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$G\alpha_{olf}$ Levels Are Regulated by Receptor Usage and Control Dopamine and Adenosine Action in the Striatum

Denis Hervé,[1] Catherine Le Moine,[2] Jean-Christophe Corvol,[1] Leonardo Belluscio,[3] Catherine Ledent,[4] Allen A. Fienberg,[5] Mohamed Jaber,[6] Jeanne-Marie Studler,[1] and Jean-Antoine Girault[1]

[1]*Institut National de la Santé et de la Recherche Médicale (INSERM) U536, Institut du Fer à Moulin, 75005 Paris, France,* [2]*Centre National de la Recherche Scientifique Unité Mixte 5541, Laboratoire d'Histologie-Embryologie, Université Victor Segalen Bordeaux 2, 33076 Bordeaux cedex, France,* [3]*Department of Neurobiology, Duke University Medical Center, Durham, North Carolina 27710,* [4]*Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucléaire, Université Libre de Bruxelles, Campus Erasme, B-1070 Bruxelles, Belgium,* [5]*Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021, and* [6]*INSERM U259, Institut François Magendie, Université Bordeaux II, 33077 Bordeaux cedex, France*

In the striatum, dopamine D_1 and adenosine A_{2A} receptors stimulate the production of cAMP, which is involved in neuromodulation and long-lasting changes in gene expression and synaptic function. Positive coupling of receptors to adenylyl cyclase can be mediated through the ubiquitous GTP-binding protein $G\alpha_S$ subunit or through the olfactory isoform, $G\alpha_{olf}$, which predominates in the striatum. In this study, using double *in situ* hybridization, we show that virtually all striatal efferent neurons, identified by the expression of preproenkephalin A, substance P, or D_1 receptor mRNA, contained high amounts of $G\alpha_{olf}$ mRNA and undetectable levels of $G\alpha_s$ mRNA. In contrast, the large cholinergic interneurons contained both $G\alpha_{olf}$ and $G\alpha_s$ transcripts. To assess the functional relationship between dopamine or adenosine receptors and G-proteins, we examined G-protein levels in the striatum of D_1 and A_{2A} receptor knock-out mice. A selective increase in $G\alpha_{olf}$ protein was observed in these animals, without change in mRNA levels. Conversely, $G\alpha_{olf}$ levels were decreased in animals lacking a functional dopamine transporter. These results indicate that $G\alpha_{olf}$ protein levels are regulated through D_1 and A_{2A} receptor usage. To determine the functional consequences of changes in $G\alpha_{olf}$ levels, we used heterozygous $G\alpha_{olf}$ knock-out mice, which possess half of the normal $G\alpha_{olf}$ levels. In these animals, the locomotor effects of amphetamine and caffeine, two psychostimulant drugs that affect dopamine and adenosine signaling, respectively, were markedly reduced. Together, these results identify $G\alpha_{olf}$ as a critical and regulated component of both dopamine and adenosine signaling.

Key words: Golf; Gs; G-protein; D_1 receptor; A_{2A} receptor; knock-out mice; striatum; dopamine; adenosine; dopamine transporter; homologous recombination

The nigrostriatal dopaminergic neurons are essential for proper motor function in both humans and rodents. In the dorsal striatum, dopaminergic influence is predominantly mediated through D_1 (D_1R) and D_2 (D_2R) dopamine receptors that stimulate and inhibit cAMP production, respectively (Sibley and Monsma, 1992; Jaber et al., 1996). cAMP signaling contributes largely to the acute effects of dopamine as well as to long-lasting changes in gene expression and synaptic plasticity (for review, see Greengard et al., 1999; Berke and Hyman, 2000). In the striatum, D_1R and D_2R are enriched in distinct populations of efferent GABAergic spiny neurons (Gerfen et al., 1990; Hersch et al., 1995; Le Moine and Bloch, 1995; Yung et al., 1996), although sensitive measurements reveal some degree of overlap in receptor distribution (Surmeier et al., 1996). The D_1R-enriched population, referred to as striatonigral, projects to the substantia nigra pars reticulata and to the entopeduncular nucleus and contains substance P and dynorphin as cotransmitters, whereas the D_2R-enriched population, referred to as striatopallidal, projects to the external globus pallidus and contains enkephalins (Beckstead and Cruz, 1986; Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990, 1991; Le Moine and Bloch, 1995). In the striatopallidal neurons, which possess little or no D_1R, adenosine A_{2A} receptors ($A_{2A}Rs$) are the main receptor type stimulating the cAMP production (Premont et al., 1977; Schiffmann et al., 1991; Svenningsson et al., 1997). Because of this "strategic" location, $A_{2A}Rs$ are potential therapeutic targets for treating psychosis and Parkinson's disease (Ledent et al., 1997; Svenningsson et al., 1999).

In spite of the strong effects of D_1R and $A_{2A}R$ on cAMP production, the striatum contains small amounts of $G\alpha_s$, the G-protein subunit responsible for adenylyl cyclase stimulation in most cell types, but high concentrations of the olfactory isoform $G\alpha_{olf}$ (Drinnan et al., 1991; Herve et al., 1993). $G\alpha_{olf}$ shares 80% amino acid identity with $G\alpha_s$ and mediates olfactory receptor signaling in the olfactory epithelium (Jones and Reed, 1989; Belluscio et al., 1998). Cocaine responses are abolished in $G\alpha_{olf}$

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Correspondence should be addressed to D. Hervé, INSERM U536, Collège de France, 11, place Marcelin Berthelot, 75231 Paris, France. E-mail: denis.herve@infobiogen.fr.

knock-out mice, indicating that $G\alpha_{olf}$ may be necessary for dopamine action (Zhuang et al., 2000), and recent data provide strong evidence that $G\alpha_{olf}$ couples $A_{2A}R$ to adenylyl cyclase (Kull et al., 2000). Moreover, we have shown that D_1R- and $A_{2A}R$-stimulated cAMP production is blocked in the striatum of $G\alpha_{olf}$ knock-out mice (Corvol et al., 2001).

The first aim of the present study was to characterize in detail the cellular localization of $G\alpha_s$ and $G\alpha_{olf}$ in the striatum using *in situ* hybridization. We then examined the functional relationship between striatal $G\alpha_{olf}$ levels and dopamine or adenosine transmission, using mutant mice in which the genes for D_1R, $A_{2A}R$, or dopamine transporter (DAT) are disrupted. Finally, we examined the functional consequences of decreasing $G\alpha_{olf}$ levels, by measuring the locomotor responses to amphetamine and caffeine in mice, which have reduced levels of $G\alpha_{olf}$. Our results indicate thus that $G\alpha_{olf}$ is a crucial site for the regulation of D_1R and $A_{2A}R$ efficacy in the striatum and a potential locus for dysfunction in dopamine and adenosine neurotransmission.

MATERIALS AND METHODS

Tissue preparation for in situ *hybridization.* Brains from adult male Sprague Dawley rats (200–280 gm; Centre d'élevage Janvier, Le Genest. Saint-Isle, France) were dissected out, frozen in liquid nitrogen, and sectioned at the level of the striatum following the atlas of Swanson (1992) into 10-μm-thick sections that were stored at −80°C until use. All experiments were performed in accordance with the guidelines of the French Agriculture and Forestry Ministry for handling animals (decree 87849, license 01499) and with the Centre National de la Recherche Scientifique approval.

Probe synthesis. ^{35}S-labeled or digoxigenin-labeled cRNA probes were prepared by *in vitro* transcription from rat or mouse ($G\alpha_s$) cDNA clones corresponding to fragments of $G\alpha_{olf}$, $G\alpha_s$, preproenkephalin A (Yoshikawa et al., 1984), substance P (Bonner et al., 1987), D_1R (Monsma et al., 1990), and choline acetyltransferase (Ibanez et al., 1991) cDNAs. The $G\alpha_{olf}$ cDNA clone corresponded to the clone 8 described by Herve et al. (1995), and the $G\alpha_s$ cDNA clone corresponded to the most 3′ 550 bp fragment of the murine $G\alpha_s$ cDNA sequence (Sullivan et al., 1986). The sequence identity between $G\alpha_{olf}$ and $G\alpha_s$ probes was <30%. Transcription was performed from 50 ng of linearized plasmid using either ^{35}S-UTP (>1000 Ci/mmol; NEN Life Science, Paris, France) or digoxigenin-11-UTP (Roche Diagnostic, Meylan, France) and SP6, T3, or T7 RNA polymerases as described (Le Moine and Bloch, 1995). After alkaline hydrolysis to obtain cRNA fragments of ~250 bp, the ^{35}S-labeled probes were purified on Sephadex-G50. The ^{35}S-labeled purified probes and the digoxigenin-labeled probes were precipitated in 3 M sodium acetate, pH 5, and absolute ethanol (0.1/2.5 vol).

In situ *hybridization: single detection of* $G\alpha_{olf}$ *and* $G\alpha_s$ *mRNAs on cryostat sections.* 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 with 0.1 M triethanolamine in 4× SSC, pH 8, for 10 min at room temperature. After dehydration, the sections were hybridized overnight at 55°C under coverslips with 10^6 cpm of ^{35}S-labeled $G\alpha_{olf}$ or $G\alpha_s$ cRNA probes in 50 μl of hybridization solution (20 mM Tris-HCl, 1 mM EDTA, 300 mM NaCl, 50% formamide, 10% dextran sulfate, 1× Denhardt's reagent, 250 μg/ml yeast tRNA, 100 μg/ml salmon sperm DNA, 100 mM DTT, 0.1% SDS, and 0.1% sodium thiosulfate). Coverslips were removed by rinsing in 4× SSC. After 20 min of RNase A treatment (20 μg/ml) at 37°C, the sections were washed sequentially in 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 were used to expose x-ray films (Kodak Biomax; Eastman Kodak, Rochester, NY) for 3–6 d, and then dipped into Ilford K5 emulsion (diluted 1:3 in 1× SSC), which was developed after an 8 week exposition and stained with toluidine blue.

In situ *hybridization: simultaneous detection of two mRNAs on the same sections.* Different combinations of cRNA probes were used for the simultaneous detection of two mRNAs on the same sections. Cryostat sections were post-fixed, acetylated, and dehydrated as described above. Combinations of ^{35}S- and digoxigenin-labeled probes (10^6 cpm of ^{35}S-labeled probe plus 10–20 ng of digoxigenin-labeled probe) were hybridized in the same hybridization solution as described above. After elimination of coverslips, the slides were treated with RNase A and washed in decreasing concentrations of SSC as mentioned above, but without DTT. At the end of the washes, the slides were cooled 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 a 5 hr incubation at room temperature with alkaline phosphatase-conjugated anti-digoxigenin antiserum (1:1000 in buffer A containing 3% normal goat serum and 0.3% Triton X-100; Roche Diagnostic), the sections were rinsed twice 5 min in buffer A, twice 10 min in 1 M STM buffer (1 M NaCl, 0.1 M Tris, and 5 mM $MgCl_2$, pH 9.5), and twice for 10 min in 0.1 M STM buffer (0.1 M NaCl, 0.1 M Tris, and 5 mM $MgCl_2$, pH 9.5). The sections were then incubated overnight in the dark at room temperature in 0.1 M STM buffer containing 0.34 mg/ml nitroblue tetrazolium and 0.18 mg/ml bromo-chloro-indolylphosphate. The sections were rinsed briefly in 0.1 M STM buffer and 2 hr in 1× SSC, dried, and dipped into Ilford K5 emulsion (diluted 1:3 in 1× SSC). After being exposed 10–14 weeks in the dark, the emulsions were developed, and the sections were mounted without counterstaining. Labeled neurons both from single-labeling and double-labeling experiments were counted on sections from three different animals as previously described on similar material (Le Moine and Bloch, 1995).

Mutant mice. Pairs of heterozygous mice with a disrupted gene of $G\alpha_{olf}$ (Belluscio et al., 1998) and a hybrid 129 and C57Bl/6 genetic background were crossed. The genomic DNA of the progeny was extracted from tail tissue, digested by *Hin*dIII, and hybridized with a $G\alpha_{olf}$ probe corresponding 1.2 kb Pst/Kpn fragment of mouse $G\alpha_{olf}$ gene located 0.5 kb upstream to the ATG codon translational start site. The probe hybridizes with fragments of 2.8 kb when the gene is mutated or 15 kb fragments in wild-type (Belluscio et al., 1998). Pairs of heterozygous mice bearing a null mutation for D_1R or DAT gene and having a hybrid 129 and C57BL/6 genetic background were mated to obtain wild-type and homozygous mutant mice and were typed by Southern blot, as described by Drago et al. (1994) or by Giros et al. (1996). Male wild-type controls and homozygous $A_{2A}R$ (Ledent et al., 1997) or CB1 cannabinoid receptor (Ledent et al., 1999) knock-out mice used for the experiments were cousins, and their common grandparents were heterozygous mutant mice backcrossed on CD1 background for 10 generations. Mice were kept in stable conditions of temperature (22°C) and humidity (60%) with a constant cycle of 12 hr light and dark and had *ad libitum* access to food and water.

Gα *protein antibodies.* Specific antibodies against $G\alpha_{olf}$ (SL48SP) were obtained by immunizing a rabbit against a recombinant $G\alpha_{olf}$ protein and by purifying the obtained serum with $G\alpha_{olf}$ and $G\alpha_s$ columns, as described previously (Corvol et al., 2001). Specific antibodies against $G\alpha_s$ (SL22AP) were raised against a $G\alpha_s$-selective peptide and affinity-purified on a peptide column (Penit-Soria et al., 1997). The other antibodies were mouse monoclonal antibodies from commercial sources against $G\alpha_o$ (clone L5.6; Neomarkers, Union City, CA), $G\alpha_{i2}$ (clone 2A.3; Neomarkers), and Gβ (clone 3, Transduction Laboratories, Lexington, KY).

Immunoblot analysis. Wild-type and mutant mice (2–10 month old, age-matched) were killed by decapitation, and their brains were immediately dissected out from the skull and frozen on dry ice. Microdiscs of tissue were punched out from frozen slices (500-μm-thick) within the striatum using a stainless steel cylinder (1.4 mm diameter). Samples were homogenized in 1% SDS, equalized for their content in protein, and analyzed by Western blot as described previously (Herve et al., 1993). Antibody dilutions were 1:1000, 1:500, 1:300, 1:600, and 1:1000 for antibodies against $G\alpha_{olf}$, $G\alpha_s$, $G\alpha_{i2}$, $G\alpha_o$, and Gβ, respectively. Antibodies were revealed by the peroxidase–chemiluminescence method (ECL; Amersham, Orsay, France) and autoradiography. The antibodies were stripped one or two times to detect other antigens on the same membranes (Erickson et al., 1982; Herve et al., 1993). Quantification was performed by optical density measurement on autoradiographic films using a computer-assisted densitometer and the NIH Image software. In each membrane, samples from control and mutant mice were alternatively loaded, and the results were normalized as percentage of the mean of controls in each membrane.

cDNA probes and Northern analysis. The $G\alpha_{olf}$ cDNA probe corresponded to a PCR fragment containing the 1156 bp of the coding region of rat cDNA clone (Jones and Reed, 1989; Herve et al., 1995) and the $G\alpha_s$ probe was the same as described above. The glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) probe was a 1.3 kbp full length of rat GAPDH cDNA (Fort et al., 1985). Total RNA was extracted by the guanidinium thiocyanate–phenol-chloroform method (Chomczynski and Sacchi, 1987) from tissue microdiscs taken on frozen slices, and Northern blots were generated as previously described (Herve et al., 1993). The filters were hybridized successively with $G\alpha_{olf}$, $G\alpha_s$, and GAPDH probes, with a dehybridation step between two hybridations consisting in the incubation of membrane in boiling 0.5% SDS. Radioactivity of positive bands was measured using Instant Imager (Packard, Downers Groves, IL). $G\alpha_{olf}$ and $G\alpha_s$ mRNA concentrations were expressed as the counts per minute ratio of $G\alpha_{olf}$/GAPDH and $G\alpha_s$/GAPDH, respectively.

(^{3}H)SCH23390, (^{125}I)iodosulpride, (^{3}H)CGS21680, and (^{3}H)WIN35428 binding. Twenty micrometer coronal brain sections from three adult heterozygous $G\alpha_{olf}$ +/− mice and three wild-type littermates were thaw-mounted onto SuperFrost microscope slides. D_1R, D_2R, A_{2A}R, and DATs were analyzed by incubating the tissue sections with 2.5 nM (^{3}H)SCH23390 (91 Ci/mmol), 0.2 nM (^{125}I)iodosulpride (2000 Ci/mmol), 5 nM (^{3}H)CGS21680 (47 Ci/mmol,) and 4 nM (^{3}H)WIN35428 (86 Ci/mmol), respectively, in conditions as previously described (Bouthenet et al., 1987; Savasta et al., 1988; Jarvis and Williams, 1989; Herbert et al., 1999). The radioligands were obtained from Amersham Pharmacia Biotech (Saclay, France) or from NEN (Boston, MA). Nonspecific binding was determined from adjacent brain sections in the presence of 1 μM SCH23390, 10 μM sulpiride, 10 μM CGS21680, and 30 μM benztropine for blocking D_1R, D_2R, A_{2A}R, and DATs, respectively. After washing in ice-cold buffer, brain sections were rapidly dried and used to expose tritium-sensitive film (Hyperfilm ^{3}H; Amersham). In autoradiographs, the binding in caudate putamen and nucleus accumbens was evaluated by measuring the optical density with a computer-assisted image analyzer and the NIH image software, and the data were expressed in femtomoles of bound ligand per milligram of tissue using standards (^{3}H-microscales; Amersham Pharmacia Biotech).

Locomotor activity. Male heterozygous $G\alpha_{olf}$ +/− mice and wild-type littermates were introduced in a circular corridor (4.5 cm width, 17 cm external diameter) crossed by four infrared beams (1.5 cm above the base) placed at every 90° (Imetronic, Pessac, France). The locomotion was counted when the animals interrupted two successive beams and, thus, had traveled one-fourth of the circular corridor. In each session, the spontaneous activity was recorded for 50 min, before the animals were injected with saline or drugs, and their activity was recorded for an additional 60 or 120 min period. In the two first sessions, the animals received saline injections (5 ml/kg, i.p.), and in the third session, they received amphetamine (1, 2, or 3 mg/kg, i.p) or caffeine (25 mg/kg, i.p.) dissolved in saline. For each session an equal number of heterozygous and wild-type mice was studied, and for each drug treatment, groups of 7–14 animals were compared. The tests were performed between 12:00 and 6:00 P.M. in stable conditions of temperature and humidity.

RESULTS

Characterization of striatal neurons expressing $G\alpha_s$ and $G\alpha_{olf}$ mRNAs

As previously reported (Drinnan et al., 1991; Herve et al., 1993; Kull et al., 2000), the striatum was the brain region in which the contrast between the levels of $G\alpha_s$ and $G\alpha_{olf}$ mRNAs was the most dramatic (Fig. 1). At low magnification, high levels of $G\alpha_{olf}$ mRNAs were observed in all the striatal areas, including caudate putamen (Cp), nucleus accumbens (NA), and olfactory tubercle (OT) (Fig. 1*A,C,E,G*), which appeared almost devoid of $G\alpha_s$ mRNAs (Fig. 1*D,F*). A strong signal for both $G\alpha_{olf}$ and $G\alpha_s$ was detected in a few regions, including piriform cortex (Pir), islands of Calleja, medial habenula (Hb), and dentate gyrus (DG) (Fig. 1*A–H*), whereas in most other brain areas an intense labeling for $G\alpha_s$ mRNAs was associated with low or no labeling for $G\alpha_{olf}$ mRNA (Fig. 1*A–H*). The use of sense probes showed no labeling for either $G\alpha_{olf}$ (Fig. 1*I*) or $G\alpha_s$ (Fig. 1*J*) mRNAs.

At the cellular level $G\alpha_{olf}$ mRNA was expressed in 96.5 ± 1.3% (n = 1250) of the striatal neurons, mostly medium-sized. Conversely, $G\alpha_s$ mRNA-positive neurons were scattered all over the striatal areas, including 13.4 ± 1.2% (n = 1300) of the caudate putamen neurons, from which approximately one-third were large-sized. Double-labeling experiments were performed using various combinations of $G\alpha_{olf}$ and $G\alpha_s$ probes labeled with ^{35}S or digoxigenin. Virtually all $G\alpha_s$ mRNA-positive neurons expressed $G\alpha_{olf}$ mRNA levels above background, whereas $G\alpha_{olf}$ mRNA was present in numerous neurons with no labeling for $G\alpha_s$ mRNA (Fig. 2*A,B*). In particular, $G\alpha_{olf}$ mRNA was present in almost all striatal medium-sized neurons, very few of which also expressed

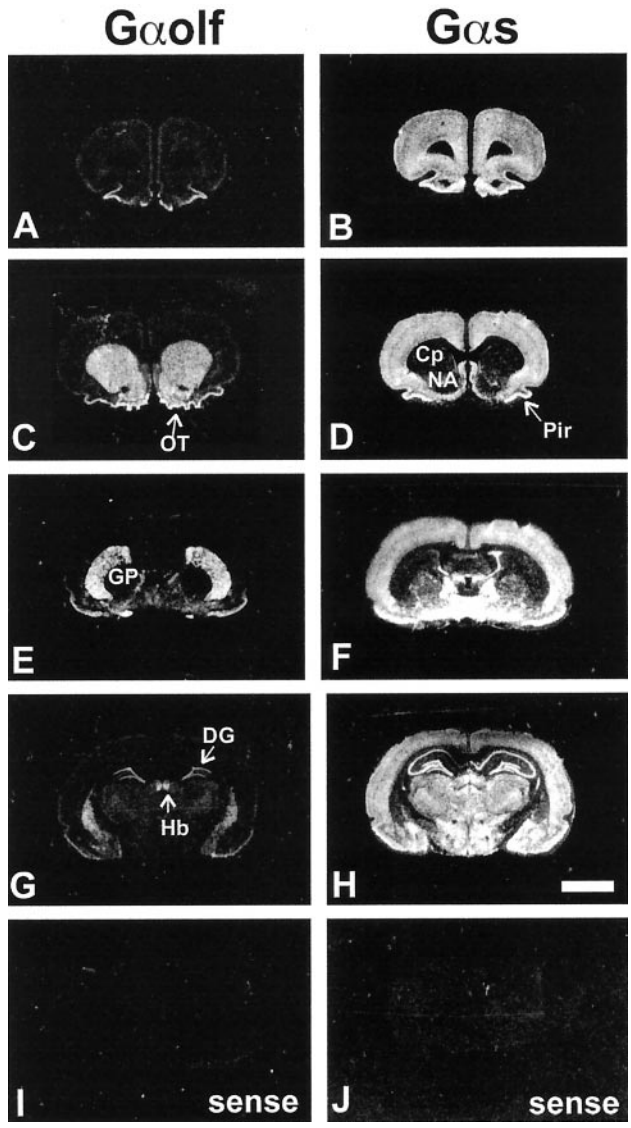

Figure 1. Distribution of $G\alpha_{olf}$ and $G\alpha_s$ mRNAs in forebrain. Negative of x-ray films exposed to rat brain adjacent sections hybridized with ^{35}S-labeled probes for $G\alpha_{olf}$ and $G\alpha_s$ mRNAs. *A*, *C*, *E*, *G*, $G\alpha_{olf}$ mRNA was highly expressed in striatal areas, including caudate putamen, nucleus accumbens, and olfactory tubercle (*C*, *E*, *G*). A signal was also found in the piriform cortex, the islands of Calleja, the medial habenula, and the dentate gyrus (*A*, *C*, *E*, *G*). All the other areas showed very low or no labeling for $G\alpha_{olf}$ mRNA. *B*, *D*, *F*, *H,* On the contrary, $G\alpha_s$ mRNA was highly expressed in many brain areas including all cortical areas, septum, globus pallidus, most of the hypothalamic and thalamic nuclei, hippocampus, and amygdala (*B*, *D*, *F*, *H*). Caudate putamen, nucleus accumbens, and olfactory tubercles showed almost no labeling, except for some sparse neurons (*D, F*). A total absence of labeling was observed in control experiments using sense probes for $G\alpha_{olf}$ and $G\alpha_s$ mRNAs (*I, J*). *Cp,* Caudate putamen; *NA,* nucleus accumbens; *OT,* olfactory tubercle; *GP,* globus pallidus; *Hb,* medial habenula; *DG,* dentate gyrus; *Pir,* piriform cortex. Scale bar, 5 mm.

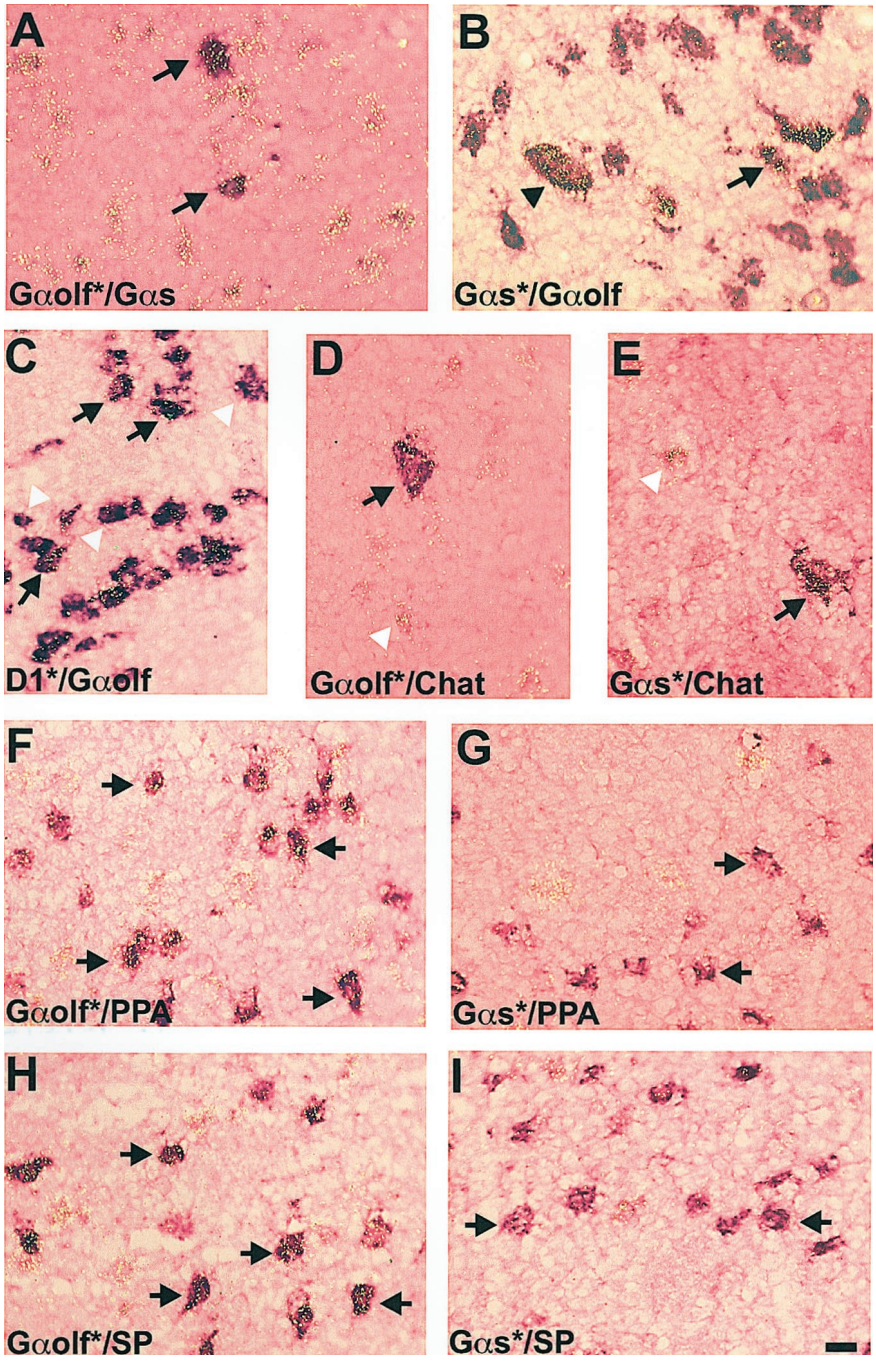

Figure 2. Phenotypical characterization of striatal neurons expressing $G\alpha_{olf}$ and $G\alpha_s$ mRNAs. Double-labeling *in situ* hybridization of rat brain sections was performed with various combinations of ^{35}S-labeled probes (indicated by *asterisks* in the figure) and digoxigenin-labeled probes. In all photomicrographs, silver grains for ^{35}S-labeling are visualized using epi-illumination and appear as *bright yellow* grains, whereas digoxigenin labeling appears as purple staining. *A,* Most $G\alpha_{olf}$-positive neurons (*silver* grains) did not contain $G\alpha_s$ mRNA (digoxigenin), which was present in only a few large- or medium-sized neurons (which also contained $G\alpha_{olf}$ mRNA; ^{35}S, *arrows*). *B,* The reverse combination of probe labeling showed that $G\alpha_{olf}$ mRNA (digoxigenin) was present in numerous medium-sized neurons, few of which also expressed significant levels of $G\alpha_s$ mRNA (^{35}S, *arrow*). Most of the large-sized neurons contained both mRNAs (*arrowhead*). *C,* Simultaneous detection of $G\alpha_{olf}$ mRNA (digoxigenin) and D_1R mRNA (*silver* grains) showed that $G\alpha_{olf}$ mRNA was colocalized with D_1R mRNA (*arrows*), but approximately half of $G\alpha_{olf}$ mRNA-positive neurons did not contain D_1R mRNA (*white arrowheads*). *D*, *E,* Double labeling for choline acetyltransferase mRNA (digoxigenin) and $G\alpha_{olf}$ mRNA (^{35}S; *D*) or $G\alpha_s$ mRNA (^{35}S; *E*). Both $G\alpha_{olf}$ and $G\alpha_s$ mRNAs were present in choline acetyltransferase-positive large neurons (*arrows*). Numerous medium-sized neurons expressed only $G\alpha_{olf}$ mRNA (*D, white arrowhead*), whereas very few expressed $G\alpha_s$ mRNA (*E, white arrowhead*). *F*, *G,* Double labeling for PPA (digoxigenin) and $G\alpha_{olf}$ mRNA (^{35}S; *F*) or $G\alpha_s$ mRNA (^{35}S; *G*). *H*, *I,* Double labeling for substance P mRNA (SP; digoxigenin) and $G\alpha_{olf}$ mRNA (^{35}S; *H*) or $G\alpha_s$ mRNA (^{35}S; *I*). $G\alpha_{olf}$ mRNA was present both in preproenkephalin A-positive (*F, arrows*) and substance P-positive (*H, arrows*) neurons, as well as in negative cells (*F, H, silver grains*). $G\alpha_s$ mRNA was mainly absent in these digoxigenin-labeled neurons (*G, I, arrows*). Scale bar, 12 μm.

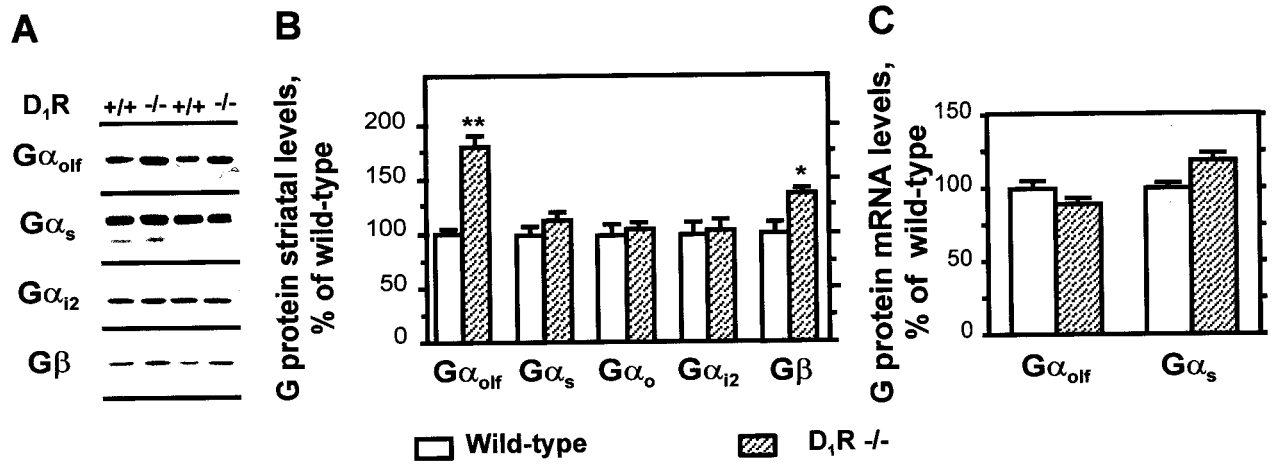

Figure 3. Levels of G-proteins in the striatum of D_1 receptor knock-out mice. *A,* Western blot analysis of representative striatal homogenates from D_1R knock-out (D_1R −/−) mice or wild-type controls (D_1R +/+), with antibodies that recognize specifically $G\alpha_{olf}$, $G\alpha_s$, $G\alpha_{i2}$, and $G\beta$. *B,* The optical density of immunoreactive bands was quantified on films and normalized taking as 100% of the mean optical density of controls on each film (see Materials and Methods). Results correspond to the mean ± SEM of data obtained in 6–16 animals studied in three independent experiments. $^*p < 0.05$ and $^{**}p < 0.01$ by two-tailed Student's *t* test. *C,* Northern analysis of RNA isolated from the striata of D_1R knock-out (D_1R −/−) mice and wild-type control (D_1R +/+) mice. In each lane, the amounts of ^{32}P-labeled $G\alpha_{olf}$, $G\alpha_s$, and GAPDH probes bound to the membranes were measured with an Instant Imager. The levels of $G\alpha_{olf}$ and $G\alpha_s$ mRNA were normalized to those of GAPDH mRNA and were expressed as a percentage of mean value measured in wild-type controls ($n = 14–16$).

high levels of $G\alpha_s$ mRNA (Fig. 2*B, arrow*). Simultaneous detection of $G\alpha_{olf}$ mRNA and D_1R mRNA showed that virtually all cells expressing D_1R mRNA were positive for $G\alpha_{olf}$ mRNA (Fig. 2*C, arrows*). However, approximately half of $G\alpha_{olf}$-positive neurons did not contain D_1R mRNA (Fig. 2*C*, *white arrowheads*). Large-sized neurons were positive for both $G\alpha_{olf}$ and $G\alpha_s$ (Fig. 2*B, arrowhead*). These large neurons, which contained $G\alpha_{olf}$ mRNA, as well as $G\alpha_s$ mRNA, were cholinergic interneurons, as demonstrated with a digoxigenin-labeled probe for choline acetyltransferase mRNA (Fig. 2*D,E, arrows*).

In the dorsal striatum, the striatopallidal and striatonigral medium-sized spiny neurons were identified using digoxigenin-labeled probes for preproenkephalin A mRNA (PPA) (Fig. 2*F,G*) or for substance P mRNA (SP) (Fig. 2*H,I*), respectively. $G\alpha_{olf}$ mRNA (^{35}S-labeled probe) was present in both preproenkephalin A- and substance P mRNA-containing neurons (Fig. 2*F,H, arrows*), whereas the levels of silver grains for $G\alpha_s$ mRNA (^{35}S-labeled probe) in these neurons were close to background (Fig. 2*G,I, arrows*).

Alterations of striatal G-protein levels in mice lacking D_1 receptors

Previous studies had shown that destruction of dopamine neurons resulted in an increase in $G\alpha_{olf}$ protein levels (Herve et al., 1993; Marcotte et al., 1994; Penit-Soria et al., 1997). The simplest explanation of these observations was that the increase in $G\alpha_{olf}$ was the consequence of the chronic absence of dopamine receptor stimulation. We therefore examined whether the complete absence of D_1R was able to alter $G\alpha_{olf}$, using mice in which the D_1R gene had been disrupted (Drago et al., 1994). As shown in Figure 3, the striatal $G\alpha_{olf}$ concentrations were higher in mutant mice than in wild-type littermates, whereas the levels of other G-protein α subunits $G\alpha_s$, $G\alpha_o$, and $G\alpha_{i2}$ were not altered (Fig. 3*A,B*). The levels of $G\beta$ subunit were also significantly increased in D_1R knock-out animals (Fig. 3*A,B*), probably reflecting the stoichiometric increase in $G\beta$ subunits participating in heterotrimeric complexes with $G\alpha_{olf}$. The lower increase in $G\beta$ (+36 ± 5%) than in $G\alpha_{olf}$ (+80 ± 10%) levels can be accounted for by the association of $G\beta$ subunits with other α subunits, which remained unaffected. Interestingly, a slight but significant increase in $G\alpha_{olf}$ protein was also detected in the striatum of heterozygous animals having only one null allele for the D_1R gene (116 ± 5% of wild-type; $n = 15$; $p < 0.05$), whereas $G\alpha_s$ protein levels were unchanged in these animals (103 ± 5% of controls).

We examined $G\alpha_{olf}$ mRNA levels by Northern blot to determine whether the increase in $G\alpha_{olf}$ protein observed in the striatum of mutant mice resulted from an increase in the transcription of the $G\alpha_{olf}$ gene and/or a stabilization of its transcript (Fig. 3*C*). At least two species of $G\alpha_{olf}$ mRNA were detected in the striatum of wild-type and mutant mice resulting from variations in the length of 5′- and 3′-untranslated regions of $G\alpha_{olf}$ mRNAs, as described previously (Herve et al., 1995). When we measured the total amount of radioactive probe hybridized with all the $G\alpha_{olf}$ mRNA species in Northern blots, the concentration of $G\alpha_{olf}$ transcripts was not significantly different in the striatum of D_1R −/− mutant mice or wild-type mice (Fig. 3*C*). When radioactivity was measured independently for the two main bands, no significant variation was observed in the striatum of D_1R-deficient mice, suggesting that the size pattern of $G\alpha_{olf}$ mRNA was unaffected in these mice (data not shown). Thus, the increase in $G\alpha_{olf}$ protein observed in the striatum of D_1R −/− mutant mice was not the consequence of alterations in $G\alpha_{olf}$ mRNA levels. $G\alpha_s$ mRNA concentrations were also not significantly altered in the striatum of D_1R knock-out mice (Fig. 3*C*).

Alterations of striatal $G\alpha_{olf}$ protein levels in mice lacking dopamine transporter

The results obtained in D_1R-deficient mice, as well as previous experiments using dopamine-depleted animals, indicated that an impairment in dopamine transmission could increase the levels of $G\alpha_{olf}$ in the striatum. To test if constitutive upregulation of dopamine neurotransmission could affect $G\alpha_{olf}$ in the opposite direction, we examined the striatal concentrations of $G\alpha_{olf}$ in mutant mice lacking DAT, responsible for the largest part of

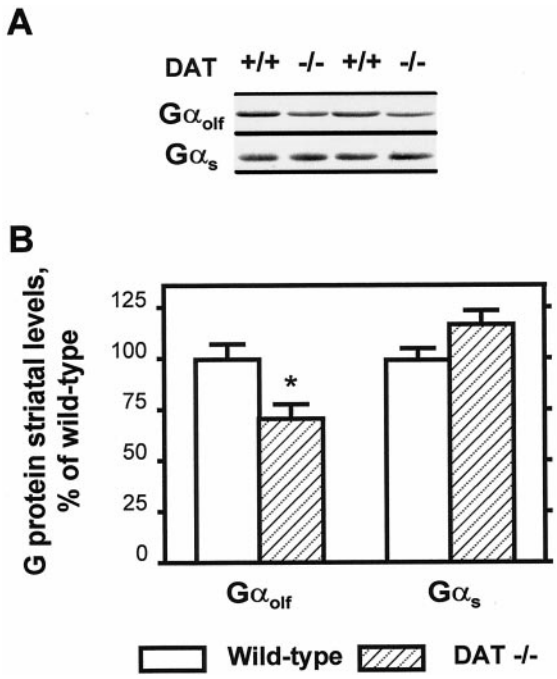

Figure 4. Levels of $G\alpha_{olf}$ and $G\alpha_s$ proteins in the striatum of dopamine transporter knock-out mice. *A,* Western blot analysis of representative striatal homogenates from DAT knock-out mice (DAT−/−) or wild-type littermates (DAT +/+). Blots were incubated with antibodies that recognize specifically $G\alpha_{olf}$ or $G\alpha_s$. *B,* The optical density of immunoreactive bands was quantified on films as in Figure 3*B* ($n = 8$; $^*p < 0.02$; two-tailed Student's *t* test).

dopamine clearance from the extracellular space (Giros et al., 1996). The levels of $G\alpha_{olf}$, but not $G\alpha_s$, were decreased in the striatum of these mice (Fig. 4). Thus, the chronic increase in the stimulation of D_1R on the one hand, and the absence of D_1R or of dopamine stimulation on the other hand, altered $G\alpha_{olf}$ protein levels in opposite directions.

Alterations of striatal $G\alpha_{olf}$ protein levels in mice lacking A_{2A} receptors

Because $G\alpha_{olf}$ was expressed in neurons containing $A_{2A}R$ and because the lack of $G\alpha_{olf}$ abolished the cAMP production induced by an A2 agonist (Corvol et al., 2001), we examined whether the absence of $A_{2A}R$ could alter $G\alpha_{olf}$ levels in a manner similar to D_1R. To evaluate this possibility, we measured $G\alpha_{olf}$ protein and other G-protein subunits in the striatum of mutant mice lacking $A_{2A}R$ (Ledent et al., 1997). By comparison to wild-type controls, an increase in $G\alpha_{olf}$ protein levels was observed in $A_{2A}R$ knock-out mice, whereas $G\alpha_s$, $G\alpha_o$, and $G\alpha_{i2}$ subunits were not significantly altered in the same striatal samples (Fig. 5*A,B*). In contrast to what was seen in D_1R knock-out mice, no change in striatal concentrations of $G\beta$ was detected in $A_{2A}R$ knock-out mice (Fig. 5*B*). This may be attributable to the fact that the increase in the concentration of $G\alpha_{olf}$ was less pronounced than in D_1R −/− mice, and not sufficient to enhance significantly the total levels of $G\beta$. As in the case of D_1R, the total amounts of $G\alpha_{olf}$ and $G\alpha_s$ mRNA (Fig. 5*C*) and the expression pattern of the various forms of $G\alpha_{olf}$ mRNA (data not shown) were not altered in $A_{2A}R$ knock-out mice.

The observed increases in $G\alpha_{olf}$ protein levels seen in D_1R and $A_{2A}R$ knock-out mice could be an unspecific consequence of the absence of any G-protein-coupled receptor. To rule out this possibility, we measured the G-protein subunits levels in knock-out mice lacking CB1 cannabinoid receptors, which are expressed at very high levels in medium-sized spiny neurons, but are negatively coupled to adenylyl cyclase unlike the D_1R and $A_{2A}R$ (Ledent et al., 1999). The lack of expression of CB1 receptors in the striatum induced no significant modification in the striatal concentrations of $G\alpha_{olf}$ (93 ± 5% of wild-type; $n = 4$). The striatal levels of $G\alpha_s$, $G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\beta$ subunits were also unchanged in these mice (data not shown). It is noteworthy that the deficiency in functional D_2R was reported to have no influence on $G\alpha_{olf}$ mRNA expression in the striatum (Zahniser et al., 2000).

Locomotor responses to amphetamine and caffeine in mice heterozygous for a null mutation of $G\alpha_{olf}$

Because alterations of dopamine or adenosine transmission affected $G\alpha_{olf}$ levels, we investigated whether changes in G-protein levels could have functional consequences on behavioral responses mediated through D_1R and $A_{2A}R$. In the striatum of mice heterozygous for a null mutation of $G\alpha_{olf}$ gene ($G\alpha_{olf}$ +/−), the levels of $G\alpha_{olf}$ were approximately half of those in wild-type mice, and the D_1R- and $A_{2A}R$-stimulated adenylyl cyclase responses were significantly reduced (Corvol et al., 2001). Thus, these mice provided an excellent model to test the effects of reduced amounts of $G\alpha_{olf}$, such as those observed in DAT-deficient mice, without the interference of other alterations of dopamine neurotransmission.

We examined the locomotor activity of $G\alpha_{olf}$ +/− mice and wild-type littermates in response to D-amphetamine and caffeine, two drugs whose actions depend on D_1R and $A_{2A}R$, respectively (Crawford et al., 1997; Ledent et al., 1997). The locomotor activity of mice was tested in three sessions, in which their basal activity was first monitored (Fig. 6*A*). During the second and third sessions, $G\alpha_{olf}$ +/− mice had a slightly lower spontaneous activity than wild-type littermates (Fig. 6*A*). The response to drugs was studied during the third session. When $G\alpha_{olf}$ +/− mice were challenged with 1, 2, or 3 mg/kg of D-amphetamine (Fig. 6*B*) or with 25 mg/kg of caffeine (Fig. 6*C*), their locomotor activity was stimulated but remained significantly lower than that of wild-type mice receiving the same treatments (Fig. 6*B,C*). Interestingly, the slight increase in locomotor activity induced by saline injection was also decreased in $G\alpha_{olf}$ +/− mice (Fig. 6*B*).

In $G\alpha_{olf}$ +/− mice, the alterations in responses to amphetamine and caffeine were not caused by changes in D_1R, D_2R, or $A_{2A}R$ levels in the caudate putamen or nucleus accumbens because the binding of (^{3}H)SCH23390, (^{125}I)iodosulpride, and (^{3}H)CGS21680 was unchanged in these brain regions (Fig. 7). The measurements of the (^{3}H)WIN35428 binding in the caudate putamen and nucleus accumbens of $G\alpha_{olf}$ +/− mice (Fig. 7) indicated also a normal density of DAT, which is known to be crucial for the psychostimulant effect of D-amphetamine in mouse (Giros et al., 1996).

DISCUSSION

Neuronal expression of $G\alpha_{olf}$ and $G\alpha_s$ in the striatum

Receptor-regulated production of cAMP in the striatum plays a central role in the physiology of the basal ganglia as well as in the long-term modifications that take place after repeated administration of drugs of abuse (for review, see Greengard et al., 1999; Berke and Hyman, 2000). The striatum contains high levels of $G\alpha_{olf}$ and very low levels of $G\alpha_s$ (Drinnan et al., 1991; Herve et al., 1993). Recent studies have demonstrated the role of $G\alpha_{olf}$ in

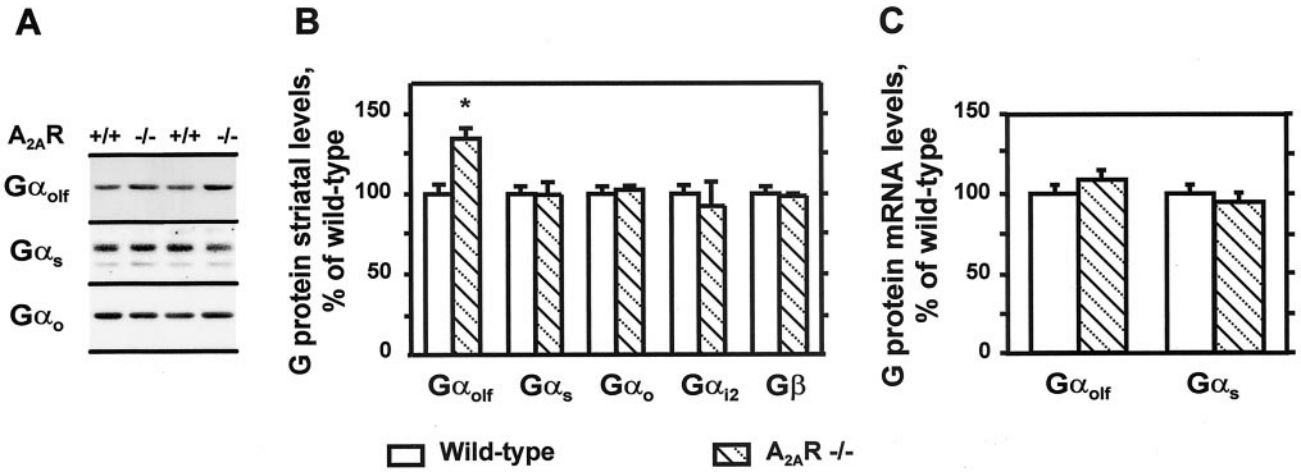

Figure 5. Levels of G-proteins in striatum of A_{2A} receptor knock-out mice. *A,* Western blot analysis of representative striatal homogenates from $A_{2A}R$ knock-out mice ($A_{2A}R$ −/−) or wild-type controls ($A_{2A}R$ +/+), with antibodies that recognize specifically $G\alpha_{olf}$, $G\alpha_s$, and $G\alpha_o$. *B,* The optical density of immunoreactive bands was quantified on films as in Figure 3*B* (n = 4–7; $^*p < 0.01$; two-tailed Student's t test). *C,* Northern analysis of RNA isolated from the striata of $A_{2A}R$ knock-out ($A_{2A}R$ −/−) mice and wild-type control ($A_{2A}R$ +/+) mice. Results were obtained as described in the legend to *C* (n = 10–11).

receptor-regulated cAMP production of the striatum and its functional consequences (Zhuang et al., 2000; Corvol et al., 2001). We confirm here that most medium-sized neurons, which correspond to GABAergic output spiny neurons and represent the vast majority of striatal neurons (Kemp and Powell, 1971), express $G\alpha_{olf}$ and very little or no $G\alpha_s$ transcripts (Kull et al., 2000). In the rat dorsal striatum these output neurons are divided in two populations identified on the basis of the neuropeptides they express (Gerfen and Young, 1988). We found that $G\alpha_{olf}$ mRNA was expressed in both types of neurons, which did not contain significant amounts of $G\alpha_s$ mRNA. Moreover, D_1R-positive neurons expressed $G\alpha_{olf}$ transcripts, but no detectable $G\alpha_s$. The rare medium-sized neurons containing $G\alpha_s$ mRNA also expressed $G\alpha_{olf}$ in double-labeling experiments. Because substance P- or preproenkephalin A-positive neurons did not express $G\alpha_s$, the medium-sized neurons containing both $G\alpha_s$ and $G\alpha_{olf}$ mRNA are likely to be aspiny interneurons (Kawaguchi et al., 1995). Interestingly, we show here that large-sized cholinergic interneurons, identified by the presence of choline acetyltransferase mRNAs, expressed both $G\alpha_{olf}$ and $G\alpha_s$ mRNAs. Striatal cholinergic neurons contain no $A_{2A}R$ (Svenningsson et al., 1997), but a few express low amounts of D_1R (Le Moine et al., 1991; Surmeier et al., 1996). Recent experiments using *in situ* hybridization have also confirmed the presence of D_5 receptor mRNA in these neurons (C. Le Moine, unpublished data), as previously indicated by single-cell RT-PCR experiments (Yan and Surmeier, 1997) and an immunohistochemical study in primate (Bergson et al., 1995). Our results indicate that both $G\alpha_{olf}$ and $G\alpha_s$ may couple D_1R and D_5 receptors to adenylyl cyclase in striatal cholinergic

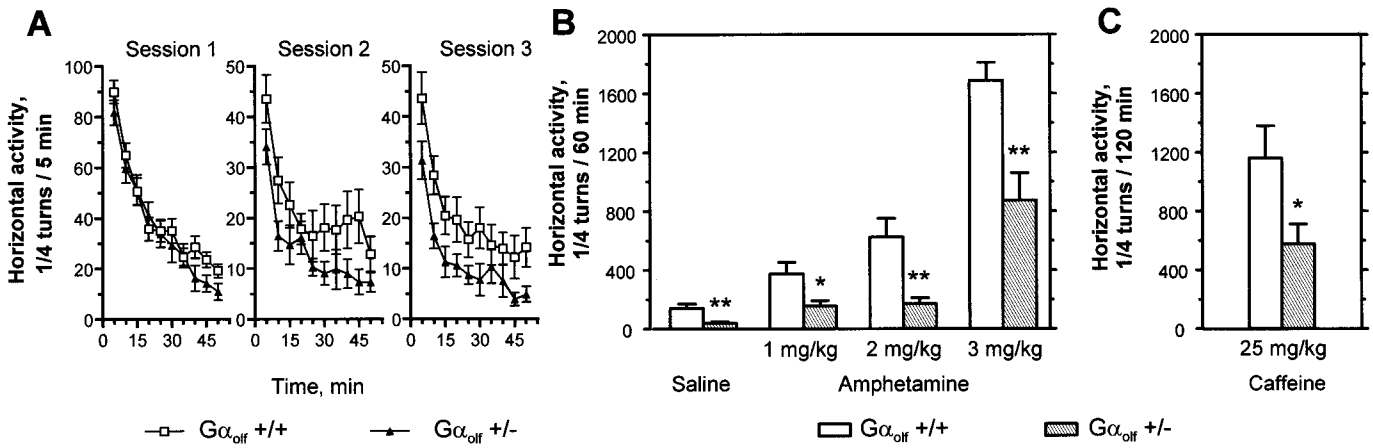

Figure 6. Effects of amphetamine and caffeine on the locomotor activity of $G\alpha_{olf}$ +/− mice. The horizontal activity of male heterozygous ($G\alpha_{olf}$ +/−) and wild-type ($G\alpha_{olf}$ +/+) mice was measured in three sessions. In each session, animals were allowed to habituate to the testing apparatus for 50 min and received saline injections in the two first sessions and amphetamine (1, 2, or 3 mg/kg) or caffeine (25 mg/kg) in the third session. *A,* Spontaneous activity of $G\alpha_{olf}$ +/− and $G\alpha_{olf}$ +/+ mice during the 50 min habituation period in the three sessions. Activities were significantly lower in mutant mice during sessions 2 and 3 (two-way ANOVA; $F_{(1,260)} = 22.3$ and $F_{(1,260)} = 33.1$, respectively; $p < 0.001$). *B,* Effects of amphetamine or saline injections on the locomotor activity of $G\alpha_{olf}$ +/− mice. Data for saline correspond to the results obtained in the second session. *C,* Effects of caffeine on the locomotor activity of $G\alpha_{olf}$ +/− mice. The results are the mean ± SEM of data obtained with 7–14 animals. $^*p < 0.05$ and $^{**}p < 0.01$, significantly different from wild-type controls with the same treatment.

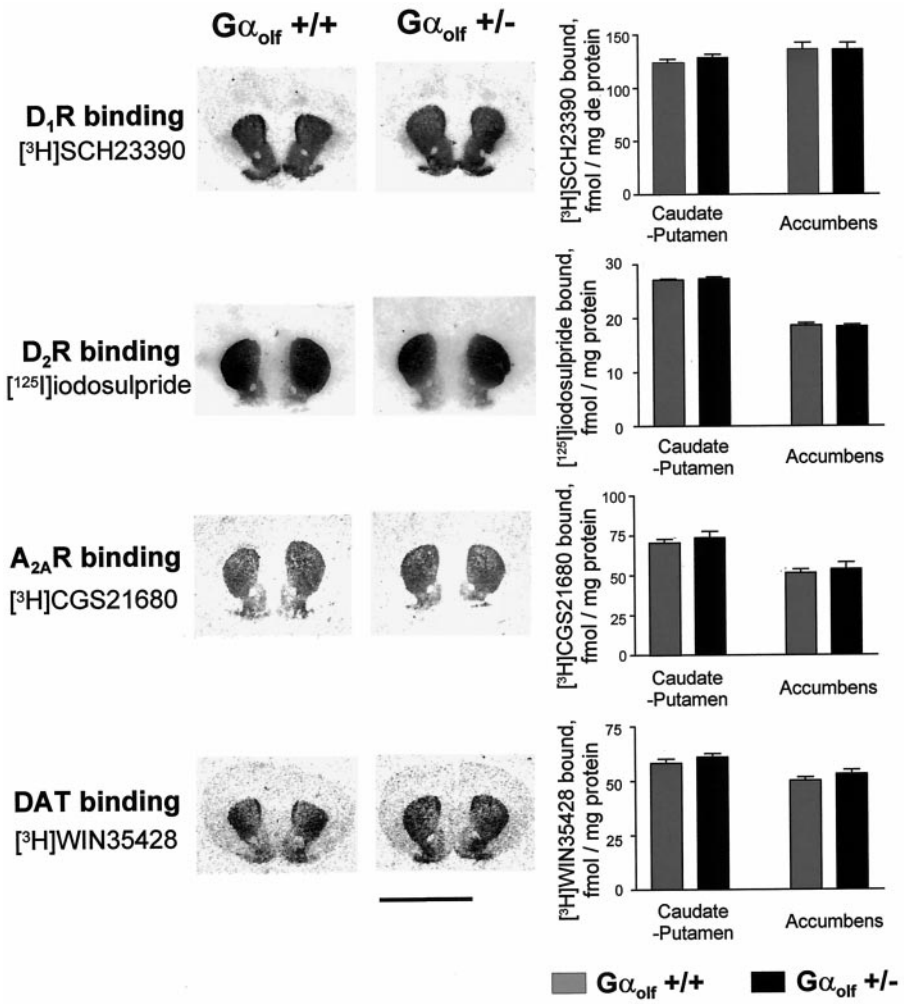

Figure 7. Lack of change in D_1, D_2, and A_{2A} receptors and in dopamine transporter in the caudate putamen and nucleus accumbens of heterozygous $G\alpha_{olf}$ knock-out mice. Coronal brain sections at the level of the striatum were incubated with (^{3}H)SCH23390, (^{125}I)iodosulpride, (^{3}H)CGS21680, or (^{3}H)WIN35428 to analyze the D_1R, D_2R, $A_{2A}R$, and DAT, respectively. The optical densities in the caudate putamen and the nucleus accumbens were measured in at least six different sections in each mouse and compared with standards. The results correspond to the mean ± SEM of data obtained in three mutant mice and three wild-type littermates. Scale bar, 5 mm.

neurons. Thus, it appears that virtually all the striatal output neurons express only $G\alpha_{olf}$, whereas interneurons express both $G\alpha_{olf}$ and $G\alpha_s$. The presence of $G\alpha_{olf}$ transcripts, but not of $G\alpha_s$, in D_1R- and $A_{2A}R$-rich output neurons, accounts for the fact that cAMP formation by stimulation of these receptors was virtually abolished in $G\alpha_{olf}$ mutant mice (Corvol et al., 2001).

Regulation of $G\alpha_{olf}$ levels in mice with genetically altered D_1 or A_{2A} signaling

In a previous study we have shown that the disappearance of striatal dopamine increased $G\alpha_{olf}$ levels by 40–50% (Herve et al., 1993; Penit-Soria et al., 1997). In mutant mice lacking either D_1R or $A_{2A}R$ we found a selective increase in $G\alpha_{olf}$ protein. Together these observations reveal that a lack of stimulation of D_1R, as well as the absence of either D_1R or $A_{2A}R$, result in an upregulation of $G\alpha_{olf}$ levels, suggesting that receptor usage may control $G\alpha_{olf}$ levels. This hypothesis is corroborated by the study of DAT −/− mice, which provide a model of chronic hyperstimulation of dopamine receptors *in vivo* (Giros et al., 1996). In these mice the levels of $G\alpha_{olf}$ protein were decreased in the striatum. These variations are reminiscent, at the level of a G-protein, of the classical "denervation hypersensitivity" and "agonist-induced desensitization," well characterized at the level of receptors (Freedman and Lefkowitz, 1996; Bloch et al., 1999).

Our results show that in $A_{2A}R$ and D_1R mutant mice, the changes in $G\alpha_{olf}$ occur at a post-transcriptional level and do not result from a change in gene expression. Preliminary evidence suggests that regulation of $G\alpha_{olf}$ levels may also be independent of cAMP-regulated protein phosphorylation pathways, because these levels were unchanged in mice lacking functional DARPP-32 (D. Herve, A. Fienberg, and J. A. Girault, unpublished observations), a key mediator in striatal cAMP-dependent protein phosphorylation (Fienberg et al., 1998). A more attractive possibility is that alterations in $G\alpha_{olf}$ protein levels result directly from changes in its rate of activation. This hypothesis is supported by several cell culture studies on $G\alpha_s$. In NG108–15 cells, stimulation of receptors activating adenylyl cyclase induced a downregulation of $G\alpha_s$ at a post-translational level, which was independent of cAMP production (McKenzie and Milligan, 1990; Adie and Milligan, 1994). Similarly, strong activation of $G\alpha_s$ by cholera toxin or by an activating mutation dramatically decreased its cellular concentration, possibly by an increased degradation (Levis and Bourne, 1992; Milligan, 1993). We suggest that analogous changes in $G\alpha_{olf}$ degradation rate may account for the changes in its levels observed here *in vivo*. Normal stimulation of D_1R and $A_{2A}R$ by endogenous dopamine and adenosine would result in a basal rate of $G\alpha_{olf}$ degradation, which is reduced in mice lacking dopamine or functional D_1R or $A_{2A}R$. Conversely, in mice lacking DAT, overstimulation of D_1R would increase $G\alpha_{olf}$ rate of degradation. According to this hypothesis, the

changes in $G\alpha_{olf}$ concentration are expected to occur only in the striatal cells affected by the mutation, i.e., striatonigral cells in mice lacking functional D_1R or DAT or striatopallidal cells in mice lacking functional $A_{2A}R$.

Role of $G\alpha_{olf}$ in locomotor activity

We have recently shown that reduced levels of $G\alpha_{olf}$ in heterozygous mutant mice ($G\alpha_{olf}$ +/−) resulted in a reduced stimulation of adenylyl cyclase by D_1R and $A_{2A}R$ in the striatum (Corvol et al., 2001). The present study demonstrates that reduced availability of $G\alpha_{olf}$ in these mice has also important functional consequences on spontaneous and drug-stimulated locomotor activity, revealing a novel phenotype of haplo-insufficiency.

The ventral striatum, including the nucleus accumbens, is involved in spontaneous and drug-stimulated motor activity (Pennartz et al., 1994). A role for D_1R in the control of locomotor activity is supported by several studies. Microinjection of D_1R antagonist in the ventral striatum of rats reduces their locomotor activity, whereas D_1R agonists have the opposite effect (Meyer, 1993). Accordingly, the decreased coupling of D_1R to adenylyl cyclase in the ventral striatum of $G\alpha_{olf}$ +/− mice could account for their slightly lower locomotor activity. By contrast it should be noted that an increase in basal activity has been reported in $G\alpha_{olf}$ −/− mice (Belluscio et al., 1998; Zhuang et al., 2000) and D_1R −/− mice (Xu et al., 1994). These surprising observations contrasted with the pharmacological evidence for a stimulatory role on motor activity of D_1R in the nucleus accumbens (Meyer, 1993; Vezina et al., 1994). These paradoxical behavioral effects in $G\alpha_{olf}$ −/− and D_1R −/− mice could be caused by differences in the methods used for measuring locomotor activity or alternatively may be the consequences of developmental changes and/or compensatory actions of other neuromodulatory systems such as serotoninergic or noradrenergic systems (Tassin et al., 1992; Trovero et al., 1992; Gainetdinov et al., 1999). Nevertheless, in both $G\alpha_{olf}$ −/− and D_1R −/− mice, the locomotor responses to cocaine or amphetamine are lost, unambiguously showing that both $G\alpha_{olf}$ and D_1R are necessary for the locomotor responses induced by these psychostimulants (Xu et al., 1994; Crawford et al., 1997; Zhuang et al., 2000). Interestingly, the acute effects of D-amphetamine on motor activity diminished in $G\alpha_{olf}$ +/− mice, further demonstrating that $G\alpha_{olf}$ levels determine the amplitude of D_1R-mediated responses.

In $G\alpha_{olf}$ +/− mice, the effects of caffeine on motor activity were reduced, indicating that $G\alpha_{olf}$ levels are an important factor for caffeine-induced behavioral response. The psychostimulant effects of caffeine are mediated through $A_{2A}R$ and are absent in mutant mice lacking these receptors (El Yacoubi et al., 2000). Caffeine is a nonselective antagonist of $A_{2A}R$, and caffeine could have a reduced effect in $G\alpha_{olf}$ +/− mice because the ongoing $A_{2A}R$ signaling, which is produced by endogenous adenosine, is blunted in these mice. This explanation is likely because endogenous levels of adenosine are sufficient to cause the activation of adenosine receptors in brain (for review, see Svenningsson et al., 1999). However, D_1R activity is required for caffeine-induced motor effect (Garrett and Holtzman, 1994), and the lower D_1R signaling in $G\alpha_{olf}$ +/− mice could also contribute to the attenuation of caffeine effects. Further experiments are needed to explore the respective contribution of these two mechanisms, both leading to a diminished effect of caffeine.

Functional implications

The present study demonstrates that experimental alterations in $G\alpha_{olf}$ concentrations affect two important types of neurotransmission in basal ganglia and provides strong evidence that $G\alpha_{olf}$ levels in striatal neurons are regulated *in vivo* by its use. Whereas much effort has been devoted to understand desensitization and downregulation of G-protein-coupled receptors *in vitro* and *in vivo* (Freedman and Lefkowitz, 1996; Bloch et al., 1999), little is known about the regulations that take place at the level of G-proteins. Our previous results in 6-hydroxydopamine-lesioned rats, as well as the present results in mice with genetically altered neurotransmission, show that a loss of D_1R activation or the absence of D_1R or $A_{2A}R$ result in an upregulation of $G\alpha_{olf}$. Thus, $G\alpha_{olf}$ constitutes an attractive locus for the regulation of D_1R and $A_{2A}R$ signaling in the striatum, either by modulation of its levels, or, possibly, by functionally relevant post-translational modifications. Alterations in $G\alpha_{olf}$ levels and/or activity are parameters that will have to be carefully considered in the function of the basal ganglia in physiological and pathological conditions. In this respect, our results introduce $G\alpha_{olf}$ heterozygous mutant mice as a potential animal model for studying striatum-dependent behavioral dysfunctions.

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