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Expression and Function of CB_1 Receptor in the Rat Striatum: Localization and Effects on D_1 and D_2 Dopamine Receptor-Mediated Motor Behaviors

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Cannabinoid CB_1 receptors are densely expressed on striatal projection neurons expressing dopamine D_1 or D_2 receptors. However, the specific neuronal distribution of CB_1 receptors within the striatum is not known. Previous research has established that the endocannabinoid system controls facilitation of behavior by dopamine D_2 receptors, but it is not clear if endocannabinoids also modulate D_1 receptor-mediated motor behavior. In the present study, we show that cannabinoid CB_1 receptor mRNA is present in striatonigral neurons expressing substance P and dopamine D_1 receptors, as well as in striatopallidal neurons expressing enkephalin and dopamine D_2 receptors. We explored the functional relevance of the interaction between dopamine D_1 and D_2 receptors and cannabinoid CB_1 receptors with behavioral pharmacology experiments. Potentiation of endogenous cannabinoid signaling by the uptake blocker AM404 blocked dopamine D_1 receptor-mediated grooming and D_2 receptor-mediated oral stereotypies. In addition, contralateral turning induced by unilateral intrastriatal infusion of D_1 receptor agonists is counteracted by AM404 and potentiated by the cannabinoid antagonist SR141716A. These results indicate that the endocannabinoid system negatively modulates D_1 receptor-mediated behaviors in addition to its previously described effect on dopamine D_2 receptor-mediated behaviors. The effect of AM404 on grooming behavior was absent in dopamine D_1 receptor knockout mice, demonstrating its dependence on D_1 receptors. This study indicates that the endocannabinoid system is a relevant negative modulator of both dopamine D_1 and D_2 receptor-mediated behaviors, a finding that may contribute to our understanding of basal ganglia motor disorders.
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INTRODUCTION

Cannabinoid CB_1 receptors are expressed in brain areas that contribute to movement such as the basal ganglia. The highest concentration of CB_1 receptors is found in the striatum, where they colocalize with dopamine D_1 and D_2 receptors in striatal neurons (Herkenham *et al*, 1990, 1991; Tsou *et al*, 1998; Hermann *et al*, 2002; Julián *et al*, 2003). However, their regional and neuronal distribution has not been established. It is known that there is a continuous release of endogenous cannabinoid CB_1 receptor agonists such as anandamide in the brain, and that these endogenous agonists exhibit neurotransmitter function (Giuffrida *et al*, 1999; Baker *et al*, 2000). The endocannabinoid system can act as a modulator of dopaminergic neurotransmission in the basal ganglia (Cadogan *et al*, 1997; Glass and Felder, 1997; Pertwee, 1999; Giuffrida *et al*, 1999; Beltramo *et al*, 2000; Gerdeman and Lovinger, 2001; Gubellini *et al*, 2002). Endogenous cannabinoids have been proposed to act in a homeostatic mechanism in the basal ganglia by activating CB_1 receptors, which appear to function as a brake on dopaminergic function in the striatum (Rodriguez de Fonseca *et al*, 1994, 1998).

Although much is known about the central effects of exogenously applied cannabinoids, the functional relevance of the endogenous cannabinoid system needs further investigation. In this context, the discovery of the highly potent CB_1 receptor antagonist, SR141716A, and the

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indirect agonist *N*-(4-hydroxyphenyl)-arachidonamide (AM404, which acts as an anandamide uptake blocker) have opened new possibilities for the identification and characterization of cannabinoid-dependent function. Particularly, the use of SR141716A has shown a close relationship between CB_1 receptors and striatal dopamine D_1, and D_2 receptor-mediated functions (Rodriguez de Fonseca *et al*, 1994, 1998). AM404, through blockade of the endocannabinoid transporters, causes accumulation of anandamide and 2-arachidonoylglycerol (2-AG), prolonging dopamine-mediated responses of endogenous cannabinoids (Beltramo *et al*, 2000; Glaser *et al*, 2003). The effects of CB_1 receptor antagonists in the striatum are proposed to be due to release from the inhibitory influence of endogenous CB_1 receptor agonists on striatal dopamine D_2 receptor function (Rodriguez de Fonseca *et al*, 1994, 1998; Giuffrida *et al*, 1999). However, several studies suggest that D_1 and CB_1 receptors also interact negatively in several rodent behaviors (Sañudo-Peña *et al*, 1998a). Determining the functional interaction between CB_1 and D_1 and D_2 receptors in the striatum is important for understanding neurochemical changes in diseases such as Parkinsonism and schizophrenia and in adaptive processes including the rewarding effects of drugs of abuse. Dopamine receptor agonists and antagonists are currently used therapeutically for these disorders and there is emerging evidence that CB_1 cannabinoid receptor antagonists have a therapeutic effect in some of these disorders as well (Fernandez-Espejo *et al*, 2005; Sañudo-Peña *et al*, 1998b).

The functional interaction between striatal CB_1 and D_1, and D_2 dopamine receptors can be studied by examining the effect of pharmacological modulation of these receptors on motor activity in rats. Because dopamine agonists injected into the striatum induce turning behavior, this behavioral response represents an index of dopaminergic imbalance between the two hemispheres (Ungerstedt and Arbuthnott, 1970; Schwarting and Huston, 1996; Gerfen *et al*, 1990; Keefe and Gerfen, 1995; Pavón *et al*, 2006). For example, unilateral intrastriatal injection of D_1 receptor agonist induces contralateral turns (Keefe and Gerfen, 1995; Pavón *et al*, 2006). D_1 and D_2 receptor functions can be assessed through specific behavioral patterns as well, since these behaviors are mostly modulated at the striatal level (McPherson and Marshall, 1996; Davidkova *et al*, 1998). Some of these responses are mediated by D_2 receptors, like oral movements (mouth fasciculation, yawning, biting, licking) while others like grooming are mediated by dopamine D_1 receptor stimulation (Molloy and Waddington, 1984; Starr and Starr, 1986a, b; McPherson and Marshall, 1996). These behaviors have been extensively studied using *in vivo* pharmacology and electrophysiology and have been shown to reflect striatal function (Aldridge and Berridge, 1998).

We hypothesized that endogenous cannabinoids modulate both D_1 and D_2 dopamine-induced motor behaviors through the interaction of CB_1 receptors. To characterize the functional neuroanatomy of cannabinoid receptor interaction in the basal ganglia, we analyzed the distribution of cannabinoid CB_1 receptors and dopamine D_1 and D_2 receptors in the striatum. To assess the functional role of endogenous cannabinoids in behavioral responses mediated by dopamine D_1 or D_2 receptors, we examined turning behavior, grooming, and oral stereotypies. We also used a dopamine D_1 receptor knockout ($D_1R^{-/-}$) mouse to support further the specificity of the responses studied.

MATERIALS AND METHODS

Animals

Studies were carried out in inbred adult male Wistar rats, ranging from 2 to 3 months old, weighing 250–300 g or in male wild-type and dopamine D_1 receptor knockout ($D_1R^{-/-}$) mice (Xu *et al*, 1994; Moratalla *et al*, 1996) derived from the mating of heterozygous mice, weighing 24–28 g. The genotype of each mouse was determined by genomic Southern blot analysis. Animals were housed in a temperature-controlled room ($22 \pm 1^{\circ}C$) on a 12-h light–dark cycle (lights on at 0800 h) with free access to food and water. The maintenance of animals and the experimental procedures were approved by the bioethical committee at the Cajal Institute and followed the guidelines from the European Union (Council Directive 86/609/EEC).

Drugs and Doses

The dopamine D_1 receptor agonists SKF38393 or SKF81297 (Tocris, Bristol, UK) dissolved in double distilled water and quinpirole (a dopamine D_2 receptor agonist, RBI, Natick, USA) dissolved in 20% ethanol were administered at 0, 0.5, and 1 μg/μl for intrastriatal injections and 5 mg/kg (SKF38393, SKF81297) or 1 mg/kg (quinpirole) for subcutaneous administration. The D_2 receptor agonist bromocriptine and CB_1 agonist HU-210 (Tocris, UK) were each dissolved in 10% ethanol with double-distilled water. Bromocriptine was administered by intrastriatal injection at 0.5 and 10 μg/μl, and HU-210 was administered at a dose of 20 μg/kg, i.p. (intraperitoneal). The CB_1 antagonist SR141716A (gift from Sanofi-Synthelabo Recherche, France), dissolved in 20% DMSO, was administered at 0, 1, and 1.5 μg/μl for intrastriatal injection or 0.3 and 1 mg/kg, i.p. AM404 (the anandamide uptake blocker, Tocris), dissolved in Tween 80 : propylen glycol : saline (5 : 5 : 90, by vol/vol), was administered at 0, 2.5, and 5 μg/μl for intrastriatal injection and 0.3 or 10 mg/kg for i.p. administration. Local injections in the striatum were performed in a volume of 1.5 μl. In each case, the same volume of appropriate vehicle was used for the 0 dose. We followed a previous method for selecting the injection site (Routtenberg, 1972). Using this method, an injection of 1.5 μl diffuses over approximately 3 mm^3, sufficient to affect a significant area of the striatum (Routtenberg, 1972). When postmortem analysis revealed injection sites that were off the target area, those animals were not included in the analysis.

Tissue Preparation for *In Situ* Hybridization

Rats were euthanized by rapid decapitation and their brains were quickly removed, frozen in dry ice and stored at $-80^{\circ}C$. Complete rostro-caudal series of coronal sections (12 μm thick) were cut in a cryostat (Leica, Wetzlar, Germany), thaw mounted onto microscope slides, air-dried and stored at $-80^{\circ}C$.

Riboprobe Synthesis and Labeling

We used the following riboprobes: a 492 bp cRNA probe complementary to rat preproenkephalin (pro-Enk) cDNA plasmid provided by Dr Sabol (NIH, Maryland, USA); a 480 bp cRNA for rat β-preprotachykinin, substance P (SP), provided by Dr James E Krause (Branford, USA); a 430 bp cRNA for rat somatostatin (Som), provided by Dr Cacicedo (Hospital Ramón y Cajal, Spain); a 700 bp cRNA for rat parvalbumin (PVB), provided by Dr Berchtold, (Universitat Zurich, Switzerland); a 694 bp cRNA for rat choline acetyltransferase (ChAT), provided by Dr Berrard (Hôpital de la Pitié Salpêtriere, Paris); a 326 bp cRNA for rat glutamic acid decarboxylase 67 (GAD67), provided by Dr Tillakaratne (University of California, USA), and a 1619 bp cRNA for the human CB_1 receptor provided by Dr Santos (Universidad Complutense de Madrid, Spain). Riboprobes were labeled with ^{35}S-isotope (^{35}S-CTP) or with digoxigenin 11-UTP to carry out dual *in situ* hybridization. Riboprobes were synthesized by *in vitro* transcription as in Julián *et al* (2003). Briefly, 1 μg of the appropriate template was reacted with 350 μCi of a ^{35}S-CTP (1000 Ci/mmol, NEN, MA, USA), 50 μM unlabeled CTP, 20 mM each of ATP, GTP, and UTP, 15–20 units of the appropriate RNA polymerase, 100 mM of dithiothreitol (DTT), and 20 units of RNasin (Promega Corporation, Madison, USA) for 1 h at 37°C. Digoxigenin-labeled probes were synthesized with 1 μg of the appropriate template, 2 μl of digoxigenin RNA labeling mix (10 ×) (Roche Molecular Biochemicals, Mannheim, Germany), 15–20 units of appropriate RNA polymerase and 20 units of RNasin and incubated for 2 h at 37°C. Labeled riboprobes were purified by ethanol precipitation and resuspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6) containing 100 mM DTT (for ^{35}S labeled probes only) and 40 units of RNase inhibitor and stored at −80°C.

In Situ Hybridization

Selected sections were fixed in 4% paraformaldehyde, acetylated, rinsed in PBS, dehydrated with ethanol, and defatted in chloroform. ^{35}S-labeled CB_1 receptor riboprobe (alone or in combination with a digoxigenin-labeled riboprobe chosen as a marker of striatal neurons) were mixed in a hybridization solution, applied to sections, and hybridized for 12 h at 60°C. Radioactive labeled probes were diluted in the hybridization solution to reach a 60 000–80 000 c.p.m./μl and digoxigenin-labeled probes were diluted 1 : 100. After hybridization, slides were rinsed in saline sodium citrate solution (SSC), treated with RNase A (100 μg/ml) and finally washed in SSC at 65°C. For single *in situ* hybridization, slides were placed in cassettes and exposed to Hyperfilm βMax (Amersham Pharmacia Biotech, Barcelona, Spain) for 3–5 days. Films were developed in D-19 (Eastman Kodak, NY, USA) and analyzed with an image analysis system. For dual *in situ* hybridization, slides were processed to detect the second mRNA labeled with digoxigenin. Slides were incubated overnight with alkaline phosphatase-conjugated polyclonal anti-digoxigenin antiserum (Roche Molecular Biochemicals) diluted 1 : 1000. The following day, slides were incubated in the dark with nitro blue tetrazolium, (NBT, 0.34 mg/ml) and 5-bromo-4-chloro-3-indolyl-phosphato (BCIP; 0.175 mg/ml). Reaction progress was monitored with a light microscope for the development of color. After exposure to Hyperfilm βMax films, selected slides were dipped in nuclear track emulsion LM1 (Eastman Kodak, New York, USA) diluted 3 : 1 in distilled water with 0.1% glycerol, stored in the dark with desiccant and developed after 2–6 weeks.

In Situ Hybridization Analysis

Quantitative analysis of CB_1 receptor mRNA on each striatal neuronal population was carried out by quantifying the number of silver grains and their distribution in digoxigenin-labeled neurons using a computer assisted image system (Qwin 500, Leica Microsistemas SA, Barcelona, Spain). Both the area of the neuronal profile (μm^2) and the number of grains present within the area were recorded and used to compute the intensity of labeling of each neuron (expressed as grains per 1.000 μm^2). For each striatal neuronal population, we quantified grains over a minimum of 2000 neurons per hemisphere. We used three animals, two coronal sections (200–300 μm apart) per animal. Statistical analysis was performed by one-way analyses of variance (ANOVA) with a repeated measures design, followed by Student's *t*-test and *post hoc* comparison with Bonferroni–Dunnet test for each population of striatal neurons to determine differences in CB_1 receptor expression. Differences with a $\sigma < 0.05$ were considered significant.

Behavioral Studies

Grooming and oral movements. Rats and mice (eight per group) were handled and placed in a glass observation box of 30 × 40 × 30 cm for a week. For behavioral studies, animals were videotaped in the familiar glass box and the time spent grooming and the number of oral stereotypies were registered by trained observers blind to the experimental conditions, as described previously (Beltramo *et al*, 2000; Giuffrida *et al*, 1999). Grooming behavior and oral movements were scored over 5 min intervals at 5, 15, 30, and 60 min after the injection. Data are presented as a sum of all time intervals (mean ± SEM). Statistical analysis was performed using one- or two-way analysis of variance (ANOVA) followed by Student or Newman–Keuls *post hoc* test. These analyses were completed using STATA program (Intercooled Stata 6.0, Stata Corporation, College Station, TX). A probability level of 5% ($p < 0.05$) was considered significant.

Rotational behavior. Local injections in the rat striatum were carried out with a guide cannula implanted a week before the experimental studies. The guide cannula (22 gauge stainless steel) was placed in the rat striatum, under anesthesia, with a Kopf stereotaxic frame, 2 mm above the corresponding infusion site (coordinates in mm from bregma and dura, AP = +0.5, $L = -3$, and $V = -5.5$; Paxinos and Watson, 2005), fastened to the skull with dental cement and fitted with a 30-gauge stainless steel obturator. Injections were performed in the home cage, replacing the obturator cannula by a 30-gauge internal cannula (Small Parts, Miami, USA) connected to a Hamilton syringe and a delivery pump. Solutions were injected over a 5 min period, and afterwards the internal cannula was

removed and the obturator cannula replaced. There were 8–10 animals in each group for behavioral studies. To study effects of compounds alone, SKF38393, quinpirole, bromocriptine, or CB_1 ligands were infused at different doses in different groups. To study the interaction between dopaminergic and cannabinoid systems we used vehicle, 0.5 or 1 μg/μl SFK38393, following a Latin square type design, changing the initial dose of dopaminergic ligand for every rat (one group of rats for each dopaminergic ligand). Vehicle, SR141716A (1.5 μg/μl) or AM404 (5 μg/μl) were injected 5 min before SKF38393. These doses elicit maximum turning behavior. If both cannabinoid ligands were injected, SR141716A was injected 5 min before AM404, and SKF38393 was injected 5 min after AM404. Locomotor directional bias was evaluated by quantifying ipsilateral and contralateral rotations induced by the infusion of compounds alone or in combination. Rotations were quantified for 60 min following injections with a rotometer system (Panlab, Barcelona, Spain). For statistical analysis we used one-way ANOVA for drugs administered alone (drug dose as factor) and two-way ANOVA for combinations of drugs (SKF38393 dose as within variable, treatment as in between factor), followed by *post hoc* comparisons with Tukey's test for drug interactions. After completion of experiments, rats were anesthetized and perfused transcardially with 4% paraformaldehyde. Brains were removed and stored in PBS at 4°C, for subsequent sectioning (50 μm). Brain sections were mounted on slides and stained with cresyl violet to examine cannula placements.

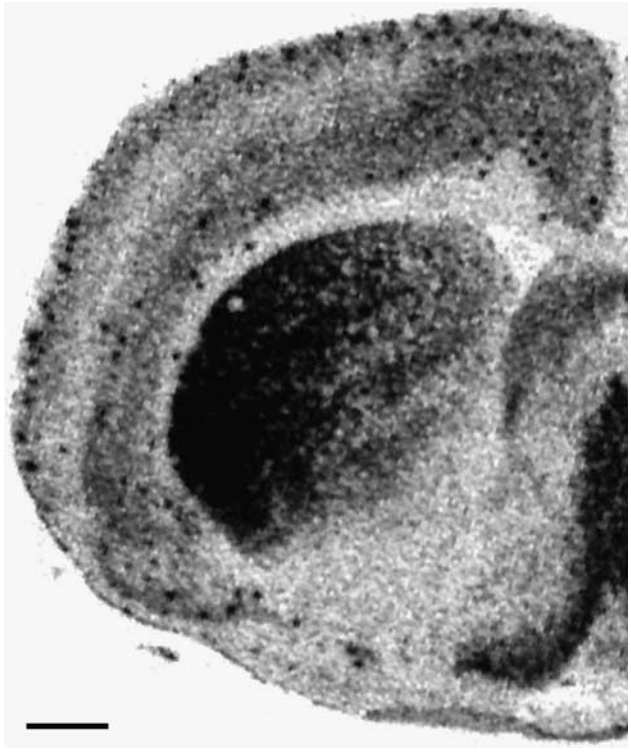

Figure 1 Distribution and expression of CB_1 receptor mRNA in the striatum. *In situ* hybridization with an ^{35}S-cRNA probe for the human CB_1 receptor in coronal sections of rat striatum. Note that the hybridization signal is not homogeneous, showing a lateromedial gradient. Scale bar, 1 mm.

RESULTS

Expression of CB_1 Receptor mRNA in Substance P- and Enkephalin-Expressing Striatal Neurons

The hybridization signal obtained with each of the riboprobes used in this study was specific and reproducible. The specificity of the probes was determined by hybridizing with labeled sense riboprobe, which did not yield any signal, and by including a 25-fold excess of cold cRNA in the hybridization solution, which obliterated the signal. Sections hybridized with the CB_1 receptor antisense riboprobe demonstrated an intense signal in the striatum consistent with our previous single-label study of CB_1 receptors in the rat (Julián *et al*, 2003) and other studies (Hermann *et al*, 2002). CB_1 mRNA expression exhibited a lateromedial gradient, more intense in the lateral striatum with a gradual decrease to a less intense signal in the medial striatum (Figure 1). Interestingly, towards the medial striatum, signal intensity was higher in patches reminiscent of striosomes, with lower signal intensity in the surrounding matrix (Julián *et al*, 2003). Similar results were obtained with emulsion-dipped slides; neurons in the lateral part of the striatum had more intense signal than those in the medial part. Signal in the nucleus accumbens was low (Figure 1). To determine whether CB_1 receptors in the striatum are coexpressed with dopamine D_1 or D_2 receptors, we conducted dual-label hybridization experiments with ^{35}S-labeled riboprobe for CB_1 receptor in combination with digoxigenin labeled riboprobes for preproenkephalin (Enk, a marker for neurons that express D_2 receptors) or β-preprotachykinin (SP, marker for neurons that express D_1 receptors). For quantitative analysis, a set of slides with sections from three different brains was prepared and hybridized simultaneously using a single batch of ^{35}S-cRNA probe for CB_1 receptor together with either Enk or SP digoxigenin-labeled probes. These slides were processed, dipped in emulsion and developed in parallel. We then counted labeled and double-labeled neurons in the dorsal striatum, which we define to include the entire caudo-putamen, excluding the nucleus accumbens. The probe for CB_1 receptor produced clusters of silver grains over almost all neurons in the striatum (Figures 1–3 and 5) that were substantially greater than the autoradiographic background density, indicating that the majority of striatal neurons express CB_1 receptors. Both types of striatal projection neurons, Enk- and SP-expressing, were labeled with approximately similar signal intensity (Figure 2a) and the CB_1 receptor silver grain distribution had a Gaussian shape in both populations (Figure 2b).

Digoxigenin-labeled probes are less sensitive than ^{35}S-labeled probes, thus we confirmed that CB_1 receptors are expressed in both types of striatal projection neurons by repeating the dual hybridization experiments using digoxigenin label for CB_1 receptors and radioactivity for Enk and SP. The results of these experiments were consistent with the results described above (Figure 3). Therefore, we conclude that CB_1 receptors are coexpressed with dopamine D_1 receptors in SP neurons and with D_2 receptors in Enk neurons.

To determine whether CB_1 receptor expression is more prominent in direct or indirect striatal pathway neurons, we

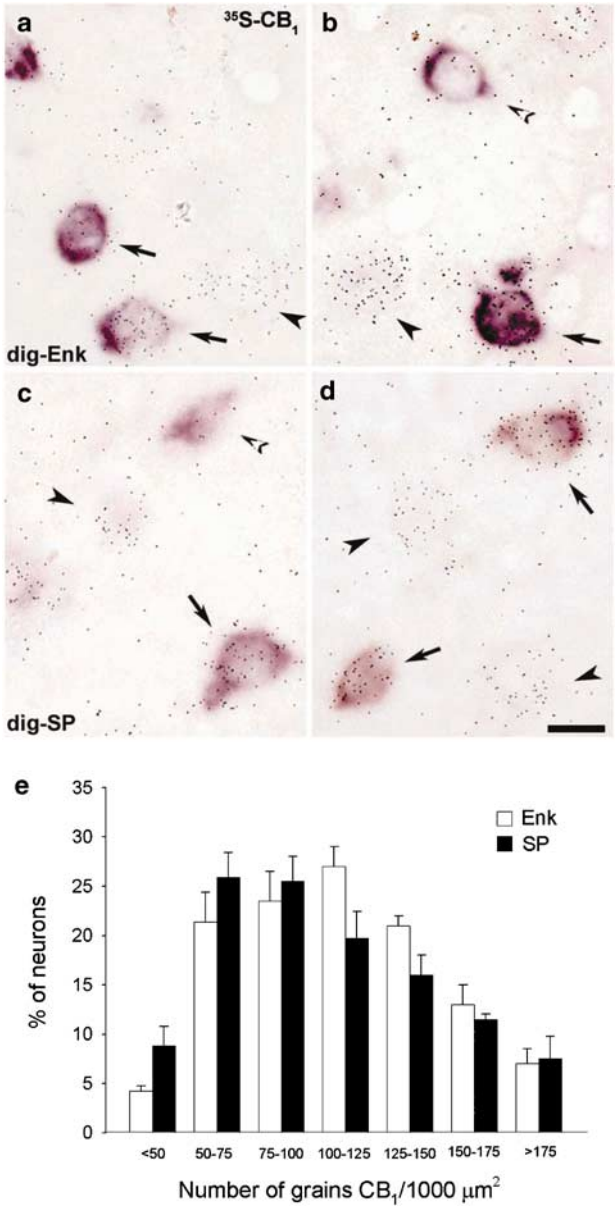

Figure 2 (a–d) Expression of CB_1 receptors in striatal projection neurons. Double *in situ* hybridization with a ^{35}S-cRNA probe for the human CB_1 receptor (detected by silver grains) in combination with digoxigenin-labeled probes for Enk and SP, markers of striatal projection neurons (detected by a blackish precipitate). (a, b) Arrows indicate neurons double-labeled for CB_1 receptors and Enk. (c, d) Arrows indicate neurons double-labeled for CB_1 receptors and SP. Note that CB_1 receptors are expressed in both Enk- and SP-positive neurons, although not all Enk-positive or SP-positive neurons expressed CB_1 receptors. Arrowheads indicate CB_1 +/Enk- in (a and b), and CB_1 +/SP- in (c and d). White arrowheads indicate neurons expressing only Enk in (a and b), or SP in (c and d). Scale bar, 10 μm. (e) Percentage of Enk- and SP-containing neurons with different degrees of CB_1 receptor labeling as measured by the number of silver grains present. Note that silver grain distribution is similar in the two populations of neurons; however, the percentage of SP-containing neurons was always higher than that of Enk neurons for each level of CB_1 receptor signal intensity.

conducted studies quantifying the percentage of ^{35}S-CB_1-positive neurons expressing enkephalin and the percentage of ^{35}S-CB_1-positive neurons expressing substance P. These studies were carried out in three different animals, with two

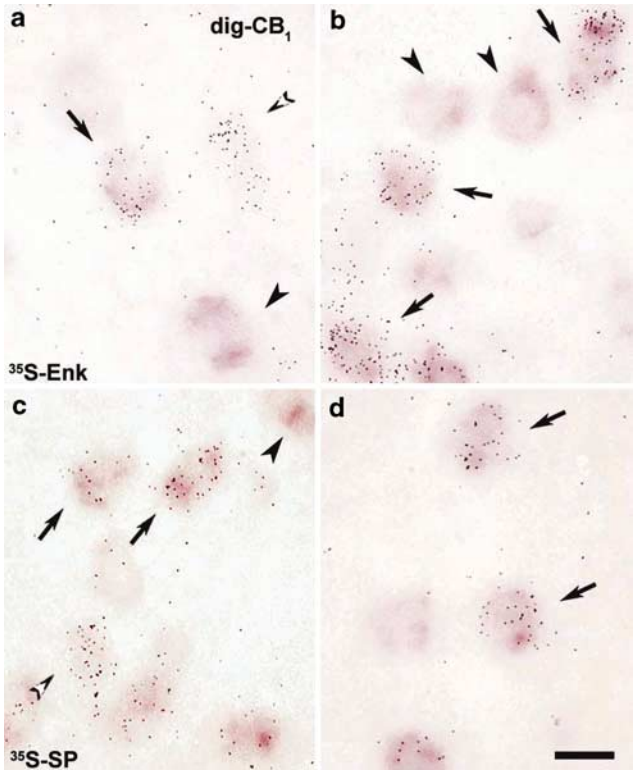

Figure 3 Reverse labeling of Enk and SP cells for the expression of CB_1 receptors to further demonstrate that CB_1 receptors are expressed in both Enk- and SP-containing striatal projection neurons. Double *in situ* hybridization with a digoxigenin-labeled probe for the human CB_1 receptor (detected by a blackish precipitate) and ^{35}S-labeled riboprobes to detect Enk and SP (silver grains). Samples of neurons double-labeled for CB_1 receptor and Enk are shown in (a and b), and for CB_1 receptor and SP are shown in (c and d), indicated by arrows. Note that not all CB_1 receptors-containing neurons expressed Enk (a and b), or SP (c and d), as indicated by arrowheads. White arrowhead in (a) indicates a Enk-positive neuron lacking CB_1 receptor signal. Scale bar, 10 μm.

coronal sections from each animal. Between 2000 and 2500 CB_1-receptor-positive neurons were counted in each striatum. Pairwise comparisons revealed that about 40% of all CB_1 receptor expressing neurons in the striatum were Enk-positive and about 60% were SP-positive. Similar percentages were found in all the striatal territories studied: dorsolateral, ventrolateral, and dorsomedial, in spite of the dorsomedial gradient in CB_1 receptor expression (Figure 4). Since there are equal number of Enk- and SP-positive neurons in the striatum (Bolam *et al*, 2000), these results indicate that CB_1 receptors are more widely coexpressed with dopamine D_1 receptors than with D_2 receptors in striatal projection neurons, regardless of the CB_1 receptor expression gradient ($p<0.001$ Student's *t*-test). We also quantified coexpression in experiments where the CB_1 receptor probe was labeled with digoxigenin and the neuropeptides were labeled with ^{35}S, yielding nearly identical results (data not shown).

Expression of CB_1 Receptors in Molecularly Identified Striatal Interneurons

To determine whether CB_1 receptors in the striatum are expressed in the interneurons and if so, in which

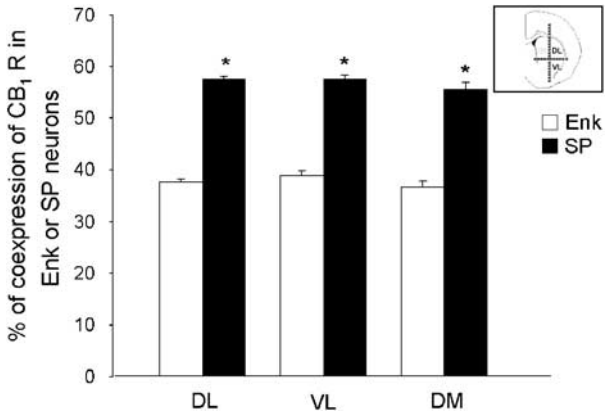

Figure 4 Quantification of striatal projection neurons expressing CB_1 receptors. Histograms illustrate the percentage of CB_1 receptor-containing neurons expressing Enk, (marker for striopallidal neurons) or SP (marker for striatonigral neurons) in different striatal regions, dorsolateral (DL), dorsomedial (DM), and ventrolateral (VL). Note that about 40% of neurons that express CB_1 receptors are indirect striatal projection neurons (labeled with Enk), while the other 60% are direct striatal projection neurons (marked with SP). $^*p < 0.05$ *vs* Enk-labeled neurons.

subpopulation, we conducted dual-label hybridization experiments with ^{35}S-labeled riboprobe for CB_1 receptor in combination with digoxigenin-labeled riboprobes for four striatal interneuronal markers: Som, PVB, ChAT, and GAD67. For quantitative analysis, a set of slides representing material from three or four different brains was prepared and hybridized simultaneously using a single batch of ^{35}S-cRNA probe for CB_1 receptor together with each of the four digoxigenin-labeled riboprobes. These slides were processed in parallel, as described above. Microscopic analysis revealed that CB_1 receptors are expressed in PVB- and GAD67-containing interneurons, with a signal intensity similar to that seen in the projection neurons (Figure 5). The majority of these two types of interneurons expressed CB_1 receptors, independent of their location within the striatum. We observed a lateromedial gradient for CB_1 receptors and for PVB, with more double-labeled cells present in the lateral striatum. Neither cholinergic nor somatostatinergic interneurons expressed CB_1 receptors: we did not find any ChAT- or SOM-positive neurons that were also positive for CB_1 receptor in any of the hemispheres examined.

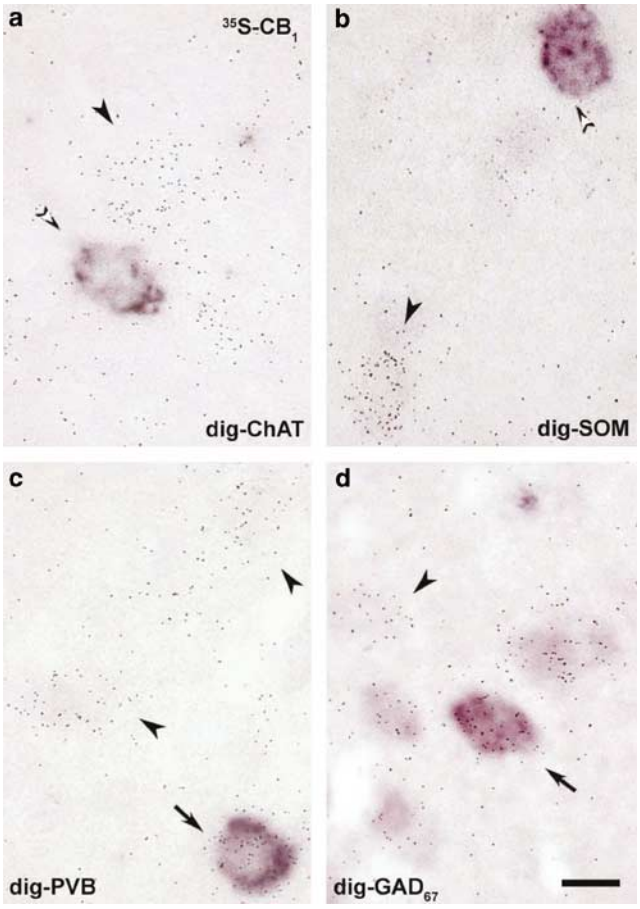

Figure 5 High-power photomicrographs illustrating the expression of CB_1 receptors in striatal interneurons. Double *in situ* hybridization with an ^{35}S-cRNA probe for the human CB_1 receptor (detected by silver grains) in combination with digoxigenin-labeled probes (blackish precipitate) for (a) choline acetyl transferase (ChAT); (b) somatostatin (SOM); (c) parvalbumine (PVB); or (d) glutamic acid decarboxylase 67 kD a (GAD_{67}). Note that CB_1 receptor mRNA is expressed in PVB and in GAD_{67} interneurons, but cholinergic or somatostatin containing neurons do not express CB_1 receptors. Arrows indicate neurons expressing CB_1 receptors and PVB (c) or CB_1 receptors and GAD_{67} (d). Arrowheads indicate neurons positive for CB_1 receptors and negative for peptides. White arrowheads indicate interneurons negative for CB_1 receptors and positive for ChAT in (a) or for SOM in (b) scale bar, 10 μm.

The Anandamide Uptake Blocker, AM404, Reduces D_1 and D_2 Receptor-Mediated Grooming, and Oral Responses

To investigate the functional significance of the anatomical colocalization of D_1 and D_2 dopamine receptors with CB_1 receptors in the striatum, we studied the effect of increasing cannabinoid tone on behaviors mediated by D_1 and D_2 receptors. Previous studies have shown that the D_1 receptor agonist SKF38393 increases grooming, while treatment with quinpirole, a D_2 preferred agonist, markedly reduced this response (Molloy and Waddington, 1987). We used the anandamide uptake blocker AM404 to elevate endogenous extracellular cannabinoid levels. AM404 has been shown to increase levels of both major endogenous cannabinoids: anandamide (Beltramo *et al*, 1997, 2000), and 2-AG (Bisogno *et al*, 2001). AM404 elicited a significant reduction in SKF38393-induced grooming but had no effect on quinpirole-induced reduction of grooming behavior (Figure 6a). These results indicate that increased anandamide levels impact grooming behavior after D_1 but not after D_2 receptor stimulation, suggesting that CB_1 receptors may have an inhibitory role in this complex motor sequence mediated by dopamine D_1 receptors. The inhibitory effect of AM404 was reversed by the cannabinoid CB_1 receptor antagonist SR141716A, which increased grooming behavior. SR141716A also reverses the quinpirole effects on grooming by antagonizing the CB_1 receptor-mediated inhibition of dopamine D_1 receptors-mediated actions (Figure 6a). In addition, the cannabinoid CB_1 receptor agonist HU-210 suppresses grooming induced by SKF38393 (Table 1), again confirming the inhibitory role of this cannabinoid receptor on dopamine D_1 receptor-induced grooming.

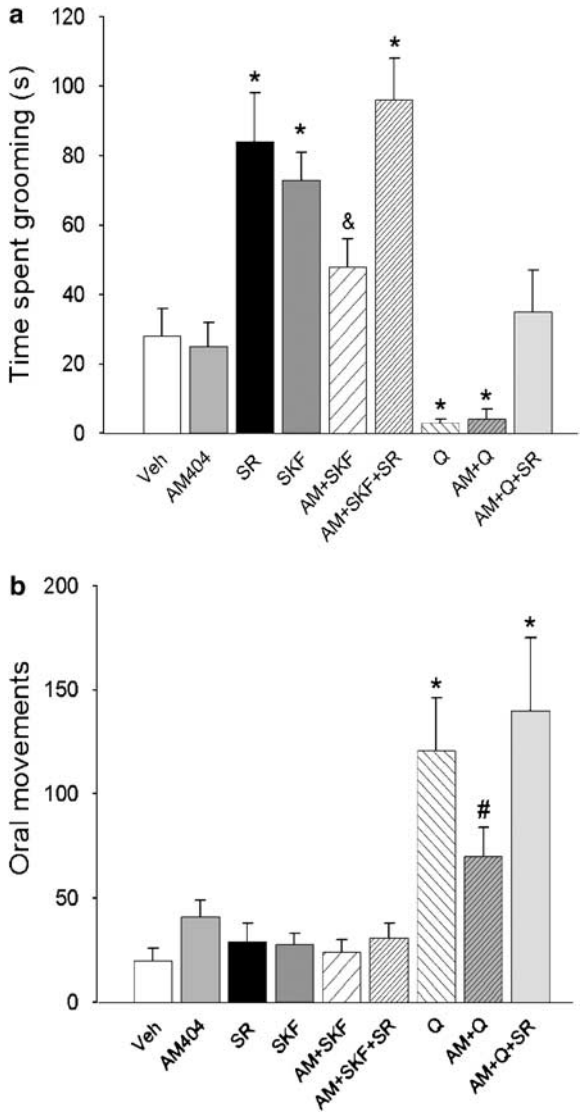

Figure 6 Pretreatment with anandamide uptake blocker AM404 counteracts dopamine D_1 or D_2 receptor-mediated behaviors. (a) Duration of grooming behaviors following administration of the dopamine D_1 receptor agonist SKF38393 (SKF, 5 mg/kg) or dopamine D_2 receptor agonist quinpirole (Q, 1 mg/kg), with or without pretreatment with AM404 (AM, 10 mg/kg) or the CB_1 receptor antagonist SR141716A (1 mg/kg). (b) Incidence of oral movements following administration of quinpirole (1 mg/kg) or SKF38393 (5 mg/kg), with or without pretreatment with AM404 (10 mg/kg) or SR141716A (1 mg/kg). $^*p<0.01$ *vs* vehicle-treated animals; $^{\&}p<0.05$ *vs* SKF; $^{\#}p<0.05$ *vs* quinpirole and vehicle, $n=8$ (Newman–Keuls' test).

We also examined oral movements, which are significantly increased following quinpirole treatment in rats (Figure 6b; $p<0.05$). AM404 and SKF81297 given alone or together had no effect on the basal level of oral movements, indicating that this behavior is regulated by the activation of dopamine D_2 receptors (Figure 6b). Interestingly, a 15-min pretreatment with AM404 significantly reduced the induction of oral movements by quinpirole. The inhibitory effect of AM404 was reversed by the cannabinoid receptor antagonist SR141716A (Figure 6b) and mimicked by the cannabinoid CB_1 receptor agonist HU-210 (Table 1). Taken together, these results indicate that CB_1 receptors have an inhibitory effect on D_2R-mediated oral behavior in rats.

Table 1 The Cannabinoid CB_1 Receptor Agonist HU-210 Blocks Both Dopamine D_1 and D_2 Receptor-Mediated Behaviours through the Activation of Cannabinoid CB_1 Receptors

	Time spent grooming (s)	Oral movements
Vehicle	28 ± 8	19 ± 5
HU-210 (HU), 20 μg/kg	5 ± 4*	3 ± 3*
SR141716A (SR), 1 mg/kg	84 ± 14*	29 ± 9
SKF38393 (SKF), 5 mg/kg	73 ± 10*	28 ± 5
HU+SKF	18 ± 9$^{\&}$	9 ± 3*
HU+SKF+SR	36 ± 10	21 ± 4
Quinpirole (Q), 1 mgkg	3 ± 1*	121 ± 25*
HU+Q	0 ± 0*	30 ± 12
HU+Q+SR	20 ± 12	76 ± 10$^{\#}$

Data represent the means ± SEM of at least 8 animals per group.
$^*p<0.05$ *vs* vehicle; $^{\#}p<0.05$ *vs* quinpirole and *vs* vehicle; $^{\&}p<0.05$ *vs* SKF.

Opposing Effects of Cannabinoids and Dopaminergic Agents on Rotation

ANOVA indicated significant dose effects after intrastriatal infusion of SKF38393 ($F(2,29)=45$, $p<0.01$), SR141716A ($F(2,29)=88$, $p<0.01$), and AM404 ($F(2,29)=65$, $p<0.01$). Thus, as it has been shown previously, intrastriatal infusion of either SKF38393, a D_1 receptor agonist, or SR141716A, a specific CB_1 receptor antagonist, significantly increased contralateral turns ($p<0.01$, Figure 7a). By contrast, intrastriatal infusion of AM404 dose-dependently increased ipsilateral turns ($p<0.05$ *vs* vehicle-treated animals), while neither dose of quinpirole (1 or 2.5 μg/μl) or bromocriptine (5 and 10 μg/μl) had any effect on turning behavior. These data indicate that motor function in the injected striatum was increased after D_1 receptor agonism or CB_1 receptor antagonism, while AM404, an indirect CB_1 agonist, causes a motor depression indicated by the direction (ipsilateral) and number of turns. Activation of D_2 receptors with either quinpirole or bromocriptine does not affect rotation (Figure 7a).

To investigate whether the cannabinoid system can also modify turning behavior induced by dopamine agonists, we increased or decreased CB_1 receptor activity and examined the effect on rotation induced by intrastriatal injection of the dopaminergic agent SKF38393 (we did not use quinpirole or bromocriptine since they had no independent effects on rotation). Two-way ANOVA revealed a significant interaction effect of SKF38393 in combination with cannabinoid ligands ($F(6,72)=34.3$, $p<0.01$). Pretreatment with the CB_1 receptor antagonist SR141716A (1.5 μg/μl) 5 min before intrastriatal injection of SKF38393 results in potentiation of contralateral turns induced by SKF38393 alone. This potentiation occurred at 1 μg/μl SKF38393 ($p<0.01$ *vs* either SR141716A or SKF38393 alone; Figure 7b). By contrast, pretreatment with AM404 (5 μg/μl) significantly reduced contralateral turning induced by 1 μg/μl SKF38393 ($p<0.05$ *vs* either AM404 or SKF38393 alone; Figure 7b). This effect was blocked when SR141716A

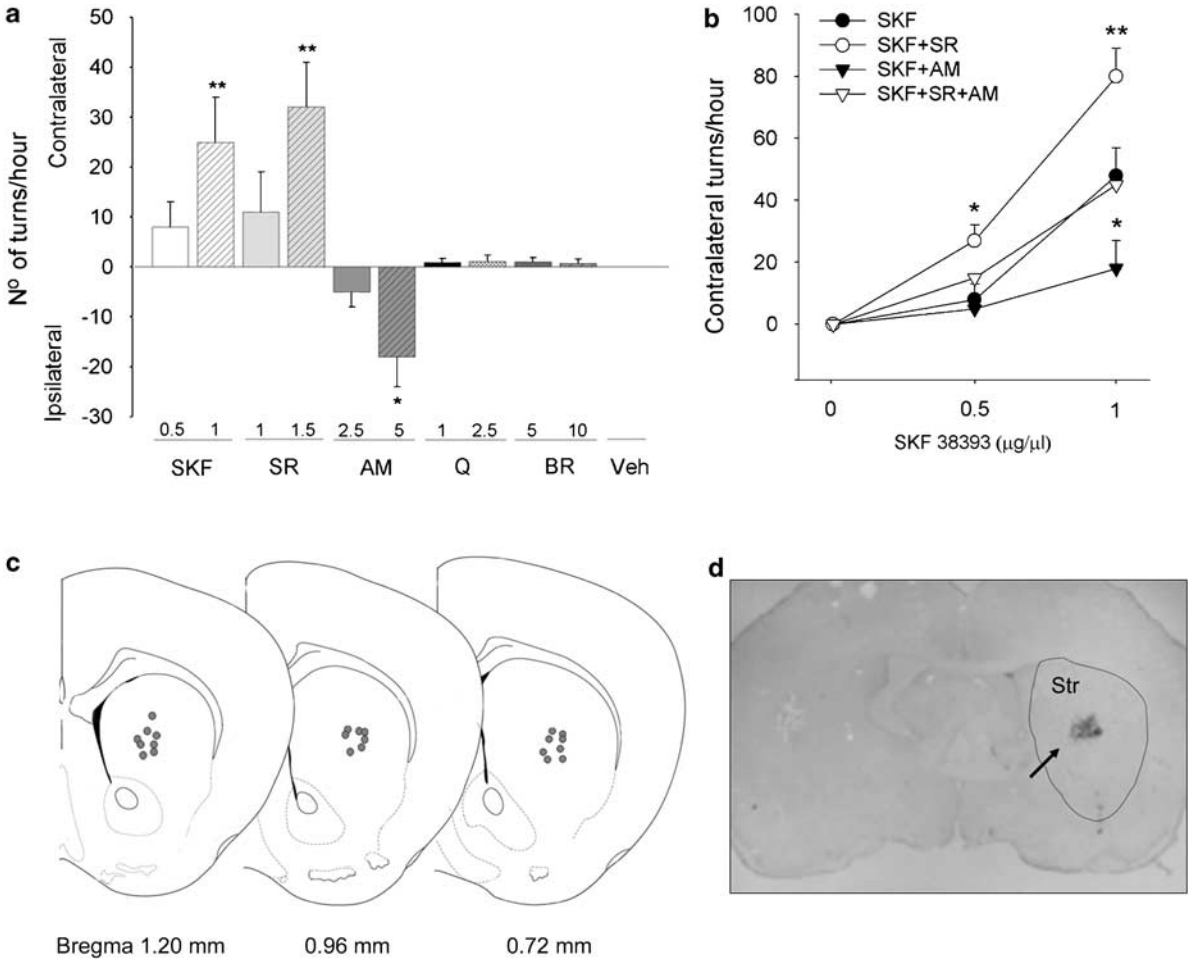

Figure 7 Effect of CB_1 receptor ligands on rotational behaviors following intrastriatal administration of D_1 and D_2 receptor agonists. (a) Rotational behavior (expressed as number of contralateral or ipsilateral turns per hour) in rats after intrastriatal injection of SKF38393 (SKF), SR141716A (SR), AM404 (AM), quinpirole (Q) or bromocriptine (BR) alone. (b) Rotational behavior following coadministration of dopamine agonists and drugs that modulate CB_1 receptor activity. Data are expressed as mean ± SEM, $^*p<0.05$, $^{**}p<0.01$ *vs* vehicle in (a) or *vs* SKF38393 alone in (b) (Student's *t*-test), $n=8$–10. Indicated doses are expressed in μg/μl. (c) The locations of infusions into left striatum are indicated on schematic sections taken from Paxinos and Watson (2005). Distance to Bregma is indicated. (d) Representative photo of an injection site in a coronal brain section stained with Nissl technique (the border of the nucleus is drawn with a dashed line). We discarded all data from animals in which histology revealed that the cannula tip was located outside the striatum. Str, striatum.

(1.5 μg/μl) was injected before AM404 (Figure 7b). Thus D_1 receptor function is enhanced by blockade of CB_1 receptors and reduced by increasing concentration of the endogenous CB_1 receptor agonist anandamide through AM404 infusion. The effect of AM404 seems to be mediated by CB_1 receptors since it is blocked by SR141716A.

For all experiments involving intrastriatal injection, we confirmed the injection site histologically and only those animals where the injection site was found to be correct were analyzed. Figure 7c illustrates the central cannula tip location in the left striatum, and Figure 7d shows a representative coronal section stained with the Nissl technique. Inspection of brain tissues revealed evidence of a small lesion and gliosis at the site of injection, although surrounding tissue was generally intact.

Studies in Wild-Type and Dopamine $D_1R^{-/-}$

To provide additional evidence for a mutual inhibitory interaction between dopamine D_1 and cannabinoid CB_1 receptors, we examined behavior in dopamine $D_1R^{-/-}$ mice. Rats and mice have a similar ratio of colocalization of mRNAs for CB_1/D_1 and CB_1/D_2 (Ana B Martin, Oscar Ortiz and Rosario Moratalla, unpublished observations). Blocking CB_1 receptors with SR141716A enhanced the duration of grooming in wild-type mice ($p<0.01$), but had no effect on grooming in dopamine $D_1R^{-/-}$ (Figure 8a). This indicates that the effect of SR141716A on grooming behavior is mediated by D_1 receptors, probably due to release of inhibitory endocannabinoid tone that modulates endogenous dopamine D_1 receptor-mediated behaviors. The D_1 agonist SKF81297 enhanced grooming in wild-type mice ($p<0.01$), but had no effect on grooming in dopamine $D_1R^{-/-}$, confirming the selectivity of SKF81927 for D_1 receptor. AM404 had no significant effect on grooming in wild type or $D_1R^{-/-}$ mice. Confirming the data shown in Figure 6, AM404 reduced the effect of SKF81927 in wild-type animals. There was no effect of either of these drugs alone or together in $D_1R^{-/-}$ mice (Figure 8).

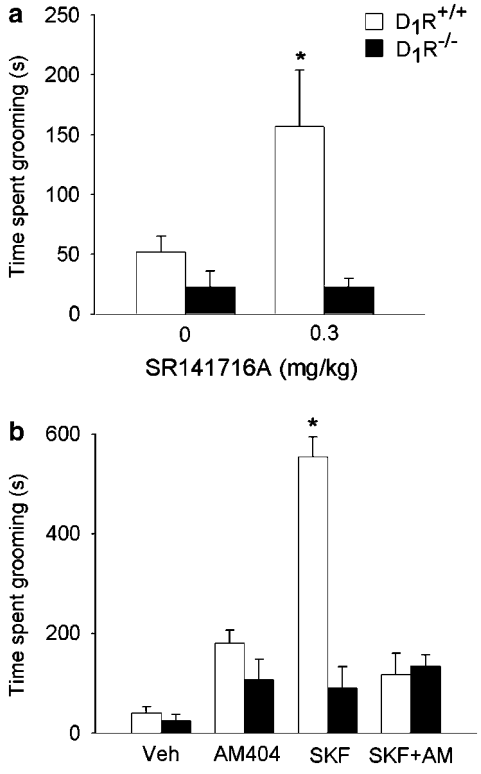

Figure 8 Cannabinoid modulation of grooming in wild-type mice is dependent on D_1 receptors. (a) Administration of the cannabinoid CB_1 receptor antagonist SR141716A (SR, 0.3 mg/kg) enhanced grooming behavior in wild-type mice, but not in $D_1R^{-/-}$ mice. (b) As expected, administration of the dopamine D_1 receptor agonist SKF81297 (5 mg/kg) enhanced grooming behavior in wild-type mice and this response was reduced by the anandamide uptake blocker AM404. Neither drug had any effect in $D_1R^{-/-}$mice. *$p < 0.01$ *vs* vehicle, $D_1R^{-/-}$ mice and SKF + AM-treated animals, $n = 8$, Newman–Keuls.

DISCUSSION

This study provides evidence that the endogenous cannabinoid system is a relevant negative modulator of dopamine D_1 and D_2 receptor-mediated behaviors through its actions on striatal neurons expressing dopamine receptors. The double-hybridization data presented in this study demonstrate that both types of striatal projection neurons as well as some interneurons in the striatum express and synthesize CB_1 receptors. The distribution of CB_1 receptors in the striatum showed a lateromedial gradient, confirming previous results (Herkenham *et al*, 1990, 1991; Tsou *et al*, 1998; Hermann *et al*, 2002; Julián *et al*, 2003). In addition, the present study reveals that the extent of CB_1 receptor mRNA expression is different in direct and indirect striatal output pathways. Our quantitative studies indicate that approximately 40% of striatal cells expressing CB_1 receptors are dopamine D_2 receptor-containing indirect projection neurons, and the remaining 60% are D_1 receptor-containing direct projection neurons. The present study also showed that CB_1 receptors are expressed by PVB and GAD67 interneurons, which are found primarily in close proximity to the cortex. Chemical stimulation of the cortex activates these interneurons, inducing expression of transcription factor genes (Berretta *et al*, 1999). Curiously enough, PVB and GAD67 interneurons express dopamine D_2 (Rivera *et al*, 2002a), but not D_1 receptors, suggesting that interaction between endocannabinoids and the dopamine system in the cortex could be via dopamine D_2 receptors on striatal interneurons. PVB neurons also express D_5 receptors (Rivera *et al*, 2002b), which are activated by D_1 receptor ligands.

The colocalization of CB_1 receptors with both dopamine D_1 and D_2 receptors indicates that these receptors may interact, potentially modifying their respective functions with important behavioral and pharmacological consequences. Supporting this notion, there are several studies suggesting the interaction between CB_1 receptors and dopamine D_2 receptors at the cellular level (Glass and Felder, 1997; Kearn *et al*, 2005). Previous reports have demonstrated a general inhibitory effect of exogenous cannabinoids on dopamine-mediated behaviors (Rodriguez de Fonseca *et al*, 1998). Activation of CB_1 receptor in the striatum is associated with a general inhibition of motor behaviors, resulting in long-term changes in striatal synaptic plasticity (Ronesi *et al*, 2004). However, there has been little information on the specific functional neuroanatomy of these interactions. We assessed some of the behavioral results of these putative interactions. Striatal dopamine D_1 and D_2 receptors are critical for striatal control of motor function. Neurons expressing D_1 receptors form the direct pathway, which projects to internal globus pallidus and substantia nigra, while neurons expressing D_2 receptors make up the indirect pathway, projecting to external globus pallidus (McKenzie *et al*, 1984; Paul *et al*, 1992; O'Connor, 1998; Nicola *et al*, 2000; Svenningsson *et al*, 2000; Onn *et al*, 2000). Dopamine is a relevant modulator of striatal excitatory inputs from the cortex, generally facilitating motor behavior (initiation, sequencing, and ending of movement, Hauber, 1998). In addition, there are several behaviors that can be elicited by specific stimulation of either dopamine D_1 or D_2 receptors. These behaviors can be used as a read-out for functional evaluation of the different striatofugal pathways (Aldridge and Berridge, 1998) and their modulation by the endocannabinoid system. In rodents, dopamine D_1 receptor stimulation elicits complex motor sequencing such as grooming behavior, while stimulation of dopamine D_2 receptors enhances horizontal locomotion and produces stereotypical oral movements. In the present study, we selected grooming and oral stereotypies as read-out behaviors for dopamine D_1 and D_2 receptor stimulation, respectively (Giuffrida *et al*, 1999; Molloy and Waddington, 1984; Starr and Starr, 1986a, b).

It is generally accepted that the endocannabinoid system in the basal ganglia plays a key role in adjusting synaptic transmission within striatal synapses, acting as a retrograde messenger on glutamatergic or gabaergic inputs, or directly modulating postsynaptic signal transduction at dopamine receptors (Glass and Felder, 1997; Mato *et al*, 2004; Rodriguez de Fonseca *et al*, 1998). Supporting this hypothesis, pharmacological stimulation of both dopamine D_1 and D_2 receptors seems to enhance anandamide production in the basal ganglia, possibly triggering negative feedback regulation of dopamine effects (Ferrer *et al*, 2003; Giuffrida *et al*, 1999). This inhibitory role on synaptic transmission is reflected in cannabinoid CB_1 receptor-

mediated inhibition of dopamine D_1 and D_2 receptor-mediated behaviors (Rodriguez de Fonseca *et al*, 1994), and the present study confirms this negative interaction on several behavioral responses. To explore the effects of endogenous cannabinoids (anandamide and 2-arachidonoylglycerol), we used AM404 to block reuptake, effectively increasing their concentrations (Beltramo *et al*, 1997, 2000; Bisogno *et al*, 2001). We found that indirect activation of CB_1 receptors by AM404 inhibits grooming, a dopamine D_1 receptor-mediated response, suggesting negative regulation of D_1 receptor responses by endogenous cannabinoids via CB_1. These data showed that dopamine D_2 receptors also appear to impact grooming behavior, because the D_2 receptor agonist quinpirole reduced grooming behavior, pointing to opposite modulation of this behavior by D_1 and D_2 receptors, as seen previously (Starr and Starr, 1986a, b). Modulation of endocannabinoid levels by AM404 did not apparently influence the inhibitory activity of quinpirole in grooming, possibly due to an already floor effect reached by quinpirole. However, administration of SR141716A reverses the suppression of grooming induced by the combined administration of quinpirole and AM404, clearly indicating the interaction of CB_1 and D_2 receptors, mutually opposing to D_1 receptor-mediated facilitation of self-grooming.

Stereotypical oral movements are a characteristic response to D_2 stimulation in rodents, and they seem to be modulated at the striatal level (McPherson and Marshall, 1996; Davidkova *et al*, 1998). Confirming previous results, we found that D_1 stimulation did not affect oral responses, while D_2 agonist clearly induced oral stereotypies in rats. Cannabinoid CB_1 receptor stimulation blocked D_2-induced oral stereotypies. This finding points to a negative interaction between D_2 and CB_1 receptors in the striatum with respect to oral stereotypies, as has been described for horizontal locomotion (Giuffrida *et al*, 1999). This has important therapeutic implications since oral stereotypies are side-effects of prolonged dopaminergic stimulation in humans including neuroleptic treatment for psychosis and levodopa therapy for Parkinson's disease. Our results suggest that CB_1 agonism has therapeutic potential for reducing the incidence of these abnormal oral responses. In this context, there is evidence that drugs that enhance the activity of the endocannabinoid system may have the capacity to suppress or prevent unwanted dyskinesias in Parkinsonian patients (Ferrer *et al*, 2003), without affecting the beneficial D_1 and D_2 effects of L-DOPA.

A potential contribution of other targets of anandamide and AM404 (eg the vanilloid VR1 receptor) to the inhibition of dopamine-mediated behaviors cannot be excluded (De lago *et al*, 2004; Tzavara *et al* 2006). However, we obtained pharmacological confirmation of the involvement of the CB_1 receptor in AM404 action on dopamine-mediated behaviors. Administration of the CB_1 agonist HU-210 suppressed both D_1 receptor-mediated grooming and D_2 receptor-mediated oral stereotypies. Furthermore, the inhibitory actions of AM404 and HU-210 were reversed by administration of the CB_1 receptor antagonist SR141716A. Thus, although activation of vanilloid receptors may exert antidopaminergic actions, our results confirm that the effects we see are mediated by cannabinoid CB_1 receptors.

At the local striatal level, our results revealed that intrastriatal D_1 (but not D_2) receptor activation enhanced motor function, leading to contralateral rotations. From a functional point of view, stimulation of D_1 receptors would resemble physiological effects of dopamine, leading to a net excitation of neurons of the motor cortex (Löschmann *et al*, 1997; Onn *et al*, 2000). In this context, D_1 receptor agonism in the striatum has been reported to stimulate motor function: intrastriatal administration of SKF38393 increased movements in rats (You *et al* 1994). We found that manipulating CB_1 function with cannabinoid ligands modified D_1-induced motor responses: CB_1 antagonism enhanced D_1-induced motor responses and CB_1 activation blocked them, again indicating a negative interaction between D_1 and CB_1 receptors. Although AM404 can also influence TRPV1 vanilloid receptors (Zygmunt *et al*, 2000), AM404-mediated effects on SKF-induced rotation were blocked by SR141716A, indicating that the AM404 effect is mediated by CB_1 receptors. As reported previously, D_2 stimulation with quinpirole or bromocriptine had no effect on rotation (Sañudo-Peña *et al*, 1998a). Many studies have shown that D_2 receptor activation only modifies turning responses in rats with unilateral striatal denervation, probably due to compensatory overexpression of D_2 receptors (El Banoua *et al*, 2004). In summary, intrastriatal infusion of cannabinoid CB_1 receptor antagonist stimulates motor activation, while CB_1 receptor agonist inhibits it. Since activation of CB_1 receptor counteracts the stimulatory effects of D_1 receptor agonists, as shown previously (Sañudo-Peña *et al*, 1998a), the effects of CB_1 receptor ligands are likely to be due to their modulation of the effects of endogenous dopamine at D_1 receptors.

We further analyzed this relationship using dopamine D_1 receptor knockout ($D_1R^{-/-}$) mice. In mice, as in rats, grooming is a characteristic behavior associated with selective stimulation of D_1 receptors (Starr and Starr, 1986a, b). Our findings confirmed that this response is activated after D_1 receptor stimulation, and disappears in mice lacking dopamine D_1 receptors. Grooming is also stimulated after CB_1 antagonism, and this effect is mediated by D_1 receptors since it does not take place in dopamine $D_1R^{-/-}$ mice. In addition, grooming is further enhanced after D_1 stimulation and CB_1 receptor blockade, indicating that D_1 and CB_1 receptors have opposing effects on grooming. Because we did not observe enhanced grooming in $D_1R^{-/-}$ mice after cannabinoid CB_1 receptor blockade we believe that the effects of CB_1 receptor blockade in wild-type mice are due to baseline cannabinoid tone that inhibits dopamine D_1-mediated behavior. We found that WT mice treated with AM404 have more grooming than control mice treated with vehicle, although this response is fourfold lower than that observed after D_1 receptor stimulation. Moreover, pretreatment with AM404 completely abolished the potent response induced by the dopamine agonist. None of these effects induced by SKF81297 were observed in $D_1R^{-/-}$ mice suggesting critical dependence on D_1 receptors. The small increase observed after AM404 in grooming behavior in both WT and $D_1R^{-/-}$ mice may be related to alternative targets implicated in grooming as indicated elsewhere (ie a potential stress response induced by AM404 injection, since stress induces grooming in mice (Kalueff and Tuohimaa, 2005).

Our study demonstrates that CB_1 mRNA is colocalized both with dopamine D_1 receptors in direct striatal projection

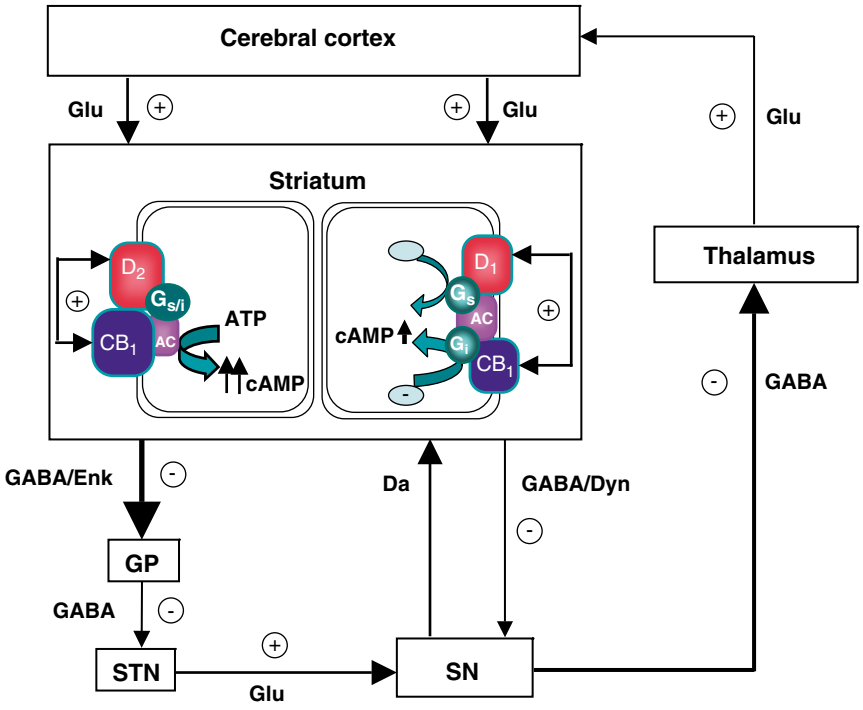

Figure 9 Diagram of the basal ganglia motor circuit. This simplified diagram shows the main connections between regions in the basal ganglia motor circuit. Cannabinoid CB_1 receptors are present on both, striatonigral gabaergic projection neurons, which also express D_1 receptors, and striopallidal gabaergic projection neurons, which express D_2 receptors. The location of CB1 receptors allows the cannabinoids to modulate both afferent glutamatergic inputs into the striatum and also the efferent inhibitory outputs from the medium-spiny projection neurons. Activation of CB_1 and D_1 receptors results in a net decrease in adenylyl cyclase activity, causing a decrease in the inhibitory activity of direct striatal projection neurons, which enhances the activity of nigral neurons and results in decreased motor response. Conversely, simultaneous activation of CB_1 and D_2 receptors stimulates adenylyl cyclase, potentiating the indirect striatal pathway neurons that in turn activate neurons of the subthalamic nuclei, also resulting in decreased movement. Globus pallidus (GP); subthalamic nucleus (STN); substantia nigra (SN).

neurons and with D_2 receptors in indirect striatal projection neurons. This allows endogenous cannabinoids acting at CB_1 receptors to modulate not only the afferent glutamatergic inputs into the striatum but also the efferent inhibitory outputs of the medium spiny neurons to their projection fields in the midbrain, as shown in the diagram of the basal ganglia motor circuit (Figure 9). These data support the hypothesis that endogenous cannabinoids act through CB_1 receptors in the striatum to inhibit dopamine-mediated motor behaviors, including dopamine D_1 receptor-mediated grooming behavior, D_1 receptor-induced turning response, and D_2 receptor-induced oral stereotypies. It has been reported that CB_1 agonists facilitate dopaminergic activity in the nucleus accumbens (French *et al*, 1997). It may seem that this contradicts our findings, but in fact, due to the complexity of basal ganglia circuitry as shown in Figure 9, it is consistent with our results. The interaction between CB_1 and D_1 receptors in dorsal striatum decreases the inhibitory input of striatal projection neurons onto dopaminergic neurons in the VTA and SN that project to the nucleus accumbens and to the striatum, enhancing their activity.

Functional interactions between dopamine D_1 and D_2 receptors, and the CB_1 receptor could occur due to direct receptor–receptor interaction (Kearn *et al*, 2005) or indirectly, via intracellular signaling pathways (Glass and Felder, 1997) or via effects on cells in different regions of the motor circuit. Since dopamine D_1 and D_2 receptors, and the CB_1 receptor all regulate adenylyl cyclase (AC), this common pathway is one likely site for interaction. Activation of CB_1 and D_1 receptors together results in a net decrease in adenylyl cyclase, causing a decrease in the inhibitory activity of direct striatal projection neurons, which enhances the activity of nigral neurons and results in decreased motor response (Figure 9). Conversely, activation of CB_1 and D_2 receptors together stimulates adenylyl cyclase (Glass and Felder, 1997), potentiating the indirect striatal pathway neurons that in turn activate neurons of the subthalamic nuclei, also resulting in decreased movement. Although this scenario is in good agreement with our results, additional work is needed to determine whether the effect of CB_1 R activation on D_1 and D_2-mediated behaviors is in fact mediated via the adenylyl cyclase signaling pathway. Whatever the mechanism, these data indicate that endogenous cannabinoids acting at striatal CB_1 receptors play a significant role in the regulation of basal ganglia motor circuits.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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