Title: Disrupted basal ganglia output during movement preparation in hemi-parkinsonian mice accounts for behavioral deficits

Abbreviated title: BG output accounts for hemi-parkinsonian deficits

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Abstract

Parkinsonian motor deficits are associated with elevated inhibitory output from the basal ganglia (BG). However, several features of Parkinson's disease (PD) have not been accounted for by this supra-inhibition framework, including the potentially therapeutically-relevant observation that movements guided by external stimuli are less impaired than otherwise-identical movements generated based on internal goals. Is this difference due to divergent processing within the BG itself, or to the recruitment of extra-BG pathways by sensory processing? In addition, surprisingly little is known about precisely when, in the sequence from selecting to executing movements, BG output is altered by PD. Here, we address these questions by recording activity in the SNr, a key BG output nucleus, in hemiparkinsonian (hemi-PD) mice performing a well-controlled behavioral task requiring stimulus-guided and internally-specified directional movements. We found that hemi-PD mice (n = 5, male) exhibited a bias ipsilateral to the side of dopaminergic cell loss, consistent with supra-inhibition of contralateral movements by BG output, and that this bias was stronger when movements were internally specified rather than stimulus guided, consistent with clinical observations in parkinsonian patients. We further found that changes in SNr activity during movement preparation could account for the ipsilateral behavioral bias, as well as its greater magnitude for internally-specified movements. These results suggest that parkinsonian changes in BG output underlying movement preparation contribute to the greater deficit in internally-specified in comparison to stimulus-guided movements.

61 **Significance Statement**

62 Parkinsonian patients exhibit the intriguing phenomenon that movements guided by
63 external stimuli are often less impaired than otherwise-identical movements generated based
64 on internal goals. For example, patients can exhibit a more normal gait when their steps are
65 guided by patterned floor tiling than when traversing a featureless floor. Whether this
66 difference in movement execution is due to distinct processing intrinsic to the basal ganglia
67 (BG) or to compensation from other motor pathways is an open question with therapeutic
68 implications. We addressed this question by recording BG output during behavior in a
69 parkinsonian mouse model. We found that mice exhibited greater impairment in internally-
70 specified than stimulus-guided movements, and that differences in BG output during
71 movement preparation could account for this effect.

72

Introduction

Parkinson's disease (PD) is a neurodegenerative disease of the basal ganglia (BG) in which motor impairments arise from disordered – typically, elevated – inhibitory BG output resulting from the loss of dopaminergic tone (DeLong, 1990; Wichmann et al., 1999; Ibanez-Sandoval et al., 2007; Utter and Basso, 2008; Wang et al., 2010a; Seeger-Armbruster and von Ameln-Mayerhofer, 2013; Brazhnik et al., 2014; Filyushkina et al., 2019; McGregor and Nelson, 2019). One predominant theoretical framework for BG pathology in PD is the "rate model", which posits that motor centers downstream of the BG are over-inhibited, leading to disordered movements (Albin et al., 1989; DeLong, 1990; Obeso et al., 2008; Utter and Basso, 2008; McGregor and Nelson, 2019; Vitek and Johnson, 2019). However, recent studies have revealed parkinsonian phenomena that the rate model would not predict, including the intriguing clinical observation that not all forms of movement are equally affected by PD: when movements are guided by external stimuli (e.g., gait matching with a rhythmic auditory stimulus or visually patterned flooring, kinematics are less impaired than for otherwise identical movements made in the absence of guiding stimuli (Glickstein and Stein, 1991; McIntosh et al., 1997; Ballanger et al., 2006; Daroff, 2008; McDonald et al., 2015; Distler et al., 2016). The primary question raised by this observation is whether parkinsonian BG output is similarly disrupted for these "stimulus-guided" and "internally-specified" movements. If so, we might infer that stimulus-guided movements are protected from PD via the recruitment of extra-BG pathways (Lewis et al., 2007; Hackney et al., 2015; Drucker et

94 al., 2019; Filyushkina et al., 2019; Chen et al., 2020). However, differences in parkinsonian
95 BG output between these forms of movements would implicate BG processing itself in this
96 behavioral phenomenon. Given that understanding the neural basis for this clinical
97 observation could be leveraged to improve treatment for PD, we sought to develop a suitable
98 experimental paradigm for examining parkinsonian BG output during stimulus-guided and
99 internally-specified movements.

100 We specifically sought to focus on parkinsonian BG output during movement
101 *preparation*, a key motor phase in which sensory and cognitive variables are integrated
102 (Cisek and Kalaska, 2010), and disruption of which is thought to underlie bradykinetic
103 parkinsonian movement (Dick et al., 1984; Jahanshahi et al., 1992; Suri et al., 1998; Berardelli
104 et al., 2001; Cutsuridis and Perantonis, 2006; Moroney et al., 2008; Wu et al., 2015; Hess and
105 Hallett, 2017). Under normal conditions, the SNr, a BG output nucleus, is strongly engaged
106 by the preparation of directional movements (Handel and Glimcher, 1999; Sato and
107 Hikosaka, 2002; Lintz and Felsen, 2016). However, while numerous studies have examined
108 parkinsonian changes in SNr activity under passive conditions or rhythmic locomotion
109 (Hutchison et al., 1994; Wichmann et al., 1999; Galati et al., 2010; Wang et al., 2010a;
110 Seeger-Armbruster and von Ameln-Mayerhofer, 2013; Brazhnik et al., 2014; Lobb and
111 Jaeger, 2015; Aristieta et al., 2016; Willard et al., 2019), this approach is insufficient for
112 differentiating how SNr activity during distinct phases of movement, including preparation,
113 is affected by PD. To address this question, we therefore recorded SNr activity during a
114 behavioral task in which mice with unilateral dopaminergic cell loss prepare, and

115 subsequently initiate, SNr-engaging directional (left or right) movements that are either
116 stimulus-guided or internally-specified (Uchida and Mainen, 2003; Thompson and Felsen,
117 2013; Lintz and Felsen, 2016). Crucially, by requiring that mice wait for a go signal before
118 initiating their movement, movement preparation is temporally isolated from initiation,
119 allowing us to dissociate the effects of PD on BG output underlying these processes.
120 We found that mice exhibited a directional bias ipsilateral to the hemisphere with
121 dopaminergic cell loss, consistent with rate model predictions, and that this bias was more
122 prominent on internally-specified than stimulus-guided trials, accordant with clinical
123 observations. Furthermore, we found that SNr activity during movement preparation was
124 altered in a manner consistent with the behavior, suggesting that reorganization of BG
125 processing by dopaminergic cell loss contributes to the greater deficit in performance of
126 internally-specified than stimulus-guided movements.
127
128
129 **Materials and Methods**
130 *Animal subjects*
131
132 All experiments were performed according to protocols approved by the University of
133 Colorado Anschutz Medical Campus Institutional Animal Care and Use Committee. Subjects
134 were male adult C57BL/6J mice (aged 7–14 months at the start of experiments; Jackson Labs)
135 housed in a vivarium with a 12-hr light/dark cycle with lights on at 5:00 am. Food (Teklad

136 Global Rodent Diet No. 2918; Harlan) was available *ad libitum*. Access to water was
137 restricted prior to the behavioral session to motivate performance; however, if mice did not
138 obtain ~1 ml of water during the behavioral session, additional water was provided for ~2–5
139 min following the behavioral session. All mice were weighed daily and received sufficient
140 water during behavioral sessions to maintain >85% of pre-water restriction weight.
141
142 For behavioral analyses, only mice that completed at least 15 pre- and 15 post-surgery
143 sessions were included (hemiparkinsonian (hemi-PD), n = 4; control, n = 4). For
144 electrophysiological analyses, mice were included if well-isolated neurons were recorded
145 during the task (hemi-PD, n = 5; control, n = 4). Some data from control mice were
146 previously published using different analyses than the current study (Lintz and Felsen, 2016).
147 For rotation assay analyses, only mice that completed at least three pre- and three-post
148 surgery rotation assay sessions were included (hemi-PD, n = 5; control, n = 3).
149
150 *Behavioral task*
151
152 Mice were trained on a task requiring stimulus-guided (SG) and internally-specified (IS)
153 movements (Fig. 1) as previously described (Lintz and Felsen, 2016). Briefly, each mouse was
154 water-restricted and trained to interact with three ports (center: odor port; sides: reward
155 ports (Fig. 2A) along one wall of a behavioral chamber (Island Motion). On each trial, the
156 mouse entered the odor port, triggering the delivery of an odor; waited 488 ± 104 ms (mean ±

157 SD) for an auditory go signal; exited the odor port; and entered one of the reward ports (Fig.
158 2A). Premature exit from the odor port resulted in the unavailability of reward on that trial.
159 Odors were comprised of binary mixtures of (-)-carvone ("Odor A") and (+)-carvone ("Odor
160 B"). On each SG trial, one of seven odor mixtures was presented via an olfactometer (Island
161 Motion): Odor A/Odor B = 95/5, 80/20, 60/40, 50/50, 40/60, 20/80, or 5/95. Mixtures in which
162 Odor A > Odor B indicated reward availability only at the left port and mixtures in which
163 Odor B > Odor A indicated reward availability only at the right port (Fig. 2B). Since we
164 surgically targeted the left hemisphere in all mice, we refer to Odor A as the "ipsilateral
165 odor" and Odor B as the "contralateral odor" (e.g., Fig. 2C). Similarly, we refer to the
166 directions "left" and "right" as "ipsilateral" and "contralateral", respectively. On trials in
167 which Odor A = Odor B (Odor A/Odor B = 50/50), the probability of reward at the ipsilateral
168 and contralateral ports, independently, was 0.5. Reward, consisting of 4 μl of water, was
169 delivered by transiently opening a calibrated water valve 10–100 ms after reward port entry.
170 Odor and water delivery were controlled, and port entries and exits were recorded, using
171 custom software (available at https://github.com/felsenlab; adapted from C. D. Brody) written
172 in MATLAB (MathWorks).

173 Mice learned to perform SG trials (Fig. 2C) within ~48 sessions (1 session/day);
174 detailed training stages are described in Stubblefield et al. (Stubblefield et al., 2013). Mice
175 required an additional ~5 sessions to learn to perform interleaved blocks of SG and IS trials.
176 On each IS trial the 50/50 mixture was presented, and reward was available only at one side
177 throughout the block (Fig. 2D). Detailed training stages for IS trials are described in Lintz &

178 Felsen (Stubblefield et al., 2013; Stubblefield et al., 2015; Lintz and Felsen, 2016). Mice
179 performed 5 blocks (SG, IS, SG, IS, SG) per session (Fig. 2B); the side associated with reward
180 switched between each IS block. Upon completing training, mice performed at least 15
181 sessions to establish pre-surgery baseline behavior, underwent surgery (see below), and
182 subsequently resumed task performance (post-surgery behavior; Fig. 1).
183
184 *Rotation assay*
185
186 The direction of spontaneous movement was assessed before and after surgery using a
187 standard rotation assay (Ungerstedt, 1976; Smith et al., 2012). Following intraperitoneal (i.p.)
188 administration of d-amphetamine (2.5 mg/kg, Sigma), mice were placed in a transparent
189 beaker with a diameter of 11.5 cm. Mice were monitored for the next 90 mins and behavior
190 recorded using an overhead camera. Rotations were analyzed from 10 to 30 mins post i.p.
191 injection. A rotation score was calculated by counting the total number of complete
192 ipsilateral (left) rotations and subtracting the total number of complete contralateral (right)
193 rotations (Fig. 3B). Repeated testing was carried out with at least 1 week between d-
194 amphetamine injections to allow for recovery.
195
196 *SNc surgery – Unilateral 6 OHDA and saline injections*
197

198 The mouse was anesthetized with isoflurane and secured in a stereotaxic device, the
199 skull was exposed with a midline incision, and a craniotomy targeting the left SNc was
200 performed, centered at 3.07 mm posterior from bregma, 1.250 mm lateral from the midline,
201 and 4.35 mm deep from cortical surface (Paxinos and Franklin, 2004). Injection volume
202 totaled 2 μL injected at target (2.43 mg 6-OHDA/mL 0.02% ascorbic acid). After suturing the
203 incision, a topical triple antibiotic ointment (Major) mixed with 2% lidocaine hydrochloride
204 jelly (Akorn) was applied to the scalp, the mouse was removed from the stereotaxic device,
205 the isoflurane was turned off, and oxygen alone was delivered to the mouse to gradually
206 alleviate anesthetic state. Mice were administered sterile isotonic saline (0.9%) for
207 rehydration and an analgesic (Ketofen; 5 mg/kg) for pain management. Analgesic and topical
208 antibiotic administration was repeated daily for up to 5 days, and mice were closely
209 monitored for any signs of distress.
210 This procedure was identical for control mice assessed on the rotation assay but with
211 saline injected instead of 6-OHDA. These mice did not undergo further surgery and were
212 used solely for rotation assay testing (Fig. 3B).
213
214 *SNr surgery – tetrode implantation*
215
216 Details of the surgical procedure are provided in Thompson and Felsen (Thompson
217 and Felsen, 2013). Briefly, following establishment of pre-surgery baseline behavior and (in
218 hemi-PD mice) following unilateral 6-OHDA injections (Fig. 1), the mouse was anesthetized

219 with isoflurane and secured in a stereotaxic device, the scalp was incised and retracted, 2
220 small screws were attached to the skull, and a craniotomy targeting the left SNr was
221 performed, centered at 3.07 mm posterior from bregma and 1.25 mm lateral from the midline
222 (Franklin and Paxinos, 2004). A VersaDrive 4 microdrive (Neuralynx), containing 4
223 independently adjustable tetrodes, was affixed to the skull via the screws, luting (3M), and
224 dental acrylic (A-M Systems). A second small craniotomy was performed in order to place
225 the ground wire in direct contact with the brain. After the acrylic hardened, a topical triple
226 antibiotic ointment (Major) mixed with 2% lidocaine hydrochloride jelly (Akorn) was
227 applied to the scalp, the mouse was removed from the stereotaxic device, the isoflurane was
228 turned off, and oxygen alone was delivered to the mouse to gradually alleviate anesthetic
229 state. Mice were administered sterile isotonic saline (0.9%) for rehydration and an analgesic
230 (Ketofen; 5 mg/kg) for pain management. Analgesic and topical antibiotic administration was
231 repeated daily for up to 5 days, and mice were closely monitored for any signs of distress.
232
233
234 *Electrophysiology*
235
236 Neural recordings were collected using four tetrodes, wherein each tetrode consisted
237 of four polyimide-coated nichrome wires (Sandvik; single-wire diameter 12.5 μm) gold
238 plated to 0.2–0.4 MΩ impedance. Electrical signals were amplified and recorded using the
239 Digital Lynx S multichannel acquisition system (Neuralynx) in conjunction with Cheetah

240 data acquisition software (Neuralynx). Tetrode depths were adjusted approximately 23 hrs
241 before each recording session in order to sample an independent population of neurons
242 across sessions. To estimate tetrode depths during each session we calculated distance
243 traveled with respect to the rotation fraction of the screw that was affixed to the shuttle
244 holding the tetrode. One full rotation moved the tetrode ~250 μm and tetrodes were moved
245 ~62.5 μm between sessions. The final tetrode location was confirmed through histological
246 assessment (see below).

247 Offline spike sorting and cluster quality analysis was performed using MClust
248 software (MClust-4.3, A.D. Redish, et al.) in MATLAB. Briefly, for each tetrode, single units
249 were isolated by manual cluster identification based on spike features derived from sampled
250 waveforms. Identification of single units through examination of spikes in high-dimensional
251 feature space allowed us to refine the delimitation of identified clusters by examining all
252 possible two-dimensional combinations of selected spike features. We used standard spike
253 features for single unit extraction: peak amplitude, energy (square root of the sum of squares
254 of each point in the waveform, divided by the number of samples in the waveform), and the
255 first principal component normalized by energy. Spike features were derived separately for
256 individual leads. To assess the quality of identified clusters we calculated two standard
257 quantitative metrics: L-ratio and isolation distance (Schmitzer-Torbert et al., 2005). Clusters
258 with an L-ratio of less than 0.75 and isolation distance greater than 6.5 were deemed single
259 units, which resulted in the exclusion of 7% of the identified clusters. Only clusters with few
260 interspike intervals less than 1.5 ms were considered for further examination. Furthermore,

261 we excluded the possibility of including data from the same neuron twice by ensuring that
262 both the waveforms and response properties sufficiently changed across sessions. If they did
263 not, we conservatively assumed that we were recording from the same neuron, and only
264 included data from one session.
265
266 *Immunohistochemistry*
267
268 Mice were overdosed with an i.p. injection of sodium pentobarbital (100 mg/kg) and
269 transcardially perfused with saline followed by ice-cold 4% paraformaldehyde (PFA) in
270 0.1 M phosphate buffer (PB). After perfusion, brains were submerged in 4% PFA in 0.1 M PB
271 for 24 hrs for post-fixation and then cryoprotected for 24 hrs by immersion in 30% sucrose in
272 0.1 M PB. The brain was encased in the same sucrose solution, and frozen rapidly on dry ice.
273 Serial coronal sections (60 μm) were cut on a sliding microtome. Fluorescent Nissl
274 (NeuroTrace, Invitrogen) was used to identify cytoarchitectural features of the SNr and
275 verify tetrode tracks and lesion damage within or below the SNr, as previously described
276 (Lintz and Felsen, 2016). In addition, coronal sections were stained for tyrosine hydroxylase
277 (TH). Following repeated soaks in PBS and blocking solution, sections were exposed to
278 primary antibody overnight (Anti-Tyrosine Hydoxylase (Rabbit) Antibody, 1:1000,
279 Rockland). Next, sections were washed in carrier solution (2x10-min) and exposed to
280 secondary antibody for 2 hrs (Goat anti-Rabbit IgG (H+L) Secondary Antibody, 1:500).
281 Images were captured with a 10x objective lens, using an LSM 5 Pascal series Axioskop 2 FS

282 MOT confocal microscope (Zeiss). For each mouse, a representative coronal section including
283 the SNc (Paxinos & Franklin) was used to quantify dopaminergic cell loss by comparing the
284 number of TH+ neurons ipsilateral and contralateral to the injection. Hemi-PD mice without
285 verified >70% dopaminergic cell loss (5/10), were excluded from the group. Secondary
286 confirmation of dopaminergic cell loss was quantified in the same manner using coronal
287 sections containing the striatum (Fig. 3A).
288
289 *Behavioral directional bias*
290
291 On SG trials, directional bias was quantified using psychometric functions fitted
292 to $p = 1/ 1 + e^{(-a - bx)}$, where x is the proportion of Odor A in the binary odor mixture, p is the
293 fraction of ipsilateral choices, and a and b are the best-fit free parameters. The slope of the
294 function is captured by b, while its directional bias was calculated as Bias = $a/b + 50$ (Felsen
295 and Mainen, 2008). Positive bias values reflect an ipsilateral (leftward) shift (Fig. 4A) relative
296 to a perfectly symmetric psychometric function, and negative values reflect a contralateral
297 (rightward) shift (Fig. 4B). To directly compare directional bias between SG and IS trials
298 within the same session (Fig. 6B), for SG and IS trials separately we calculated the difference
299 between the fraction of trials for which ipsilateral was the correct choice and the fraction of
300 actual ipsilateral choices. Sessions were included in this analysis if the mouse completed at
301 least 25 trials of each direction (ipsilateral and contralateral) and type (SG and IS) trials.
302

303 *Neuronal direction preference*

304

305 We used an ROC-based analysis to quantify the selectivity of single neurons for
306 movement direction (Green DM, 1966; Lintz and Felsen, 2016). This analysis calculates the
307 ability of an ideal observer to classify whether a given firing rate was recorded in one of two
308 conditions (i.e., preceding ipsilateral (left) or contralateral (right) movement). We defined
309 "preference" as $2(ROC_{area} - 0.5)$, a measure ranging from –1 to 1, where –1 signifies the
310 strongest possible preference for ipsilateral, 1 signifies the strongest possible preference for
311 contralateral, and 0 signifies no preference (Feierstein et al., 2006; Lintz and Felsen, 2016).
312 For example, if the firing rate of a given neuron is generally higher preceding ipsilateral than
313 contralateral movements, that neuron is assigned a preference < 0. Statistical significance was
314 determined with a permutation test: we recalculated the preference after randomly
315 reassigning all firing rates to either of the two groups, repeating this procedure 500 times to
316 obtain a distribution of values, and calculated the fraction of random values exceeding the
317 actual value. We tested for significance at $\alpha = 0.05$. Trials in which the movement time
318 (between odor port exit and reward port entry) was > 1.5 s were excluded from all analyses.
319 Neurons with < 25 trials of each trial type under comparison (ipsilateral SG, contralateral SG,
320 ipsilateral IS, or contralateral IS) or with a firing rate < 2.5 spikes/s for either trial type under
321 comparison, were excluded from all analyses.

322

323 *Shift function*

We used a shift function to quantify if and how two distributions differ (Rousselet et al., 2017). Briefly, using a Harrell-Davis quantile estimator (Harrell and Davis, 1982), distributions were divided into 10 equal parts by 9 "deciles." For example, the 1st decile is the value below which 10% of the values lie while the 9th decile is the value below which 90% of values lie. The shift function compares a given decile in distribution A with its corresponding decile in distribution B. Corresponding deciles were determined to be significantly different if the confidence interval of their differences, calculated by sampling the difference between bootstrapped distributions 200 times, did not cross 0.

Activity change during epoch of interest compared to baseline

We calculated the normalized response (NR) for each neuron as NR = Ft/Fb where Ft is the median firing rate in the "test" window (either movement preparation epoch or movement initiation epoch) and Fb is the median firing rate in the "baseline" window. Since the structure of our task does not include a natural "baseline" epoch – i.e., in which the mouse is in a motionless state unaffected by task demands – our baseline window was defined as the time of odor port entry to reward port exit (i.e., the duration of the whole trial; (Lintz and Felsen, 2016). Neurons with Fb < 2.5 spikes/s were excluded from analyses. Statistical significance was determined using a pairwise t-test to compare Ft and Fb from the same trial. Neurons with NR < 1 ($p < 0.05$) were defined as "Decreasing" and neurons with

345 NR > 1 ($p < 0.05$) were defined as "Increasing"; all other neurons were categorized as "No Δ"
346 (Fig. 8; Table 1). Note that, by convention, a decreasing neuron that decreases more for
347 contralateral than ipsilateral movement would be considered to have an ipsilateral direction
348 preference (as calculated above), because firing rate is higher for ipsilateral movement (Sato
349 and Hikosaka, 2002; Lintz and Felsen, 2016).
350
351 *Statistical Analysis*
352
353 In general, MATLAB was used for all statistical analyses except for χ^2 analyses, which
354 were performed in R. Distributions were tested for normality and unless all data sets under
355 comparison were normally distributed, non-parametric statistical tests were used. For
356 consistency, all graphical representations of central tendency are medians, independent of
357 whether parametric or non-parametric statistical tests were used.
358
359

360 **Results**

361

362 *Effect of unilateral dopaminergic cell loss on stimulus-guided and internally-specified*

363 *movements*

364

365 To examine how parkinsonian conditions affect stimulus-guided (SG) and internally-

366 specified (IS) movements and their underlying BG output, we first trained mice on a

367 behavioral task designed to elicit these forms of movements (Figs. 1, 2A,B) (Lintz and Felsen,

368 2016). Briefly, on each trial of the task, the mouse was presented with a binary odor mixture

369 at a central port, waited for an auditory go signal, and moved to the ipsilateral (left) or

370 contralateral (right) reward port for a water reward (Fig. 2A). Each daily experimental

371 session consisted of interleaved blocks of SG and IS trials (Fig. 2B; Materials and

372 Methods)(Lintz and Felsen, 2016). On SG trials, the dominant component of the odor

373 mixture – which varied by trial – determined the side at which reward was delivered, while

374 on IS trials, a balanced mixture of the two odors preceded delivery of reward at the same side

375 throughout the block (Fig. 2B; Materials and Methods). Consistent with previous results

376 (Lintz and Felsen, 2016; Lintz et al., 2019), the direction of movement on SG trials was

377 selected based on the stimulus (Fig. 2C) while the direction of movement on IS trials was

378 selected based on recent trial history (Fig. 2D).

379 Upon achieving proficient performance on the task (Fig. 2C,D; Materials and

380 Methods), mice received 6-OHDA injections to one SNc to unilaterally ablate dopaminergic

381 neurons (Ungerstedt, 1976; Chang et al., 2006; Israel and Bergman, 2008; Avila et al., 2010;
382 Smith and Heuer, 2011; Brazhnik et al., 2012; Seeger-Armbruster and von Ameln-
383 Mayerhofer, 2013; Brazhnik et al., 2014) and were implanted with a chronic tetrode drive
384 targeting the ipsilateral SNr to record BG output (Fig. 1). Only mice with > 70%
385 histologically-confirmed dopaminergic cell loss (examined following all behavioral and
386 recording experiments; Fig. 1, Fig. 3A) were included in the "hemi-PD" group for all
387 subsequent analyses (5/10 mice; Materials and Methods). Confirming the validity of our
388 hemi-PD model, hemi-PD mice exhibited a greater ipsilateral bias than control mice (saline
389 delivered to SNc) on a standard rotation assay ($p = 0.0357$, 1-tailed Wilcoxon rank sum test;
390 Fig. 3B; Materials and Methods), and the mean firing rate of SNr neurons was higher ($p =$
391 0.0157, 1-tailed Wilcoxon rank sum test; Fig. 3C), consistent with rate-model predictions and
392 previous reports (Sanderson et al., 1986; Hutchison et al., 1994; Wichmann et al., 1999;
393 Chang et al., 2006; Brazhnik et al., 2014; Lobb and Jaeger, 2015).
394 We next examined whether unilateral dopaminergic cell loss affected the SG and IS
395 movements required by our task (Fig. 1). Given the rate-model prediction that SNr-recipient
396 nuclei for contralateral movement are over-inhibited (Albin et al., 1989; DeLong, 1990;
397 Obeso et al., 2008; Utter and Basso, 2008; McGregor and Nelson, 2019; Vitek and Johnson,
398 2019), and supported by our findings of greater ipsilateral bias on the rotation assay (Fig. 3B)
399 and an increase in mean SNr activity (Fig. 3C), we expected hemi-PD mice to exhibit an
400 ipsilateral bias for both SG and IS movements. To quantify the extent of directional bias on
401 SG trials, for each mouse we estimated the pre-surgery and post-surgery bias based on a

402 logistic function fit separately to the choices from all sessions before and all sessions after
403 surgery, respectively (Fig. 4A,B; Materials and Methods). In hemi-PD mice, we found that
404 choices after surgery were biased ipsilaterally compared to before surgery ($p = 0.0478$, 1-
405 tailed unpaired t-test; Fig. 4A,C), while no post-surgery change in bias was observed in
406 control mice ($p = 0.186$, 1-tailed unpaired t-test; post-surgery change in ipsilateral bias was
407 greater for hemi-PD than control mice, $p= 0.0468$, 1-tailed unpaired t-test; Fig. 4B,C).
408 Consistent with their ipsilateral directional bias, hemi-PD mice exhibited a larger increase in
409 reaction time (defined as the time from go signal to reward port entry) post-surgery for
410 contralateral than ipsilateral movements ($p = 0.0143$, 1-tailed Wilcoxon rank sum test; Fig.
411 4D,F). While control mice also exhibited longer reaction times post-surgery, as expected
412 given the additional weight of the chronic recording drive, the increase was not greater for
413 contralateral than ipsilateral movements ($p = 0.385$, 1-tailed unpaired t-test; Fig. 4E,G).
414 We observed similar effects of unilateral dopaminergic cell loss on IS movements (Fig.
415 5). For these trials, we quantified bias by separately examining the fraction of ipsilateral
416 choices during blocks in which reward was delivered at the ipsilateral port ("ipsilateral
417 blocks") and at the contralateral port ("contralateral blocks"). On ipsilateral blocks, both
418 groups of mice nearly always chose the ipsilateral port (median fraction ipsilateral choices:
419 hemi-PD pre-surgery = 0.88, post-surgery = 0.85; control pre-surgery = 0.89, post-surgery =
420 0.87; Fig. 5A,B). On contralateral blocks, however, hemi-PD mice were more likely to
421 choose the ipsilateral port after than before surgery (median pre-surgery = 0.12, post-surgery
422 = 0.31; $p = 0.0115$, 1-tailed paired t-test; Fig. 5A,C), while control mice did not exhibit this

423 change (median pre-surgery = 0.12, post-surgery = 0.18; $p = 0.100$, 1-tailed paired t-test; post-
424 surgery change in ipsilateral bias was greater for hemi-PD than control mice, $p = 0.0298$, 1-
425 tailed unpaired t-test; Fig. 5B,C). We also found the same effect on reaction times on IS trials
426 as we did on SG trials: hemi-PD (but not control) mice exhibited a greater post-surgery
427 increase in reaction time on contralateral than ipsilateral trials (hemi-PD: $p = 0.0143$, 1-tailed
428 Wilcoxon rank sum test; control: $p = 0.434$, 1-tailed unpaired t-test; Fig. 5D-G). Thus, on
429 both SG and IS trials, unilateral dopaminergic cell loss resulted in fewer and slower
430 contralateral movements, consistent with rate-model predictions.

431 We next asked whether this effect was greater in magnitude, indicative of greater
432 impairment, on SG or IS trials. Given that PD patients are less impaired when performing
433 movements guided by external stimuli (Glickstein and Stein, 1991; Ballanger et al., 2006;
434 Daroff, 2008; McDonald et al., 2015; Distler et al., 2016; Tekriwal et al., 2018), we expected
435 unilateral dopaminergic cell loss to have a greater impact on IS trials. To examine this
436 possibility, we directly compared, within each session, behavior on SG and IS trials. We first
437 compared the percent correct, as a measure of overall performance on these two trial types.
438 We found that, before surgery, hemi-PD mice performed better (higher percent correct) on
439 IS trials, consistent with the observation that the correct choice remained the same
440 throughout the block (Fig. 6A). However, post-surgery, mice exhibited a significant shift
441 toward better relative performance on SG trials ($p = 6.52 \times 10^{-15}$, 1-tailed Wilcoxon rank sum
442 test; Fig. 6A). We next compared directional bias on SG and IS trials by calculating the
443 difference between the fraction of trials for which ipsilateral was the correct choice and the

444 fraction of actual ipsilateral choices, separately for SG and IS blocks within each session. We
445 found that, pre-surgery, hemi-PD mice were slightly more ipsilaterally biased on IS than SG
446 trials, but post-surgery they exhibited a greater ipsilateral bias on IS than SG trials ($p = 0.0300$, 1-tailed unpaired t-test; Fig. 6B). Finally, we compared median reaction times
447
448 between SG and IS trials within each session, separately for ipsilateral and contralateral
449 movements. To control for the potential effects of discrimination difficulty on reaction time,
450 we only included "easy discrimination" SG trials (95:5, 80:20, 20:80, 5:95)(Lintz and Felsen,
451 2016) in this analysis. We found that, pre-surgery, reaction times in both directions tended
452 to be somewhat shorter on IS than SG trials, consistent with previous findings (Lintz and
453 Felsen, 2016) ($p_{pre_ipsi} = 1.25 \times 10^{-12}$; $p_{pre_contra} = 4.65 \times 10^{-12}$; 1-tailed Wilcoxon sign rank test;
454 Fig. 6C). Post-surgery, reaction times for ipsilateral movements exhibited this same pattern,
455 but there was no difference in reaction times between contralateral SG and IS movements
456 ($p_{post_ipsi} = 6.10 \times 10^{-5}$; $p_{post_contra} = 0.0901$, 1-tailed Wilcoxon sign rank test). Together, these
457 analyses demonstrate that IS trials are relatively more affected than SG trials by unilateral
458 dopaminergic cell loss.
459
460 *Effect of unilateral dopaminergic cell loss on basal ganglia output*
461
462 We sought to determine whether behavioral differences between SG and IS trials
463 could be accounted for by activity in the SNr, which is known to play a role in the
464 movements elicited by this task (Lintz and Felsen, 2016). In the same mice described in the

465 above behavioral results, we used chronically implanted tetrodes to record from 183 SNr
466 neurons in hemi-PD mice (n = 5) and 285 neurons in control mice (n = 4) that met our
467 analysis criteria (Materials and Methods). We focused on activity underlying movement
468 preparation, which has been implicated in parkinsonian motor deficits (Dick et al., 1984;
469 Jahanshahi et al., 1992; Suri et al., 1998; Berardelli et al., 2001; Cutsuridis and Perantonis,
470 2006; Moroney et al., 2008; Wu et al., 2015; Hess and Hallett, 2017). Consistent with
471 previous results (Lintz and Felsen, 2016), we observed that SNr activity recorded from hemi-
472 PD mice was often modulated during the "movement preparation epoch" (from 100 ms after
473 the odor valve was opened until the go signal) and depended on movement direction (Fig.
474 7A). We first asked whether firing rate during this epoch differed between hemi-PD and
475 control mice, similar to the difference we and others have observed in baseline activity (Fig.
476 3C)(Hutchison et al., 1994; Wichmann et al., 1999; Galati et al., 2010; Wang et al., 2010a;
477 Seeger-Armbruster and von Ameln-Mayerhofer, 2013; Brazhnik et al., 2014; Lobb and
478 Jaeger, 2015; Aristieta et al., 2016; Willard et al., 2019). The rate model would predict
479 elevated SNr activity in hemi-PD mice, consistent with the ipsilateral bias that we observed
480 given that SNr activity inhibits downstream motor centers that primarily mediate
481 contralateral movement. However, we found no overall difference between groups for
482 movement in either direction (p_{ipsi}= 0.0520; p_{contra}= 0.0954, 1-tailed Wilcoxon rank sum tests;
483 Fig. 7B,C). The ipsilateral bias exhibited by hemi-PD mice on SG and IS trials therefore
484 cannot be explained simply by an *absolute* increase in SNr activity during movement
485 preparation.

486 We next examined whether unilateral dopaminergic cell loss affected the *relative*
487 activity of individual neurons between ipsilateral and contralateral movements (Fig. 7A),
488 which could also potentially account for the ipsilateral behavioral bias. We therefore
489 calculated the "direction preference" of each neuron, which quantifies the difference in
490 firing rate during a specified epoch between ipsilateral and contralateral movements, and
491 ranges from -1 (higher firing rates for ipsilateral movements) to 1 (higher firing rates for
492 contralateral movements), where 0 represents no preference (Materials and Methods)(Green
493 DM, 1966; Feierstein et al., 2006; Lintz and Felsen, 2016). We found that the populations of
494 neurons recorded in hemi-PD and control mice each exhibited a range of preferences, with
495 some neurons preferring ipsilateral movement (Fig. 7D, cyan; $p < 0.05$, permutation test;
496 Materials and Methods) and some preferring contralateral movement (Fig. 7D, magenta; $p <$
497 0.05, permutation test). However, the distributions of preferences exhibited by neurons
498 recorded in hemi-PD and control mice differ ($p = 7.31 \times 10^{-4}$, 2-sample Kolmogorov-Smirnov
499 test) in two key respects.
500 First, the population of SNr neurons in hemi-PD mice exhibited weaker direction
501 preference than the population in control mice. We quantified this difference with several
502 complementary analyses. A smaller proportion of neurons in hemi-PD mice (52/183, 28%;
503 Table 1) than control mice (199/285, 70%; Table 1) exhibited a significant direction
504 preference ($p = 2.20 \times 10^{-16}$, $\chi 2$-test = 147, df = 1). When the entire population in hemi-PD
505 and control mice is considered (i.e., including neurons with and without a significant
506 direction preference), the strength of the preference, independent of sign, was smaller in

507 hemi-PD mice (median = 0.0815) than control mice (median = 0.179; $p = 1.87\times 10^{-12}$, 1-tailed
508 Wilcoxon rank sum tests). Finally, we used a shift function analysis to identify the deciles in
509 which the distributions differed (Rousselet et al., 2017) (Materials and Methods). This
510 analysis revealed that preferences were closer to 0 in hemi-PD than control mice in the 3
511 deciles representing the most ipsilateral preferences and in the 3 deciles representing the
512 most contralateral preferences, consistent with weaker direction preference in hemi-PD
513 mice. Together, these analyses indicate that unilateral dopaminergic cell loss results in a
514 fundamental disruption of the representation of movement direction that is normally
515 observed in the SNr (Handel and Glimcher, 1999; Berardelli et al., 2001; Lintz and Felsen,
516 2016).

517 Second, the population of SNr neurons in hemi-PD mice, but not control mice,
518 exhibited a slight bias towards contralateral preferences. While the entire distribution of
519 preferences in hemi-PD mice was not contralaterally skewed (median = 0.136; $p = 0.368$, 1-
520 tailed Wilcoxon sign-rank test), of the neurons that exhibited a significant preference (Fig.
521 7D, magenta and cyan, Table 1), more were contralateral-preferring (32/52, magenta; Table
522 1) than ipsilateral-preferring (20/52, cyan; $p = 0.0155$, $\chi 2$-test = 4.65 df = 1; Table 1). In
523 contrast, in control mice we found roughly equal proportions of contralateral-preferring
524 (105/199, magenta; Table 1) and ipsilateral-preferring (94/199, cyan; Table 1) neurons ($p =$
525 0.158, $\chi 2$-test = 1.01, df = 1; Fig. 7D, Table 1). This analysis indicates that, in the population
526 of SNr neurons in which direction preference is spared, unilateral dopaminergic cell loss
527 results in more neurons exhibiting higher activity for contralateral than ipsilateral

528 movements, consistent with the ipsilateral behavioral bias that we observed (Figs. 4,5, Table
529 1).
530 To gain insight into the reorganization of BG output induced by unilateral
531 dopaminergic cell loss, we next examined these systematic differences in preferences
532 between hemi-PD and control mice within functional classes of SNr neurons (Fig. 8, Table
533 1). Separate subpopulations of SNr neurons are known to exhibit increases or decreases in
534 activity as movements are prepared and initiated (Handel and Glimcher, 1999; Sato and
535 Hikosaka, 2002; Lintz and Felsen, 2016); these subpopulations presumably play different
536 functional roles. We therefore categorized neurons into one of three classes based on
537 whether their activity during movement preparation increased, decreased, or did not change,
538 relative to baseline (Table 1; Materials and Methods). We first noticed a clear difference in
539 the proportion of neurons in each class between hemi-PD and control groups ($p = 1.32 \times 10^{-}$
540 ^{11}, $\chi 2$-test = 50.108, df = 2; n = 183 neurons, 5 hemi-PD mice; n = 285 neurons, 4 control
541 mice; Fig. 8A, Table 1). Specifically, we found that a higher proportion of neurons recorded
542 in hemi-PD mice (54.1%) than in control mice (40.0%) exhibited decreased activity, and a
543 corresponding lower proportion of neurons recorded in hemi-PD mice (19.7%) than in
544 control mice (44.9%) exhibited increased activity (Fig. 8A, Table 1; Materials and Methods).
545 In addition, our finding that fewer neurons in hemi-PD than control mice exhibited a
546 significant direction preference held true across all 3 subpopulations of neurons (Fig. 8A,
547 Table 1). Finally, we found that the contralateral bias in the hemi-PD group among neurons
548 exhibiting a direction preference was largely due to those that exhibited decreased activity

549 during movement preparation (contralateral:ipsilateral ratio = 25:7, $p = 1.07 \times 10^{-5}$, $\chi 2$-test =
550 18.1, df = 1; Fig. 8A, Table 1); this subpopulation in the control group did not show this effect
551 (contralateral:ipsilateral ratio = 36:42; p = 0.788, $\chi 2$-test = 0.641, df = 1; Fig. 8A, Table 1).
552 Thus, unilateral dopaminergic cell loss resulted in a larger proportion of SNr neurons that
553 release downstream motor centers from inhibition, with a greater effect on ipsilateral than
554 contralateral movements, accounting for the relationship between SNr activity and the
555 ipsilateral behavioral bias.

556 Thus far we have focused on SNr activity during the movement preparation epoch. As
557 our task is designed to separate movement preparation from initiation, we were able to
558 extend our analyses to movement initiation. We expected to observe similar changes during
559 movement initiation, consistent with previous studies (Kravitz et al., 2010; Wang et al.,
560 2010b; Abedi et al., 2013; Freeze et al., 2013). We therefore repeated our firing rate, direction
561 preference and functional class analyses for the period from the go signal until 100 ms after
562 odor port exit, when the movement is initiated. As with the movement preparation epoch,
563 we found no overall difference in firing rate during movement initiation between the hemi-
564 PD and control groups for movement in either direction (p_{ipsi}= 0.193; p_{contra}= 0.103, 1-tailed
565 Wilcoxon rank sum tests). Next, as with movement preparation, we found that a smaller
566 proportion of neurons in hemi-PD than control mice exhibited a direction preference during
567 movement initiation ($p = 4.41 \times 10^{-5}$, $\chi 2$-test = 20.0, df = 2; Fig. 8B), but our shift function
568 analysis comparing the distributions of direction preferences in hemi-PD and control mice
569 revealed only one differing decile (8_{th} decile). Consistent with this result, we found no

570 difference between hemi-PD and control mice in the proportion of neurons that increased or
571 decreased activity during movement initiation compared to baseline ($p = 0.459$, $\chi2$-test =
572 1.56, df = 2; n = 183 neurons, 5 hemi-PD mice; n = 285 neurons, 4 control mice; Fig. 8B,
573 Table 1). Thus, dopaminergic cell loss appears to affect BG output more during movement
574 preparation than during movement initiation in the context of our behavioral task.
575 Finally, we examined whether the changes in SNr activity during movement
576 preparation associated with unilateral dopaminergic cell loss were consistent with the
577 stronger ipsilateral behavioral bias on IS compared to SG trials (Fig. 6). For example, the
578 representative neuron shown in Fig. 9A appears to exhibit a contralateral preference,
579 consistent with an ipsilateral behavioral bias, on IS but not SG trials. To examine this
580 phenomenon across the population, we compared direction preference during the movement
581 preparation epoch between IS and SG trials within the same session (Fig. 9B). In the hemi-
582 PD group, we found that preference was significantly greater (i.e. more contralateral) on IS
583 than SG trials ($p = 0.00390$, 1-tailed Wilcoxon sign rank test, n = 158 neurons; Fig. 9B). We
584 also observed a significant difference in control mice ($p = 0.0180$, 2-tailed Wilcoxon sign
585 rank test; n = 285 neurons), but in the opposite direction (i.e. more ipsilateral). Together,
586 these analyses of SNr activity show that changes in BG output in hemi-PD mice are
587 consistent with their overall ipsilateral behavioral bias (Figs. 4,5), as well as their stronger
588 bias on IS than SG trials (Fig. 6).
589
590 **Discussion**

591

592 In this study, we primarily sought to determine whether BG output could explain the
593 greater impairment in IS than SG movements under parkinsonian conditions. By recording
594 SNr activity in hemi-PD mice performing SG and IS movements, we found that unilateral
595 dopaminergic cell loss alters the relationship between SNr activity and movement direction
596 as movements are prepared (Figs. 7D, 8, Table 1), consistent with the ipsilateral bias in
597 behavior (Figs 4, 5). While we did not observe an absolute increase in preparation-related
598 SNr activity in hemi-PD mice (Fig. 7B,C), as predicted by classical models of parkinsonian
599 BG activity (DeLong, 1990; McGregor and Nelson, 2019), our findings are consistent with a
600 rate-model framework in which greater SNr output inhibits downstream motor nuclei
601 mediating contralateral movements (DeLong, 1990; McGregor and Nelson, 2019; Vitek and
602 Johnson, 2019). In contrast, neural activity during movement initiation was little changed
603 between hemi-PD and control groups (Fig. 8B), suggesting that parkinsonian conditions
604 affect BG output subserving movement preparation more than initiation, consistent with
605 studies in PD patients (Jahanshahi et al., 1992; Beiser and Houk, 1998; Suri et al., 1998;
606 Cutsuridis and Perantonis, 2006; Moroney et al., 2008; Wu et al., 2015).

607 While unilateral dopaminergic cell loss resulted in an ipsilateral bias on both SG and
608 IS trials (Figs. 4 and 5), we found that the effect was larger on IS trials (Fig. 6). This
609 difference was reflected in the activity of SNr neurons, which exhibited a stronger
610 contralateral preference (i.e., higher activity on contralateral than ipsilateral trials) on IS
611 than SG trials (Fig. 9). This finding is consistent with our previous work showing that SNr

activity is more sensitive to movement direction under IS than SG conditions (Lintz and Felsen, 2016), which suggests that the cognitive processes associated with IS movements are more BG-dependent than those associated with SG movements. One key factor that may explain the difference in BG-dependence between IS and SG trials is the influence of prior choices and outcomes, which are crucial for determining the correct direction of movement on IS, but not SG, trials. The SNr receives direct excitatory input from the pedunculopontine tegmental nucleus (Scarnati et al., 1984; Beninato and Spencer, 1987), which encodes prior choices and outcomes (Thompson and Felsen, 2013); SNr activity itself reflects prior choices (Lintz and Felsen, 2016), and in general the BG are thought to bias activity in downstream motor centers towards movements associated with larger rewards (Hikosaka et al., 2000; Sato and Hikosaka, 2002; Watanabe et al., 2003; Kawagoe et al., 2004; Hikosaka et al., 2006). The disruption of BG signaling by unilateral dopaminergic cell loss may therefore affect the (adaptive) influence of priors on movement selection (Perugini et al., 2016), resulting in a greater deficit on IS than SG trials. The importance of the BG in representing priors is also consistent with our finding that SNr activity related to movement preparation, which is influenced by priors, was more affected than activity related to movement initiation, which is not.

In addition to the shift in the population of neurons in hemi-PD mice towards contralateral preference consistent with the ipsilateral behavioral bias, a large proportion of neurons exhibited no relationship between activity and movement direction (gray bars in Figs. 7D, 8A, Table 1). Further, we found that more neurons in hemi-PD mice exhibited a

633 decrease than an increase in activity during movement preparation compared to baseline
634 activity, which was not the case in control mice (Fig. 8A, Table 1), perhaps due to the
635 increased baseline activity in hemi-PD mice (Fig. 3C). While these finding do not directly
636 relate to our primary behavioral readout of directional bias, they suggest a profound
637 reorganization of BG output under parkinsonian conditions. How this reorganization could
638 contribute to other features of parkinsonian behavior can be examined in future studies.
639 While we have demonstrated a clear link between the electrophysiological and
640 behavioral effects of unilateral dopaminergic cell loss, it is worth considering potential
641 caveats in interpreting our results. First, the hemi-PD mice included in our study all
642 exhibited an ipsilateral behavioral bias. Although our electrophysiological results are
643 consistent with this bias, we would be able to draw stronger conclusions about the
644 relationship between BG output and behavior with a set of mice exhibiting a wider range of
645 behavioral biases. Indeed, one mouse that was excluded from our hemi-PD group due to
646 insufficient dopaminergic cell loss (< 70%; Fig. 3A) exhibited a significant *contralateral* bias
647 on both SG and IS trials ($SG_{\Delta \text{ Directional bias}} = -12.9$, see Fig. 2C for comparisons; $IS_{\Delta \text{ Fraction ipsi. choices}} =$
648 -0.0792 , see Fig. 3C for comparisons). When we analyzed the SNr recordings from this
649 mouse, we found that the neurons with a significant direction preference (74/83 neurons)
650 largely exhibited an *ipsilateral* preference during the movement preparation epoch
651 (ipsilateral:contralateral ratio = 59:15, $p = 7.74 \times 10^{-13}$, $\chi 2$-test = 50.0, df = 1, see Fig. 8A, Table
652 1 for comparisons). While we can only speculate about the potential compensatory
653 adaptations that emerge in the BG with relatively moderate dopaminergic cell loss, the fact

654 that the direction of behavioral bias remains consistent with neural direction preference
655 further supports our finding that BG output mediates the effect of parkinsonian conditions
656 on behavior.

657 Second, olfactory deficits are a known hallmark of PD (Hawkes and Shephard, 1993;
658 Hawkes, 1995; Tarakad and Jankovic, 2017; Tekriwal et al., 2017). Given our use of an
659 olfactory task, is it possible that the behavioral effects under parkinsonian conditions are due
660 to sensory and not motor deficits. We suggest that this is unlikely for two reasons: 1) on IS
661 trials the olfactory cue was not informative about reward location, 2) and on SG trials a
662 deficit in olfactory discrimination would be reflected in a flattening, rather than a shift, in
663 the psychometric function, which we did not observe.

664 Finally, while the hemi-PD model provides a powerful approach for examining the
665 neural basis of parkinsonian movement (Ungerstedt, 1976; Avila et al., 2010; Galati et al.,
666 2010; Brazhnik et al., 2012; Brazhnik et al., 2014; Dorval and Grill, 2014), PD typically
667 presents with bilateral dopaminergic cell loss. It is possible that dopaminergic input from the
668 spared SNc may compensate for the unilateral insult, and other differences between the
669 model and the clinical condition must be considered. However, our comparison between
670 ipsilateral and contralateral movements, at the behavioral and neurophysiological levels, was
671 well suited to the hemi-PD model, and we suggest that our results can cautiously inform the
672 reorganization of BG output that occurs in PD.

673 In conclusion, we found that the behavioral effects of unilateral dopaminergic cell
674 loss, including differences between stimulus-guided and internally-specified movements, can

675 be accounted for by changes in SNr activity during movement preparation. While our results
676 could not be explained by the simplest prediction of the classical rate model that BG output
677 is tonically elevated by dopaminergic cell loss, they were consistent with a model in which
678 output during movement preparation determines movement direction. Future studies can
679 examine how the BG interacts with other motor systems to differentially mediate stimulus-
680 guided and internally-specified movements under parkinsonian conditions.

681

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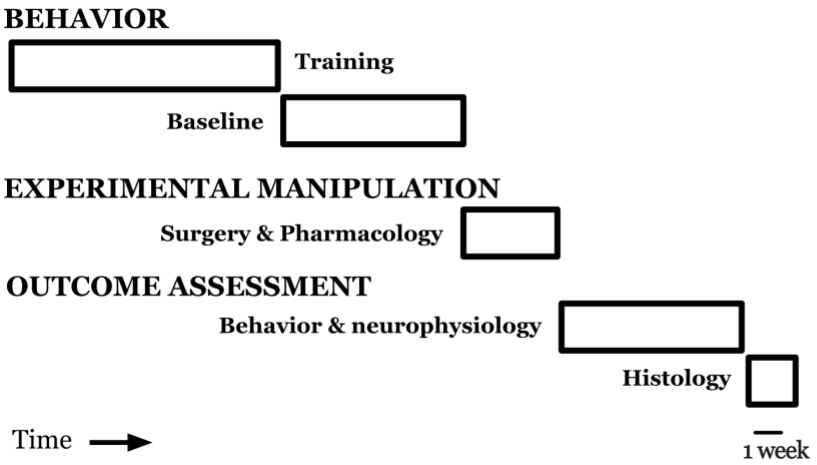
880 **Figures**



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882 **Figure 1.** Experimental timeline.

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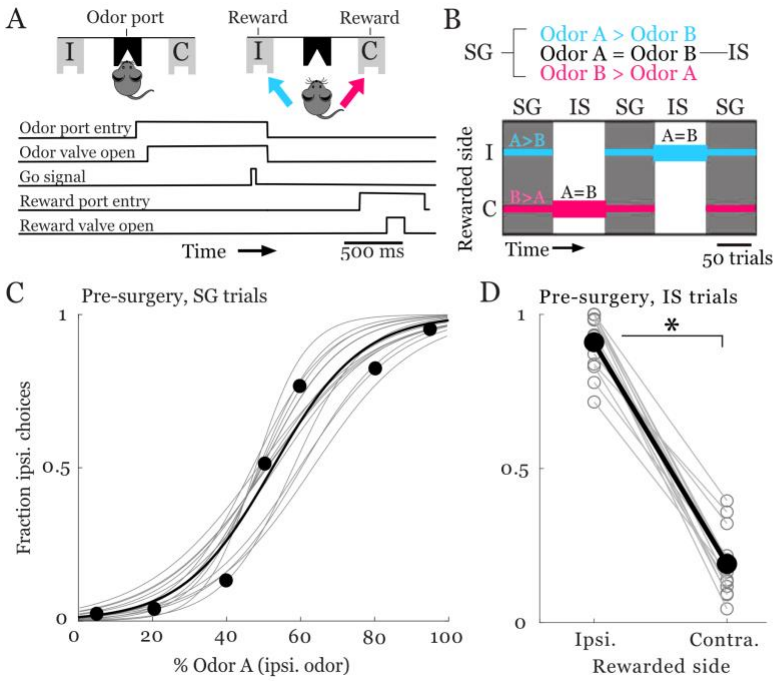

Figure 2. Behavioral task and baseline performance. ***A***, Port locations (top) and timing of task events (bottom). Ipsilateral (I) and contralateral (C) are defined relative to the side of brain targeted for surgery (always left). Cyan represents ipsilateral choices, magenta represents contralateral choices. ***B***, Odor mixtures on SG (stimulus-guided) and IS (internally-specified) trials (top) and interleaving of SG and IS blocks within a session (bottom). Horizontal cyan and magenta lines indicate which port(s) were rewarded in each block: On SG trials, ipsilateral side was rewarded when Odor A > Odor B (cyan), contralateral side was rewarded when Odor B > Odor A (magenta), and either side was equally likely to be rewarded when Odor A = Odor B; on all IS trials, Odor A = Odor B and only one side was rewarded throughout the block. ***C***, Baseline (pre-surgery) performance on SG trials for representative mouse subsequently assigned to the hemi-PD group. Gray lines show best-fit logistic functions (Materials and Methods) for each session (n = 17). Circles

show average across sessions; solid line shows best-fit logistic function to all choices across sessions. ***D***, Baseline (pre-surgery) performance on IS trials for representative mouse subsequently assigned to hemi-PD group. Gray lines link ipsilateral- and contralateral-rewarded IS blocks within the same session (n = 15). Black circles indicate medians. Mouse chose the ipsilateral port more often on ipsilateral-rewarded blocks ($p = 3.05 \times 10^{-5}$, 1-tailed Wilcoxon signed rank test).

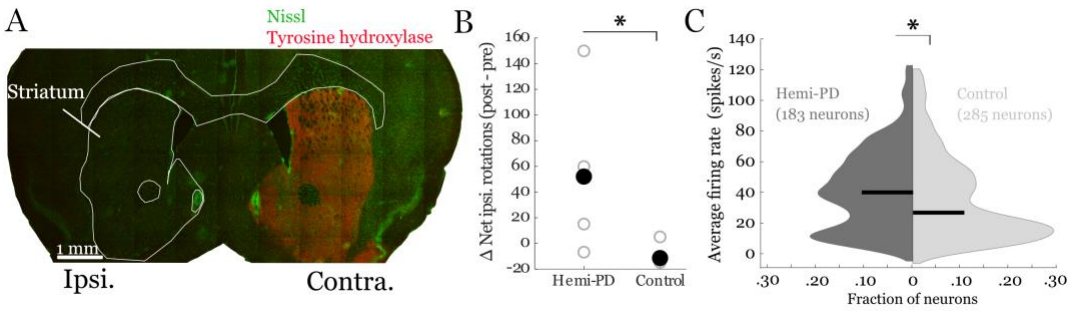

Figure 3. Validation of hemi-PD mouse model. ***A***, Representative coronal section (0.97 mm anterior to Bregma) in hemi-PD mouse. 6-OHDA was delivered to the ipsilateral SNc. Green, Nissl; red, tyrosine hydroxylase. ***B***, Behavior in rotation assay. Beginning 10 min. following delivery of d-amphetamine (IP, 2.5 mg/kg), net ipsilateral rotations (# ipsilateral - # contralateral) were counted for 20 min. Within mouse (n = 5 hemi-PD; n = 3 control (saline delivered to SNc instead of 6-OHDA)) change in net ipsilateral rotations between pre- and post-surgery sessions was calculated. Gray, each mouse; black, median. Hemi-PD mice exhibited more net ipsilateral rotations following surgery ($p = 0.0357$ 1-tailed Wilcoxon rank sum test). ***C***, Baseline activity (odor port entry to reward port exit in each trial) of SNr neurons during task performance in hemi-PD (dark gray) and control (light gray) mice. Black lines, medians. Median baseline SNr activity was higher in hemi-PD mice ($p = 0.0157$, 1-tailed Wilcoxon rank sum test).

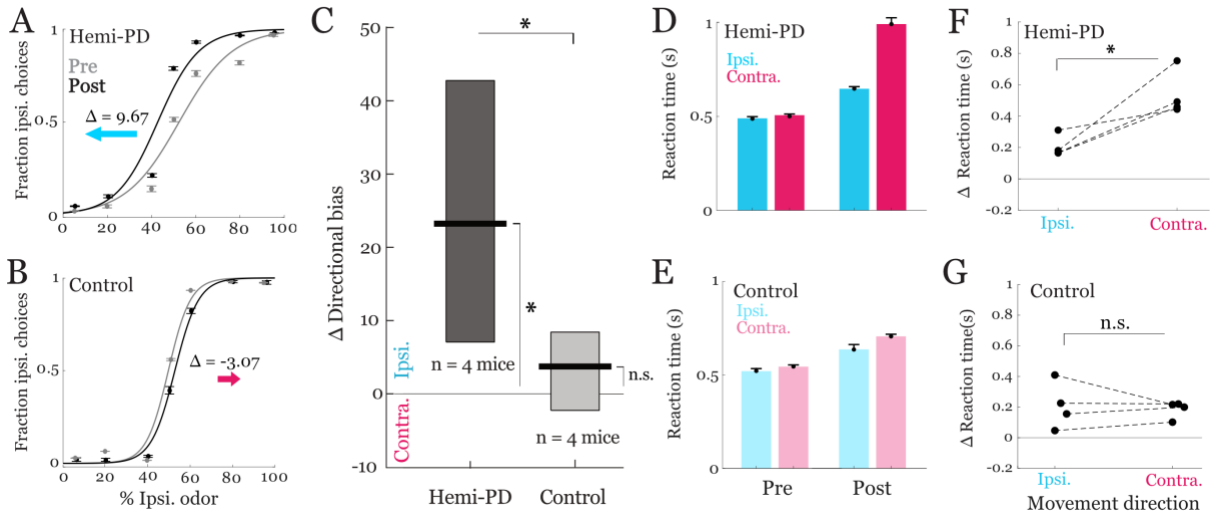

Figure 4. Effect of hemi-parkinsonian state on stimulus-guided (SG) movements. ***A***, Choices and best-fit logistic functions for SG trials in all pre- (gray, n = 30) and post- (black, n = 42) surgery sessions for a representative hemi-PD mouse. Error bars, ±SEM. Leftward arrow indicates ipsilateral directional bias post-surgery relative to pre-surgery, quantified with logistic fits (Materials and Methods). ***B***, As in *A*, for a representative control mouse (tetrode drive implanted to SNr; n = 17 pre-surgery and 33 post-surgery sessions). ***C,*** Change in directional bias (post-surgery – pre-surgery) was more ipsilateral for hemi-PD than control mice (p = 0.0468, 1-tailed unpaired t-test); ipsilateral change in directional bias was significant for hemi-PD (p = 0.0478, 1-tailed t-test) but not control mice (p = 0.186, 1-tailed t-test). Horizontal bars, medians; boxes, 25th and 75th percentiles. ***D,*** Median reaction time (from go signal to reward port entry) shown separately for pre-surgery (n = 16 sessions) and post-surgery (n = 12 sessions) ipsilateral and contralateral SG trials for the same mouse as in *A*. Only correct trials are included; all choices on 50/50 trials are considered correct. (Error bars, ±2 SEM). ***E,*** As in *D*, for a representative control mouse (n = 17 pre-surgery and 33 post-

951 surgery sessions; same representative mouse as in *B*). ***F*,** Change in median reaction time

952 (post-surgery – pre-surgery) was larger for contralateral than ipsilateral correct SG trials in

953 hemi-PD mice (p = 0.0143, 1-tailed Wilcoxon rank sum test). ***G*,** As in *F*, for control mice (p =

954 0.385, 1-tailed unpaired t-test).

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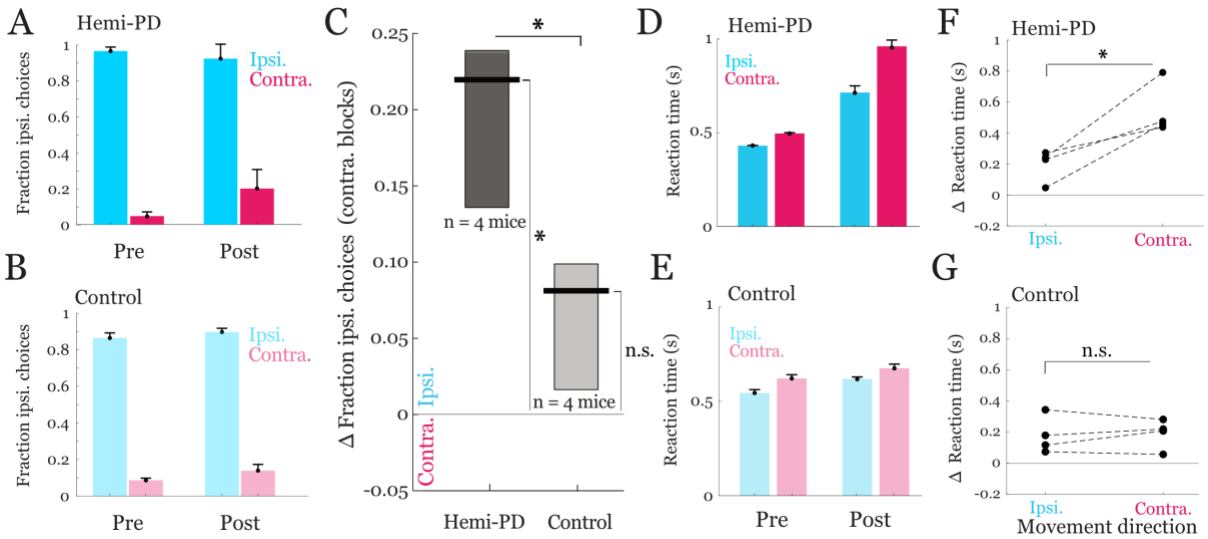

Figure 5. Effect of hemi-parkinsonian state on internally-specified (IS) movements. **A.** Separately for IS trials in which the ipsilateral and contralateral side was rewarded, median fraction ipsilateral choices for pre-surgery (n = 65 sessions) and post-surgery (n = 22 sessions) sessions are shown for a representative hemi-PD mouse. Fraction of ipsilateral choices increased post-surgery relative to pre-surgery on trials in which the contralateral side was rewarded, but not on trials in which the ipsilateral side was rewarded (Error bars, ±2 SEM). ***B,*** As in *A*, for a representative control mouse (tetrode drive implanted; n = 14 pre-surgery and 13 post-surgery sessions) ***C.*** Change in fraction ipsilateral choices on trials in which contralateral side was rewarded (post-surgery – pre-surgery) was more ipsilateral for hemi-PD than control mice (p = 0.0298, 1-tailed unpaired t-test); ipsilateral change was significant for hemi-PD (p = 0.0115, 1-tailed t-test) but not control mice (p = 0.1004, 1-tailed t-test). Horizontal bars, medians; boxes, 25th and 75th percentiles. ***D,*** Median reaction time (from go signal to reward port entry) shown separately for pre-surgery (n = 65 sessions) and post-

981 surgery (n = 22 sessions) ipsilateral and contralateral IS trials for the same mouse as in *A*).

982 Only correct trials are included. (Error bars, ±2 SEM). ***E,*** As in *D*, for a representative control

983 mouse (n = 14 pre-surgery and 13 post-surgery sessions, same representative mouse as in *B*).

984 ***F,*** Change in median reaction time (post-surgery – pre-surgery) was larger for contralateral

985 than ipsilateral IS trials in hemi-PD mice (p = 0.0143, 1-tailed Wilcoxon rank sum test). ***G,***

986 As in *F*, for control mice (p = 0.434, 1-tailed unpaired t-test).

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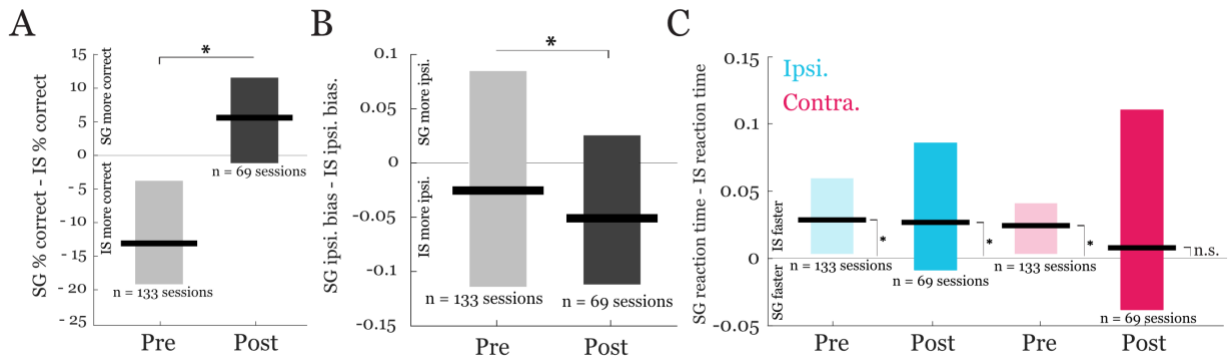

Figure 6. Direct comparison between stimulus-guided (SG) and internally-specified (IS) behavior in hemi-PD mice. ***A,*** Within-session comparison of % correct between SG and IS trials for pre- and post-surgery sessions for hemi-PD mice (n = 4). All 50/50 SG trials were considered correct. Pre-surgery performance was better on IS trials; post-surgery (hemi-parkinsonian) performance was better on SG trials ($p = 6.52 \times 10^{-15}$, 1-tailed Wilcoxon rank sum test). Horizontal bars, medians; boxes, 25th and 75th percentiles. (Controls, not pictured, p = 0.405, 1-tailed Wilcoxon rank sum test, n = 153 pre-surgery sessions, n = 48 post-surgery sessions). ***B,*** Within-session comparison of fraction of ipsilateral choices between SG and IS trials for all hemi-parkinsonian sessions, see Materials and Methods for calculation details. Hemi-parkinsonian behavior was more ipsilaterally biased on IS than SG trials as compared to pre-surgery (p = 0.0300, 1-tailed unpaired t-test) (Controls, not pictured, p = 0.707, 1-tailed unpaired t-test, n = 153 pre-surgery sessions, n = 48 post-surgery sessions). ***C,*** Within-session comparison of reaction time between SG and IS trials for all hemi-parkinsonian sessions. In pre-surgery sessions, reaction times were faster on IS than SG trials for both directions. In hemi-parkinsonian sessions, reaction times were faster on IS than SG ipsilateral

trials, but did not differ between IS and SG contralateral trials. To control for multiple comparisons, we first ran a Kruskal-Wallis as an ANOVA measure. If this test rejected the null that all groups are from the same distribution, we then investigated specific comparisons based on our planned analyses ($p = 9.83 \times 10^{-7}$, Kruskal-Wallis test; $p_{pre_ipsi} = 1.25 \times 10^{-12}$, 1-tailed Wilcoxon sign rank test; $p_{post_ipsi} = 6.10 \times 10^{-5}$, 1-tailed Wilcoxon sign rank test; $p_{pre_contra} = 4.65 \times 10^{-12}$, 1-tailed Wilcoxon sign rank test; $p_{post_contra} = 0.0901$, 1-tailed Wilcoxon sign rank test). (Controls, not pictured, $p = 5.11 \times 10^{-6}$, Kruskal-Wallis test; $p_{pre_ipsi} = 9.95 \times 10^{-10}$, 1-tailed Wilcoxon sign rank test; $p_{post_ipsi} = 7.27 \times 10^{-7}$, 1-tailed Wilcoxon sign rank test; $p_{pre_contra} = 7.12 \times 10^{-23}$, 1-tailed Wilcoxon sign rank test; $p_{post_contra} = 2.41 \times 10^{-4}$, 1-tailed Wilcoxon sign rank test).

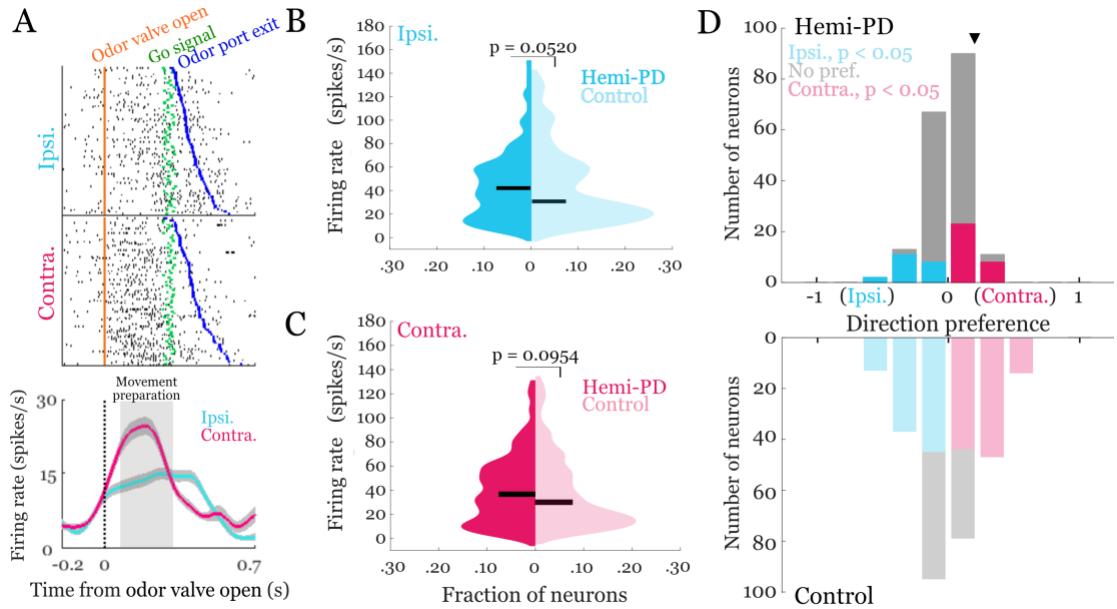

Figure 7. SNr activity during movement preparation in behaving hemi-PD and control mice. ***A***, Rasters (top) and peri-event time histograms (bottom) for an example neuron from a hemi-PD mouse aligned to odor valve open and segregated by choice. Histograms are smoothed with a Gaussian filter; shading, ±SEM. ***B,C***, Mean firing rate during movement preparation epoch (between odor valve open and go signal) did not differ between populations of neurons in hemi-PD and control mice on ipsilateral (*B*) or contralateral (*C*) trials (p_{ipsi}= 0.0520; p_{contra}= 0.0954, 1-tailed Wilcoxon rank sum tests; n = 183 neurons in 5 hemi-PD mice; 285 neurons in 4 control mice). Horizontal bars, medians. ***D***, Distribution of direction preferences for population of neurons in hemi-PD mice (top) had a smaller range than in control mice (bottom) ($p = 7.31 \times 10^{-4}$, 2-sample Kolmogorov-Smirnov test), and more neurons exhibited a significant preference in control than in hemi-PD mice ($p = 2.20 \times 10^{-16}$, $\chi 2$-test). Arrowhead, example neuron shown in *A*.

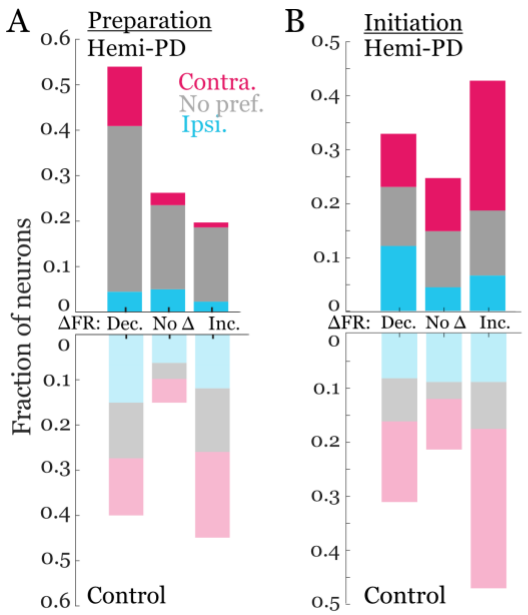

Figure 8. Functional classes of SNr neurons during movement preparation and initiation in behaving hemi-PD and control mice. ***A***, Fraction of neurons with a given direction preference segregated by whether their average activity during the movement preparation epoch (beginning 100 ms after the odor valve opens and ending with the go signal) significantly increased (Inc.), decreased (Dec.) or did not change (No Δ) relative to average baseline firing rate, for hemi-PD (top) and control (bottom) mice. Proportion of neurons exhibiting an increase, a decrease, and no change differs between hemi-PD and control mice ($p = 1.32 \times 10^{-11}$, $\chi 2$-test = 50.108, df = 2). Similarly, proportion of ipsilateral, contralateral, and no direction preference neurons differed between hemi-PD and control mice ($p = 2.2 \times 10^{-16}$, $\chi 2$-test = 149.58, df = 2). ***B***, Fraction of neurons with a given direction preference

segregated by whether their activity during the movement initiation epoch (beginning with the go signal and ending 100 ms after odor poke out) increased, decreased or did not change relative to pre-surgery, for hemi-PD (top) and control (bottom) mice. Proportion of neurons exhibiting an increase, a decrease, and no change did not differ between hemi-PD and control mice ($p = 0.459$, $\chi2$-test = 1.5586, df = 2; n = 183 neurons in 5 hemi-PD mice; 285 neurons in 4 control mice); proportion of ipsilateral, contralateral, and no direction preference neurons differed between hemi-PD and control mice ($p = 4.413 \times 10^{-5}$, $\chi2$-test = 20.049, df = 2).

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Hemi-PD	Decreased FR	No Δ FR	Increased FR	Total
Contralateral	25	5	2	32 (17.5%)
Ipsilateral	7	9	4	20 (10.9%)
Non-selective	67	34	30	131 (71.6%)
Total	99 (54.1%)	48 (26.2%)	36 (19.7%)	183 neurons

Control	Decreased FR	No Δ FR	Increased FR	Total
Contralateral	36	15	54	105 (36.8%)
Ipsilateral	42	18	34	94 (33.0%)
Non-selective	36	10	40	86 (30.2%)
Total	114 (40%)	43 (15.1%)	128 (44.9%)	285 neurons

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1084 **Table 1:** Direction preference and activity change from baseline for all SNr neurons recorded

1085 from hemi-PD (upper) and control (lower) mice.

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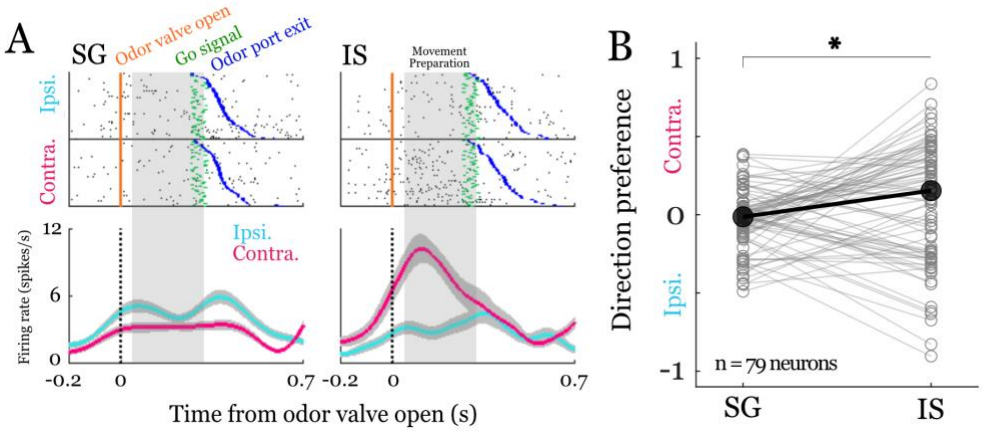

Figure 9. SNr activity on stimulus-guided (SG) and internally-specified (IS) trials in hemi-PD mice. ***A*,** Rasters (top) and peri-event histograms (bottom), on stimulus-guided (SG, left) and internally-specified (IS, right) trials, for an example neuron from a hemi-PD mouse aligned to odor valve open and segregated by choice. Histograms are smoothed with a Gaussian filter; shading, ±SEM. ***B*,** Direction preference on SG and IS trials for population of SNr neurons in hemi-PD mice. Each neuron is represented by a pair of connected gray symbols; only neurons with significant preference ($p < 0.05$) on SG and/or IS trials are shown. Preference was more contralateral on IS than SG trials ($p = 0.0132$, 1-tailed Wilcoxon sign rank test, $n = 79$). Black symbols, medians.