Supporting Information

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SI Results

Selective Absence of Glucocorticoid Receptors (GRs) in the Microglia of $GR^{LysMCre}$ Mice and Resultant GR Levels After Acute 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) Treatment. We examined whether the absence of microglial GR impacts the overall levels of GR after nigrostriatal pathway injury. GR mRNA levels in the substantia nigra (SN) and striatum at 1 and 2 d after MPTP intoxication were modulated similarly in mutant $GR^{LysMCre}$ mice as in the wild-type mice (Fig. S1*D* cf. Fig. 1*E*). Nigral GR protein level was reduced 2 d after MPTP treatment both in control $GR^{loxP/loxP}$ and mutant animals (Fig. S1*E*, $P = 0.04$). This decrease in GR protein level in both controls and mutants could reflect DN loss and is in contrast to mRNA level at the same time point (Fig. 1*E*), suggesting that de novo GR protein synthesis may not follow the immediate changes in mRNA level in, for example, proliferating microglia, induced by degenerating DNs. In striatum (where there is no cell loss) GR protein level is augmented 2 d after MPTP intoxication in $GR^{loxP/loxP}$ mice but not in $GR^{LysMCre}$ mutants, suggesting that up-regulation of GR may be occurring in striatal cells immediately after MPTP injections (Fig. S1*E*).

Selective Absence of GR in the Microglia of $GR^{LysMCre}$ Mice and Resultant Changes in Dopaminergic Nerve Terminals Parameters. The analysis of dopamine (DA) nerve terminal parameters showed that the striatal DA level in $GR^{LysMCre}$ mutants was slightly lower compared with $GR^{loxP/loxP}$ controls after MPTP treatment (Fig. S2*A*). Results of specific [^{3}H]DA uptake in synaptosomes prepared from striata of saline- and MPTP-treated mice showed that MPTP decreases the DA transporter (DAT) activity by as much as 10-fold. Although the specific [^{3}H] DA uptake in control and mutants was similar 7 d after MPTP injections, a further significant decrease in DA uptake in mutants compared with $GR^{loxP/loxP}$ controls was found at day 21 (Fig. S2*B*), suggesting that a progressive neurodegeneration takes place in the $GR^{LysMCre}$ mutants. Western blot analysis of tyrosine hydroxylase (TH) and DAT protein levels showed strong decrease that was similar in control and mutant mice (Fig. S2 *C* and *D*).

Levels of Pro- and Anti-Inflammatory Genes and Upstream Activators of Innate Immunity Are Modulated by Microglial GR After MPTP-Induced DN Injury. Ample evidence exists for the role of TNF-α, inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), IL-1β, and IL-6 in DN degeneration in Parkinson disease (PD) (1), and, as expected, their mRNA levels were significantly increased in the $GR^{loxP/loxP}$ controls 24 h after acute MPTP treatment (Fig. S5). The gene regulatory effect of microglial GR was analyzed in terms of a relative change in mRNA level in $GR^{LysMCre}$ mice acutely treated with MPTP with respect to similarly treated $GR^{loxP/loxP}$ mice. The most pronounced effect was on TNF-α mRNA, in which the expression level in the mutants increased significantly in the SN (×4.2) and in the striatum (×3.7) (Fig. S6*A*). The expression of ICAM-1 was also analyzed because its up-regulation in astrocytes has been described in PD (2). A significant increase in ICAM (×5–7) was found after MPTP treatment both in the SN and striatum of control mice (Fig. S5). Yet in $GR^{LysMCre}$ mutants, there was a further increase, especially in the SN (2.5-fold) (Fig. S6*A*). In contrast, the expression levels of IL-6, IL-9, and pro–IL-18 were lower in the MPTP-treated $GR^{LysMCre}$ mutants (×0.6–0.8 of the levels in $GR^{loxP/loxP}$ controls) (Fig. S6*B*).

Genes coding for anti-inflammatory mediators IL-1R2 and MAPK phosphatase 1 (MKP-1) are established GR-inducible target genes (3). It is worth noting that their expression was markedly up-regulated after MPTP treatment in $GR^{loxP/loxP}$ control mice (Fig. S5*B*), suggesting that the MPTP-induced inflammatory response is not only associated with a detrimental proinflammatory response but also with the induction of factors that can balance the intensity of the inflammatory burden. Importantly, we noted a significant decrease in the expression level of these molecules in $GR^{LysMCre}$ mutant mice compared with their control littermates 24 h after MPTP treatment (Fig. S6*C*).

SI Materials and Methods

Postmortem Tissue. Human SN and putamen samples from postmortem control subjects and clinically diagnosed PD patients were obtained as described in Salazar et al. (4). For immunoblot experiments, two groups of human striatal specimens from control subjects and PD patients were used [mean ± SEM; group 1 ($n = 4$): control, age 76 ± 2.8 y, 3.0 ± 1.0 h postmortem delay; PD, age 78 ± 0.5 y, 10.0 ± 6.2 h postmortem delay; group 2 ($n = 3$): control, age 82 ± 1.5 y, 7.8 ± 3.7 h postmortem delay; PD, age 79 ± 1.2 y, 8.3 ± 5.0 h postmortem delay).

Plasma Cortisol and Corticosterone (CS) Levels. For cortisol levels, plasma from PD patients or healthy subjects was collected by centrifugation from whole blood (blood withdrawn at 10 AM) and stored at −80 °C until use. Plasma cortisol levels were assessed by electrochemiluminescence immunoassay (ECLIA) using a Modular Analytics E170 (Roche Diagnostics Laboratory) according to the manufacturer's instructions. Signed informed consent was obtained from all subjects before any procedure. The study was carried out in accordance with the Declaration of Helsinki, clinical good practice, and local laws and was approved by the local ethics committee.

For CS levels, mice, housed undisturbed, were decapitated between 8 and 9 AM, and the trunk blood was rapidly collected in chilled tubes containing EDTA, centrifuged (1,200 × *g*, 4 °C, 15 min), and the plasma was stored at −80 °C until analysis. Plasma CS levels were determined by radioimmunoassays (Immunochem CS Kit; MP Biomedicals) according to the manufacturer's instructions.

Animal Housing, MPTP, and CS Treatment. All studies were performed in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* and the European Communities Council Directive 86/609/EEC. $GR^{loxP/loxP}$, $GR^{LysMCre}$, and GR^{DATCre} mice were weaned at 21–25 d of age, group-housed under a controlled photoperiod (12-h light/12-h dark cycle; lights on at 7 AM) at constant room temperature (22 °C), and given access to food and water ad libitum.

For acute treatment, wild type, $GR^{LysMCre}$ and GR^{DATCre} mutants, and their control littermates $GR^{loxP/loxP}$ were given four injections of 20 mg/kg i.p. MPTP·HCl (Sigma Aldrich) at 2-h intervals, whereas for subchronic treatment, mice were injected once daily for 5 consecutive d with 30 mg/kg MPTP·HCl. In parallel, mice were also injected with saline. An increased mortality was observed in $GR^{LysMCre}$ mutants (≈40% for $GR^{LysMCre}$ vs. 15% for $GR^{loxP/loxP}$ mice). After the last acute MPTP or saline injection, $GR^{loxP/loxP}$ and $GR^{LysMCre}$ mice were given, in their drinking water, either 0.45% β-cyclodextrin or β-cyclodextrin plus CS (35 μg/mL; both Sigma) dissolved by bath sonication (5).

Primary Microglia Cultures, Treatment, and Transient Transfections. Microglial cell cultures were prepared essentially as described in ref. 6. Briefly, cerebral cortices were dissected from P1 pups (wild type) or $GR^{LysMCre}$ and $GR^{loxp/loxp}$. The tails were used for Cre genotyping. Glial cells were grown in DMEM supplemented with 10% FCS, penicillin, and streptomycin. After 12–14 d, microglia were harvested by shaking the culture dishes and pelleted by centrifugation of the supernatants (1,000 × *g*, 15 min, 4 °C). Microglia at $2–3 \times 10^5$ density were plated in 12-well precoated poly-DL-ornithine (1.5 μg/mL) dishes containing DMEM supplemented with 4% FCS. Medium was changed 4 h after plating and, where necessary, cells were pretreated with dexamethasone (100 nM) for 2 h. Cell were treated with lipopolysaccharide (LPS; 100 ng/mL) or 3 μM 1-methyl-4-phenylpyridinium (MPP+) for indicated times (all reagents were from Sigma Aldrich). For double immunofluorescence experiments, microglia were cultured on precoated poly-DL-ornithine glass coverslips.

For transient transfection assays, microglial cells ($\sim 2 \times 10^5$ cells per well) were plated in 24-well poly-DL-ornithine–coated dishes. At 24 h later, cells in each well were cotransfected with 0.6 μg of pGL4.3 *luc*2P/NF-κB-RE vector (Promega) expressing synthetic firefly luciferase gene driven by NF-κB enhancer control elements and, to control for transfection efficiency, 0.3 μg of *Renilla*-TK-luciferase vector (a kind gift from Dr. A. Joliot, Collège de France, Paris). The cells were transfected for 3 h with Lipofectamine 2000 reagent (Invitrogen) according to manufacturer's instructions. At 16 h posttransfection, microglia were treated with LPS or LPS plus dexamethasone for 30 min as described above. The cells were lysed 30 min after treatments and assayed for both firefly and *Renilla* luciferase activities. The relative light units obtained from *Renilla* luminescence for each condition was used for normalization of luciferase luminescence values.

Tissue Preparation and Immunoblot Analysis. Human and mouse striata and mouse SN were homogenized in 3 vols of Laemmli buffer containing 2% SDS, 5% β-mercaptoethanol, 62.5 mM Tris (pH 6.8), 10% glycerol, and a mixture of protease inhibitors (Roche). Samples were boiled for 5 min and centrifuged at 7,500 × *g* for 10 min at 4 °C, and supernatants were stored at −80 °C. Total protein concentration used was as follows: 5 μg for TH, GFAP, and DAT analysis samples; 50 and 10 μg, respectively, for human and mouse striatal GR and TLR9 analysis; and 40 and 20 μg, respectively, for mouse tissue and microglia cells for phosphorylated NF-κB analysis. The samples were loaded on Novex NuPage 4–12% Bis-Tris gradient gels (Invitrogen), After transfer onto a nitrocellulose membrane, the blots were probed with anti-TH (1/1,000; Chemicon), anti-GFAP (1/500; Sigma), anti-DAT (1/500; Chemicon), anti-GR (1/750; Santa Cruz Biotechnology), anti-TLR9 (1/500; Imgenex), anti-Ser^{276} NF-κB (1/500), anti-Ser^{536}NF-κB, (1/500), anti-p65 NF-κB (1/1,000) (Cell Signaling), or anti-actin (1/1,000; Sigma) antibodies. After incubation with secondary peroxidase-conjugated antibodies (GE Healthcare) diluted at 1:2,000, signals were visualized by using ECL or ECL-plus detection kits (GE Healthcare).

Immunostaining. The animals were anesthetized and rapidly perfused transcardially with an ice-cold solution of 4% paraformaldehyde (PFA) in PBS, postfixed overnight in fresh 4% PFA/PBS solution, and cryoprotected with 30% sucrose in PBS. Striatal and mesencephalic brain regions were cut by using a freezing microtome into 20-μm coronal sections that were collected in 10 regularly spaced series. In the cases when the vibratome was used (in TH and GFAP immunofluorescence experiments), brains in 4% PFA/PBS solution were cut into regularly spaced 30-μm striatal and mesencephalic sections. All sections were stored in PBS/0.4% sodium azide solution at 4 °C until use. For immunohistochemistry, the sections were rinsed in PBS, treated with 1% H_2O_2/PBS for 20 min to remove endogenous peroxide activity, and blocked in 4% goat serum (Sigma-Aldrich) in PBS/0.2% Triton X-100 (PBST) for 2 h. Sections were incubated with the following primary antibodies for 24–48 h at 4 °C: mouse anti-TH (1/1,000; Chemicon), rabbit polyclonal anti-Iba1 (1/500; Wako Chemicals), rabbit polyclonal anti-GFAP (1/500; Sigma), rat anti-F4/80 (both 1/200; Serotec), rabbit polyclonal anti-GR (M-20; Santa Cruz), and mouse anti-GR (3D5; Abcam) antibodies. After incubation with appropriate anti-mouse or anti-rabbit biotinylated secondary antibodies diluted 1/400 (Vector Laboratories) for 2 h, the antibodies were revealed by the ABC method according to manufacturer's instructions (Vector Laboratories) with 3,3-diaminobenzidine (DAB) (Sigma) as the peroxidase substrate. For TH-IR quantification, the sections were counterstained with Nissl solution. For double immunofluorescence experiments, the experimental protocol was essentially the same except that the secondary antibodies used were anti-rabbit CY3 (Vector Laboratories), anti-mouse or rat Alexa 488 (Invitrogen) at 1/400 dilution.

Image Analysis and Cell Counting. The immunofluorescent labeling of TH, GR, GFAP, and Iba1 was analyzed by using a Leica TCS SP2 confocal microscope. To quantify GR in microglia, regularly spaced sections, at 200-μm intervals, of mesecephalon covering the whole SN (from rostral pole of the SN to the locus coeruleus) and striatum (+1.54 to –0.58 mm relative to bregma) were analyzed. Sequential images from processed 20-μm *Z* stacks were obtained with ×63 objective and comprised four different fields of the same section. The confocal images of microglia from all of the sections containing the nuclear marker DAPI and that showed or did not show GR localization were quantified.

Quantification of DN loss and of GFAP+ and Iba+ glia was done stereologically as described previously (7, 8) on regularly spaced DAB sections of SN and striatum, as above, by bright-field microscopy using a Nikon microscope (Eclipse, ×20 objective) equipped with a semiautomatic stereology system (Mercator software; Explora Nova VisioScan T4.18 system) that allowed for cell counts and section thickness verification. The genotype of mice was unknown to the investigator at the time of quantification. TH optical density was determined on TH+ DAB-labeled striatal sections that were exposed to bright-field illumination under controlled room temperature using the Mercator software package. The microglial cell soma size was determined on Iba+ DAB images of microglia from five to six fields of SN section (×40 objective) using ImageJ software.

[^{3}H]DA Uptake. After the dissection of striata, the synaptosomal P1 fraction was prepared by homogenization and centrifugation in ice-cold lysis buffer (10 mM Tris, pH7.5, and 0.32 M sucrose). The synaptosomal pellet was resuspended in Krebs Ringer phosphate buffer, pH 7.4 (16 mM NaH_2PO_4, 16 mM Na_2HPO_4, 119 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.3 mM EDTA, and 5.6 mM glucose), and the specific DA uptake was measured by incubating the synaptosomes with 200 nM [^{3}H]DA (66 Ci/mmol; GE Healthcare) and in parallel with specific DA uptake inhibitor GBR12909 (1 μM).

HPLC Analysis of Levels of MPP+, DA and Its Metabolites, and Related Monoamines. For MPP+ levels, mice were killed after a single i.p. injection of 30 mg/kg MPTP·HCl. Striata were rapidly dissected and processed for HPLC using EL detection (+650 mV). The levels of striatal DA and its metabolites as well as serotonin and noradrenaline were measured as described in Alvarez-Fischer et al. (8).

RT–Quantitative PCR (qPCR). For human samples, RNA was extracted with TRIzol (Invitrogen). For mice, 24 or 48 h after acute MPTP treatment, the striata and SN regions were rapidly dissected by punch at −5 to −10 °C in sterile conditions, and

tissue was kept in RNAlater (Qiagen). Total RNA was prepared by using the lipid RNA extraction kit (Qiagen). RNA integrity and concentration were determined by using Agilent gel and Agilent apparatus. On average, the RNA integrity number (RIN) values ranged between 8 and 9. Total RNA (1 μg) was used for cDNA synthesis (SuperScriptII; Invitrogen), and qPCR experiments were carried out on a Biorad Icycler. The HPRT gene was used as an internal control. qPCR primer sequences are available on request.

Coimmunoprecipitation. Nuclear extracts were prepared from microglial cultures that were trypsinized and pelleted. The cells were resuspended in 400 μL of ice-cold buffer A [10 mM Hepes (pH 8), 10 mM KCl, 0.5 mM DTT, 1.5 mM Mg_2Cl, 0.2% sodium orthovanadate, and protease inhibitor mixture (Roche)] and kept on ice for 10 min and vortexed for 15 s. The cells were centrifuged for 1 min at 10,000 × *g*. The pellet was resuspended in 100 μL of cold RIPA buffer [50 mM Hepes (pH 8), 150 mM KCl, 0.5% Na deoxycholate, 0.5 mM EDTA, 0.5 mM EGTA, sodium orthovanadate, and protease inhibitor mixture], subjected to gentle shaking for 15 min at 4 °C, frozen/thawed, and centrifuged at 4 °C, 10,000 × *g* for 15 min. The supernatants were assayed for protein concentration (Biorad) and stored at –80 °C until use. For coimmunoprecipitation, Protein A Sepharose beads (Sigma) calibrated with RIPA buffer (no phosphatase or protease inhibitors, 30-μL slurry) were incubated with 2 μg of anti-GR antibody (BuGR2; Abcam) overnight on a rotating wheel and then washed five times with 500 μL of cold RIPA buffer. The anti-GR antibody/Protein A Sepharose complex was incubated with 50 μg of nuclear extracts (precleared with Protein A Sepharose) on a rotating wheel for 3 h at 4 °C. The suspension was pelleted, and beads were washed five times with cold RIPA buffer. To the beads, Laemmli sample buffer was added, denatured at 95 °C for 5 min, and loaded onto NuPage 4–12% gels. The association of GR with p65 NF-κB was revealed by using anti-p65 NF-κB antibody (1/1,000 dilution).

Statistical Analysis. Data are expressed as mean ± SEM. Statistical analysis was performed by using one- or two-way ANOVA and Mann–Whitney tests unless specified. To test for the effect of the genotype when the primary effect of MPTP treatment was found to be significant, post hoc comparisons between $GR^{LysMCre}$ and $GR^{fl/fl}$ groups were made using a Bonferroni/Dunn test. Differences of $P < 0.05$ were considered statistically significant. Statistical analyses were carried out using StatView 5.0 software.

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Fig. S1. Selective absence of GR in microglial cells in $GR^{LysMCre}$ mice and the resultant overall GR levels after MPTP treatment. (*A*) Double immunofluorescence labeling of GR (red) and microglial marker F4/80 (green) of primary microglial cultures prepared from cortex of P1 $GR^{loxP/loxP}$ control or GR^{LysMC} mutant pups. (Bar = 20 μm.) (*B*) Immunoblot analysis of GR protein in two different $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutant microglial cultures showing absence of GR protein in mutants. The blots were rehybridized with anti-actin antibody. (*C*) Confocal micrographs of double immunofluorescence staining of GR in microglia using Iba-1 as microglial marker and TH as DN marker in SN of $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutant mice. Nuclear GR staining depicts its colocalization with ToPro, a specific nuclear stain, shown by arrows. GR is labeled in Iba1+ cells in control and not mutant, whereas both the control and mutant show GR labeling in DNs. (Bar = 10 μm.) (*D*) qPCR of GR in SN and striatum of $GR^{LysMCre}$ mice 1 and 2 d after MPTP treatment. (*E*) Immunoblot results of GR protein in SN and striatum of $GR^{loxP/loxP}$ and $GR^{LysMCre}$ mutants analyzed 48 h after saline injections or acute MPTP intoxication. The band intensities were quantified (a.u., arbitrary units) by using Gel Analyst software and adjusted according to actin results. $^{*}P < 0.05$, saline vs. MPTP (n = 3–5).

Fig. S2. Effects of microglial GR on MPTP-induced changes in dopaminergic nerve terminals in striatum after acute MPTP toxicity. (*A*) Striatal DA levels in controls and mutants 7 d after MPTP treatment. The results are calculated as percentage change in concentration from the values of control and mutant mice injected with saline. The DA levels in saline-injected mice were 5.89 ± 0.3 and 6.62 ± 0.4 ng/mg wet tissue for $GR^{loxP/loxP}$ and $GR^{LysMCre}$, respectively (*n* = 8). (*B*) Specific [^{3}H]DA uptake in synaptosomes prepared from striatum of control and mutant $GR^{LysMCre}$ mice either after saline (S) or MPTP injections at days shown. Note that MPTP causes about a 10-fold decrease in uptake activity, which is decreased significantly in mutants at day 21. ***$P < 0.001$ $GR^{loxP/loxP}$ vs. $GR^{LysMCre}$ MPTP mice (*n* = 6–12). (*C* and *D*) Western blot analysis of TH and DAT protein levels in striatum in $GR^{loxP/loxP}$ and $GR^{LysMCre}$ mice at 7 d (*C*) and 21 d (*D*) after either saline (S) or MPTP treatment, with actin used as a loading control. A representative experiment is shown. Blots were quantified, and the results are expressed as arbitrary units (a.u.). MPTP reduces TH levels by ~60% and DAT levels by 85% (*n* = 5).

Fig. S3. (*A*) MPP+ levels in striata of $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutant mice 90 min after a single 30 mg/kg MPTP·HCl injection showing no difference in MPTP metabolism in mutants compared with controls. (*B*) TNF-α mRNA levels as analyzed by qPCR after 1 h of LPS (100 ng/mL) or MPP+ (3 μM) treatment of in vitro microglia cultures prepared from $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutant P1 pups. MPP+ treatment does not induce TNF-α mRNA in either control or in mutant cultures. RQV, realtive quantitative value.

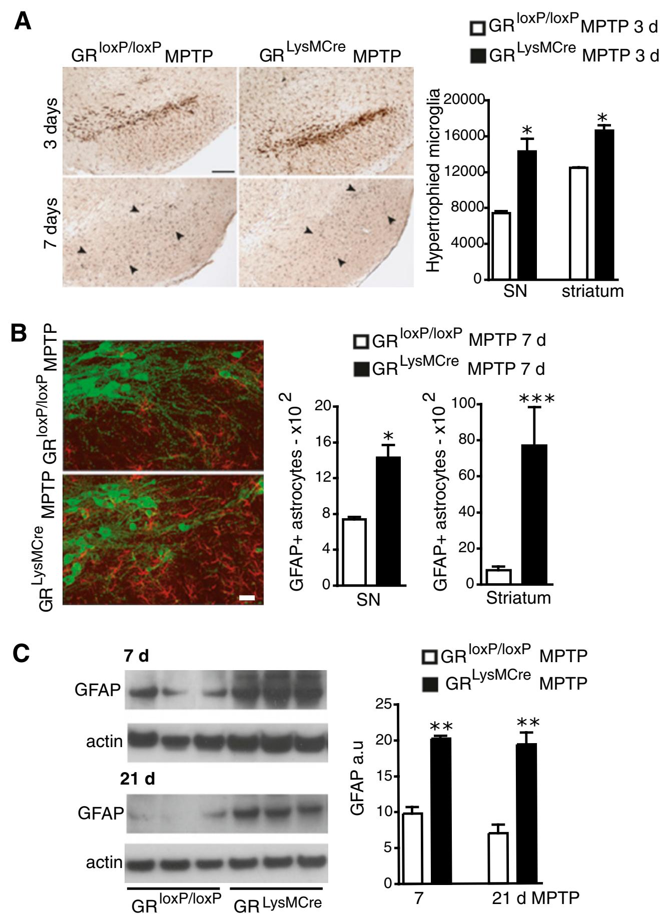

Fig. S4. Microglial and astroglial reactivity in GR^{LysMCre} mutant and control mice after acute MPTP treatment. (*A*) Immunohistochemistry of Iba-1 in SN of control and GR^{LysMCre} mutant mice 3 and 7 d after MPTP treatment. The microglial activation seen in the SN pars compacta at 3 d after MPTP has almost disappeared at day 7. (Bar = 200 μm.) Quantification of hypertrophied microglia in SN and striatum at 3 d shows a significant increase in GR^{LysMCre} mutants. $*P <$ 0.05 GR$^{loxP/loxP}$ vs. GR^{LysMCre} MPTP mice at 3 d. (*B*) Increased GFAP+ astrocytes in GR^{LysMCre} mutants after MPTP treatment as depicted by a confocal image of GFAP+ astrocytes (red) and TH-IR neurons (green) in the SN. (Bar = 20 μm.) The quantification of GFAP+ astrocytes after immunohistochemistry in the SN and striatum after MPTP treatment shows a significant increase in GR^{LysMCre} mutants, particularly in the striatum. $*P < 0.05$, $***P < 0.001$ GR$^{loxP/loxP}$ vs. GR^{LysMCre} MPTP mice at 7 d. (*C*) A representative Western blot experiment of striatal GFAP protein levels analyzed 7 and 21 d after MPTP treatment. The results were quantified by using actin as loading control. $**P < 0.01$ ($n = 4$).

Fig. S5. qPCR analysis of changes in expression of pro- and anti-inflammatory genes mediated by acute MPTP intoxication in $GR^{loxp/loxp}$ control mice. The relative quantitative values (RQV) denote changes in mRNA level 24 h after MPTP injections in $GR^{loxp/loxp}$ mice relative to values obtained in saline-injected $GR^{loxP/loxP}$ mice. $*P < 0.05$, $**P < 0.01$ $GR^{loxP/loxP}$ control saline vs. MPTP treated mice ($n = 4$).

Fig. S6. qPCR examination of modulations in the levels of inflammatory mediators in $GR^{LysMCre}$ mutant mice compared with $GR^{loxP/loxP}$ controls in the SN and striatum after acute MPTP treatment. Results of relative changes in the levels of inflammatory mediators in $GR^{LysMCre}$ mutants as compared with $GR^{loxP/loxP}$ controls in the SN and striatum (STR) 24 and 48 h after acute MPTP treatment. PCR values are presented as relative quantitative values (RQV). All of the proinflammatory mediators shown in *A* are up-regulated in $GR^{LysMCre}$ mutants compared with $GR^{loxP/loxP}$ controls, whereas *B* shows the interleukin genes that are down-regulated in MPTP-treated $GR^{LysMCre}$ mutants with respect to MPTP-treated $GR^{fl/fl}$ controls. (*C*) Analysis of two known anti-inflammatory GR target genes, MKP-1 and IL-1R2, in $GR^{LysMCre}$ mutants relative to $GR^{loxP/loxP}$ control mice in the SN and striatum (STR). $*P < 0.05$, $**P < 0.01$ $GR^{loxP/loxP}$ control vs. $GR^{LysMCre}$ mutant MPTP mice (n = 4).

Fig. S7. Regulation of NF-κB by microglial GR (*A Upper*) Coimmunoprecipitation of GR and p65 NF-κB. Primary microglial cultures were treated for 30 min with LPS (100 ng/mL) or LPS in dexamethasone (100 nM) pretreated (2 h) cells. GR complex was immunoprecipitated from nuclear extracts of microglia and its association with p65 NF-κB revealed by immunoblot. *IgG. (*Lower*) GR protein (input) in the nuclear extracts is shown. The experiment was repeated with similar results. (*B*) NF-κB reporter activity was measured in control and mutant primary microglial cells that had been cotransfected with pGL4.3*luc*2P/NF-κB-RE and p*Renilla*-TK-luciferase and subsequently treated with LPS or LPS plus dexamethasone. The luciferase luminescence values reflecting NF-κB reporter activity were normalized with respect to *Renilla* luminescence. The data are presented as fold changes from three independent experiments. *$P < 0.05$, control LPS vs. control LPS plus dexamethasone, Student's paired *t* test. (*C*) Phopho-Ser^{276} and Ser^{536} NF-κB in whole-cell microglial lysates of $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutants treated for 30 min with LPS or LPS in the presence of dexamethasone. The anti–phospho-Ser^{276} NF-κB antibody reacts with phospho-Ser^{276} p65 as well as phospho-Ser^{337} p105 subunits. (*D*) Phopho-Ser^{276} and Ser^{563} NF-κB proteins in SN and striatal lysates prepared from $GR^{loxP/loxP}$ control and $GR^{LysMCre}$ mutant mice injected with saline or 48 h after MPTP intoxication. p65 NF-κB and actin levels show the specificity of changes in phosphorylated NF-κB (*n* = 3–5). The bands were quantified relative to actin values. *$P < 0.05$, $GR^{loxP/loxP}$ control vs. $GR^{LysMCre}$ mutants treated with MPTP.

Table S1. Characteristics of subjects for the analysis of plasma cortisol levels

Table S1

PD severity was assessed by the motor score of the Unified Parkinson's Disease Rating Scale (UPDRS; range 0–108) and the Hoehn and Yahr score (0: no symptom; 1: unilateral symptoms; 2: bilateral symptoms, no impairment of balance; 3: balance impairment, physically independent; 4: severe disability, still able to walk or stand unassisted; 5: needing a wheelchair or bedridden unless assisted).