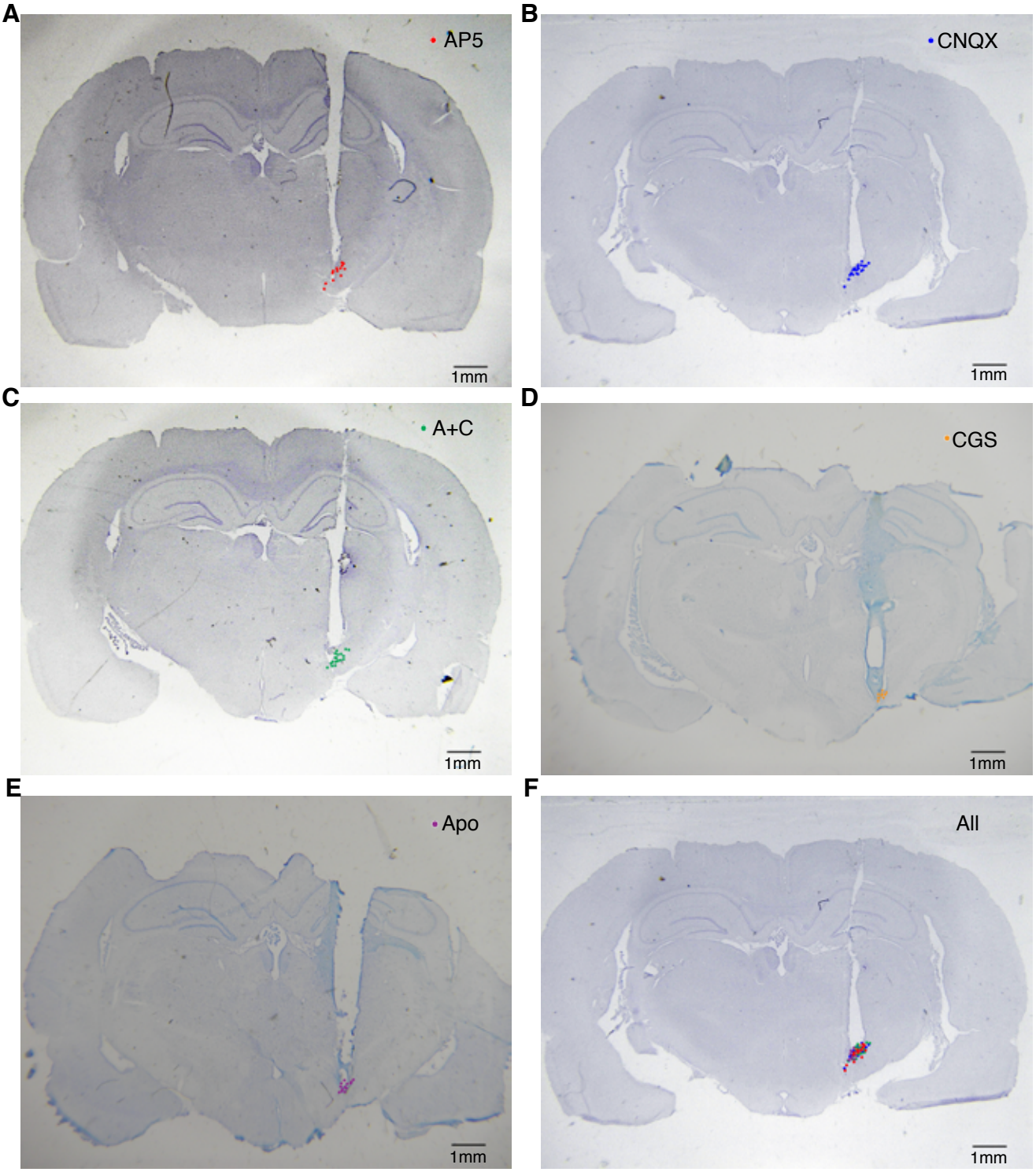
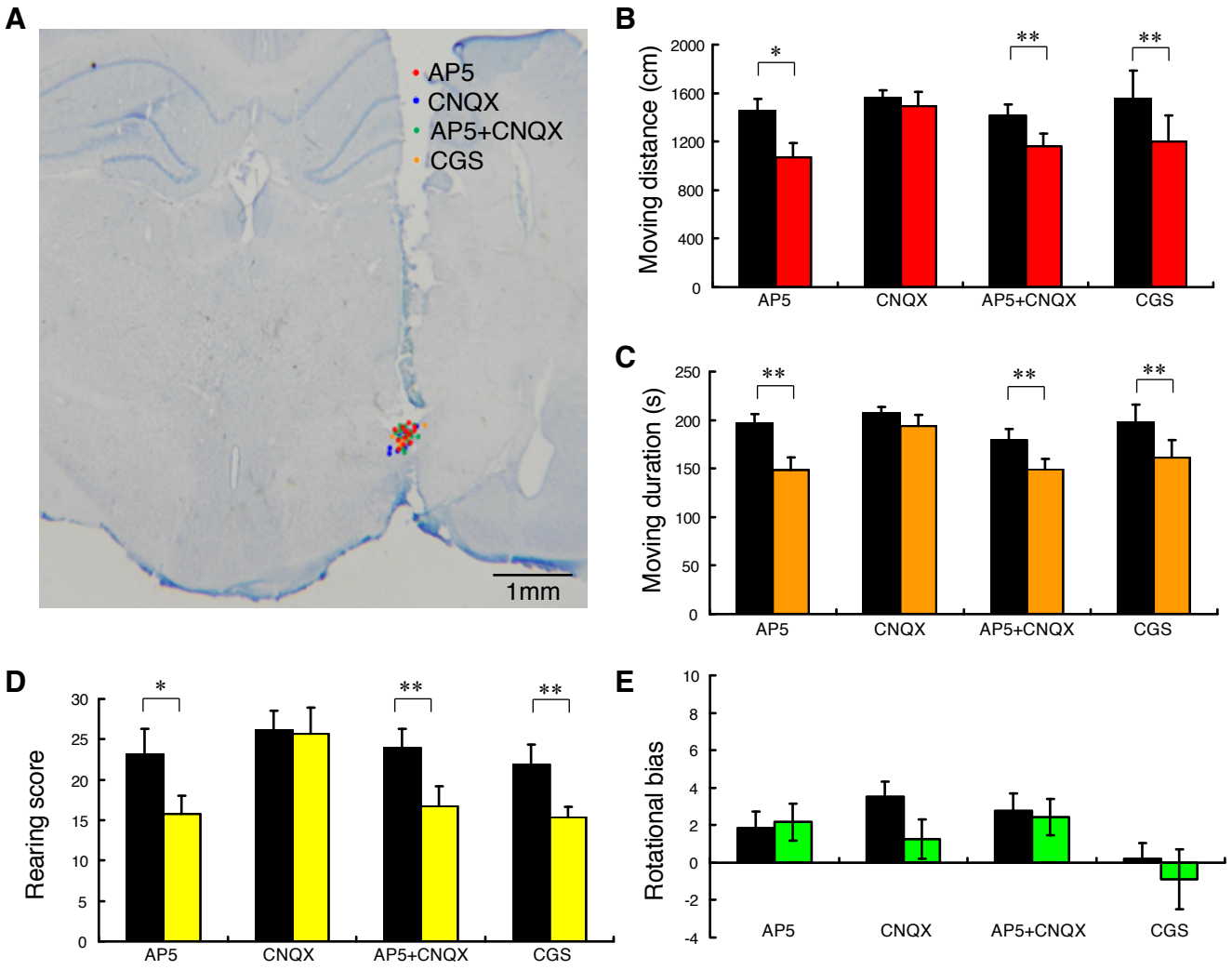
Supplemental Figure 1



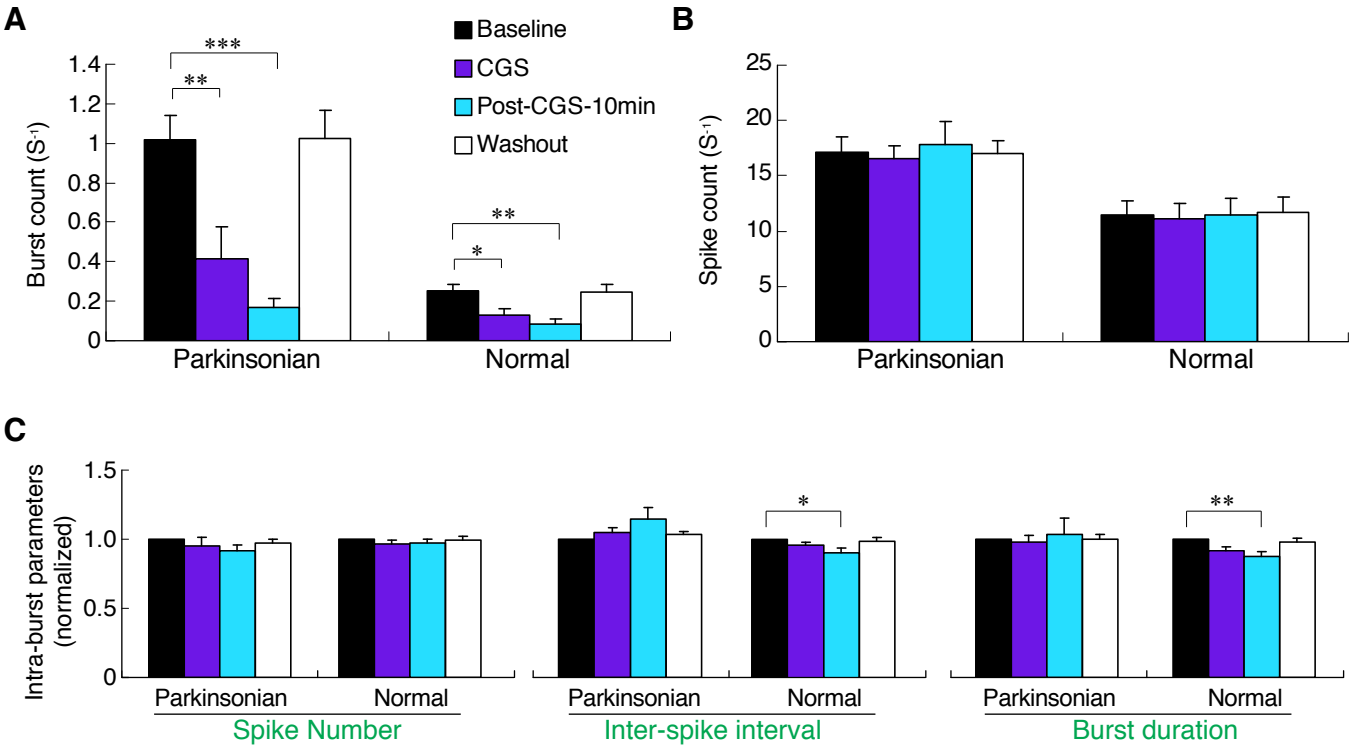
Supplemental Figure 1. The locations of microinfusion cannulae. Each dot represents the cannula tip of a given rat in Figure 2. The locations of cannula tips in each group of parkinsonian rats are summarized in (**A-E**), and put together in (**F**). The tips are clustered along the upper border or inside STN.

Supplemental Figure 2



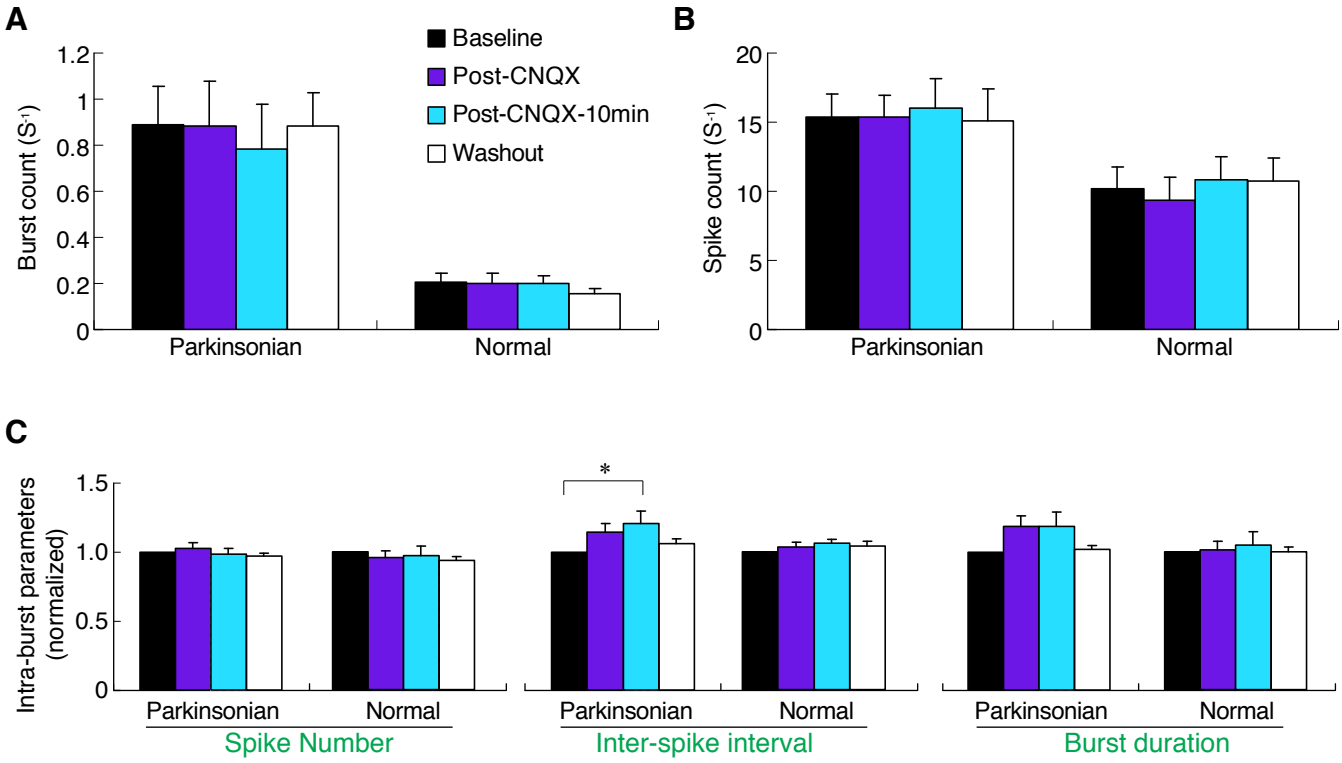
Supplemental Figure 2. The effect of glutamate receptor antagonists on the locomotor behaviors in normal rats. Each drug was locally infused into STN (colored bars), with a paired control of aCSF microinfusion (black bars). The parameters and the experimental procedures are the same as those for the parkinsonian rats in Fig. 2. (**A**) The location of the cannula tips in normal rats. (**B-D**) AP5 reduces moving distance (**B**), moving duration (**C**), and rearing score (**D**) in a subtle but significant degree (n = 12, P = 0.028, 0.009, and 0.022, respectively). Another NMDA receptor blocker, CGS (n = 10, P = 0.005, 0.007, and 0.008, respectively), and co-administration of AP5 and CNQX (n = 14, P = 0.006, 0.006, and 0.008, respectively) also have similar effects. On the other hand, CNQX alone fails to reveal significant effects (n = 11). (**E**) There is no rotational bias observed with drug infusion in the normal rats. All statistics are conducted by non-parametric Wilcoxon Signed Rank Test. Data represent mean ± s.e.m., *P < 0.05, **P < 0.01.

Supplemental Figure 3



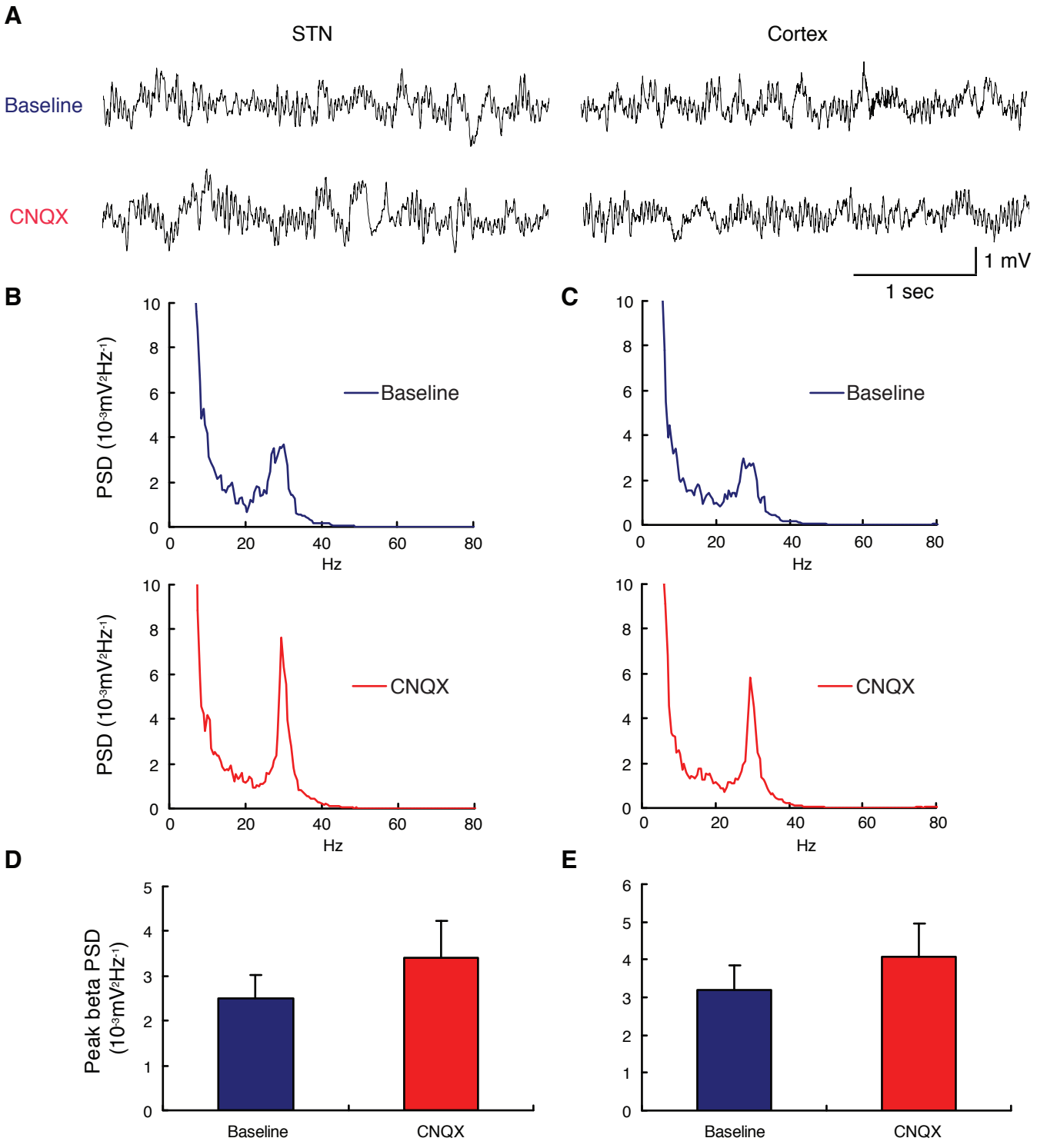
Supplemental Figure 3. The effect of CGS on subthalamic discharges. CGS was slowly infused into STN while single-unit firings of subthalamic neurons were recorded. The parameters and the experimental procedures are the same as those for AP5 in Fig 3. (**A**) CGS has a prominent burst-suppressing effect in both parkinsonian (n = 12 units in 5 rats, P = 0.004 and <0.001 in post-CGS and post-CGS-10min conditions, respectively) and normal rats (n = 10 units in 4 rats, P = 0.03 and 0.002 respectively). (**B**) Similar to AP5, CGS dose not change the subthalamic discharges in the spike mode (parkinsonian group, n = 12 units in 3 rats; normal group, n = 9 units in 3 rats). (**C**) The intra-burst profiles remain unchanged in parkinsonian rats, while inter-spike interval and burst duration are mildly reduced in normal rats. All statistics are conducted by one-way ANOVA with *post-hoc* Dunnett's correction. Data represent mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001.

Supplemental Figure 4



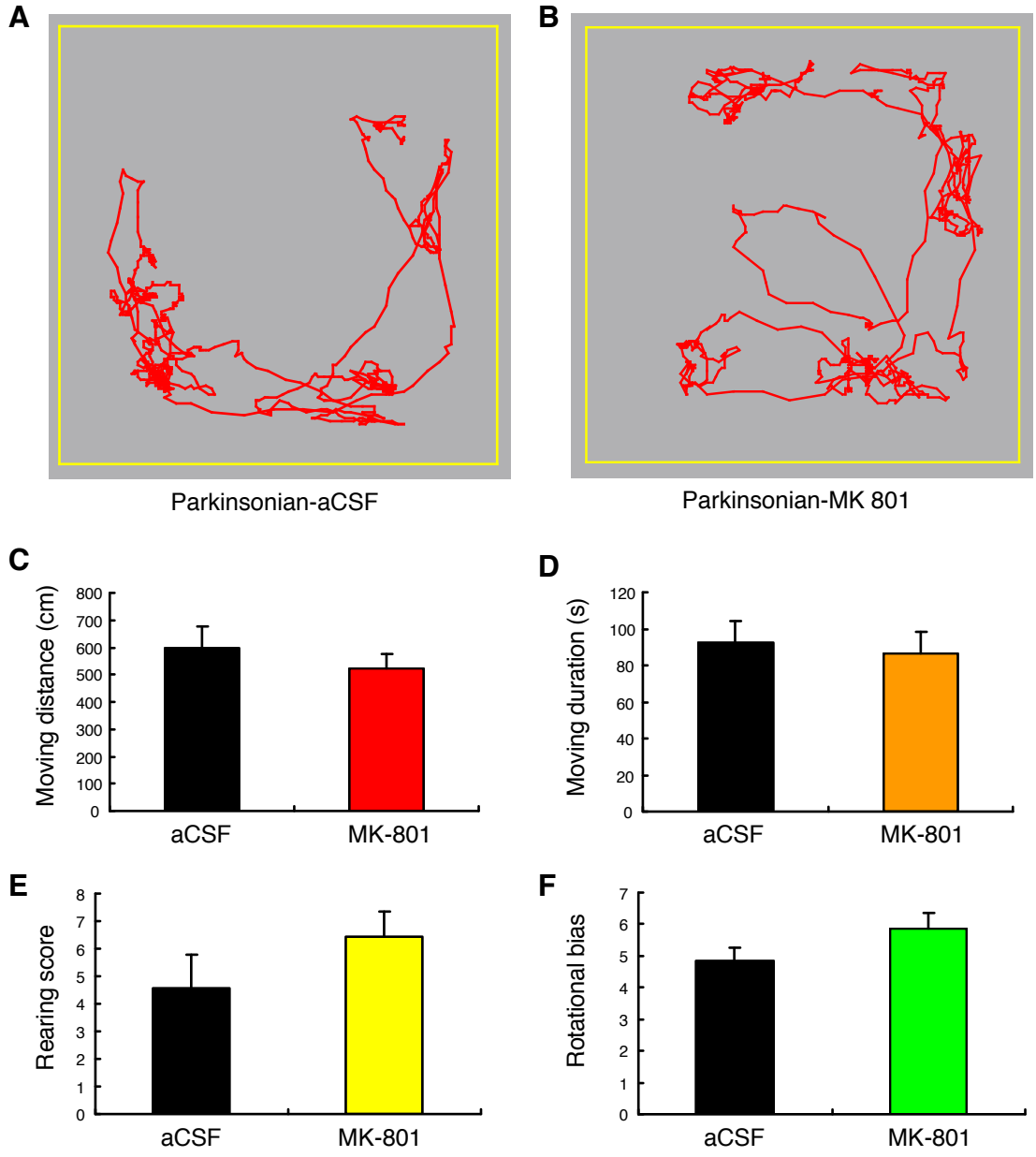
Supplemental Figure 4. The effect of CNQX on subthalamic discharges. (**A**) Infusion of CNQX (2mM) into STN dose not change the burst rates of subthalamic single units in parkinsonian and normal rats (parkinsonian group, n = 12 in 3 rats; normal group, n = 8 in 3 rats). (**B**) CNQX dose not change subthalamic firings in the spike mode (parkinsonian group, n = 15 in 3 rats; normal group, n = 12 in 3 rats). (**C**) The intra-burst parameters mostly remain unchanged except for the inter-spike interval in parkinsonian rats (P = 0.037). All statistics are conducted by one-way ANOVA with *post-hoc* Dunnett's correction. Data represent mean ± s.e.m., *$P < 0.05$.

Supplemental Figure 5



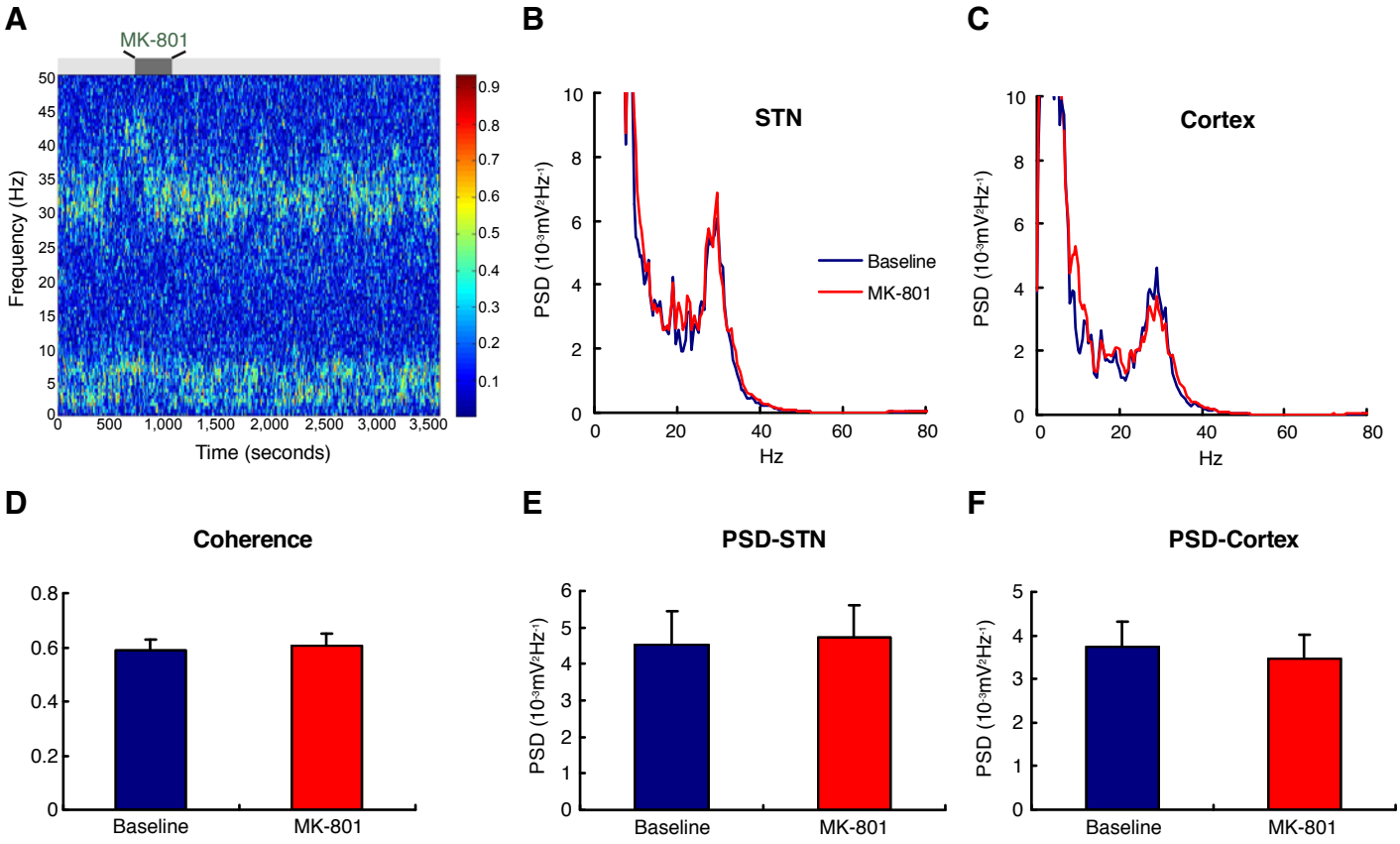
Supplemental Figure 5. The effect of CNQX on *in situ* synchronization of STN and premotor cortex. The results from STN are shown in the left column, and those from premotor cortex are presented on the right side. (**A**) Raw sweeps of LFP in parkinsonian rats. There is no significant change between baseline and subthalamic CNQX microinfusion in STN or premotor cortex. (**B** and **C**) Representative PSD distribution in a rat. In both STN and premotor cortex, a PSD peak in beta frequencies is noted in baseline and cannot be suppressed by intra-subthalamic CNQX. Instead, CNQX may even tend to narrow and sharpen the beta PSD distribution. (**D** and **E**) Quantitative measurement of peak PSD in beta frequencies. Although not statistically significant, CNQX tends to increase peak PSD power in both STN (n = 12, P = 0.065) and premotor cortex (P = 0.071). All statistics are conducted by non-parametric Wilcoxon Signed Rank Test. Data represent mean ± s.e.m..

Supplemental Figure 6



Supplemental Figure 6. The effect of MK-801 on the locomotor behaviors in parkinsonian rats. The parameters and the experimental procedures are the same as those in Figure 2. (**A** and **B**) Sample traces of locomotor activities in a rat with subthalamic microinfusion of aCSF or MK-801, a pore blocker of opened NMDA receptor. (**C-F**) MK-801 fails to show significant effect ($n = 7$, $P > 0.1$ in all conditions) in any of the quantitative parameters of locomotor activities (**C-E**) and asymmetric movements (**F**). All statistics are conducted by non-parametric Wilcoxon Signed Rank Test. Data represent mean ± s.e.m..

Supplemental Figure 7



Supplemental Figure 7. The effect of MK-801 on cortico-subthalamic and *in situ* synchronization. The experimental procedures are the same as those in Figure 4 and Figure 5. A representative time-frequency coherence plot of cortico-subthalamic synchronization (**A**), as well as PSD distributions of STN (**B**) and premotor cortex (**C**) in a parkinsonian rat. (**D-F**) Quantitative analysis of coherence and peak PSD in STN and cortex. MK-801 dose not address noticeable changes in synchronization-based pathophysiology in PD. ($n = 9$, $P > 0.2$ in all conditions). All statistics are conducted by non-parametric Wilcoxon Signed Rank Test. Data represent mean ± s.e.m..

LEGEND OF SUPPLEMENTAL VIDEO

Supplemental Video 1. Overactivation of cortico-subthalamic axons induce parkinsonian behavior. The Thy1::ChR2 mouse was implanted with fiber optic cannula at the right STN. Illumination of the STN, which stimulates cortico-subthalamic axons, instantaneously turns a normal mouse into hemiparkinsonian one. The behaviors readily return to normal when the light is off. Note that the shelter around the fiber optic cannula was removed for video demonstration, so the illumination and behavioral changes can be observed simultaneously.

SUPPLEMENTAL METHODS

Animal materials and preparation of parkinsonian model. For the parkinsonian rat model, we used 6-OHDA to produce hemiparkinsonian rats. The animals were pretreated with desipramine hydrochloride (25 mg/kg, i.p., Sigma-Aldrich) 30-minutes before 6-OHDA exposure to protect nonadrenergic neurons. Each rat was then anesthetized with chloral hydrate (280-350 mg/kg, i.p., Sigma-Aldrich), and placed into a stereotactic instrument with the skull exposed. The hole was drilled and a brain-lesioning cannula (30-gauge, Braintree Scientific) was slowly placed in unilateral substantia nigra pars compacta with the coordinates AP -2.8 mm, L 2.0 mm, D 8.0 mm from the bregma point. The cannula was left in place for 5 minutes, and 8 μg of 6-OHDA (dissolved in 4 μl of normal saline with 0.01% ascorbic acid and protected from light, Sigma-Aldrich) was slowly infused with the speed of 0.5 μl/min for 8 minutes. The cannula was left in place for another 10 minutes and then slowly withdrawn. The wound was closed by 4/0 nylon monofilament. After 7 to 10 days, the rats were tested by subcutaneous injection of apomorphine (0.05 mg/kg s.c.). Only those rats with strong rotational behaviors (more than 25 turns in 5 minutes) toward the side contralateral to the 6-OHDA lesioning were considered as validated parkinsonian rats and retained for further electrophysiological and/or behavioral recordings 2 to 4 weeks later.

Open-field test of animal behaviors. The animals were brought into a quiet room with dim light 2 hours before the test in the dark cycle. For conditioning, each rat or mouse was put into a square arena (size 45 cm x 45 cm for rats and 30 cm x 60 cm for mice) made of plexiglass for 5 minutes every other day until the behavioral profiles were stabilized several days later. For the neurotransmitter experiment, aCSF or neurotransmitter receptor blocker was infused with the speed of 0.4 ul/min for 8 minutes (3 minutes prior and 5 minutes during the behavior recording). The animal was put into the arena,

equipped with a video recording and tracking system (Ethovision 3.0, Noldus, Netherland) above the arena, and recorded for 5 minutes. For each set of experiment, each animal received either aCSF or the neurotransmitter blocker randomly in the first run, and the other one in the second run. The two runs were separated by least 1.5 hours to avoid carry-over effect. For the experiments with subcutaneous saline or apomorphine, the animals were put into the arena 20 minutes after the injection. 3 parameters, including moving distance, moving duration and rearing score were extracted automatically from the Ethovision system to represent the moving abilities. These parameters indicated the total moving distance, total non-freezing time, and the number of events the rat reared freely in a 5-minute recording. The rotation bias, head position bias and net asymmetric climbing were selected to quantify the asymmetric movements. The rotational bias was manually calculated by counting the rotational behaviors in the video. We added +1 to the rotational bias if the rats rotated 360^0 toward the side of 6-OHDA lesioning (or fiber optic implantation), and -1 if they rotated away from the side of lesioning. Head position bias was the number of head tilts ($>15^0$ deviation from the midline) toward the side of 6-OHDA lesioning subtracted by the number of contralateral deviation. Climbing on the wall with one forelimb was identified as asymmetric climbing. The number of climbings with non-affected forelimb subtracted by the climbings with the affected one defined the net asymmetric climbing. The result was presented as the ratio of net asymmetric climbing divided by total climbing numbers (which also include non-asymmetric climbings). For optogenetic experiments, a 473 nm diolaser (Crystalaser) was connected to the fiber optic cannula through optic fiber cord and adjust the light intensity to 4 mW. During the experiments, the fiber cord was attached to the cannula mounted on the mouse skull, and the mouse was put in to the 40 cm x 20 cm arena for 90 seconds. We turned the laser on in the middle 30 seconds, while the first and the last 30 seconds were self-controls in light-off state. During light-on state, the duty cycle was 50% and the illumination was set in pseudo-randomized order between 8-20

Hz to avoid a rate-dependent effect. The frequency range was designed to generate burst-firing rate mimicking parkinsonian STN (0.8-1 Hz, **Figure 3C**) according to the burst generating probability by arbitrary stimulation of naïve cortico-subthalamic pathway (**Figure 6, C** and **D**). The recording system and behavioral parameters were the same as those in the rat experiments, except the additional parameter of peak velocity, which can also be reliably measured in the arena large enough to the size of a mouse.

Continuous in vivo single-unit recording. We recorded single-unit firings of STN in anesthetized rats. The stainless electrodes were inserted perpendicularly into the dorsal border of STN (AP -3.8 mm, Lat 2.4 mm, D 6.5 mm) as a starting point for single-unit recordings. The electrodes were slowly moved deeper into STN until the maximal recordable units were reached. We first got a 5-minute record of baseline firings, and then one of the tested drugs was slowly infused into STN through the cannula with a speed of 0.2 μl/min for 5 minutes. We recorded the subthalamic discharges for 5 minutes immediately (post-drug) and 10-minute (post-drug-10min) after termination of drug infusion. Because the drug effect typically lasted around 40 minutes, we sampled the washout condition 1 hour after the termination of drug infusion. The electrical signals were filtered (300-3,000 Hz band-pass filter), amplified (20,000x), and digitized (sampling rate: 40,000 Hz) with a microelectrode amplifier (Model 3600, A-M System).

Implantations of electrodes for local field potentials. Each rat was anesthetized with chloral hydrate, mounted on stereotactic instrument with skull exposed. A bundle of 6 insulated stainless electrodes (diameter 0.002 inch, No. 304, California Fine Wire Company) on a 10-pin connector (Omnetics Connector Corporation) were inserted perpendicularly in STN ipsilateral to the 6-OHDA lesioning. The

insertion was performed according to the protocol of single-unit recordings to ensure best positioning of the electrodes by direct visualization of the single-unit firings in STN. Two stainless screws were fixed on the skull according to the coordinates of premotor cortex on both sites (AP -2.7 mm, L ±2.0 mm) in contact with the dura mater. The one ipsilateral to the 6-OHDA lesioning was wired to the connector as the epidural electrode of premotor cortex. One screw was fixed in the nasal bone (AP -5.7 mm, L 0 mm) and wired as ground electrode. Another two screws were fixed on the skull in cerebellar areas (AP 12 mm, L ±2 mm), with the ipsilateral one wired as reference electrode. For those rats designed to evaluate the effect of glutamate receptor blockers, another non-metallic microinfusion cannula (PlasticsOne) was place into STN with a 30-degree tilt in the sagittal plane. The tip of the cannula was withdrawn by 0.1 mm from the STN coordinate, which was still inside STN but would avoid direct contact between cannula and electrodes. The electrodes, connector and the cannula were fixed with bone cement on the skull, and the wound was closed with 4/0 nylon monofilament. The experiments and recordings were performed 1 to 2 weeks after the surgery, when the body weight and health condition of the rats were back to the pre-surgical state.

Recordings of cortical and subthalamic local field potentials. We recorded the LFPs from epidural and subthalamic electrodes simultaneously. The rat was placed in an open-field arena in dim-light, and a wired headstage was connected to the connector on the head. The animal was left freely movable, while electrical signals from premotor cortex and STN were simultaneously recorded for an hour. For those rats designed to evaluate the effect of neurotransmitter blockers, the drug was locally infused into STN (0.2 μl/min) for 5 minutes in middle (13^{th} minute to 18^{th} minute) of the 1-hour recording. The electrical signals were filtered (0.3-100 Hz band-pass filter), amplified (2,000x), and digitized (sampling rate: 512 Hz) through a microelectrode amplifier (Model 3600, A-M System). The signals from the epidural

electrode were used as the LFPs of the premotor cortex. Signals from each one of the six channels in STN were subtracted from the remaining five channels one by one. Signals from the pair with the best quality were used as the LFPs in STN. At the end of the final section, the rat was anesthetized with overdosed chloral hydrate, and negative currents of 1 mA were injected through the recording electrodes for 10 seconds. The rats were perfused with formaldehyde and the brain was removed for histological verification.

Data processing of continuous and evoked single-unit recordings. The electrical signals recorded from either continuous or evoked single-unit recordings were post-processed in a procedure described in our previous works (25). Spikes were detected under a threshold algorithm of the negative peaks (SciWorks 6.0, DataWave Techologies). The detected spikes were sorted by spike-sorting tools under principle component analysis (PCA) algorithm. To ensure the data quality, we only included those units with signal-to-noise ratio greater than 3 and the coefficient of variation (CV) greater than 0.8 in all 5 spike profiles (including peak amplitudes, volley amplitudes, peak time, spike height, and spike widths). The valid single-units then went through burst detection with criteria defined by interval algorithms: maximal interval to start a burst: 20 ms, minimal interval to end a burst: 20 ms, minimal number of spikes in a burst: 4. Single-units which included at least one burst were defined as burst mode, and those did not reveal any burst were defined as spike mode. We quantified the burst-firing rates of units in the burst mode, and the spike-firing rates of units in the spike mode. We also quantified three intra-burst parameters including spike count in each burst (spike number), intra-burst inter-spike interval, and burst duration for the bursting units. We analyzed these profiles with protocols of continuous or evoked single-unit recordings, and made a comparison before and after the application of different glutamate receptor antagonists. For the burst analysis for evoked single-unit recordings, the first 150

ms in the 400-ms-sweep were further divided into 3 segments (50 ms each). The burst was counted in one of the segments if the burst initiated in the time window of that segment. We chose 50-ms window to categorize the sweeps based on the characters of evoked subthalamic discharges. The first 50 ms represented the firings related to cortico-subthalamic pathway (29, 30, 35), the second part was closely related to the indirect pathway (30, 36), and the last part reflected the background condition.

Data processing of local field potentials. The recorded LFPs from premotor cortex and STN were post-processed by Matlab 7.4 (Mathworks, Natick). LFP signals were characterized in frequency domain by power spectrum density (PSD) function (PSD by Welch's method with Hanning windowing, sampling rate: 512 Hz in data block of 2 seconds, giving the resolution of 0.5 Hz and half of the data overlap in each step). For time-frequency analysis, each PSD data point was constructed from a 10-second window and one-second shift for the next data point until the completion of the 1-hour recording. Coherence analysis between cortex and STN is a function of frequency defined by squared cross-spectrum between the two signals, divided by the auto-spectrum of each signal. The value of coherence is between 0 to 1, while one indicates that one signal can be linearly transformed into the other one in the given frequency, and zero means completely lack of the linear relationship. The coherence data were constructed with the same windowing, fractioning, overlapping and time-shifting methodology as PSD. The data were organized in the time-frequency plot for direct visualization. The peak PSD and maximal mean coherence was calculated in the beta frequency ranges between 20-40 Hz at baseline (sampled from 5^{th} to 10^{th} minute). For the group designed to evaluate the effect of neurotransmitter blockers, another set of coherence data were sampled 10 minutes after the completion of drug-application (e.g. sampled from 28^{th} to 33^{th} minute).

Verification of the recording sites and 6-OHDA lesions. For single-unit and LFP recordings, the electrophysiological verification was first achieved by the unique single-unit firing patterns in STN during the recordings or electrode implantations. 3 to 5 electrodes of the bundle are typically within STN and showed adequate firing pattern. In parkinsonian group, the effect of 6-OHDA lesioning was verified by the rotational behaviors in apomorphine test (more than 25 turns in 5 minutes). Those failed to show adequate firing patterns or rotational behaviors were excluded from further experiments. The failure rate of 6-OHDA lesioning is less than 10 percent. Moreover, post-mortem histological verification was performed to reassure the electrodes are correctly implanted. Purssian blue staining and hematoxylin counter-staining were used to examine the position of the electrodes. Tyrosine hydroxylase immunohistochemistry was also used to verify the unilateral loss of dopaminergic neurons in behavioral-confirmed parkinsonian rats (**Figure 1A**). The position of the drug-infusing (**Figure 1B; Supplemental Figure 1 and 2A**) or fiber optic cannula (**Figure 8B**) was also confirmed by hematoxylin staining. Data were collected only from the animals with correctly placed electrodes and cannulae. The data that fulfilled the verification criteria were all included in the analysis without further selection.