Differential Synaptic Input to External Globus Pallidus Neuronal Subpopulations In Vivo

Graphical Abstract

Highlights
- In vivo whole-cell recordings were performed in the mouse GPe
- Recorded neurons were classified as prototypic or arkypallidal cells
- The subpopulations differed in their synaptic inputs and sensory responses
- Arkypallidal cells integrate inputs from direct, indirect, and hyperdirect pathways

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In Brief
Ketzef and Silberberg describe the membrane properties, synaptic inputs, and sensory responses of prototypic and arkypallidal cells of the external globus pallidus using in vivo whole-cell recordings and optogenetics. They show differential integration of direct, indirect, and hyperdirect basal ganglia pathways performed by the respective cell types.
Differential Synaptic Input to External Globus Pallidus Neuronal Subpopulations In Vivo

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SUMMARY

The rodent external globus pallidus (GPe) contains two main neuronal subpopulations, prototypic and arky pallidal cells, which differ in their cellular properties. Their functional synaptic connectivity is largely unknown. Here we studied the membrane properties, synaptic inputs, and sensory responses of these subpopulations in the mouse GPe. We performed in vivo whole-cell recordings in GPe neurons and used optogenetic stimulation to dissect their afferent inputs from the striatum and subthalamic nucleus (STN). Both GPe subpopulations received barrages of excitatory and inhibitory inputs during slow wave activity and responded to sensory stimulation with distinct multiphasic patterns. Prototypic cells synaptically inhibited arky pallidal and prototypic cells. Both GPe subpopulations received synaptic input from STN and striatal medium spiny neurons (MSNs). Although STN and indirect pathway MSNs strongly targeted prototypic cells, direct pathway MSNs selectively inhibited arky pallidal cells. We show that GPe subtypes have distinct connectivity patterns that underlie their respective functional roles.

INTRODUCTION

The external globus pallidus (GPe) is a central part of the basal ganglia (BG). The activity of GPe cells is altered following dopamine depletion (Mallet et al., 2008; Nini et al., 1995; Raz et al., 2000), suggesting that they have an important role in Parkinson’s disease. The GPe is composed of continuously active GABAergic cells and is traditionally considered part of the indirect pathway of the BG (Albin et al., 1989; DeLong, 1990). As part of the indirect pathway, the GPe projects to the output structures of the BG, the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi) but also to the subthalamic nucleus (STN) and striatum. The cardinal input to the GPe is GABAergic inhibition from striatopallidal medium spiny neurons (MSNs) of the indirect pathway; however, the GPe is also reciprocally connected to the STN (Kita et al., 1983; Kita and Kitai, 1991; Robledo and Feger, 1990) and receives input from striatonigral MSNs (Cazorla et al., 2014; Kawaguchi et al., 1990; Wu et al., 2000). Thus, the GPe is positioned as a hub that connects the main BG pathways: the direct, indirect, and hyperdirect pathways (Mathai and Smith, 2011; Nambu et al., 2002). Additionally, the GPe has been shown to project to the cortex and thalamus (Abecassis et al., 2020; Mastro et al., 2014; Saunders et al., 2015), suggesting that it has a far more central role in BG function than previously appreciated.

The GPe was traditionally regarded as a homogeneous nucleus, but already in early studies in non-human primates (DeLong, 1971), different firing patterns of GPe cells were described. It is now clear that GPe cells are divided into at least two main subpopulations with distinct electrophysiological properties, developmental origins, molecular markers, and projection targets (Abdi et al., 2015; Dodson et al., 2015; Hernández et al., 2015; Mallet et al., 2012; Mastro et al., 2014). The majority of GPe cells, the prototypic cells, conform to the classic description of GPe cells; they are high-frequency spiking cells that project to downstream nuclei, with a small portion of them also projecting to the striatum (Bevan et al., 1998; Dodson et al., 2015; Mallet et al., 2012; Mastro et al., 2014; Saunders et al., 2016). Prototypic cells express the transcription factor NK2 homeobox 1 (NKX2.1) (Abdi et al., 2015; Dodson et al., 2015) and, to a lesser degree, also parvalbumin (PV) and LIM homeobox 6 (LHX6) (Abdi et al., 2015; Dodson et al., 2015; Mallet et al., 2015; Mastro et al., 2014, 2017). The other main class of GPe cells consists of arky pallidal cells, which are a smaller group of relatively less active cells that project extensively to the striatum (Mallet et al., 2012). These cells express the transcription factor for head box protein P2 (FoxP2) (Abdi et al., 2015; Dodson et al., 2015), the neuropeptide precursor preproenkephalin (PPE) (Hoover and Marshall, 2002; Mallet et al., 2012) and the neuronal PAS domain protein 1 (NPas1) (Hernández et al., 2015).

The marked differences between the two main subtypes of GPe cells suggest that they have different functional roles (Mallet et al., 2016); however, the synaptic organization of the respective subtypes is largely unknown. Characterizing the functional connectivity of the different GPe subpopulations is essential for understanding their respective functional roles, especially in sensorimotor processing but also in other brain functions and dysfunctions. Here we studied the GPe in vivo whole-cell...
recordings, enabling us to characterize the membrane properties, synaptic input, and sensory integration of identified prototypic and arkypallidal cells in the intact brain. We quantified the afferent inputs to the respective cell types from the STN and striatum as well as the intra-pallidal connectivity between them.

RESULTS

Whole-cell recordings were obtained from cells in the GPe of anesthetized mice (Figures 1A and 1B); they indicated the existence of at least two main GPe neuronal subtypes. Most recorded cells were spontaneously active with high firing rates (Figures 1C and 1F), had depolarized membrane potential values (Figures 1C and 1E), and paused or reduced their firing rate during cortical slow wave “up states” (Figure 1C). These cells were identified as prototypic GPe cells (n = 53 cells) using two independent measures. Prototypic cells were negative to post hoc immunostaining for FoxP2 (Figure 1C, left) or responded positively to photostimulation (Figure S1) during recordings using the “optopatcher” (Katz et al., 2013; Ketzef et al., 2017) in Channelrhodopsin (ChR2)-expressing cells of NKX2.1-ChR2 (21 cells from 19 mice) or PV-ChR2 mice (3 cells in 3 mice). A smaller fraction of recorded cells was silent (see example in Figure S4) or fired at lower average frequencies (Figures 1D and 1F), had more hyperpolarized membrane potential

Figure 1. Membrane Properties of GPe Cells Recorded Using In Vivo Whole-Cell Patch Clamp Recordings

(A) A scheme describing the experimental setup, including LFP cortical recordings (S1 LFP) and GPe whole-cell recordings (Vm).

(B) Example of a GPe neuron labeled with biocytin following whole-cell recordings (scale bar, 500 μm). The right panel shows the recovered cell at higher magnification (scale bar, 25 μm).

(C) Left panel: identification of prototypic cells according to their lack of expression of FoxP2 (Scale bar, 25 μm, as indicated in D). The cell is stained with cy2 streptavidin (cy2) following biocytin filling. Right panel: prototypic cells (black) fire at high frequencies and slow or pause their firing when the cortex (S1 LFP, top trace) is engaged in an up state.

(D) Left panel: identification of arkypallidal cells according to their expression of FoxP2; arkypallidal cells are positive to FoxP2. Scale bar, 25 μm. The cell is stained with cy3 streptavidin (cy3). Right panel: arkypallidal cells (turquoise) typically depolarize during cortical up states.

(E) Membrane potential histogram for the prototypic and arkypallidal cells presented in (C) and (D). (F) Inter-spike interval (ISI) histogram for the cells presented in (C) and (D). (E and F) The counts are normalized to the maximum value.

(G) Left panel: dendrogram showing the classification of 286 recorded GPe cells. Note the marking of the molecularly identified cells to the right of the dendrogram. Inset: pie chart representing the distribution of recorded cells. Right panel: membrane properties of the GPe cells according to their classification by membrane potential, spontaneous firing frequency, and sag ratio. Prototypic cells are shown in black and arkypallidal cells in turquoise. Cx, cortex; ST, striatum; GPe, external globus pallidus; LV, lateral ventricle; Vm, membrane potential; LFP, local field potential; S1, primary somatosensory cortex.

***p < 0.001. Data are presented as mean ± SEM. Statistical test: Mann-Whitney/two-sample t test. See also Figures S1–S4.
Electrophysiological Properties of GPe Cells In Vivo

We used whole-cell recordings in vivo to extract and compare the membrane properties of 89 molecularly identified prototypic and arkypallidal cells. Prototypic (P) cells were significantly more depolarized than arkypallidal (A) cells (P, −46.11 ± 0.47; A, −60.09 ± 1.00 mV; p < 0.001, Mann-Whitney test), fired spontaneously at higher average rates (P, 13.31 ± 1.01; A, 0.74 ± 0.25 Hz; p < 0.001, Mann-Whitney test), and had larger sag ratios (P, 1.10 ± 0.01; A, 1.06 ± 0.01; p < 0.001, Mann-Whitney test) (n = 53 prototypic and 36 arkypallidal cells; Figure S2). Other membrane properties, such as input resistance (P, 0.23 ± 0.01; A, 0.23 ± 0.01 GigaOhm [GΩ], p = 0.89, two-sample t test) were not different between the groups. Because there were cases in which the cells were not found conclusively post hoc, we performed unsupervised clustering, as described previously for slice recordings (Abrahao and Lovinger, 2018).

We thus classified 286 recorded GPe cells in an unbiased manner based on their electrophysiological properties as recorded in vivo (Figure 1G). The clustered data showed a division into two main groups that corresponded to the molecularly defined subgroups of prototypic and arkypallidal cells (Figures 1G and S2). The two groups differed in various electrical properties, including subthreshold properties and features of their action potential (AP) firing (Figure 1G). Prototypic cells fired more regularly (lower inter-spike interval coefficient of variance [CV_{is}] than arkypallidal cells (P, 0.99 ± 0.04; A, 1.30 ± 0.06; p < 0.001, Mann-Whitney test) and had smaller AP amplitudes (P, 44.48 ± 1.05; A, 55.18 ± 1.58 mV; p < 0.001, Mann-Whitney test). These properties accompanied the significant differences observed in their membrane potential (P, −46.05 ± 0.24; A, −58.76 ± 0.54 mV; p < 0.001, Mann-Whitney test), average spontaneous firing frequency (P, 13.89 ± 0.55; A, 0.99 ± 0.16 Hz; p < 0.001, Mann-Whitney test), and sag ratio (P, 1.12 ± 0.01; A, 1.05 ± 0.01; p < 0.001, Mann-Whitney test), already described for identified cells (n = 191 prototypic cells, n = 95 arkypallidal cells; Figure 2D). Input resistance (P, 0.23 ± 0.01; A, 0.22 ± 0.01 GΩ; p = 0.212, two-sample t test), AP half-width (P, 0.59 ± 0.02; A, 0.68 ± 0.04 ms), and AP afterhyperpolarization (P, −6.04 ± 0.17; A, −6.19 ± 0.33 mV) did not differ between the classified groups (p > 0.061, Mann-Whitney test). Although the hierarchical cluster analysis supports two GPe populations, further subdivisions can be explored. In our data, we could find some difference between possible prototypic subgroups, but these differences were not correlated with previously described molecular subtypes (some examples are presented in Figure S3).

Both GPe Cell Types Receive Excitatory and Inhibitory Inputs during Slow Wave Activity

The opposite modulation of GPe cell types during up states (recorded in cortical local field potential [LFP]; Figure 1) could be due to differences in their membrane properties (Figure 1) as well as differences in the synaptic inputs they receive during up states. To explore these two possibilities, we manipulated the membrane potential of recorded cells by injecting negative and positive holding currents via the patch pipette (Figure S4). When hyperpolarizing prototypic cells, the activity coinciding with the cortical up states reversed polarity and was now depolarizing, occasionally enabling firing. This indicates that, during up states, prototypic cells do not only receive inhibitory input but also an excitatory component. Conversely, depolarization of arkypallidal cells reversed their modulation during cortical up states, which resulted in hyperpolarization and reduction in firing rate, as observed in prototypic cells (Figures 2 and S4). These data suggest that, during slow wave activity, prototypic and arkypallidal cells receive a barrage of mixed excitatory and inhibitory inputs and that the modulation of their spiking during up states depends on their respective membrane potentials and baseline activity levels.

Although both cell types receive inhibitory and excitatory inputs during up states, the respective magnitudes of these components could differ and shape the responses in a cell-type-specific manner. To quantify such differences, we calculated the correlation coefficient between the cortical LFP and the membrane potential for all 286 recorded GPe cells (Figure 2). The up and down states were apparent in the cortical LFP recordings, and their modulation of the membrane potential was extracted from whole-cell recordings of GPe cells. Performing cross-correlation between these two simultaneously acquired traces enabled us to extract the polarity (whether cells depolarized or hyperpolarized during up states) and magnitude of modulation (STAR Methods). The correlation coefficient of classified prototypic and arkypallidal cells differed in magnitude and polarity (Figures 2A–2C; P, −0.09 ± 0.03; A, 0.39 ± 0.03; p < 0.001, Mann-Whitney test). Although almost all arkypallidal cells (97%) had positive correlation coefficients, indicating depolarization during up states, prototypic cells were, on average, only weakly and negatively modulated by cortical activity (the average correlation coefficient was slightly negative). This analysis shows that the GPe subpopulations respond differently to the barrages of synaptic input during cortical slow wave oscillations.

In a subset of cells, we recorded the ongoing activity of the same cells at different membrane potential values by injecting different holding currents via the patch pipette (Figures 2D, 2E, and S4), enabling us to calculate the correlation coefficient for each condition. We could then approximate the membrane potential at which correlation coefficient values changed from positive to negative, indicating a “functional reversal potential” for the compound inputs during up states, serving as an indirect measure of the excitation-inhibition balance. The resulting value was more depolarized for arkypallidal cells compared with prototypic cells (P, −52.24 ± 0.93; A, −46.17 ± 1.21 mV; p < 0.001, two-sample t test, n = 55 prototypic and n = 23 arkypallidal cells). Most recorded cells, regardless of their type, were found to be positively correlated with the cortical LFP during negative current.
Figure 2. Modulation of Membrane Potential Dynamics in GPe Cells during Slow Wave Activity

(A) Examples of traces recorded in prototypic (black) and arkypallidal cells (turquoise) without modulation of membrane potential by current injection ($I_{in} = 0$) and their correlation to the LFP recorded in S1. Note the pink-shaded area showing the cortical up state and the corresponding activity in the whole-cell recorded GPe neuron.

(B) Correlation coefficient of the cortical LFP and the cell activity filtered between 0.4–1.6 Hz, organized according to the classifier into the two types of GPe cells. Darker circles correspond to the examples in (A).

(C) Distribution of the correlation coefficient values according to membrane potential (left) and spontaneous firing frequency (right) for prototypic and arkypallidal cells. Vertical lines in each graph indicate the mean value per group.

(D) Examples of traces recorded in GPe cells injected with holding currents: hyperpolarized prototypic cells (left) and depolarized arkypallidal cells (right). Note the change in polarity of the correlation coefficient values for each cell type compared with (A). The pink-shaded areas show an example of the cortical up state and the corresponding activity in the recorded neuron.

(E) Correlation coefficients of all cells that were recorded in at least 3 different holding currents.

(F) Extrapolation of the membrane potential value in which the correlation coefficient shifts from positive to negative values, organized according to the classifier.

(G) Distribution of all correlation coefficient values recorded in all protocols (with and without membrane potential modulation by current injections). The plot is superimposed by sigmoidal fitting between the maximum and minimum values of the correlation coefficient, 1 and $-1$. CC, correlation coefficient. Prototypic cells are represented in black and arkypallidal cells in turquoise. ***p < 0.001. Data are presented as mean ± SEM. Statistical test: Mann-Whitney. See also Figures S3 and S4.
injections and, vice versa, negatively correlated with the cortical LFP during positive current injections (Figure 2G).

These results suggest that prototypic and arkypallidal cells receive different compositions of excitatory and inhibitory synaptic inputs during slow wave activity. The modulation of their respective activities is therefore determined by cell-type-specific membrane properties and synaptic inputs. We next aimed to dissect the synaptic inputs to the respective GPe cell types from the local GPe circuitry, the STN, and the two types of striatal MSNs.

**Prototypic Cells Inhibit Arkypallidal and Prototypic Cells**

Intra-pallidal connectivity may have an important role in GPe function, especially because of the perisomatic location of the local inhibitory synapses (Gross et al., 2011). Anatomical data (Sadek et al., 2007) and ex vivo recordings in slices (Bugaysen et al., 2013) have revealed only sparse connectivity among GPe cells, but it has been predicted that a primary intra-pallidal synaptic pathway exists from prototypic to arkypallial cells (Nevado-Holgado et al., 2014). To study the synaptic interactions between GPe cells, we virally expressed ChR2 in the GPe of Nkx2.1-Cre mice (Figures 3A and 3B). During *in vivo* recordings, we activated cells with an optic fiber placed dorsally to the GPe (Figures 3A and 3B). ChR2-expressing prototypic cells responded to photostimulation with strong, reliable, and sustained depolarization, superimposed by APs, even with very low light intensities (less than 0.2 mW; Figures 3D and 3G). The onset of light responses indicated direct optogenetic excitation of recorded prototypic cells (0.42 ± 0.05 ms, n = 20; Figure 3H). In contrast, arkypallidal cells were strongly inhibited by photostimulation (−9.59 ± 2.33 mV when cells were held at −45 mV with current injections, n = 7; Figures 3F, 3G, and 3I), with onset delays suggesting monosynaptic inhibition (5.00 ± 0.54 ms; Figure 3H). The reversal potential of inhibitory responses was approximately −75 mV, corresponding to the expected value for GABAergic inhibition (Figure S5). We also recorded from prototypic cells (FoxP2 negative) that were not virally transduced and not excited by light activation (Figures 3E, 3G, and 3J). Photostimulation of neighboring prototypic cells induced inhibitory synaptic responses in these cells with similar amplitudes (−7.20 ± 1.49 mV, n = 5, p = 0.621, two-sample t test; Figures 3E, 3G, and 3J) and onset delays (5.12 ± 0.88 ms, p = 0.977, two-sample t test; Figure 3H) to arkypallidal cells, showing the existence of inhibition among prototypic cells. The amplitudes and onset delays of photostimulated cells (prototypic cells expressing ChR2) were significantly different from the values recorded in synaptically inhibited cells (arkypallidal cells depolarized by current injections and prototypic cells that did not express ChR2; p < 0.001, two-sample t test, 20 photostimulated and 12 synaptically inhibited cells). These results show that prototypic cells provide strong and reliable inhibition to arkypallidal cells and are also interconnected among themselves. It also suggests that, in addition to afferent excitation, arkypallidal cells are disinhibited during up-states because of the reduction in spiking of prototypic cells.

**Arkypallidal Cells Sparsely Target Other GPe Cells**

The connectivity between arkypallidal and prototypic cells or of arkypallidal cells among themselves was not predicted to be high (Nevado-Holgado et al., 2014). To examine this, we expressed ChR2 in the GPe of FoxP2-Cre mice (Figure 4A). Because FoxP2 is also expressed by striatal MSNs, we used very small volumes of injected virus to minimize possible contamination. When recording *in vivo* (Figure 4B), we did not find any synaptic responses to photostimulation of the infected arkypallidal cells (P, n = 0 of 20; A, n = 0 of 3; Figures 4C–4E). To verify our findings, we recorded in the slice preparation *ex vivo* (Figure 4F). All virus-expressing cells tested responded to photostimulation with depolarization and firing (n = 6/6; Figure 4G), with onset latency of 0.33 ± 0.07 ms. Cells that did not express ChR2 but were juxtaposed to transduced fibers did not respond to photostimulation (n = 0 of 6; Figure 4H). Thus, our findings, *in vivo* and *ex vivo*, show very low connectivity from arkypallidal to prototypic cells and among arkypallidal cells. These data, combined with the strong connectivity observed from Nkx2.1-Cre cells (Figure 3), point towards a highly non-reciprocal connectivity between the prototypic and arkypallidal populations.

**The STN Provides Afferent Excitatory Input to Both GPe Cell Types**

The STN is reciprocally connected to the GPe and has been considered to be the main source of excitatory input to the GPe (Kita et al., 1983; Kita and Kitai, 1991; Pamukcu et al., 2020). To study the STN inputs to the GPe, we expressed ChR2 in STN neurons using retrograde viral transduction in vglut2-Cre mice (Figure 5A; STAR Methods). We then recorded the responses of GPe cells to photostimulation of STN cells through a fiber placed above the STN (Figures 5A and 5B). All recorded GPe cells responded to STN photostimulation (Figure 5C); however, there were differences in responses properties. The amplitude of the initial response phase was not significantly different between prototypic and arkypallidal cells (P, 13.62 ± 1.43; A, 9.12 ± 1.59 mV; n = 19 prototypic and 6 arkypallidal cells, p = 0.055, two-sample t test; Figures 5D–5F), nor was there a difference in onset delays (P, 4.75 ± 0.14; A, 4.35 ± 0.1 ms; p = 0.14, two-sample t test; Figure 5G). In contrast, photostimulation with a 500 ms light pulse induced sustained depolarization in prototypic cells (Figure 5D) but only a transient response in arkypallidal cells (Figure 5E). The response amplitudes measured at the end of the light pulse were strongly reduced in arkypallidal cells compared with their initial response amplitudes (start, 9.12 ± 1.59; end, 3.49 ± 1.02 mV; p < 0.001, paired-sample t test) but not in prototypic cells (start, 13.62 ± 1.43; end, 14.32 ± 1.59 mV; p = 0.61, paired-sample t test; Figure 5F). Photostimulation of the STN while holding GPe cells at −45 mV resulted in continuous depolarization of prototypic cells (Figure 5H), in contrast to brief depolarization followed by sustained hyperpolarization of arkypallidal cells (Figure 5I), likely originating from activated prototypic cells (Figure 3). Nevertheless, photostimulation of the STN with a high-frequency train (20 pulses at 20 Hz; Figures 5J and 5K) induced depolarizing responses that enabled firing of APs in both cell types. These results show that, although the STN provides excitatory synaptic input to both GPe populations, the effect of this input is cell-type-specific and shaped by intra-pallidal connectivity.
Target Selectivity in Striatopallidal Inhibition

The major source of inhibition to the GPe is attributed to the striatum and particularly to indirect pathway MSNs (iMSNs), however, axon collaterals of direct pathway MSNs (dMSNs) were also shown to project to the GPe (Cazorla et al., 2014; Kawaguchi et al., 1990; Wu et al., 2000). In order to understand how the two types of MSNs inhibit the GPe subpopulations, we expressed ChR2 in dMSNs or iMSNs, placed an optic fiber in the dorsal striatum, and recorded the synaptic responses in GPe cells to striatal photostimulation. To study the inputs of
iMSNs to the GPe, we expressed ChR2 in iMSNs virally using D2-Cre or A2A-Cre mice (Figures 6A and 6B) or in D2-Cre mice crossed with ChR2 reporter mice (Ai32; Figure S6). As expected, in all experimental groups, prototypic cells were strongly inhibited by photostimulation of iMSNs (Figures 6C–6E). It was thus expected that inhibition of prototypic cells may cause depolarization of arkypallidal cells via disinhibition (Figure 4); however, in all cases (Figure 6C), arkypallidal cells responded to photostimulation by initial hyperpolarization (Figures 6F–6I), indicating direct inhibition by iMSNs. The initial hyperpolarization was often followed by delayed depolarization (Figure 6H), likely originating from reduced hyperpolarization of neighboring prototypic cells (Figure 6D). Such a biphasic response was not seen in prototypic cells. The amplitude of inhibitory responses (Figure 6J) was larger in prototypic cells compared with arkypallidal cells held at a similar membrane potential using current injections (P = 19.12 ± 0.54; A = −8.90 ± 1.37 mV; p < 0.001, two-sample t test), and the onset delay (Figure 6K) was not different (P = 0.34 ± 0.35; A = 8.60 ± 0.43 ms; p = 0.051, two-sample t test; cells recorded in A2a-Cre or D2-Cre, n = 27 prototypic, n = 11 arkypallidal cells). Similar results were obtained in D2-ChR2 mice using the same experimental configuration (Figure S6). These data suggest that, although prototypic and arkypallidal cells receive inhibition from iMSNs, this inhibition is biased toward prototypic cells and can induce delayed disinhibition of arkypallidal cells.

To study the inputs from dMSNs to GPe cells, we virally expressed ChR2 in the striatum of D1-Cre mice and positioned an optic fiber in the dorsolateral striatum (Figures 7A and 7B). Of 33 prototypic cells recorded, only 6 (18.18%) showed measurable responses to photostimulation (Figures 7C–7F). In contrast, all arkypallidal cells were inhibited by dMSN photostimulation, as seen by short-latency hyperpolarization of their membrane potential and suppression of spiking (n = 5; Figures 7C and 7G–7I). The amplitude of inhibitory responses was larger in arkypallidal than in prototypic cells (P = 0.58 ± 0.24; A = −10.99 ± 2.62 mV; p < 0.001, Mann-Whitney test, n = 33 prototypic and 5 arkypallidal cells; Figure 7J). However, there was no difference in onset delay (P = 0.42; A = 8.16 ± 1.14 ms; p = 0.99, two-sample t test, n = 6 prototypic and 5 arkypallidal cells; Figure 7K). These results indicate a strong bias in dMSNs input to the GPe, with strong and prevental inhibition of arkypallidal cells and only sparse and weak inhibition of prototypic cells.

Figure 4. Arkypallidal Cells Provide Only Sparse Input to Other GPe Cells

(A) A scheme of the experimental setup: viral injections in the GPe (left) and photostimulation and ex vivo whole-cell recordings (right).

(B) Sagittal (left; scale bar, 1 mm) and coronal (right; scale bar, 1 mm) sections showing virus expression and fiber location.

(C) Pie charts representing the distribution of prototypic (gray) and arkypallidal (faint turquoise) cells not responding directly or indirectly (synaptically) to photostimulation.

(D) Example of traces recorded in prototypic cells (black) during photostimulation.

(E) Top panel: images illustrating the recorded arkypallidal cell stained with cy3-streptavidin (cy3), its neighboring virus-transduced fibers (YFP), and staining for FoxP2 confirming the cell as an arkypallidal cell. Scale bar, 20 μm. Bottom and right panels: examples of traces recorded in arkypallidal cells (turquoise) at rest (left) or depolarized (right) during photostimulation. (D and E) Traces are presented as raw traces in faint color overlaid with the average trace in darker color.

(F) ex vivo validation of the results obtained in vivo. Left panel: a scheme representing photostimulation and ex vivo whole-cell recording in the GPe. Right panel: cells expressing the virus responded to photostimulation with depolarization, superimposed with AP firing. Bottom panel: differential interference contrast (DIC), fluorescence, and merged images of the recorded cell. Also shown is a pie chart depicting the number of cells recorded.

(F and G) 10-Hz photostimulation (8 pulses of 2 ms each and a recovery pulse 0.5 s after the last pulse) was applied. Data are presented as raw traces in faint color overlaid with the average trace in darker color.

Prototypic cells are represented in black, arkypallidal cells in turquoise, and ChR2-negative cells in purple.
Distinct Sensory Responses in Arkypallidal and Prototypic Cells

To study the functional effect of excitatory and inhibitory synaptic inputs on the GPe subpopulations, we characterized the responses of GPe cells to tactile stimulation delivered as brief whisker deflections (Figure 8A). Bilateral whisker stimulation induced a multiphasic response in all prototypic and arkypallidal cells (n = 170 cells; Figures 8B and 8D–8F). The responses typically started with depolarization, followed by hyperpolarizing and depolarizing phases. This sequence was triphasic in most cases but could be followed by delayed secondary components. The triphasic pattern suggests that the response to sensory stimulation is shaped by excitatory and inhibitory inputs converging on the cells in a specific sequence. Response onset was not different between the subgroups (P, 26.45 ± 0.84; A, 25.71 ± 1.60 ms; p = 0.214, Mann-Whitney test, n = 97 prototypic cells, n = 73 arkypallidal cells; Figure 8C). We next defined the three response phases based on the subsequent depolarization and hyperpolarization peaks (Figures 8D and 8E). The amplitudes and temporal properties of the sensory response phases differed significantly between prototypic and arkypallidal cells (p < 0.001, two-way repeated-measures ANOVA followed by Tukey test for pairwise comparison; Figures 8D and 8E). The first phase depolarized more (P, 1.59 ± 0.11; A, 6.91 ± 0.50 mV; p < 0.001) and lasted longer (P, 10.69 ± 0.61; A, 29.81 ± 2.23 ms; p < 0.001) in arkypallidal cells, whereas the second phase hyperpolarized prototypic cells more (P, −12.47 ± 0.51; A, −3.99 ± 0.54 mV; p < 0.001) and peaked earlier (P, 33.08 ± 0.95; A, 43.88 ± 2.91 ms; p = 0.012). The third phase significantly depolarized prototypic cells (P, 10.51 ± 0.46; A, 3.76 ± 0.45 mV; p < 0.001) and peaked later in these cells (P, 80.85 ± 5.63; A, 69.30 ± 4.47 ms; p = 0.007). These results indicate that, although both GPe subpopulations receive convergent excitatory and inhibitory inputs during sensory stimulation, these inputs, together with the membrane properties of the GPe subpopulations, generate distinct response patterns.

Our results show that the different GPe cell types receive input from the STN and striatum; however, pronounced target preference manifested in the photostimulation response amplitudes.
kinetics, and connection probabilities. Interestingly, arkypallidal cells receive reliable synaptic inputs from the STN, dMSNs, and iMSNs, linking them to the three main BG pathways. Last, we show that the differences in membrane and synaptic properties between the GPe subpopulations are also mirrored by distinct responses to sensory stimulation.

**DISCUSSION**

In this study, we used in vivo whole-cell patch-clamp recordings in mice to study the membrane properties, network connectivity, and sensory integration in GPe cells. To our knowledge, this is the first report of such recordings in the GPe, enabling study of
Figure 7. dMSNs Target Arkypallidal Cells and Almost Avoid Prototypic Cells

(A) A scheme of the experimental setup: viral injections in the striatum (left), photostimulation in the striatum, and whole-cell recording in the GPe (right).

(B) Sagittal (left; scale bar, 1 mm) and coronal (right; scale bar, 0.5 mm) sections showing viral transduction in the dorsal striatum, optic fiber location, and typical projection of dMSNs to the GPi and SNr.

(C) Pie chart representation of the proportion of cells responding to dMSN light activation.

(D) Responses of prototypic cells to 500 ms light stimulation of striatal dMSNs. The magenta dashed area is expanded to the right of the trace.

(E) Responses of prototypic cells to 20-Hz light stimulation of dMSNs. Baseline membrane potential is as indicated in (D).

(F) Same as in (D) but for depolarized arkypallidal cells.

(G) Same as in (E) but for depolarized arkypallidal cells.

(H) Same as in (D) but for arkypallidal cells at rest.

(I) Same as in (E) but for arkypallidal cells at rest.

(J) Light response amplitude in prototypic and arkypallidal cells held at similar membrane potentials.

(K) Onset delay of the response to photostimulation in prototypic and arkypallidal cells held at similar membrane potentials.

Prototypic cells are shown in black and arkypallidal cells in turquoise. ***p < 0.001. ST, striatum; GPe, external globus pallidus; SNr, substantia nigra pars reticulata. Data are presented as mean ± SEM. Statistical tests: two-sample t test/Mann-Whitney.
sub- and suprathreshold activity in the intact brain. Combining whole-cell recordings with optogenetics, we characterized the membrane properties and afferent inputs to the different GPe subpopulations. We show that, during slow wave activity, prototypic and arkypallidal cells receive barrages of excitatory and inhibitory inputs that, together with their electrophysiological properties, pattern their spontaneous activity. The local GPe circuitry is dominated by unilateral inhibition from prototypic cells to all other GPe cell types, which can be recruited by external input. Both GPe subpopulations receive input from the STN and striatum, but there is a clear target preference in these afferent inputs, in particular the very weak input to prototypic cells from dMSNs. Notably, arkypallidal cells receive direct pathway information from dMSNs, indirect pathway information from iMSNs, and hyperdirect pathway input from the STN, placing them as integrators of the three main BG pathways. Finally, we show that GPe cells of both subpopulations respond to tactile sensory stimulation but with distinct multiphasic patterns.

We identified cells as prototypic or arkypallidal cells primarily according to their expression of the molecular markers FoxP2 and NKX2.1, dividing our recorded cells into prototypic and arkypallidal cells without further subdivision. FoxP2 is a molecular marker for the arkypallidal cells that is rarely co-expressed with NKX2.1 or PV (Abdi et al., 2015; Dodson et al., 2015; Hernández et al., 2015). The activity of molecularly identified GPe cells acquired using whole-cell in vivo recordings was similar to that described for extracellular recordings (Abdi et al., 2015; Dodson et al., 2015; Mallet et al., 2012). As reported previously (Mallet et al., 2012), some arkypallidal cells were completely silent under control conditions. We used electrophysiological data from molecularly identified cells to classify a larger number of GPe cells recorded in vivo. As expected, we found differences in the firing frequency and regularity between prototypic and arkypallidal cells, as described with extracellular recordings (Abdi et al., 2015; Dodson et al., 2015; Mallet et al., 2012). We also found significant differences in their membrane properties, some of which have been described using ex vivo recordings (Abdi et al., 2015; Abrahao and Lovinger, 2018; Hernández et al., 2015; Mastro et al., 2014).

Our data show that arkypallidal cells receive strong, reliable, and unidirectional inhibition from prototypic cells (Figures 3 and 4), implying that they are under tonic inhibition that is transiently relieved when prototypic cells are inhibited. We also show that prototypic cells are interconnected by inhibitory synapses (Figure 4); however, the organization and functional role of this recurrent inhibition is still unclear and will be subject to future

Figure 8. Responses to Sensory Stimulation in Prototypic and Arkypallidal Cells
(A) Sensory stimulation was evoked by applying bilateral air puffs to the whiskers.
(B) Responses to whisker stimulation in prototypic (black, left) and arkypallidal (turquoise, right) cells. Single responses are presented in faint color, and the average traces of these cases are presented in darker colors. Inset: averaged response to ~20 repetitions of whisker stimulation. The orange arrow indicates the air puff trigger time point.
(C) Onset delay taken from the trigger time point.
(D) Comparison of the amplitude of the response to sensory stimulation phases in prototypic and arkypallidal cells. Inset: scheme describing the measured response phases amplitude.
(E) Comparison of the response to sensory stimulation peak times in prototypic and arkypallidal cells. Inset: scheme describing the measured response phases peak times.
(F) Number of cells in each GPe subgroup recorded in this protocol.
Prototypic cells are represented in black and arkypallidal cells in turquoise. *p < 0.05, **p < 0.01, ***p < 0.001. Statistical test: repeated-measures ANOVA followed by Tukey test for pairwise comparison.
studies. Arkypallidal cells did not provide local inhibition within the GPe in our experimental setup. This could be a result of the cautious approach to minimize the expression of the ChR2 virus outside of the GPe. However, ex vivo validation of our in vivo observations supports low local arkypallidal-mediated inhibition, as suggested recently (Aristieta et al., 2020). The afferent pathways to the GPe have been described previously (Albin et al., 1989; Cazorla et al., 2014; DeLong, 1990; Kawaguchi et al., 1990; Kita et al., 1983; Robledo and Feher, 1990; Wu et al., 2000); however, their effect on the different GPe subpopulations was unknown. We showed that the STN provides excitatory input to prototypic and arkypallidal cells; however, its effect on the activity of the respective subpopulations was cell-type-specific. Excitation of arkypallidal cells was curtailed by strong inhibition from prototypic cells, resulting in only a brief response at the onset of STN activation. The differential responses to STN photostimulation may also reflect differences in other synaptic properties of STN input to the GPe subpopulations.

Striatal input was also biased with respect to the postsynaptic GPe cell type. As expected, iMSNs strongly inhibited prototypic cells; however, they also provided reliable but weaker inhibition to arkypallidal cells. Surprisingly, we found that dMSNs target only a small fraction of prototypic cells and with much weaker inhibition compared with arkypallidal cells (Figure 7). This target selectivity in the GABAergic inhibition from dMSNs to arkypallidal cells is intriguing in the face of the selective excitation of prototypic cells by substance P (Mizutani et al., 2017). Other inputs to the GPe, such as from cortical regions (Abecassis et al., 2020; Karube et al., 2019), were not explored in this study but are also likely to shape GPe activity.

Prototypic cells project downstream and inhibit BG output structures but also other structures, such as the STN (Bevan et al., 2002; DeLong, 1990; Smith et al., 1998), striatum (Bevan et al., 1998; Mallet et al., 2012; Mastro et al., 2014; Saunders et al., 2016), thalamic nuclei (Hazarati and Parent, 1991; Mastro et al., 2014), and the substantia nigra pars compacta (Mastro et al., 2014; Paladini et al., 1999). Within the GPe, they exert local inhibition onto arkypallidal cells as well as themselves (Figure 3). Our findings show that prototypic cells are indeed targeted by iMSNs (Albin et al., 1989; Loopuitt and van der Kooy, 1985) and the STN (Kita et al., 1983; Robledo and Feher, 1990), as shown previously. STN activation results in reliable excitation of prototypic cells that, in turn, would inhibit downstream BG targets. iMSN activation strongly inhibits prototypic cells, disinhibiting the STN as well as neighboring arkypallidal cells. Our observations are therefore in line with the canonical role of prototypic GPe cells in the indirect pathway (Chiken et al., 2008; Nambu et al., 2000; Ozaki et al., 2017; Sano et al., 2013). The contribution of prototypic cell activation on structures outside of the BG was not explored further in this study.

The projection from the GPe to striatum differs between prototypic and arkypallidal cells. Arkypallidal cells have been shown to target striatal interneurons and MSNs (Mallet et al., 2012), whereas prototypic cells mainly target interneurons (Bevan et al., 1998; Saunders et al., 2016). Fractions of both GPe subtypes have been shown to express Npas1 (Abrahao and Lo-
Detailed methods are provided in the online version of this paper and include the following:

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### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.neuron.2020.11.006.

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### AUTHOR CONTRIBUTIONS

M.K. and G.S. conceived and planned the experiments. M.K. performed the in vivo experiments and analyzed the data. G.S. performed the ex vivo experiments and analyzed the data. M.K. and G.S. wrote the manuscript.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Cy3 conjugated streptavidin | Jackson ImmunoResearch Laboratories | RRID: AB_2337244 |
| Cy2 conjugated streptavidin | Jackson ImmunoResearch Laboratories | RRID: AB_2337246 |
| Anti-FOXP2 antibody | abcam | Cat#ab16046; RRID: AB_2107107 |
| Cy5 AffiniPure Donkey Anti-Rabbit IgG (H+L) | Jackson ImmunoResearch Laboratories | RRID: AB_2340607 |
| Bacterial and Virus Strains |        |            |
| AAV5.EF1.dflox.hChR2(H134R)-mCherry.WPRE.hGH | Karl Deisseroth | Cat# 20297; RRID:Addgene_20297 |
| AAV5.EF1.dflox.hChR2(H134R)-eYFP.WPRE.hGH | Karl Deisseroth | Cat# 20298; RRID:Addgene_20298 |
| rgAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA | Karl Deisseroth | Cat# 20298; RRID:Addgene_20298 |
| Chemicals, Peptides, and Recombinant Proteins |        |            |
| Isoflurane, Forene | AbbVie AB (Apoteket) | Cat#506949 |
| Ketamine, Ketaminol Vet | Intervet AB (Apoteket) | Cat#511485 |
| Medetomidine, Dormitor Vet | Orion Pharma AB (Apoteket) | Cat#015602 |
| Sodium pentobarbital | APL | Cat#338327 |
| Temgesic | Indivior Europe Limited (Apoteket) | Cat#521634 |
| Experimental Models: Organisms/Strains |        |            |
| Mouse: B6:129S-Gt(Rosa)26Sce1tm1(CAG-COP4-H134R/EYFP)Lev/J | the Jackson laboratory | RRID:IMSR_JAX:012569 |
| Mouse:B6.FVB(Cg)-Tg(Drd1-cre)EY217Gsat/Mmucd | GENSAT | RRID:MMRRC_034258-UCD |
| Mouse:B6.FVB(Cg)-Tg(Drd2-cre)ER44Gsat/Mmucd | GENSAT | RRID:MMRRC_032108-UCD |
| Mouse:STG Tg(Adora2a-cre)KG139Gsat/Mmucd | GENSAT | RRID:MMRRC_031168-UCD |
| Mouse:Sc17ap1m2(cre)Lev/J | the Jackson laboratory | RRID:IMSR_JAX:016963 |
| Mouse:B6:129P2-Pvalbtm1(cre)Arbr/J | the Jackson laboratory | RRID:IMSR_JAX:008069 |
| Mouse:C57BL/6J-Tg(Nkx2-1-cre)2Sand/J | the Jackson laboratory | RRID:IMSR_JAX:008661 |
| Mouse: B6.Cg-Foxp2tm1.1(cre/GFP)Rpa/J | the Jackson laboratory | RRID:IMSR_JAX:0030541 |
| Software and Algorithms |        |            |
| Spike2 | CED | N/A |
| SPSS | IBM | N/A |

RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gilad Silberberg (gilad.silberberg@ki.se).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
Custom-written MATLAB code and data for this study are available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All experiments were performed according to the guidelines of the Stockholm municipal committee for animal experiments under an ethical permit to G.S. (N12/15). Adult D1-Cre (EY217 line), D2-Cre (ER44 line), Adora2a-Cre (KG139 line, GENSAT), v glut2-Cre
MICROPIPETTE PULLER P-1000 (Sutter Instruments). Pipettes (6-9 MOhm, borosilicate, Hilgenberg), back-filled with intracellular solution: 0.3-0.5 M \( \text{gluc} \), 5K Cl, 10Hepes, 4Mg-ATP, 0.3GTP, 10Na2-phosphocreatine, and 0.2-0.3% biocytin (pH = 7.25, negative pressure till cell opening was evident. Recordings were performed in current clamp mode. Intracellular solution contained (Slc17a6tm2(cre)Lowl/J), PV-Cre (B6;129P2-Pvalbtm1(cre)Arbr/J), FoxP2-Cre (B6.Cg-Foxp2tm1(cre/GFP)Rpa/J) and NKX2.1-Cre mice (C57BL/6-Tg(Nkx2-1-cre)2Sand/J, the Jackson laboratory) were used for virus injections. In some cases, Cre lines were crossed with the Channelrhodopsin (ChR2)-YFP reporter mouse line (Ai32, the Jackson laboratory) to induce expression of ChR2 in a specific cell population. Both male and female mice were used in this study, but no obvious differences were observed between sexes. Mice were housed under a 12-hour light-dark cycle with food and water ad libitum. All experiments were carried out during the light phase.

METHOD DETAILS

Virus injections
Mice, 6-8 weeks old, were anesthetized with isoflurane and placed in a stereotaxic frame (Stoelting). Craniotomy coordinates and injections volumes: Striatum (AP: 0.7, ML: 2.25, DV –3.1) 0.5 - 1 \( \mu \text{L} \) of virus. GPe (AP: –0.35, ML: 2.15, DV: –3.65) 0.05 - 0.25 \( \mu \text{L} \) of virus. Viruses: AAV5.EF1.dflox.hChR2(H134R)-mCherry.WPRE.hGH or AAV5.EF1.dflox.hChR2(H134R)-eYFP.WPRE.hGH, rgAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHPa, addgene. Injections were done using a micropipette at 0.1 \( \mu \text{L} \) min\(^{-1}\) (Quintessential Stereotaxic Injector, Stoelting). The pipette was held in place for 5 min before being slowly retracted from the brain. Temgesic was applied after surgery (0.1 mg/Kg).

In vivo recordings
Experiments were conducted as described previously (Ketzef et al., 2017; Reig and Silberberg, 2014), briefly, 2-3 months old mice, usually 3 weeks following virus injections, were anesthetized by intraperitoneal injection of ketamine (75mg/kg) and medetomidine (1 mg/kg) diluted in 0.9% NaCl. To maintain mice under anesthesia, a third of the dose of Ketamine was injected intraperitoneally approximately every 2 hours or in cases when the mouse showed response to pinching or changes in EcoG patterns. Mice were tracheotomized, placed in a stereotaxic frame and received oxygen enriched air throughout the recording session. Core temperature was monitored with feedback-controlled heating pad (FHC) and was kept on 36.5 ± 0.5 °C. The skull was exposed and 3 craniotomies were drilled (Osada success 40): for cortical LFP recordings, GPe intracellular recordings and for optic fiber placement. Sensory cortex craniotomy coordinates: 1.5 mm posterior to bregma, 3.25 mm lateral to mid sagittal suture. A bipolar tungsten electrode with impedances of 1-2 M\( \Omega \) was inserted 1 mm deep from the surface. Signals were amplified using a Differential AC Amplifier model 1700 (A-M Systems) and digitized at 20 KHz with CED and Spike 2 parallel to whole-cell recording. An optic fiber was inserted for activation of input population. A craniotomy was drilled and the fiber was inserted (AP, ML, DV in mm) to either ST (0.7, 2.25, –2), STN (– 2, 1.5, –4.5) or above GPe (–0.35, 2.2, –2.75). For patch clamp recordings, the craniotomy was performed 0.3-0.5 posterior to, and 4.25 mm lateral to bregma, and the dura was removed. Patch pipettes were pulled with a Flaming/Brown micropipette puller P-1000 (Sutter Instruments). Pipettes (6-9 MOhm, borosilicate, Hilgenberg), back-filled with intracellular solution, were inserted with a ~1200 mbar positive pressure to a depth of about 2.8 mm from the surface, after which the pressure was reduced to 25-35 mbar. From that point the pipette was advanced in 1 \( \mu \text{m} \) steps in depth (32 degrees angle), in voltage clamp mode. When a cell was encountered, the pressure was removed to form a Gigaseal, followed by application of a ramp of increasing negative pressure till cell opening was evident. Recordings were performed in current clamp mode. Intracellular solution contained (in mM): 130 K-glutconate, 5 KCl, 10 HEPES, 4 Mg-ATP, 0.3 GTP, 10 Na2-phosphocreatine, and 0.2 - 0.3% biocytin (pH = 7.25, osmolality~285 mOsm). The exposed brain was continuously covered by 0.9% NaCl to prevent drying. Signals were amplified using MultiClamp 700B amplifier (Molecular Devices) and digitized at 20 KHz with a CED acquisition board and Spike 2 software (Cambridge Electronic Design). The membrane potential of a cell was the peak value of the all point membrane potential histogram collected for over a minute. The spontaneous firing frequency was calculated for a similar period of time. Inter-spike interval coefficient of variance (CV\(_{\text{ISI}}\)) was calculated as the variance of the Inter-spike interval normalized to the average Inter-spike interval. Input resistance was calculated as the slope of the steady-state voltage responses to current injection (–100 pA to 0 pA in steps of 20 pA for 5 s each). For each current injection, values during cortical up and down states were extracted separately. Sag was extracted from hyperpolarization protocol in which the cell was first not injected with any current, establishing baseline, followed by injection of –100 pA for 5 s. The sag was taken as the maximal drop in voltage at the beginning of the hyperpolarizing step. Sag ratio was the result of diving the sag by the steady-state voltage responses of the hyperpolarizing step. AP half width was calculated at half distance between membrane potential baseline and average AP peak value. Afterhyperpolarization (AHP) was measured between the membrane potential baseline to the average AP hyperpolarization peak value. For all these properties only recordings that were stable for a duration of at least a minute were taken; i.e., no drift in membrane potential or change in AP amplitude during the recording.

For classifying the membrane properties, we used data obtained from 286 GPe cells that included the molecularly identified cells. Unsupervised hierarchical cluster analysis was performed using Ward’s cluster method and Euclidean distance for parameters that were non-normally distributed. Since we could molecularly verify 2 clusters by the presence of the identified cells and the maximal drop in distance seen in the dendrogram divided the data into 2 groups, in most cases, the comparison is carried out between 2 groups.
Correlation coefficient was calculated from the filtered squared LFP signal recorded in S1 and the corresponding cell membrane potential in zero lag. Signals were filtered between 0.4 – 1.6 Hz using a second-order Butterworth filter (Abdi et al., 2015) before the correlation coefficient was extracted.

**Optogenetic stimulation**
Blue light (470 nm, mightex) was delivered through a cannula inserted to the craniotomy at these coordinates (AP, ML, DV in mm) to either ST (0.7, 2.25, −2.25), STN (−2, 1.5, −4.5) or above GPe (−0.35, 2, −2.75). Light activation protocols were triggered by spike2 program. Maximal LED light intensity at the tip of the cannula was 2 mW. All light stimulations were presented as percentages of the maximal light intensity. In subset of experiments we used the optopatcher (Katz et al., 2013; Ketzef et al., 2017) for identification of the cells recorded online.

**Whisker stimulation**
Air puffs were delivered by a picospritzer unit (Picospritzer III, Parker Hannifin) through plastic tubes (1 mm diameter) positioned up to a centimeter from the whiskers. Bilateral air puff stimulations (15-20 psi, 15 ms) were delivered at 0.2 Hz and at least 20 responses were acquired. The response properties (onset delay, phase amplitude and peak time) were extracted from the average trace of the responses. The baseline membrane potential was calculated at the first 10 ms following the trigger time point. The phases of the sensory response were defined as following: the first phase was between the onset and the first depolarizing peak, while the 2nd and 3rd phases were defined between the local maximum and minimum (hence hyperpolarizing, and alternatively, minimum and maximum, hence depolarizing) points in the response trace (See Figure 8D).

**Ex vivo slice recordings**

**Slice preparation**
2-3 months old mice, around 3 weeks following virus injections, were anaesthetized with isoflurane (VM Pharma AB, Sweden) prior to being decapitated, whereupon brains were extracted while submerged in ice cold cutting solution consisting of (in mM): KCl 2.5, NaH2PO4 1.25, CaCl2 0.5, MgCl2 7.5, Glucose 10, NaHCO3 25, and Sucrose 205. Parasagittal sections of 250 μm thickness were cut using a VT1200S Vibratome (Leica, Japan) at an angle of 10° and subsequently left to recover for 30 minutes in 35 °C artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125, KCl 2.5, MgCl2 1, NaH2PO4 1.25, CaCl2, Glucose 25, and NaHCO3 25. Slices were maintained at room temperature for recovery until recording at 35 °C. Cutting solution and ACSF were continuously infused with carbogen (95% O2, 5% CO2) throughout the procedure.

**Patch clamp electrophysiology**
Borosilicate glass pipettes were pulled using a P1000 Micropipette Puller (Sutter Instrument, U.S.A.) at a resistance of 6 – 8 MΩ and filled with an intracellular solution containing (in mM): K-glucuronate 105, KCl 30, Na2-Phosphocreatine 10, HEPES 10, ATP-Mg 4, GTP-Na 0.3. Neurons were selected by Infrared-Differential Interference Contrast (IR-DIC) imaging on a BX51WI (Olympus, Japan) upright microscope using a 40x water-immersion objective. Wide-field fluorescent imaging was used to localize afferent fibers or cells expressing the virus. Once a whole-cell patch was achieved, light evoked responses were recorded at a holding potential of −75 mV in current-clamp mode using a MultiClamp 700B amplifier (Molecular Devices, U.S.A.), digitized at 10 KHz on an ITC-18 (HEKA, U.S.A.) and acquired with Igor Pro 6.3 (Wavemetrics, U.S.A). Stimulation was applied through an ocular-mounted blue LED producing 6.4 mW light under the objective, controlled through a SLA-1200-2 LED driver (Mightex, U.S.A.).

**Cell labeling and immunohistology**
During the recordings, the cells were loaded with biocytin (Sigma). At the end of the recording session, the mouse received an overdose of sodium pentobarbital (200 mg/kg I.P.), and transcardially perfused with 4% PFA in 0.01M phosphate buffer (PBS) pH 7.4. The brain was removed and kept for additional 2 hours in the fixative, after which it was transferred to 0.01 M PBS. The brain was transferred to and kept in 12% sucrose solution in 0.01M PBS overnight and < 20 μm cryo-sections were produced. Sections were mounted on microscope gelatin coated slides and incubated for 2 hours in room temperature with cy2 / cy3 conjugated streptavidin (1:1000, Jackson ImmunoResearch Laboratories) in staining solution (1% BSA, 0.1% NaDeoxycholate and 0.3% triton in 0.01 PBS). After washes in PBS, slides were mounted on fluorescence microscope in order to locate the recorded cells. If a cell has been found, following the biocytin staining, we stained for FoxP2 (Rabbit anti mouse, Abcam) expression (1:1000 in staining solution) overnight at 4 °C, followed by 2 hours incubation with secondary antibody (cy5 conjugated donkey anti rabbit, Jackson ImmunoResearch Laboratories, 1:500 in staining solution). Photomicrographs of the results were taken with Olympus XM10 (Olympus Sverige AB, Stockholm, Sweden) digital camera. All the cells recorded were found in the dorsal portion of the GPe, and between the anterior posterior bregma distances −0.22 to −0.70.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Statistical analysis**
Data are represented as mean ± SEM. The ns represent cells. Data distributions were first checked for normality (Shapiro-Wilk test) and analyzed accordingly. Normally distributed data were tested by one way ANOVA followed by post hoc Tukey’s test analysis for
multiple comparisons, and the unpaired and paired two-sample Student’s t test was used for two group comparisons. Non-normally distributed data were analyzed by Kruskal-Wallis test for multi-group comparisons followed by Mann-Whitney for two group comparison. Confidence level was set to 0.05. All statistical analyses were done in SPSS (IBM). Statistical tests are reported in the figure legends. Experiments were not included if there was no virus expression, placement of fiber was not correct, or recordings were not stable, as described in the ‘in vivo recording’ section.