA3 significantly different from CA1 by ANOVA followed by Bonferroni post hoc test ($P < 0.01$; 0.001 respectively).

may form another focal point for the activation of brain circuits. Indeed, all hippocampal regions show increased c-fos and RGS2, with the highest inductions observed in the dentate gyrus (DG) at 2 h postinjection (Tables 2 and 3, Fig. 8). The DG receives a slight dopaminergic innervation from the ventral tegmental area (Swanson, 1982; Gasbarri et al., 1997), which when deleted may lead to the sensitization of D1 receptors in the DG. Alternatively, as discussed above for the cortex, the depletion of other catecholamines such as norepinephrine by the 6-OHDA lesion may have an indirect effect on the sensitivity of dopamine receptors. Noradrenergic fibers densely innervate the DG and to a lesser extent the hippocampus proper (Haring and Davis, 1985), therefore an indirect effect of altered noradrenergic innervation may contribute to the D1/D5 induced gene upregulation in these structures. Due to strong associational connections within the DG and the CA3 regions as well as the strong intrahippocampal connections from the DG to the CA3 and from the CA3 to the CA1, the activation of sensitized hippocampal neurons can readily influence other hippocampal neurons. Further, once the hippocampal formation on one side of the brain is stimulated, it can influence other brain regions via intrinsic

or efferent hippocampal projections (reviewed in Witter and Amaral, 2004). Primarily, the CA3 region may be crucial for transmitting excitatory stimuli to the hippocampus of the intact hemisphere as it sends projections to the contralateral CA3 and CA1 regions (Swanson et al., 1978; Witter and Amaral, 2004). Moreover, certain efferent projections of the hippocampal formation and entorhinal cortex (EC) may contribute to c-fos and RGS2 induction patterns in the basal forebrain. In this context, the lateral septum (Swanson and Cowan, 1977; van Groen and Wyss, 1990) and the nucleus accumbens (van Groen and Wyss, 1990; Totterdell and Meredith, 1997) may be mentioned.

Different patterns of bilateral gene induction of c-fos compared with RGS2 in D1 challenged hemiparkinsonian rats

The bilateral gene induction patterns of c-fos and RGS2 are not identical. For instance, c-fos upregulation in the striatum is higher in the lesioned hemisphere compared with the contralateral hemisphere, while no interhemispheric differences are found for striatal RGS2 induction (Fig. 3, Tables 2 and 3). This difference is intriguing since RGS2 and c-fos are generally induced by the same stimuli. A plausible explanation is given by Andersson et al. (2001), who have shown that gene induction pathways in the striatum are modified as a result of the 6-OHDA denervation. With this assumption, c-fos responds more strongly and to this alternative gene induction mechanism in the lesioned hemisphere than via the normal pathways in the non-lesioned hemisphere. Accordingly, RGS2 responds equally to both mechanisms. Despite the fact that neuronal activation as indicated by c-fos is relatively low in the intact striatum, the relatively high levels of RGS2 seem to indicate that signaling regulation activities are also high. Since D1 receptors are not supersensitized in the intact striatum, this signaling homeostasis activity may be directed at other adenylyl cyclase linked striatal receptors such as metabotropic glutamate receptors or adrenergic receptors. Further studies will be required to verify this hypothesis.

Another main difference between c-fos and RGS2 induction is their expression in the CA1 region relative to upregulation levels seen in the DG and CA3. The DG is the brain region showing the highest gene induction levels for both c-fos and RGS2, while both genes are upregulated in the CA3 in a manner similar to the EC (EC). However, for RGS2, induction in CA1 is similar to that observed in the DG while c-fos induction in CA1 is more comparable to levels seen in CA3 (Fig. 8). It seems most likely that differences in nature of the innervation of CA1 neurons compared with CA3 neurons may account for this difference. First, CA1 receives a strong input from the reuniens thalamic nucleus (Wouterlood et al., 1990). Second, CA1 also integrates both perforant path inputs from the EC and Schaffer collateral inputs from the CA3. These pathways have different effects on NMDA- and AMPA-mediated neurotransmission (Otmakhova et al., 2002) which may lay at the basis for the difference in induction magnitude between

c-fos and RGS2 in CA1. This discrepancy may indicate that while neuronal activation in the CA1 region is relatively low (as indicated by c-fos levels), CA1 cells seem nevertheless to be engaged in relatively strong signaling regulation activities (as may be indicated by RGS2 levels). Further investigations are needed to characterize the nature of these putative regulation activities.

c-fos/RGS2 induction patterns and behavior in D1 challenged hemiparkinsonian rats

It is interesting to note that although SKF82958 is ranked among the most potent D1 agonists to stimulate adenylyl cyclase in the striatum (O'Boyle et al., 1989; Gnanalingham et al., 1995), it is ranked as one of the lowest potent D1 agonists in inducing circling behavior in hemiparkinsonian rats (Gnanalingham et al., 1995). This is in contrast to the partial D1 agonist SKF38393 which is relatively ineffective compared with dopamine and SKF82958 at stimulating cAMP production but is significantly more effective at inducing contralateral rotations in rats with a unilateral 6-OHDA lesion (O'Boyle et al., 1989; Gnanalingham et al., 1995). In our experimental groups, we observed that the rotational behavior that occurs shortly after SKF82958 injection in the hemiparkinsonian rats was interspersed with bouts of head rearing behavior reminiscent of stereotypy induced in normal rats by psychostimulants such as amphetamine. The head rearing which interrupts the circling may be a behavioral indication of the bilateral neuronal activation observed in the present study. Acute amphetamine induces not only stereotypy, but also striatal c-fos and RGS2 (Graybiel et al., 1990; Burchett et al., 1998). In hemiparkinsonian rats given the partial D1 agonist SKF38393, upregulations of c-fos and RGS2 in the striatum are only observed ipsilateral to the lesion and this corresponds to relatively strong contralateral rotations (Morelli et al., 1992; Berke et al., 1998; Taymans et al., 2004). In the present study, the bilateral activation of dopaminergic circuits shown by the bilateral induction of c-fos and RGS2 is reflected behaviorally by reduced behaviors indicative of a unilateral stimulation (i.e. fewer rotations) and the presence of some behavior typical for a bilateral stimulation (i.e. head rearing).

The findings presented here may be relevant for the development of new symptomatic therapies for Parkinson's disease. Although L-DOPA is generally regarded as the drug which best suppresses parkinsonian symptoms (Jankovic, 2000), specific agonists of dopamine receptors have regularly been considered as alternatives for improvement. Indeed, D1 agents have been shown to decrease Parkinsonism signs in primates to a degree comparable to levodopa (for review Mailman et al., 2001). SKF82958 itself has been shown to have weak antiparkinsonian activity in primates (Rupniak et al., 1992). Generally, antiparkinsonian drugs are tested primarily for their ability to restore motor function in Parkinsonism, functions subserved by the basal ganglia-thalamocortical circuits. The fact that a D1 agonist here is shown to potentially activate also hippocampal circuits is interesting in light of recent findings that PD is associated with hippocampal

atrophy and that these hippocampal changes are predictive of cognitive impairments (Camicioli et al., 2003; Bruck et al., 2004). Though speculative, it would be interesting to investigate whether the activation of hippocampal circuits by D1 agonists shows cognitive benefits in Parkinsonism.

CONCLUSION

In summary, the present report shows that unilaterally sensitized D1 receptors can activate the rat brain bilaterally by polysynaptic contacts. Way stations accounting for this bilateral activation include not only the basal ganglia-thalamocortical circuits but also hippocampal circuits. The induction patterns of the activity marker genes used, RGS2 and c-fos, were mostly comparable except in some regions, such as the intact striatum or the CA1 region, which showed relatively low c-fos induction together with relatively high RGS2 induction, possibly indicating that these regions are engaged in unusually strong signaling regulation activities.

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