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1 **Aversion encoded in the subthalamic nucleus**

2

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11

12 **Abstract**

13 Activation of the subthalamic nucleus (STN) is associated with the stopping of ongoing  
14 behavior via the basal ganglia. However, we recently observed that optogenetic STN  
15 excitation induced a strong jumping/escaping behavior. We hypothesized that STN activation  
16 is aversive. To test this, place preference was assessed. Optogenetic excitation of the STN  
17 caused potent place aversion. Causality between STN activation and aversion has not been  
18 demonstrated previously. The lateral habenula (LHb) is a critical hub for aversion.  
19 Optogenetic stimulation of the STN indeed caused firing of LHb neurons, but with delay,  
20 suggesting the involvement of a polysynaptic circuit. To unravel a putative pathway, the  
21 ventral pallidum (VP) was investigated. VP receives projections from the STN and in turn  
22 projects to the LHb. Optogenetic excitation of STN-VP terminals caused firing of VP neurons  
23 and induced aversive behavior. This study identifies the STN as critical hub for aversion,  
24 potentially mediated via an STN-VP-LHb pathway.

25

26 **Total word count 4994.**

27

28 **Introduction**

29 The subthalamic nucleus (STN) is a small and bilaterally positioned nucleus which exerts  
30 excitatory influence over the indirect pathway of the basal ganglia (Kita, 1994; Kita and Kitai,  
31 1987). The STN has long been associated with motor and cognitive processes (Bonnievie  
32 and Zaghoul, 2019; Schmidt and Berke, 2017; Weintraub and Zaghoul, 2013). Aberrant

33 STN activity is observed in patients with Parkinson's disease and obsessive-compulsive  
34 disorder (OCD), but can be corrected by high-frequency electrical stimulation of the STN, so  
35 called deep brain stimulation (DBS), which successfully alleviates both motor deficiency in  
36 Parkinson's disease and compulsivity in OCD (Benabid et al., 2009; Blomstedt et al., 2015;  
37 Mallet et al., 2008). However, while STN-DBS is highly beneficial to many severely afflicted  
38 individuals, a number of adverse side-effects have been reported, including low emotional  
39 state (Péron et al., 2013; Temel et al., 2006). This has not been easily understood, but high-  
40 lights the need for revealing the full repertoire of regulatory roles executed by the STN.

41 Reward and punishment motivate learning through complex neuronal circuits (Jean-Richard-  
42 Dit-Bressel et al., 2018). Studies of rewarding and aversive stimuli were long focused on  
43 dopamine neurons in the ventral tegmental area (VTA) (Barker et al., 2016; Bromberg-Martin  
44 et al., 2010; Lammel et al., 2014; Wise, 2004). However, with the seminal discovery that  
45 glutamatergic neurons of the lateral habenula (LHb) respond to reward in an opposite  
46 manner as dopamine neurons, LHb has gained considerable attention as an important brain  
47 area in the regulation of aversive responses and negative reward-processing (Matsumoto  
48 and Hikosaka, 2007). LHb neurons are activated both by aversive stimuli and the omission of  
49 an expected reward (Gao et al., 1996; Matsumoto and Hikosaka, 2007). Further, LHb  
50 hyperactivity has been associated with depression and negative symptoms of addiction (Hu,  
51 2016).

52 The LHb receives regulatory input from several sources, many identified over the past few  
53 years using optogenetic stimulations. For example, optogenetic excitation of glutamatergic  
54 projections from the VTA and the lateral hypothalamus was shown to induce aversion  
55 (Lazaridis et al., 2019; Lecca et al., 2017; Root et al., 2014; Stamatakis et al., 2016), as did  
56 also stimulation of afferent fibers from the entopeduncular nucleus (EP) (Shabel et al.,  
57 2012; Stephenson-Jones et al., 2016). In addition, optogenetic stimulation of glutamatergic  
58 neurons in the ventral pallidum (VP) was recently shown to evoke post-synaptic activity in the  
59 LHb and to drive place avoidance (Faget et al., 2018; Tooley et al., 2018). Thus, through  
60 various sources of excitatory input, activation of the LHb mediates aversive behavior.

61

62 The STN projects directly to several of the structures recognized in aversive processing,  
63 including the EP and VP, both directly connected to the LHb (Faget et al., 2018; Shabel et  
64 al., 2012; Stephenson-Jones et al., 2016; Tooley et al., 2018). However, while ample studies  
65 have addressed pallidal and habenular structures using optogenetics leading towards  
66 increased understanding of their role in affective functions, only a limited set of optogenetic  
67 studies have yet investigated the STN. Based on the strong interest in STN-DBS,

68 optogenetics in the STN has mainly been used to address motor functions in the context of  
69 Parkinson's disease (Gradinaru et al., 2009; Sanders and Jaeger, 2016; Tian et al., 2018;  
70 Yoon et al., 2016, 2014). Further, one recent study identified that optogenetic excitation of  
71 the STN interrupted ongoing licking behavior in mice (Fife et al., 2017). STN neurons  
72 express the *Pitx2* gene, which allows recombination of floxed alleles using Pitx2-Cre  
73 transgenic mice (Martin et al., 2004). Confirming the selectivity of the Pitx2-Cre transgene in  
74 directing opsin expression to the STN, we have previously shown that photostimulation of the  
75 STN results in post-synaptic currents and glutamate release in basal ganglia target areas  
76 (Schweizer et al., 2016, 2014; Viereckel et al., 2018). In a recent report, we show that  
77 optogenetic excitation and inhibition result in opposite locomotor effects (Guillaumin et al.,  
78 2020). Surprisingly, we also observed that STN excitation could induce an avoidance-like  
79 behavior. When placed in an open and novel arena, mice displayed a strong escaping  
80 behavior, characterized by attempts to jump out of the apparatus. This unexpected outcome  
81 was evident immediately upon STN excitation (Guillaumin et al., 2020).

82 Based on these observations, we hypothesized that the STN is engaged in aversive  
83 behavior. To test this, optogenetic stimulations followed through with electrophysiological  
84 recordings and place preference analyses were implemented in Pitx2-Cre mice. Our results  
85 identify place avoidance upon selective activation of either the STN itself or STN terminals in  
86 the VP, and also demonstrate multi-synaptic STN-communication with the LHb. The findings  
87 contribute to the current decoding of the neurocircuitry of aversive behavior.

88

## 89 **Results**

### 90 **STN neurons follow 20 Hz optogenetic stimulation.**

91 Optogenetics allows spatially and temporally precise control of neuronal activation which  
92 under experimental conditions enables the correlation between distinct neuronal activation  
93 and measurable behaviors in freely-moving animals (Deisseroth, 2011). To allow optogenetic  
94 control of STN neurons, Pitx2-Cre mice were bilaterally injected with adeno-associated  
95 (AAV) virus to express a construct encoding either Channelrhodopsin-2 (ChR2) fused with  
96 eYFP (so called Pitx2/ChR2-eYFP mice) or, as controls, eYFP alone (Pitx2/eYFP; control  
97 mice) (Figure 1A). Bilateral optic cannulas were placed above the STN (Figure 1A).  
98 Histological analyses and *in vivo* extracellular recordings were performed to validate the  
99 approach, and injections and cannulas placement were confirmed after completed behavioral  
100 analyses.

101 Mice that displayed strong cellular eYFP labeling throughout the extent of the STN and in  
102 which optic cannulas could be confirmed as positioned immediately above the STN, were

103 included in the statistical analyses of the electrophysiological and behavioral experiments  
104 (Figure 1B). In addition to the STN, the para-subthalamic nucleus (pSTN), associated with  
105 the medial STN, was eYFP-labeled to minor extent. Addressing eYFP-positive terminals in  
106 target areas of the STN, these were detected in the GP (GP externa in humans), EP,  
107 substantia nigra *pars reticulata* (SNr), substantia nigra *pars compacta* (SNc) and VP,  
108 confirming that Pitx2-Cre-positive neurons of the STN reach these target areas (Figure 1B).

109 To ensure connectivity, *in vivo* single cell electrophysiological recordings upon optogenetic  
110 stimulation of the STN were performed (Figure 1C,D). First, an optotagging protocol (Figure  
111 1E) was used to stimulate and record within the STN. To observe the reaction of STN  
112 neurons to photostimulation, peri-stimulus time histograms (PSTH protocol, 0.5 Hz, 5 ms bin  
113 width, 5-8 mW) were created by applying a 0.5 Hz stimulation protocol for at least 100  
114 seconds. Action potentials in ChR2-positive STN cells were successfully evoked by STN  
115 photostimulation (Figure 1E).

116 Once neuronal activity of STN neurons returned to baseline, a photostimulation protocol  
117 intended for behavioral experiments was validated (Behavior protocol, 20 Hz, 5 ms pulses, 5-  
118 8 mW; 10 seconds) which increased the frequency and firing rate of STN neurons for the  
119 whole duration of the stimulation, after which they returned to normal (Figure 1F).

120 The excitability of STN neurons was next confirmed by patch-clamp recordings in the STN of  
121 Pitx2/ChR2-eYFP mice with the optic fiber placed above the recording site (Figure 1G).  
122 ChR2-eYFP expression was strong in the STN (Figure 1H), and all of the STN neurons  
123 tested responded to continuous (Figure 1I) or 20 Hz trains (Figure 1J) of light stimulation by  
124 sustained ChR2-mediated currents. When light stimulation was applied in brain slices from  
125 Pitx2/eYFP, no current were observed in STN neurons (n=3, data not shown).

126 Having confirmed optogenetics-driven activation of STN neurons, behavioral effects upon  
127 optogenetic STN activation were next tested.

### 128 **Activation of STN neurons induces place avoidance**

129 To experimentally test the hypothesis that the STN drives affective behavior, we applied  
130 optogenetic excitation in the real-time place preference paradigm (RT-PP) and the elevated  
131 plus maze (EPM), representing validated tests for aversion and anxiety. First, Pitx2/ChR2-  
132 eYFP and control mice were analyzed in the RT-PP. Here, the mouse moves around freely in  
133 a three-compartment apparatus, in which entry into one of two main compartments (A or B)  
134 is paired with photostimulation of the STN. A photostimulation-neutral, transparent, corridor  
135 (neutral compartment) connects the main compartments, allowing free access to the whole  
136 apparatus. In this setup, an aversive response to photostimulation would be displayed as  
137 avoidance of entry into the light-paired compartment, and hence avoidance of activation of

138 the STN. Further, if the association is strong, this would be expected to give rise to similar  
139 behavior even during days after the experience of STN activation due to conditioning effect  
140 (Figure 2A). Pitx2/ChR2-eYFP mice showed an active avoidance of STN-photostimulation by  
141 spending significantly less time in the light-paired compartment (A) than the unpaired  
142 compartment (B) both during the two days of real-time exposure to photostimulation (Days 3,  
143 4), and also during the conditioned test days (Days 5, 6) (Figure 2B,C). Control mice showed  
144 an absence of stimulation-dependent response throughout the trials (Figure 2B).

145 Next, to test the ability for reversal of the avoidance behavior, a strategy was implemented in  
146 which the previously light-unpaired compartment was paired with photostimulation, and the  
147 stimulation protocol was repeated. On the first reversal day (Day 7), Pitx2/ChR2-eYFP mice  
148 responded by spending time in all three compartments which resulted in lack of significant  
149 preference for any compartment. On the second day (Day 8), mice again spent significantly  
150 more time in the unpaired compartment (A). On the final conditioned test day (Day 9),  
151 Pitx2/ChR2-eYFP mice spent a similar amount of time in the main compartments A and B  
152 (Figure 2B,C).

153 Avoidance towards the light-paired compartment was corroborated by faster and shorter  
154 distance moved compared to the non-light paired compartment across all conditioning days,  
155 except Day 7 when no specific compartment was preferred or avoided (Figure 2D,E). Control  
156 mice spent a comparable amount of time in A and B regardless of which compartment the  
157 stimulation was applied in, and showed no significant effects in distance moved or speed  
158 (Figure 2D,E). These findings identified a direct correlation between STN activation and  
159 place avoidance both upon direct STN stimulation and upon exposure to aversion-paired  
160 cues.

### 161 **Aversion induced by optogenetic STN activation stronger than inherent aversion for** 162 **unsheltered exposure**

163 To assess a putative anxiogenic component and to further pinpoint the nature of the aversive  
164 response observed in the RT-PP, mice were next analyzed in two versions of the EPM, the  
165 regular EPM and a modified EPM setup established to compare any aversion to STN-  
166 stimulation with a naturally occurring aversive situation (Figure 3A,D).

167 The regular EPM is commonly used to assess anxiety. Here, the innate response of mice is  
168 their aversion towards the exposed open arms and a center zone, resulting in an avoidance  
169 of these areas, and consequently, a preference for the sheltered space provided by the  
170 closed arms. In the current experiment, STN-photostimulation was given throughout the trial  
171 (Figure 3A). Similar as controls, Pitx2/ChR2-eYFP mice spent most of the time in the

172 sheltered space provided by the closed arms where duration was significantly higher  
173 compared to exposed open arms and the center (Figure 3B). Number of entries into either  
174 the open or closed arms was not significantly different between Pitx2/ChR2-eYFP and  
175 control mice, but for both groups, the number of entries into the closed arms was statistically  
176 higher than entry into open arms (Figure 3C). Since Pitx2/ChR2-eYFP mice showed the  
177 same behavior as control mice, the results suggest that activation of the STN is not  
178 associated with altered response towards the naturally aversive area, which they actively  
179 avoid both in presence and absence of STN stimulation. These results suggest that the  
180 aversive behavior upon STN-activation is likely not related to an anxiogenic effect.

181 Next, we re-designed the EPM test to directly compare the optogenetically induced aversive  
182 behavior with the naturally aversive context offered by the exposed open arm and center  
183 zone. In this EPM-avoidance setup, STN-photostimulation was activated directly upon entry  
184 into one of the two closed arms and disabled by leaving them, while entry into open arms or  
185 the center zone had no effect on STN-photostimulation (Figure 3D). The effect was striking;  
186 Pitx2/ChR2-eYFP mice actively tried to avoid the previously preferred closed arms, showed  
187 increased number of entries in all arms, and spent significantly more time in the open arms  
188 and in the center (Figure 3E,F). These findings demonstrated that the aversive effect induced  
189 by STN activation was stronger than the naturally aversive effect provided by unsheltered  
190 exposure. Thus, while not confirming STN activation as anxiogenic, analysis in both the RT-  
191 PP and EPM-avoidance paradigms demonstrated a causal relationship between STN  
192 activation and aversive behavior.

### 193 **STN photoactivation induces excitatory post-synaptic responses in the LHb neurons** 194 **revealing poly-synaptic connectivity**

195 The LHb has recently emerged as an important brain area for aversive behavior, firmly  
196 demonstrated by optogenetic analyses of the LHb itself as well as projections to the LHb  
197 (Faget et al., 2018; Lazaridis et al., 2019; Lecca et al., 2017; Shabel et al., 2012;  
198 Stephenson-Jones et al., 2016; Tooley et al., 2018). While STN activation has not previously  
199 been associated with aversion, it has been shown that high frequency stimulation of the STN  
200 in rats induces c-Fos expression in LHb (Tan et al., 2011) and modulation of LHb neuron  
201 activity (Hartung et al., 2016).

202 The potential impact of STN-photostimulation on LHb activity was assessed using *in vivo*  
203 extracellular recordings in Pitx2/ChR2-eYFP (Figure 4A). An optic fiber was positioned above  
204 the STN (Figure 4C), and a stimulation protocol (PSTH 0.5 Hz, 5 ms pulses, 5-8 mW; at least  
205 100 seconds) was implemented which evoked an excitatory response in 50% of the recorded  
206 LHb neurons (onset latency =10.08 ms +/- 0.81 ms) (Figure 4B). Pontamine staining and

207 neurobiotin-marked neurons confirmed the positioning of the recording electrodes within the  
208 LHb and enabled the reconstruction of neuronal positions (Figure 4D,E). No eYFP-positive  
209 fibers were detected in the habenular area of Pitx2/ChR2-eYFP (Figure 4E), confirming the  
210 absence of a direct connection between the STN and the LHb. These findings indicate a  
211 multi-synaptic glutamatergic pathway between the STN and the LHb.

### 212 **Selected optogenetic activation STN-VP projections results in VP firing**

213 Several projections to the LHb are involved in aversion. With the lack of direct projection from  
214 the STN to LHb, we decided to assess the putative role of the VP as the bridge between the  
215 STN and LHb in the observed STN-driven aversion. While the STN projects to several basal  
216 ganglia structures, the ventral pallidal target area, VP, was of particular interest as it sends  
217 projections to both the VTA and LHb (Root et al., 2015; Tripathi et al., 2013). Further, the VP  
218 was recently proposed to play a role in integrating rewarding and aversive information (Creed  
219 et al., 2016; Itoga et al., 2016). Of mixed neurotransmitter phenotype, specifically the  
220 glutamatergic neurons of the VP were recently identified as sending direct projections to the  
221 LHb (Faget et al., 2018; Tooley et al., 2018). In addition, it has been shown that these VP  
222 neurons receive input from the STN, among several regions (Tooley et al., 2018).

223 Focusing on the VP, to address the effect of STN optogenetic activation on the excitability of  
224 VP neurons, sagittal slices from Pitx2/ChR2-eYFP and control mice were prepared for whole-  
225 cell patch-clamp recordings. STN inputs were stimulated with 473 nm laser pulses through  
226 an optic fiber placed above the VP (Figure 5A). VP neurons were all recorded in the area just  
227 below the anterior commissure, a region rich in ChR2-eYFP-expressing STN axon terminals  
228 (Figure 5B-C). The impact of the activation of the STN-VP pathway was first assessed in cell-  
229 attached configuration. Trains of stimulation at 20 Hz induced a strong increase in the firing  
230 activity of all recorded VP compared to basal firing activity (Figure 5D;  $p < 0.05$ ), suggesting a  
231 powerful excitatory drive from the STN on VP neurons. Next, STN-VP synaptic transmission  
232 was recorded in whole-cell voltage-clamp configuration which confirmed the glutamatergic  
233 nature of this pathway (Figure 5E). Altogether, these data support the strong impact of the  
234 STN-VP pathway on the excitability of VP neurons.

### 235 **Activation of STN-VP terminals induces place avoidance**

236 Finally, to investigate if selective activation of the STN-VP pathway can drive aversive  
237 behavior, optic cannulas were bilaterally implanted above the VP in a new batch of Pitx2-Cre  
238 mice virally injected into the STN (following the terminology used above, these mice are  
239 referred to as Pitx2/ChR2-eYFP/VP and Pitx2/eYFP/VP, respectively) (Figure 6A). This  
240 allowed behavioral testing in the same RT-PP and EPM paradigms as used for the STN-  
241 photostimulation, but now upon photostimulation directed to the ChR2-eYFP-positive STN-

242 terminals in the VP. Pitx2/ChR2-eYFP/VP mice showed an active avoidance behavior to VP-  
243 photostimulation by spending significantly less time in the light-paired compartment (A) than  
244 the unpaired compartment (B) both during the two days of real-time exposure to  
245 photostimulation (Days 3, 4), and also during the first conditioned test days (Days 5) (Figure  
246 6B). Control mice showed an absence of stimulation-dependent response throughout the  
247 trials (Figure 6B,C).

248 Upon reversal of light-pairing (Days 7, 8), Pitx2/ChR2-eYFP/VP mice reversed their  
249 preference by again spending significantly more time in the light-unpaired compartment (A).  
250 The preference for the unpaired compartment was maintained on the final conditioned test  
251 day (Day 9) when no light was applied (Figure 6B,C). Avoidance towards the light-paired  
252 compartment was confirmed by shorter distance moved compared to the non-light paired  
253 compartment across all test days similarly to what observed upon STN optogenetic  
254 stimulation while, in the other hand, speed was not affected (Figure 6D,E).

255 These results suggest that the STN-VP pathway is involved in the aversive response  
256 observed upon optogenetic STN activation.

### 257 **Activation of STN-VP terminals reduces the natural preference for a sheltered** 258 **environment**

259 Also the EPM protocols were used to analyse the Pitx2/ChR2-eYFP/VP mice (Figure 7A). A  
260 similar response was observed upon VP-stimulation as was seen using STN-stimulation.  
261 That is, in the regular EPM, photoactivation of the terminals in the VP did not induce any  
262 visible difference between Pitx2/ChR2-eYFP/VP and control mice. Pitx2/ChR2-eYFP/VP  
263 mice spent most of the time in the closed arms compared to open arms and center (Figure  
264 7B). The number of entries into either the open or closed arms were not significantly different  
265 between Pitx2/ChR2-eYFP/VP and control mice (Figure 7C). These results were in  
266 accordance with those observed with STN activation.

267 Next, we tested the Pitx2/ChR2-eYFP/VP mice in the re-designed EPM-avoidance paradigm  
268 (Figure 7D), where VP-photostimulation was activated directly upon entry into one of the two  
269 closed arms and disabled by leaving them. As observed upon STN-photostimulation,  
270 activation of STN-VP terminals in the VP induced a visible reduction of the time spent in the  
271 closed arms with an increase of the time spent in the open arms and center. Pitx2/eYFP/VP  
272 control mice spent significant more time in the closed arms compared to open arms and  
273 center, while Pitx2/ChR2-eYFP/VP mice spent a similar amount of time in the three  
274 compartments, annulling the natural preference for the closed arms (Figure 7E). Despite an  
275 increased tendency in the number of entries comparing Pitx2/ChR2-eYFP/VP with  
276 Pitx2/eYFP/VP control mice, no significant difference was detected (Figure 7F). In summary,

277 while mice did not show an anxiogenic phenotype, optogenetic stimulation of STN terminals  
278 in the VP was sufficient to drive avoidance behavior.

279

## 280 **Discussion**

281 Three distinct sets of optogenetics-based experiments in mice expressing ChR2 selectively  
282 in the STN enabled the identification of a previously unknown role of this clinically relevant  
283 brain area in aversion: First, we identify that photostimulation of the STN drives aversive  
284 behavior. Second, STN-photostimulation drives an excitatory response in LHb neurons.  
285 Third, photostimulation of STN-terminals in the VP results in significant firing, and induces a  
286 similar aversive response as when the STN itself is stimulated. Further, as no direct  
287 projection is observed between the STN and LHb, and with a delay in the observed evoked-  
288 firing of the LHb, the current results support an indirect connection between the STN and  
289 LHb via the VP. The identification of a causal role of the STN in driving aversion opens up for  
290 new possibilities in the current understanding and on-going decoding of aversive behavior.

291 The role of selective STN excitation in mediating emotional valence and motivated behaviors  
292 has so far been poorly investigated. Aversive stimuli, such as the threat of a predator or the  
293 perception of an imminent dangerous situation induce the activation of a “survival state”  
294 designed to avoid or reduce the possible harmful outcome. In this context, aversive learning  
295 allows the animals to detect the aversive stimulus and learn to actively avoid it, while  
296 aversive Pavlovian conditioning serves to associate neutral stimuli to the hostile situation and  
297 environment reducing the probability of a related behavior being expressed (Jean-Richard-  
298 Dit-Bressel et al., 2018). Obtaining a reward or avoiding punishment shapes decision-making  
299 and motivates learning with several brain regions playing a part in such vital responses  
300 (Jean-Richard-Dit-Bressel et al., 2018). Using a recently validated optogenetic approach in  
301 *Pitx2*-Cre mice in which STN neurons can be selectively excited and thus activated, our  
302 findings now identify aversive responses upon STN activation, possibly by acting as an  
303 upstream component of the LHb. Indeed, STN excitation caused an evoked excitation of LHb  
304 neurons, demonstrating a common circuitry, but also found the association indirect. With the  
305 observed STN-mediated excitation of VP neurons followed through with behavioral aversion,  
306 the VP is here suggested as a brain area connecting the STN and LHb. However, given the  
307 multitude of structures that communicate with the STN, additional experiments will be  
308 required to fully pinpoint the STN circuitry of aversion. *Pitx2* gene expression is characteristic  
309 of the STN, with expression starting early in STN development and persisting throughout life  
310 (Dumas and Wallén-Mackenzie, 2019; Martin et al., 2004; Schweizer et al., 2016; Skidmore  
311 et al., 2008). In previous work, we could verify the selectivity of the *Pitx2*-Cre transgene in

312 driving floxed opsin constructs to the STN, with photostimulation-induced post-synaptic  
313 currents observed in pallidal (globus pallidus) and nigral (SNr) target areas (Viereckel, 2018,  
314 Schweizer 2014). Thus, in addition to the post-synaptic response of VP neurons identified  
315 here, also other STN target areas might be involved in mediating the observed behavioral  
316 avoidance. While further investigations will be needed to fully outline the neurocircuitry of  
317 STN-mediated aversion, the current findings clearly show that STN excitation caused both  
318 immediate place avoidance and cue-induced avoidance behavior, thus directly linking STN  
319 activation to aversion-processing. In this context, the STN, which in turn receives inputs from  
320 cortical regions supporting hedonic processing (Degos et al., 2008; Frankle et al., 2006;  
321 Haynes and Haber, 2013; Isaacs et al., 2018), might play a modulatory role in decision-  
322 making in response to aversive conditions.

323 In a recent study, we could experimentally confirm the long-assumed role of the STN in  
324 locomotion by demonstrating directly opposite motor effects by optogenetic excitation and  
325 inhibition of the STN (Guillaumin et al., 2020). We found that STN excitation was generally  
326 correlated with significant reduction in locomotor activity, while STN inhibition enhanced  
327 locomotion, just as classical models of the regulatory role of the STN in basal ganglia motor  
328 loops propose. However, we also found that these correlations were not true for all types of  
329 behavior. Upon STN-photostimulation in the open field test, jumping and self-grooming  
330 behaviors were induced, not reduced. Curiously, this was only observed in a non-challenging  
331 environment, not when mice were engaged in complex motor tasks. While jumping and  
332 grooming are distinctly measurable motor output, they might represent an emotional  
333 manifestation (Smolinsky et al., 2009; Wahl et al., 2008). In fact, the mice attempted to jump  
334 out of the experimental test arena as if to escape the stimulation. In a follow-up pilot study,  
335 we observed the mice in their natural home-cage environment. STN-photostimulation was  
336 turned on by remote control, and only in short periods. Immediately upon STN-stimulation,  
337 *Pitx2/ChR2-eYFP* mice responded with self-grooming and attempts to escape the home-  
338 cage. Self-grooming was mostly observed when the lid was on, preventing escape, while in  
339 an open cage, most of the mice escaped by jumping over the walls (unpublished  
340 observations).

341 These behavioral manifestations suggested to us that the mice experience STN activation as  
342 strongly unpleasant. Identifying a causal role for the STN in neurocircuitry of aversion could  
343 help the understanding of aversive conditions, including some of the adverse side-effects  
344 observed upon STN-DBS. In the present study, we decided to fully explore these striking  
345 observations. By implementing the RT-PP paradigm along with two different setups of the  
346 EPM, the current results now allow both the pin-pointing of place aversion upon STN  
347 activation, and also the segregation between aversion and anxiety. Further, in these

348 experimental arenas, mice move freely between compartments in which they receive, or do  
349 not receive, optogenetic stimulation. As the mouse chooses where it will position itself  
350 throughout the test session, there is no driving force for escape. However, our current  
351 analyses clearly show that when allowed this choice, mice actively avoid areas in which they  
352 receive stimulation of either the STN itself or STN-terminals within the VP. While mice do not  
353 show measurable anxiety, significant place avoidance was evident in both the RT-PP and the  
354 EPM-avoidance tests, and this avoidance behavior was strong enough to compete out the  
355 natural aversion for an open unsheltered space.

356 STN activation has not been experimentally associated with aversion before. Instead, a main  
357 brain area for aversion is the LHb. During the past years, several different glutamatergic  
358 afferents to the LHb have proven responsible for mediating aversive behavior. For instance,  
359 optogenetic stimulation of glutamatergic neurons of the EP (Shabel et al., 2012; Stephenson-  
360 Jones et al., 2016), VP (Faget et al., 2018; Tooley et al., 2018) and lateral hypothalamus  
361 (Lazaridis et al., 2019; Lecca et al., 2017) projecting to the LHb all induce place avoidance.  
362 Now, our findings identifying optogenetically induced aversion in the RT-PP and EPM  
363 avoidance tests upon stimulation of either the STN itself or its terminals in the VP, place the  
364 STN as a distinct brain nucleus responsible for the control of aversion by the recruitment of  
365 VP neurons. Using *in vivo* extracellular recordings, we confirmed the activational response of  
366 the STN neurons themselves upon photostimulation, and also demonstrated that stimulation  
367 of STN glutamatergic neurons drive neuronal activity in the LHb, while patch-clamp results  
368 identify excitatory post-synaptic response in the VP. Together, these electrophysiological and  
369 behavioral findings provide strong support for an STN-VP-LHb pathway for aversion. Our  
370 findings are in accordance with recent observations identifying that activation of  
371 glutamatergic projection from several regions to the LHb triggers place avoidance as well as  
372 activation of LHb activity. Further, our results allow the identification of one more component  
373 to the aversion circuitry, and establish the STN as putative upstream regulator of the VP-LHb  
374 pathway.

375 Curiously, the VP is mostly a GABAergic structure, but recent studies demonstrated that it  
376 also contains glutamatergic neurons projecting to the LHb (Faget et al., 2018; Tooley et al.,  
377 2018). The glutamatergic population of the VP was recently shown to receive projections  
378 from the STN, among several areas, and selective stimulation of glutamatergic VP neurons  
379 was demonstrated to evoke EPSCs in the LHb and induce place avoidance (Tooley et al.,  
380 2018). The glutamatergic population of the VP receives input from different cortical and  
381 midbrain regions (Knowland et al., 2017; Tooley et al., 2018), and it remains to establish  
382 which of these upstream structures, if any, is responsible for their activation in avoidance  
383 behavior. However, our present results pin-point the STN as one distinct subcortical regulator

384 of VP activity. Further, the findings identify that the multisynaptic STN-VP-LHb circuit is  
385 composed mainly of glutamatergic connections, which provides new insight into the role of  
386 excitatory pathways in aversive behavior.

387 The clinical importance of the STN highlights the need for decoding the role of this small  
388 nucleus in behavioral regulation. STN-DBS treatment for motor dysfunction in advanced  
389 stage Parkinson's disease is a long established and highly successful clinical intervention  
390 (Benabid et al., 2009). More recently, STN-DBS has also been approved as treatment for  
391 highly refractory and treatment-resistant OCD, another disorder in which the STN shows  
392 aberrant activity (Klavir et al., 2009; Mallet et al., 2008; Winter et al., 2008). However,  
393 considering that numerous adverse side-effects of STN-DBS have been reported, including  
394 low mood state, depression, personality changes and even suicide (Péron et al., 2013;  
395 Temel et al., 2006), manipulating the STN might come with a certain risk. While this issue  
396 has been challenging to resolve, any direct causality between STN excitation and behavioral  
397 aversion in mice is clearly crucial to take into consideration as putatively important not only  
398 experimentally, but also clinically.

399 The present results underscore the importance of decoding the full repertoire of behavioral  
400 regulation executed by the STN not least to improve treatment prediction and outcome in any  
401 intervention aiming to manipulate the STN and its pathways. Beyond Parkinson and OCD,  
402 STN-DBS has recently gained attention as treatment for additional neuropsychiatric  
403 disorders, including addiction (Pelloux and Baunez, 2013). This interest is strongly based on  
404 findings that STN-DBS reduces symptoms of the Dopamine dysregulation syndrome in  
405 Parkinson's disease (Bandini et al., 2007; Broen et al., 2011; Knobel et al., 2008; Smeding et  
406 al., 2006). Also studies in rodents showing that experimental STN-DBS induces different  
407 motivational responses depending on the nature of the reinforcers (Baunez et al., 2002;  
408 Darbaky et al., 2005; Rouaud et al., 2010), and can reduce addictive behaviors (Lardeux and  
409 Baunez, 2008; Rouaud et al., 2010; Wade et al., 2017), have high-lighted the STN in  
410 addiction. However, it has also been shown that STN lesioning in rats led to altered  
411 emotional state in response to various rewarding and aversive stimuli (Pelloux et al., 2014).  
412 Taken together, both experimental and clinical data high-light the STN structure as important  
413 in affective processing.

414 Here, we describe the identification of the STN as a non-canonical source of negative  
415 emotional value. Well embedded within the brain circuitry of negative reinforcing properties,  
416 the results point towards a pivotal role of the clinically relevant STN in aversive learning.  
417 Evidently, aberrant activity of an STN-VP-LHb circuitry, as well as its manipulation, could  
418 result in both beneficial and detrimental effects. Further studies will be needed to pinpoint the

419 nature of all pathways emerging from the STN that have an impact on rewarding vs aversive  
420 responses. In a recent transcriptomics-histological effort, we identified gene expression  
421 patterns that define distinct spatio-molecular domains within the STN structure (Wallén-  
422 Mackenzie et al., 2020). Based on the current finding that STN encodes aversive behavior,  
423 future experimental approaches should make an effort to correlate specific domains within  
424 the STN with behavioral output. Such anatomical-functional mapping within the STN  
425 structure would be important not least to aid towards improving spatial selectivity in STN-  
426 based interventions.

427

## 428 **METHODS SECTION**

### 429 **Animal housing and ethical permits**

430 Pitx2-Cre\_C57BL/6NTac transgenic mice were bred in-house and housed at the animal  
431 facility of Uppsala University before and during behavioral experiments or at University of  
432 Bordeaux after virus injections for *in vivo* electrophysiological experiments. Mice had access  
433 to food and water *ad libitum* in standard humidity and temperature conditions and with a 12  
434 hour dark/light cycle. PCR analyses were run to confirm the genotype of Pitx2-Cre positive  
435 mice from ear biopsies. All animal experimental procedures followed Swedish (Animal  
436 Welfare Act SFS 1998:56) and European Union Legislation (Convention ETS 123 and  
437 Directive 2010/63/EU) and were approved by local Uppsala or Bordeaux (N°50120205-A)  
438 Ethical Committee.

### 439 **Stereotaxic virus injection and optic cannula implantation**

440 *Virus injection:* Stereotaxic injections were performed on anesthetized Pitx2-Cre mice  
441 maintained at 1.4-1.8 % (0.5-2 L/min, isoflurane-air mix v/v). Before starting the surgery, and  
442 24h post-surgery, mice received subcutaneous injection of Carprofen (5 mg/Kg, Norocarp). A  
443 topical analgesic, Marcain (1.5 mg/kg; AstraZeneca), was locally injected on the site of the  
444 incision. After exposing the skull, holes were drilled in the skull for virus injections and skull  
445 screws implantations for behavioral experiments. Mice were injected in the STN bilaterally  
446 with a virus containing either a Cre-dependent Channelrhodopsin (rAAV2/EF1a-DIO-  
447 hChR2(H134R)-eYFP) named Pitx2/ChR2-eYFP, or an empty virus carrying only a  
448 fluorescent protein (rAAV2/EF1a-DIO-eYFP) named Pitx2/eYFP, respectively  $3.8 \times 10^{12}$  virus  
449 molecules/mL,  $2.7 \times 10^{12}$  virus molecules/mL;  $4.6 \times 10^{12}$  virus molecules/mL (viruses purchased  
450 from UNC Vector Core, Chapel Hill, NC, USA) at the following mouse brain coordinates (from  
451 Paxinos and Franklin, 2013): anteroposterior (AP) = -1.90 mm, mediolateral (ML) = +/- 1.70  
452 mm from the midline and 250 nL of virus was injected with a NanoFil syringe (World  
453 Precision Instruments, Sarasota, FL, USA) at two dorsoventral levels (DV) = -4.65 mm and -  
454 4.25 mm from the dura matter at 100 nL.min<sup>-1</sup>.

455 *Optic cannula implantation:* Optic cannulas (Doric Lenses) were implanted directly after  
456 completion of virus injections. Two skull screws were implanted in the skull to hold the optic  
457 cannula-cement-skull complex. Primers were then applied and harden with UV light  
458 (Optibond). Optic cannulas were implanted bilaterally above the STN (coordinates: AP = -  
459 1.90 mm, ML = +/-1.70 mm from the midline DV = -4.30 mm) or the VP (coordinates: AP =  
460 +0.45 mm, ML = +/-1.55 mm from the midline, DV = -4.00 mm), and fixed with dental cement.  
461 1 mL of saline was injected subcutaneously at the end of the surgery.

#### 462 **Single-cell extracellular recordings**

463 *Surgery:* *In vivo* single cell extracellular recordings started at least 4 weeks after virus  
464 injections. Mice were anesthetized with a mix isoflurane-air (0.8-1.8 % v/v) and placed in a  
465 stereotaxic apparatus. Optic fiber, optrode and glass micropipettes coordinates were AP = -  
466 1.90 mm, ML = +/- 1.70 mm and DV = -4.30 mm for the STN and AP = -1.60 mm, ML = +/-  
467 0.50 mm, DV = -2.00 to -3.30 mm for the LHb.

468 *STN Optotagging:* A custom-made optrode was made with an optic fiber (100  $\mu$ m diameter,  
469 Thorlabs) connected to a laser (MBL-III-473 nm-100 mW laser, CNI Lasers, Changchun,  
470 China) mounted and glued on the recording glass micropipette which was filled with 2%  
471 pontamine sky blue in 0.5  $\mu$ M sodium acetate (tip diameter 1-2  $\mu$ m, resistance 10-15 M $\Omega$ ).  
472 The distance between the end of the optic fiber and the tip of the recording pipette varied  
473 between 650 nm and 950 nm. Extracellular action potentials were recorded and amplified  
474 with an Axoclamp-2B and filtered (300 Hz/0.5 kHz). Single extracellular spikes were collected  
475 online (CED 1401, SPIKE2; Cambridge Electronic Design). The laser power was measured  
476 before starting each experiment using a power meter (Thorlabs). The baseline was recorded  
477 for 100 seconds for each neuron before starting any light stimulation protocols which were  
478 set and triggered with Spike2 software. Light protocol consisted in a peristimulus time  
479 histogram (PSTH, 0.5 Hz, 5 ms pulse duration, 5-8 mW) for at least 100 seconds followed,  
480 after returned to baseline, by a "behavioral" protocol corresponding to the parameters used  
481 for behavioral experiments (20 Hz, 5 ms pulse duration, 5-8 mW).

482 *LHb recordings:* An optic fiber and a recording pipette filled with either 2% pontamine sky  
483 blue or 2% neurobiotin were respectively positioned in the STN and LHb. For each LHb  
484 neurons, a PSTH was recorded upon STN optogenetic stimulation (PSTH 0.5 Hz, 5 ms  
485 pulses, 5-8 mW) for at least 100 seconds. Neurons are considered as excited during the  
486 PSTH protocol when, following the light pulses centered on 0, the number of spikes/5ms bin  
487 is higher than the baseline (-500 ms to 0 ms) plus two times the standard deviation. Injection  
488 of neurobiotin (2% neurobiotin in 0.5 M acetate sodium) by juxtacellular iontophoresis was  
489 performed in some cases for precise spatial identification of excited neurons.

## 490 **Ex vivo electrophysiology**

491 STN and VP neurons were recorded in acute brain slices, prepared as previously described  
492 (Dupuis et al., 2013; Froux et al., 2018). Briefly, Pitx2/eYFP and Pitx2/ChR2-eYFP mice (>  
493 12 months) were deeply anesthetized with an i.p. injection of ketamine/xylazine (75/10  
494 mg/kg) mixture and then perfused transcardially with cold (0-4°C) oxygenated (95% O<sub>2</sub> - 5%  
495 CO<sub>2</sub>) artificial cerebrospinal fluid (ACSF), containing the following (in mM): 230 sucrose, 26  
496 NaHCO<sub>3</sub>, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 0.5 CaCl<sub>2</sub>, 10 MgSO<sub>4</sub> and 10 glucose (pH~7,35). The brain  
497 was quickly removed, glued to the stage of a vibratome (VT1200S; Leica Microsystems,  
498 Germany), immersed in the ice-cold ACSF and sectioned into 300 µm thick parasagittal  
499 slices. Slices were transferred for 1 hour to a standard oxygenated ACSF solution, warmed  
500 (~35°C) containing (in mM unless otherwise stated): 126 NaCl, 26 NaHCO<sub>3</sub>, 2.5 KCl, 1.25  
501 NaH<sub>2</sub>PO<sub>4</sub>, 2 CaCl<sub>2</sub>, 2 MgSO<sub>4</sub>, 10 glucose, 1 sodium pyruvate and 4.9 µM L-gluthathione  
502 reduced. Single slices were then transferred to a recording chamber, perfused continuously  
503 with oxygenated ACSF (without sodium pyruvate and L-gluthathione) heated at 32-34°C, and  
504 visualized using infrared gradient contrast video microscopy (Ni-e workstation, Nikon) and a  
505 60X water-immersion objective (Fluor APO 60X/1.00 W, Nikon).

506 Recordings from individual STN and VP neurons were performed with patch electrodes  
507 (impedance, 3–8 MΩ) obtained from borosilicate glass capillaries (GC150F10; Warner  
508 Instruments, Hamden, CT, USA) pulled with a horizontal puller (P-97; Sutter Instruments,  
509 Novato, CA, USA). For cell-attached and whole-cell voltage-clamp recordings, pipettes were  
510 filled with K-Gluconate-based internal solution containing (in mM): 135 K-gluconate, 3.8  
511 NaCl, 1 MgCl<sub>2</sub>.6H<sub>2</sub>O, 10 HEPES, 0.1 EGTA, 0.4 Na<sub>2</sub>GTP, 2 Mg<sub>1.5</sub>ATP, 5 QX-314 and 5.4  
512 biocytin (pH=7.2, ~292 mOsm). Recordings were obtained using a Multiclamp 700B amplifier  
513 and Digidata 1440A digitizer controlled by Clampex 10.2 (Molecular Devices LLC). Signals  
514 were sampled at 20 kHz and low-pass filtered at 4 kHz. Series resistance was monitored  
515 throughout the experiment by voltage steps of -5 mV. Data were discarded when the series  
516 resistance changed by >20%. Biocytin-filled neurons were identified. For *ex vivo* optogenetic  
517 stimulation, a LED laser source (Prizmatix, Israel) connected to optic fiber (Ø: 500 µm) was  
518 placed above the brain slice. Light intensities ranged from 4mW to 90mW at the tip of the  
519 optic fiber depending of the type experiments. For cell body stimulation, continuous 100ms  
520 long duration light stimulation was applied at low (4mW) and high (90 mW) intensities. To  
521 evoke synaptic transmission, single pulses or train of stimulation (800 pulses at 20Hz) of 1ms  
522 duration at full power (90mW) were used in order to maximize axon terminal depolarization  
523 and efficient release of neurotransmitter. After electrophysiological recordings, slices were  
524 fixed overnight in 4% paraformaldehyde, and maintained in PBS-azide at 0.2% at 4°C until  
525 immunohistochemical processing.

## 526 **Histological analysis**

527 Following behavioral analyses, mice were deeply anesthetized and perfused transcardially  
528 with phosphate-buffer-saline (PBS) followed by ice-cold 4% formaldehyde. Brains were  
529 extracted and 60  $\mu\text{m}$  sections were cut with a vibratome.

530 Fluorescent immunohistochemistry was performed to enhance the eYFP signal. All mice  
531 were analysed. After rinsing in PBS, sections were incubated for 90 min in PBS 0.3% X-100  
532 Triton containing 10% blocking solution (normal donkey serum) followed by an incubation  
533 with primary antibody (chicken anti-GFP 1:1000, cat. no. ab13970, Abcam), diluted in 1%  
534 normal donkey serum in PBS, overnight at 4°C. Next day, sections were rinsed in PBS plus  
535 0.1% Tween-20 solution and incubated for 90 min with secondary antibody diluted in PBS  
536 (A488 donkey anti-chicken 1:1000, cat. no. 703-545-155, Jackson Immunoresearch). After  
537 rinsing in PBS containing 0.1% Tween-20, sections were incubated for 30 min with DAPI  
538 diluted in distilled water (1:5000). Sections were mounted with a Fluoromount aqueous  
539 mounting medium (Sigma, USA) and cover-slipped. Sections were scanned with  
540 NanoZoomer 2-0-HT.0 (Hamamatsu) scanner and visualized with NDPView2 software  
541 (Hamamatsu).

542 For *in vivo* electrophysiological experiments, a deposit was made at the last recording  
543 coordinates by pontamine sky blue iontophoresis ( $-20\ \mu\text{A}$ , 25 min). Brains were then  
544 collected after 0.9% NaCl and 4% PFA transcardial perfusion. 60  $\mu\text{m}$  brain sections were cut  
545 with a vibratome at the levels of the STN and the LHb, mounted with Vectashield medium  
546 (Vector Laboratories), coverslipped and visualized with an epifluorescent microscope to  
547 confirm eYFP expression, recording pipette and optic fiber positions. Neurons injected with  
548 neurobiotin were revealed by first incubating the brain slices in a solution of  
549 saturation/permeabilization (1X PBS-Triton X-100 0.3% and 1% BSA) for one hour at room  
550 temperature followed by an incubation overnight at room temperature in a solution containing  
551 1X PBS-Triton X-100 0.3% (v/v; Sigma-Aldrich) and Streptavidine-557 (1:500; R&D System  
552 #NL 999).

553 To reveal biocytin-filled neurons after *ex vivo* recordings, brain slices were incubated  
554 overnight in a solution of PBS 0.01 mM/Triton™ X100 0.3% (v/v; Sigma-Aldrich) containing  
555 streptavidine-557 (1/1000; Cat# NL999; R&D system). Fluorescent immunohistochemistry was  
556 used to enhance eYFP signal as described above but with a mouse anti-GFP (1/600;  
557 Cat#11814460001, Roche or Cat#A11122; Life technologies) primary antibody and an Alexa-  
558 Fluor Donkey anti-Mouse 488 (1/500; Cat#A21202, Life technologies) as secondary  
559 antibody. The slices were then washed in PBS 0.01 mM before mounting onto slides in  
560 Vectashield medium (CAT#H-1000, Vector laboratories). Images were acquired with a

561 fluorescence microscope (Axio Imager 2, Zeiss) or a confocal microscope (Leica, SP8) and  
562 were analysed with Fiji.

### 563 **Behavioral testing**

564 After approximately four weeks of recovering from surgery, mice were tested in validated  
565 behavioral tests by an experimenter blind to experimental groups, during which the same  
566 stimulation protocol was used for Pitx2/ChR2-eYFP, Pitx2/ChR2-eYFP/VP and respective  
567 controls: 473 nm light, 5 mW, 20 Hz, 5 ms pulse delivered by a MBL-III-473 nm-100 mW  
568 laser (CNI Lasers, Changchun, China). Duration and condition of stimulation are specified for  
569 each test. After completed behavioral tests, mice were sacrificed and brains analysed  
570 histologically and for assessment of optic cannula position. Mice in which the optic cannulas  
571 were not in correct position were excluded from the analysis. For each test, results were  
572 obtained from groups of mice that followed the same battery of experiments. The initial  
573 sample size was determined based by previous published studies with similar experimental  
574 design.

### 575 **Habituation**

576 Three weeks after surgery and before the first behavioral test, all mice were handled and  
577 habituated to the experimental room and to the optic cables to reduce the stress during the  
578 day of the experiment. Before each behavioral test, mice were acclimatized for 30 minutes in  
579 the experimental room.

### 580 **Real-time place preference (RT-PP)**

581 A three-compartment apparatus (Spatial Place Preference Box, Panlab, Harvard Apparatus)  
582 was used for the RT-PP test. The apparatus is composed of two compartments with different  
583 visual and tactile cues for both floors and walls and a connecting corridor (neutral  
584 compartment) with transparent walls and floor. The study was carried out throughout 8  
585 consecutive days preceded by one "*Habituation*" day. On each day of the experiment,  
586 subjects were placed in a neutral cage for at least 3 minutes to recover after connecting the  
587 optic cables. Mice were subsequently moved into the transparent corridor of the apparatus  
588 where, after 30-50 s, the doors were removed and animals allowed to freely explore the  
589 apparatus. The position of the mouse was detected by a camera positioned above the  
590 apparatus. On "*Habituation*", "*Pre Test*" and "*Conditioning Place Test*" days (15 min), mice  
591 were connected to the optic fiber but no light was paired to any chamber. On the "*Real Time*  
592 *Conditioning*" days (30 min), one chamber was randomly chosen as a light-paired chamber  
593 (counterbalanced across animals) while the other one was subsequently assigned as light-  
594 paired chamber on the "*Reverse Real Time Conditioning*" days (30 min). Every time the  
595 subject entered the light-paired chamber, the laser was activated according to the stimulation

596 protocol. Cumulative duration of the time spent in each compartment was recorded by using  
597 the Ethovision XT13.0/14.0 tracking software (Noldus Information Technology, The  
598 Netherlands). Mice that showed strong initial preference during the “Pre Test” for either one  
599 of the two compartments (<25% or >75% of time spent) were excluded from the statistical  
600 analysis.

### 601 **Elevated plus maze (EPM)**

602 EPM analysis was carried out in an apparatus with the shape of a plus, made of two open  
603 arms (35 cm length) and two closed arms (35 cm length) with walls (15 cm high) and crossed  
604 in the middle to create a center platform. The EPM apparatus adjusted for mice is elevated  
605 50 cm from the floor. Mice were placed in a neutral cage for 5 minutes to recover from optic  
606 cable connection. Mice were next placed individually in the center of the maze facing one of  
607 the open arms and allowed to freely explore the apparatus for 10 minutes with light delivered  
608 for the total duration of the test according to the stimulation protocol. The results of the test  
609 were recorded with a camera placed above the EMP arena. Time spent in arms and number  
610 of entries in arms were manually scored by experimenter blind to experimental groups  
611 through subsequent video analysis.

### 612 **Modified version of the EPM (EPM avoidance)**

613 The re-designed version of the EPM test aims to assess the aversive effect induced by the  
614 photostimulation in comparison to the natural aversion experienced in the open arms of the  
615 apparatus. In the EPM avoidance test, photostimulation was activated upon entry into any of  
616 the closed arms, and disabled by leaving it. In contrast, visiting the open arms or occupying  
617 the center zone had no effect on photostimulation. The animal was placed in the center zone  
618 of the arena and allowed to freely explore the apparatus for 15 min. Time spent in each  
619 compartment and number of entries were recorded by the Ethovision XT13.0/14.0 tracking  
620 software (Noldus Information Technology, The Netherlands).

### 621 **Statistics**

622 Data are expressed on the plots as means  $\pm$  SEM. For behavioral analysis, repeated  
623 measures two-way ANOVA were performed followed by Bonferroni's or Tukey's multiple  
624 comparisons where appropriate. For single cell extracellular recordings, Friedman test was  
625 performed followed by Dunn's multiple comparisons. For *ex vivo* electrophysiology, paired t-  
626 test was used (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla  
627 California USA). See Supplementary file 1.

628

629

630 **Figure legends**

631 **Figure 1. Photostimulation in the STN in Pitx2-ChR2-eYFP mice excites STN neurons**

632 (A) Graphical representation of stereotaxic bilateral injection into the STN of an rAAV  
633 carrying a Cre-dependent Channelrhodopsin 2 (ChR2) fused with eYFP (ChR2-eYFP), or  
634 eYFP alone, into Pitx2-Cre mice (Pitx2/ChR2-eYFP mice and Pitx2/eYFP control mice,  
635 respectively) followed by canula implantation above the STN. (B) Coronal sections showing  
636 eYFP fluorescence (green) in STN neurons and its target areas projections in a  
637 representative Pitx2/ChR2-eYFP mouse; from left to right: STN cell bodies, STN target areas  
638 projections in the VP, GP, EP, SNr and SNc; nuclear marker (DAPI, blue); scale, 250  $\mu$ m. (C)  
639 Procedure for STN optotagging experiments. (D) eYFP-positive neurons (green) in the STN;  
640 pontamine blue sky deposit (red dot) at the last recorded coordinate; nuclear marker (DAPI,  
641 blue) (scale, 200  $\mu$ m). (E) Averaged PSTH of STN-excited neurons upon light stimulation  
642 (0.5 Hz, 5 ms pulse duration, 5-8 mW). (F) Example of an excited STN neuron during a  
643 behavioral protocol (20 Hz, 5 ms pulse duration, 5-8 mW) with the frequency and the  
644 recording trace. (G) Frequency of STN neurons before, during and after 10 s light stimulation  
645 following the behavioral protocol (20 Hz, 5 ms pulse duration, 5-8 mW); data expressed as  
646 mean  $\pm$  SEM, n=7, both \*p=0.15. (H) Graphical representation of a para-sagittal slice  
647 obtained from a Pitx2/ChR2-eYFP mouse with a patch-clamp electrode and an optic fiber  
648 placed above the STN. (I) Confocal images depicting a STN neuron filled with biocytin (BC)  
649 and expressing the ChR2-eYFP construct (visualized by eYFP). Scale bar: 10 $\mu$ m. (J)  
650 Representative example of typical ChR2-mediated currents induced by a 1 s blue light ( $\lambda$  =  
651 470 nm) pulses at intensities of 4mW and 90 mW, respectively. (K) ChR2-mediated currents  
652 induced by a train of blue light of 40 s duration at 20 Hz (individual light pulses of 1 ms).  
653 Abbreviations: STN, subthalamic nucleus; VP, ventral pallidum; GP, globus pallidus, EP;  
654 entopeduncular nucleus; SNr, substantia nigra *pars reticulata*; SNc, substantia nigra *pars*  
655 *compacta*.

656 **Figure 2. Optogenetic activation of STN neurons induces place aversion**

657 (A) Graphical representation of the real-time place preference (RT-PP) setup in which  
658 Pitx2/ChR2-eYFP and control mice were tested. (B) Percentage of time that Pitx2/ChR2-  
659 eYFP and control mice spent in each compartment expressed as mean  $\pm$  SEM. Dark blue filled  
660 circles (compartment A) and dark blue filled squares (compartment B) indicate  
661 photostimulation in that compartment. (Left panel) n=12 Pitx2/ChR2-eYFP, compartment A  
662 vs. compartment B, \*\*\*p<0.001; (Right panel) n=10 Pitx2/eYFP controls. (C) Representative  
663 occupancy heat-maps for Pitx2/ChR2-eYFP mice show less time spent in the  
664 photostimulation-paired chamber than in the unpaired chamber. (D) Distance moved in each  
665 compartment expressed as mean  $\pm$  SEM throughout the course of the experiment. Light blue

666 filled histograms indicate photostimulation in that compartment. (Left panel), \*\*\* $p < 0.001$ .  
667 (Right panel)  $n = 10$  Pitx2/eYFP control mice. (E) Speed in each compartment expressed as  
668 mean  $\pm$  SEM. (Left panel)  $n = 12$  Pitx2/ChR2-eYFP mice, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .  
669 (Right panel)  $n = 10$  Pitx2/eYFP control mice.

670 **Figure 3. Aversion induced by optogenetic STN activation stronger than inherent**  
671 **aversion for unsheltered exposure**

672 (A) Schematic representation of the elevated plus maze (EPM) in which Pitx2/ChR2-eYFP  
673 and control mice were tested; STN-photostimulation delivered for 10 minutes throughout the  
674 entire test arena. (B) Percentage of time spent in arms expressed as mean  $\pm$  SEM,  $n = 17$   
675 Pitx2/ChR2/eYFP, open arms vs. closed arms \*\*\* $p < 0.001$ ; center vs. closed arms,  
676 \*\*\* $p < 0.001$ ;  $n = 11$  Pitx2/eYFP mice, open arms vs. closed arms, \*\*\* $p < 0.001$ ; center vs. closed  
677 arms, \*\*\* $p < 0.001$ . (C) Number of entries in arms expressed as mean  $\pm$  SEM,  $n = 17$   
678 Pitx2/ChR2/eYFP mice, open arms vs. closed arms \* $p < 0.05$ ;  $n = 11$  Pitx2/eYFP, open arms  
679 vs. closed arms, \*\* $p < 0.01$ . (D) Graphical representation of a modified version of the EPM  
680 test and representative heat map showing occupancy. (E) Time spent in arms expressed as  
681 mean  $\pm$  SEM,  $n = 14$  Pitx2/ChR2/eYFP, open arms vs. closed arms, \* $p < 0.05$ ;  $n = 11$   
682 Pitx2/eYFP, open arms vs. closed arms, \*\*\* $p < 0.001$ ; center vs. closed arms, \*\*\* $p < 0.001$ ;  
683 Pitx2/ChR2/eYFP vs. Pitx2/eYFP, open arms \* $p < 0.05$ , closed arms \*\*\* $p < 0.001$ , center  
684 \*\*\* $p < 0.001$ . (F) Number of entries in arms expressed as mean  $\pm$  SEM, Pitx2/ChR2/eYFP vs.  
685 Pitx2/eYFP, open arms \*\*\* $p < 0.001$ ; Closed arms \*\* $p < 0.01$ .

686 **Figure 4. STN-photoactivation in Pitx2-ChR2-eYFP mice induces excitatory post-**  
687 **synaptic responses in neurons of the lateral habenula (LHb)**

688 (A) Procedure of LHb recordings experiments with STN-photostimulation in Pitx2/ChR2-  
689 eYFP mice. (B) Close-up of the averaged PSTH of LHb-excited neurons upon STN  
690 photostimulation and overlap of 10 traces of a LHb neuron centred on the 5 ms light pulse.  
691 (C) Representative image of coronal section showing eYFP fluorescence (green) in STN cell  
692 bodies of Pitx2/ChR2/eYFP mice with optic cannula track above the STN; nuclear marker  
693 (DAPI, blue); scale, 250  $\mu$ m. (D) Reconstructed mapping of LHb recorded neurons ( $N = 44$   
694 neurons recorded in 3 mice). Dark blue dots indicate LHb neurons excited during the  
695 stimulation protocol; light blue dots correspond to excited neurons outside of the LHb; grey  
696 dots correspond to neurons which did not respond to the light. (E) Excited LHb neuron  
697 injected with neurobiotin. No eYFP-positive fibers in the LHb.

698 **Figure 5. Selective optogenetic activation of projections from the STN to the ventral**  
699 **pallidum (VP) results in firing of VP neurons**

700 (A) Graphical representation of a para-sagittal slice obtained from a Pitx2/ChR2-eYFP/VP  
701 mouse with a patch-clamp electrode and optic fiber placed above the ventral pallidum (VP).  
702 (B) Epifluorescence image depicting the globus pallidus (GP) and VP. VP neurons were  
703 recorded below the anterior commissure (ac). (C) Confocal images showing a VP neuron in a  
704 Pitx2/ChR2-eYFP/VP mouse filled with biocytin (BC); surrounded by STN axon terminals  
705 expressing ChR2-eYFP (eYFP). Scale bar: 10 $\mu$ m. (D) Representative firing rate of a VP  
706 neuron during ON/OFF periods of light stimulation (20Hz, 1ms, for 40 s) and corresponding  
707 population data (n=5, \*p<0.05, paired t-test). Note the strong increase of firing produced by  
708 light stimulation of STN axon terminals. (E) Typical light-evoked excitatory synaptic current  
709 (EPSC) which is abolished by AMPA/NMDA receptors antagonists (DNXQ, 20 $\mu$ m; D-APV,  
710 50 $\mu$ m). (F) Population graph of light-evoked EPSCs (n=6). (F) Graph showing the  
711 pharmacological blockade of light-evoked EPSC (n=2) by DNQX and D-APV.

712 **Figure 6. Selective photoactivation within the ventral pallidum (VP) of STN-VP**  
713 **projections is sufficient to drive place aversion**

714 (A) Graphical representation of the real time place preference (RT-PP) experiment in which  
715 Pitx2/ChR2-eYFP/VP and corresponding control mice were tested. (B) Percentage of time  
716 spent in each compartment express as mean  $\pm$  SEM. Dark blue filled circles (compartment A)  
717 and dark blue filled squares (compartment B) indicate photostimulation in that compartment.  
718 (Left panel) n=4 Pitx2/ChR2/eYFP/VP, compartment A vs. compartment B, \*\*\*p<0.001;  
719 (Right panel) n=3 Pitx2/eYFP/VP controls. (C) Representative occupancy heat-maps for  
720 Pitx2/ChR2-eYFP mice less time spent in the photostimulation-paired chamber than in the  
721 unpaired chamber. (D) Distance moved in each compartment expressed as mean  $\pm$  SEM  
722 throughout the course of the experiment. Light blue filled histograms indicate  
723 photostimulation in that compartment. (Left panel) n=4 Pitx2/ChR2/eYFP/VP mice,  
724 compartment A vs. compartment B,\*\*\*p<0.001. (Right panel) n=3 Pitx2/eYFP/VP control  
725 mice. (E) Speed in each compartment expressed as mean  $\pm$  SEM. (Left panel) n=4  
726 Pitx2/ChR2/eYFP/VP mice. (Right panel) n=3 Pitx2/eYFP/VP control mice.

727 **Figure 7. Optogenetic activation within the ventral pallidum (VP) of STN-VP terminals**  
728 **reduces the natural preference for a sheltered environment**

729 (A) Schematic representation of elevated plus maze (EPM) with photostimulation delivered  
730 for 10 minutes in the entire arena. (B) Percentage of time spent in arms expressed as mean  
731  $\pm$  SEM, n=4 Pitx2/ChR2/eYFP/VP mice, open arms vs. closed arms, \*\*\*p<0.001; center vs.  
732 closed arms, \*\*\*p<0.001; n=3 Pitx2/eYFP/VP control mice, open arms vs. closed arms,  
733 \*\*\*p<0.001; center vs. closed arms, \*\*\*p<0.001. (C) Number of entries in EPM arms  
734 expressed as mean  $\pm$ SEM. (D) Graphical representation of a modified version of the EPM  
735 test and representative occupancy heat map. (E) Time spent in EPM arms expressed as

736 mean  $\pm$ SEM, n=3 Pitx2/eYFP/VP, open arms vs. closed arms, \*\*p<0.01; center vs. closed  
737 arms, \*\*p<0.01. (F) Number of entries in arms expressed as mean  $\pm$ SEM.

738

### 739 **Supplementary file 1**

740 Detailed description of the statistics used for the different experiments.

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748

### 749 **References**

750 Bandini, F., Primavera, A., Pizzorno, M., Cocito, L., 2007. Using STN DBS and medication  
751 reduction as a strategy to treat pathological gambling in Parkinson's disease. *Parkinsonism &*  
752 *Related Disorders* 13, 369–371. <https://doi.org/10.1016/j.parkreldis.2006.07.011>

753 Barker, D.J., Root, D.H., Zhang, S., Morales, M., 2016. Multiplexed neurochemical signaling  
754 by neurons of the ventral tegmental area. *Journal of Chemical Neuroanatomy* 73, 33–42.  
755 <https://doi.org/10.1016/j.jchemneu.2015.12.016>

756 Baunez, C., Amalric, M., Robbins, T.W., 2002. Enhanced Food-Related Motivation after  
757 Bilateral Lesions of the Subthalamic Nucleus. *The Journal of Neuroscience* 22, 562–568.  
758 <https://doi.org/10.1523/JNEUROSCI.22-02-00562.2002>

759 Benabid, A.L., Chabardes, S., Mitrofanis, J., Pollak, P., 2009. Deep brain stimulation of the  
760 subthalamic nucleus for the treatment of Parkinson's disease 8, 15.

761 Blomstedt, P., Sjöberg, R.L., Hansson, M., Bodlund, O., Hariz, M.I., 2015. Deep Brain  
762 Stimulation in the Treatment of Obsessive-Compulsive Disorder. *FOCUS* 13, 213–221.  
763 <https://doi.org/10.1176/appi.focus.130216>

764 Bonnevie, T., Zaghoul, K.A., 2019. The Subthalamic Nucleus: Unravelling New Roles and  
765 Mechanisms in the Control of Action. *The Neuroscientist* 25, 48–64.  
766 <https://doi.org/10.1177/1073858418763594>

767 Broen, M., Duits, A., Visser-Vandewalle, V., Temel, Y., Winogrodzka, A., 2011. Impulse  
768 control and related disorders in Parkinson's disease patients treated with bilateral subthalamic  
769 nucleus stimulation: A review. *Parkinsonism & Related Disorders* 17, 413–417.  
770 <https://doi.org/10.1016/j.parkreldis.2011.02.013>

- 771 Bromberg-Martin, E.S., Matsumoto, M., Hikosaka, O., 2010. Dopamine in Motivational  
772 Control: Rewarding, Aversive, and Alerting. *Neuron* 68, 815–834.  
773 <https://doi.org/10.1016/j.neuron.2010.11.022>
- 774 Creed, M., Ntamati, N.R., Chandra, R., Lobo, M.K., Lüscher, C., 2016. Convergence of  
775 Reinforcing and Anhedonic Cocaine Effects in the Ventral Pallidum. *Neuron* 92, 214–226.  
776 <https://doi.org/10.1016/j.neuron.2016.09.001>
- 777 Darbaky, Y., Baunez, C., Arecchi, P., Legallet, E., Apicella, P., 2005. Reward-related  
778 neuronal activity in the subthalamic nucleus of the monkey: *NeuroReport* 16, 1241–1244.  
779 <https://doi.org/10.1097/00001756-200508010-00022>
- 780 Degos, B., Deniau, J.-M., Le Cam, J., Mailly, P., Maurice, N., 2008. Evidence for a direct  
781 subthalamo-cortical loop circuit in the rat. *European Journal of Neuroscience* 27, 2599–2610.  
782 <https://doi.org/10.1111/j.1460-9568.2008.06229.x>
- 783 Deisseroth, K., 2011. Optogenetics. *Nature Methods* 8, 26–29.  
784 <https://doi.org/10.1038/nmeth.f.324>
- 785 Dumas, S., Wallén-Mackenzie, Å., 2019. A VGLUT2-positive continuum proceeds  
786 differentiation into heterogeneous mes- and di-encephalic dopamine and glutamate neurons.
- 787 Dupuis, J.P., Feyder, M., Miguez, C., Garcia, L., Morin, S., Choquet, D., Hosy, E., Bezdard,  
788 E., Fisone, G., Bioulac, B.H., Baufreton, J., 2013. Dopamine-Dependent Long-Term  
789 Depression at Subthalamo-Nigral Synapses Is Lost in Experimental Parkinsonism. *Journal of*  
790 *Neuroscience* 33, 14331–14341. <https://doi.org/10.1523/JNEUROSCI.1681-13.2013>
- 791 Faget, L., Zell, V., Souter, E., McPherson, A., Ressler, R., Gutierrez-Reed, N., Yoo, J.H.,  
792 Dulcis, D., Hnasko, T.S., 2018. Opponent control of behavioral reinforcement by inhibitory  
793 and excitatory projections from the ventral pallidum. *Nature Communications* 9.  
794 <https://doi.org/10.1038/s41467-018-03125-y>
- 795 Fife, K.H., Gutierrez-Reed, N.A., Zell, V., Bailly, J., Lewis, C.M., Aron, A.R., Hnasko, T.S.,  
796 2017. Causal role for the subthalamic nucleus in interrupting behavior. *eLife* 6.  
797 <https://doi.org/10.7554/eLife.27689>
- 798 Frankle, W.G., Laruelle, M., Haber, S.N., 2006. Prefrontal Cortical Projections to the  
799 Midbrain in Primates: Evidence for a Sparse Connection. *Neuropsychopharmacology* 31,  
800 1627–1636. <https://doi.org/10.1038/sj.npp.1300990>
- 801 Froux, L., Le Bon-Jego, M., Miguez, C., Normand, E., Morin, S., Fioramonti, S., Barresi,  
802 M., Frick, A., Baufreton, J., Taupignon, A., 2018. D5 dopamine receptors control  
803 glutamatergic AMPA transmission between the motor cortex and subthalamic nucleus.  
804 *Scientific Reports* 8. <https://doi.org/10.1038/s41598-018-27195-6>
- 805 Gao, D.M., Hoffman, D., Benabid, A.L., 1996. Simultaneous Recording of Spontaneous  
806 Activities and Nociceptive Responses from Neurons in the Pars Compacta of Substantia Nigra  
807 and in the Lateral Habenula. *European Journal of Neuroscience* 8, 1474–1478.  
808 <https://doi.org/10.1111/j.1460-9568.1996.tb01609.x>
- 809 Gradinaru, V., Mogri, M., Thompson, K.R., Henderson, J.M., Deisseroth, K., 2009. Optical  
810 Deconstruction of Parkinsonian Neural Circuitry. *Science* 324, 7.

- 811 Guillaumin, A., Serra, G.P., Georges, F., Wallén-Mackenzie, Å., 2020. Optogenetic excitation  
812 and inhibition of the subthalamic nucleus (STN) induce opposite motor effects (preprint).  
813 bioRxiv. <https://doi.org/10.1101/2020.07.08.193359>
- 814 Hartung, H., Tan, S.K.H., Temel, Y., Sharp, T., 2016. High-frequency stimulation of the  
815 subthalamic nucleus modulates neuronal activity in the lateral habenula nucleus. *European*  
816 *Journal of Neuroscience* 44, 2698–2707. <https://doi.org/10.1111/ejn.13397>
- 817 Haynes, W.I.A., Haber, S.N., 2013. The Organization of Prefrontal-Subthalamic Inputs in  
818 Primates Provides an Anatomical Substrate for Both Functional Specificity and Integration:  
819 Implications for Basal Ganglia Models and Deep Brain Stimulation. *Journal of Neuroscience*  
820 33, 4804–4814. <https://doi.org/10.1523/JNEUROSCI.4674-12.2013>
- 821 Hu, H., 2016. Reward and Aversion. *Annual Review of Neuroscience* 39, 297–324.  
822 <https://doi.org/10.1146/annurev-neuro-070815-014106>
- 823 Isaacs, B.R., Forstmann, B.U., Temel, Y., Keuken, M.C., 2018. The Connectivity Fingerprint  
824 of the Human Frontal Cortex, Subthalamic Nucleus, and Striatum. *Frontiers in Neuroanatomy*  
825 12. <https://doi.org/10.3389/fnana.2018.00060>
- 826 Itoga, C.A., Berridge, K.C., Aldridge, J.W., 2016. Ventral pallidal coding of a learned taste  
827 aversion. *Behavioural Brain Research* 300, 175–183.  
828 <https://doi.org/10.1016/j.bbr.2015.11.024>
- 829 Jean-Richard-Dit-Bressel, P., Killcross, S., McNally, G.P., 2018. Behavioral and  
830 neurobiological mechanisms of punishment: implications for psychiatric disorders.  
831 *Neuropsychopharmacology* 43, 1639–1650. <https://doi.org/10.1038/s41386-018-0047-3>
- 832 Kita, H., 1994. Physiology of Two Disynaptic Pathways from the Sensori-Motor Cortex to the  
833 Basal Ganglia Output Nuclei, in: Percheron, G., McKenzie, J.S., Féger, J. (Eds.), *The Basal*  
834 *Ganglia IV, Advances in Behavioral Biology*. Springer US, Boston, MA, pp. 263–276.  
835 [https://doi.org/10.1007/978-1-4613-0485-2\\_28](https://doi.org/10.1007/978-1-4613-0485-2_28)
- 836 Kita, H., Kitai, S.T., 1987. Efferent projections of the subthalamic nucleus in the rat: Light  
837 and electron microscopic analysis with the PHA-L method. *Journal of Comparative*  
838 *Neurology* 260, 435–452. <https://doi.org/10.1002/cne.902600309>
- 839 Klavir, O., Flash, S., Winter, C., Joel, D., 2009. High frequency stimulation and  
840 pharmacological inactivation of the subthalamic nucleus reduces ‘compulsive’ lever-pressing  
841 in rats. *Experimental Neurology* 215, 101–109.  
842 <https://doi.org/10.1016/j.expneurol.2008.09.017>
- 843 Knobel, D., Aybek, S., Pollo, C., Vingerhoets, F.J.G., Berney, A., 2008. Rapid Resolution of  
844 Dopamine Dysregulation Syndrome (DDS) After Subthalamic DBS for Parkinson Disease  
845 (PD): A Case Report. *Cognitive and Behavioral Neurology* 21, 187–189.  
846 <https://doi.org/10.1097/WNN.0b013e318185e6e2>
- 847 Knowland, D., Lilascharoen, V., Pacia, C.P., Shin, S., Wang, E.H.-J., Lim, B.K., 2017.  
848 Distinct Ventral Pallidal Neural Populations Mediate Separate Symptoms of Depression. *Cell*  
849 170, 284–297.e18. <https://doi.org/10.1016/j.cell.2017.06.015>

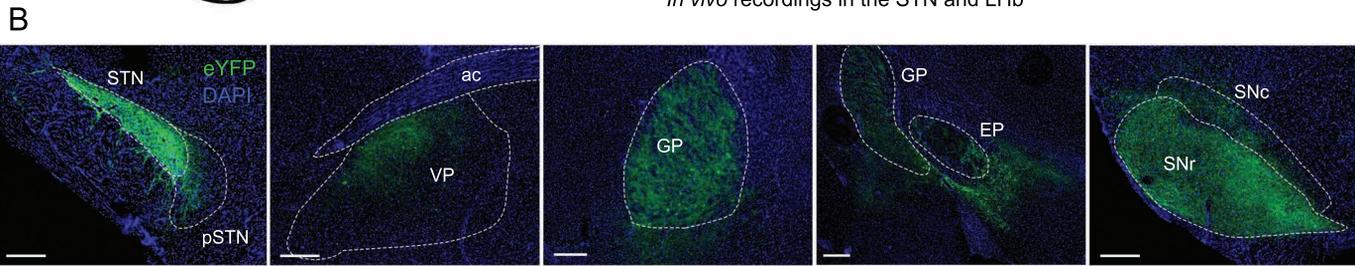
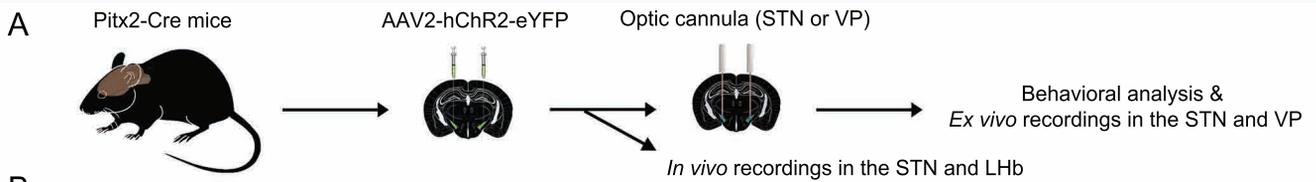
- 850 Lammel, S., Lim, B.K., Malenka, R.C., 2014. Reward and aversion in a heterogeneous  
851 midbrain dopamine system. *Neuropharmacology*, NIDA 40th Anniversary Issue 76, 351–359.  
852 <https://doi.org/10.1016/j.neuropharm.2013.03.019>
- 853 Lardeux, S., Baunez, C., 2008. Alcohol Preference Influences the Subthalamic Nucleus  
854 Control on Motivation for Alcohol in Rats. *Neuropsychopharmacology* 33, 634–642.  
855 <https://doi.org/10.1038/sj.npp.1301432>
- 856 Lazaridis, I., Tzortzi, O., Weglage, M., Märtin, A., Xuan, Y., Parent, M., Johansson, Y.,  
857 Fuzik, J., Fürth, D., Fenno, L.E., Ramakrishnan, C., Silberberg, G., Deisseroth, K., Carlén,  
858 M., Meletis, K., 2019. A hypothalamus-habenula circuit controls aversion. *Molecular*  
859 *Psychiatry* 24, 1351–1368. <https://doi.org/10.1038/s41380-019-0369-5>
- 860 Lecca, S., Meye, F.J., Trusel, M., Tchenio, A., Harris, J., Schwarz, M.K., Burdakov, D.,  
861 Georges, F., Mameli, M., 2017. Aversive stimuli drive hypothalamus-to-habenula excitation  
862 to promote escape behavior. *eLife* 6. <https://doi.org/10.7554/eLife.30697>
- 863 Mallet, L., Polosan, M., Jaafari, N., Baup, N., Welter, M.-L., Fontaine, D., Montcel, S.T. du,  
864 Yelnik, J., Chéreau, I., Arbus, C., Raoul, S., Aouizerate, B., Damier, P., Chabardès, S.,  
865 Czernecki, V., Ardouin, C., Krebs, M.-O., Bardinet, E., Chaynes, P., Burbaud, P., Cornu, P.,  
866 Derost, P., Bougerol, T., Bataille, B., Mattei, V., Dormont, D., Devaux, B., Vérin, M.,  
867 Houeto, J.-L., Pollak, P., Benabid, A.-L., Agid, Y., Krack, P., Millet, B., Pelissolo, A., 2008.  
868 Subthalamic Nucleus Stimulation in Severe Obsessive–Compulsive Disorder. *New England*  
869 *Journal of Medicine* 359, 2121–2134. <https://doi.org/10.1056/NEJMoa0708514>
- 870 Martin, D.M., Skidmore, J.M., Philips, S.T., Vieira, C., Gage, P.J., Condie, B.G., Raphael, Y.,  
871 Martinez, S., Camper, S.A., 2004. PITX2 is required for normal development of neurons in  
872 the mouse subthalamic nucleus and midbrain. *Developmental Biology* 267, 93–108.  
873 <https://doi.org/10.1016/j.ydbio.2003.10.035>
- 874 Matsumoto, M., Hikosaka, O., 2007. Lateral habenula as a source of negative reward signals  
875 in dopamine neurons. *Nature* 447, 1111–1115. <https://doi.org/10.1038/nature05860>
- 876 Pelloux, Y., Baunez, C., 2013. Deep brain stimulation for addiction: why the subthalamic  
877 nucleus should be favored. *Current Opinion in Neurobiology* 23, 713–720.  
878 <https://doi.org/10.1016/j.conb.2013.02.016>
- 879 Pelloux, Y., Meffre, J., Giorla, E., Baunez, C., 2014. The subthalamic nucleus keeps you high  
880 on emotion: behavioral consequences of its inactivation. *Frontiers in Behavioral Neuroscience*  
881 8. <https://doi.org/10.3389/fnbeh.2014.00414>
- 882 Péron, J., Frühholz, S., Vérin, M., Grandjean, D., 2013. Subthalamic nucleus: A key structure  
883 for emotional component synchronization in humans. *Neuroscience & Biobehavioral Reviews*  
884 37, 358–373. <https://doi.org/10.1016/j.neubiorev.2013.01.001>
- 885 Root, D.H., Mejias-Aponte, C.A., Qi, J., Morales, M., 2014. Role of Glutamatergic  
886 Projections from Ventral Tegmental Area to Lateral Habenula in Aversive Conditioning.  
887 *Journal of Neuroscience* 34, 13906–13910. [https://doi.org/10.1523/JNEUROSCI.2029-](https://doi.org/10.1523/JNEUROSCI.2029-14.2014)  
888 14.2014

- 889 Root, D.H., Melendez, R.I., Zaborszky, L., Napier, T.C., 2015. The ventral pallidum:  
890 Subregion-specific functional anatomy and roles in motivated behaviors. *Progress in*  
891 *Neurobiology* 130, 29–70. <https://doi.org/10.1016/j.pneurobio.2015.03.005>
- 892 Rouaud, T., Lardeux, S., Panayotis, N., Paleressompouille, D., Cador, M., Baunez, C., 2010.  
893 Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation. *Proceedings*  
894 *of the National Academy of Sciences* 107, 1196–1200.  
895 <https://doi.org/10.1073/pnas.0908189107>
- 896 Sanders, T.H., Jaeger, D., 2016. Optogenetic stimulation of cortico-subthalamic projections is  
897 sufficient to ameliorate bradykinesia in 6-ohda lesioned mice. *Neurobiology of disease* 95,  
898 225–237.
- 899 Schmidt, R., Berke, J.D., 2017. A Pause-then-Cancel model of stopping: evidence from basal  
900 ganglia neurophysiology. *Philosophical Transactions of the Royal Society B: Biological*  
901 *Sciences* 372, 20160202. <https://doi.org/10.1098/rstb.2016.0202>
- 902 Schweizer, N., Pupe, S., Arvidsson, E., Nordenankar, K., Smith-Anttila, C.J.A., Mahmoudi,  
903 S., Andren, A., Dumas, S., Rajagopalan, A., Levesque, D., Leao, R.N., Wallen-Mackenzie,  
904 A., 2014. Limiting glutamate transmission in a Vglut2-expressing subpopulation of the  
905 subthalamic nucleus is sufficient to cause hyperlocomotion. *Proceedings of the National*  
906 *Academy of Sciences* 111, 7837–7842. <https://doi.org/10.1073/pnas.1323499111>
- 907 Schweizer, N., Viereckel, T., Smith-Anttila, C.J.A., Nordenankar, K., Arvidsson, E.,  
908 Mahmoudi, S., Zampera, A., Warner Jonsson, H., Bergquist, J., Levesque, D., Konradsson-  
909 Geuken, A., Andersson, M., Dumas, S., Wallen-Mackenzie, A., 2016. Reduced  
910 Vglut2/Slc17a6 Gene Expression Levels throughout the Mouse Subthalamic Nucleus Cause  
911 Cell Loss and Structural Disorganization Followed by Increased Motor Activity and  
912 Decreased Sugar Consumption. *eNeuro* 3. <https://doi.org/10.1523/ENEURO.0264-16.2016>
- 913 Shabel, S.J., Proulx, C.D., Trias, A., Murphy, R.T., Malinow, R., 2012. Input to the Lateral  
914 Habenula from the Basal Ganglia Is Excitatory, Aversive, and Suppressed by Serotonin.  
915 *Neuron* 74, 475–481. <https://doi.org/10.1016/j.neuron.2012.02.037>
- 916 Skidmore, J.M., Cramer, J.D., Martin, J.F., Martin, D.M., 2008. Cre fate mapping reveals  
917 lineage specific defects in neuronal migration with loss of Pitx2 function in the developing  
918 mouse hypothalamus and subthalamic nucleus. *Molecular and Cellular Neuroscience* 37, 696–  
919 707. <https://doi.org/10.1016/j.mcn.2007.12.015>
- 920 Smeding, H.M.M., Goudriaan, A.E., Foncke, E.M.J., Schuurman, P.R., Speelman, J.D.,  
921 Schmand, B., 2006. Pathological gambling after bilateral subthalamic nucleus stimulation in  
922 Parkinson disease. *Journal of Neurology, Neurosurgery & Psychiatry* 78, 517–519.  
923 <https://doi.org/10.1136/jnnp.2006.102061>
- 924 Smolinsky, A.N., Bergner, C.L., LaPorte, J.L., Kalueff, A.V., 2009. Analysis of Grooming  
925 Behavior and Its Utility in Studying Animal Stress, Anxiety, and Depression, in: Gould, T.D.  
926 (Ed.), *Mood and Anxiety Related Phenotypes in Mice*. Humana Press, Totowa, NJ, pp. 21–36.  
927 [https://doi.org/10.1007/978-1-60761-303-9\\_2](https://doi.org/10.1007/978-1-60761-303-9_2)
- 928 Stamatakis, A.M., Van Swieten, M., Basiri, M.L., Blair, G.A., Kantak, P., Stuber, G.D., 2016.  
929 Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral

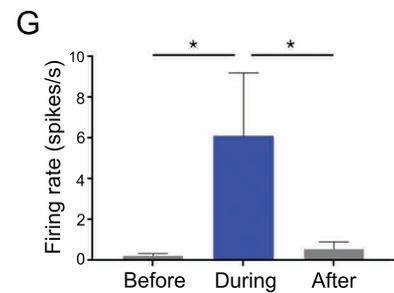
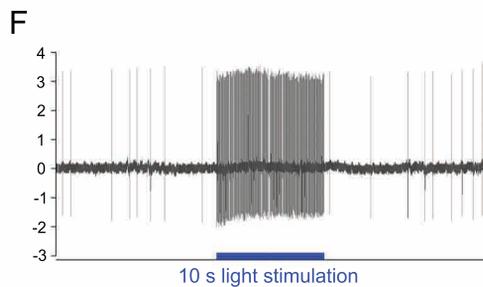
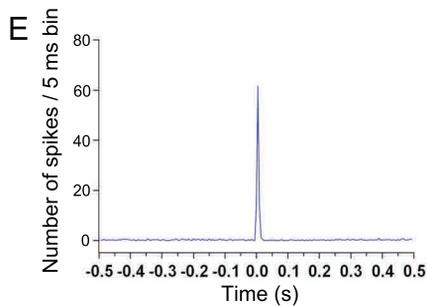
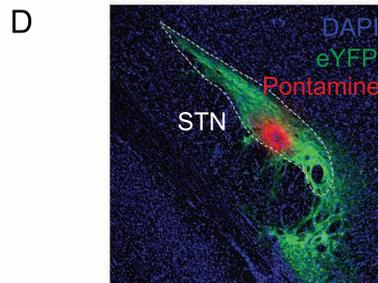
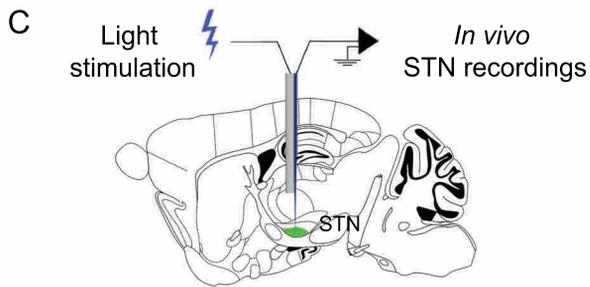
- 930 Habenula Regulate Feeding and Reward. *Journal of Neuroscience* 36, 302–311.  
931 <https://doi.org/10.1523/JNEUROSCI.1202-15.2016>
- 932 Stephenson-Jones, M., Yu, K., Ahrens, S., Tucciarone, J.M., van Huijstee, A.N., Mejia, L.A.,  
933 Penzo, M.A., Tai, L.-H., Wilbrecht, L., Li, B., 2016. A basal ganglia circuit for evaluating  
934 action outcomes. *Nature* 539, 289–293. <https://doi.org/10.1038/nature19845>
- 935 Temel, Y., Kessels, A., Tan, S., Topdag, A., Boon, P., Visser-Vandewalle, V., 2006.  
936 Behavioural changes after bilateral subthalamic stimulation in advanced Parkinson disease: a  
937 systematic review. *Parkinsonism & related disorders* 12, 265–272.
- 938 Tian, J., Yan, Y., Xi, W., Zhou, R., Lou, H., Duan, S., Chen, J.F., Zhang, B., 2018.  
939 Optogenetic Stimulation of GABAergic Neurons in the Globus Pallidus Produces  
940 Hyperkinesia. *Frontiers in Behavioral Neuroscience* 12.  
941 <https://doi.org/10.3389/fnbeh.2018.00185>
- 942 Tooley, J., Marconi, L., Alipio, J.B., Matikainen-Ankney, B., Georgiou, P., Kravitz, A.V.,  
943 Creed, M.C., 2018. Glutamatergic Ventral Pallidal Neurons Modulate Activity of the  
944 Habenula–Tegmental Circuitry and Constrain Reward Seeking. *Biological Psychiatry* 83,  
945 1012–1023. <https://doi.org/10.1016/j.biopsych.2018.01.003>
- 946 Tripathi, A., Prensa, L., Mengual, E., 2013. Axonal branching patterns of ventral pallidal  
947 neurons in the rat. *Brain Structure and Function* 218, 1133–1157.  
948 <https://doi.org/10.1007/s00429-012-0451-0>
- 949 Viereckel, T., Konradsson-Geuken, Å., Wallén-Mackenzie, Å., 2018. Validated multi-step  
950 approach for in vivo recording and analysis of optogenetically evoked glutamate in the mouse  
951 globus pallidus. *Journal of Neurochemistry* 145, 125–138. <https://doi.org/10.1111/jnc.14288>
- 952 Wade, C.L., Kallupi, M., Hernandez, D.O., Breyse, E., de Guglielmo, G., Crawford, E.,  
953 Koob, G.F., Schweitzer, P., Baunez, C., George, O., 2017. High-Frequency Stimulation of the  
954 Subthalamic Nucleus Blocks Compulsive-Like Re-Escalation of Heroin Taking in Rats.  
955 *Neuropsychopharmacology* 42, 1850–1859. <https://doi.org/10.1038/npp.2016.270>
- 956 Wahl, K., Salkovskis, P.M., Cotter, I., 2008. ‘I wash until it feels right.’ *Journal of Anxiety*  
957 *Disorders* 22, 143–161. <https://doi.org/10.1016/j.janxdis.2007.02.009>
- 958 Wallén-Mackenzie, Å., Dumas, S., Papathanou, M., Martis Thiele, M.M., Vlcek, B., König,  
959 N., Björklund, Å.K., 2020. Spatio-molecular domains identified in the mouse subthalamic  
960 nucleus and neighboring glutamatergic and GABAergic brain structures. *Communications*  
961 *Biology* 3(338). <https://doi.org/10.1038/s42003-020-1028-8>
- 962 Weintraub, D.B., Zaghoul, K.A., 2013. The role of the subthalamic nucleus in cognition.  
963 *Reviews in the Neurosciences* 24. <https://doi.org/10.1515/revneuro-2012-0075>
- 964 Winter, C., Flash, S., Klavir, O., Klein, J., Sohr, R., Joel, D., 2008. The role of the  
965 subthalamic nucleus in ‘compulsive’ behavior in rats. *European Journal of Neuroscience* 27,  
966 1902–1911. <https://doi.org/10.1111/j.1460-9568.2008.06148.x>
- 967 Wise, R.A., 2004. Dopamine, learning and motivation. *Nature Reviews Neuroscience* 5, 483–  
968 494. <https://doi.org/10.1038/nrn1406>

- 969 Yoon, H.H., Min, J., Hwang, E., Lee, C.J., Suh, J.-K.F., Hwang, O., Jeon, S.R., 2016.  
970 Optogenetic Inhibition of the Subthalamic Nucleus Reduces Levodopa-Induced Dyskinesias  
971 in a Rat Model of Parkinson's Disease. *Stereotactic and Functional Neurosurgery* 94, 41–53.  
972 <https://doi.org/10.1159/000442891>
- 973 Yoon, H.H., Park, J.H., Kim, Y.H., Min, J., Hwang, E., Lee, C.J., Francis Suh, J.-K., Hwang,  
974 O., Jeon, S.R., 2014. Optogenetic Inactivation of the Subthalamic Nucleus Improves Forelimb  
975 Akinesia in a Rat Model of Parkinson Disease: *Neurosurgery* 74, 533–541.  
976 <https://doi.org/10.1227/NEU.0000000000000297>
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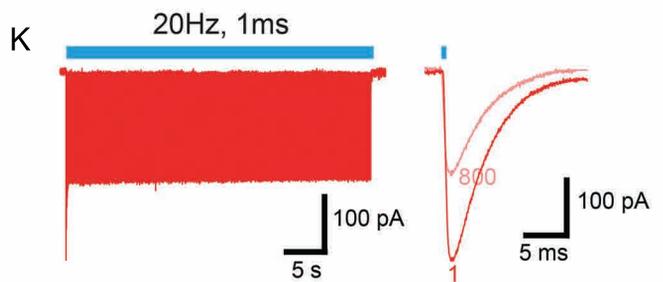
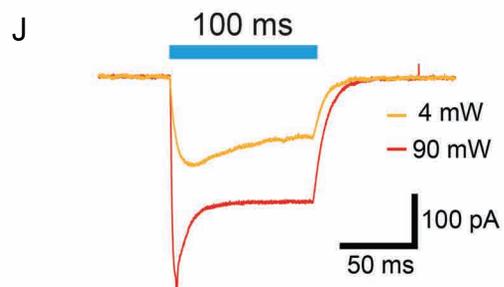
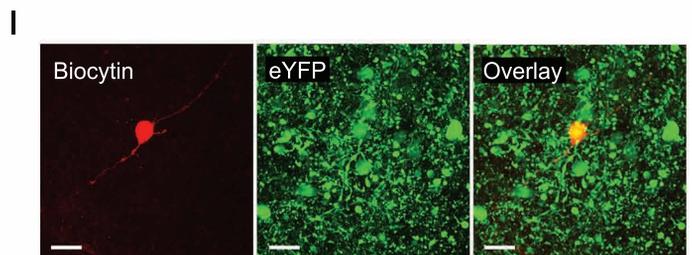
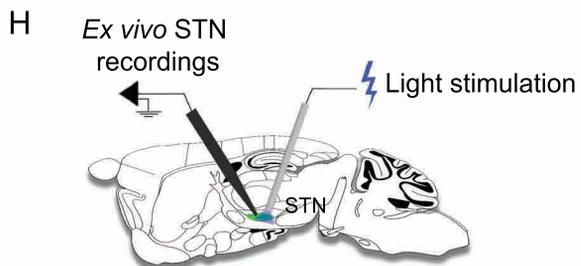
# Experimental protocol and histological analysis



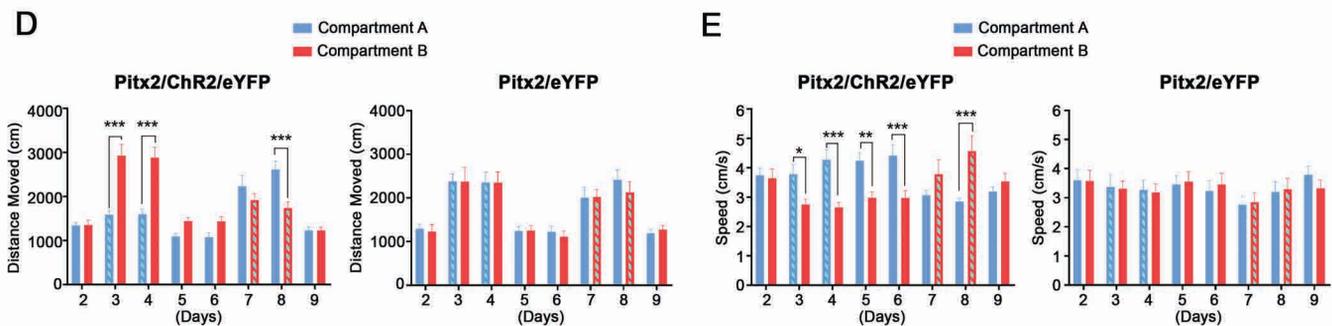
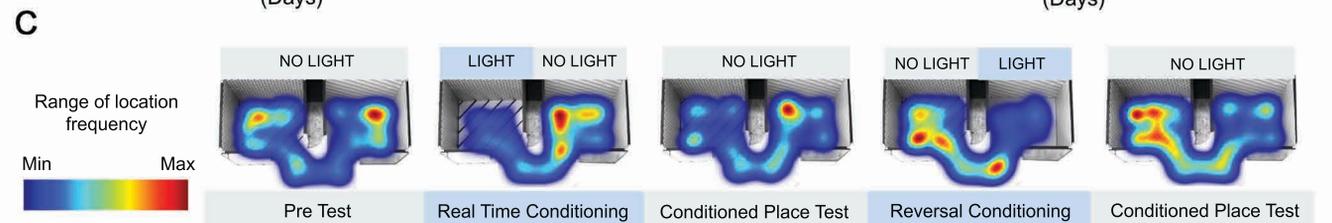
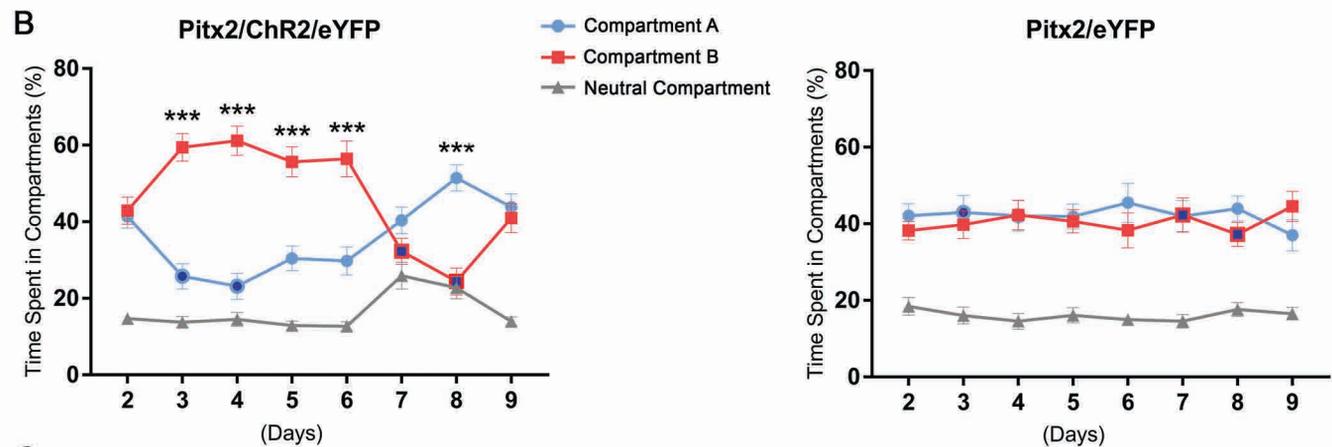
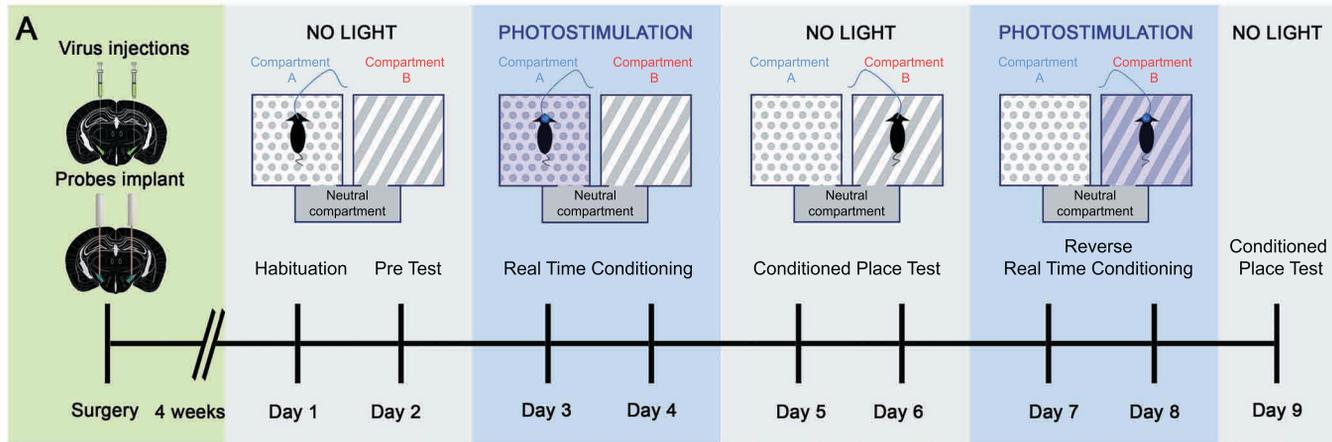
## *In vivo* electrophysiological recordings in the STN



## *Ex vivo* electrophysiological recordings in the STN

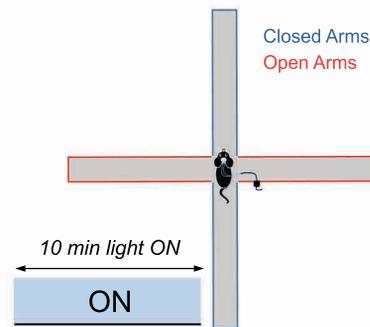


# Real-Time Place Preference (RT-PP) : STN

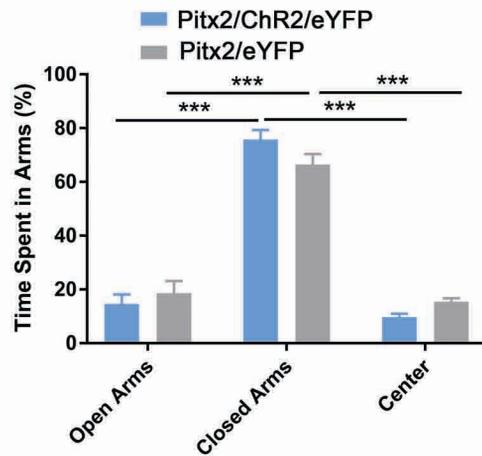


# Elevated Plus Maze (EPM) : STN

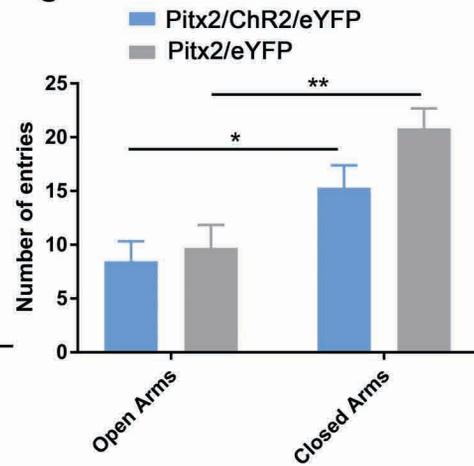
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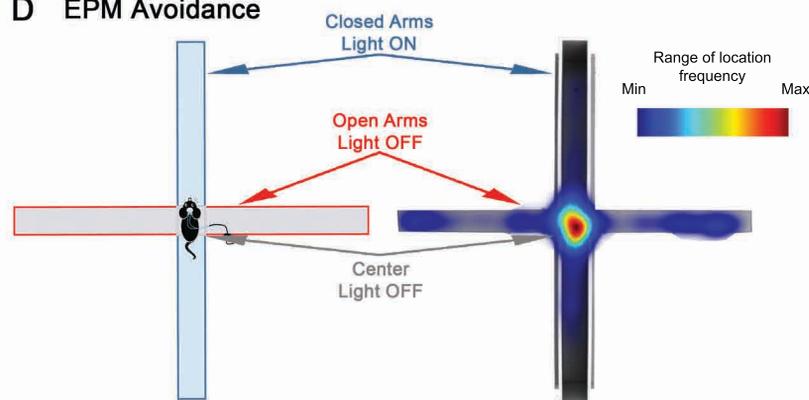
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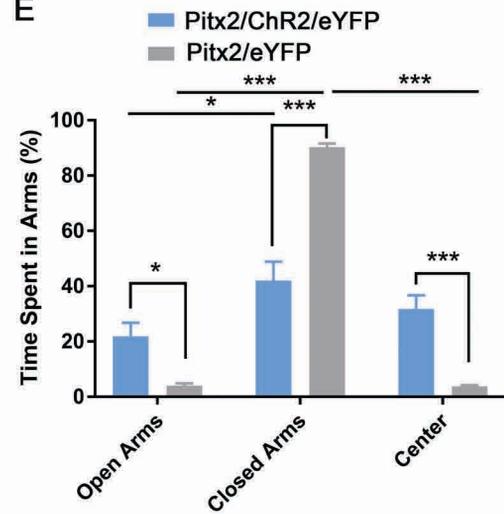
## C



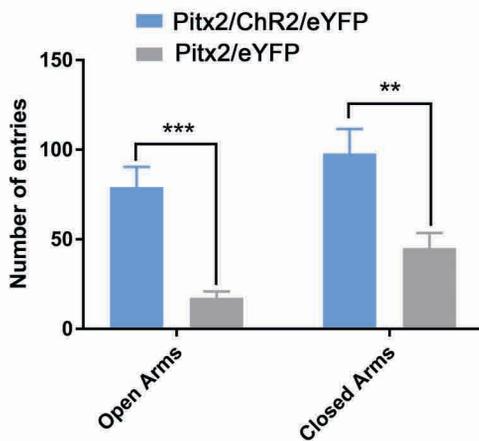
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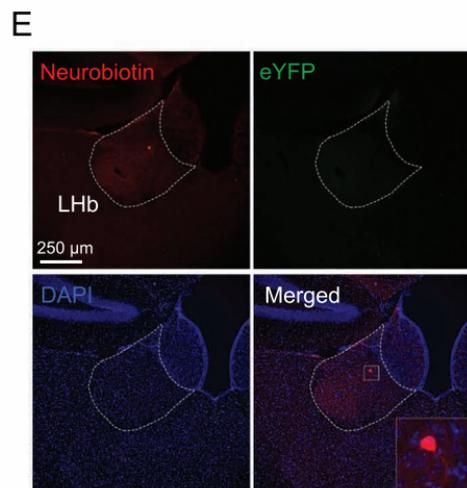
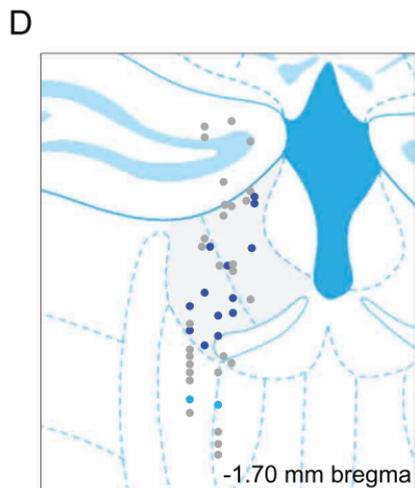
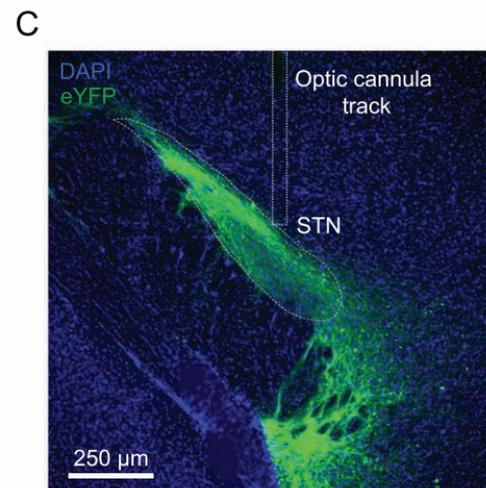
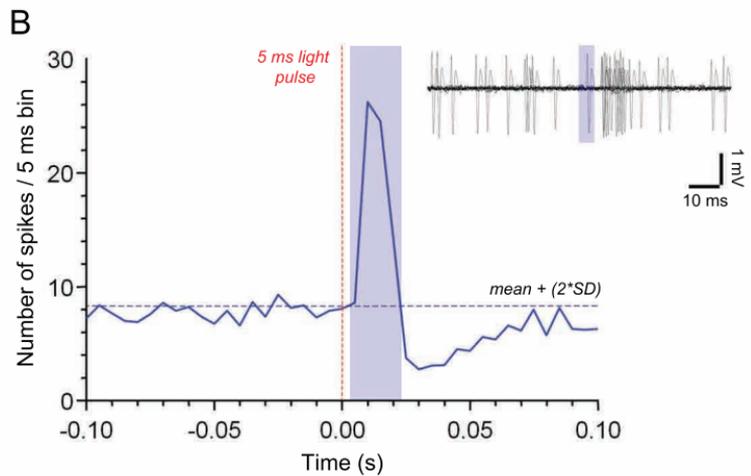
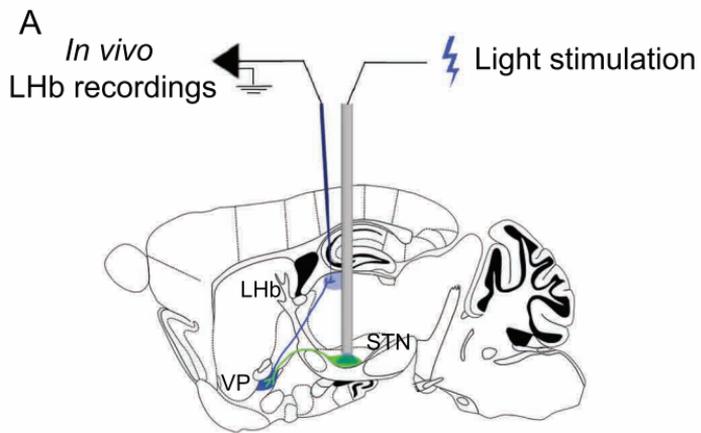


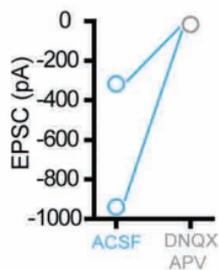
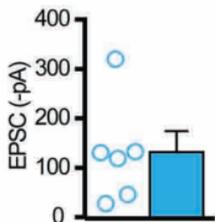
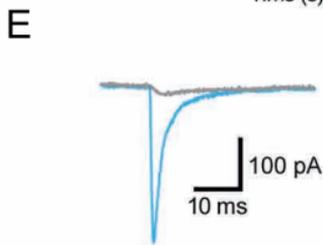
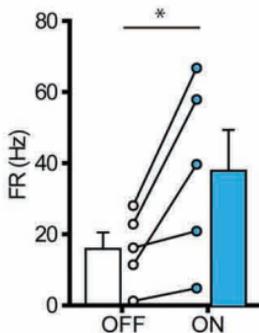
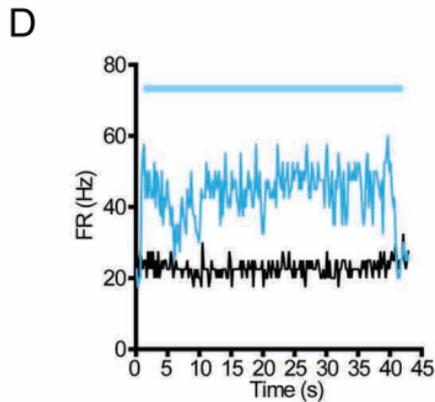
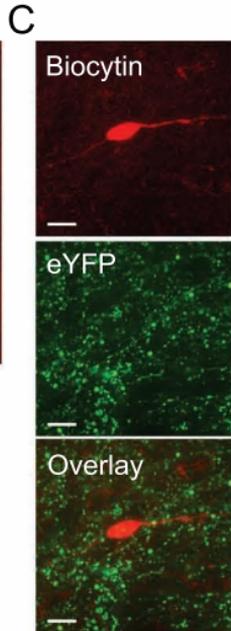
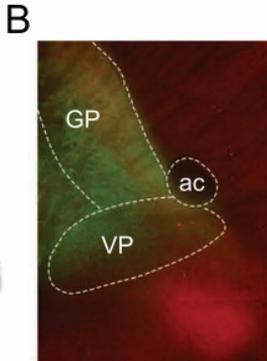
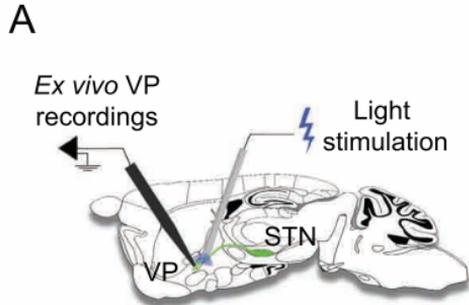
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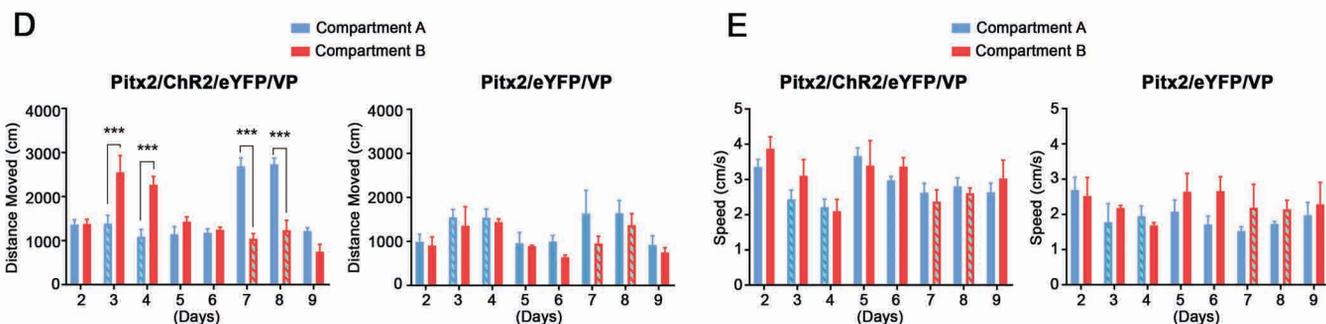
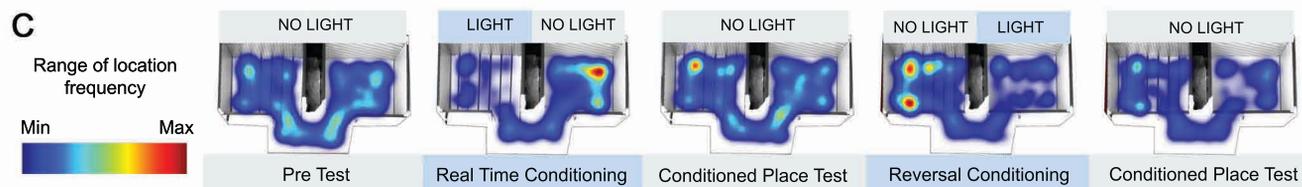
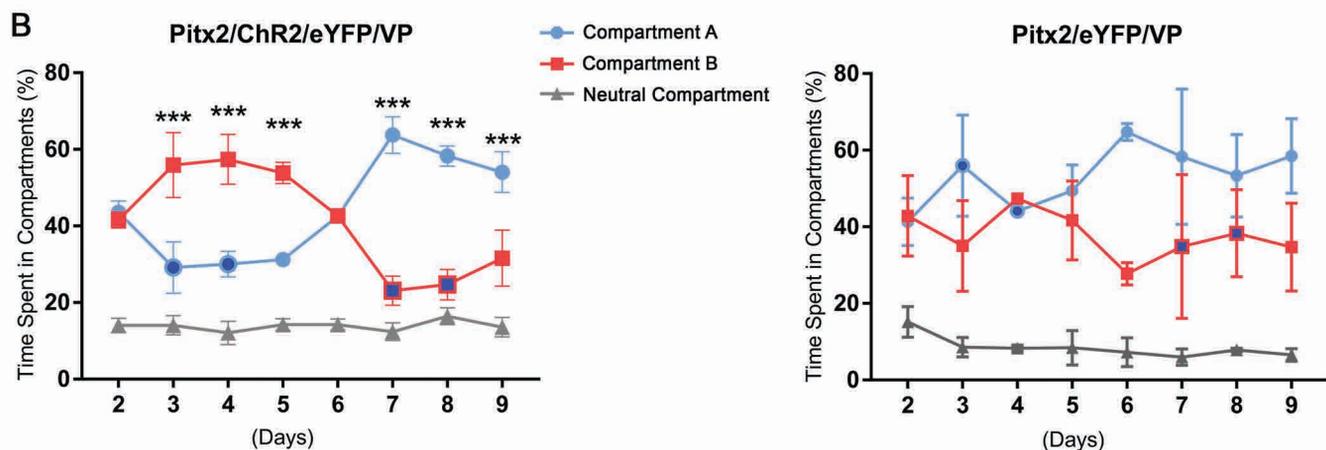
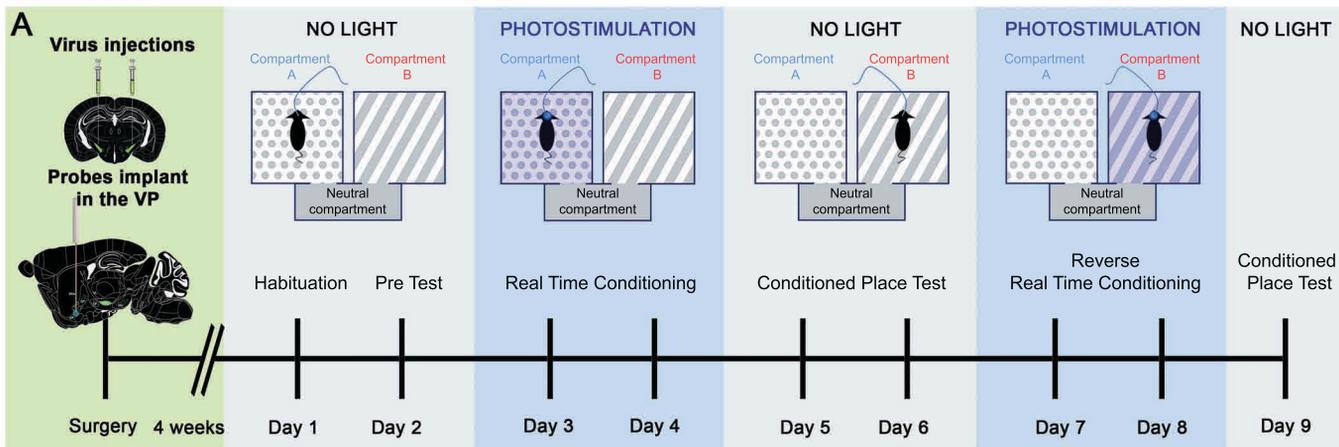
## F





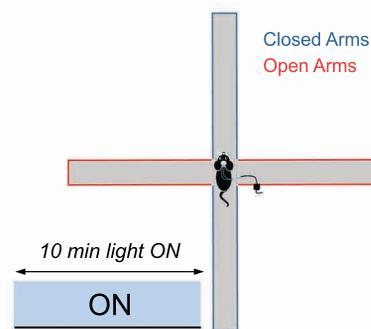


# Real-Time Place Preference (RT-PP) : STN-VP

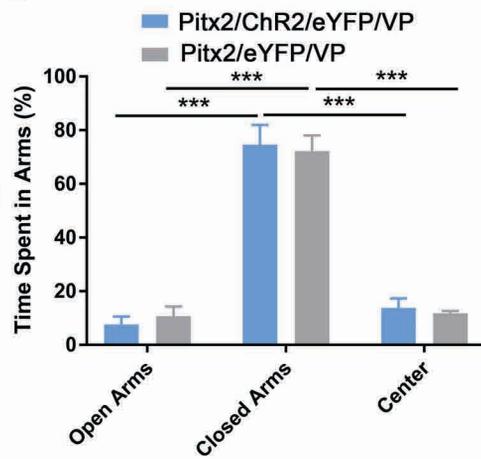


# Elevated Plus Maze (EPM) : STN-VP

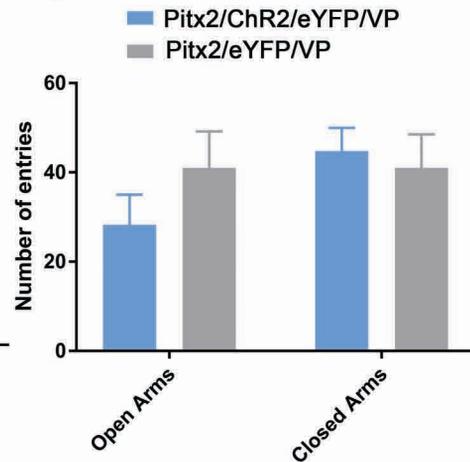
## A Regular EPM



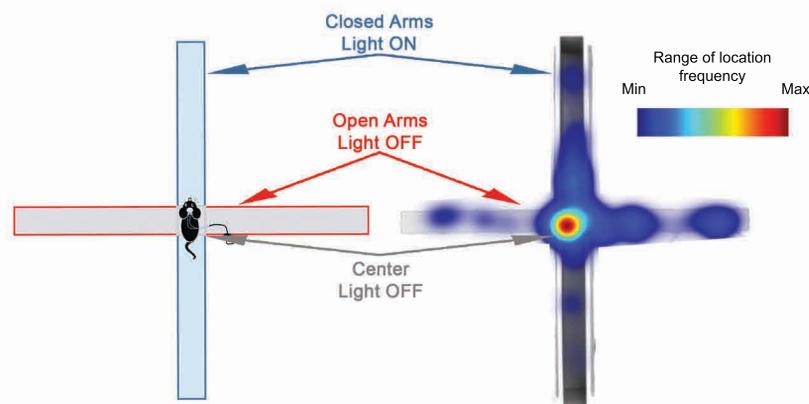
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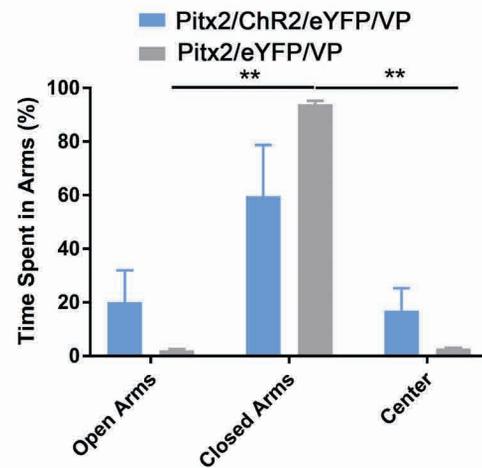
## C



## D EPM Avoidance



## E



## F

