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Ultrastructural localization and function of dopamine D1-like receptors in the substantia nigra pars reticulata and the internal segment of the globus pallidus of parkinsonian monkeys

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Abstract

The motor symptoms of Parkinson's disease (PD) are commonly attributed to striatal dopamine loss, but reduced dopamine innervation of basal ganglia output nuclei, the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) may also contribute to symptoms and signs of PD. Both structures express dopamine D1 and D5 receptors under normal conditions, and we have recently demonstrated that their local activation reduces neuronal discharge rates and enhances bursts and oscillatory activity in both nuclei of normal monkeys [M.A. Kliem *et al.* (2007) *J. Neurophysiol.*, 89, 1489–1500]. Here, we determined the ultrastructural localization and function of D1-like receptors in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated parkinsonian monkeys. In both normal and MPTP-treated monkeys, most of the D1 and D5 receptor immunoreactivity was associated with unmyelinated axons, but we also found significant postsynaptic D5 receptor immunostaining in dendrites of GPi and SNr neurons. A significant proportion of axonal D1 immunostaining was bound to the plasma membrane in both normal and MPTP-treated monkeys. Local microinjections of the D1 / D5 receptor agonist SKF82958 significantly reduced discharge rates in GPi and SNr neurons, while they increased burst firing and oscillatory activity in the 3–15-Hz band in SNr, but not in GPi, of parkinsonian monkeys. Together with our recent findings from normal monkeys, these data provide evidence that functional D1 / D5 receptors are expressed in GPi and SNr in both normal and parkinsonian states, and that their activation by endogenous dopamine (under normal conditions) or dopamine receptor agonists (in parkinsonism) may regulate basal ganglia outflow.

Keywords

D1; D5; globus pallidus; MPTP; Parkinson's disease; substantia nigra

Introduction

The substantia nigra pars compacta (SNc) projects to the striatum (Bernheimer *et al.*, 1973; Hornykiewicz & Kish, 1987), and supplies dopamine to the basal ganglia output nuclei, the internal pallidal segment (GPi) and the substantia nigra pars reticulata (SNr; Bernheimer *et al.*, 1973; Geffen *et al.*, 1976; Cheramy *et al.*, 1981; Smith *et al.*, 1989; Pifl *et al.*, 1990; Robertson *et al.*, 1991; Schneider & Rothblat, 1991; Whone *et al.*, 2003). In GPi, dopamine is released from terminals of the nigropallidal projection which, in monkeys, arises from a population of SNc neurons that are partly different from those giving rise to the nigrostriatal pathway (Smith *et al.*, 1989; Jan *et al.*, 2000), while dopamine reaches SNr cells through dendritic release from ventral tier SNc neurons (Bjorklund & Lindvall, 1975; Nieoullon *et al.*, 1978; Arsenault *et al.*, 1988).

Dopaminergic transmission is mediated through D1-like (D1LRs; Clark & White, 1987; Neve, 1997) and D2-like (D2LRs; Neve, 1997) G-protein-coupled dopamine receptors. The D1LRs, the focus of this report, are comprised of D1 and D5 subtypes, and are positively coupled to adenylyl cyclase (Kebabian & Calne, 1979; Gingrich & Caron, 1993). D1LR ligand binding is expressed at high concentrations in the monkey GPi and SNr (Richfield *et al.*, 1987; Besson *et al.*, 1988). Previous rodent studies have demonstrated that D1 receptors are presynaptically located in axons and terminals of γ -aminobutyric acid (GABA)ergic striatopallidal and striatonigral projections (Barone *et al.*, 1987; Fremeau *et al.*, 1991; Levey *et al.*, 1993; Yung *et al.*, 1995; Caille *et al.*, 1996), but the location of D1 receptors has not been studied in detail in primates (Levey *et al.*, 1993; Betarbet & Greenamyre, 2004; Paspalas & Goldman-Rakic, 2005). In addition, very little is known about the ultrastructural localization of D5 receptors in the basal ganglia (Bergson *et al.*, 1995; Ciliax *et al.*, 2000; Khan *et al.*, 2000). In our recent electrophysiological and pharmacological studies, we showed that local activation or blockade of D1LRs modulates neuronal discharge in the basal ganglia output nuclei of normal monkeys (Bergson *et al.*, 1995; Kliem *et al.*, 2007), likely due to modulation of GABA release from striatal projections to these nuclei, although postsynaptic effects cannot be ruled out (Zhou *et al.*, 2009).

Studies of changes in D1LR expression in the basal ganglia of dopamine-depleted animals have provided conflicting results. For instance, some authors have shown that the level of D1 receptor mRNA is decreased in striatonigral projection neurons of dopamine-depleted rats (Gerfen *et al.*, 1990) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Morissette *et al.*, 1996), while other *in situ* hybridization studies in MPTP-treated monkeys resulted in opposite findings along with the demonstration that D1LR binding in basal ganglia output nuclei does not significantly change in these animals (Gnanalingham *et al.*, 1993; Betarbet & Greenamyre, 2004). Potential dopamine depletion-related changes in the localization of D1LR protein immunoreactivity and D1LR function in the SNr and GPi have not been documented in non-human primate models of Parkinson's disease (PD). To address this issue and complement our recent findings in normal monkeys (Kliem *et al.*, 2007), we undertook a detailed ultrastructural analysis of the localization of D1 and D5 receptors, and characterized the function of D1LRs in GPi and SNr of MPTP-treated parkinsonian monkeys. The results of this study have been presented in abstract forms (Kliem *et al.*, 2006, 2009).

Materials and methods

Animals

Nineteen Rhesus monkeys (*Macaca mulatta*, 3–10 kg) were used in this study. Three of these animals were used for electrophysiological recording of GPi and SNr neurons, while brain tissue from the remaining animals was used for immunocytochemical studies. Eight of these animals were normal, and 11 were treated with MPTP. The animals were housed under conditions of protected contact housing, with free access to standard primate chow and water. All experimental protocols were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Anonymous, 1996) and the PHS Policy on Humane Care and Use of Laboratory Animals (amended 2002), and were approved by the Animal Care and Use Committee of Emory University.

General outline of procedures

The effects of local microinjections of the specific D1LR receptor agonist SKF82958 on GPi and SNr activity were studied with electrophysiological methods in MPTP-treated animals ($n = 3$). Electron microscopic (EM) immunoperoxidase studies of D1 and D5 receptor immunoreactivity were carried out on GPi and SNr sections from normal ($n = 8$) and MPTP-treated monkeys ($n = 8$). In addition, immunogold studies were performed in both structures to assess the degree of plasma membrane expression of D1 receptor immunoreactivity. These were successfully completed in four normal and four parkinsonian monkeys.

Electrophysiology experiments

Recording chamber placement—The animals were first trained to sit in a primate chair, to adapt to the laboratory, and to permit handling by the experimenter. We then placed two stainless steel chambers (inner diameter = 16 mm; Crist Instruments, Hagerstown, MD, USA) for chronic access over trephine holes under aseptic conditions and isoflurane anesthesia (1–3%). One chamber was stereotactically aimed at the SNr in the parasagittal plane ($A = 9$, $L = 5$, $D = 0$; Horsley–Clarke coordinate system) at a 25° angle, posterior to the vertical. A second chamber was directed at the GPi in the coronal plane ($A = 12$, $L = 10$, $D = 4$) at a 40° angle. The chambers were both positioned over the right hemisphere and affixed to the skull with dental acrylic (Atlanta Dental Supply, Duluth, GA, USA). A metal head holder (Crist Instruments) was embedded in the acrylic cap to permit head stabilization during recording procedures.

MPTP administration—The MPTP-treated monkeys used in the electrophysiological experiments had previously been used in a similar study in the normal state (Kliem *et al.*, 2007). MPTP was administered by injection into the right common carotid artery after occlusion of the external carotid artery on the same side (0.5–0.7 mg / kg per injection, 1 mg / mL, infused over 10 min; Sigma, St Louis, MO, USA) under sterile conditions and isoflurane gas anesthesia. Monkey F received one injection, monkey I received two injections 14 days apart, and monkey E received three injections spaced by at least 14 days. Monkey E also received additional systemic injections of MPTP (0.25 mg / kg i.m.), 4 months after the intracarotid treatment (Eberling *et al.*, 1998). The recording experiments started at least 3 weeks after the last MPTP injection. At the time of recording, all three

animals showed moderate to severe parkinsonian motor signs on the side contralateral to the MPTP injections, including bradykinesia, rigidity, postural instability and flexed limb posture. They continued to feed and groom themselves. The stability of their parkinsonian motor signs was documented with biweekly observations throughout the recording sessions (for details, see Soares *et al.*, 2004), as described in the next section.

Behavioral assessment method—A variety of behavioral methods was used to measure the degree of impairment induced by MPTP and to confirm stable parkinsonism before and during recording sessions, and at least 8 weeks for animals used exclusively for D1 and / or D5 receptor distribution and subcellular localization analysis. Parkinsonian motor signs were documented through observations of spontaneous cage behavior. A computer-assisted behavioral scoring system was used to quantify behavioral changes. For this, limb movements were documented over a 20-min time period. This system has been used and validated in other studies (Bergman *et al.*, 1990; Wichmann *et al.*, 2001; Soares *et al.*, 2004). Briefly, a computer keyboard key was assigned to a limb, and each time the animal moved a limb the key was pressed for the duration of the movement. The ratio of the arm movements was calculated as an index of the degree of disability.

An automated activity-monitoring system was also used. The observation cage was equipped with eight infrared beams (Banner Engineering, Minneapolis, MN, USA) arranged in a square formation on two adjacent sides of the cage (back and side). The animal's behavior was also videotaped. A computer system was attached and logged the timing of beam crossings. Off-line, the total activity counts within a 20-min period were calculated.

Most of the animals were taught to perform a food-retrieval task to quantify bradykinesia. A food treat was placed in a well. The entry and exit from the well was monitored by two infrared beams, and the timing of the infrared beam disruptions was recorded to computer disk. In each experimental session, 10 trials per side were completed.

Finally, a rating scale was used to determine the degree of behavioral change induced by MPTP treatment for both injection protocols. Nine criteria were used to assess parkinsonian motor signs (gross motor activity, balance, posture, arm bradykinesia, arm hypokinesia, leg bradykinesia, leg hypokinesia, arm tremor, leg tremor), each on a scale of 0–3 (normal / absent to severe), yielding a maximum score of 27. Animals received additional injections of MPTP if a score of < 10 was recorded. Dystonia and chorea were rated separately with a four-point scale (arm dystonia, leg dystonia, dyskinetic arm movements, dyskinetic leg movements), yielding a maximum score of 12. Stereotypy and rotational behaviors were rated, yielding a maximal score of 6.

All observations were performed at the same time of day. The behavioral assessment methods were used biweekly before and after MPTP injections, and throughout the recording experiments.

Electrophysiological mapping procedures—After stabilization of parkinsonian motor signs, the boundaries of GPi and SNr were identified using extracellular single-cell recordings with tungsten microelectrodes (Frederick Haer, Bowdoinham, ME, USA;

impedance 0.5–1.0 M Ω at 1 kHz). For each electrode penetration, a 20-gage steel guide tube (Small Parts, Miami Lakes, FL, USA) was used to penetrate the dura, and a microdrive (MO-95B, Narishige, Tokyo, Japan) to subsequently lower electrodes into the brain. The microdrive had an X–Y platform to determine the coordinates inside the recording chamber, and a linear potentiometer coupled to the vertical cylinder that moves the electrode and provides us with a readout of the position of the electrode tip. The electrical neuronal signal was amplified (DAM-80 amplifier, World Precision Instruments, Sarasota, FL, USA), filtered (400–10 000 Hz; Krohn-Hite, Brockton, MA, USA), displayed on a digital oscilloscope (DL1640; Yokogawa, Tokyo, Japan) and made audible via an audio amplifier. GPi and SNr neurons were easily identified by their characteristic high-frequency discharge rates (Starr *et al.*, 2000).

Injection / recording procedures—A combination microelectrode recording–injection system (Kliem & Wichmann, 2004) was used to assess the effects of a D1LR agonist on GPi and SNr neurons in MPTP-treated monkeys. This system allows us to study the effects of drugs on the activity of neurons in the vicinity of the injection site (Kita *et al.*, 2004, 2006; Kliem & Wichmann, 2004; Galvan *et al.*, 2005; Kliem *et al.*, 2007). A standard tungsten microelectrode (see above) was positioned alongside fused silica tubing (inner diameter = 40 μ m; outer diameter = 120 μ m; Polymicro Technologies, Phoenix, AZ, USA) inside a polyimide sleeve (outer diameter = 0.5 mm; MicroLumen, Tampa, FL, USA) and secured with epoxy glue. The tip of the microelectrode extended 50–100 μ m beyond the tip of the silica tubing. A 10-mm section of 23-gage stainless steel tubing (Small Parts) was held in place over the silica tubing at the proximal end with epoxy glue, in order to provide a tight link to a micro-T connector (CMA, Solna, Sweden) via a ‘flexible connector’ (inner diameter = 0.020; Tygon tubing; Saint-Gobain, Akron, OH, USA). Gas-tight syringes (1 mL; CMA) were connected to the inlets of the micro-T, and controlled remotely by a dual syringe infusion pump (CMA / 102) to infuse sub-microliter quantities of solutions.

Prior to the injections, the selective D1LR agonist, SKF82958 (3 μ g / μ L; Sigma-Aldrich) was dissolved in artificial cerebrospinal fluid [comprised of (in mM): NaCl, 143; KCl, 2.8; CaCl₂, 1.2; MgCl₂, 1.2; Na₂HPO₄, 1; pH 7.2–7.4] and 0.1% ascorbic acid solution, placed in a sonicator and filtered (pore size = 0.2 μ m; Fisher Scientific). Vehicle was infused for control experiments. The injection–recording device was lowered into the brain using the microdrive and guide tube (see above). Cells were recorded for at least 1 min with the pump in the ‘OFF’ position, providing us with a record of the neuron’s baseline firing activity. While we continued to record, the pump was switched to the ‘ON’ position for drug infusion (rate = 0.25 μ L / min; 2 min) and then switched off again. The post-infusion period lasted for at least 4 min. The recorded neuronal signals were amplified and stored to computer disk using the CED data acquisition system (Spike2, CED; Cambridge, UK). Given the very small volume of drug infused, the drug effects are not likely to extend more than 200–300 μ m beyond the tip of the recording electrode (personal observation; Kita *et al.*, 2004).

The animals were awake throughout all of the recording–injection experiments. Recording sessions were separated by at least 24 h. In some sessions, more than one injection was done. In these rare instances, the injections were spatially separated by at least 500 μ m, and at least 30 min apart. Cumulative effects of these drug infusions were not seen.

Termination of the experiment—After completion of the recording experiments, the animals were killed with an overdose of sodium pentobarbital (100 mg / kg, i.v.), and then transcardially perfused with oxygenated Ringer's solution followed by 2 L of fixative [4% paraformaldehyde, 0.1% glutaraldehyde in phosphate buffer (PB; 0.1 M, pH 7.2)]. The brains were then removed from the skull and cut into 10-mm-thick blocks containing GPi or SNr. The tissue blocks were further cut into 60- μ m-thick sections with a freezing microtome, and stained with Cresyl violet to verify electrode locations. Other sections were immunostained for tyrosine hydroxylase (TH; see procedure below) to confirm the loss of dopaminergic neurons in the SNc, and the loss of striatal dopamine innervation. The sections were mounted on slides and analysed at the light microscopic level (LM).

Analysis of electrophysiological data—Only neurons in which the reconstruction of the electrode tracks, based on recording tip position measurements during the experimental sessions and postmortem histology, showed that they were within the confines of GPi or SNr were used. An off-line template-matching spike-sorting routine with subsequent principal component analysis (Spike2, CED) was used to determine inter-spike intervals (ISIs). ISI distribution histograms were examined for quality control.

The subsequent analysis was carried out in Matlab (MathWorks, Natick, MA, USA). As the first step, we calculated discharge rate readouts in 1-s bins. The median of these values in the pre-infusion data segment was defined as the baseline discharge rate of the neuron. An injection was classified as having had an effect if the discharge rate significantly differed from the baseline discharge rate for at least 60 s, with an onset of the effect within 240 s from the start of the injection. These parameters were chosen based on previous studies (Kliem & Wichmann, 2004; Kliem *et al.*, 2007). The 'effect period' was defined as the time period in which the discharge rate of a neuron showed the greatest magnitude of change or was maximally affected by the drug injection. The 'maximal' effect refers to the time point after the drug injection at which the greatest magnitude of change (either an increase or decrease) from the baseline time period was recorded. For statistical analysis, this epoch contained a 60-s data segment that started 30 s before and ended 30 s after the maximal effect. In most cases, significant drug effects continued until the end of the record. For non-responding cells, a 60-s data segment starting 120 s after the beginning of the injection was selected for statistical analysis (see below).

We statistically compared the median baseline discharge rate with the median discharge rate during the effect epochs. The median discharge during the effect epoch was also expressed as a percentage of the baseline discharge rate. We report medians and 25th and 75th percentiles of these percentages in the summary analyses in the Results.

We also calculated burst indices for the baseline and effect epochs, using the Poisson 'surprise' method (Legendy & Salcman, 1985). A 'surprise' value of 3 was chosen (Aldridge & Gilman, 1991; Wichmann & Soares, 2006) to calculate burst indices (i.e. the number of spikes found in bursts divided by the total number of spikes for the record). In addition, changes in oscillatory discharges were assessed with power spectral methods. Power spectra were calculated from the ISI data, as described elsewhere (Soares *et al.*,

2004). In each cell, the raw spectra were integrated in the 1–3-Hz, 3–8-Hz, 8–15-Hz and > 15-Hz ranges, and the resultant values expressed as a fraction of the total power.

Analysis of drug effects: normal vs. MPTP—The effects of D1LR agonist infusions on firing rate, bursts and oscillations (3–8- and 8–15-Hz ranges) in the parkinsonian state were compared with those recorded in the normal state. For this, we used the findings from our recent study collected from the GPi and SNr of normal monkeys (Kliem *et al.*, 2007). We used normalized data (percent change from baseline for each cell) for the comparisons between groups.

Statistics—We used non-parametric methods throughout this study. Mann–Whitney tests were used to compare the discharge rates in the baseline period with those in the drug effect period for individual experiments, and the normalized data describing drug effects in the normal state (Kliem *et al.*, 2007) and MPTP-treated condition. The Wilcoxon signed rank test was used to compare the data collected before and after drug or vehicle injections for neurons in SNr and GPi. *P*-values of < 0.05 indicated significance.

Immunocytochemical experiments

Tissue preparation and histology—For this portion of the study, brain tissue from 16 rhesus monkeys, different from those used in the electrophysiological experiments, was used. Eight animals received MPTP. Six of these animals received systemic injections of MPTP (i.m., 0.2 mg / kg / week), while two other monkeys received an intracarotid injection of MPTP (see above) followed by a series of i.m. injections (0.2 mg / kg) until they reached a parkinsonian state that remained stable for a minimum of 8 weeks (documented with the behavioral assessment methods mentioned above). The total amount of MPTP injected per animal ranged between 7 and 36.5 mg / kg. These animals were later killed with an overdose of sodium pentobarbital (100 mg / kg, i.v.) and perfused with a mixture of paraformaldehyde (4%) and glutaraldehyde (0.1%). Tissue blocks were cut in 60- μ m-thick sections with a vibrating microtome and processed for LM or EM immunocytochemistry to visualize D1 and D5 receptor immunoreactivity (see below; Charara *et al.*, 2004). Some sections were also processed to reveal TH (1 : 1000; Millipore, Temecula, CA, USA) immunostaining at the LM level to confirm striatal dopamine denervation. All MPTP-treated monkeys used in this study showed an almost complete loss of TH immunoreactivity in the dorsal striatum and severe depletion in the number of TH-immunoreactive SNc neurons in both hemispheres (Fig. 1).

Before immunocytochemical processing, sections prepared for EM or LM procedures were rinsed in phosphate-buffered saline (PBS; 0.01 M, pH 7.4), incubated in 1% sodium borohydride solution in PBS (20 min), rinsed in PBS, treated with a cryoprotectant solution (EM only), frozen at –80°C, thawed, and finally rinsed again in PBS.

Immunoperoxidase localization of D1 and D5 receptors

Primary antisera: Two affinity-purified highly specific antibodies were used in this study. Although the transmembrane regions of D1 and D5 receptors are highly homologous, these receptors differ significantly at the third intracellular loop and carboxy-terminus (Sunahara

et al., 1991; Tiberi *et al.*, 1991). We used highly specific monoclonal D1 receptor antibodies (1 : 75; Sigma-Aldrich, St Louis, MO, USA; Levey *et al.*, 1993), which were raised against a 97-amino acid peptide in the carboxy-terminus. This antibody has been extensively used and well characterized in both rodents and primates using Western immunoblot techniques, transfected cells and preabsorption control experiments (Levey *et al.*, 1993; Betarbet & Greenamyre, 2004; Paspalas & Goldman-Rakic, 2005).

We also used a selective and thoroughly characterized D5 receptor polyclonal antiserum [1 : 500, made by one of the authors (Z.U.K.)] raised against a 10-amino acid peptide in the carboxy-terminus of D5 receptor protein (residues 428–438; Sunahara *et al.*, 1991; Tiberi *et al.*, 1991; Khan *et al.*, 2000). The characterization and specificity tests of this antibody, using immunoprecipitation, immunoblots and immunocytochemistry techniques, have been published by Dr Khan and colleagues (2000). Briefly, the immunoblot showed reactivity of a single polypeptide band of 47 kDa, the expected molecular weight of the D5 receptor based on cloning studies, in rat brain tissue that was subsequently abolished by pre-absorption of the D5 receptor antibody with a cognate peptide. Moreover, this D5 receptor antibody binds to recombinant cells that were transfected with D5 complementary DNA, while no immunoreactivity was observed in cells that expressed other dopamine receptors (Khan *et al.*, 2000).

Immunoperoxidase procedure: Sections were pre-blocked in a solution containing 10% normal goat serum (NGS) and 1% bovine serum albumin (BSA) in PBS at room temperature for 1 h. The sections were then incubated for 2 days in primary antibody solution at 4°C. For LM processing, 0.3% Triton X-100 was added to the pre-incubation and primary antibody solutions, and kept overnight at room temperature (or 48 h at 4°C). The sections were then incubated in a solution containing biotinylated goat anti-rat (D1 receptor immunohistochemistry), anti-rabbit (D5 receptor immunohistochemistry) or anti-horse (TH visualization) IgGs (Vector Labs, Burlingame, CA, USA; 1 : 200), and avidinbiotin-peroxidase complex solution (Vectastain Standard Kit, Vector Labs; Hsu *et al.*, 1981; 1 : 100). Immunoreactive agents were diluted in PBS containing 1% NGS, 1% BSA and 0.3% Triton X-100 (LM only). Sections were rinsed in PBS and Tris buffer (0.5 M, pH 7.6) before being transferred to a solution containing imidazole (0.01 M; Fisher Scientific, Hampton, NH, USA), hydrogen peroxide (0.006%) and 3-3'-diaminobenzidine tetrahydrochloride (0.025%; Sigma-Aldrich) for 10 min.

After washes in PBS and PB (0.1 M, pH 7.4), the sections were postfixated in 1% osmium tetroxide (20 min), rinsed in PB and dehydrated in an increasing gradient of ethanol. Uranyl acetate (1%) was added to the 70% alcohol step in the gradient to improve contrast in the EM analysis. Next, the sections were exposed to propylene oxide before being embedded in epoxy resin (Durcupan, ACM; Fluka, Ft. Washington, PA, USA), mounted on microscope slides and placed in an oven (60°C) for 2 days.

Blocks of GPi and SNr were taken from the microscope slides and glued with cyanoacrylate on the top of resin blocks. Ultrathin sections (60 nm) were taken from the surface of the blocks using an ultramicrotome (Leica Ultracut T2; Nussloch, Germany), collected onto

Pioloform-coated single copper grids, stained with lead citrate (5 min; Reynolds, 1963) and examined with an EM (Zeiss EM 10C; Thornwood, NY, USA).

Immunogold procedure: To further characterize the subcellular location of axonal D1 receptor immunoreactivity, we used the pre-embedding immunogold technique. Although slightly less sensitive, this method offers a higher level of spatial resolution than the immunoperoxidase method.

The GPi and SNr tissue from eight animals was used for the immunogold localization of the D1 receptor. Sections were processed for the pre-embedding immunogold method as described in previous studies from our laboratory (Hubert & Smith, 2004; Galvan *et al.*, 2006). Briefly, they were pre-incubated for 1 h in a solution containing 5% milk and 1% BSA in PBS, followed by several rinses in TRIS-buffered saline-gelatin. Next, the sections were exposed to solutions containing 1% milk, 1% BSA and the primary antibody. Afterwards, the sections were rinsed and transferred to a solution containing goat anti-rat IgGs (1 : 100), conjugated to 1.4-nm gold particles, before exposure to the HQ kit (Nanoprobes) for silver intensification of the gold particles (5–10 min). The sections were then rinsed with 2% aqueous sodium acetate buffer and PB, and treated with osmium tetroxide (0.5% in PB, 0.1 M, pH 7.4) for 10 min. The remainder of the tissue preparation was the same as described above for the immunoperoxidase reaction.

Qualitative analysis—Sections immunolabeled for the D5 receptor were examined with a Leica DMRB microscope, photographed at a magnification of 1.6–20× (camera: Leica DC500; Varshaw Scientific, Atlanta, GA, USA) and captured with computer software (Leica IM50; v.1.20). They were then transferred to Adobe Photoshop (Adobe Systems, San Jose, CA, USA), and adjusted for brightness and contrast.

Quantitative ultrastructural analysis—Ultrathin sections were examined with a Zeiss EM10C EM. Randomly selected areas containing immunoperoxidase or gold particle labeling were scanned and photographed at a magnification of 16 000–25 000× (camera: DualView 300W; Gatan, Pleasanton, CA, USA; controller software: Digital Micrograph Software; Gatan, Warrendale, PA, USA; v. 3.6.5). Some micrographs were adjusted for brightness and contrast with either Digital Micrograph or Adobe Photoshop.

Analysis of immunoperoxidase data: From a series of 30–50 EMs per block, immunoreactive elements were categorized as axons, terminals, dendrites or glial processes based on ultrastructural criteria described by Peters *et al.* (1991). These micrographs covered a total surface area of approximately 734.1–1223.5 μm^2 of either nigral or pallidal tissue per animal. The number of specific immunoreactive structures (such as axons or dendrites) was expressed as a proportion of the total number of all labeled elements in SNr or GPi. The mean percentages ($\pm$ SD) of labeled elements were compared between normal and MPTP-treated monkeys.

Analysis of immunogold data: Silver-intensified gold labeling was identified as large (10–30 nm) electron-dense particles that were classified as ‘plasma membrane-bound’ (MB) if they were apposed to, or in close contact with, the plasma membrane, or ‘intracellular’ (IN)

if they were not in direct contact with the plasma membrane. The proportions of MB and IN particles in GPi and SNr were calculated and statistically compared between normal and MPTP-treated monkeys using the Chi-square test. *P*-values of < 0.05 indicated significance.

Results

Electrophysiological effects of D1LR agonist injections

We assessed the effects of local micro-injections of the SKF82958 on the activity of 19 SNr and 25 GPi neurons in the immediate vicinity of the injection sites in MPTP-treated monkeys. The data collected from the three animals used in this part of the study were pooled. We tested neurons throughout the full rostrocaudal and mediolateral extents of both nuclei. Because of the relatively small sample size, we did not determine whether the drug-mediated effects were regionally specific within GPi or SNr. Control injections of vehicle had no effect on neuronal activity ($n = 5$; data not shown). We also compared the drug effects recorded in parkinsonian monkeys (present study) with those previously recorded in the GPi and SNr of normal animals (Kliem *et al.*, 2007).

Effects of D1LR activation in MPTP-treated monkeys—The infusion of SKF82958 ($3 \mu\text{g} / \mu\text{L}$) reduced the discharge rate of most neurons in SNr (14 / 19) and GPi (19 / 25), while a few cells did not respond (5 / 19 in SNr, 4 / 25 in GPi; Fig. 2). The remaining two cells in GPi responded with an increase in neuronal discharge. The median discharge rate of SNr cells decreased from 38.1 spikes / s (25.1–43.7; 25th–75th percentile) at baseline to 22.0 spikes / s (13.0–33.7) after drug exposure, reducing the firing of individual SNr cells by a median of 36% (12–72%; $P < 0.005$, Wilcoxon signed-rank test; Fig. 2). The effect reached its maximum with a median latency of 373 s (215.3–446.6 s). In GPi, the median discharge rate was 64.6 spikes / s (41.9–98.1) at baseline, and 28.5 spikes / s (19.7–58.1) after exposure to SKF82958, reducing the firing of GPi cells by a median of 62% (25–79%; $P < 0.001$, Wilcoxon signed-rank test; Fig. 2). In this case, the maximal effect was seen at a median latency of 257.2 s (227.7–409.2). No behavioral effect was observed after these small drug injections.

The analysis of burst discharges revealed that D1LR agonist exposure increased the median proportion of spikes within bursts in GPi from 14.8% (8.3–19.4) to 21.0% (14.3–24.8), and in SNr from 15.2% (9.3–21.5) to 18.1% (13.3–28.7; Fig. 3). Compared with the cell's baseline burst index, the median increase amounted to 31.9% (9.6–94.2%; $P < 0.001$) in GPi and no significant change (–42.7 to 121.1%, n.s.) in SNr.

Statistical comparisons of the normalized integrated power spectra showed that exposure to SKF82958 increased the median power in GPi discharge in the 8–15-Hz band after drug exposure from 6.3% (5.3–6.8) to 6.6% (5.9–7.2; $P < 0.05$; Fig. 3). No significant changes occurred in the other frequency bands, or in SNr firing.

Comparison of effects of D1LR activation in normal vs. MPTP-treated monkeys—The magnitude of the effect of D1LR agonist infusions on the median proportion of spikes within bursts was significantly larger ($P < 0.05$) in the GPi of parkinsonian monkeys than in normal animals (data from Kliem *et al.*, 2007; Fig. 4B).

However, no significant difference in the effect of D1LR agonist infusions on burst discharges was found in the SNr between normal and MPTP-treated monkeys (data not shown). Similarly, no significant difference was found in the D1LR agonist-mediated effects on firing rates or integrated power spectra in GPi or SNr of normal vs. parkinsonian monkeys (Fig. 4A).

Localization of D1 and D5 receptor immunoreactivity in GPi and SNr

In order to provide a substrate for the interpretation of the physiological data described above and in our previous study (Kliem *et al.*, 2007), we analysed the ultrastructural localization of D1 and D5 receptor immunoreactivity in GPi and SNr of normal and MPTP-treated monkeys. Because there was no obvious regional heterogeneity in the distribution of either receptor subtype in GPi and SNr, data collected from different regions within these structures were pooled.

Ultrastructural localization of D1 receptors—The overall distribution of D1 receptor immunoreactivity in GPi and SNr did not differ significantly between normal and parkinsonian monkeys (Figs 5 and 8). The D1 receptor labeling was almost exclusively found in unmyelinated axonal segments in the SNr of normal (89%, ± 5.2 ; $n = 4$) and MPTP-treated monkeys (88.8%, ± 5.9 ; $n = 3$). A similar pattern of labeling was found in GPi (90.4%, ± 2.2 ; $n = 3$ in normal vs. 90.2%, ± 0.40 ; $n = 3$ in MPTP-treated monkeys). Glial processes did not display immunoreactivity for D1 receptors in either condition.

Localization of D5 receptors—At the LM level, the GPi and SNr were found to contain a significant number of D5 receptor-immunoreactive neurons and moderate neuropil labeling in both normal and MPTP-treated monkeys (Fig. 6), with no obvious difference in the overall pattern of labeling between the two groups of animals. In contrast, there was a substantial loss of D5 receptor-immunoreactive neurons in the ventral tier of the SNc, while neurons in the dorsal tier of the SNc were relatively spared in MPTP-treated monkeys (Fig. 6), indicating the strong expression of D5 receptors immunoreactivity in midbrain dopaminergic neurons. The D5 receptor immunoreactivity was located mainly in cell bodies and proximal dendrites of GPi and SNr neurons, without any obvious change in the pattern of staining between the normal and MPTP-treated animals (Fig. 6). No specific elements could be recognized in the neuropil of either structure at the LM level.

At the EM level, the pattern of D5 receptor labeling resembled that of D1 receptors, i.e. the majority of D5 receptor immunoreactivity was confined to unmyelinated axons (Figs 7 and 8) in GPi (72.0%, ± 8.5 ; $n = 3$ in normal vs. 67.0%, ± 3.7 ; $n = 3$ in MPTP-treated monkeys) and SNr (74.3%, ± 17.6 ; $n = 3$ in normal vs. 74.8%, ± 5.8 ; $n = 3$ in MPTP-treated monkeys), with no significant difference between normal and MPTP conditions (Fig. 8). However, in both experimental groups, dendrites, terminals and glial processes accounted for a significantly larger proportion of D5 receptor-immunoreactive elements than in the D1 receptor-immunostained material (Fig. 7). In some cases, the immunoperoxidase labeling of dendrites was confined to a small sector of the labeled elements, while in others, a dense and diffuse immunoreactivity completely filled the labeled dendrites (Fig. 7). Labeled glial

processes were often found in the vicinity of the rosettes of striatal-like terminals around single dendrites (Fig. 7C).

Immunogold localization of D1 receptors—The pattern of D1 receptor immunogold labeling was consistent with the immunoperoxidase data. In normal and MPTP-treated monkeys, most gold particles were found in small unmyelinated axons (Fig. 5), with about half of the gold particles bound to the plasma membrane in GPi (IN = 627, MB = 627, $n = 3$ in normal; IN = 469, MB = 836, $n = 3$ after MPTP) and in SNr (IN = 556, MB = 507, $n = 3$ in normal; IN = 366, MB = 648, $n = 2$ after MPTP; Fig. 9), and only scarce postsynaptic labeling. The total number of gold particles (n) and axons (ax) examined: GPi, normal ($n = 1303$, ax = 1048), GPi, MPTP ($n = 1305$, ax = 1156), SNr, normal ($n = 1063$, ax = 922) and SNr, MPTP ($n = 1014$, ax = 825). The proportion of MB immunoreactive axonal processes significantly increased after MPTP treatment in GPi (48.1%, $n = 3$ in normal; 64.1%, $n = 3$ after MPTP; $P < 0.05$) and SNr (47.7%, $n = 3$ in normal; 63.9%, $n = 2$ after MPTP; $P < 0.05$).

Discussion

These results, together with our recent study in normal monkeys (Kliem *et al.*, 2007), demonstrate that D1LR activation reduces neuronal discharge rates in GPi and SNr, along with changes in bursts and oscillatory activity in MPTP-treated monkeys. The ultrastructural data suggest that the D1LR-mediated changes in neuronal discharge of basal ganglia output neurons are likely due to activation of presynaptic D1 and D5 receptors located on the plasma membrane of striato-GPi and striatonigral axons, although postsynaptic D5 receptors may also play a role. Our findings also show a significant increase in the relative expression of D1 receptor immunoreactivity on the plasma membrane of axons in both GPi and SNr of parkinsonian monkeys, suggesting that changes in trafficking, synthesis or turnover of presynaptic axonal D1 receptors may occur in the GPi and SNr of parkinsonian monkeys.

D1 and D5 receptors in the monkey GPi and SNr

Our results show that most D1 and D5 receptors in GPi and SNr of normal and MPTP-treated monkeys are located in unmyelinated axons. Taking into consideration the large density of axonal labeling and the fact that some of the immunoreactive terminals displayed the ultrastructural features of striatal-like GABAergic boutons (Smith *et al.*, 1998), it is likely that most immunoreactive unmyelinated axons in GPi and SNr are pre-terminal axonal segments of striatopallidal and striatonigral projections, although other inputs cannot be ruled out (Kliem *et al.*, 2007).

In line with our findings, previous ultrastructural studies in normal rodents (Yung *et al.*, 1995) and monkeys (Levey *et al.*, 1993; Caille *et al.*, 1996) have also described D1 receptor expression in striatal axons in GPi and SNr. It was interesting to find that most receptor labeling was confined to unmyelinated axons instead of axon terminals, a feature that applies to many pre-synaptic G-protein-coupled or ionotropic receptors, including kainate receptors, group II metabotropic glutamate receptors and GABA-B receptors (Fisher & Alger, 1984; Huntley *et al.*, 1993; Petralia *et al.*, 1994; Clarke *et al.*, 1997; Rodriguez-Moreno *et al.*, 1997; Chittajallu *et al.*, 1999; Galvan *et al.*, 2006). The significance of such

axonal labeling remains poorly understood, but our immunogold data show that about half of the D1 receptor immunoreactivity in axons is bound to the plasma membrane in normal monkeys, suggesting that these receptors may bind extracellular dopamine, potentially regulating striatofugal GABAergic transmission. Furthermore, our quantitative analysis of D1 receptor immunogold data showed an increase in the relative expression of D1 receptor labeling on the plasma membrane over the intracellular compartment in unmyelinated axons in GPi and SNr of parkinsonian monkeys, suggesting that the trafficking, synthesis or turnover of these axonal presynaptic receptors may be influenced by the relative level of extracellular dopamine, although this remains to be confirmed with direct measurements of dopamine levels in the basal ganglia output nuclei of normal and parkinsonian monkeys (Bernheimer *et al.*, 1973; Hornykiewicz & Kish, 1987).

The location and function of D5 receptors in GPi and SNr are not as well characterized as that of D1 receptors, in large part due to the limited specificity of D5 receptor antibodies and the lack of drugs that can differentiate D1 from D5 receptors. *In situ* hybridization and immunohistochemical studies have reported D5 receptor mRNA and protein expression in the rat and monkey GPi and SNr (Bergson *et al.*, 1995; Choi *et al.*, 1995; Ciliax *et al.*, 2000; Khan *et al.*, 2000). Qualitative EM studies have found D5 receptors at both pre- and postsynaptic locations in the rodent and monkey SNr (Bergson *et al.*, 1995; Ciliax *et al.*, 2000; Khan *et al.*, 2000), but these studies did not provide quantitative information on the relative distribution of D5 receptor-immunoreactive elements in basal ganglia nuclei under normal or parkinsonian conditions. As was the case for D1 receptor immunoreactivity, we found most D5 receptor labeling in unmyelinated axons in GPi and SNr, suggesting that presynaptic D5 receptors regulate synaptic transmission in the basal ganglia output nuclei. However, in contrast to the pattern of D1 receptor labeling, a larger proportion of D5 receptor immunostaining was also found in dendrites and glial processes, providing a substrate for D1LR-mediated postsynaptic effects. Based on our LM observations showing a significant loss of D5 receptor-immunoreactive cell bodies in the ventral tier of the SNc, it appears that most of the dendritic D5 receptor labeling described in our EM analysis is associated with nigrostriatal dopaminergic neurons. If this is the case, dopamine release in the SNr and GPi could impact the activity of basal ganglia output neurons through regulation of GABA transmission along the striatofugal axons via activation of presynaptic D1 / D5 receptors, but could also modulate the activity of nigrostriatal dopaminergic neurons, and possibly dopamine release in the SNr and GPi via activation of D5 receptors located on dendrites of SNc neurons.

Effects of D1LR activation in GPi and SNr under normal and parkinsonian conditions

Based on previous studies, it is likely that one of the effects of D1LR activation in GPi and SNr is to increase GABA release from terminals of the striatopallidal and striatonigral projections (Timmerman & Westerink, 1995; Ferre *et al.*, 1996; Rosales *et al.*, 1997; Matuszewich & Yamamoto, 1999; Trevitt *et al.*, 2002; Kliem *et al.*, 2007). Increased GABA release also likely underlies the prominent reduction of discharge rates in SNr and GPi neurons after local application of D1LR agonists in these nuclei (Waszczak & Walters, 1983, 1986; Floran *et al.*, 1990; Aceves *et al.*, 1995; Timmerman & Westerink, 1995; Ferre *et al.*, 1996; Timmerman & Abercrombie, 1996; Rosales *et al.*, 1997; Trevitt *et al.*, 2002;

Windels & Kiyatkin, 2006; Kliem *et al.*, 2007). However, the mechanism(s) by which D1LR activation increases GABA release from striatopallidal and striatonigral terminals are not clear. It has been speculated that D1LR activation may affect GABA release independent of striatal activity (Kliem *et al.*, 2007), supported by SNr recordings in a rat brain slice preparation that showed that the frequency of spontaneous miniature inhibitory postsynaptic potentials increases after D1LR activation (Radnikow & Misgeld, 1998).

The contribution of other intrinsic mechanisms, such as D1 receptor-mediated regulation of glutamate release from the STN, in SNr and GPi neurons cannot be completely ruled out. D1 and D5 receptor proteins and mRNA have, indeed, been found in monkey and rodent STN neurons (Flores *et al.*, 1999; Ciliax *et al.*, 2000) and, in normal rats, D1LR activation modulates glutamate release in the SNr (Rosales *et al.*, 1997; Hatzipetros & Yamamoto, 2006; Ibanez-Sandoval *et al.*, 2006).

Previous *in vivo* studies in dopamine-depleted rats have shown that systemically administered D1LR agonists lead to a reduction of SNr activity (Waszczak *et al.*, 1984; Weick & Walters, 1987). In the present study, we show that D1LR agonists, applied locally in GPi or SNr, have the same effect in dopamine-depleted monkeys, thereby providing further evidence for functional D1LRs in basal ganglia output nuclei in both normal and parkinsonian conditions. Although a significant decrease of endogenous dopamine throughout the basal ganglia output structures is expected in PD (SNr; Bernheimer *et al.*, 1973; Geffen *et al.*, 1976; Cheramy *et al.*, 1981; Smith *et al.*, 1989; Pifl *et al.*, 1990, 1992; Robertson *et al.*, 1991; Schneider & Rothblat, 1991; Whone *et al.*, 2003), the increased expression of axonal MB D1 receptor immunoreactivity in the parkinsonian condition may compensate, in part, for this loss.

In fact, these changes in the plasma membrane expression of D1 receptors in the parkinsonian condition may explain some of the discrepancies between localization and functional data previously reported on D1 receptors in rodent models of PD. For instance, despite the decreased expression of D1 receptor mRNA in striatonigral projection neurons (Gerfen *et al.*, 1990) of unilateral 6-hydroxydopamine-treated rats, D1LR-mediated changes in GABA release in slices of the SNr are not significantly different from controls, thereby suggesting that D1LR function in basal ganglia output nuclei is unaffected in the parkinsonian state (Floran *et al.*, 1990; Aceves *et al.*, 1995). On the other hand, intranigral injections of a D1LR agonist enhanced contralateral rotations in dopamine-depleted rats compared with normal animals (Yurek, 1997), indicating that some functional changes to the D1LR system may occur after dopamine denervation. It is also worth remembering that the outcome of experiments investigating the effects of D1LR ligands in parkinsonism may be influenced by parkinsonism-related changes in GABAergic transmission (e.g. changes in GABA-A receptor density or affinity; see above; Pan *et al.*, 1985; Gnanalingham & Robertson, 1993; Calon *et al.*, 1995; Chadha *et al.*, 2000a, b).

In MPTP-treated monkeys, D1LR binding or protein expression either does not change or is slightly upregulated in GPi and SNr (Gnanalingham *et al.*, 1993; Betarbet & Greenamyre, 2004; Aubert *et al.*, 2005). Our functional data demonstrate that the D1LR-mediated reduction of firing rates in GPi is not significantly different from controls. However, D1LR

agonist infusions significantly increased burst activity in the GPi, but not in the SNr, of MPTP-treated monkeys compared with controls (Kliem *et al.*, 2007), supporting the notion that D1LR function may be differentially affected in the two basal ganglia output nuclei in parkinsonism. Similar to our findings from normal monkeys (Kliem *et al.*, 2007), we found that D1LR activation also increases the proportion of spikes in bursts and oscillations in the 8–15-Hz band (at least in GPi) in MPTP-treated monkeys. The changes in bursting and oscillatory activities are somewhat paradoxical because they resemble pathological findings obtained from animal models and patients with PD (Murer *et al.*, 1997; Bergman *et al.*, 1998; Wichmann *et al.*, 1999; Levy *et al.*, 2001; Brown, 2003; Wichmann & Soares, 2006). Increased bursting after local D1LR activation may be partly mediated by rebound excitation that follows GABA-mediated hyperpolarization, as previously shown in rat brain slices of GP and STN (Nambu & Llinas, 1994; Overton & Greenfield, 1995; Beurrier *et al.*, 1999, 2000; Bevan *et al.*, 2002; Kass & Mintz, 2006), though such observations must be confirmed in primate GPi and SNr neurons. Our findings, therefore, suggest that the changes in dopamine innervation and the resulting effects on D1LR activation in GPi and SNr in parkinsonism are unlikely to contribute to the increased burst firing and oscillations that characterize this condition.

Possible behavioral and therapeutic effects of activation of D1 / D5 receptors in GPi and SNr

Systemic administration of D1LR agonists has anti-parkinsonian effects in MPTP-treated monkeys (Blanchet *et al.*, 1996; Goulet & Madras, 2000), while local D1LR blockade in the rat SNr impairs motor activity (Trevitt *et al.*, 2001; Bergquist *et al.*, 2003), and increases electromyographic activity and rigidity (Hemsley & Crocker, 2001). Behavioral and biochemical studies in rats with partial dopaminergic depletion have also demonstrated that dopamine loss in the SNr impairs motor functions, and that increased nigral dopamine release can counteract motor impairment (Andersson *et al.*, 2006). Less is known about the effects of dopamine loss in GPi in parkinsonism. A reduction of dopamine has been reported in the GPi of parkinsonian patients (Bernheimer *et al.*, 1973) and MPTP-treated monkeys (Piffl *et al.*, 1992). Positron emission tomography studies have demonstrated a decrease in ¹⁸F-dopa uptake in GPi of patients with advanced parkinsonism (Whone *et al.*, 2003). These studies suggest that nigral and pallidal D1LRs activation may regulate motor behaviors. Dopamine replacement therapies aimed at the SN, such as grafts of embryonic mesencephalic tissue containing dopaminergic cells (Starr *et al.*, 1999) or the infusion of glia-derived neurotrophic factor, indeed ameliorate parkinsonian motor signs in MPTP-treated monkeys (Gash *et al.*, 1996; Gerhardt *et al.*, 1999). Conceivably, D1LR activation and the associated reduction in firing rates contributes to these beneficial effects. This evidence, however, has to be weighed against our data showing an increase in burst firing and oscillations after local D1LR activation in the monkey GPi and SNr, and the disappointing results of preliminary clinical trials of D1LR agonist testing in patients with PD (Mailman *et al.*, 2001). As activation of presynaptic D1 receptors and postsynaptic D5 receptors may have opposite effects, the development of allosteric modulators that display D1 receptor agonistic or D5 receptor antagonistic properties may be a worthwhile strategy of treating parkinsonism (Conn *et al.*, 2009).

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Abbreviations

BSA	bovine serum albumin
D1LR	D1-like G-protein-coupled dopamine receptors
D2LR	D2-like G-protein-coupled dopamine receptors
EM	electron microscopic
GABA	γ -aminobutyric acid
GPI	internal globus pallidus
IN	intracellular
ISI	inter-spike interval
LM	light microscopic
MB	plasma membrane-bound
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NGS	normal goat serum
PB	phosphate buffer
PBS	phosphate-buffered saline
PD	Parkinson's disease
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
TH	tyrosine hydroxylase

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Fig. 1.

LM showing TH immunostaining in substantia nigra (SN) and striatum of normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. Strong TH labeling is found in SN and ventral tegmental area (VTA) neurons in normal animals (A), but not in MPTP-treated monkeys (B). The caudate (CD) and putamen (Put) also show a significant reduction in TH labeling in MPTP-treated monkeys (D) compared with controls (C).

Abbreviations: AC, anterior commissure; GPe, external globus pallidus; IC, internal capsule; SNr, substantia nigra pars reticulata. Scale bars: 1 mm.

Fig. 2.

Effects of SKF82958 on firing rates. D1LR activation reduced the firing rate of an internal globus pallidus (GPi) neuron recorded after MPTP treatment (A). The solid line represents the median discharge rate during the baseline period, while dotted lines indicate 25th (bottom) and 75th (top) percentiles. The thick line at the bottom of the plot indicates the time and length of drug infusion. Changes in discharge rates of GPi ($n = 25$) and substantia nigra pars reticulata (SNr; $n = 19$) neurons after MPTP treatment (B). For each cell in an experimental group, the discharge rate during the effect epoch was calculated and compared with the baseline discharge rate. The changes in discharge rate are expressed as a percent change from baseline for each experiment. Medians of these percentages are reported here along with the 25th and 75th percentiles (top and bottom of boxes), and minima and maxima (top and bottom of error bars). $*P < 0.05$, Wilcoxon signed rank test.

Fig. 3.

Changes in discharge patterns of internal globus pallidus (GPI; $n = 25$) and substantia nigra pars reticulata (SNr; $n = 19$) neurons after infusions of SKF82959 in MPTP-treated monkeys. The changes in burst incidence (A) and changes in integrated power spectra in the 8–15-Hz band (B) are expressed as a percent change from baseline for each experiment. Medians of these percentages are reported here along with the 25th and 75th percentiles (top and bottom of boxes), and minima and maxima (top and bottom of error bars). * $P < 0.05$, Wilcoxon signed rank test.

Fig. 4.

Comparison of the effects of SKF82958 on firing rates and bursts in GPi between normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. The changes in discharge rates (A) and bursts (B) between normal (Kliem *et al.*, 2007) and MPTP-treated monkeys ($n = 25$) are expressed as a percent change from baseline for each experiment. Medians of these percentages are reported here along with the 25th and 75th percentiles (top and bottom of boxes), and minima and maxima (top and bottom of error bars). * $P < 0.05$, Mann–Whitney test.

Fig. 5.

Localization of D1 receptors in internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr) of normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. D1 receptor-immunolabeled unmyelinated axons (Ax) are shown in the GPi and SNr of normal and MPTP-treated monkeys (A–C). An immunoreactive myelinated axon (M.Ax) is also depicted in GPi (A). D1 receptor immunogold labeling is apposed to the plasma membrane (arrows in D–F) or intracellular (arrowheads in D–F) in unmyelinated axons that travel through GPi and SNr of normal and MPTP-treated monkeys (D–F). Scale bars: 0.50 μ m. Den, dendrites.

Fig. 6.

LM showing cellular D5 receptor immunostaining in internal globus pallidus (GPi) and substantia nigra (SN) of normal and 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP)-treated monkeys. Strong D5 receptor labeling is found in cell bodies of ventral tier substantia nigra pars compacta (SNc) neurons in normal animals (A), which is subsequently lost after MPTP treatment (B). Both dorsal and ventral tier SNc neurons display strong D5 receptor immunoreactivity in normal animals (C), whereas a significant reduction in the density of D5 receptor-containing neurons was found in the ventral tier of the SNc in MPTP-treated cases (D). No significant difference in the pattern of D5 receptor immunoreactivity was found in the substantia nigra pars reticulata (SNr) and GPi of normal (E, G) and MPTP-treated (F, H) monkeys. Abbreviations: CP, cerebral peduncle; SNc-d, substantia nigra pars compacta-dorsal; SNc-v, substantia nigra pars compacta-ventral. Scale bars: 0.5 mm (A, B), 50 μ m (C–H).

Fig. 7.

Localization of D5 receptor immunoreactivity in internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr) of normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. (A–D) D5 receptor immunolabeling in dendrites (Den), unmyelinated axons (Ax) and terminals (Ter) in GPi (A and B) and SNr (C and D) of normal and MPTP-treated monkeys. Occasional glial labeling is also depicted in the SNr (arrowhead in C). Scale bars: 0.50 μ m.

Fig. 8.

Comparative distribution of D1 (A) and D5 (B) receptors in axons, dendrites, terminals and glia (B only) in internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr) of normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. Data are expressed as percentages of immunolabeled elements. Each bar represents the mean $\pm$ SD.

Fig. 9.

Proportion of plasma membrane-bound (MB) and intracellular (IN) D1 receptor immunogold labeling in internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr) of normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. Data are expressed as relative percentages of total immunogold particles in immunolabeled unmyelinated axons. Each bar represents the mean $\pm$ SD. The asterisks indicate significant differences in the percentages of MB gold particles labeling between normal and MPTP-treated conditions. $*P < 0.05$, Chi-square test.