

Topographic connectivity and cellular profiling reveal detailed input pathways and functionally distinct cell types in the subthalamic nucleus

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1 **Summary:** The subthalamic nucleus (STN) controls psychomotor activity and is an efficient
2 therapeutic deep brain stimulation target in Parkinson's disease patients. Despite evidence
3 indicating position-dependent therapeutic effects and distinct functions within the STN, input
4 circuit and cellular profile in the STN remain largely unclear. Using advanced neuroanatomical
5 techniques, we constructed a comprehensive connectivity map of the indirect and hyperdirect
6 pathways in both the mouse and human STN. Our detailed circuit- and cellular-level connectivity
7 revealed a topographically graded organization with three convergent types of indirect and
8 hyperdirect-pathways. Furthermore, we identified two functional types of glutamatergic STN
9 neurons (parvalbumin, PV +/- neurons) segregated with a topographical distribution.
10 Glutamatergic PV+ STN neurons contribute to burst firing. We confirmed synaptic connectivity
11 from indirect and hyperdirect pathways to both PV+ and PV-. These data suggest a complex
12 interplay of information integration within the basal ganglia underlying coordinated movement
13 control and therapeutic effects.

Keywords: Subthalamic nucleus; connectivity; indirect and hyperdirect pathways; cell type; firing pattern;

14 **Introduction**

15

16 The subthalamic nucleus (STN), a key element of the basal ganglia (BG), plays a central role in
17 psychomotor control and serves as the most efficient therapeutic target of deep brain stimulation
18 (DBS) in Parkinson's disease patients (Benabid et al., 2009; Bergman et al., 1990; Bevan, 2017;
19 Eisenstein et al., 2014; Group, 2001; Herzog et al., 2004; Kopell et al., 2006; Limousin et al.,
20 1995; Lozano and Lipsman, 2013). Within the STN, two principal tracts modulate the activity of
21 the BG: the indirect and hyperdirect pathways. The indirect pathway carries most GABAergic
22 inputs to the STN from external globus pallidus (GPe) neurons (Mallet et al., 2012; Smith et al.,
23 1998), while the hyperdirect pathway carries most glutamatergic inputs to the STN (**Figure 1A**)
24 (Monakow et al., 1978; Nambu et al., 2002; Smith et al., 1998). The balance of synaptic excitation
25 and inhibition is critical to the operation of brain microcircuits, and this balance is often perturbed
26 in diseases (Chu et al., 2017; Cobos et al., 2005; del Pino et al., 2013; Fan et al., 2012; Kopell et
27 al., 2006; Turrigiano, 2011; Yizhar et al., 2011). The effects of STN DBS appear to involve an
28 interplay of converging inhibitory indirect and excitatory hyperdirect pathways, as well as multiple
29 interactions from molecular and cellular processes that translate into global network effects
30 (Gradinaru et al., 2009; Hashimoto et al., 2003; Hemptinne et al., 2015; Li et al., 2012; Stefani et
31 al., 2005; Windels et al., 2000). Although there is evidence indicating heterogeneous firing
32 patterns, distinct functional movement control, and electrode position-dependent therapeutic
33 effects including side-effects of DBS within the STN (Herzog et al., 2004; Kaku et al., 2019; Kim et
34 al., 2015; Mallet et al., 2012; Mosher et al., 2021; Wodarg et al., 2012), detailed topographical
35 profiling of connectivity, cytoarchitecture, and physiological properties remain incomplete. Thus,
36 comprehensive information regarding these input patterns as well as cellular profiles of the STN
37 is critical for deepening our understanding of normal movement control, Parkinson's disease, and
38 DBS. Here, using advanced neuroanatomical techniques, we developed detailed three-
39 dimensional (3D) descriptions of the convergent connectivity in the indirect and hyperdirect
40 pathways, as well as molecular and cellular profiles of STN circuitry. Our analysis includes a

41 comparison of ultrahigh-resolution fiber tractography in the human STN with respect to the
42 effectiveness of STN DBS in Parkinson's disease patients. Our circuit-level connectivity revealed
43 a topographically graded organization, with overlaps and no evident anatomical boundaries, and
44 direct cortico-pallidal connection in both the mouse and human STN. Together with cellular-level
45 connectivity analysis, we found, at least, three convergent types of indirect and hyperdirect-
46 pathway (GPe-only, STN-only, and both) suggesting complex signal integration, such as a
47 classical convergence of inhibition and excitation, feedforward inhibition, and collateral
48 convergence. These circuit features imply functional computational complexity and delicate
49 control. Furthermore, we identified two functional types of glutamatergic STN parvalbumin (PV)
50 +/- neurons that integrate information from both indirect and hyperdirect pathways, are
51 segregated along a topographical distribution. Glutamatergic PV+ neurons show phasic burst
52 firing and are particularly prevalent in the dorsolateral and middle STN, whereas PV- neurons fail
53 to generate burst firing and prevalent in the ventromedial and middle STN. In addition, we
54 confirmed synaptic connectivity from indirect and hyperdirect pathways to PV+/- neurons by
55 combining retrograde virus injections and mammalian GFP reconstitution across synapses
56 (mGRASP) (Kato et al., 2014; Kim et al., 2012). Our analysis corrects errors and adds a critical
57 new level of detail to understanding STN circuitry.

58 **Results**

59

60 **Indirect pathway in the STN**

61 To comprehensively analyze the organization of the indirect pathway in the mouse STN (IP_{STN}),
62 we employed a mesoscopic approach with the following components: 1) topographic labeling of
63 subsets of GPe neurons with adeno-associated virus (AAV) expressing green or red fluorescent
64 proteins (sfGFP or tdTomato), 2) computational reconstruction of axonal projection patterns, and
65 3) connecto-informatics of the GPe-STN circuit (**Figure 1; Figure S1–3; see STAR Methods**). To
66 obtain IP_{STN} data, we conducted 73 injections into the GPe, serially imaged the GPe and STN,
67 and then aligned our images to the Allen Mouse Brain Common Coordinate Framework (CCF v3,
68 <https://mouse.brain-map.org>; **Figure 1A; Figure S1A; Movie S1**). After filtering our data to
69 remove artifactual and weak projection signals, the results from 55 injections covering 84% of the
70 GPe volume were further analyzed for IP_{STN} patterns (**Figure 1B; Figure S1B, C; Table S1**).
71 Injections and axonal projections were reconstructed onto the segmented GPe and STN,
72 respectively (**Figure S2**).

73 To investigate IP_{STN} connectivity patterns, we constructed heatmaps of GPe injection and
74 STN projection (IP_{STN}) distributions along the whole-brain anatomic axes, that is, anterior-
75 posterior (AP), medial-lateral (ML), and dorsal-ventral (DV; **Figure 1C; Figure S3A, B**). The
76 centrality of the signal distribution was used to identify injection and projection sites, and to
77 subsequently measure their correlation. These heatmaps show that while projection signals were
78 dispersed throughout the STN, injection signals were restricted to specific topographic loci in the
79 GPe, which is desirable for fine connectivity mapping. Reconstructed STN projection patterns
80 show a graded distribution with a notable degree of overlap, rather than divisible sub-territories
81 (**Figure 1C–E; Figure S2, 3**) (Lambert et al., 2012; Mallet et al., 2007; Parent and Hazrati, 1995;
82 Plantinga et al., 2018). This gradient was predominantly oriented along the ventromedial to
83 dorsolateral axis, corresponding with the geometric longitudinal axis of the STN, termed “STN
84 axis1”, and also along the transverse direction, perpendicular to the longitudinal axis, termed

85 “STN axis2” (**Figure 1D, and 1F**). Although the centroid-based measurement effectively
86 represents the projection sites, it may not fully portray the varying spatial pattern of broadly
87 dispersed axonal projections. Therefore, we used multidimensional scaling (MDS) that visualizes
88 the level of similarity of projection patterns in a 3D space, termed “pattern space” (see **STAR**
89 **Methods**). Projections in a pattern space generated by MDS were continuously arranged with
90 little or no noticeable segregation along two major dimensions which were significantly correlated
91 with the STN axes (**Figure 1G**). Consistent with the centroid analysis, MDS confirmed graded
92 topographic organization of IP_{STN} along the geometrical axes of the STN (STN axes 1 and 2). We
93 then investigated the topographic relationships between inputs originating in the GPe and their
94 projection targets by pairwise comparison of each GPe input locus and its corresponding
95 projection locus in the STN along STN axes 1 and 2. Our analysis of input and projection
96 distributions in the indirect pathway indicated that injection sites in the GPe are topographically
97 aligned linearly along two directions, matching the geometric longitudinal and transverse axes of
98 the GPe itself (**Figure 1H**; see **STAR Methods**). Interestingly, along STN axis2, the input-
99 projection pattern was reversely related, revealing a “cross-over” projection pattern from the GPe
100 to the STN. Our detailed analysis of GPe-STN connectivity clearly shows graded IP_{STN}
101 organization along geometric axes, with generally extensive overlaps and an absence of sharp
102 boundaries.

103

104 **Hyperdirect pathway in the STN**

105 Next, to comprehensively assess the organization of the hyperdirect pathway in the STN (HP_{STN}),
106 we extracted data from the Allen Mouse Brain Connectivity Atlas dataset (AMBCA,
107 <https://connectivity.brain-map.org>) (Oh et al., 2014) regarding axonal projections following
108 isocortical injections. Our data-filtering criteria included detectable STN projection signals and
109 contamination of cortical injection (**Figure 2A**; **Figure S1A and 4A–C**; see **STAR Methods**). The
110 resulting dataset included 176 injections with AAV-expressing fluorescent proteins throughout the
111 isocortex, covering 71% of the volume of layer (L) 5 in wild-type C57BL/6J mice and nine

112 transgenic Cre lines shown to have L5-specific expression of Cre (**Figure 2B; Table S2, 3**).
113 Additionally, our own retrograde tracer experiments using retro-AAV or -Lenti systems (Kato et
114 al., 2014; Tervo et al., 2016) confirmed that HP_{STN} arises in various cortical subregions, mainly
115 from a subpopulation of corticofugal neurons in the ipsilateral L5, which is consistent with
116 previous observations (Kita and Kita, 2012) (**Figure S4D**).

117 Information flow from the cortex to the BG is believed to progress through a hierarchical
118 series of sensorimotor, association, and limbic areas, supporting the tripartite STN model
119 (Lambert et al., 2012; Mallet et al., 2007; Parent and Hazrati, 1995; Plantinga et al., 2018).
120 However, much of the data leading to this view regarding the subdivisions of the STN are
121 qualitative, variable, fragmented, and difficult to reconcile, owing to the technical limitations of
122 neuroanatomical research (Alkemade and Forstmann, 2014; Keuken et al., 2012; Lambert et al.,
123 2015). Therefore, to update this view of the STN, we first performed an inclusive analysis at the
124 level of region-to-region connectivity of the HP_{STN}. We found that STN projection patterns from
125 each cortical subregion exhibited distinct yet extensively overlapping projection patterns (**Figure**
126 **2C**). For instance, projections from motor cortical areas such as the primary and secondary motor
127 areas (MOp and MOs) broadly targeted the center region of the STN, while those from sensory
128 areas such as primary and supplemental somatosensory areas (SSp and SSs) targeted the
129 dorsolateral STN (anatomic abbreviations and functional groups shown in **Table S2**). Association
130 areas, such as the retrosplenial (RSP), visual (VIS), and visceral (VISC) area, and limbic areas,
131 such as anterior cingulate (ACA) and orbital (ORB) area, contained dorsolateral and ventromedial
132 STN projections spanning the central part of STN, respectively. Overall, our analysis of STN
133 projection patterns showed much broader coverage, with more extensive overlap than previously
134 thought (Lambert et al., 2012; Mallet et al., 2007; Parent and Hazrati, 1995; Plantinga et al.,
135 2018). Furthermore, within motor and sensory cortices, we found that individual injections
136 revealed variable and distinct yet overlapping projections in the STN (**Figure 2D**). Hence, we
137 investigated the organization of the HP_{STN} using non-biased techniques, such as a voxel-based
138 analysis that disregards anatomically defined cortical subregions. Similar to our analysis of the

139 IP_{STN} described above, we examined the distributions of cortical inputs and STN projections along
140 the whole-brain anatomic axes (AP, ML, and DV). We found that the HP_{STN} had a graded
141 distribution with a notable degree of overlap along STN axis1 (**Figure 2E**). Furthermore, MDS
142 analysis indicated that the 3D projection patterns of the HP_{STN} were gradually distributed along
143 STN axis1 and axis2 (**Figure 2F**). Together, these results indicate that the HP_{STN} has graded
144 organization along geometric axes, with overlaps and an absence of sharp boundaries, which
145 prompts re-evaluation of the conventional view of a functionally subdivided STN determined by
146 cortical input patterns.

147

148 **Hyperdirect pathway in the GPe**

149 We expanded our analysis of the hyperdirect pathway to include projection patterns in the GPe.
150 Based on previous reports (Abecassis et al., 2020; Karube et al., 2019; Naito and Kita, 1994;
151 Winnubst et al., 2019) showing the existence of direct cortico-pallidal connections, we questioned
152 whether the hyperdirect pathway providing direct cortico-pallidal projections into the GPe (called
153 HP_{GPe}) collaterally provides HP_{STN} , and how HP_{GPe} displays topographic distributions. First, we
154 injected retro-AAV-iCre and Cre-dependent AAV-JxON-tdTomato into the GPe and MOp,
155 respectively, and then cleared the tissue. Our results revealed strong collateral projections from
156 labeled MOp neurons in both the GPe and STN with additional downstream targets in the
157 substantia nigra (SN) and superior colliculus (SC), but few or no targets in contralateral cortical
158 areas (**Figure 3A**). As with the HP_{STN} , we observed graded organization within the HP_{GPe} . The
159 HP_{GPe} appeared to be topographically organized with fidelity to the whole-brain anatomic axes
160 and segmented into a tripartite form. This was especially the case with respect to inputs arising in
161 association and limbic areas, which is comparable to the HP_{STN} (**Figure 3B**). This organization
162 might arise through the innate geometric situation of the GPe matched with the whole-brain
163 anatomic axes and/or the larger receptive volume of the GPe compared with the STN. Next, we
164 conducted pairwise topographic matching between the loci of cortical inputs and projections in the
165 GPe (HP_{GPe}). We found two major axes of topographic organization of hyperdirect pathway inputs

166 and the HP_{GPe} that were correlated and reversely correlated along STN axis1 and axis2,
167 respectively (**Figure 3C, D; Figure S5A**). These gradient axes of the HP_{GPe} appeared to match
168 the geometric axes of the GPe, which is similar to the gradient axes observed for input into the
169 indirect pathway. To examine the relationship between the two pathways along these gradient
170 axes, we performed a paired comparison between the IP and HP patterns. For this analysis, we
171 measured the similarity between the pathways in the STN and GPe, respectively. The HP_{STN} and
172 IP_{STN} projection patterns were used to examine the gradient similarity in the STN, while the HP_{GPe}
173 and indirect pathway input (IP_{INPUT}) were used for that in the GPe. We then calculated the
174 correlation between these pairwise similarities in the STN and GPe. We found strong similarities
175 in STN projection patterns between the IP_{STN} and HP_{STN} when the IP_{INPUT} and HP_{GPe} locus were
176 located close together (**Figure 3E**).

177

178 **Complex integration of the indirect and hyperdirect pathways in the STN**

179 Given our findings that the IP_{STN} , HP_{STN} , and HP_{GPe} are topographically organized with gradient
180 convergence at the mesoscopic level, we next performed a detailed analysis of HP connectivity at
181 the cellular level. To dissect HP connectivity types, we first labeled cortical neurons innervating
182 HP_{STN} and/or HP_{GPe} by injecting retro-AAV-tdTomato and -sfGFP in the STN and GPe,
183 respectively. We identified three types of cortical neurons in L5 of MOp: Those innervating only
184 HP_{STN} , only HP_{GPe} , and both $HP_{GPe/STN}$ (**Figure 3F**). Additionally, by data-mining single-neuron
185 reconstruction data from the MouseLight project (<http://www.mouselight.janelia.org>) (Winnubst et
186 al., 2019), we confirmed the existence of these three types of cortical neurons (**Figure 3G;**
187 **Figure S5B, C**). Neurons innervating the HP_{STN} are predominantly pyramidal tract (PT) neurons,
188 while those innervating the HP_{GPe} are both PT and intratelencephalic (IT) neurons, with a subset
189 (~15 %) that collaterally innervate the $HP_{GPe/STN}$. Notably, the axonal density of neurons
190 innervating the $HP_{GPe/STN}$ in the STN is 3.7-fold higher than that in the GPe. Consistent with our
191 mesoscopic retrograde tracer data, shown in **Figure 3A**, we found that cortical neurons
192 innervating the $HP_{GPe/STN}$ send collateral innervations to multiple brain areas such as the striatum

193 (STR), SN, SC, and others, which suggests that these neurons may help orchestrate movement.
194 Although we analyzed a small set of reconstructed single neurons, their projection patterns were
195 consistent with our mesoscopic, topographic analysis of the HP_{GPe} and HP_{STN} (**Figure S5D**). Our
196 results indicate that at least three types of signals from the indirect and hyperdirect pathways in
197 the STN are integrated, including a classical form of convergence of inhibition and excitation,
198 feedforward inhibition, and collateral convergence (parallel after-discharge-like circuitry; **Figure**
199 **3H**). In addition, we confirmed HP projections into the human STN at the macroscopic scale by
200 7T-MRI-based *in vivo* ultrahigh-resolution fiber tractography of a healthy human brain (**Figure S6**;
201 **Table S4**; see **STAR Methods**). Our seed-based probabilistic tractography of the HP revealed a
202 direct cortico-pallidal connection, the HP_{GPe}, and a collateral HP_{GPe/STN} in the human brain, similar
203 to those we observed in mice (**Figure S6B, C**). We divided cortical subregions into three groups:
204 motor, limbic, and association (further divided into prefrontal- and rest-association) areas (**Table**
205 **S2**). HP projections from the association, motor, and limbic areas were only somewhat
206 segregated in the ventromedial, dorsolateral, and ventrolateral areas, respectively, appearing
207 along the longitudinal axis of the STN (axis1). However, as we observed in the mouse HP_{STN},
208 there was a high degree of overlap and no evident anatomical borders or clear subdivisions
209 (**Figure S7D–H**). We also found the HP_{STN}, HP_{GPe}, and collateral HP_{GPe/STN} organization in the
210 human brain to be consistent with the complex signal integration patterns in the mouse. In
211 agreement with our mouse connectivity maps, our analysis of human 7T MRI-based tractography
212 data revealed considerable and gradual overlap along the longitudinal axis of the human STN.
213 Together, these results suggest that graded convergence of IP_{STN} and HP_{STN} is organized along
214 the STN geometric axes, and that GPe neurons innervating the IP_{STN} might be activated by the
215 same HP_{STN}. These circuit features imply functional computational complexity and delicate
216 control, updating classical models (DeLong and Wichmann, 2015) with the idea that the indirect
217 and hyperdirect pathways participate in relaying and integrating information needed for
218 coordinated movement.

219

220 Cellular composition of the STN

221 The way in which STN neurons process convergent inputs from the IP and HP depends critically
222 on the molecular and cellular properties of these neurons. The STN is generally described as a
223 homogenous glutamatergic nucleus of the BG. To test this, we used serial single-molecule
224 fluorescence in situ hybridization (smFISH) with a custom computational analysis for cell-type
225 markers and receptors to examine the topographic molecular and cellular profile of the STN
226 (**Figure 4A**; see **STAR Methods**). Most STN neurons identified by the neuronal marker *Rbfox3*
227 co-expressed the glutamatergic marker *Slc17a6*, while very few neurons co-expressed the
228 GABAergic marker *Slc32a1*. Approximately half of the STN cells expressed glia-associated genes
229 (*Aqp4*, *Mbp*, and *Tmem119*) (**Figure S7**). Thus, our serial smFISH, immunostaining, and
230 topographic analysis confirmed that most neurons in the STN are purely glutamatergic. Previous
231 anatomical studies have reported the presence of parvalbumin-expressing (PV+) neurons in the
232 STN (Alkemade et al., 2019; Hontanilla et al., 1998; Lévesque and Parent, 2005; Wu et al.,
233 2018). PV expression is conventionally interpreted to reflect a GABAergic subpopulation (Hu et
234 al., 2014), which is inconsistent with our serial smFISH results and previous studies (Lévesque
235 and Parent, 2005; Roccaro-Waldmeyer et al., 2018). Accordingly, we conducted detailed cellular
236 characterization of PV+ STN neurons. First, we performed triple smFISH for *Pvalb*, *Slc17a6*, and
237 *Slc32a1*. We found that *Pvalb*+ neurons in the STN exclusively co-express glutamatergic marker
238 *Slc17a6*, but not GABAergic marker *Slc32a1*, whereas those in the other brain areas such as
239 hippocampus, cortex, and zona incerta co-express *Slc32a1* (**Figure 4B–D**; **Figure S8A**). Notably,
240 quantitative analysis of the topographic distribution of *Pvalb*+ neurons indicated that
241 glutamatergic PV+ neurons were particularly prevalent in the dorsolateral and middle STN
242 (**Figure 4C, D**). Our serial multiplex-smFISH data provide direct evidence that PV+ STN cells are
243 glutamatergic with topographic segregation in the STN (Hontanilla et al., 1998; Wu et al., 2018)
244 and suggest that parvalbumin cannot reliably serve as a marker for GABAergic interneurons,
245 together with previous findings (Jinno and Kosaka, 2004; Shang et al., 2015; Wallace et al.,
246 2017).

247 To examine further physiological properties of PV+ STN neurons, we used PV-IRES-
248 Cre::Ai6 mice expressing ZsGreen in PV+ cells. We first confirmed that PV+ neurons in the STN
249 of these mice were immunohistochemically positive for glutamate and negative for GABA (**Figure**
250 **5A**). By clearing parasagittal sections (2.5 mm in thickness) that embrace the entire STN without
251 making physical cuts, we 3D-mapped total PV+ neurons in the STN and found topographically
252 segregated distribution in the dorsolateral and middle STN, consistent with serial-smFISH data
253 (**Figure 5B; Movie S2**). Because Parkinsonian symptoms are correlated with an increase in burst
254 firing in STN neurons, we next recorded the firing patterns of glutamatergic PV+/- STN neurons
255 using PV-IRES-Cre::Ai6 mice. Both PV+ and PV- neurons displayed similar spontaneous firing
256 and other basic properties including input resistance (**Table S5**). However, only PV+ neurons
257 showed phasic burst firing, which we refer to a firing pattern, composing of fast firing (an
258 interspike interval, ISI, of less than 10 ms) and a pause; even current injection could not evoke
259 bursts from PV- neurons (**Figure 5C-F; Figure S8B-E; Table S6; see STAR Methods**). Burst
260 and tonic firing patterns were observed at an equivalent membrane potential range (-58 to -45
261 mV) (**Figure S8E**), suggesting that burst firing was not due to differences in the membrane
262 potential *per se*, even though firing patterns in STN neurons can be affected by membrane
263 potentials (Kass and Mintz, 2006). Injection of negative current pulses evoked significantly
264 smaller hyperpolarization-induced depolarizing sags in PV+ vs. PV- neurons, which was
265 consistent with their burst properties (**Figure 5F**). Taken together, our results provide clear
266 evidence that there are at least two functional types of glutamatergic neurons in the STN, and
267 that glutamatergic PV+ neurons contribute to burst firing in the dorsolateral and middle STN. This
268 is compatible with previous studies of spatial distribution of firing patterns by intraoperative
269 microelectrode recording within the human STN showing the occurrence of burst firing in in the
270 dorsal STN while tonic firings towards the ventral STN (Kaku et al., 2019).

271

272 **Cell type-dependent synaptic connectivity**

273 Our analysis defined new functional cell types and established wiregrams for the IP_{STN} and
274 HP_{STN}, allowing us to refine our analysis of synaptic connectivity in the IP and HP to be cell type-
275 specific. Thus, we tested whether the IP_{STN} and HP_{STN} form PV_± cell-specific synaptic
276 connections, and whether the HP_{GPe} forms synapses on the two major GABAergic cell types in
277 the GPe, that is, prototypic neurons projecting to the STN and arkypalidal neurons projecting to
278 the striatum (Mallet et al., 2012; Mastro et al., 2014). By combining retrograde virus injections and
279 mammalian GFP reconstitution across synapses (mGRASP) (Kato et al., 2014; Kim et al., 2012),
280 which is an advanced method for accurately detecting synaptic connections, we mapped synaptic
281 connectivity of these four cell-types in IP_{STN}, HP_{STN}, and HP_{GPe} (**Figure 6A-F**; see **STAR**
282 **Methods**). We identified synaptic connections in STN PV₊ and PV₋ neurons originating in both
283 the IP_{STN} and HP_{STN}, by injecting AAV expressing the pre- and post-synaptic mGRASP
284 components into the GPe (for IP_{STN}), cortex (for HP_{STN}), and STN, respectively. STN PV_±-
285 neurons were distinguished by injection of AAV expressing iRFP in a Cre-dependent manner into
286 the PV-Cre mouse line. We also observed synaptic connections in both GPe prototypic and
287 arkypalidal neurons (labeled by a combination of retro-Lentivirus expressing iCre injected into the
288 STN and STR, respectively, and AAV expressing Cre-On-postsynaptic mGRASP into the GPe)
289 projecting from the HP_{GPe} (labeled by injecting presynaptic mGRASP in the cortex). Our analysis
290 revealed that axonal projections in both the STN and GPe from the IP_{STN} and HP_{STN}, and from the
291 HP_{GPe}, respectively, indeed serve as inputs. Furthermore, our results indicated that all four cell
292 types in the STN and GPe (STN PV₊, PV₋, GPe arkypalidal, and prototypic neurons)
293 communicate with each other and receive synaptic information from the cortex. Thus, in terms of
294 cortex-GPe-STN circuitry, our data indicate that “everyone talks and everyone listens.” Next, in
295 terms of connectivity patterns, the two types of STN neurons integrate excitatory and inhibitory
296 signals (E/I) from the IP_{STN} and HP_{STN}. We examined the differential expression of glutamate and
297 GABA receptors in STN PV_± neurons. To this end, we performed serial triple smFISH for *Gria1*
298 (encoding GluA1), *Gabra1* (GABA_A α1 subunit), and *Pvalb*, and quantitatively compared their
299 expression with respect to the topographically graded innervations of the IP_{STN} and HP_{STN} (**Figure**

300 **6G-I**). Our co-expression plot of *Gria1* and *Gabra1* showed a strong correlation between these
301 genes, indicating that most STN neurons integrate both excitatory and inhibitory inputs at the
302 single-cell level. Notably, the total expression levels of *Gria1* and *Gabra1* formed a gradient
303 along the STN axis1 with higher and lower expression in the ventromedial and dorsolateral STN,
304 respectively, similar to the expression pattern of *Slc17a6* (**Figure 4C, and 6H, I**). At the cellular
305 level, the *Gria1* to *Gabra1* expression ratio was approximately similar in the dorsolateral PV+, but
306 this expression ratio was significantly higher in ventromedial PV- neurons (**Figure 6I**). Taken
307 together, our results indicate that two types of glutamatergic PV+/- STN neurons, which integrate
308 information from both the IP_{STN} and HP_{STN}, and fire in different patterns, are segregated along a
309 topographical distribution and express different ratios of genes encoding E/I receptors. Although
310 further direct evidence is needed, these findings may represent a step forward in characterizing
311 the E/I imbalance of BG circuits in Parkinson's disease models, as well as the effects of DBS.
312 Interestingly, when we analyzed DBS results with respect to electrode position within specific
313 STN structures from 26 Parkinson's disease patients who had received bilateral STN DBS
314 implementation together with previous studies (Herzog et al., 2004; Mallet et al., 2012; Wodarg et
315 al., 2012), we found that patients showing DBS-associated movement improvement had
316 electrodes placed in the dorsolateral and middle STN, with somewhat greater improvement
317 associated with electrode placement towards the middle of STN axis1 (**Figure S9**). This part of
318 the STN has been reported to receive direct inputs from motor cortical areas and to contain PV+
319 glutamatergic neurons (Alkemade et al., 2019; Eisenstein et al., 2014; Herzog et al., 2004;
320 Lévesque and Parent, 2005; Mallet et al., 2007; Wodarg et al., 2012). The correlation among
321 position-dependent STN DBS effects and connectivity patterns led us to hypothesize that distinct
322 features of connectivity and cellular composition in the dorsolateral and middle STN influence the
323 impact of DBS.

324 In Sum, our comprehensive profiling of circuit- and cellular-level connectivity of the
325 indirect and hyperdirect pathways revealed a topographically graded organization with three
326 convergent types of indirect and hyperdirect-pathway (GPe-only, STN-only, and both) in the STN.

327 Topographically distinct molecular, cellular, and physiological properties together with the circuit
328 features imply functional computational complexity and delicate control. Our results suggest a
329 complex interplay of information within the basal ganglia further on than the general consensus of
330 parallel segregated input patterns with homogeneous cytoarchitecture and physiology. Our new
331 view of the indirect and hyperdirect pathways, cytoarchitecture, and physiology properties
332 substantially updates classical models and suggests, from a structural basis, a way to view
333 relayed and integrated information flow underlying coordinated movement controls and STN DBS
334 effects (**Figure 7**).

335 **Discussion**

336 Understanding how the STN controls motor functions, and identifying the circuit-level
337 mechanisms underlying the effects of STN-DBS requires detailed convergent connectivity profiles
338 for the main inhibitory and excitatory inputs in the STN. Furthermore, molecular profiles of cell
339 types and neurotransmitter receptors in the STN are needed to infer the various interacting
340 molecular and cellular processes that contribute to these mechanisms. In this study, we
341 constructed a new comprehensive map of connectivity of the inhibitory indirect and excitatory
342 hyperdirect pathways in the mouse and human STN. We identified glutamatergic PV+ STN
343 neurons that show distinct burst firing patterns and topographical segregation in the dorsolateral
344 and middle STN. Cell type-specific synaptic connectivity mapping revealed that both STN PV+
345 and PV- neurons receive synaptic inputs from both the IP_{STN} and HP_{STN} and that both GPe
346 prototypic and arkypalidal neurons receive synaptic inputs from the HP_{GPe} . Thus, “everyone talks,
347 and everyone listens” in cortex-GPe-STN circuitry. These data provide new insights into the basic
348 organization of the BG network and represent an anatomic and functional guide for further
349 dissection of BG functions and DBS effects. Detailed knowledge regarding the anatomy and
350 connectivity pattern of cortico-BG circuits is essential for understanding the psychomotor activity
351 and pathophysiology associated with a wide range of neurological and neuropsychiatric diseases.

352 The cortex is widely considered to serve as the initial input to the BG through cortico-
353 striatal pathways that convey sensorimotor, associative, and limbic information. The information
354 from these three functional domains is thought to be transmitted to the downstream structures of
355 the BG in a segregated manner, which supports the tripartite model (Lambert et al., 2012; Mallet
356 et al., 2007; Parent and Hazrati, 1995; Plantinga et al., 2018). However, our data indicate that
357 topographically graded organization exists in both the indirect and hyperdirect pathways in the
358 STN, with continuum overlaps, an absence of anatomical boundaries, and direct cortico-pallidal
359 connections. Although previous studies have demonstrated that the coexistence of clearly
360 segregated and overlapping input patterns from the cortex to the BG (Draganski et al., 2008), in

361 the present study we constructed a large-scale map of the indirect and hyperdirect pathways,
362 signifying a new understanding of input organization in the STN. In addition, our detailed analysis
363 of direct cortico-pallidal connections together with recent findings regarding the complexity of the
364 GPe (Abecassis et al., 2020; Mallet et al., 2012; Mastro et al., 2014, 2017) updates current views
365 of the BG network, in which the GPe is considered to be a simple relay nucleus providing a
366 unidirectional flow of information from the input structures (such as the striatum and STN) to the
367 BG output structures (such as the GPi and SNr). These data, together with our single-cell
368 reconstruction and cell type-specific synaptic connectivity analyses, suggest further complexity of
369 BG functional organization through three types of information integration (**Figure 3H**), in addition
370 to reciprocal and loop connections within the BG (Hegeman et al., 2016; Mallet et al., 2012). For
371 instance, beyond the STN-GPe network, the hyperdirect pathway directly and/or collaterally
372 innervates the STN and the striatum as well as two types of GABAergic GPe neurons (prototypic
373 and arkypallidal), thus providing HP-driven feedforward inhibition. This circuit-level complexity
374 implies the existence of anatomical substrates for precisely coordinated regulations of motor as
375 well as nonmotor functions governed by the BG.

376 It is important to note that, as with any work, the present study has several potential
377 limitations and strengths. First, at the mesoscopic level of mouse connectivity, projection signals
378 labeled using cytosolic fluorescence proteins (FPs) include both axons from passing fibers and
379 synaptic terminals. Like previous studies that found cytoplasmic FP signals to strongly
380 correspond to synaptically-targeted FP signals after removing the axons of passage (Harris et al.,
381 2019; Hunnicutt et al., 2016; Oh et al., 2014), we found no significant difference in the
382 topographic organization of the indirect and hyperdirect pathways after removing the axons of
383 fasciculated passing fibers observed in some areas i.e., the dorsal part of the GPe, and the
384 anterior/posterior ends of the STN (**Figure S4E**; see **STAR Methods**). Second, to test the
385 possibility that our discovery of a topographic gradient in the STN might be the product of graded
386 injections, we examined a dataset showing overlapping axonal projections in the STN that were
387 innervated from separate injections, so that no overlap in these injection sites could be

388 guaranteed. An additional statistical analysis confirmed that neighboring yet non-overlapping
389 injections in different functional subregions led to overlapping projection patterns in the STN
390 (**Figure S4F**). These results support the existence of graded organization in the STN, and
391 challenge the previous models of clear segregation, such as the tripart model. Third, at the
392 microscopic level, although mGRASP-assisted circuit mapping provided convincing data
393 regarding the cell types in the STN and GPe that receive synaptic inputs from the indirect and
394 hyperdirect pathways, further fine-scale mapping of the topographic organization of convergent
395 synaptic connectivity is necessary. Finally, for assessing human connectivity, we used diffusion-
396 weighted image (DWI)-based tractography. This method carries some degree of inherent
397 ambiguity, such as that related to the validity of tract reconstruction and orientation (Maier-Hein et
398 al., 2017; Petersen et al., 2019). However, substantial scientific innovations have aimed to
399 overcome the limitations of this technique. Regardless, it is currently the only tool for mapping
400 human brain connectivity *in vivo*, and the data regarding overlapping patterns were highly
401 consistent with our mouse data, which were obtained using advanced and accurate techniques,
402 as well as with data from previous reports (Plantinga et al., 2016). Overall, our data represent a
403 translational comparison of connectivity patterns in the STN across species.

404 Previous studies suggested the existence of glutamatergic PV+ neurons in STN and their
405 topographical organization at coarse level (Hontanilla et al., 1998; Roccaro-Waldmeyer et al.,
406 2018; Wu et al., 2018) based on immunohistochemistry with anti-PV or indirect comparison of
407 separate conventional *in situ* hybridization datasets for PV and vGluT2, which makes it difficult to
408 map a precise topographic distribution of STN PV+ neurons. Here, we directly identified
409 topographically localized glutamatergic PV+ neurons in the STN using serial multiplex smFISH.
410 They are a main contributor to burst firing, which indicates a potential key role as generators and
411 modulators of β -band (15-30 Hz) oscillations in the BG. As excess burst firing and β -band
412 oscillations are signatures of Parkinson's disease and these pathophysiological activities are
413 decreased by STN DBS therapy, these burst firing PV+ STN neurons may be implicated in
414 Parkinson's disease symptoms (Kühn et al., 2004; Steigerwald et al., 2008). By mapping the

415 distributions of major excitatory and inhibitory transmitter receptors in the STN, we found distinct
416 topographic patterns of expression level. These patterns suggest the existence of a functional
417 layer of differential molecular and cellular receptibility on the top of neural connectivity.
418 Interestingly, the E/I receptor expression ratio is equivalent in dorsolateral PV+ STN neurons, but
419 higher in ventromedial PV- STN neurons. These results raise the important question of whether
420 cell type-specific changes in E/I receptor expression emerge in dopamine-depleted states.
421 Further studies designed to interrogate alterations in synaptic connectivity, receptor expression,
422 and glutamatergic PV+ STN neuronal firing patterns in models of Parkinson's disease will provide
423 important new insights regarding the coordinated regulation of specialized PV+ neurons in the BG
424 network.

425 Our systematic connectivity maps, superimposed upon regions with distinct functional
426 cellular heterogeneity, suggest a complex interplay of information within the BG. Our new view of
427 the indirect and hyperdirect pathways (**Figure 7**) substantially updates classical models and
428 proposes, from a structural basis, a new take on relayed and integrated information flow
429 underlying coordinated movement controls and the effects of STN DBS. Further studies of other
430 inputs (e.g. serotonergic and cholinergic) into the STN as well as its outputs will facilitate the
431 elucidation of the mechanisms of the STN functions and complex effects of DBS.

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434 course of the investigation.

435

436

437 **Author Contributions**

438 H.L. performed mouse connectivity experiments and imaging. H.J. performed statistical analysis
439 with mouse connectivity. D.K. and Z.-H.C. provided and analyzed human 7T MRI data. H.L. and
440 Jiwon Kim performed in situ hybridization and immunohistochemistry. K.T.-Y. conducted and
441 analyzed electrophysiological data. H.J., Jiwon Kim and L.F. analyzed molecular profiling data.
442 H.P., Y.L. and S.H.P. performed DBS surgery and collected clinical data. H.J., W.C.O., H.P., and
443 S.H.P. analyzed DBS data. H.L., H.J., D.K., Jiwon Kim., K.T.-Y., H.P., and Jinhyun Kim wrote the
444 original draft. All of the authors reviewed the manuscript. H.L., H.J. and Jinhyun Kim conceived
445 and designed the study.

446

447

448 **Competing Interest Statement**

449 Authors declare no competing interests.

450

451

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Figure Titles and Legends

Figure 1. Graded topography of indirect pathway in STN. **(A)** Pipeline of indirect pathway (IP_{STN}) mapping data generation and processing. Scale bar, 250 μ m. **(B)** Spatial locations (left) and coverage (right) of the GPe injections ($n = 55$). **(C)** Heatmap showing GPe inputs and IP_{STN} signal distribution for each injection experiment (rows) along anatomical axes (columns), sorted by the GPe injection centroid along the AP. **(D)** Illustration of STN geometric axis1 and axis2. **(E)** Heatmap of IP_{STN} and GPe inputs signal distribution, sorted by projection centroid along STN axis1. **(F)** Representative images of the GPe injection sites (left) and serial coronal images of corresponding IP_{STN} showing graded topographic organization along STN axis1 (right, top) and axis2 (right, bottom). Heatmap showing signal distribution for each slice along the white line, parallel to the corresponding STN axis. The distance from the bregma indicated at the corner. Scale bar, 250 μ m. **(G)** Left: IP_{STN} projection patterns (dots) plotted in a pattern space generated via multidimensional scaling (MDS). Right: dimensions 1 and 2 in the pattern space were significantly correlated with STN axis1 and axis2 (arrows in the MDS plot), respectively (right). **(H)** Topographic organization of the IP_{STN} . Left: GPe injection sites and IP_{STN} centroids superimposed onto coronal 3D views of the GPe and STN. Colors indicate the IP_{STN} centroid location along axis1, with the corresponding GPe inputs gradient direction (arrows). Right: correlations between GPe inputs and IP_{STN} along GPe gradient axes and STN axes. Correlations were calculated via Pearson's r .

Figure 2. Graded topography of hyperdirect pathway in STN. (A) Representative axonal projections in the STN (HP_{STN}) from an MOp injection (inset) extracted from AMBCA datasets. Scale bar, 250 μm . **(B)** Top: coverage (left) and spatial locations of the hyperdirect cortical injection site (right) across the isocortex ($n = 176$). Bottom: Bar plot of STN projection power for each subregion, an estimated STN projection volume assuming the subregion is fully labelled, shown in log-scale. Grouped functional parts of cortical subregions are color-coded (See **Table S3** for functional classification). **(C)** Digitally reconstructed serial STN projection density patterns of the averaged HP_{STN} from each grouped functional part and constituting cortical subregion at 25- μm intervals. The number in parentheses denotes the number of localized injections within the subregion. Subregions without localized injections are not shown. **(D)** Representative set of intra-subregion injections within the MO (top) and SS (bottom), and corresponding serial STN projection density at 50- μm intervals. **(E)** Heatmap showing cortical inputs and HP_{STN} signal distribution along anatomical axes (columns). Cortical injection experiments (rows) were grouped into three functional parts and sorted according to the AP of the injection centroid in each group. **(F)** Left: HP_{STN} projection patterns in the pattern space (same as in **Figure 1G**). Right: Dimensions 1 and 2 in the HP_{STN} pattern space were also significantly correlated with STN axis1 and axis2 (arrows in the MDS plot). Correlations were calculated by Pearson's r .

Figure 3. Complex integration of indirect and hyperdirect pathways in STN. (A) Left: illustration of the hyperdirect pathway with a direct pallidal connection and schematic of the injection strategy for labelling collateral hyperdirect projections (retroAAV-iCre and Cre-dependent switch-on AAV-tdTomato were injected in the GPe and MOp, respectively). Right: z-projection image of a cleared parasagittal section (2.5 mm thick) showing hyperdirect projections both in the GPe and the STN, and the trajectories of the HP with two downstream targets (arrows). Scale bar, 250 μ m. **(B)** Heatmap showing cortical inputs and HP_{GPe} signal distribution along anatomical axes (columns). Cortical injection experiments (rows) were grouped into three functional parts and sorted according to the AP of the injection centroid in each group. **(C)** Heatmap of sorted HP_{STN} with cortical inputs and HP_{GPe} signal distribution along the STN axis1 and corresponding cortex and GPe gradient axis. Rows are sorted by HP_{STN} axial location along STN axis1. **(D)** Left: illustration of the topographic organization of the HP_{STN}, HP_{GPe}, and cortical inputs with STN geometric axes and corresponding gradient axes of the HP_{GPe} and cortical inputs (arrows). Right: linear relationship between axial locations of the HP_{GPe} (top), HP_{STN} (bottom), and cortical inputs. **(E)** Topographic relationship between the IP and HP. Left: the projection similarity between IP_{STN}-HP_{STN} was inversely correlated with the distance between IP_{INPUT}-HP_{GPe}. Each dot represents a pair of IP and HP experiments ($n = 55 \times 176$). Middle: Scatterplots of IP_{STN} and HP_{STN} density from three representative pairs showing similar (1), intermediate (2), and different (3) IP_{STN} and HP_{STN} projections. Right: Prediction of HP_{GPe}-HP_{STN} based on GPe input-IP_{STN} topographic organization resulted in significantly lower mean squared error (MSE) compared with randomizations ($***p < 0.001$, two-sided Student t-test). Red error bars represent the mean with s.e.m, while the gray area represents the 5th to 95th percentile of randomization. **(F)** Left: Injection sites of retroAAV expressing tdTomato and sfGFP in the GPe (top) and STN (bottom), respectively. Right: Magnified image of the MOp showing labeled GPe (red)- and STN (green)-projecting cells in L5, with co-labeled (yellow) cells representing collateral GPe/STN-projecting neurons. Scale bar 1 mm and 100 μ m (enlarged view). **(G)** Analysis of single cell reconstruction data from the MouseLight database. Left: representative MOs neuron (AA0772) collaterally

projecting to the GPe and STN. Right: cortical neuronal population innervating the HP_{GPe} (64%), HP_{STN} (20%), and collaterally innervating the $HP_{GPe/STN}$ (15%). **(H)** Schematic illustration of three types of signal integration in the STN from indirect and hyperdirect pathways. Correlations were calculated via Pearson's r .

Figure 4. Glutamatergic STN PV+ neurons. (A) Top: pipeline of serial smFISH data generation and processing. Bottom: example serial smFISH image of STN with the distance from the bregma indicated at the corner. **(B)** Left: example smFISH image of the STN (inset) from coronal brain slice with *Pvalb*, *Slc17a6*, and *Slc32a1*. Right: magnified views of the regions indicated by white boxes in the dorsolateral (top) and ventromedial (bottom) STN (left panel) show that STN neuronal population co-express *Pvalb* and *Slc17a6*, but not *Slc32a1*. Scale bar, 1 mm (left), 250 μ m (left, inset), and 10 μ m. **(C), (D)** Topographic distribution of glutamatergic PV+ neurons in the STN. Representative smFISH images cropped to show the STN (**C**, left), and quantification of cells expressing each gene along STN axis1 shown with the mean ($n = 2$) and s.e.m. (**C**, right). The pseudocolor arrow represents STN axis1. Co-expression plot of *Pvalb* with *Slc17a6* (**D**, left) and *Slc32a1* (**D**, right), showing the number of mRNA puncta per cell for each gene. Each dot represents a cell, colored according to location along STN axis1. Dashed lines represent the thresholds for positive cells. Double negative cells are not shown. Scale bar, 250 μ m.

Figure 5. Firing patterns of STN PV+/- neuron. **(A)** Left: representative image of the STN (inset) from coronal brain slice of PV-IRES-Cre::Ai6 mouse line, showing PV+ neurons. Right: magnified immunofluorescence image of STN cells show STN PV+ neurons co-labeled with anti-Glutamate (top) but not with anti-GABA (bottom). Scale bar, 1 mm (left), and 250 μm (left, inset) and 20 μm (right). **(B)** Distribution of PV+ neurons in the STN using automatic cell detection from PV-IRES-Cre::Ai6 mouse. Scale bar, 250 μm . **(C)** Electrophysiological properties of STN PV+/- neurons. Representative tonic (bottom) and burst (top) firing with enlarged traces shown in boxes, recorded from STN PV+/- neurons using the PV-IRES-Cre::Ai6 mouse line. Interspike interval (ISI) of first two, 2nd and 3rd, last two spikes, and average of all spikes shown in the boxes; 7.7, 5.9, 7.7, and 6.5 ms for PV+ burst firing; 50.3, 51.4, 46.7, and 47.5 ms for PV- tonic firing neuron. **(D)** ISI histogram of spontaneous firing patterns (burst PV+ and tonic PV+/- neurons, n = 12, 14, and 20, respectively). Insets in the top panels show magnified ISI histogram during 0-150 ms (shaded area). Bar graph shows the proportion of burst firing cells (right). **(E)** ISI histogram of firing patterns during a rebound depolarization following the removal of a hyperpolarizing current injection (left), and during depolarizing current injection (right; see **Methods**) **(F)** The hyperpolarization-induced depolarizing sag of PV+/- neurons, marked by an arrow (left), was significantly smaller than that in PV+ neurons (right). Data are mean \pm s.e.m. Mann-Whitney U test with $p < 0.01$. ISI of first two, 2nd and 3rd, last two spikes, and average of all spikes; 4.2, 3.5, 8.9, and 4.6 ms for PV+ burst firing, 12.4, 12.5, 55.1, 24.2 ms for PV- tonic firing neuron.

Figure 6. Cell type-dependent synaptic connectivity. **(A)** Scheme of tested cell type-specific connections in the HP_{GPe} (1, 2), HP_{STN} (3, 4), and IP_{STN} (5, 6). **(B-E)**, Labeling strategies for testing synaptic connections listed in **(A)** using retrograde virus and mGRASP system. Presynaptic-mGRASP was injected into either the cortex (1–4) or the GPe (5–6). Projection-dependent GPe cell types (arkypallidal and prototypic) were labeled with retro-lenti virus expressing Cre in the Striatum and STN, respectively (1, 2), followed by Cre-ON postsynaptic-mGRASP in the GPe. A mixture of postsynaptic-mGRASP and Cre-ON fluorescent protein was injected into the STN of PV-IRES-Cre mice to distinguish STN PV[±] neurons (3–6). **(F)** Postsynaptic neurons (top) and enlarged image of dendrite (bottom), marked by white box. Green mGRASP signals along the dendrites of post-synaptic neurons (red) were detected in all tested sets, indicating the synaptic connections. Scale bar, 10 μm (top), and 2 μm (bottom). **(G)** Representative coronal smFISH image of the STN with *Pvalb*, *Gria1*, and *Gabra1*. Enlarged views of the regions indicated by white boxes in the dorsolateral (top) and ventromedial (bottom) STN (left panel) showing co-expression of *Gria1* and *Gabra1*. Scale bar, 1 mm (left), 250 μm (left, inset) and 20 μm (right). **(H)** Topographic distribution along STN axis1 of cells expressing *Gria1* and *Gabra1*. Quantification of cells expressing each gene along STN axis1 (left) and a co-expression plot (right) showing that most STN cells co-express *Gria1* and *Gabra1*. Data are represented as means (n = 6) with s.e.m. **(I)** Left: Relative expression level of *Gria1* and *Gabra1* in PV[±] neurons. Middle: *Gria1* to *Gabra1* ratio in PV[±] neurons exhibit distinct topographic pattern along STN axis1. Right: the *Gria1* to *Gabra1* ratio was significantly lower in STN PV[±] cells (permutation test, n = 10,000, ****p < 0.0001). Data are represented as means (n = 6) with s.e.m.

Figure 7. Schematic of basal ganglia network with detailed topographic connectivity, cytoarchitecture, and firing pattern of the STN. (A) Summary of connectivity, cellular and physiological profiles (top) and cell-type specific synaptic connectivity (bottom) of the STN. Topographical organization of three convergent types of indirect and hyperdirect pathways is represented in gradual color change and arrows. Representative traces of two firing patterns, burst and tonic firing, illustrate topographic distribution of functionally distinct cell-types along dorsolateral and ventromedial parts of the STN, respectively. **(B)** Schematic of basal ganglia network with updated circuit- and cellular-level connectivity found in this study (represented in colored line). Three convergent hyperdirect and indirect pathways related to the STN are shown in solid lines.

458

459 **STAR Methods**

460

461 **Mice**

462 C57BL/6J, PV-IRES-Cre (B6;129P2-Pvalb^{tm1(cre)Arbr}/J, JAX stock no. 017320), Ai6 (B6.Cg-
463 Gt(ROSA)26Sor^{tm6(CAG-ZsGreen1)Hze}/J, JAX stock no. 007906), and double-transgenic PV-IRES-
464 Cre::Ai6 mice were used in this study. While eight 10-week-old mice were used for most
465 experiments, five 7-week-old mice were used for electrophysiology. All experiments were
466 conducted in accordance with protocols approved by the Institutional Animal Care and Use
467 Committee at the Korea Institute of Science and Technology and the National Institutes of Health
468 guidelines for animal care and use.

469

470 **Stereotaxic injection**

471 Iontophoresis of recombinant adeno-associated virus (rAAV) vectors was performed by applying
472 a positive current for 5 min using a Digital Midgard Precision Current Source (Stoelting). For
473 indirect pathway mapping experiments, rAAV2/1.CAG.sfGFP.WPRE or
474 rAAV2/1.CAG.tdTomato.WPRE (average titer from seven batches: 1.7×10^{13} vg/ml) was injected
475 via a 3 μ A current into the GPe. Unilateral dual-injections were performed in 35 mice with a total
476 of 55 injections (**Table S1**). For HP_{STN} labeling, retroAAV-expressing iCre,
477 rAAV2.CAG.iCre.WPRE packaged with a rAAV2-retro helper (Addgene, 51904 and 81070)(Kato
478 et al., 2014; Tervo et al., 2016), was injected via a 5 μ A current into the STN of Ai6 mice. For
479 other surgeries, pressure injections were performed at 20–40 nl per min using a Nanoliter injector
480 and a Micro-4 syringe pump (World Precision Instruments). For cortico-pallidal pathway labeling,
481 rAAV2/retro.CAG.iCre.WPRE and rAAV2/1.CAG.JxON.tdTomato.WPRE (100 nl each) were
482 injected into the GPe and ipsilateral primary motor cortex (MOp). For cortico-pallidal pathway
483 dissection, rAAV2/retro.CAG.sfGFP.WPRE and rAAV2/retro.CAG.tdTomato.WPRE (100 nl each)
484 were injected into the STN and the GPe, respectively. For synaptic connectivity mapping,
485 improved mGRASP (Kim et al., 2012) combined with a retro-Lenti system (Kato et al., 2014) or

486 transgenic mouse line was used. For mapping cell type-specific synaptic connectivity, pre- and
487 post-synaptic mGRASP components were injected into the cortex/GPe and STN. mGRASP
488 technology has been demonstrated to be an accurate synaptic detector and has been
489 successfully employed in the mapping of fine-scale synaptic connectivity in multiple cell types in
490 various circuits (Druckmann et al., 2014; Feng et al., 2014; Kim et al., 2012; Kwon et al., 2018;
491 Mukherjee et al., 2020; Song et al., 2018). To validate synaptic connections in hyper-/indirect
492 pathways using mGRASP, rAAV2/1.CAG.pre-mGRASPi.OLLAS.WPRE was injected into the
493 MOp (600 nl) or GPe (400 nl), respectively. For post-mGRASP labeling specific to GPe cell types,
494 50 nl of retroLenti/FuG-E.CAG.iCre.WPRE was injected into the striatum or STN, and 100 nl of
495 rAAV2/1.CAG.JxON.post-mGRASP.2A.dTomato.WPRE (Addgene, 34913) was injected into the
496 GPe. For post-mGRASP labeling specific to STN cell types, a mixture of rAAV2/1.CAG.post-
497 mGRASP.2A.dTomato.WPRE (Addgene, 34912) and rAAV2/1.CAG.JxON.iRFP670.WPRE (4:1
498 ratio) was injected into the STN of PV-IRES-Cre mice (50 nl). The following coordinates (in mm)
499 were used for the GPe: -0.22 anterioposterior from bregma (AP), +2.0 mediolateral from bregma
500 (ML), -3.5 dorsoventral from dura (DV); STN: -1.65 AP, +1.6 ML, -4.55 or -4.65 DV (for PV-IRES-
501 Cre); MOp: +2.0 AP, +1.75, +2.0, and +2.25 ML, -0.8 and -0.9 DV; and striatum: +0.5 AP, +2.0
502 ML, -3.0, -2.5, and -2.0 DV. The viral vectors were packaged using vector cores at the University
503 of Pennsylvania or the University of North Carolina at Chapel Hill.

504

505 **Tissue preparation**

506 Brains were prepared as previously described(Feng et al., 2014). For GABA
507 immunofluorescence, 4% paraformaldehyde in 0.1M phosphate buffer with pH 7.4 (4% PFA)
508 containing 0.3% glutaraldehyde was used for fixation. For immunofluorescence, 50- μ m sections
509 were permeabilized in 1% Triton X-100 in tris-buffered saline (TBS) and blocked in 5% normal
510 goat serum and 0.4% Triton X-100 in TBS. The sections were incubated with primary antibody
511 for 1–2 days at 4 °C. After washing, the sections were incubated with secondary antibody for 2–3
512 h at room temperature and counterstained with DAPI, except for in the mGRASP experiments.

513 Sections were mounted with mounting media (Invitrogen, Prolong Diamond Antifade or Vector
514 Labs, VectaShield). In this study, we used the following antibodies: rat anti-OLLAS (Novus,
515 NBP1-06713, 1:1000), guinea pig anti-NeuN (Sigma-Aldrich, ABN90P, 1:1000), rabbit anti-
516 glutamate (Sigma-Aldrich, G6642, 1:500-1000), rabbit anti-GABA (Sigma-Aldrich, A2052, 1:500-
517 1000), Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen, A11008, 1:200), Alexa Fluor 555 goat
518 anti-guinea pig IgG (Invitrogen, A21435, 1:200), Alexa Fluor 633 goat anti-rabbit IgG (Invitrogen,
519 A21071, 1:500), and Alexa Fluor 633 goat anti-rat IgG (Invitrogen, A21094, 1:1000).

520 For tissue clearing, we modified the CLARITY method (Tomer et al., 2014). Briefly, PFA-
521 fixed brains were cut as parasagittal sections (2.5 mm) containing the GPe and STN. The
522 sections were incubated for at least 2 days in a hydrogel solution with the following composition
523 (in %): 1 acrylamide, 4 PFA, and 0.25 VA-044 initiator in PBS. Following degassing and gel
524 polymerization at 37 °C, the sections were incubated in SmartClear Clearing Buffer Type A
525 (LifeCanvas Technologies) at 37 °C and in EasyIndex (LifeCanvas Technologies) at room
526 temperature.

527

528 **Microscopy**

529 Wide-field images were obtained using an Axioscan Z1 slide scanner (Carl Zeiss Microscopy)
530 equipped with a 20X 0.8 NA Plan-Apochromat air lens. Confocal images were acquired using an
531 LSM 780 confocal microscope (Carl Zeiss Microscopy). mGRASP images were obtained at 0.6
532 μm depth intervals using a 40x 1.4 NA Plan Apochromat oil lens with a 2-fold digital zoom. For
533 cleared tissue imaging, each sample was mounted with EasyIndex. Cleared brain images were
534 acquired at 8.0 μm depth intervals using a 20x 1.0 NA Plan-Neofluar optimized for CLARITY (Carl
535 Zeiss Microscopy). Single-molecule fluorescence *in situ* hybridization (smFISH) images were
536 acquired at 1 or 2 μm depth intervals using a 40x 1.3 NA oil immersion lens.

537

538 **Indirect projection mapping pipeline**

539 The MATLAB (<https://www.mathworks.com>) version of the BaSiC (Peng et al., 2017) algorithm
540 was applied to each fluorescent channel of image tiles (2040 px x 2040 px) using the default
541 parameters for shading correction. Using a custom built GUI, image series were manually
542 inspected for 1) missing or damaged tissue and 2) mispositioning, including flipping and rotation.
543 Image series with severe artifacts in either the GPe or STN were consequently removed from the
544 study. Human experts manually annotated the locations of the injection sites to encompass the
545 cell bodies of labeled neurons, and a morphological operation was performed to generate a
546 smooth binary injection mask. The injection volume of the filtered dataset ($n = 55$) averaged
547 $0.1403 \pm 0.0797 \text{ mm}^3$ ($0.0546 \pm 0.031 \text{ mm}^3$ within the GPe).

548 The custom signal detection algorithm was applied to each image to extract positive
549 signals from the background autofluorescence. The autofluorescence distribution of each image
550 series was estimated from the non-signal regions contralateral to the injection site, and the mean
551 was subsequently subtracted from the image. A median filter (3 x 3) was applied to reduce the
552 noise. Candidate signal objects were first detected using the autofluorescence intensity profile
553 and classified into three groups based on the intensity. Low- and medium-intensity objects were
554 combined with the detection result from the edge detection filter ($k_{\text{width}} = 10, 30$). The binary signal
555 masks generated from the detection results were used to classify each pixel as either signal or
556 background, and the density within a $10 \mu\text{m} \times 10 \mu\text{m}$ grid was computed.

557 Images were first pre-aligned sequentially to minimize the inter-sectional difference in the
558 DAPI signal. Stacked image volumes were aligned to the Allen Common Coordinate Framework
559 (CCF v3) (Wang et al., 2020) via iterative 3D global and two-dimensional (2D) slice affine
560 alignment using elastix (Klein et al., 2010). A population average model from 35 aligned volumes
561 was generated following the Allen informatics data pipeline (Kuan et al., 2015), then registered to
562 the CCF v3 using the Symmetric Normalization (SyN) transformation model (Avants et al., 2008)
563 from Advanced Neuroimaging Tools (ANTs, <https://github.com/ANTsX/ANTs>). 3D deformable
564 transformation from the population-averaged model to the CCF v3 was applied to each DAPI-
565 based volume and the corresponding signal image was used to generate a $25 \mu\text{m}$ isotropic 3D

566 registered volume. Injection and projection density signals within the GPe and STN were
567 segmented using 3D region annotation from the Allen Mouse Brain Atlas and stored in a
568 vectorized form. Signals in the GPe were downsampled to an isotropic voxel-size of 50 μm to
569 facilitate computation. Signal volumes were computed as the sum of the signal density within the
570 target structure, multiplied by the voxel size.

571 To limit false positive projection signals, image series with (1) low injection or projection
572 volume ($< 0.001 \text{ mm}^3$) or (2) ectopic injections at the central nucleus of the amygdala (CeA) or
573 substantia innominata (SI) were removed.

574

575 **Hyperdirect projection data from the AMBCA**

576 Using the API for the Allen Mouse Brain Connectivity Atlas (AMBCA, [https://connectivity.brain-](https://connectivity.brain-map.org)
577 [map.org](https://connectivity.brain-map.org)), we downloaded the dataset of isocortical injections with GPe or STN projections ($n_{\text{gpe}} =$
578 1270 , $n_{\text{stn}} = 851$, at the time of our analysis). The voxel resolution for isotropic volume was set to
579 50 μm for injections and the GPe projection density, and 25 μm for the STN projection density.
580 Injection sites were inspected to remove experiments in which more than 10% of the injection
581 volume was located outside the isocortex. The minimum projection volume for both the GPe and
582 STN was set to 0.001 mm^3 . We included nine transgenic Cre-lines with at least five datasets
583 (Table S3) in addition to a dataset for wild-type C57BL/6J, which result in 176 datasets in total
584 (181 for HP_{STN}).

585 Trainable WEKA Segmentation (http://fiji.sc/Trainable_Weka_Segmentation) plugin for
586 Fiji was used to identify and subtract passing fibers in the GPe and STN. The WEKA
587 Segmentation model was trained using set of manually annotated labeled images containing
588 labels for 1) densely populated thick fasciculated axon fibers, 2) fine axon projections, and 3)
589 background. Among 86 datasets with densely populated projection signal (density > 0.5) in GPe,
590 25% were randomly selected for training dataset ($n_{\text{train}} = 22$). Selection of filters and parameters
591 were same as described in Hunnicutt *et al.*(Hunnicutt et al., 2016). After training, the classifier was
592 applied to remaining dataset and generated a probability map for each label. To minimize the

593 artifacts from thick fasciculate fibers, conservative segmentation criteria was set to subtract as
594 much passing fiber signal as possible. Default classification threshold was set to $P_{fiber} > 0.25$ for
595 fasciculated fiber, and $P_{signal} > 0.7$ for axon signal classification. Custom built GUI in MATLAB was
596 used to inspect the classification result, and to manually tune the segmentation threshold for each
597 data if needed.

598

599 **Single-neuron reconstruction data from the MouseLight database**

600 Single neuron reconstruction data were obtained from the MouseLight database using Neuron
601 Browser (<http://www.mouselight.janelia.org>). The search query was set to include only neurons
602 for which the soma was located within the isocortex and any axonal structures were in either the
603 GPe or STN (**Figure S5B**), which resulted in 142 reconstructed neurons. Neurons whose soma
604 was located in the left hemisphere were mirrored onto the right hemisphere. Reconstructed
605 neurons without ipsi-lateral projections to the GPe or STN were removed, and the remaining
606 neurons ($n = 69$) were classified by projection pattern: (1) IT types showing contralateral
607 projections ($n = 24/69$); (2) Collateral $HP_{STN/GPe}$ ($n = 10/69$); (3) HP_{STN} only ($n = 15/69$); and (4)
608 HP_{GPe} only ($n = 20/69$).

609 Normalized projection density was computed as the length of axons within the region
610 normalized by the region volume. To visualize the topographical organization (Figure S5D), only
611 neurons with axonal projections longer than 100 μm in both the GPe and STN were shown.

612

613 **Analysis of mouse hyper/indirect pathway topographic organization**

614 The projection centroid was defined as the weighted centroid of the 3D density signal within a
615 target region. The distance between two projections was measured using the pairwise cosine
616 distance between two vectorized projections. STN geometric axis1 and axis2 were defined as the
617 principal axis and its perpendicular, respectively, in a 2D projection of the STN along the AP axis.

618 For the signal distribution heatmap, reconstructed signals were digitally resliced along a
619 designated axis and the fraction of signal volume within each slice to the total signal volume was

620 computed. The similarities between signal distributions were measured using the Pearson
621 correlation between the signal centroid location along each direction.

622 We applied classical multi-dimensional scaling (MDS) (Kruskal and Wish, 1978) to
623 visualize the pairwise projection distance relationships between STN projections in the “pattern
624 space”. Robust Canonical Correlation Analysis (rCCA) (Branco et al., 2005) was performed to
625 identify the graded topographic organization between the locations of each projection in the
626 pattern space and the injection centroid in the anatomical space. MDS embeddings were then
627 rotated so that the major axes corresponded to the canonical variates. Gradient axes in the cortex
628 and the GPe corresponding to the STN geometric axes were computed via ridge regression on
629 the STN projection centroid location along the gradient axis and input centroid coordinates.

630 Injections for which the majority (> 80%) of the injection volume was contained within the
631 primary injection structure were classified as localized injections and used to investigate
632 projection patterns from a single cortical subregion. Projection density signals from localized
633 injections into the target structure were rescaled to [0, 1], then averaged to generate a
634 characteristic projection. Note that the resulting characteristic projection may reflect only the
635 spatial pattern of the projection, and its intensity may not be comparable to characteristic
636 projections from other regions.

637 The projection power of a functional subregion, which represents an estimated projection
638 volume assuming the subregion is fully labeled, was computed using non-negative ridge
639 regression on the projection volume with the vector containing the injection volume within each
640 subregion normalized by its volume, as a regressor.

641 The topographic relationship between IP_{STN} - IP_{INPUT} was modeled using a multivariate
642 linear model with the IP_{STN} centroid locations along two gradient axes as observations and the
643 IP_{INPUT} injection centroids as predictors. The same model was assessed using HP_{STN} centroids as
644 inputs and HP_{GPe} centroids as targets. Error was measured according to the distance between the
645 actual and predicted HP_{GPe} centroids. Permutation tests were used to compare the means of
646 observed error against the error from 10,000 randomizations, where for each HP_{STN} , the

647 corresponding HP_{GPe} was reassigned by shuffling. Two-sided t-tests were used to compare the
648 means of the two groups.

649 In addition, to test whether nearby cortical injections targeting different functional
650 subregions resulted in clearly segregated STN projection patterns, we measured the STN
651 projection pattern distances between pairs of experiments using the following criteria: 1) the two
652 injection volumes are localized within different functional tripartite cortical subregions; 2) the
653 injection volumes exhibit no overlap in the reference space; 3) the distance between injection
654 centroids is greater than 1 mm and less than 1.5 mm. We compared the distribution and mean of
655 the STN projection distances from pairs of nearby injections targeting different subregions and
656 pairs with the same input subregion using two-sided t-tests.

657

658 **Serial Single-Molecule Fluorescence In Situ Hybridization**

659 Mouse brains were collected and immediately frozen in -80 °C 100% ethanol. Coronal sections
660 (20 µm) containing the STN were collected using a cryostat (Leica) and mounted on SuperFrost
661 microscope slides (FisherBrand). Serial smFISH was performed using the RNAscope multiplex
662 fluorescent kit (ACD bio, 320850) according to the manufacturer's protocols. Brain sections were
663 fixed in 4% PFA and dehydrated serially in ethanol (50%, 70%, 100%, and 100%). Sections were
664 incubated in proteinase IV and rinsed with PBS. Probes were applied for 2 h at 40 °C and
665 amplifiers 1–4 were subsequently applied at 40 °C. We used probes for *Aqp4* (catalog no.
666 417161), *Gabra1* (435451), *Gria1* (421931), *Mbp* (451491), *Pvalb* (421931), *Rbfox3* (313311),
667 *Slc17a6* (319171), *Slc32a1* (319191), and *Tmem119* (472901). Sections were counterstained
668 with DAPI.

669 A nucleus detection pipeline, as previously described (Feng et al., 2019), was trained on
670 30 images containing DAPI-stained cell nuclei (~180 per image) that were manually labeled by
671 human experts. To detect mRNA puncta, we used a splitting strategy with a marker-controlled
672 watershed approach and a variational Bayesian Gaussian mixture model (Feng et al., 2012). The

673 detected mRNA puncta were assigned to the nearest detected nuclei with a maximum distance of
674 1 μm .

675 Human experts manually registered each smFISH image section to a corresponding slice
676 in Allen CCF v3, using a GUI that was custom designed in MATLAB, via rigid transformation
677 (reflections, translation, and rotations). Support Vector Machine was then used to determine the
678 threshold for positive cell classification. A classification model was trained on 30 randomly
679 selected and manually labeled cells per image section, using the number, size, and intensity of
680 puncta as the input.

681

682 **Electrophysiological recording**

683 Mice were anesthetized with halothane and transcardially perfused with an ice-cold dissection
684 solution containing (in mM): 228 Sucrose, 2.5 KCl, 7 MgSO₄, 0.5 CaCl₂, 1.25 NaH₂PO₄, 26
685 NaHCO₃, and 11 glucose, oxygenated with 95% O₂ and 5% CO₂. The brains were quickly
686 removed and parasagittal slices (250 μm) containing the STN were prepared using a vibratome
687 (Leica VT 1200S) in cold dissection solution. Slices were then transferred into extracellular
688 solution containing (in mM): 125 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1.25 NaH₂PO₄, 26
689 NaHCO₃, and 11 glucose, oxygenated with 95% O₂ and 5% CO₂, in which they were constantly
690 kept before being moved to the recording chamber which also contained the extracellular
691 solution. Chemicals for electrophysiological analyses were obtained from Sigma or Wako Pure
692 Chemical Industries unless otherwise specified.

693 Whole-cell patch clamp recordings were made from ZsGreen-positive or ZsGreen-
694 negative neurons in the STN of PV-IRES-Cre::Ai6 mice. Patch pipettes (resistance 5-6 M Ω) were
695 filled with a solution containing (in mM): 130 potassium gluconate, 2 NaCl, 4 MgCl₂, 4 Na₂ATP,
696 0.4 NaGTP, 20 HEPES (pH 7.2), and 0.25 EGTA. Recordings in the cell-attached configuration
697 were sometimes conducted before making whole-cell recordings. Neurons in the STN were
698 visualized directly using a BX61WI microscope (Olympus), and those expressing ZsGreen were
699 identified by a FV1000 confocal microscope (Olympus). Membrane potentials were recorded in

700 current-clamp mode either without current injection, or by giving depolarizing (100 pA) or
701 hyperpolarizing (-200 pA) current steps for 500 ms, using a Multiclamp 700B amplifier and
702 pClamp software (Molecular Devices). Data were accepted if the input membrane resistance was
703 > 100 M Ω , firing action potentials had an amplitude of at least 50 mV, and the
704 afterhyperpolarizing potential was < -50 mV. Analyses of electrophysiological parameters were
705 performed using OriginPro (<https://www.originlab.com>) and Clampex
706 (<https://www.moleculardevices.com>) software. The peak of afterhyperpolarizing potential was
707 considered as membrane potential during firing. The parameter values for action potentials were
708 equivalent to a previous study (Beurrier et al., 1999). Statistical significance was evaluated using
709 the Mann-Whitney U test. In this study, to examine two neuronal firing patterns (tonic and burst
710 firing), we classified burst firing according to the presence of, at least, one of the following firing
711 patterns in three sequential recording conditions: 1) When recording without current injection,
712 spontaneous firing shows fast burst firing (< 10 ms inter-spike intervals, ISI, on average) followed
713 by a pause. Similar to the previously reported burst firing in the STN (Beurrier et al., 1999; Huang
714 et al., 2017; Kass and Mintz, 2006), the membrane potential tends to be depolarized during burst
715 firing, and tends to be drastically hyperpolarized at the start of a pause; 2) when recording during
716 a 250-ms period of rebound depolarization following the removal of a hyperpolarizing current
717 injection (-200 pA), the firing frequency is fast (< 10 ms ISI on average) and the membrane
718 potential tends towards depolarization, followed by drastic hyperpolarization (> 15 pA) and
719 quiescence of firing; 3) when recording during a 500-ms depolarizing current injection (100 pA),
720 the firing frequency is fast (< 10 ms ISI on average) and the membrane potential tends towards
721 depolarization at the beginning of a depolarizing current injection, followed by quiescence of firing
722 for more than 50 ms (e.g., the top-right trace in **Figure S9C**). The firing properties used for this
723 classification were compared between burst PV+, tonic PV+, and tonic PV- neurons. We noted
724 that the properties of burst firing neurons generally differed from those of tonic firing neurons and
725 that there was a clear tendency for firing to slow down during bursts, as reflected by the
726 significant difference between the first or second ISI and the last ISI.

727

728 **Human 7T-DTI tractography**

729 T2*-weighted imaging (T2*WI) with 0.2 mm in-plane resolution and diffusion-weighted imaging
730 (DWI) along 64 directions with a b-value of 2000 s/mm² and b0 with 1.8 mm isotropic resolution
731 were obtained from a healthy 30-year-old adult male using a 7.0T MRI scanner (Magnetom,
732 Siemens) and a homemade 8-channel radiofrequency head coil (Gachon University) (Cho et al.,
733 2014). A 3D T1-weighted whole brain scan was obtained for anatomical reference using a 3.0T
734 MRI scanner (Magnetom Verio, Siemens).

735 The DWI data were preprocessed to correct geometric distortions (Oh et al., 2012).
736 Axonal fiber orientation information was calculated using a constrained spherical deconvolution-
737 based diffusion model in the MRtrix3 toolbox (<https://www.mrtrix.org>) (Tournier et al., 2010).
738 Whole-brain streamline tractography was performed using the probabilistic algorithm, iFOD1, as
739 implemented in the MRtrix3 and visualized by track-density imaging (TDI) (Calamante et al.,
740 2010) with a 0.2 mm isotropic resolution. Stacked T2*-WIs were interpolated to an isotropic 0.2
741 mm resolution and then co-registered to the b0 volume image of the DWI data using SPM12
742 (<https://www.fil.ion.ucl.ac.uk/spm/software>). The regions-of-interest, including the STN and GPe,
743 were manually traced using the MRtrix3, and the 3D Slicer (<http://www.slicer.org>) was used for
744 3D modeling. T1-images were registered to the b0 volume image of DWI scans via affine
745 transformation and annotated using the human Brainnetome atlas (Fan et al., 2016), mapped
746 using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu>).

747 Probabilistic constrained spherical deconvolution-based fiber tracking was performed
748 using the iFOD2 algorithm (Oh et al., 2012). Optimal parameters were determined such that the
749 resulting streamlines were visually similar to the combined subset of streamlines filtered
750 according to anatomical constraints (Desmond et al., 1995). An additional five parameter
751 combinations with values close to the selected parameters were also used. Whole-track and end-
752 point TDI maps were computed using tracks that terminated within the STN.

753 The normalized average density of tracks in a region was defined as the average track
754 intensity of all voxels within the TDI map of the target subregion. The spatial overlap was defined

755 as $\frac{\sum_{|A \cap B|} \min(A_i, B_j)}{\sum_{|A \cup B|} \max(A_i, B_j)}$, where A_i and B_j are the voxel intensities from two subregions.

756

757 **Human deep brain stimulation**

758 This study included 26 patients who received bilateral STN DBS implantation between March
759 2005 and December 2008 at the Movement Disorder Center of Seoul National University Hospital
760 (SNUH). The criteria were electrical stimulation of the left STN with a mono-polar electrode
761 configuration and follow-up period of more than 3 years.

762 Pre-operative MRI images were obtained using a 1.5 T Signa MRI system (General
763 Electric Medical Systems). STN DBS implantation was performed using a Leksell frame and
764 SurgiPlan with microelectrode recording and macro-stimulation under local anesthesia, as
765 previously described (Kim et al., 2009, 2010). The dorsolateral portion of the STN was targeted
766 using MRI images, with theoretical STN coordinates (4 mm anterior from the midcommissural
767 point, 10–12 mm lateral from the midline, and 3–4 mm below the intercommissural line).
768 Permanent DBS electrode (Medtronic, DBS 3389) placement was determined to avoid damage to
769 adjacent vessels, ventricles, and sulci.

770 Three-dimensional spiral stereotactic CT scans (Philips, Brilliance CT 64-channel) with 1
771 mm thick slices were taken more than 1 month after surgery as previously described (Paek et al.,
772 2008). The post-operative CT images were co-registered to pre-operative MRI images that were
773 normalized to the ICBM 152 2009b nonlinear asymmetric T2 template (Fonov et al., 2011). The
774 electrode trajectories were pre-reconstructed from post-operative CT images and manually
775 adjusted. All normalization and registration processes were done using Lead-DBS (Horn and
776 Kühn, 2015). Electrode trajectories were plotted onto images from the Schaltenbrand and
777 Wahren human brain atlas for 2D visualization (Schaltenbrand and Wahren, 1998).

778 Neurologists evaluated the patients using the Unified Parkinson Disease Rating Scale
779 (UPDRS) before and at 12, 24, 36, and 60 (if applicable) months after surgery. Motor symptom
780 improvement was measured as a post-operative change in the summed UPDRS motor score
781 (UPDRS III) of the right limb.

782

783 **Data Availability**

784 The datasets generated and analyzed supporting the findings of this study are available from the
785 corresponding author upon reasonable request.

786

787 **Code availability**

788 Custom code used in this study are available from the corresponding author upon request.

789

790

791 **Supplemental Information**

792 **9 Figures; 6 Tables; 2 Movies**

793

Data	Title	Related to
Figure. S1	Mesoscale connectivity data processing pipeline	Figure 1
Figure. S2	Overview of individual indirect pathway dataset	Figure 1
Figure. S3	Graded topographic organization of indirect pathway	Figure 1
Figure. S4	Characteristics of hyperdirect pathway dataset	Figure 2
Figure. S5	Topographic organization of hyperdirect pathway	Figure 3
Figure. S6	Topographic analysis of human hyperdirect pathway using 7T-MRI	Figure 3
Figure. S7	Cellular composition of the STN by serial-smFISH	Figure 4
Figure. S8	Electrophysiological properties of STN PV+ neurons	Figure 5
Figure. S9	Electrode position-dependent therapeutical effects in clinical DBS trials	Main text
Table S1	GPe injection summary	Figure 1
Table S2	Abbreviations	Figure 2
Table S3	List of transgenic Cre-lines used for hyperdirect pathway	Figure 2
Table S4	7T-MRI parameters	Figure 3
Table S5	Electrophysiological parameters of STN PV+/- neurons	Figure 5

Table S6	Firing properties of burst and tonic firing neurons	Figure 5
Movie S1	Aligned and registered 3D reconstructed whole brain slices using optimized mesoscopic mapping pipeline	Figure 1
Movie S2	High resolution imaging and reconstruction of cleared STN of PV-IRES-Cre::Ai6 mice	Figure 5

794

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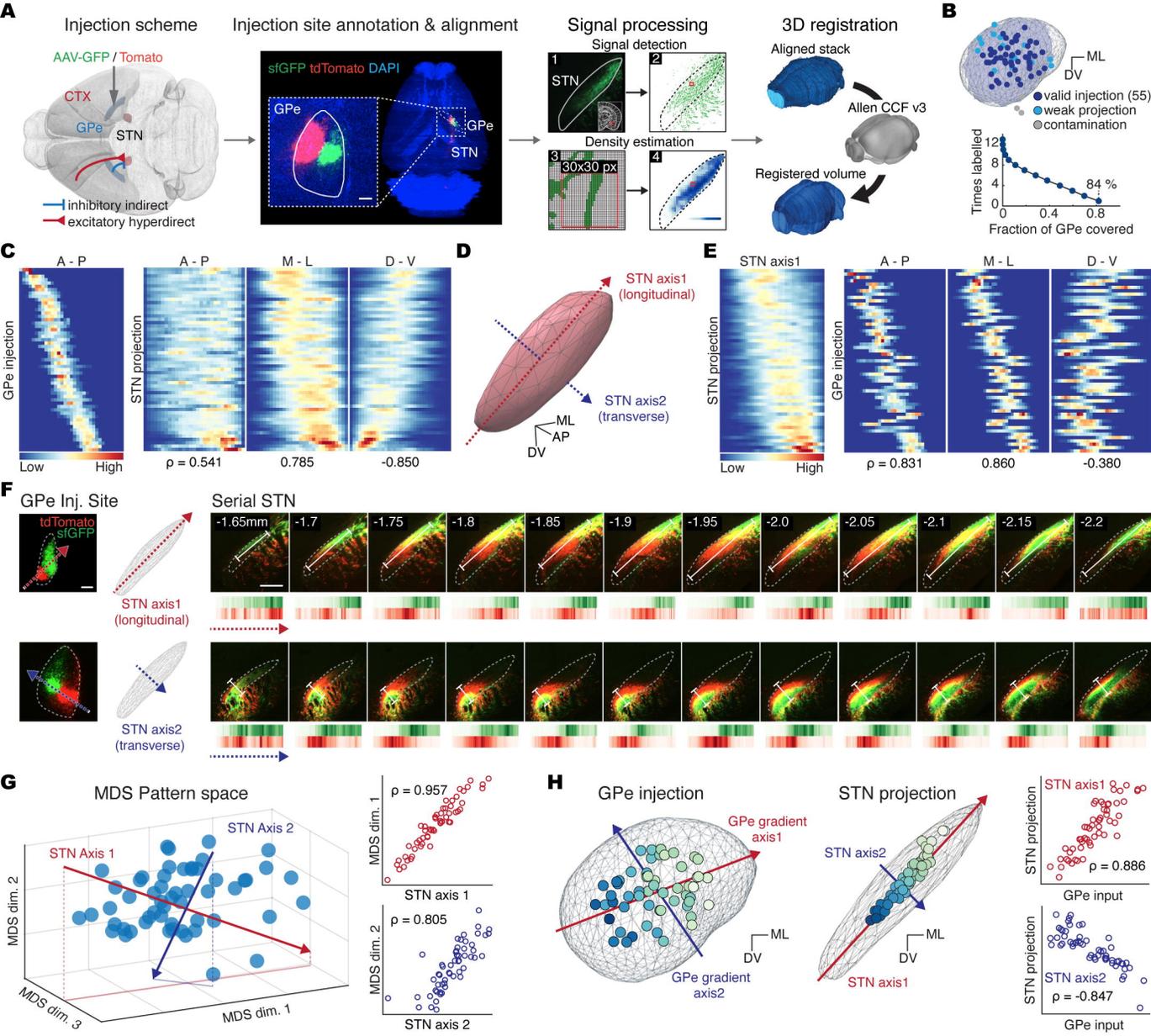
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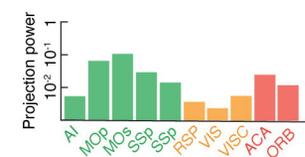
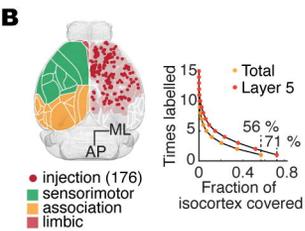
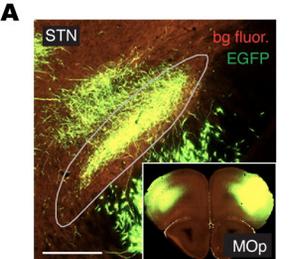
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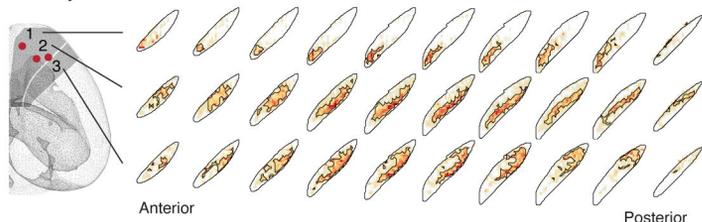
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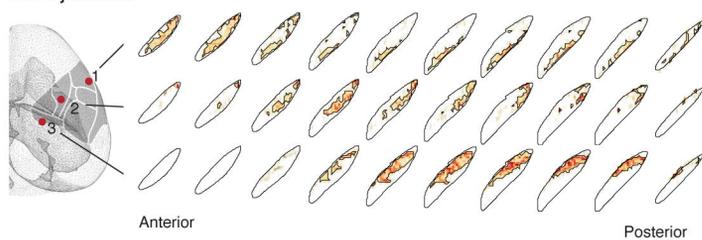


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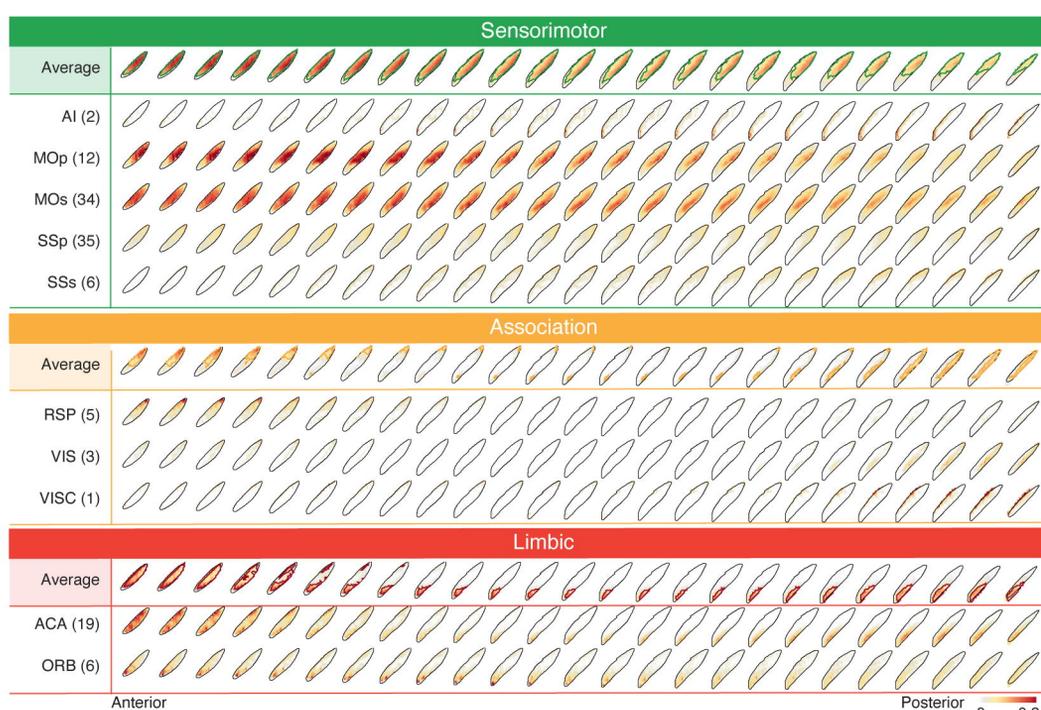
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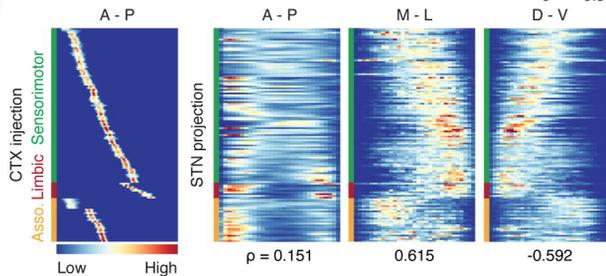
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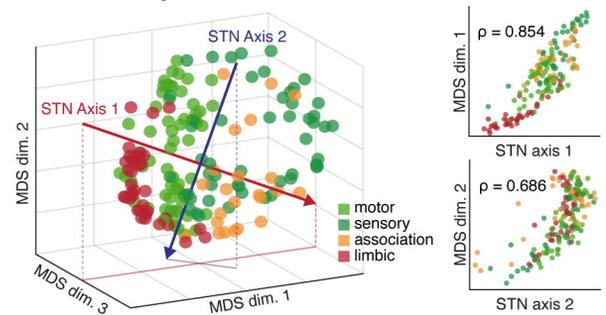
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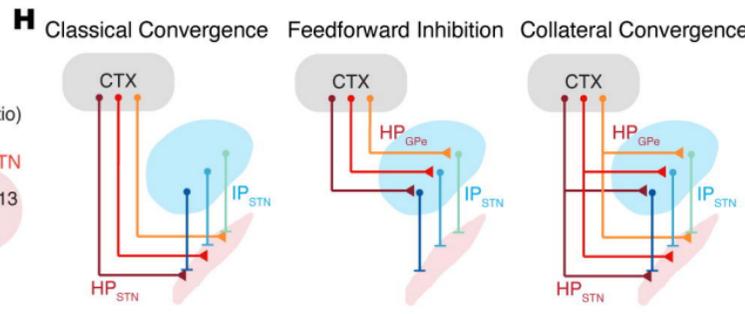
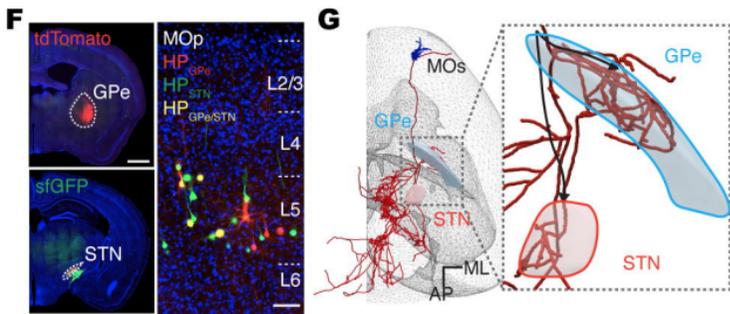
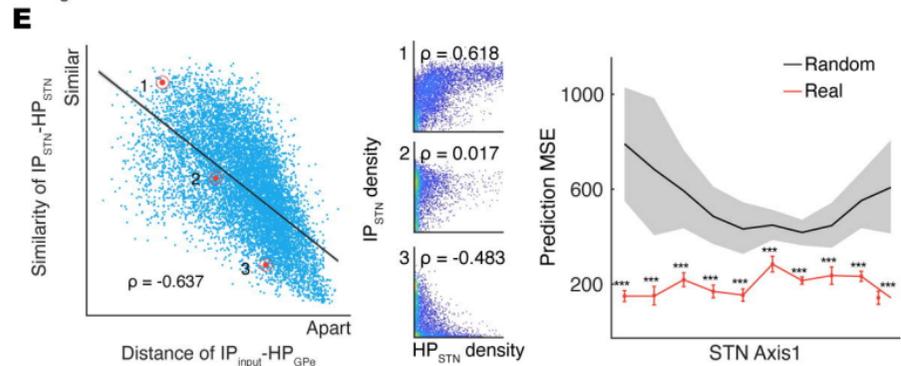
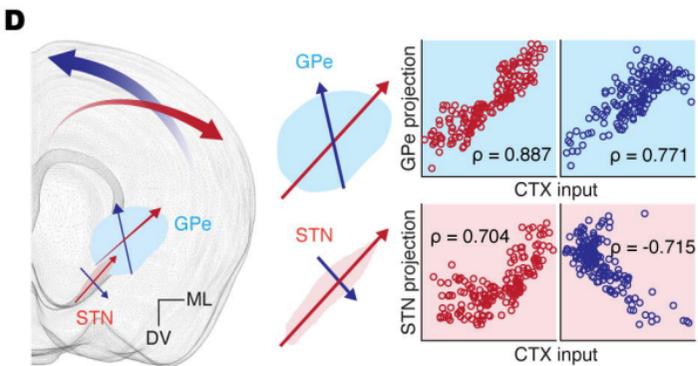
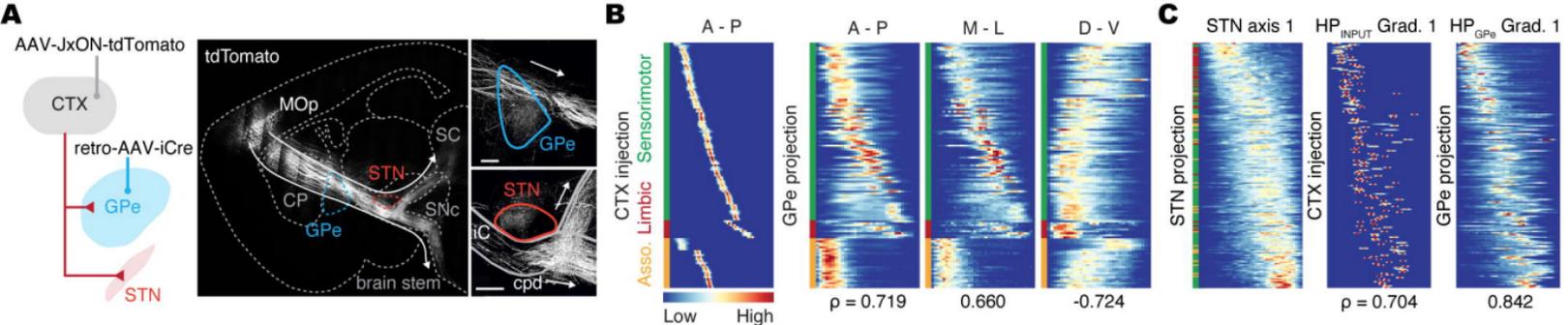


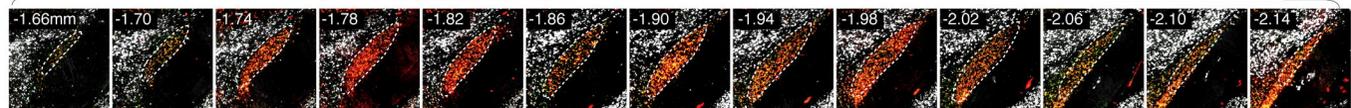
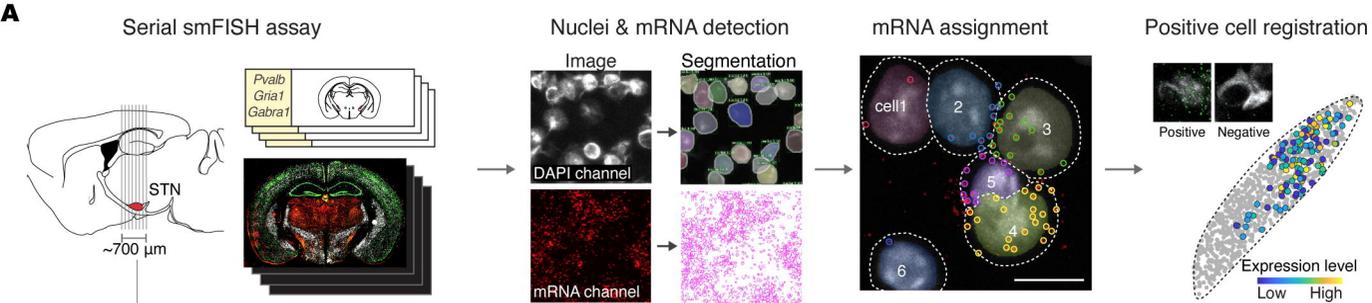
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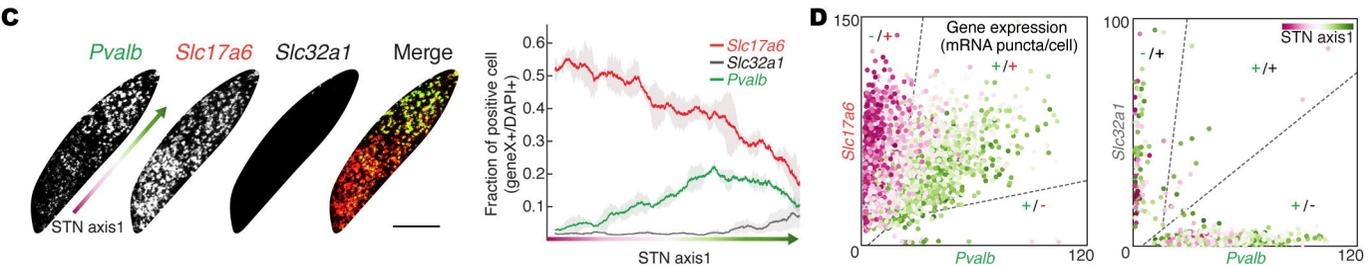
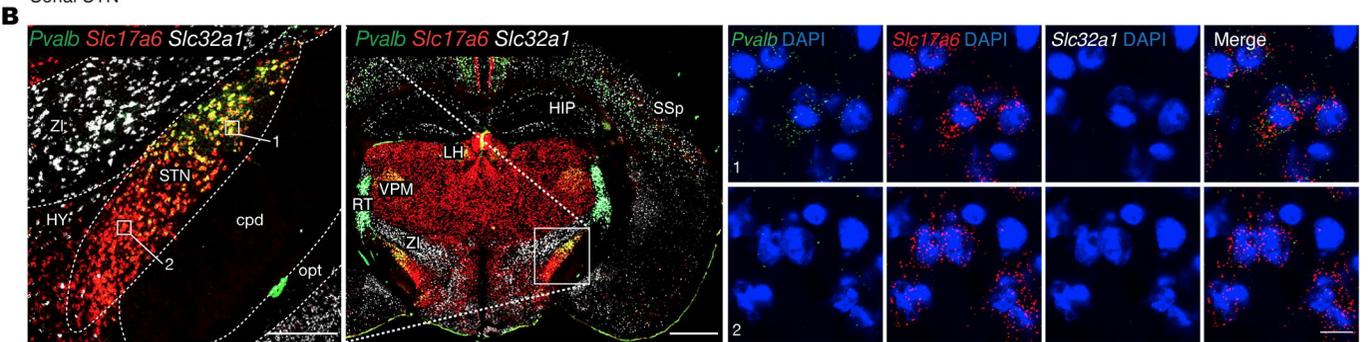
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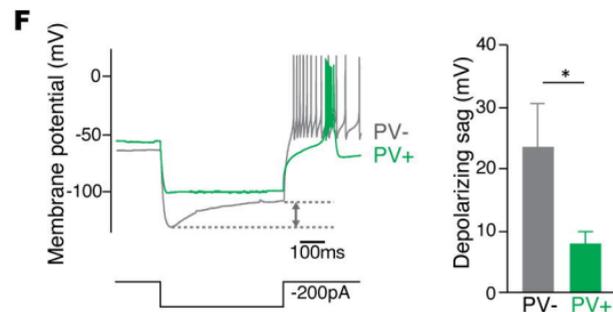
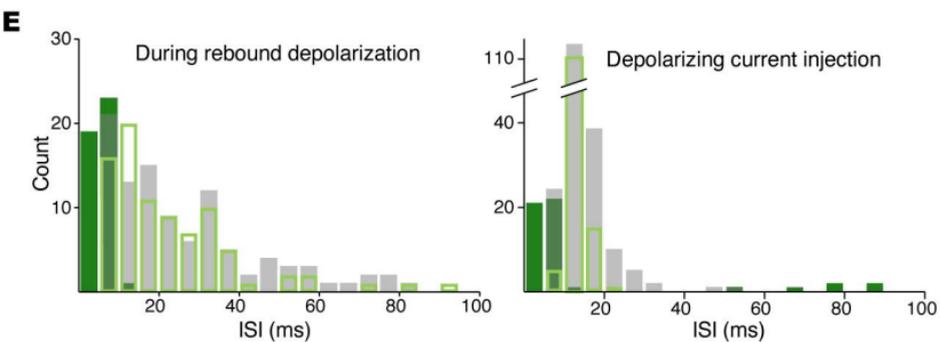
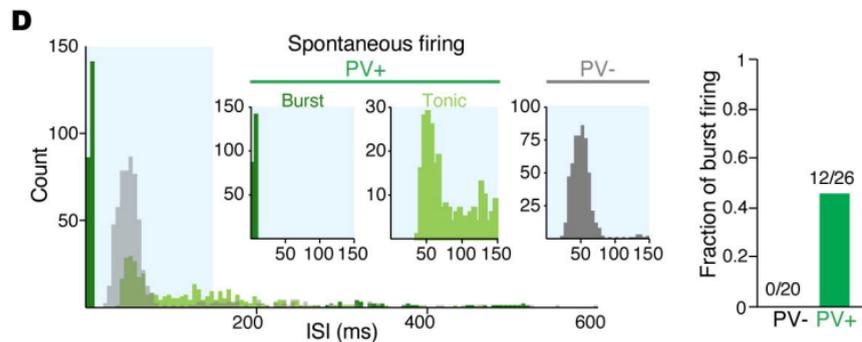
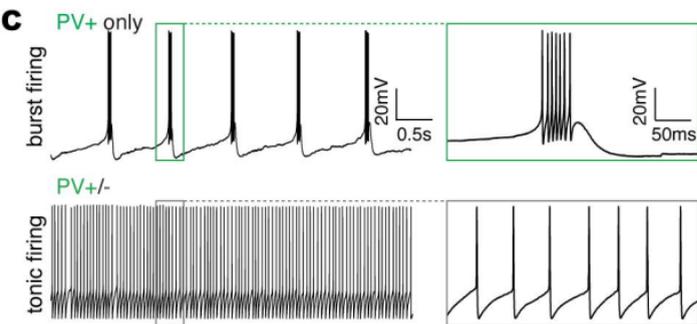
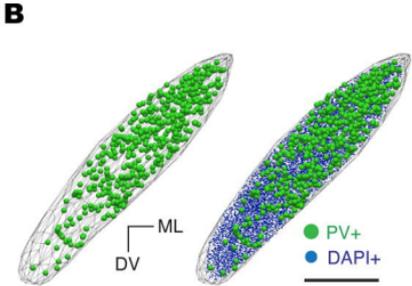
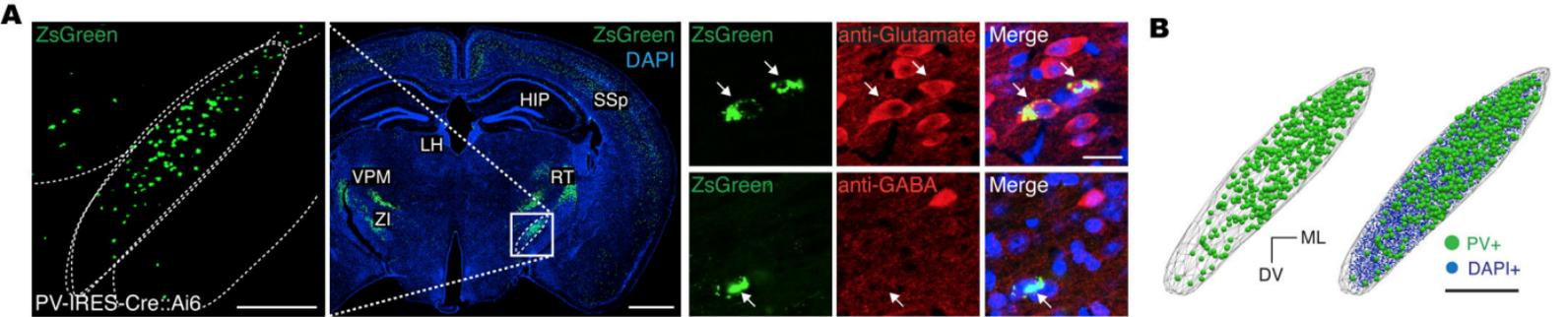


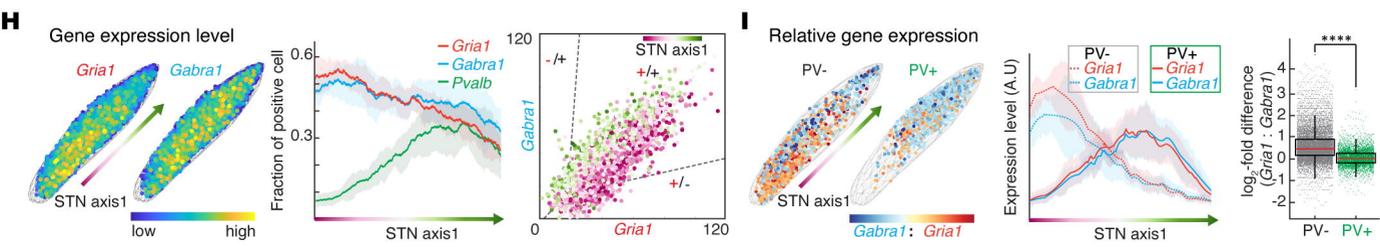
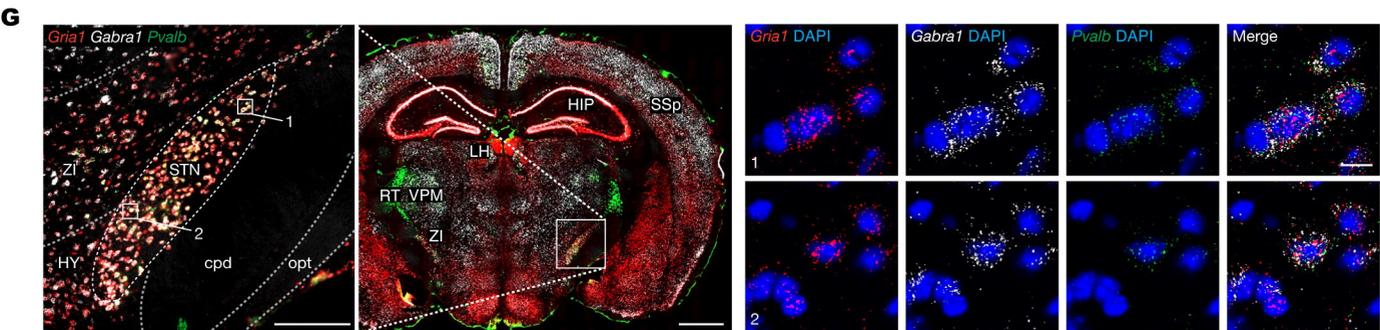
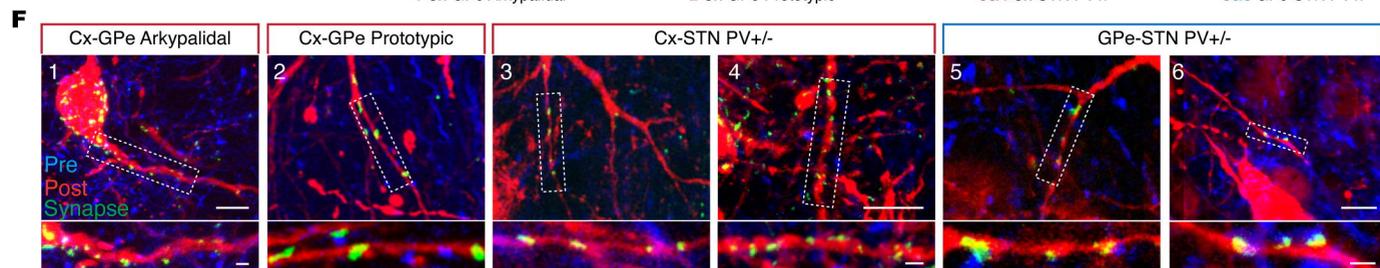
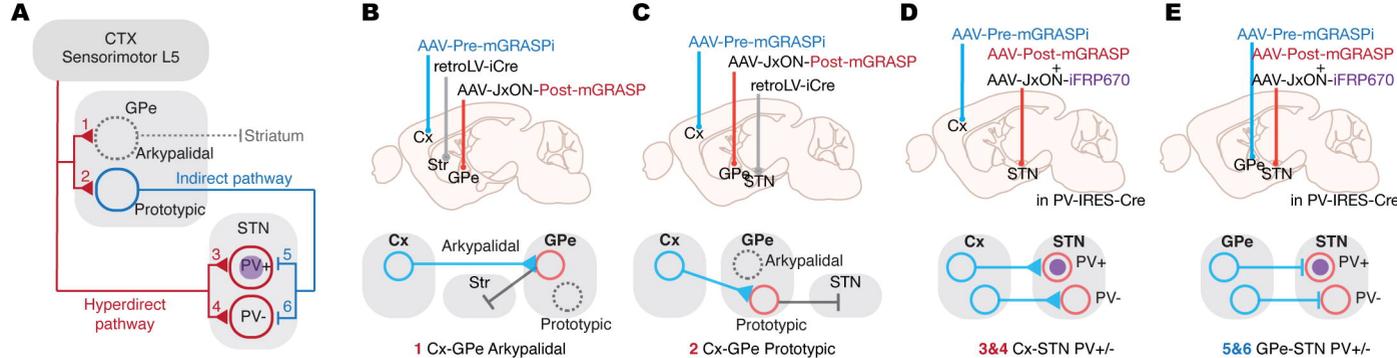


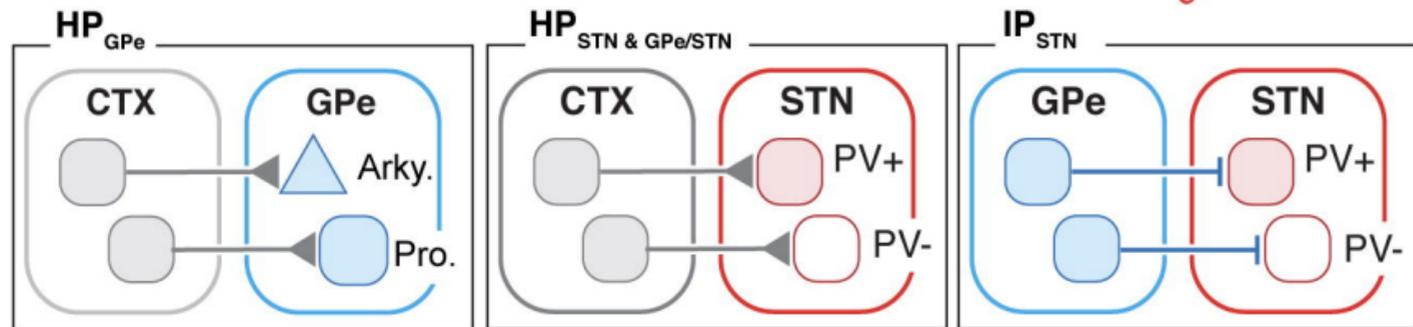
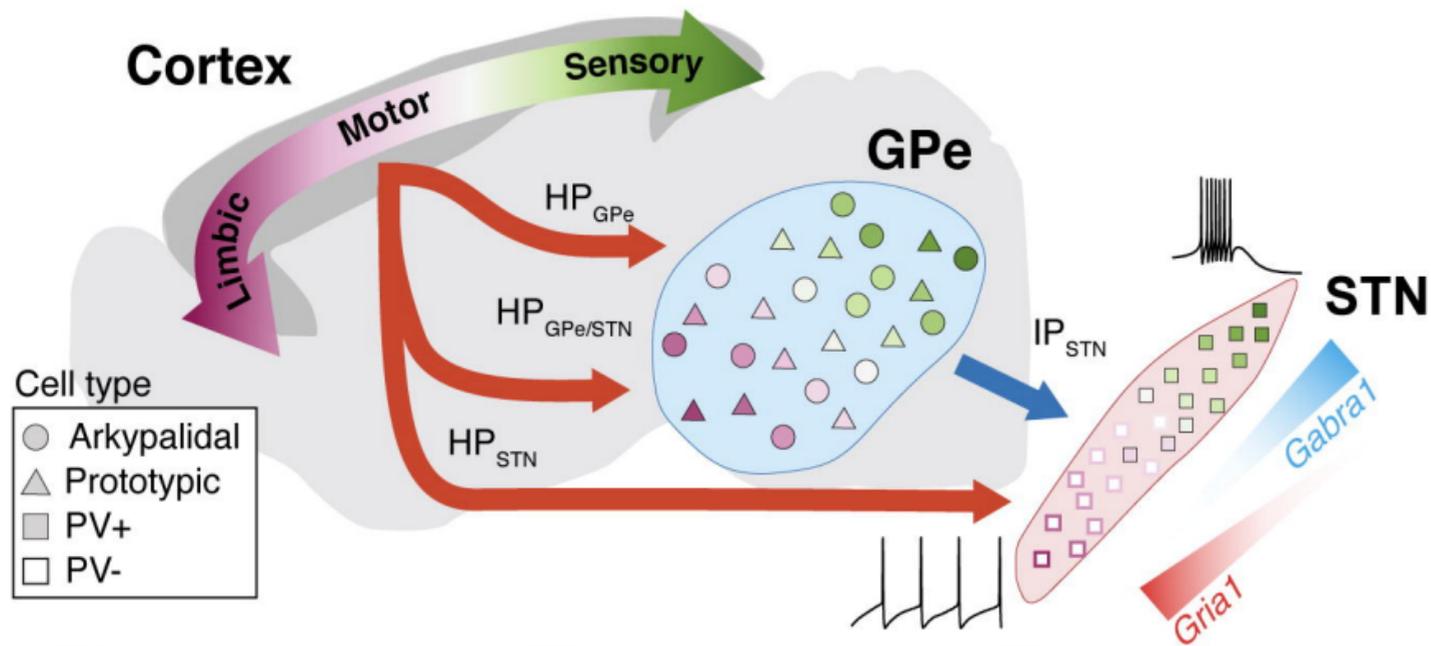


Serial STN







A**B**