Dear Editor,

Motor control as a function of the basal ganglia circuit is crucial for every aspect of life and movement disorders, such as Parkinson’s disease (PD). In PD, the progressive denervation of dopamine in the dorsal striatum leads to inhibition of the direct pathway and facilitation of the indirect pathway and results in activation of the subthalamic nucleus (STN) and globus pallidus internus (GPI), two important nuclei in the motor loop of basal ganglia. Indeed, manipulating STN or GPI via deep brain stimulation (DBS) can correct motor symptoms of both PD patients and animal models. STN-DBS greatly suppresses the resting tremor and reduces dopaminergic medications, but increases the risk of falls, whereas GPI-DBS mainly benefits dyskinesia and gait. These observations suggest that other circuit in addition to STN–GPI circuit also plays a role in motor control.

We initially mapped the STN-projecting nuclei with a series of viral tracing studies. First, adeno-associated virus (AAV) expressing enhanced yellow fluorescent protein (EYFP) was injected into the unilateral mouse STN (Supplementary Fig. S1a, b). Besides the brain areas well known to receive STN projections, like globus pallidus externa (GPe) and GPI (Supplementary Fig. S1c), the EYFP-positive fibers were also evident in the anterior thalamic nucleus (ANT) (Fig. 1a), a nucleus known to project to cingulate cortex and important for voluntary movement. To further examine this previously unidentified STN–ANT circuit, we injected trans-monosynaptic AAV expressing Cre recombinase or AAV harboring Dio-EYFP into STN or ANT, respectively, (Supplementary Fig. S1d, top and middle, left) to mark the ANT neurons that receive projections from STN. The EYFP-positive cells in ANT were co-labeled with NeuN, a neuronal marker (Supplementary Fig. S1d, bottom, left). Moreover, retrograde viral tracing experiments also confirmed the STN–ANT connection (Supplementary Fig. S1e). Next, whether the STN–ANT circuit is functionally monosynaptic was tested by optogenetics and whole-cell recording (Fig. 1c, left). Optical stimulation of STN-projecting fibers on ANT slice evoked excitatory postsynaptic currents (EPSCs) on ANT neurons, with an average latency of 4.81 ± 0.34 ms and amplitude of 50.27 ± 13.69 pA (Supplementary Fig. S1f). The evoked EPSCs were sensitive to CNQX (Supplementary Fig. S1f, right), but insensitive to tetrodotoxin and 4-aminopyridine (Fig. 1c, right). Altogether, these results suggested an undiscovered, monosynaptic, and excitatory projection from STN to ANT.

Since the activity of excitatory STN neurons was increased in PD and STN-projecting ANT neurons are mainly excitatory, we wondered whether the activity of ipsilateral ANT was increased in PD model rodents. We established hemiparkinsonian rodent models by injecting 6-OHDA into the unilateral dorsal striatum (mice) or medial forebrain bundle (MFB) (rats) to induce a distinct loss of dopamine neurons in unilateral substantia nigra compacta (SNc) (Supplementary Fig. S2a). In hemiparkinsonian model mice, c-fos expression was clearly enhanced in ipsilateral, but not in contralateral ANT. While lesion of ipsilateral STN by ibotenic acid (IBO) reversed the enhanced c-fos expression (Supplementary Fig. S2b–d). To characterize this increased activity in vivo, we then did multichannel electrophysiological recordings in freely behaving rats (Supplementary Fig. S2e). The firing rate of the neurons in the ipsilateral ANT was greatly increased than that in the contralateral ANT of PD model rats (Fig. 1d). A distinct band of local field potential (LFP) activity in the beta range (15–35 Hz) (Supplementary Fig. S2f, g), regarded as a pathophysiological marker of PD motor deficits, was observed in ipsilateral ANT. The total power of LFP between 15–35 Hz was markedly increased in five out of nine PD model rats (Fig. 1e) compared with that in five normal rats.

To investigate the role of enhanced neural activity in STN–ANT in motor control of PD model mice (Supplementary Fig. S3a), we applied a balance beam test and apomorphine (APO)-induced rotations (Supplementary Fig. S3b). Lesion of ANT by IBO in PD model mice dramatically improved motor performance with a decrease of time for passing through the beam and the number of contralateral rotations (Fig. 1f). Consistently, suppressing ANT (Supplementary Fig. S3c, d) or STN–ANT circuit via optogenetics ameliorated the motor deficits (Fig. 1g, h and Supplementary Fig. S3e, f).

We next studied how the enhanced STN–ANT neural activity leads to chronic motor abnormalities in PD model mice. As ANT neurons received glutamatergic inputs from STN and glutamate receptors are important for the establishment and maintenance of synaptic activity, we initially examined the activities of AMPAR and NMDAR, two important glutamate receptors, in ANT and STN–ANT circuit. The AMPAR/NMDAR current ratio was largely increased both in ANT and STN–ANT circuit (Supplementary Fig. S4a, b and Fig. 1i), indicating that the excitatory synaptic transmission was greatly enhanced in STN–ANT circuit of PD model mice. It is known that the increased expression of GluR2-lacking AMPARs (GluR1 is the primary component) on the postsynaptic membrane is essential for long-term synaptic potentiation. Indeed, the rectification index of AMPARs, reflecting the GluR2-lacking AMPARs on the membrane, both in ANT and STN–projecting ANT neurons of PD model mice were markedly increased (Supplementary Fig. S4c and Fig. 1j), indicating the accumulation of GluR2-lacking AMPARs on the membrane of the ipsilateral ANT neurons in PD model mice. Furthermore, previous reports suggested that GluR1 membrane expression is regulated by phosphorylation of its intracellular carboxy-terminal motif. The phosphorylation sites usually include serine 831 (S831), phosphorylated by CaMKII or protein kinase C, and serine 845 (S845), phosphorylated by protein kinase A (PKA). Using phosphorylation
site-specific antibodies, we found that GluR1-S845, but not GluR1-
S831, was greatly increased in ipsilateral ANT, whereas the
expression of total AMPAR-GluR1 was not changed. Applying H-
89, a specific PKA inhibitor, into the ipsilateral ANT or ANT slice
blocked the increased GluR1-S845 phosphorylation (Fig. 1k),
brought the increased AMPAR/NMDAR ratio to the control levels
(Fig. 1l, left and Supplementary Fig. S4d). Moreover, delivery of H-
89 into ipsilateral ANT alleviated motor de-

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treated mice (Fig. 1, middle and right, Supplementary Fig. S4e) and MPTP-treated mice (Fig. 1m). To specifically prevent the phosphorylation of GluR1-S845, we designed TAT-S845, a cell-permeable peptide that contained a sequence spanning the PKA phosphorylation site in GluR1 (Supplementary Fig. S4f). Application of TAT-S845 reversed the increase of AMPAR/NMDAR current ratio to the control levels and alleviated motor deficits of PD model mice (Fig. 1n).

Together, the present experiments showed that the synaptic plasticity of STN–ANT circuit controls the motor behaviors in PD model rodents (Fig. 1o). The newly identified and characterized STN–ANT circuit may represent a circuit that precisely relays motor signals from the basal ganglia (STN) to the cingulate cortex for sensory-motor integration and synaptic plasticity in this circuit participates in the regulation of the motor deficits in the PD models. Dissecting the role of STN–ANT circuit will provide new insights into ETX- and 4-AP-induced rotational behaviors in PD and that synaptic plasticity in the STN–ANT circuit controls the motor behaviors in PD model rats.

DATA AVAILABILITY
The datasets in this study are available from the corresponding author upon reasonable request.

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ADDITIONAL INFORMATION
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