Abnormal Cortico-Basal Ganglia Neurotransmission in a Mouse Model of 1-DOPA-Induced Dyskinesia

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1-3,4-dihydroxyphenylalanine (1-DOPA) is an effective treatment for Parkinson’s disease (PD); however, long-term treatment induces 1-DOPA-induced dyskinesia (LID). To elucidate its pathophysiology, we developed a mouse model of LID by daily administration of 1-DOPA to PD male ICR mice treated with 6-hydroxydopamine (6-OHDA), and recorded the spontaneous and cortically evoked neuronal activity in the external segment of the globus pallidus (GPe) and substantia nigra pars reticulata (SNr), the connecting and output nuclei of the basal ganglia, respectively, in awake conditions. Spontaneous firing rates of GPe neurons were decreased in the dyskinesia-off state (24 h after 1-DOPA injection) and increased in the dyskinesia-on state (20–100 min after 1-DOPA injection while showing dyskinesia), while those of SNr neurons showed no significant changes. GPe and SNr neurons showed bursting activity and low-frequency oscillation in the PD, dyskinesia-off, and dyskinesia-on states. In the GPe, cortically evoked late excitation was increased in the PD and dyskinesia-off states but decreased in the dyskinesia-on state, while in the SNr, cortically evoked inhibition was largely suppressed, and monophasic excitation became dominant in the PD state. Chronic 1-DOPA treatment partially recovered inhibition and suppressed late excitation in the dyskinesia-off state. In the dyskinesia-on state, inhibition was further enhanced, and late excitation was largely suppressed. Cortically evoked inhibition and late excitation in the SNr are mediated by the cortico-striato-SNr direct and cortico-striato-GPe-subthalamo-SNr indirect pathways, respectively. Thus, in the dyskinesia-on state, signals through the direct pathway that release movements are enhanced, while signals through the indirect pathway that stop movements are suppressed, underlying LID.

Key words: basal ganglia; direct and indirect pathways; external segment of the globus pallidus; extracellular recording; 1-DOPA-induced dyskinesia; substantia nigra pars reticulata

Significance Statement

Parkinson’s disease (PD) is caused by progressive loss of midbrain dopaminergic neurons, characterized by tremor, rigidity, and akinesia, and estimated to affect around six million people worldwide. Dopamine replacement therapy is the gold standard for PD treatment; however, control of symptoms using 1-3,4-dihydroxyphenylalanine (1-DOPA) becomes difficult over time because of abnormal involuntary movements (AIMs) known as 1-DOPA-induced dyskinesia (LID), one of the major issues for advanced PD. Our electrophysiological data suggest that dynamic changes in the basal ganglia circuitry underlie LID; signals through the direct pathway that release movements are enhanced, while signals through the indirect pathway that stop movements are suppressed. These results will provide the rationale for the development of more effective treatments for LID.

Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder caused by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and characterized by tremor, rigidity, akinesia, and non-motor symptoms (Albin et al., 1989; DeLong, 1990; Fahn, 2003). Administration of 1-3,4-dihydroxyphenylalanine (1-DOPA) compensates for reduced dopamine and ameliorates PD symptoms (Cotzias et al., 1969; Fahn, 2003). However, disease progression and long-term 1-DOPA treatment induce motor fluctuations and dyskinesia in the majority of PD patients within 10 years, and make the control of PD symptoms difficult (Ahlskog and Muenter, 2001; Cenci, 2014). 1-DOPA-induced dyskinesia (LID) characterized by choreiform and dystonic
movements (Fahn, 2000; Obeso et al., 2000a; Bezard et al., 2001; Cenci and Lundblad, 2006) limits the quality of life of PD patients, and its control is a major issue for advanced PD. Once LID appears, it occurs in every L-DOPA administration (Cenci and Lundblad, 2006; Jenner, 2008), suggesting latent and irreversible changes after long-term L-DOPA treatment.

Molecular and biochemical changes have been reported in LID (Cenci, 2007, 2014), including excessive release of dopamine from serotonergic terminals, supersensitivity of postsynaptic dopaminergic receptors, changes of intracellular signaling in striatal (Str) neurons, and abnormal cortico (Cx)-Str plasticity (Picconi et al., 2003, 2011). Synaptic connectivity and dendritic morphology of Str projection neurons were also altered (Fiebinger et al., 2014; Nishijima et al., 2014; Suárez et al., 2014, 2016). On the other hand, studies at the circuit level have been rather limited. Recent imaging studies showed that LID induced differential activity changes in Str direct-pathway (Strd) and indirect-pathway (Stri) neurons (Parker et al., 2018; Ryan et al., 2018), and their selective optogenetic/chemogenetic activation exacerbated or alleviated LID (Alaccer et al., 2017; Hernández et al., 2017; Perez et al., 2017; Girasole et al., 2018; Ryan et al., 2018; Keifman et al., 2019).

To elucidate the pathophysiology of LID systematically at the circuit level, here we recorded neuronal activity in the external segment of the globus pallidus (GPe) and substantia nigra pars reticulata (SNr) using a mouse model of LID in awake conditions. The GPe is the connecting nucleus of the basal ganglia (BG), while the SNr together with the internal segment of the globus pallidus (GPI) is the output nucleus (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990). Their activity was altered in animal models and human patients of movement disorders (Filion and Tremblay, 1991; Hutchinson et al., 1997; Boraud et al., 1998, 2001; Wichmann et al., 1999; Lozano et al., 2006; Obeso et al., 2000b; Levy et al., 2001; Heimer et al., 2002, 2006; Wichmann and Soares, 2006).

We paid special attention to their responses to motor Cx stimulation, a triphasic response composed of early excitation, inhibition, and late excitation in the GPe and SNr/GPi in the normal state. Each component is mediated by the Cx-subthalamic (STN)-GPe/SNr/GPi, Cx-Str-GPe/SNr/GPi, and Cx-Str-GPe-STN-GPe/SNr/GPi pathways, respectively, based on the following findings: (1) blockade of the STN suppressed both early excitation and late excitation in the GPe/SNr/GPi; (2) local GABA_A receptor blockade diminished inhibition in the GPe/GPi; (3) ablation of Str-GPe neurons diminished inhibition and late excitation in the GPe and late excitation in the SNr (Ryan and Clark, 1991; Maurice et al., 1999; Nambu et al., 2000, 2002; Kita et al., 2004; Tachibana et al., 2008; Sano et al., 2013). Response pattern changes in the GPe and SNr/GPi reflect neurotransmission efficacy through the BG circuitry. Moreover, in voluntary movements, activity originating in the Cx is transmitted through the BG circuitry and reaches the SNr/GPi. Thus, Cx stimulation can mimic such information processing through the BG. Indeed, Cx-evoked response patterns are altered in movement disorders and could explain their pathophysiology (Nambu et al., 2000; Chiken et al., 2008, 2015; Kita and Kita, 2011; Nishiyayashi et al., 2011; Sano and Nambu, 2019). To reveal latent changes after chronic L-DOPA treatment, we also examined the dyskinesia-off state when the effect of L-DOPA was washed out, and LID was not observed.

Materials and Methods

Animals

Twelve adult male ICR mice (SLC; −10 weeks old, 36–42 g before the operation) were used. The experimental procedures were approved by the Institutional Animal Care and Use Committee of National Institutes of Natural Sciences. All experiments were conducted according to the National Institutes of Health Guide for Care and Use of Laboratory Animals. Mice were housed under a 12/12 h light/dark cycle, with free access to water and food. Before experiments, mice were handled daily to become accustomed to the experimenters.

Experimental design

Animals were separated into two groups (Fig. 1A). The vehicle (sham-lesioned group, six mice) or 6-hydroxydopamine (6-OHDA; 6-OHDA-lesioned group, six mice) was injected unilaterally to the medial forebrain bundle (MFB) of the right hemisphere (Fig. 1A, day −14). Fourteen days after vehicle/6-OHDA injection (day 0), the first cylinder test was conducted to evaluate PD motor features. The first surgery to mount a head holder on the mouse’s head (day −3) and the second surgery to chronically implant stimulating electrodes in the motor Cx (day 3) were performed 3 d before and 3 d after the cylinder test, respectively. Then, spontaneous activity and Cx-evoked responses of GPe and SNr neurons were recorded in the sham-lesioned (“control”) state and 6-OHDA-lesioned (“PD”) state groups (from day 11 to 42) in the awake state. After recording in the control or PD state, all mice received daily injections of L-DOPA (10 mg/kg, i.p.) for 11 d (from day 42 to 52). Abnormal involuntary movements (AIMs) were examined before and after L-DOPA treatment every other day. The second cylinder test was performed to evaluate PD motor features after the termination of chronic L-DOPA treatment (day 53). Then, recordings of the activity of GPe and SNr neurons were restarted in both the sham-lesioned (“L-DOPA-off” states) and 6-OHDA-lesioned (“dyskinesia-off” states) groups (from day 56 to 95) along with acute L-DOPA treatment (10 mg/kg, i.p.). When recordings were not performed, L-DOPA treatment continued two to four times per week to maintain AIMs scores. After the final recording (day 100), animals were perfused, and recording sites in the GPe and SNr and dopaminergic neurons in the SNC were examined histologically.

Vehicle or 6-OHDA injection

6-OHDA was injected into the MFB according to standard methods (Francardo et al., 2011; Thiele et al., 2011; Fig. 1A, day −14); 30 min before vehicle or 6-OHDA injection, desipramine hydrochloride (25 mg/kg, i.p., norepinephrine reuptake inhibitor; Sigma-Aldrich) was administered to mice to increase the selectivity of 6-OHDA-induced lesions. Vehicle (0.02% acetic acid dissolved in saline) or 6-OHDA-hydrobromide (3 mg/ml in 0.02% ascorbic acid dissolved in saline; Sigma-Aldrich) was prepared immediately before surgeries. Each mouse was anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (5 mg/kg, i.p.) and held in the stereotaxic apparatus. The skin of the head was incised, and a small hole was made on the xylazine hydrochloride (5 mg/kg, i.p.) and held in the stereotaxic apparatus. The incised skin was closed with surgical clips (Reflex Clip, WPI).

First surgery for head fixation

Three days before the first cylinder test (Fig. 1A, day −3), the surgical operation to mount a head holder on the mouse’s head was performed as described previously (Chiken et al., 2008; Sano et al., 2013). Each mouse was anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (5 mg/kg, i.p.) and held in the stereotaxic apparatus. The skull was widely exposed and covered with bone adhesive resin (Bistite II, Tokuyama Dental). A small U-shape head holder made...
of acetal resin was attached to the skull with acrylic resin (Unifast II, GC).

Cylinder test to evaluate PD motor features
Fourteen days after vehicle or 6-OHDA injection (Fig. 1A, day 0), PD motor features were evaluated using the cylinder test (Schallert et al., 2000; Tillerson et al., 2001; Meredith and Kang, 2006). Each mouse was placed inside a transparent acrylic cylinder (inner diameter 10 cm, height 20 cm) and videotaped for 10 min without previous habituation to the cylinder. Two mirrors were placed side-by-side, forming a 90° angle with each other, behind the cylinder to allow views from all sides of the cylinder. Mice showed exploratory behavior by rearing and leaning on the wall of the cylinder with their forelimbs. The numbers of rotations (≥180°) during 10 min and of contacts with their right or left forelimb...
for the first 5 min were counted (Cenci and Lundblad, 2007; Francardò et al., 2011; Alcacer et al., 2017). A contralateral forelimb use score was calculated with the number of wall contacts by the left forelimb (contra-
lateral to the lesion, affected side) as a percentage of the total number of wall contacts by the right or left forelimb. If mice showed no impair-
ment in the usage of the left forelimb compared with that of the right
forelimb (contralateral forelimb use score \( \geq 20\% \)), additional injections
of 6-OHDA were performed. In the present study, all mice received
6-OHDA injections twice and showed significant impairment. Another
cylinder test was performed after chronic \( \alpha \)-DOPA treatment (day 53) to
examine its long-term therapeutic effects on PD motor features.

Second surgery for implantation of stimulating electrodes
Three days after the first cylinder test (Fig. 1A, day 3), the second surgery
was performed to chronically implant stimulating electrodes in the
motor Cx. The mouse was anesthetized with ketamine hydrochloride
(50–100 mg/kg, i.p.) and then held in the stereotaxic apparatus with its
head restrained using the U-shape head holder. Part of the skull in the
right hemisphere, ipsilateral to the vehicle/6-OHDA injection side, was
removed to access the motor Cx, GPe, and SNr. Two pairs of bipolar
stimulating electrodes (50-\( \mu \)m diameter Teflon-coated tungsten wires,
tip distance 300–400 \( \mu \)m) were implanted chronically into the orofacial
and forelimb regions of the primary motor Cx, and fixed using acrylic
resin (Chiken et al., 2008; Sano et al., 2013). Somatotopy of these regions
was confirmed by intracranial microstimulation (a train of 10 pulses at
333 Hz, 200-\( \mu \)s duration, \( \leq 35 \mu \)A).

Neuronal activity recording in the control or PD state
After recovery from the second surgery (Fig. 1A, from day 11 to 42),
the awake mouse in the control or PD state was positioned painlessly in a
stereotaxic apparatus using the U-shape head holder (Chiken et al.,
2008, 2015; Sano et al., 2013). For single-unit recording, a glass-coated
tungsten microelectrode (0.5 or 1.0 M\( \Omega \) at 1 kHz; Alpha Omega) was
inserted vertically into the GPe (target area; posterior 0.3–0.7 mm and
lateral 1.6–2.4 mm from bregma) or SNr (posterior 2.9–3.3 mm and lateral
1.5–1.9 mm from bregma) through the dura mater using a hydraulic
microdrive. Signals from the microelectrode were amplified and filtered
(0.3–5.0 kHz). Unit activity was isolated, converted to digital data with a
homemade time-amplitude window discriminator, and sampled at 2.0 kHz
using a computer with LabVIEW 7.1 software (National Instruments) for
off-line data analysis. Spontaneous discharges were recorded for 50 s. The
responses to Cx-electrical stimulation (200-\( \mu \)s duration, monophasic single
pulse at 0.7 Hz, 50-\( \mu \)A strength) through the stimulating electrodes
implanted in the motor Cx were examined by constructing peristimulus
time histograms (PSTHs; bin width of 1 ms; prestimulus, 100 ms; poststim-
ulus, 900 ms) for 100 stimulation trials.

Chronic \( \alpha \)-DOPA treatment
After finishing the recording session in the control or PD state, sham-
lesioned and 6-OHDA-lesioned groups were treated daily with \( \alpha \)-DOPA
for 11 d (Fig. 1A, from day 42 to 52). They received an i.p. injection of a mixture of 10 mg/kg \( \alpha \)-DOPA (Dopastop, Ohara Pharmaceutical)
and 15 mg/kg benserazide (peripheral \( \alpha \)-DOPA decarboxylase inhibitor;
Sigma-Aldrich) dissolved in saline.

Behavioral test measuring AIMs
One day before and every other day during chronic \( \alpha \)-DOPA treatment
(Fig. 1A, from day 42 to 52), each mouse was placed inside a transparent
acrylic cage (width 26 cm, depth 18 cm, and height 13 cm) and video-
taped for 1 min at 20-min intervals during 120 min. LID was assessed by
using the AIMs scale described below, which is similar to that used in
human dyskinesia (Cenci and Lundblad, 2007; Francardò et al., 2011; Thiele et al., 2011), for 1 min before (at 0 min) and every 20 min after (at
20, 40, 60, 80, 100, and 120 min) \( \alpha \)-DOPA injection. Based on the topo-
graphical distribution, AIMs were classified into four subtypes: axial (lat-
eral flexion and axial rotation of the neck and trunk toward the side
contralateral to the lesioned hemisphere), forelimb (repetitive rhythmic
jerky movements or dystonic posturing of the forelimb on the side con-
tralateral to the lesioned hemisphere), orolingual (repetitive and vacuous
chewing movements of the jaw with or without tongue protrusion), and
locomotive (increased rotational activity toward the contralateral side of
the lesion with tactile contacts of at least three paws with the floor). Each
subtype was scored on a scale from 0 to 4 (0, absent; 1, occasional; 2, fre-
quent; 3, continuous; 4, continuous and not interruptible by outer
stimuli). Total AIMs scores at each time point were calculated by the
sum of AIMs scores in the four different body parts (maximum, 16).
Total AIMs scores of the day were calculated by the sum of
AIMs scores at each time point after \( \alpha \)-DOPA injection (six points in
total; maximum, 96).

Neuronal activity recording in the \( \alpha \)-DOPA-on/off or dyskinesia-off/on
states
After measuring AIMs scores, neuronal activity recording in the
\( \alpha \)-DOPA-on/off or dyskinesia-off/on states was restarted (Fig. 1A, from
day 56 to 95). Spontaneous discharges and the responses to Cx stimula-
tion were recorded in the GPe and SNr in awake conditions as described
above. Data recorded long (>24 h) after the previous \( \alpha \)-DOPA treatment
and before the next \( \alpha \)-DOPA treatment (Fig. 1A, inset images) were
described as \( \alpha \)-DOPA-off (sham-lesioned group) or dyskinesia-off
(6-OHDA-lesioned group) when acute \( \alpha \)-DOPA effects were washed
out, and no AIMs were observed in the 6-OHDA-lesioned group. The
dyskinesia-off state corresponds to the latent state of LID after long-term
\( \alpha \)-DOPA treatment.

After the recording, the awake mouse was kept in the stereotaxic appa-
ratus, and \( \alpha \)-DOPA (10 mg/kg with benserazide, i.p.) was injected through
an elaster needle, which had been inserted into the peritoneal cavity before the experiment. Then, another recording was performed from 20 to 100 min after acute \( \alpha \)-DOPA injection when the 6-OHDA-
lesioned group showed AIMs (Fig. 1A, inset images). Data recorded dur-
ing this period were described as \( \alpha \)-DOPA-on (sham-lesioned group) or
dyskinesia-on (6-OHDA-lesioned group).

Histology
After the final recording (Fig. 1A, day 100), GPe and SNr recording sites
were marked by passing cathodal DC (20 \( \mu \)A for 20 s) through the rec-
ording electrode. Mice were then deeply anesthetized with sodium pen-
tobarbital (100 mg/kg, i.p.) and perfused transcardially with 0.01 M
PBS containing 10% formalin at 4°C overnight and cryoprotected in graded
series (40 \( \mu \)m) were cut with a freezing microtome and collected in 0.01 M
PBS as previously described (Sano et al., 2013). Brain sections con-
taining the motor Cx, GPe, or SNr were mounted on MAS-coated
slide glasses (Matsunami Glass), air-dried, and stained with Neutral
Red. The recording sites in the GPe and SNr were confirmed accord-
ing to the lesions made by cathodal DC and the traces of electrode
tracks.

The sections containing the SNc were used to evaluate dopaminergic
neurons in the lesioned hemisphere as described previously (Sano et al.,
2015). Free-floating sections were incubated with primary antibody
against mouse tyrosine hydroxylase (TH; 1:1000, Merck) at 4°C over-
night, and visualized with biotinylated secondary antibody (1:500;
Vector Laboratories) using an ABC method (Vectorstain Elite ABC kit,
Vector Laboratories). The sections were mounted, air-dried, cover-
slipped, and examined under a light microscope.

Analysis of electrophysiological data
Spontaneous discharge rates were calculated from continuous digitized
recordings for 50 s. Other parameters characterizing firing patterns were
calculated from the first 30 s of the same recordings; the coefficient
of variation (CV) of interspike intervals (ISIs), the burst index defined as
the ratio of the mean and the mode of ISIs, and the percentage of num-
ber of spikes in bursts detected by Poisson surprise method \( \text{surprise value} \approx \log_{10}(P) \geq 2 \), the minimum number of spikes during bursts was 3,\nLegéndy and Salcman, 1985; Chiken et al., 2008, 2015; Sano et al., 2013].

Autocorrelograms (bin width of 0.5 ms) were calculated from contin-
uous digitized recordings for 50 s and smoothed with a Gaussian filter
(\( \sigma = 1.6 \text{ ms} \)). The mean value and SD were calculated between 0.1 and
of 4096 bins (2048 ms) with 50% overlapping (Tachibana et al., 2011). The shuffled PSD was also calculated from a shuffled spike train, which was constructed by combining randomly shuffled short segments of the original spike train (one-half of the mean ISIs in length). The original PSD was divided by the shuffled PSD to compensate for the spectral distortion at low frequencies and averaged among neurons.

The responses to Cx stimulation were analyzed by using PSTHs. Cx stimulation typically induced a triphasic response composed of early excitation, inhibition, and late excitation in GPe and SNr neurons. The mean value ($\mu_{\text{baseline}}$) and SD ($\text{SD}_{\text{baseline}}$) of the discharge rate during 100 ms preceding the onset of stimulation were considered as the baseline discharge rate, and statistical significance level was set as $\mu_{\text{baseline}} \pm 1.65 \times \text{SD}_{\text{baseline}}$ (corresponding to $p = 0.1$, two-tailed t test). If two consecutive bins during 3–7 ms (five bins) for early excitation, three consecutive bins during 5–19 ms (15 bins) for inhibition, and three consecutive bins during 12–42 ms (31 bins) for late excitation exceeded the significance level, the response was judged significant (corresponding to $p = 0.04$ for early excitation, $p = 0.013$ for inhibition, and $p = 0.029$ for late excitation after Bonferroni’s correction, two-tailed t test). Once significant bins were detected, the response was considered continuing unless two consecutive bins fell below the significance level (within $\mu_{\text{baseline}} \pm 1.65 \times \text{SD}_{\text{baseline}}$). The starting and end points were defined as the first and last bins of the response, respectively. In case that early excitation and late excitation were merged without inhibition, the end point of early excitation and the starting point of late excitation were set to 17 and 18 ms, respectively. In case that early excitation and late excitation were used, the end point of late excitation were set to 17 and 18 ms, respectively. The duration and amplitude were set to zero.

For population PSTHs, the PSTH of each neuron with a significant response to Cx stimulation was averaged within the same condition and smoothed using a Gaussian filter ($\sigma = 1.6$ ms). For heat maps, the PSTH of each neuron was standardized using the Z-score ($z_i = (y_i - \mu_{\text{baseline}})/\text{SD}_{\text{baseline}}$), where $y_i$ is the firing rate at time $t_i$. The time points, and by sorted the response patterns and the latency of the first component of Cx-evoked responses. Electrophysiological data were analyzed using IgorPro 7 (Wavemetrics), OriginPro 2018 (Lightstone), and MATLAB R2016b (MathWorks) software.

### Results

#### Motor behaviors before and after chronic l-DOPA treatment

Vehicle (six mice) or 6-OHDA (six mice) was injected into the right MFB. PD motor features were examined using the cylinder test after 14 d, and 6-OHDA was additionally injected into all 6-OHDA-lesioned mice. 6-OHDA-lesioned mice (Fig. 1A, day 0) showed significant ipsilateral rotational behavior (ipsilateral rotation, $10.5 \pm 4.0$ turns; contralateral rotation, 0 turn; $F_{(1,20)} = 23.94, p < 0.001); one-way ANOVA with Tukey’s post hoc test), while sham-lesioned mice did not (ipsilateral rotation, 1.5 ± 1.8 turns; contralateral rotation, 3.3 ± 1.5 turns). 6-OHDA-lesioned mice also showed a significant reduction in the usage of the forelimb contralateral to the lesion in wall contacts (contralateral forelimb use, 16.5 ± 14.6%; $F_{(3,20)} = 5.12, p = 0.0045$), while all sham-lesioned mice showed similar usage of both forelimbs (contralateral forelimb use, 53.3 ± 39.4%).

#### Spontaneous activity changes of GPe neurons

The mean firing rate of GPe neurons in PD was not significantly different from that in the control state ($69.3 \pm 18.9$ Hz) was not significantly different from the PD state ($67.3 \pm 18.0$ Hz, $p > 0.99$) states. The mean firing rate of GPe neurons in the PD state (69.3 ± 18.9 Hz) was not significantly different from 2672 - J. Neurosci., March 24, 2021 - 41(12):2668–2683

### Statistical analysis

Two-way repeated-measures ANOVA with Dunn’s multiple comparison test was defined as a PD model.

### Spontaneous activity changes of GPe neurons

The mean firing rate of GPe neurons (significant interaction between lesion group × l-DOPA treatment, $F_{(1,20)} = 13.86, p < 0.001$; two-way ANOVA; Fig. 2A) was $67.9 \pm 18.2$ Hz in the control state, and it remained unchanged in the l-DOPA-off (68.2 ± 21.1 Hz; $p > 0.99$; Kruskal–Wallis test with Dunn’s multiple comparison test) and l-DOPA-on (67.7 ± 18.0 Hz; $p > 0.99$) states. The mean firing rate of GPe neurons in the PD state (69.3 ± 18.9 Hz) was not significantly different from that in the control state ($p > 0.99$), whereas that was significantly lower in the dyskinesia-off state (59.8 ± 19.3 Hz) and higher in the dyskinesia-on state (83.8 ± 23.2 Hz; l-DOPA-off vs dyskinesia-off, $p = 0.035$; PD vs dyskinesia-off, $p = 0.004$; l-DOPA-on vs dyskinesia-off, $p = 0.04$).
The firing patterns were also examined by the CV of ISIs, the burst index, and spikes in burst of GPe and SNr neurons in each state (control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group and PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group; \( p < 0.05 \), \( \approx p < 0.01 \) significantly different from each other; cyan \( \approx \), \( ** \)two-way ANOVA with Kruskal–Wallis test with Dunn’s multiple comparison test; magenta \( \approx \), \( ** \)two-way ANOVA with Bonferroni’s post hoc test. \( n \), number of neurons; \( m \), number of mice.

Figure 2. Spontaneous activity in the GPe and SNr. A, B, Box plots (the median, first and third quartiles, and minimum and maximum excluding any outliers outside 1.5 times the interquartile range from the upper and lower quartiles) and means (rectangles) of firing rate, CV of ISIs, burst index, and spikes in burst of GPe (A) and SNr (B) neurons are shown in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group and PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group; \( p < 0.05 \), \( \approx p < 0.01 \) significantly different from each other; cyan \( \approx \), \( ** \)two-way ANOVA with Kruskal–Wallis test with Dunn’s multiple comparison test; magenta \( \approx \), \( ** \)two-way ANOVA with Bonferroni’s post hoc test.

Typical examples of digitized spikes and smoothed autocorrelograms in each state are shown in Figure 3A. GPe neurons in the control, L-DOPA-off, L-DOPA-on, and dyskinesia-on states fired with weak regularity, while such regularity disappeared in the PD and dyskinesia-off states. The percentage of GPe neurons that exhibited regular firings was much smaller in the PD (9%) and dyskinesia-off (3%) states as compared with the sham-lesioned group (control vs PD, \( \chi^2 = 30.71 \), \( p < 0.001 \); L-DOPA-off vs dyskinesia-off, \( \chi^2 = 63.94 \), \( p < 0.001 \); \( \chi^2 \) test with Bonferroni’s correction; Table 1), but not in the dyskinesia-on state (50%; L-DOPA-on vs dyskinesia-on, \( \chi^2 = 2.76 \), \( p = 0.097 \)). Strong regularity appeared in the dyskinesia-on state (Fig. 3A), the number of peaks of autocorrelograms (significant effect of lesion group, \( F_{(1,183)} = 4.13 \), \( p < 0.043 \); two-way ANOVA; Table 1) was larger (\( p < 0.001 \); Bonferroni’s post hoc test), and the mean time of the first peak (significant effect of lesion group, \( F_{(1,183)} = 55.81 \), \( p < 0.001 \); significant effect of
Table 1. Regularity of spontaneous activity of GPe and SNr neurons

<table>
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<tr>
<th></th>
<th>Sham lesioned</th>
<th>6-OHDA lesioned</th>
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<tbody>
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<td></td>
<td>Control</td>
<td>L-DOPA-off</td>
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<tr>
<td><strong>GPe</strong></td>
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<tr>
<td>n, number of neurons</td>
<td>n = 100</td>
<td>n = 108</td>
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<tr>
<td>m, number of mice</td>
<td>m = 3</td>
<td>m = 4</td>
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<tr>
<td>Number of regular-firing neurons (%)⁶</td>
<td>45 (45%)</td>
<td>53 (49%)</td>
</tr>
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<td>Number of peaks of autocorrelograms⁵</td>
<td>1.8 ± 0.8</td>
<td>1.9 ± 0.8</td>
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<tr>
<td>Time of the 1st peak (ms)⁵</td>
<td>16.8 ± 4.3</td>
<td>13.4 ± 4.2²⁵</td>
</tr>
<tr>
<td><strong>SNr</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, number of neurons</td>
<td>n = 84</td>
<td>n = 85</td>
</tr>
<tr>
<td>m, number of mice</td>
<td>m = 4</td>
<td>m = 4</td>
</tr>
<tr>
<td>Number of regular-firing neurons (%)⁶</td>
<td>46 (55%)</td>
<td>49 (38%)</td>
</tr>
<tr>
<td>Number of peaks of autocorrelograms⁵</td>
<td>2.1 ± 0.9</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Time of the 1st peak (ms)⁵</td>
<td>14.8 ± 3.0</td>
<td>13.7 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SD, *p < 0.01 significantly different from the corresponding states of the sham-lesioned group, ##p < 0.01 significantly different from the control state, $p < 0.01$ significantly different from the PD and dyskinesia-off states, ²x² test with Bonferroni’s correction, ³two-way ANOVA with Bonferroni’s post hoc test.

Figure 3. Spontaneous firing patterns of GPe neurons. A, Digitized spikes and smoothed autocorrelograms of spontaneous activity in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (left), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (right). The mean value (continuous gray line) and p = 0.001 levels (dashed gray lines) are indicated. B, Averaged PSD among neurons in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (left), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (right). Arrows, activity changes between different states; *peaks around 1 Hz.
L-DOPA treatment, $F(2,183) = 5.014, p = 0.008$) was shorter ($p < 0.001$), suggesting regular high-frequency firings. The mean time of the first peak was also shorter in the PD state ($p < 0.001$), although the number of neurons with regular firings were small.

In the sham-lesioned group, L-DOPA treatment significantly shortened the mean time of the first peak (control vs L-DOPA-off, control vs L-DOPA-on, $p < 0.001$).

Population PSDs of GPe neurons revealed that 60- to 80-Hz activity was high, while 10- to 40-Hz activity was low in the control and L-DOPA-off states (Fig. 3B, left). In the L-DOPA-on state, 10- to 50-Hz activity was increased (Fig. 3B, left, upward arrow). In all three states in the 6-OHDA-lesioned group, 0- to 40-Hz activity was increased (Fig. 3B, right, upward arrow), achieving peaks around 1 Hz (Fig. 3B, right, *), while 50- to 90-Hz component was decreased (Fig. 3B, right, downward arrow).

**Figure 4.** Spontaneous firing patterns of SNr neurons. A, Digitized spikes and smoothed autocorrelograms. B, Averaged PSD. *peaks around 1 Hz.

Spontaneous activity changes of SNr neurons

Spontaneous firing rates and patterns of SNr neurons were examined in Figure 2B. The mean firing rates of SNr neurons (significant interaction between lesion group $\times$ L-DOPA treatment, $F(2,183) = 3.844, p = 0.022$; two-way ANOVA; Fig. 2B) were 63.3 $\pm$ 15.8 Hz in the control, 63.0 $\pm$ 16.4 Hz in the L-DOPA-off, and 65.2 $\pm$ 19.3 Hz in the L-DOPA-on states and remained unchanged in the 6-OHDA-lesioned group (PD, 60.8 $\pm$ 15.9 Hz, $p > 0.99$; dyskinesia-off, 63.9 $\pm$ 13.8 Hz, $p > 0.99$; dyskinesia-on, 55.9 $\pm$ 16.4 Hz, $p = 0.17$; Kruskal–Wallis test with Dunn’s multiple comparison test). The CV of ISIs (significant interaction between lesion group $\times$ L-DOPA treatment, $F(2,183) = 10.55, p < 0.001$; two-way ANOVA; Fig. 2B) and burst index (significant effect of lesion group, $F(1,463) = 36.64, p < 0.001$; Bonferroni’s post hoc test) were larger in the PD and dyskinesia-on states as compared with the sham-lesioned group ($p < 0.001$). In addition, the percentages of spikes in bursts (significant effect of lesion group, $F(1,463) = 69.00, p < 0.001$) in the 6-OHDA-lesioned group were larger in all three states ($p < 0.001$).

Typical examples of digitized spikes and smoothed autocorrelograms in each state are shown in Figure 4A. SNr neurons fired with weak regularity in control, L-DOPA-off, and L-DOPA-on states (Fig. 4A, left), while such regularity disappeared in PD, dyskinesia-off, and dyskinesia-on states. More than 50% of SNr
Figure 5. Responses of GPe neurons evoked by motor Cx stimulation. A, The stimulation (Cx) and recording (GPe) sites are depicted with the BG circuitry. Red, cyan, and light-green lines represent glutamatergic excitatory, GABAergic inhibitory, and dopaminergic projections, respectively. In the Str, d and i represent direct-pathway (Strd) and indirect-pathway (Stri) neurons, respectively. Cx-evoked response in the GPe is typically composed of early excitation (Ex), inhibition (inh), and following late excitation (ii), which are mediated by the Cx-STN-GPe, Cx-Stri-GPe, and Cx-Strd-GPe-STN-GPe pathways, respectively. B, PSTHs of the typical response of GPe neurons to Cx stimulation in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (left), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (right). Cx stimulation was delivered at time 0 (arrows) for 100 stimulation trials. The mean value of responses of GPe neurons was standardized using the Z-score and displayed in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (upper), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (lower). Ex, excitation; inh, inhibition. Ex-inh-ex means a triphasic response composed of early excitation, inhibition, and following late excitation. Cx-evoked response in the GPe is typically composed of early excitation (Ex), inhibition (inh), and following late excitation (ii), which are mediated by the Cx-STN-GPe, Cx-Stri-GPe, and Cx-Strd-GPe-STN-GPe pathways, respectively. B, PSTHs of the typical response of GPe neurons to Cx stimulation in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (left), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (right). Cx stimulation was delivered at time 0 (arrows) for 100 stimulation trials. The mean value of responses of GPe neurons was standardized using the Z-score and displayed in the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group (upper), and in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group (lower). Ex, excitation; inh, inhibition. Ex-inh-ex means a triphasic response composed of early excitation, inhibition, and following late excitation. 

Changes of Cx-evoked responses of GPe neurons

Next, we examined the responses of GPe neurons induced by Cx stimulation by constructing PSTHs (Fig. 5A). The typical response pattern of GPe neurons in the control state of the sham-lesioned group was a triphasic response composed of early excitation, inhibition, and late excitation (Fig. 5B, control), as reported previously (Chiken et al., 2008, 2015; Sano et al., 2013; Sano and Nambu, 2019). Each component in the GPe is mediated by the Cx-STN-GPe, Cx-Stri-GPe, and Cx-Strd-GPe-STN-GPe pathways, respectively (Fig. 5A; Ryan and Clark, 1991; Nambu et al., 2000; Kita et al., 2004; Sano et al., 2013). This typical response pattern was not changed in the L-DOPA-off and L-DOPA-on states (Fig. 5B, L-DOPA-off, L-DOPA-on). These findings were also confirmed in population PSTHs (Fig. 5C, left), bar graphs categorizing response patterns (Fig. 5D, upper), heat map visualization (Fig. 5E, upper), and quantitative analyses (GPe, sham lesioned; Table 2). Similar to the sham-lesioned group, the most common response pattern in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group was a triphasic response (Fig. 5B, PD, dyskinesia-off, dyskinesia-on, D, lower). However, late excitation was strengthened in the PD and dyskinesia-off states, and weakened in the dyskinesia-on state (Fig. 5B, PD, dyskinesia-off, dyskinesia-on). Population PSTHs (Fig. 5C, right) and heat maps (Fig. 5E, lower) depict these differences in late excitation.

Quantitative analyses confirmed these changes (GPe, 6-OHDA lesioned; Table 2). Both the duration and amplitude of
Changes of Cx-evoked responses of SNr neurons

We also examined Cx-evoked responses of SNr neurons (Fig. 6A). The typical response pattern of SNr neurons in the control state of the sham-lesioned group was a triphasic response composed of early excitation, inhibition, and late excitation (Fig. 6B, control), as reported previously (Sano et al., 2013; Sano and Nambu, 2019). Each component in the SNr/Gpi is mediated by the Cx-STN-SNr/Gpi hyperdirect, Cx-STr–SNr/Gpi direct, and Cx-Str–Gpi-STN-SNr/Gpi indirect pathways, respectively (Fig. 6A; Maurice et al., 1999; Nambu et al., 2000; Tachibana et al., 2008; Sano et al., 2013). This typical response pattern was not changed in the L-DOPA-off and L-DOPA-on states (Fig. 6B, L-DOPA-off, L-DOPA-on). These findings were also confirmed in population PSTHs (Fig. 6C, left), bar graphs (Fig. 6D, upper), heat maps (Fig. 6E, upper), and quantitative analyses (SNr, sham lesioned; Table 2).

On the other hand, in the PD state of the 6-OHDA-lesioned group, monophasic excitation was the typical response pattern (Fig. 6B, PD). Population PSTHs also showed monophasic excitation (Fig. 6C, right, PD). Excitation without inhibition, such as monophasic early excitation, biphasic excitation, and monophasic late excitation, became dominant (80%; Fig. 6D, PD, warm colors), and responses with inhibition, such as a triphasic response, inhibition followed by excitation, excitation followed by inhibition, and monophasic inhibition, was reduced in the PD state (20%; $\chi^2 = 51.24$, $p < 0.001$, $\chi^2$ test with Bonferroni’s correction; Fig. 6D, PD, cold colors). In the heat map, it is apparent that monophasic excitation was dominant, and a fraction of biphasic responses composed of early excitation and the following inhibition was observed (Fig. 6E, PD).

After chronic L-DOPA treatment, in the dyskinesia-off state, excitation without inhibition (Fig. 6B, dyskinesia-off, C, right, dyskinesia-off) remained dominant (65%; Fig. 6D, dyskinesia-off, warm colors). However, responses with inhibition and without any late excitation, such as excitation followed by inhibition and monophasic inhibition (15%; Fig. 6D, dyskinesia-off, greenish colors), were increased in number compared with the PD state (3%; $\chi^2 = 6.54$, $p = 0.015$). These changes were observed as a small deflection in the falling phase of population PSTHs (Fig. 6C, right, dyskinesia-off, red arrowhead). In the heat map, inhibition was increased, although monophasic excitation remained (Fig. 6E, dyskinesia-off).

In the dyskinesia-off state, the typical response pattern was a biphasic response composed of excitation followed by inhibition (Fig. 6B, dyskinesia-on). Responses with inhibition and without late excitation (44%; Fig. 6D, dyskinesia-on, greenish colors) were increased in number compared with PD and dyskinesia-off states ($\chi^2 = 34.98$, $p < 0.001$). Meanwhile, responses without inhibition (32%; Fig. 6D, dyskinesia-on, warm colors) were late excitation (duration, significant interaction between lesion group $\times$ L-DOPA treatment, $F_{(2,505)} = 29.04$, $p < 0.001$; amplitude, $F_{(2,505)} = 17.6$, $p < 0.001$; two-way ANOVA) were significantly larger in the PD state and smaller in the dyskinesia-off state (duration, control vs PD, $p = 0.011$; L-DOPA-on vs dyskinesia-off, $p < 0.001$; PD vs dyskinesia-off, $p < 0.001$; Tachibana et al., 2008) test, with Dunn’s multiple comparison test; amplitude, control vs PD, $p = 0.011$; L-DOPA-on vs dyskinesia-off, $p = 0.001$; Kruskal–Wallis test with Dunn’s multiple comparison test; amplitude, control vs PD, $p = 0.011$; L-DOPA-on vs dyskinesia-off, $p = 0.001$; PD vs dyskinesia-off, $p < 0.001$; Kruskal–Wallis test with Dunn’s multiple comparison test; amplitude, control vs PD, $p = 0.011$; L-DOPA-on vs dyskinesia-off, $p = 0.001$; PD vs dyskinesia-off, $p < 0.001$; dyskinesia-off vs dyskinesia-off, $p = 0.001$). The amplitude of early excitation (significant effect of lesion group, $F_{(1,505)} = 23.77$, $p = 0.011$; two-way ANOVA) was smaller in the dyskinesia-off and dyskinesia-on states in the 6-OHDA-lesioned group as compared with the sham-lesioned group (L-DOPA-off vs dyskinesia-off, $p = 0.021$; L-DOPA-on vs dyskinesia-off, $p < 0.001$; Bonferroni’s post hoc test).

Changes of Cx-evoked responses of SNr neurons

We also examined Cx-evoked responses of SNr neurons (Fig. 6A). The typical response pattern of SNr neurons in the control state of the sham-lesioned group was a triphasic response composed of early excitation, inhibition, and late excitation (Fig. 6B, control), as reported previously (Sano et al., 2013; Sano and Nambu, 2019). Each component in the SNr/Gpi is mediated by the Cx-STN-SNr/Gpi hyperdirect, Cx-STr–SNr/Gpi direct, and Cx-Str–Gpi-STN-SNr/Gpi indirect pathways, respectively (Fig. 6A; Maurice et al., 1999; Nambu et al., 2000; Tachibana et al., 2008; Sano et al., 2013). This typical response pattern was not changed in the L-DOPA-off and L-DOPA-on states (Fig. 6B, L-DOPA-off, L-DOPA-on). These findings were also confirmed in population PSTHs (Fig. 6C, left), bar graphs (Fig. 6D, upper), heat maps (Fig. 6E, upper), and quantitative analyses (SNr, sham lesioned; Table 2).

Table 2. Response parameters of GPe and SNr neurons to Cx stimulation

<table>
<thead>
<tr>
<th></th>
<th>Sham lesioned</th>
<th>6-OHDA lesioned</th>
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<tr>
<td></td>
<td>Control</td>
<td>L-DOPA-off</td>
<td>L-DOPA-on</td>
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<tr>
<td>GPe</td>
<td></td>
<td></td>
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<tr>
<td>n, number of neurons</td>
<td>n = 82</td>
<td>n = 92</td>
<td>n = 87</td>
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<td>m, number of mice</td>
<td>m = 3</td>
<td>m = 4</td>
<td>m = 3</td>
</tr>
<tr>
<td>Early excitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (ms)$^a$</td>
<td>4.3 ± 2.3</td>
<td>4.0 ± 2.1</td>
<td>5.2 ± 6.4</td>
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<tr>
<td>Amplitude (spikes)$^a$</td>
<td>90.0 ± 56.0</td>
<td>90.3 ± 58.7</td>
<td>95.7 ± 55.9</td>
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<tr>
<td>Inhibition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (ms)$^a$</td>
<td>7.3 ± 3.6</td>
<td>8.0 ± 3.0</td>
<td>8.3 ± 2.3</td>
</tr>
<tr>
<td>Amplitude (spikes)$^a$</td>
<td>−45.0 ± 25.0</td>
<td>−52.2 ± 24.0</td>
<td>−50.3 ± 20.9</td>
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<tr>
<td>Late excitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (ms)$^b$</td>
<td>36.2 ± 32.4</td>
<td>43.0 ± 34.1</td>
<td>38.8 ± 26.4</td>
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<tr>
<td>Amplitude (spikes)$^b$</td>
<td>495.6 ± 483.6</td>
<td>571.1 ± 497.2</td>
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<td>SNr</td>
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<tr>
<td>n, number of neurons</td>
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<td>n = 56</td>
<td>n = 47</td>
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<td>m, number of mice</td>
<td>m = 4</td>
<td>m = 4</td>
<td>m = 4</td>
</tr>
<tr>
<td>Early excitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (ms)$^a$</td>
<td>5.0 ± 3.1</td>
<td>4.4 ± 3.5</td>
<td>4.6 ± 33</td>
</tr>
<tr>
<td>Amplitude (spikes)$^a$</td>
<td>92.4 ± 73.4</td>
<td>94.8 ± 110.7</td>
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<td>Inhibition</td>
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<td></td>
</tr>
<tr>
<td>Duration (ms)$^b$</td>
<td>4.7 ± 4.5</td>
<td>5.1 ± 5.4</td>
<td>5.7 ± 4.6</td>
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<tr>
<td>Amplitude (spikes)$^b$</td>
<td>−26.3 ± 26.7</td>
<td>−292 ± 30.4</td>
<td>−321 ± 26.3</td>
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<td>Late excitation</td>
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<td></td>
<td></td>
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<tr>
<td>Duration (ms)$^a$</td>
<td>13.4 ± 10.5</td>
<td>15.7 ± 19.0</td>
<td>16.0 ± 16.1</td>
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<td>Amplitude (spikes)$^a$</td>
<td>215.2 ± 187.5</td>
<td>202.5 ± 226.5</td>
<td>209.9 ± 203.4</td>
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</tbody>
</table>

$^a$Values are means ± SD; $p < 0.05$, $**p < 0.01$ significantly different from the corresponding states of the sham-lesioned group, $***p < 0.01$ significantly different from the PD state, $\#\#p < 0.01$ significantly different from the dyskinesia-off state; two-way ANOVA (no interaction between lesion group $\times$ L-DOPA treatment) followed by Bonferroni’s post hoc test; two-way ANOVA (significant interaction between lesion group $\times$ L-DOPA treatment) followed by Kruskal–Wallis test with Dunn’s multiple comparison test.

Figure 6: Some figures and text are not directly translatable into a natural language representation, such as images of graphs and tables. The natural text is extracted from the surrounding text without these figures and tables.
reduced in the dyskinesia-on state compared with the PD and dyskinesia-off states (p < 0.001, \( \chi^2 = 34.98 \)). Population PSTHs revealed that the averaged response in the dyskinesia-on state was excitation followed by inhibition without late excitation (Fig. 6C, right, dyskinesia-on). In the heat map, inhibition appeared to be further increased, and late excitation was mostly lost (Fig. 6E, dyskinesia-on).

Quantitative analysis (SNr, 6-OHDA lesioned; Table 2) revealed that the duration and amplitude of inhibition (duration, significant interaction between lesion group \( \times \) L-DOPA treatment, \( F_{(2,326)} = 15.45, p < 0.001; \) amplitude, \( F_{(2,326)} = 11.07, p < 0.001; \) two-way ANOVA) were significantly smaller in the PD state (duration, \( p = 0.005; \) amplitude, \( p = 0.004; \) Kruskal–Wallis test with Dunn’s multiple comparison test) and returned to the control levels in the dyskinesia-off state. The duration and amplitude of inhibition were further increased in the dyskinesia-on state (duration, PD vs dyskinesia-on, \( p < 0.001; \) dyskinesia-off vs dyskinesia-on, \( p < 0.001; \) amplitude, PD vs dyskinesia-on, \( p < 0.001; \) dyskinesia-off vs dyskinesia-on, \( p < 0.001; \) Bonferroni’s post hoc test), reflecting the dominance of inhibition in the dyskinesia-on state. The duration and amplitude of late excitation (duration, significant effect of lesion group, \( F_{(1,326)} = 17.62, p < 0.001; \) amplitude, \( F_{(1,326)} = 28.67, p < 0.001; \) two-way ANOVA) was significantly smaller in the dyskinesia-off state (duration, \( p = 0.038; \) amplitude, \( p = 0.039; \) Bonferroni’s post hoc test) and dyskinesia-on state (duration, \( p < 0.001; \) amplitude, \( p < 0.001; \) states, as compared with the sham-lesioned group.

Location of recorded GPe and SNr neurons

Recording sites were plotted in the representative frontal planes of the GPe and SNr with different symbols based on Cx-evoked response patterns (Fig. 7). In the GPe, we mainly recorded in the middle and lateral parts in both the sham-lesioned and 6-OHDA-lesioned groups (Fig. 7A), which corresponds to the somatomotor region of the GPe (Chiken et al., 2008, 2015; Sano et al., 2013). In the control, L-DOPA-off, and L-DOPA-on states of the sham-lesioned group, the most common response pattern was a triphasic response composed of early excitation, inhibition, and late excitation (Fig. 7A, left, blue circles). This common response pattern remained unchanged in the PD, dyskinesia-off, and dyskinesia-on states of the 6-OHDA-lesioned group, with similar distribution in the GPe (Fig. 7A, right, blue circles).

In the SNr, we mainly recorded in the dorso-lateral part in both the sham-lesioned and 6-OHDA-lesioned groups (Fig. 7B), which corresponds to the somatomesencephalic region of the SNr (Sano et al., 2013). In the sham-lesioned group, the most common response pattern was a triphasic response (Fig. 7B, left, blue circles). In the PD state, the most common response pattern was excitation without inhibition (Fig. 7B, right, PD, warm colors) and remained unchanged in the dyskinesia-off state (Fig. 7B, right, dyskinesia-off). In the dyskinesia-on state, the most common response pattern was inhibition without late excitation, such as excitation-inhibition and monophasic inhibition (Fig. 7B, right, dyskinesia-on, greenish colors). SNr neurons with these response patterns were similarly distributed to those in the sham-lesioned group, suggesting that SNr neurons in the same area showed different response patterns between the control, PD, dyskinesia-off, and dyskinesia-on states.

Discussion

To elucidate the pathophysiology of LID, we recorded neuronal activity in the GPe and SNr of model mice. (1) There were
almost no differences in the spontaneous firing rates and patterns, and Cx-evoked responses of GPe and SNr neurons between the control, L-DOPA-off, and L-DOPA-on states. (2) Spontaneous firing rates of GPe neurons were decreased in the dyskinesia-off state and increased in the dyskinesia-on state, while those of SNr neurons showed no changes. (3) GPe and SNr neurons generally increased bursting activity and low-frequency oscillation around 1 Hz in the PD, dyskinesia-off, and dyskinesia states. (4) In the GPe, Cx-evoked late excitation was increased in the PD and dyskinesia-off states, but decreased in the dyskinesia-on state. (5) In the SNr, Cx-evoked inhibition was largely suppressed in the PD state, but enhanced in the dyskinesia-on state, and Cx-evoked late excitation was suppressed in the dyskinesia-off and dyskinesia-on states, indicating specific changes to LID.

Spontaneous activity changes in the GPe and SNr
Dopaminergic inputs inhibit Str neurons through dopamine D2 receptors (D2Rs) and stimulate Str neurons through dopamine D1 receptors (D1Rs; Figs. 5A, 6A; Albin et al., 1989; DeLong, 1990; Gerfen et al., 1990). Thus, loss of dopamine in PD would increase the activity of Str neurons and conversely decrease the activity of Str neurons, resulting in hypoactivity of GPe neurons and hyperactivity of SNr/GPi neurons. On the other hand, excessive dopamine input would induce hyperactivity of GPe neurons and hypoactivity of SNr/GPi neurons, resulting in LID (Crossman, 1990; Bezard et al., 2001) in PD patients (Hutchinson et al., 1997; Lozano et al., 2000) and monkeys (Filion et al., 1991; Papa et al., 1999; Boraud et al., 2001). However, recent studies in PD do not support these spontaneous activity changes (Wichmann et al., 1999; Wichmann and Soares, 2006; Galvan et al., 2010; Tachibana et al., 2011). The present study also showed no firing rate changes of SNr neurons in the PD, dyskinesia-off, and dyskinesia-on states (Fig. 2).

Bursts and oscillatory activity in the BG were reported to be correlated with PD motor features (Wichmann et al., 1999; Heimer et al., 2002; Wichmann and Soares, 2006; Tachibana et al., 2011). Low-frequency (0.5–4 Hz) oscillatory activity was also reported in dopamine-depleted states (Walters et al., 2007; Arienta et al., 2016; Whalen et al., 2020). Low-frequency oscillations were found in the STN of dyskinetic PD patients (Foffani et al., 2005; Alonso-Frech et al., 2006) and in the SNr of a rat LID model (Meissner et al., 2006). In the present study, we found firing pattern changes (Figs. 3, 4), but they were not specific to LID.

Changes of Cx-evoked responses in the GPe
In the GPe, Cx stimulation induces early excitation, inhibition, and late excitation, which is mediated by the Cx-STN-GPe, Cx-Str-GPe, and Cx-Str-GPe-STN-GPe pathways, respectively (Figs. 5A, 8, GPe, normal). In the present study, 6-OHDA lesion strengthened Cx-evoked late excitation in GPe neurons, which persisted after chronic L-DOPA treatment in the dyskinesia-off state, but was lessened in the dyskinesia-on state (Fig. 5; Table 2), suggesting that neurotransmission through the Cx-Str-GPe-STN-GPe pathway is upregulated in the PD and dyskinesia-off states, and downregulated in the dyskinesia-on state (Fig. 8, GPe). Similar changes were reported in rodent PD models (Kita and Kita, 2011; Sano and Nambu, 2019). A plausible mechanism is alteration in the Cx-Str neurotransmission; following loss of inhibitory dopamine signals through D2Rs, Str neurons increased their intrinsic excitability (Suárez et al., 2016; Parker et al., 2018; Ryan et al., 2018), and in the dyskinesia-on state, decreased their activity (Parker et al., 2018; Ryan et al., 2018) or returned to the control excitability (Suárez et al., 2016).

However, in the present study, Cx-evoked inhibition in the GPe mediated by the Cx-Str-GPe pathway did not show evident...
changes in the PD and dyskinesia-off states (Fig. 5; Table 2), probably because (1) Str\textsubscript{1} neurons, which were fully activated in the control state, could not further inhibit GPe activity, and/or (2) increased late excitation may mask increased inhibition. Decreased early excitation in the dyskinesia-off state (Table 2) may reflect increased inhibition. Another plausible mechanism is that dopamine depletion strengthens GPe-STN neurotransmission directly (Fan et al., 2012) or indirectly through the Cx-STN projections (Chu et al., 2015, 2017).

Changes of Cx evoked responses in the SNr

In the SNr, Cx stimulation induces early excitation, inhibition, and late excitation, which is mediated by the Cx-STN-SNr\textsubscript{hyperdirect}, Cx-Str\textsubscript{4}-SNr/GPi, and Cx-Str\textsubscript{1}·GPe-STN-SNr/GPi\textsubscript{indirect} pathways, respectively (Figs. 6A, 8, SNr/GPi, normal). Cx-evoked inhibition in SNr neurons was lost in the PD state, partially recovered in the dyskinesia-off state, and strongly enhanced in the dyskinesia-on state (Fig. 6; Table 2), suggesting that inhibitory inputs through the Cx-Str\textsubscript{4}-SNr/GPi\textsubscript{direct} pathway are downregulated in the PD state, partially recovered in the dyskinesia-off state, and upregulated in the dyskinesia-on state (Fig. 8, SNr/GPi). Similar changes were reported in PD rodents and in D1Rs knockdown mice (Kita and Kita, 2011; Chiken et al., 2015; Sano and Nambu, 2019). These changes could be attributed to activity changes of Str\textsubscript{4} neurons. Dopamine depletion reduced the activity of Str\textsubscript{4} neurons (Mallet et al., 2006; Parker et al., 2018; Ryan et al., 2018) and decreased their spine densities (Suárez et al., 2014, 2016). In the dyskinesia-off state, the activity of Str\textsubscript{4} neurons was increased (Fieblinger et al., 2014; Suárez et al., 2016), and in the dyskinesia-on state, was further increased (Parker et al., 2018; Ryan et al., 2018). Str\textsubscript{4} activity has a causative role in LID; optogenetic activation of Str\textsubscript{4} neurons or both Str\textsubscript{4} and Str\textsubscript{1} neurons (Hernández et al., 2017; Perez et al., 2017; Girasole et al., 2018; Keifman et al., 2019) or activation of D1Rs (Darmopil et al., 2009) induced LID. Increased GABA release from the Str\textsubscript{4}-SNr terminals may also contribute to the increased Cx-induced inhibition (Yamamoto et al., 2006; Borgkvist et al., 2015).

In the PD state, early excitation mediated by the Cx-STN-SNr\textsubscript{hyperdirect} pathway and late excitation mediated by the Cx-Str\textsubscript{1}·GPe-STN-SNr/GPi\textsubscript{indirect} pathway fused together in the SNr, forming monophasic excitation (Fig. 8, SNr/GPi, PD). In the dyskinesia-off state, late excitation in SNr neurons was reduced (Fig. 6; Table 2), probably because increased late excitation in the GPe could depress late excitation in SNr neurons through the inhibitory GPe-SNr and/or GPe-STN-SNr pathways (Fig. 8, SNr/GPi, dyskinesia-off). In the dyskinesia-on state, Cx-evoked late excitation in SNr neurons was further depressed (Fig. 6; Table 2), which could be explained by the downregulation of the Cx-Str\textsubscript{4}-GPe-STN pathway as discussed above (Fig. 8, SNr/GPi, dyskinesia-on).

In the present study, each connection of Cx-BG pathways was not directly evaluated. Further studies using pathway specific manipulation are necessary to clarify the origin of changes in Cx-evoked responses.

Pathophysiology of PD and LID

We have proposed a dynamic model of the Cx-BG network (Nambu et al., 2000, 2002, 2015) to explain the control mechanism of voluntary movements (Fig. 8, normal). First, Cx-induced early excitation (magenta) in the SNr/GPi through the Cx-STN-
SNR/GPi hyperdirect pathway resets on-going activity in the thalamus and Cx. Second, inhibition (blue) in the SNR/GPi through the Cx-Sn-rSNr/GPi direct pathway disinhibits thalamus/Cx, and releases intended movements at an appropriate timing. Finally, late excitation (green) in the SNr/GPi through the Cx-Sn-GP-e-STN-SN/GP/i indirect pathway inhibits thalamus/Cx and stops movements. Based on this model and the present study, we can explain the pathophysiology of PD and LID (Fig. 8).

(1) In the PD state (PD untreated), inhibition in the SNr/GPi mediated by the direct pathway is suppressed, and early excitation mediated by the hyperdirect pathway and late excitation mediated by the indirect pathway become dominant. Thus, signals through the direct pathway cannot release an appropriate movement at an appropriate timing, resulting in akinesia.

(2) In the dyskinesia-off state (PD treated/LID latent), inhibition through the direct pathway is partially recovered, and late excitation through the indirect pathway is suppressed in the SNr/GPi. This may be a latent change in LID. In the present study, some PD motor features, such as abnormal rotational behaviors, were reduced in the dyskinesia-off state.

(3) In the dyskinesia-on state (PD treated/LID manifest), L-DOPA application further enhances inhibition and depresses late excitation in the SNr/GPi. This situation means that signals to release movements through the direct pathway are enhanced, while signals to stop movements through the indirect pathway are suppressed. Thus, unintended movements can be easily released at random timing and cannot be easily stopped once they are released, which is the manifestation of dyskinesia.

Clinical significance

LID is one of the major side effects after long-term L-DOPA treatment, and its control is a major issue for advanced PD. Increased inhibition mediated by the direct pathway and loss of late excitation mediated by the indirect pathway in the SNr/GPi seem to play a causative role in LID (Fig. 8). Restoring neurotransmission through the indirect pathway and/or suppressing neurotransmission through the direct pathway (Darmopil et al., 2009) may be useful to suppress LID, suggesting a future therapeutical strategy for LID.

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