Dopamine–Adenosine Interactions in the Striatum and the Globus Pallidus: Inhibition of Striatopallidal Neurons through Either D_2 or A_{2A} Receptors Enhances D_1 Receptor-Mediated Effects on *c-fos* Expression

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D_1 receptors located on striatonigral neurons and D_2 receptors located, together with A_{2A} receptors, on striatopallidal neurons are known to interact functionally. Using *in situ* hybridization, we examined the effects of D_1 and D_2 agonists and of an A_{2A} antagonist on *c-fos* mRNA in identified striatal neurons and in globus pallidus. The full D_1 agonist, SKF 82958 (1 mg/kg), induced a homogenous increase of *c-fos* mRNA in the striatum. This increase occurred to a similar extent in D_1 and D_2 receptor-containing striatal neurons. Conversely, the D_2 agonist, quinelorane (2 mg/kg), decreased *c-fos* mRNA in these populations but increased it in globus pallidus. The adenosine A_{2A} receptor antagonist, SCH 58261 (5 mg/kg), also decreased *c-fos* mRNA in D_2 receptor-containing neurons in striatum but did not affect pallidal *c-fos* mRNA. Concomitant administration of either D_1 plus D_2 agonists or D_1 agonist plus A_{2A} antagonist caused a potentiation of *c-fos* mRNA in striatal neurons expressing the D_1 receptor and in globus pallidus. However, only the combination of D_1 and D_2 agonists modified the *c-fos* mRNA expression to a "patchy" distribution. Our data show that (1) *c-fos* expression can be activated through D_1 and *inhibited* through A_{2A} or D_2 receptors in both striatal output pathways in normal rats, and (2) D_2 receptor stimulation as well as A_{2A} receptor blockade can interact with D_1 receptor activation to potentiate *c-fos* expression in the striatum and the globus pallidus. The data also suggest that the topological alteration of *c-fos* expression after coadministration of D_1 and D_2 agonists involves D_2 receptors located on interneurons or presynaptically on dopaminergic nerve terminals.

Key words: In situ *hybridization; phenotypical characterization; immediate early gene; dopamine–adenosine interactions; synergistic effects; striatal output pathways; globus pallidus*

The basal ganglia are involved in the integration of sensorimotor, associative, and limbic information to produce motor behaviors. The central component of these structures, the striatum, integrates excitatory glutamatergic inputs from cortex, thalamus, and limbic areas, with dopaminergic inputs from mesencephalon. It is composed of a large proportion of medium-sized spiny output neurons (95%) and of interneurons (5%). Striatal output neurons are GABAergic and project to either substantia nigra (pars reticulata) or globus pallidus and differ in their neuropeptide content: the striatonigral pathway contains substance P/dynorphin and the striatopallidal enkephalin (for review, see Graybiel, 1990; Gerfen and Wilson, 1996).

Dopamine regulates striatal neurotransmission via two types of receptor families, D_1-type (D_1 and D_5) and D_2-type (D_2, D_3, D_4) receptors, which have distinct pharmacological profiles and mechanisms of transduction (Creese et al., 1983; Jaber et al., 1996). It has been suggested that dopamine differentially regulates the two striatal output pathways and that a balanced control is essential for the proper function of the extrapyramidal motor system (for review, see Alexander and Crutcher, 1990; Gerfen, 1992). Accordingly, several anatomical studies have demonstrated a segregation of D_1 and D_2 receptors, respectively, in striatonigral/substance P and striatopallidal/enkephalin neurons (Gerfen et al., 1990; Le Moine et al., 1990a, 1991; Hersch et al., 1995; Le Moine and Bloch, 1995, 1996; Yung et al., 1996). However, many physiological data indicate synergistic effects after coactivation of D_1- and D_2-type receptors (for review, see Waddington and Daly, 1993; White and Hu, 1993).

In the basal ganglia A_{2A} receptors are restricted to striatopallidal/D_2-containing neurons and, in contrast to D_2 receptors, are not present on dopaminergic nerve terminals and are virtually absent from cholinergic interneurons (Schiffmann et al., 1991; Fink et al., 1992; Augood and Emson, 1994; Svenningsson et al., 1997). An alternative way to investigate how D_1/D_2 interactions occur is to study how adenosine modulates neurotransmission via adenosine A_{2A} receptors and how they can be involved in interactions with D_1 receptor-mediated effects. Indeed, it has been shown that dopamine acting on D_2 receptors and adenosine acting on A_{2A} receptors have opposing actions on neurotransmitter release, gene expression, and several motor behaviors (for

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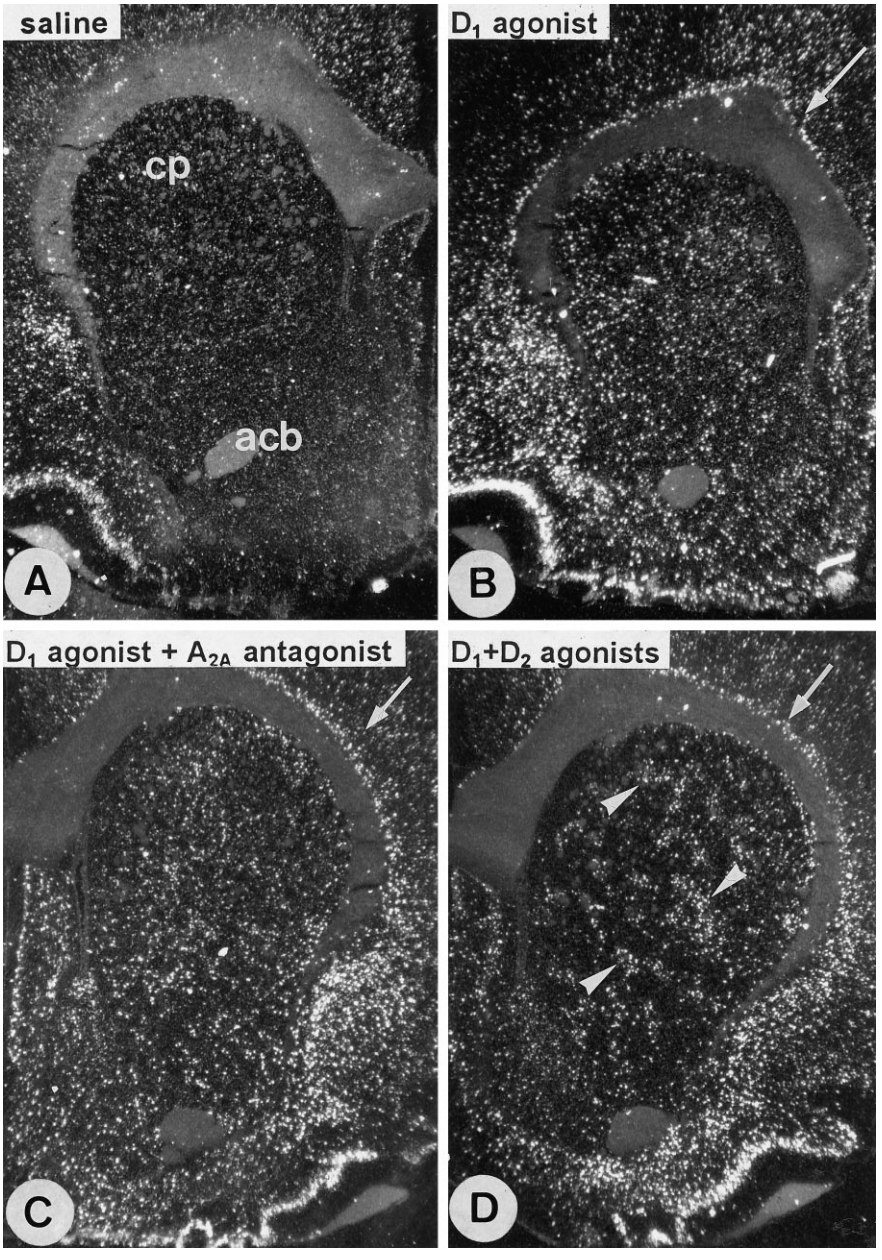

Figure 1. D_1/D_2 and D_1/A_{2A} receptor interactions on *c-fos* expression. Dark-field photomicrographs after *in situ* hybridization with a ^{35}S-labeled riboprobe show the localization of *c-fos* mRNA-containing neurons in the striatum after saline (*A*), D_1 agonist SKF-82958 (*B*), D_1 agonist SKF-82958 + A_{2A} antagonist SCH-58261 (*C*), and D_1 agonist SKF-82958 + D_2 agonist quinelorane (*D*). Under basal conditions (*A*) *c-fos* mRNA-containing neurons are few and scattered in the caudate putamen (*cp*) and the nucleus accumbens (*acb*). *c-fos* is induced after the D_1 agonist both in the caudate putamen and the nucleus accumbens (*B*). As compared with D_1 agonist + A_{2A} antagonist (*C*), the combined treatment with D_1 + D_2 agonists potentiates the D_1-induced expression of *c-fos* with a heterogeneous "patchy" pattern (*arrowheads* in *D*). Cortical expression of *c-fos* in layer VIb is seen clearly after D_1 agonist alone or in combination with either A_{2A} antagonist or plus D_2 agonists (*arrows* in *B–D*). Magnification, 11×.

review, see Ferré et al., 1992; Ongini and Fredholm, 1996). Accordingly, selective A_{2A} antagonists share with D_2 agonists the ability to potentiate motor effects induced by D_1 receptor agonists as well as D_1-induced *c-fos* expression in dopamine-depleted striatum (Jiang et al., 1993; Pinna et al., 1996; Pollack and Fink, 1996).

In this context, detailed analysis of the modulation of D_1 or D_2 agonist-mediated effects by an A_{2A} antagonist may help to elucidate the D_1/D_2 interactions in the basal ganglia. We therefore used sensitive *in situ* hybridization with riboprobes to examine how pharmacological treatments involving dopamine or adenosine receptors might up- or downregulate the expression of *c-fos* in the basal ganglia. In particular, *c-fos* expression was studied in phenotypically identified striatal neurons, with double-labeling, after challenges with selective compounds acting at D_1, D_2, and A_{2A} receptors given alone or in combination.

MATERIALS AND METHODS

Pharmacological manipulations and tissue preparation. All experiments have been performed in accordance with the guidelines of the French Agriculture and Forestry Ministry (decree 87849, license 01499) and with the Centre National de la Recherche Scientifique approval. Adult male Sprague Dawley rats (200–280 gm) (Iffa Credo, France) were maintained in standard housing conditions several days before the experiments. Animals were treated with systemic injections of saline (NaCl 0.9%); ±SKF-82958 (Research Biochemicals, Natick, MA), a full dopamine receptor agonist that has a 200-fold selectivity for D_1 over D_2 receptors (Andersen and Jansen, 1990); quinelorane or LY-163,502 (Research Biochemicals), a dopamine receptor agonist that conversely shows at least a 50-fold selectivity for D_2 over D_1 receptors (Bymaster et al., 1986; Andersen and Jansen, 1990); or SCH-58261 (Schering-Plough, Milan, Italy), an adenosine receptor antagonist that is 60-fold selective for A_{2A} over A_1 receptors (Zocchi et al., 1996). All rats had been handled the day before the injection and had received two injections. The different treatment groups were as follows: saline plus saline (n = 5), quinelorane 2 mg/kg plus saline (n = 4), SKF-82958 0.5 mg/kg plus saline (n = 3), SKF-82958 1 mg/kg plus saline (n = 5), SKF-82958 2 mg/kg plus saline

Table 1. Density of neurons containing *c-fos* mRNA after D_1 or/and D_2 agonists and A_{2A} antagonist, alone or in combination

Treatment group	*n*	Caudate putamen	Globus pallidus
Saline (*a*)	6	35.25 ± 4.34	24.20 ± 3.40
Quinelorane (*b*)	5	8.55 ± 1.70*^{a}	42.20 ± 4.00*^{a}
SKF-82958 (*c*)	4	132.75 ± 15.30*^{a}	12.50 ± 1.60
SCH-58261 (*d*)	4	16.70 ± 1.50*^{a}	18.25 ± 4.20
SKF-82958 + quinelorane	5	156.20 ± 6.50*^{b,ns,c}	122.30 ± 17.60*^{b,c}
SKF-82958 + SCH-58261	4	183.90 ± 6.30*^{c,d}	69.80 ± 13.90*^{c,d}
Quinelorane + SCH-58261	5	17.35 ± 1.39*^{b,ns,d}	60.30 ± 8.10*^{d,ns,b}

Rats were treated with saline (NaCl 0.9%), with the D_2 agonist quinelorane (2 mg/kg), with the D_1 agonist SKF-82958 (1 mg/kg), with the A_{2A} antagonist SCH 58261 (5 mg/kg), or various combinations: SKF-82958 (1 mg/kg) + quinelorane (2 mg/kg), SCH 58261 (5 mg/kg) + SKF-82958 (1 mg/kg), and quinelorane (2 mg/kg) + SCH 58261 (5 mg/kg). *c-fos* mRNA was detected with single *in situ* hybridization (exposure times, 7 weeks). Values represent the mean ± SEM of the number of *c-fos* mRNA-containing neurons per mm^2. Two-way ANOVA, followed by *post hoc t* tests corrected for the experiment-wise alpha level (Bonferroni correction). The results of the global ANOVA were for quinelorane/SKF-82958 interaction: $F_{(1,16)} = 11.48$, $p < 0.005$ for caudate putamen (CP) and $F_{(1,16)} = 23.87$, $p < 0.001$ for globus pallidus (GP); for SKF-82958/SCH-58261 interaction: $F_{(1,14)} = 19.02$, $p < 0.001$ for CP and $F_{(1,14)} = 19.84$, $p < 0.001$ for GP; for quinelorane/SCH-58261 interaction: $F_{(1,16)} = 21.27$, $p < 0.001$ for CP and $F_{(1,16)} = 5.163$, $p < 0.05$ for GP. For the multiple *post hoc t* tests Bonferroni correction, an asterisk indicates relevant significant differences between indicated groups ($p < 0.05$).

($n = 2$), SCH-58261 5 mg/kg plus saline ($n = 4$), SKF-82958 1 mg/kg plus quinelorane 2 mg/kg ($n = 5$), SKF-82958 1 mg/kg plus SCH-58261 5 mg/kg ($n = 4$), or quinelorane 2 mg/kg plus SCH-58261 5 mg/kg ($n = 5$). SKF-82958 and quinelorane were dissolved in saline, whereas SCH-58261 was dissolved in saline/5% Tween 80 after careful sonication. Drugs were injected intraperitoneally, 0.5 ml per injection, and the rats were decapitated 1 hr after the injections. The brains were dissected out, frozen over liquid nitrogen, and then sectioned into 10 μm sections, collected on gelatin-coated slides, and stored at −80°C until used.

Probe synthesis. ^{35}S-labeled cRNA probes were prepared by *in vitro* transcription from cDNA clones corresponding to fragments of the rat *c-fos* cDNA (Curran et al., 1987) (a gift from Dr. T. Curran, Roche Institute of Molecular Biology, Nutley, NJ), rat D_1 and D_2 dopamine receptor cDNAs (Monsma et al., 1989, 1990) (a gift from Dr. D. Sibley, National Institute of Health, NINDS, Bethesda, MD), and rat μ-opioid receptor cDNA (Thompson et al., 1993) (a gift from Dr. S. J. Watson, University of Michigan, Ann Arbor, MI). Transcriptions were performed from 50 ng of linearized plasmid, using either ^{35}S-UTP (>1000 Ci/mmol; DuPont de Nemours, Les Ulis, France) or digoxigenin-11-UTP (Boehringer Mannheim, Meylan, France) and SP6, T3, or T7 RNA polymerases as described by Le Moine and Bloch (1995). After alkaline hydrolysis to obtain 250 bp cRNA fragments, the ^{35}S-labeled probes were purified on G50-Sephadex. The ^{35}S-labeled probes and the digoxigenin-labeled probes were precipitated in 3 M sodium acetate/absolute ethanol (0.1:2.5, v/v), pH 5.

Single detection of c-fos *mRNA on cryostat sections.* Sections were hybridized as described by Le Moine and Bloch (1995, 1996) with minor modifications. Cryostat sections were post-fixed in 4% paraformaldehyde (PFA) for 5 min at room temperature, rinsed twice in 4× SSC, and placed into 0.25% acetic anhydride in 0.1 M triethanolamine/4× SSC, pH 8, for 10 min at room temperature. After dehydration, the sections were hybridized overnight at 55°C with 10^6 cpm of ^{35}S-labeled cRNA probe in 50 μl of hybridization solution (20 mM Tris-HCl, 1 mM EDTA, 300 mM NaCl, 50% formamide, 10% dextran sulfate, 1× Denhardt's, 250 μg/ml yeast tRNA, 100 μg/ml salmon sperm DNA, 100 mM DTT, 0.1% SDS, and 0.1% sodium thiosulfate). After 20 min of RNase A treatment (20 mg/ml), the sections were washed with 2× SSC (5 min, twice), 1× SSC (5 min), 0.5× SSC (5 min) at room temperature, and rinsed in 0.1× SSC at 65°C (30 min, twice) before dehydration (the latter SSC washes contained 1 mM DTT). Sections either were exposed on x-ray films (Kodak BIOMAX, Rochester, NY) for 3–6 d or dipped into Ilford K5 emulsion, exposed for 7 weeks, developed, and stained with toluidine blue.

Simultaneous detection of c-fos *mRNA with D_1 or D_2 mRNAs on cryostat sections.* Two combinations of probes were used for the simultaneous detection of two mRNAs on a single section: a ^{35}S-labeled *c-fos* probe in combination with digoxigenin-labeled D_1 or D_2 probes. Cryostat sections were pretreated as mentioned above. After dehydration the sections were hybridized overnight at 55°C with a combination of ^{35}S- and digoxigenin-labeled probes (10^6 cpm of ^{35}S-labeled probe and 10–20 ng of digoxigenin-labeled probe in 50 μl of hybridization solution). After 20 min of RNase A treatment at 37°C (20 μg/ml), the slides were washed in various concentrations of SSC as mentioned above, but without DTT. After washing, the sections were put in 0.1× SSC at room temperature and then processed directly for detection of the digoxigenin signal. The sections were rinsed twice for 5 min in buffer A (1 M NaCl, 0.1 M Tris, and 2 mM $MgCl_2$, pH 7.5) and then for 30 min in buffer A containing 3% normal goat serum and 0.3% Triton X-100. After 5 hr of incubation at room temperature with alkaline phosphatase-conjugated anti-digoxigenin antiserum (Boehringer Mannheim; 1:1000 in buffer A, 3% normal goat serum, and 0.3% Triton X-100), the sections were rinsed in buffer A (5 min, twice) and then for 10 min twice in STM buffer (1 M NaCl, 0.1 M Tris, and 5 mM $MgCl_2$, pH 9.5), and finally for 10 min twice in 0.1 M STM buffer, pH 9.5 (0.1 M NaCl, 0.1 M Tris, and 5 mM $MgCl_2$, pH 9.5). Then the sections were incubated overnight in the dark at room temperature in 0.1 M STM buffer, pH 9.5, containing 0.34 mg/ml nitroblue tetrazolium and 0.18 mg/ml bromo-chloro-indolylphosphate. They were rinsed in 0.1 M STM buffer, pH 9.5, and then in 1× SSC, dried, and dipped into Ilford K5 emulsion (diluted 1:3 in 1× SSC). After being exposed for 10 weeks in the dark, the sections were developed and mounted without counterstaining.

Counting of labeled neurons. Labeled neurons both from single-labeling and double-labeling experiments (exposure times: 7 weeks for single *in situ* hybridization and 10 weeks for double *in situ* hybridization) were counted as previously described on similar material (Le Moine and Bloch, 1995). Accordingly, a labeled neuron corresponded to a density of silver grains at least twofold higher than background. One section per animal was analyzed for counting in single *in situ* hybridization, and one section per animal was counted for the double labeling. The densities of *c-fos* mRNA-containing neurons were studied in the striatum (+1 mm from bregma) and globus pallidus (−0.8 mm from bregma) according to Swanson (1992). The areas examined were 2–4 mm^2 for the caudate putamen and 1.5–2 mm^2 for the globus pallidus. The labeled neurons were counted using an image analyzer system for cartography (HISTO 200, Biocom, Les Ulis, France). For double *in situ* hybridization, quantification was performed only on the sections with simultaneous detection of *c-fos* and D_2 mRNAs, and the *c-fos* mRNA-labeled neurons were divided into two populations: the D_2 mRNA-positive (+) and D_2 mRNA-negative (−) neurons. The densities of *c-fos*-expressing neurons (number of *c-fos* mRNA-positive neurons per mm^2) were pooled and averaged for each group, and statistical analysis was performed by a two-way ANOVA, followed by *post hoc t* tests corrected for the experiment-wise α level by the Bonferroni correction.

RESULTS

Effects of D_1 and D_2 agonists on *c-fos* expression in the striatum and in the globus pallidus

Under control conditions (i.e., saline-treated rats), neurons containing *c-fos* mRNA were observed in several cortical areas, especially the endopiriform and piriform cortices, in the septum and in the caudate putamen and nucleus accumbens (Fig. 1). The densities of *c-fos*-positive neurons (mean ± SEM) were 35.25 ± 4.34 per mm^2 in the caudate putamen and 24.2 ± 3.4 per mm^2 in the globus pallidus (Table 1).

One hour after administration of the D_1 agonist SKF-82958 at the dose of 1 mg/kg, the number of *c-fos* mRNA-containing neurons dramatically increased in the caudate putamen (+277%) and the nucleus accumbens (Figs. 1, 2, Table 1). An increase also was found in the cortex (with a particularly high concentration in layer VIb) and in the septum (Fig. 1). By contrast, the number of *c-fos* mRNA-containing neurons tended to decrease (by 48%, $p = 0.08$) in the globus pallidus (Fig. 3, Table 1). In all of the examined areas, the effects of SKF-82958 were similar over the dose range tested (0.5–2 mg/kg; data not shown).

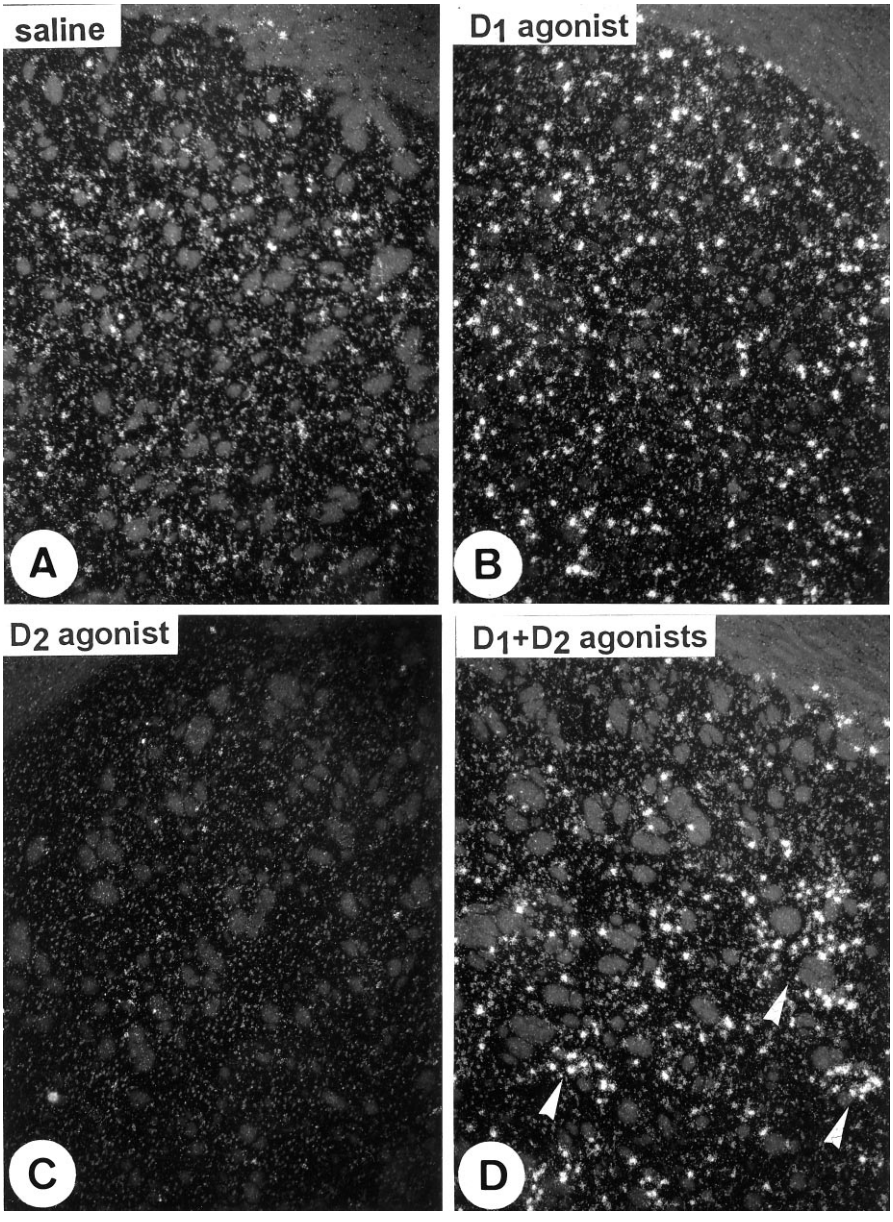

Figure 2. D_1- and D_2-mediated regulation of *c-fos* expression in the caudate putamen. Dark-field photomicrographs from single *in situ* hybridization with a ^{35}S-labeled riboprobe show *c-fos* mRNA after treatments with D_1 and D_2 agonists alone or in combination. The D_1 agonist SKF-82958 increases the number of *c-fos*-positive neurons (*B*), whereas the D_2 agonist quinelorane *decreases* it (*C*), as compared with saline-treated rats (*A*). Association of D_1 and D_2 agonists changes the D_1-induced *c-fos* expression into a heterogeneous "patchy" pattern (*arrowheads* in *D*). Quantitative data are listed in Table 1. Magnification, 40×.

Conversely, the D_2 agonist quinelorane, at the dose of 2 mg/kg, caused a *decrease* in the number of *c-fos* mRNA-containing neurons in the caudate putamen (−75%, Table 1). Detection of such a decrease is directly related to our ability to consistently detect and quantify *c-fos* mRNA in basal conditions by using sensitive riboprobes (Fig. 2). In contrast, the density of labeled neurons in the globus pallidus was increased after treatment with the D_2 agonist (+74%) (Fig. 3, Table 1).

When quinelorane (2 mg/kg) was coadministered with SKF-82958 (1 mg/kg), the density of *c-fos*-labeled neurons in the caudate putamen and the nucleus accumbens was increased to the same extent as after SKF-82958 alone (Table 1). However, as shown in Figures 1 and 4, the homogenous distribution of the *c-fos* mRNA-containing neurons after SKF-82958 treatment was heterogeneous ("patchy") after coadministration of the two drugs. Comparison on adjacent sections shows that the distribution of *c-fos* mRNA after D_1 plus D_2 agonists was parallel to the distribution of μ-opioid receptor mRNA (Fig. 4). At the same time, in the globus pallidus, the coadministration of both SKF-82958 (1 mg/kg) and quinelorane (2 mg/kg) increased by 190% the density of *c-fos*-labeled neurons as compared with quinelorane alone (Fig. 3, Table 1).

Effects of an A_{2A} antagonist alone or in combination with a D_1 agonist on *c-fos* expression in the striatum and in the globus pallidus

The adenosine A_{2A} antagonist SCH-58261 had similar effects to the D_2 agonist quinelorane in the striatum. Treatment with SCH-58261 at a dose of 5 mg/kg induced a *decrease* in the density of *c-fos*-labeled neurons in the caudate putamen (−53%). In contrast to quinelorane, it had no effect on the density of labeled neurons in the globus pallidus (Fig. 5, Table 1). The coadministration of SKF-82958 (1 mg/kg) and SCH-58261 (5 mg/kg) induced a further increase in the density of *c-fos* mRNA-containing neurons in the caudate putamen (+38%) as compared with SKF-82958 alone (Fig. 5, Table 1). The distribution pattern of the *c-fos*-labeled neurons after the coadministration was homoge-

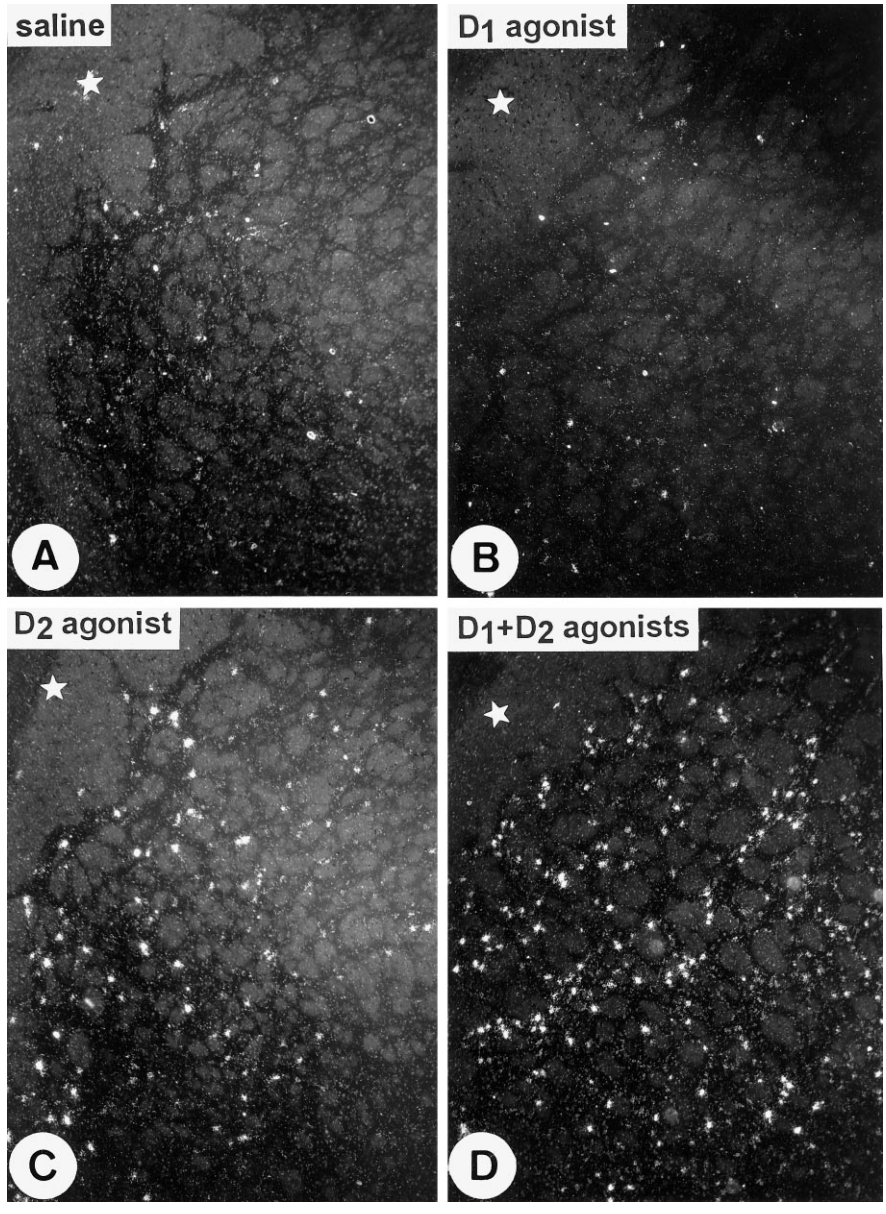

Figure 3. D_1- and D_2-mediated regulation of *c-fos* expression in the globus pallidus. Dark-field photomicrographs from single *in situ* hybridization with a ^{35}S-labeled riboprobe show *c-fos* mRNA after treatments with D_1 and D_2 agonists alone or in combination. The level of *c-fos* mRNA observed under basal conditions in *A* is increased after the D_2 agonist (*C*), whereas it tends to decrease with the D_1 agonist (*B*). Combined treatment with both D_1 and D_2 agonists potentiated the D_2-mediated induction of *c-fos* in the globus pallidus (*D*). *Stars* indicate the internal capsule. Quantitative data are listed in Table 1. Magnification, 40×.

neous in the striatum and not patchy, as seen after D_1 plus D_2 agonists (Figs. 1, 2, 4, 5).

In the globus pallidus the coadministration of SKF-82958 with SCH-58261 induced a dramatic increase in the density of labeled neurons as compared with the saline-treated rats (+188%) but also as compared with SKF-82958 alone (+458%) (Fig. 5, Table 1).

Effects of an A_{2A} antagonist alone or in combination with a D_2 agonist on *c-fos* expression in the striatum and in the globus pallidus

As mentioned above, the D_2 receptor agonist quinelorane (2 mg/kg) decreased the density of *c-fos* mRNA-containing neurons in the caudate putamen and increased it in the globus pallidus, whereas the A_{2A} receptor antagonist SCH 58261 (5 mg/kg) affected *c-fos* mRNA expression only in the caudate putamen, where it caused a *decrease* in the density of labeled neurons (Table 1). The coadministration of D_2 agonist and A_{2A} antagonist significantly counteracted the decrease induced by quinelorane in the caudate putamen (from −75 to −52%). No synergistic effect of the two drugs on *c-fos* expression was found in the globus pallidus as compared with quinelorane alone (Table 1).

Phenotypical identification of the *c-fos* mRNA-containing neurons in the caudate putamen after D_1 and D_2 agonists, given alone or in combination

To examine in which type of striatal neurons the above-mentioned changes in *c-fos* expression occurred, we used double-labeling experiments with probes for either D_1 or D_2 receptor mRNA, together with a probe for *c-fos* mRNA. Because the results, analyzed in two separate experiments (as illustrated in Fig. 6), were identical, quantitative data were generated only from *c-fos* plus D_2 mRNAs simultaneous detection (Table 2). Therefore, in the following, D_2 mRNA-negative (−) neurons are referred to as D_1 mRNA-positive (+) neurons on the basis of both experiments and previously published data (Le Moine and Bloch, 1995, 1996).

Figure 6 shows that administration of the D_1 agonist SKF-82958 (1 mg/kg) increased the number of both D_1 and D_2 mRNA-containing neurons that express *c-fos* mRNA (Table 2). Con-

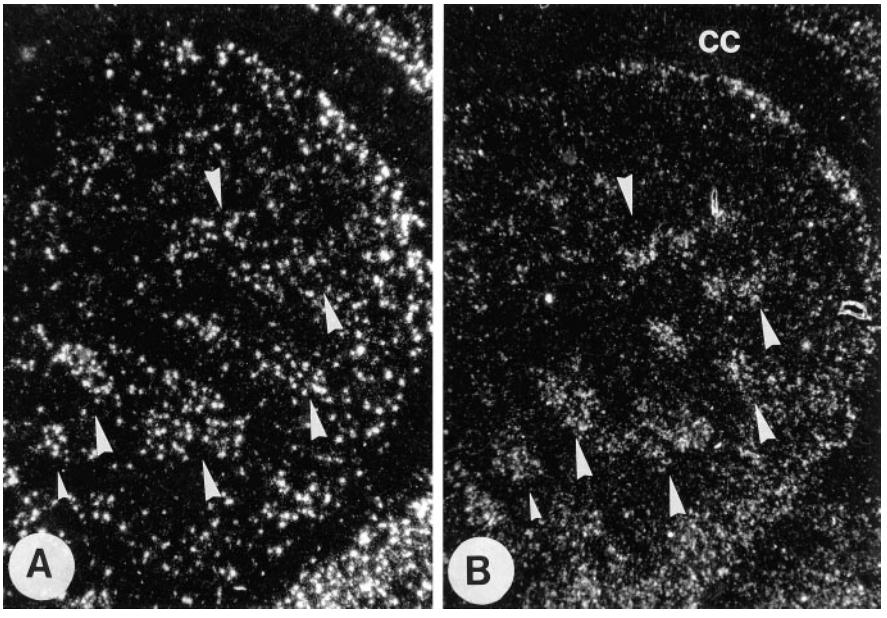

Figure 4. Striatal *c-fos* expression in patches after combined treatment with D_1 and D_2 agonists. Dark-field photomicrographs after *in situ* hybridization with ^{35}S-labeled riboprobes on adjacent sections show that the "patches" of *c-fos* mRNA-containing neurons (*arrowheads* in *A*) correspond to patches of μ-opioid receptor mRNA expression in the striatum (*arrowheads* in *B*). Also note the concomitant expression of *c-fos* and μ-mRNA in the subcallosal patch. *cc*, Corpus callosum. Magnification, 23×.

versely, the D_2 agonist quinelorane (2 mg/kg) decreased the density of *c-fos*-labeled neurons both for D_1 and D_2 mRNA-containing neurons (Table 2). The coadministration of D_1 and D_2 agonists had opposite effects on *c-fos* expression in these two populations because it induced an increase in the density of *c-fos*-labeled neurons containing D_1 mRNA and a decrease in the density of *c-fos*-labeled neurons containing D_2 mRNA, as compared with the D_1 agonist alone (Fig. 6, Table 2). Indeed, in SKF-82958 treated rats 53% of the *c-fos* expressing neurons were D_1 mRNA-positive, whereas in rats treated by SKF-82958 plus quinelorane, the proportion of these neurons reached 91% (Table 2). Note here and below that the relative changes observed in the density of *c-fos*-labeled neurons in the caudate putamen are comparable to what was observed in the single-labeling experiments and summarized in Table 1.

Phenotypical identification of the *c-fos* mRNA-containing neurons in the caudate putamen after A_{2A} antagonist and D_1 agonist, given alone or in combination

Similar experiments, performed with the A_{2A} antagonist SCH 58261 (5 mg/kg), showed a decrease in the density of *c-fos*-labeled neurons and in D_2 mRNA-containing neurons, but not in D_1 mRNA-containing neurons (Table 2). As mentioned above, the D_1 agonist SKF-82958 increased the density of *c-fos*-labeled neurons both in D_1 and D_2 mRNA-positive neurons (Table 2). The coadministration of the D_1 agonist and the A_{2A} antagonist potentiated the increase in the density of *c-fos*-labeled neurons that were positive for D_1 mRNA but had no effect on the density of *c-fos* labeled in D_2 mRNA-containing neurons, as compared with the D_1 agonist alone (Table 2).

Phenotypical identification of the *c-fos* mRNA-containing neurons in the caudate putamen after A_{2A} antagonist and D_2 agonist, given alone or in combination

The density of D_2 mRNA-positive neurons that express *c-fos* mRNA was lower in SCH-58261 (−60%) and quinelorane-treated animals (−97%) as compared with saline (Table 2). At the same time, quinelorane—and not SCH-58261—induced a reduction of *c-fos* in neurons positive for D_1 mRNA (−84.5%). When SCH-58261 and quinelorane were coadministered, there was no synergistic effect on *c-fos* expression in the D_1-containing nor in the D_2-containing neurons (Table 2).

DISCUSSION

Individual and synergistic effects of dopamine D_1 and D_2 receptor agonists and of an adenosine A_{2A} receptor antagonist on *c-fos* expression were analyzed in the striatum and globus pallidus. Our data, summarized in Figure 7, show that (1) *c-fos* expression can be either activated through D_1 and *inhibited* through A_{2A} or D_2 receptors in the two striatal output pathways in normal rats, and (2) D_2 receptor stimulation as well as A_{2A} receptor blockade can interact with D_1, but not D_2, receptor activation to potentiate *c-fos* expression in both the striatum and the globus pallidus.

Effect of D_2 and D_1 agonists given alone on *c-fos* expression in the striatum

Selective activation of D_2 receptors by the D_2 agonist produced a significant decrease in the number of striatal neurons expressing *c-fos* in the caudate putamen. The decrease was found in both D_1- and D_2-positive neurons. In D_2-containing neurons this decrease may be explained by the fact that dopamine is likely to have an inhibitory action on striatopallidal neurons via postsynaptic D_2 receptors (Gerfen et al., 1990). Conversely, the D_2 agonist effect on *c-fos* in D_1-containing neurons might be related to activation of presynaptic D_2 autoreceptors located on dopaminergic terminals, because this strongly decreases striatal dopamine release (Imperato et al., 1988; Suaud-Chagny et al., 1991) and thereby the D_1-mediated activity in striatonigral neurons. Decreases of mRNA coding for the immediate early gene NGFI-A (*zif 268*) have been described after treatment with drugs acting on D_2 or A_{2A} receptors (Gerfen et al., 1995; Svenningsson et al., 1995), but we describe here for the first time the D_2-mediated inhibition of *c-fos* expression in the two striatal output neurons.

The full D_1 agonist SKF-82958 increased *c-fos* expression in the striatum in normal rats, as previously reported by Wang and McGinty (1996). A strong induction of *c-fos* expression in the D_1 rich cortical layer VIb (Gaspar et al., 1995) also was found. Interestingly, *c-fos* mRNA increased to a similar extent in D_1- and D_2-containing neurons in the striatum. The stimulation of *c-fos*

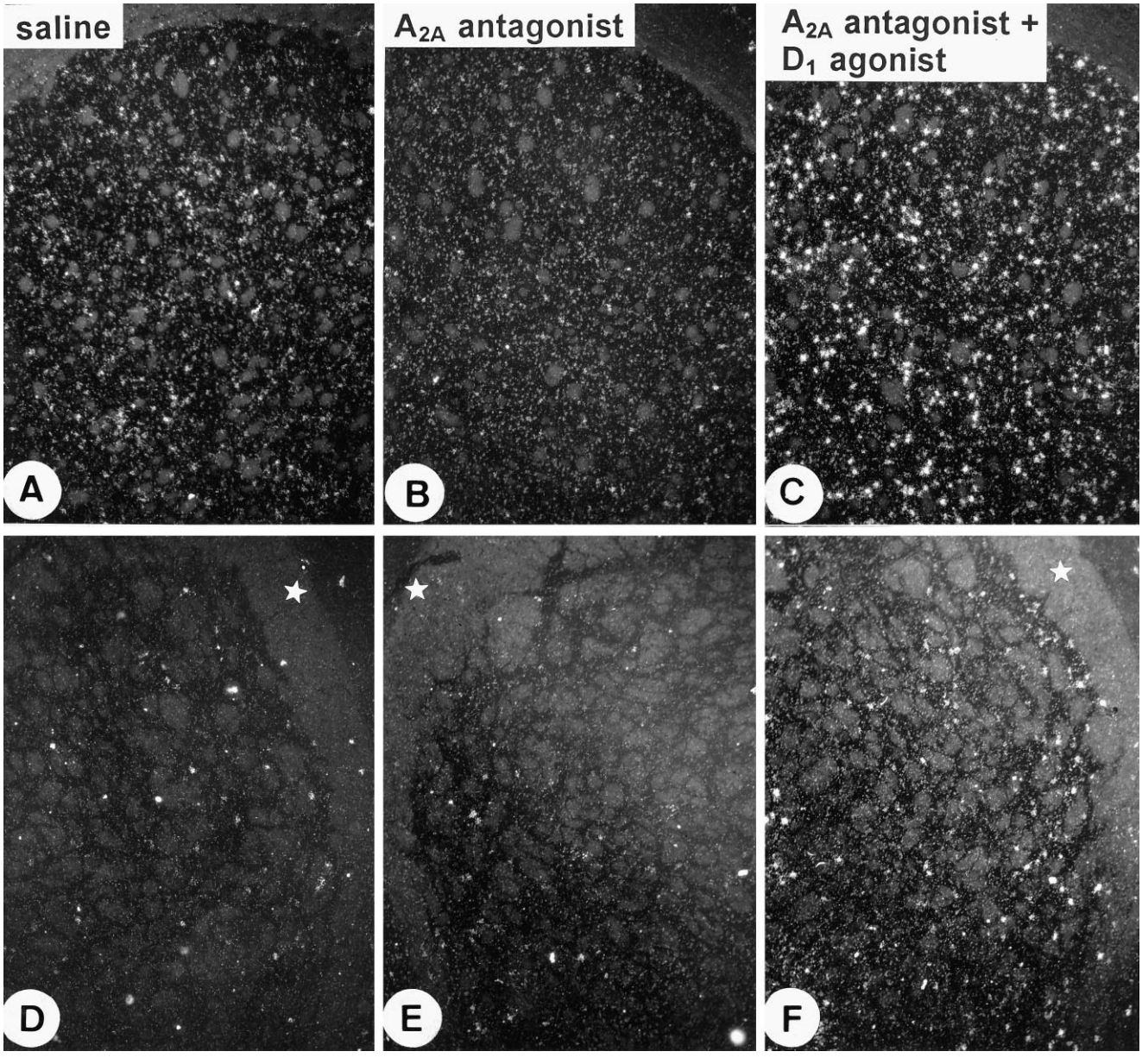

Figure 5. Effect of the A_{2A} antagonist alone or in combination with the D_1 agonist on *c-fos* expression in the caudate putamen (*A–C*) and the globus pallidus (*D–F*). Dark-field photomicrographs from single *in situ* hybridization with a ^{35}S-labeled riboprobe show the basal levels of *c-fos* mRNA in the caudate putamen (*A*) and in the globus pallidus (*D*). The A_{2A} antagonist SCH-58261 alone *decreases* the number of *c-fos*-positive neurons in the caudate putamen (*B*) but has no effect on the globus pallidus (*E*). Coadministration of the D_1 agonist, together with the A_{2A} antagonist, induces *c-fos* both in the caudate putamen (*C*) and in the globus pallidus (*F*) with a synergistic effect, as compared with the D_1 agonist alone (see also Table 1). *Stars* indicate the internal capsule. Quantitative data are listed in Table 1. Magnification, 40×.

expression in D_1-positive neurons was expected, because many studies have demonstrated that the dopamine-mediated induction of striatal Fos is dependent on D_1 activation [see Hughes and Dragunow (1995) and references therein]. The increased number of D_2-positive neurons expressing *c-fos* after SKF-82958 was unexpected. In previous studies, using the partial D_1 agonist SKF-38393, researchers observed *c-fos* induction only in the D_1 receptor-containing striatonigral neurons (Robertson et al., 1990; Gerfen et al., 1995). However, these studies were performed in animals with nigrostriatal lesions, and we therefore suggest that *c-fos* induction by the D_1 agonist in striatopallidal neurons requires intact nigrostriatal neurons. We hypothesize that the D_1 agonist, when injected systemically, acts on D_1 receptors located on striatonigral terminals (Caillé et al., 1996) and stimulates GABA release (Cameron and Williams, 1993), which in turn inhibits nigrostriatal neurons and decreases the extracellular striatal dopamine level (Suaud-Chagny et al., 1992). This effect would be indirectly responsible for an increase of *c-fos* in striatopallidal neurons. Nevertheless, cholinergic interneurons expressing D_5 (C. Le Moine, unpublished results) in addition to D_2 receptors (Le Moine et al., 1990b) and corticostriatal glutamatergic neurons (Gaspar et al., 1995) also may be involved in this D_1-dependent *c-fos* activation in the D_2-containing neurons (Berretta et al., 1992).

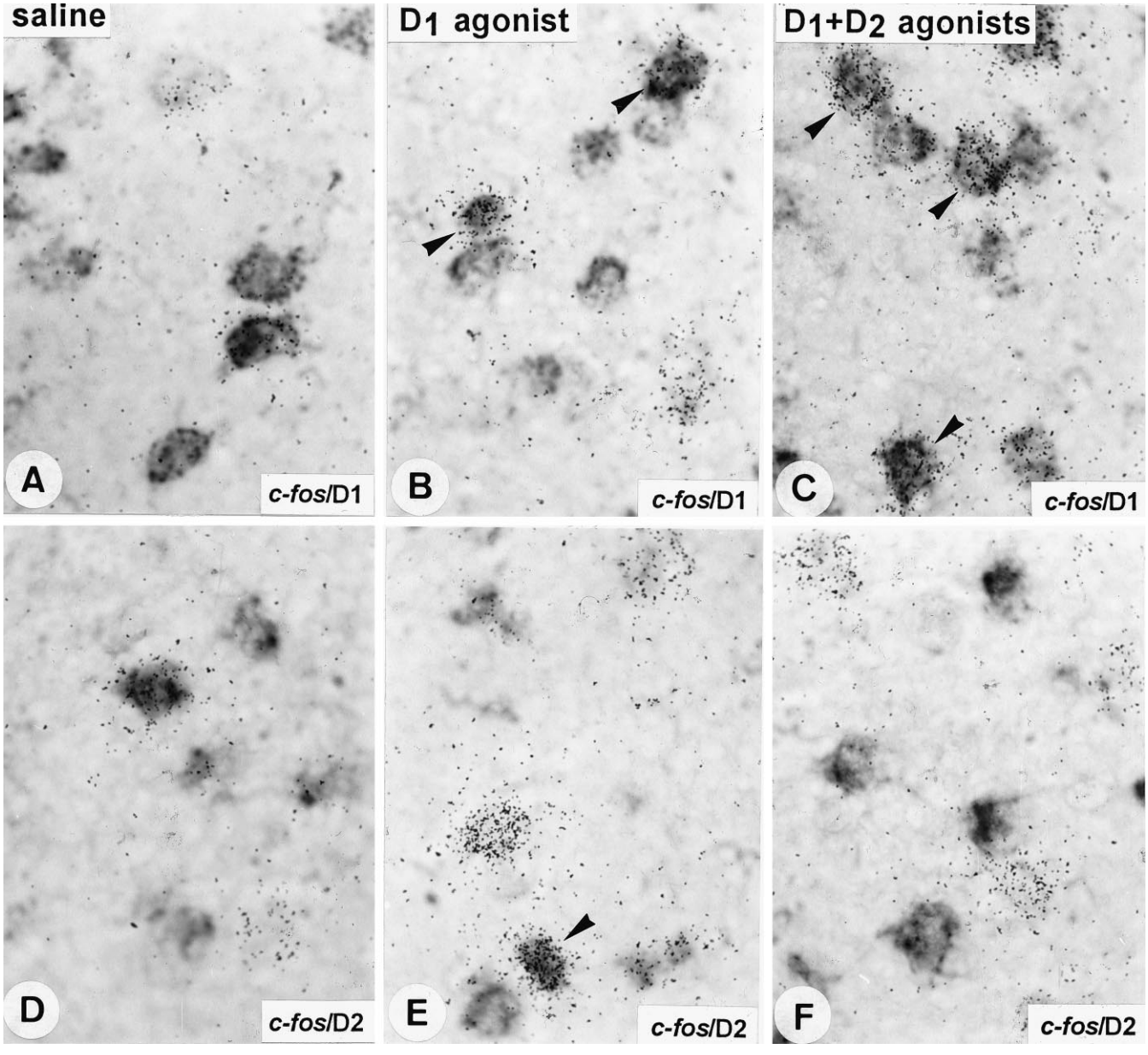

Figure 6. Phenotypical characterization of the striatal neurons expressing *c-fos* after D_1 and D_2 agonists, alone or in combination. Double *in situ* hybridization detects D_1 or D_2 receptor mRNA with digoxigenin-labeled riboprobe (stained cells), together with *c-fos* mRNA, with a ^{35}S-labeled riboprobe (silver grains). *A* and *D* show that *c-fos* mRNA is present both in D_1 mRNA-containing (*A*) and D_2 mRNA-containing (*D*) neurons under basal conditions. The D_1 agonist SKF-82958 increases *c-fos* expression both in D_1 mRNA-containing neurons (*arrowheads* in *B*) and in D_2 mRNA-containing neurons (*arrowhead* in *E*). As compared with the D_1 agonist alone, coadministration of D_1 and D_2 agonists potentiates the increase of *c-fos* expression in D_1 mRNA-containing neurons (*arrowheads* in *C*) and decreases it in D_2 mRNA-containing neurons. Quantitative data are listed in Table 2. Magnification, 640×.

Effect of combined D_1 and D_2 agonists on *c-fos* expression in the striatum

Thus, the effects of D_1 or D_2 agonists probably can be attributed to both direct postsynaptic effects and indirect effects mediated by the mesencephalic dopamine neurons. However, when these drugs are combined, the effects of endogenous dopamine are likely to be masked. Indeed, in the striatum, combined treatment with D_1 and D_2 agonists potentiated *c-fos* expression in D_1-containing neurons but inhibited it in D_2-containing neurons. The fact that the combined treatment induces *c-fos* at 92% in D_1-containing neurons is consistent with data obtained in conditions that enhance extracellular dopamine concentration (Graybiel et al., 1990; Young et al., 1991; Moratalla et al., 1993; Jaber et al., 1995; Wang et al., 1995; Chergui et al., 1996).

Effect of an A_{2A} antagonist alone or in combination with D_1 or D_2 agonists in the striatum

A_{2A} and D_2 receptors regulate pallidal GABA release in an opposite manner (Ferré et al., 1993; Mayfield et al., 1993, 1996) and are colocalized in striatopallidal neurons, but not in interneurons nor on nigrostriatal terminals (Schiffmann et al., 1991; Fink et al., 1992; Augood and Emson, 1994; Svenningsson et al., 1997). Therefore, studying the effects of A_{2A} receptors on striatal neurotransmission may be of interest not only to better under-

Table 2. Density of D_1− or D_2 striatal neurons expressing *c-fos* mRNA after D_1 or/and D_2 agonists and A_{2A} antagonist, alone or in combination

Treatment group	*n*	Fos+/D_2− neurons	Fos+/D_2+ neurons
Saline (*a*)	5	34.2 ± 4.6	26.7 ± 3.7
Quinelorane (*b*)	4	5.3 ± 1.7^{*a}	0.75 ± 0.4^{*a}
SKF-82958 (*c*)	5	84.2 ± 14.8^{*a}	73.0 ± 10.4^{*a}
SCH-58261 (*d*)	4	22.5 ± 3.8^{ns,a}	10.8 ± 3.1^{*a}
SKF-82958 + quinelorane	5	233.5 ± 25.3^{*b,c}	22.4 ± 2.5^{*b,c}
SKF-82958 + SCH-58261	4	166.6 ± 18.0^{*c,d}	65.6 ± 7.7^{*d,ns,c}
Quinelorane + SCH-58261	5	10.8 ± 2.3^{*b,ns,d}	1.5 ± 0.5^{*ns,b,d}

Rats were treated with saline (NaCl 0.9%), with the D_2 agonist quinelorane (2 mg/kg), with the D_1 agonist SKF-82958 (1 mg/kg), with the A_{2A} antagonist SCH 58261 (5 mg/kg), or various combinations: SKF-82958 (1 mg/kg) + quinelorane (2 mg/kg), SCH 58261 (5 mg/kg) + SKF-82958 (1 mg/kg), and quinelorane (2 mg/kg) + SCH 58261 (5 mg/kg). *c-fos* mRNA was detected with double *in situ* hybridization (exposure times, 10 weeks). Values represent the mean ± SEM of the number of *c-fos* mRNA-containing neurons per mm^2. Two-way ANOVA, followed by *post hoc t* tests corrected for the experiment-wise alpha level (Bonferroni correction). The results of the global ANOVA were for quinelorane/SKF-82958 interaction: $F_{(1,15)} = 31.55$, $p < 0.001$ for D_2-negative neurons and $F_{(1,15)} = 4.155$ for D_2-positive neurons; for SKF-82958/SCH-58261 interaction: $F_{(1,14)} = 15.45$, $p < 0.001$ for D_2-negative neurons and $F_{(1,14)} = 0.35$ for D_2-positive neurons. For the multiple *post hoc t* tests Bonferroni correction, an asterisk indicates relevant significant differences between indicated groups ($p < 0.05$).

stand adenosinergic modulation but also to delineate effects specifically related to an altered activity of striatopallidal neurons. We show here that the A_{2A} antagonist SCH-58261 shared with the D_2 agonist the ability to decrease *c-fos* expression in the striatum. This decrease occurred only in D_2-containing neurons, suggesting that this effect is mainly postsynaptic. Indeed, unlike the D_2 agonist, the A_{2A} antagonist does not affect dopamine release (Ferré et al., 1993). This supports the idea that endogenous adenosine acting at A_{2A} receptors regulates the constitutive expression of immediate early genes in the striatum (Svenningsson et al., 1995).

Coadministration of the A_{2A} antagonist with the D_1 agonist potentiated the D_1-induced increase in *c-fos* expression in D_1-containing neurons, like treatment with D_1 and D_2 agonists. However, this combination, unlike the D_1 plus D_2 combination, caused no inhibition of D_1-mediated *c-fos* induction in D_2-containing neurons. This suggests that regulation of *c-fos* by dopamine is more potent than A_{2A}-mediated effects on these neurons in our conditions. Whereas the D_1/D_2 combined treatment produced a change of the initial homogeneous striatal expression of *c-fos* into a "patchy" pattern, as previously described (Paul et al., 1992; Wang and McGinty, 1996), the pattern of *c-fos* expression after the D_1/A_{2A} combination was homogeneous in the striatum. These results suggest that D_2 receptors located postsynaptically on striatopallidal neurons, like the A_{2A} receptors, are involved in the quantitative enhancement of *c-fos* mRNA in striatal neurons, whereas D_2 receptors located presynaptically or on interneurons might be involved more specifically in differential dopaminergic regulations between the patch/matrix compartments.

D_1/D_2 and D_1/A_{2A} interactions in the globus pallidus

In accordance with previous immunohistochemical studies (Robertson et al., 1992; Marshall et al., 1993), we show here an increase of *c-fos* expression in the globus pallidus after administration of the D_2 agonist. A strong tendency for a decrease of *c-fos* expression was found after D_1 agonist treatment, although not significant in our statistical conditions. This tendency might be attributable to the D_1-mediated *c-fos* expression in striatopallidal neurons. Taken together, these data suggest that stimulation of D_1 and D_2 receptors has opposite effects on pallidal neurons also.

The combined treatment with D_1 and D_2 agonists potentiated the increase in *c-fos* expression induced by the D_2 agonist alone, as previously shown (Paul et al., 1992, 1995; Marshall et al., 1993). This agrees with electrophysiological data showing that the D_1 plus D_2 coactivation is required for the maximal excitatory effect,

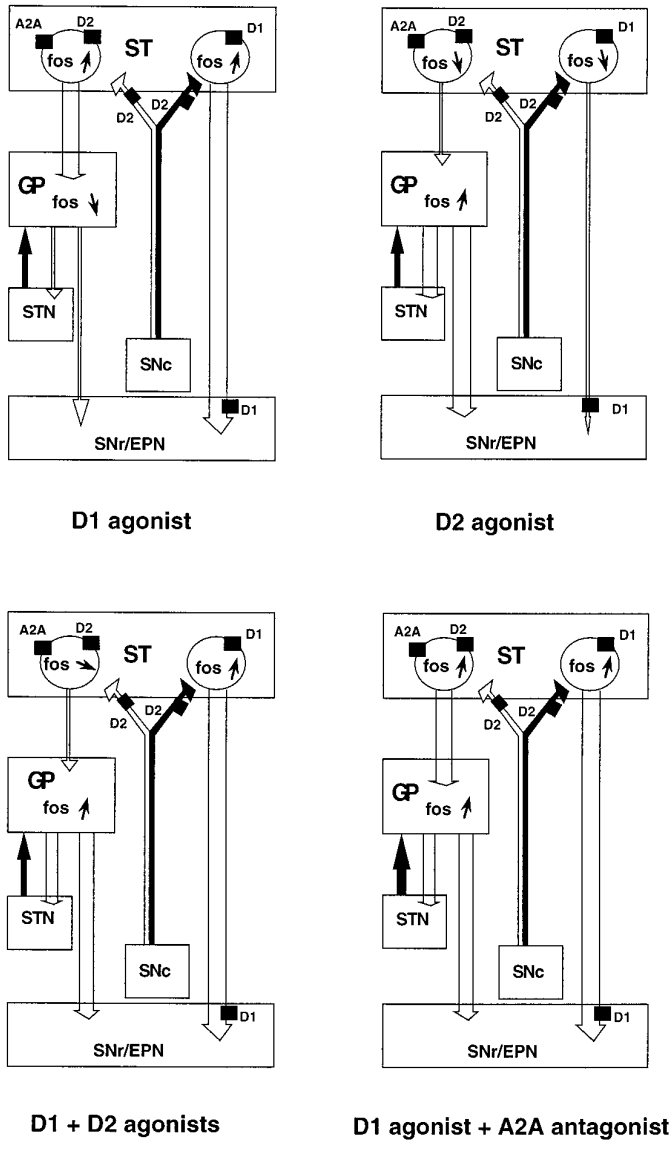

Figure 7. Schematic representation of the interactions in the basal ganglia after treatments with D_1 and D_2 agonists or combined treatment with D_1 + D_2 agonist or D_1 agonist + A_{2A} antagonist. The variations of expression of *c-fos* mRNA as compared with basal conditions are indicated inside the structure or the neuronal populations that we have studied. *Dark arrows* represent excitatory pathways, and *white arrows* represent inhibitory pathways. The *thickness* of the *arrows* changes according to the supposed neuronal activity in the different pathways. *ST*, Striatum; *GP*, globus pallidus; *SNc*, substantia nigra pars compacta; *SNr/EPN*, substantia nigra pars reticulata/entopeduncular nucleus; *STN*, subthalamic nucleus; *fos*, *c-fos* mRNA.

demonstrating a potentiated effect mediated by D_1 receptors on D_2 receptor-activated responses (Walters et al., 1987). There was also a strong induction of *c-fos* expression after combined treatment, using the A_{2A} antagonist together with the D_1 agonist. Interestingly, coadministration of A_{2A} antagonist together with the D_2 agonist had no synergistic effects on *c-fos* expression in the globus pallidus. This implies that, despite their coexpression and their well established interactions (Ferré et al., 1992, 1993), the D_2 and A_{2A} receptors are not solely the key for adenosine/dopamine interactions in the basal ganglia. Instead, our findings suggest that the most important functional interactions may be between drugs that affect A_{2A} and dopamine receptors in distinct neuronal populations. This conclusion also has implications for our understanding of the D_1/D_2 interactions.

Disinhibition of striatopallidal neurons is one of the mechanisms whereby *c-fos* is induced in globus pallidus. However, if *c-fos* expression can correlate with the activity of striatopallidal neurons, these neurons are likely to be stimulated rather than inhibited by combined treatments with D_1 plus D_2 agonists or D_1 agonist plus A_{2A} antagonist. Thus, the increase in pallidal *c-fos* expression may be attributable to the involvement of additional inputs to the globus pallidus. This may be attributable to an increased activity in the excitatory input from subthalamic nucleus. It has been found that NMDA receptor antagonists inhibit the induction of pallidal Fos immunoreactivity after combined administration of D_1 and D_2 agonists (Paul et al., 1992, 1995). Thus, it might turn out that concomitant stimulation of an excitatory input and inhibition of striatopallidal neurons act in synergy to increase *c-fos* in globus pallidus.

Conclusion

Although *c-fos* generally is used as a neuronal activation marker, we demonstrate here that basal *c-fos* expression is *up*regulated by a D_1 agonist but *down*regulated by a D_2 agonist or an A_{2A} antagonist. This suggests that *c-fos* mRNA levels may be used as an indicator of *inhibition* as well as activation of a neuronal pathway. Synergistic effects have been observed in the striatal output pathways after coadministration of D_1 plus D_2 agonists or D_1 agonist plus A_{2A} antagonist, providing evidence for important interactions between these parallel pathways. This work gives a basis for further investigations to elucidate the mechanisms whereby these synergistic effects occur, especially in the globus pallidus.

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