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DOI: 10.1038/s41467-018-05847-5

Region-specific and state-dependent action of striatal GABAergic interneurons

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Striatum processes a wide range of functions including goal-directed behavior and habit formation, respectively encoded by the dorsomedial striatum (DMS) and dorsolateral striatum (DLS). GABAergic feedforward inhibition is known to control the integration of cortical information by striatal projection neurons (SPNs). Here we questioned whether this control is specific between distinct striatal functional territories. Using opto-activation and opto-inhibition of identified GABAergic interneurons, we found that different circuits are engaged in DLS and DMS, both ex vivo and in vivo: while parvalbumin interneurons efficiently control SPNs in DLS, somatostatin interneurons control SPNs in DMS. Moreover, both parvalbumin and somatostatin interneurons use a dual hyperpolarizing/depolarizing effect to control cortical input integration depending on SPN activity state: GABAergic interneurons potently inhibit spiking SPNs while in resting SPNs, they favor cortical activity summation via a depolarizing effect. Our findings establish that striatal GABAergic interneurons exert efficient territory-specific and state-dependent control of SPN activity and functional output.
Cerebral cortex and basal ganglia are tightly interconnected structures involved in goal-directed behavior and procedural learning\(^1-3\). Striatum, the main input nucleus of basal ganglia, receives massive convergent glutamatergic inputs from the whole cortex and distinct inputs from the different cortical areas form distinct functional territories within the striatum\(^4-7\). Two major functional territories are the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS), which corresponds to the sensorimotor territory and is involved in habit formation\(^8\). The two territories also interact with each other since in the same behavioral task involving procedural learning, DMS and DLS neurons are both activated, but preferentially at different phases of the task and at different stages of the learning course\(^9,10\). Both territories then relay the information toward the output structures of basal ganglia (internal part of the globus pallidus and the substantia nigra pars reticulata (SNr)).

DMS and DLS are functionally distinct, although the composition and the properties of their microcircuits appear similar. Since striatum has no evident anatomical boundaries, functional differences of the distinct striatal regions could arise from their distinct incoming cortical inputs. The composition of the striatal circuits could also define specific functional regions. Striatal neuronal circuits are composed of a majority of striatal projection neurons (SPNs), and a variety of GABAergic interneurons, which are also efficiently recruited by cortical afferents\(^11-14\) and exert a strong feedforward inhibition on SPNs\(^15-17\). The role of striatal interneurons is highlighted by the consequences of global alteration in GABAergic circuits, which alters synaptic plasticity\(^18,19\) and leads to severe motor deficits that are particularly exemplified in the context of dystonia or Tourette Syndrome\(^20\). The two most extensively described interneuron subtypes in striatum are the parvalbumin (PV)-expressing cells (fast-spiking interneurons) and the somatostatin/neuropeptide Y/nitric oxide synthase (SOM/NPY/NOS)-expressing cells (persistent and low-threshold spiking cells).

Here we questioned whether PV and SOM interneurons could play a role in the distinct properties of DMS and DLS. Using in vivo multi-channel recordings associated with optogenetics, we found that opto-inhibition of PV or SOM cells in DMS or DLS differentially control SNr activity. We explored this functional dichotomy within the striatum and found that PV cells control the activity of SPNs in DLS while SOM cells control SPNs in DMS. This dichotomy is based on a marked heterogeneity in the anatomical distribution, connectivity and electrophysiological properties of PV and SOM cells in DLS and DMS. Interestingly, our results show that the territory specificity of GABAergic microcircuits translates to the trans-striatal transfer of information of cortical inputs to the nigral output of the striatum. We also described that both PV and SOM interneurons mediate a dual hyperpolarizing depolarizing control of SPNs that depends on SPN activity state, with the depolarizing effect favoring cortical integration. Our findings therefore demonstrate that the selective feedforward control of cortical inputs by GABAergic interneurons is specific to the striatal functional territories and to the SPN activity state.

Results

**SOM and PV cells in DMS and DLS differentially affect SNr spontaneous activity.** SPNs act as coincidence detectors of coherent cortical activity, extract pertinent information from background noise and relay signals towards the main basal ganglia output structure, the SNr. We used SNr spontaneous activity as a readout of striatal output modulation by striatal interneurons. We first examined the effect of an opto-inhibition of SOM and PV interneurons in DMS or DLS onto SNr spontaneous activity (Fig. 1a). To do so, we recorded extracellular activity of SNr units in vivo in urethane-anesthetized SOM::Arch3 and PV::Arch3 mice that selectively express Arch3 in SOM and PV cells, respectively (Fig. 1a, b and Supplementary Fig. 1). SNr units were identified by their high spontaneous spiking frequency (median (interquartile range (IQR)): 18.7 (10.3) Hz, n = 239 units) and their regularity (coefficient of variation of the inter-spike intervals (CV-ISI), median (IQR): 0.41 (0.22)) (Fig. 1c). In control conditions (i.e., without opto-inhibition), no difference was found between the distribution of the spontaneous firing rates of SNr units recorded in SOM::Arch3 vs. PV::Arch3 mice (n = 130 units from 17 SOM::Arch3 mice, n = 109 units from 13 PV::Arch3 mice, p = 0.2044, Fig. 1c). Using optic fibers implanted in the DMS or DLS, we tested the effect of opto-inhibition of SOM and PV cells in the two striatal territories (Fig. 1a, b). We found that all conditions (SOM and PV in DMS and DLS) could efficiently increase SNr activity (Fig. 1d–f and Supplementary Fig. 2). However, we found a selective contribution of SOM and PV opto-inhibition to SNr activity depending on the targeted striatal territory (Fig. 1d, e). First, the proportion of modulated SNr units was significantly higher when opto-inhibiting PV interneurons than SOM interneurons in both DLS and DMS (50.0% of significantly modulated units in PV-DLS, n = 68 vs. 8.7% in SOM-DLS, n = 69, p = 0.0001; 32.9%, n = 79 in PV-DMS vs. 14.7%, n = 109 in SOM-DMS, p = 0.0079), suggesting a stronger weight of PV interneurons compared to SOM interneurons. Second, we found a stronger impact of PV opto-inhibition in DLS than in DMS (p = 0.0422) (Fig. 1d–f), suggesting a territory specificity in the modulation of SNr activity. These effects were confirmed at the population level (Fig. 1f and Supplementary Fig. 2), where silencing PV or SOM striatal interneurons induced an overall increase in SNr activity in all conditions (SOM-DMS: median (IQR) change +1.7 (6.1)%, p = 0.0001; PV-DMS: +4.1 (8.3)%, p < 0.0001; SOM-DLS: +1.0 (5.5)%, p = 0.0196; PV-DLS: +9.1 (15.4)%, p < 0.0001). The comparison of effects of opto-inhibition between striatal interneurons and territories on the SNr firing rate confirmed the stronger effect of silencing PV compared to SOM interneurons in both territories (DMS: p = 0.0208; DLS: p = 0.0001), and the stronger effect of PV interneurons in the DLS compared to the DMS (p = 0.0087) (Fig. 1f). These differential effects did not result from an anatomical bias in the DLS or DMS connectivity of the targeted SNr region since we did not observe a segregation of significantly modulated units or an effect depending on the recording location (Supplementary Fig. 2).

Together, these in vivo data suggest that SOM and PV interneurons of the dorsal striatum exert differential effects on the spontaneous activity of the SNr. In addition, these effects depend on their location in striatal territories (DMS vs. DLS).

**Selective inhibitory weight on spiking SPNs in DMS and DLS.** To investigate the cause of this differential effect, we dissected the effects of SOM and PV interneuron opto-inhibition onto the SPNs in DMS and DLS. To this aim, we characterized the effect of striatal GABAergic circuits locally in the striatum by performing ex vivo experiments using brain slices preserving layer 5 cortical connections from cingulate cortex to DMS or from somatosensory cortex to DLS (see Methods). We first confirmed that cortical layer 5 pyramidal cells directly contact both types of striatal interneurons as previously described\(^11-14,21\) (Fig. 2). We found that PV cells are tightly locked to the timing of cortical stimulations and display reproducible evoked responses, while SOM cells show more variability in their responses to cortical inputs in...
both territories (Fig. 2a–c). We built input/output curves for SOM and PV interneurons and for SPN evoked responses by gradually increasing the stimulation of cortical afferents (Fig. 2d–f). In the two territories, 100% of PV and SOM interneurons were efficiently activated by cortical inputs and, in both DMS and DLS, most PV or SOM interneurons in the brain express Arch3; Fig. 3a (in which SOM::Arch3 mice (n = 130 units from 17 mice) in DLS, n = 69 units in DLS, n = 79 units for PV::Arch3 mice in DMS, n = 68 in DLS). Proportion of SNr units displaying a significant modulation of their firing in response to opto-inhibition of PV or SOM interneurons in DMS and DLS (total n = 109 units for SOM::Arch3 in DMS, n = 69 in DLS, n = 79 units for PV::Arch3 mice in DMS, n = 68 in DLS). Median (IQR) change in firing rate of all recorded SNr units in response to opto-inhibition of PV (green) and SOM (purple) interneurons in the DMS and DLS. Indicates a significant effect across the population of recorded SNr units for PV-DLS (**p < 0.05, ***p < 0.001, Wilcoxon signed rank test corrected for multiple comparisons). In e and f, results in the 4 conditions were compared using a generalized linear (e) or a linear (f) model (see Methods) followed by post-hoc tests, *p < 0.05, **p < 0.01, ***p < 0.001. For the 3D images in a, brain and brain structures are captured from the Allen Institute for Brain Science’s Mouse Brain Atlas (© 2004 Allen Institute for Brain Science, Allen Mouse Brain Atlas available from: mouse.brain-map.org/) using Brain Explorer®.
probability of SPN-DMS ($p = 0.005, n = 10$), whereas the spiking probability of SPN-DLS remained unaffected ($p = 0.1571, n = 9$).

The picture was reversed for PV cells since their opto-inhibition led to an increase in spiking probability of SPN cells in DLS ($p = 0.0087, n = 14$) but not in DMS ($p = 0.4040, n = 8$) (Fig. 3b, c). These results were confirmed with the opposite strategy, by activating SOM and PV interneurons while stimulating cortical afferents using SOM::ChR2 or PV::ChR2 mice (Fig. 3d and Supplementary Figs 5 and 6) or virally expressed with AAV-DIO-

![ChR2-mCherry in SOM-Cre and PV-Cre mice](Supplementary Fig. 4, f). We mimicked brief (a single spike induced by 5 ms light pulse, Fig. 4b) or long (bursts of spikes induced by 300 ms light pulse, Fig. 4c) activation of GABAergic circuits. We found that SPN firing frequency was significantly decreased by brief opto-stimulation of both SOM and PV cells in DMS and DLS ($F_{2, 14} = 9.151, p < 0.0001$) but with different magnitudes depending on the striatal territories. In DMS, SOM cell activation exerted stronger inhibition than PV cell activation (SOM opto-stimulation, normalized frequency before vs. during opto-stimulation was $0.75 \pm 0.04, n = 16$, PV opto-stimulation, normalized frequency was $0.85 \pm 0.03, n = 16, p = 0.0258$). In DLS, PV cells induced a stronger decrease in frequency in SPNs than SOM cells (PV: $0.61 \pm 0.04, n = 12$, SOM: $0.78 \pm 0.03, n = 14, p = 0.0014$) (Fig. 4b). Using the viral infection strategy, we observed similar results, indicating that only local GABAergic circuits are responsible for the inhibitory weight specificity (Supplementary Fig. 7). These findings were confirmed using longer duration (300 ms) opto-stimulation of both PV and SOM cells in DMS and DLS ($F_{3, 14} = 9.108, p < 0.0001$). SOM-DMS cells exerted a stronger inhibition onto SPN firing frequency than PV-DMS cells (frequency ratio before vs. during opto-stimulation, **p < 0.001.**
Selective modulation of cortically-evoked spiking activity by SOM and PV cells. 

**Experimental set up:** Electrical stimulations were applied in the cortex and evoked APs were recorded in SPNs, in control or with specific opto-inhibition of PV cells or SOM cells in transgenic mice PV::Arch3 and SOM::Arch3 (top) or PV-Cre and SOM-Cre mice virally infected with AAV-Flex-Arch-tomato (bottom) (Arch3 activation for 300 ms with a 570 nm LED).

The spiking probability of SPNs was compared between interleaved control and opto-inhibition conditions in DMS (b) and DLS (e) using transgenic mice (top, black) or virally infected interneurons (bottom, red). Individual experiments and averaged normalized spiking probability are represented. Opto-inhibition of SOM cells leads to a significant increase in spiking probability in DMS (n = 15, p = 0.0063 for transgenic and n = 8, p = 0.033 for virus experiments) and no difference in DLS (n = 10, p = 0.2567 for transgenic and n = 6, p = 0.55479 for viruses). For PV cells, the effect is opposite, opto-inhibition of PV cells significantly increase the spiking probability in DLS (n = 16, p = 0.0288 for transgenic and n = 5, p = 0.0063 for viruses), while their opto-inhibition has no effect in DMS (n = 7, p = 0.1854 for transgenic and n = 9, p = 0.8007 for viruses). 

Experimental set up: electrical stimulations were applied in the cortex and evoked APs were recorded in SPNs, in control or with specific opto-activation of PV cells or SOM cells with ChR2 activation in transgenic mice PV::ChR2 and SOM::ChR2 (top) or PV-Cre and SOM-Cre mice virally infected with AAV-Flex-ChR-mCherry (bottom) (for 5 ms using a 470 nm excitation LED). 

The spiking probability of SPNs was compared between the control and opto-activation conditions in DMS (e) and DLS (f) using transgenic mice (top, black) or virally infected interneurons (bottom, red). Individual experiments and averaged normalized spiking probability are represented. SOM opto-activation leads to a significant decrease in spiking probability in DMS (n = 7, p = 0.0002 for transgenic mice and n = 7, p = 0.0002 for viral experiments) and no difference in DLS (n = 6, p = 0.9114 for transgenic and n = 5, p = 0.9980 for viruses). Opto-activation of PV cells significantly decrease the spiking probability in DLS (n = 9, p = 0.0059 for transgenic and n = 7, p = 0.0023 for viruses) but not in DMS (n = 9, p = 0.9816 for transgenic and n = 6, p = 0.4518 for viruses). Paired tests, *p < 0.05, **p < 0.01, ***p < 0.001.

These data demonstrate that SOM cells have a stronger inhibitory weight in DMS while PV cells control more efficiently SPN firing rate in DLS. Remarkably, in both DMS and DLS, the inhibitory effect of PV cells was independent on the initial SPN firing rate.
While this decrease was significantly higher for PV cells (green) activation in DLS territory (p = 0.0014, n = 14 SOM cells and n = 12 PV cells), while this decrease was significantly higher for PV cells (green) activation in DLS territory (p = 0.0014, n = 12 PV cells). c) Left: Representative trace of discharge frequency inhibition by a brief (5 ms) opto-activation of PV-DLS cells. Right: Frequency inhibition ratio (frequency during LED ON vs. before LED ON, mean ± SEM) in PV-DLS cells induced by 5 ms light pulse. Frequency decrease was significantly higher for SOM cell (purple) activation in DMS territory (p = 0.0258, n = 16 SOM cells and n = 16 PV cells), while this decrease was significantly higher for PV cells (green) activation in DLS territory (p = 0.0014, n = 14 SOM cells and n = 12 PV cells). d) Correlation of PV (green) and SOM (purple) cell inhibitory weight (discharge frequency ratio) and initial SPN discharge frequency. There is a significant correlation for SOM cells in both DMS (r^2 = 0.3481, Pearson’s correlation, p = 0.0437, n = 14) and DLS (r^2 = 0.4648, p = 0.0127, n = 14), and there was no significant correlation for PV cells (r^2 = 0.097 for DMS and r^2 = 0.1739 for DLS); *p < 0.05, **p < 0.01

**Fig. 4** Differential local inhibitory weight of SOM and PV cells on firing SPNs. a) In the DMS and DLS, SPNs are recorded while either PV cells or SOM cells are opto-activated with ChR2 with a 470 nm excitation LED. Effect of PV or SOM cells on SPNs was evaluated in active SPNs, i.e., while inducing spiking activity in SPNs by depolarizing current steps (500 ms in b or 1 s in c and d). b) Left: Representative trace of discharge frequency inhibition by a brief (5 ms) opto-activation of PV-DLS cells. Right: Frequency inhibition ratio (frequency during LED ON vs. before LED ON, mean ± SEM) in SPNs induced by 5 ms light pulse. Frequency decrease was significantly higher for SOM cell (purple) activation in DMS territory (p = 0.0258, n = 16 SOM cells and n = 16 PV cells), while this decrease was significantly higher for PV cells (green) activation in DLS territory (p = 0.0014, n = 14 SOM cells and n = 12 PV cells). c) Left: Representative trace of discharge frequency inhibition by a brief (5 ms) opto-activation of PV-DLS cells. Right: Frequency inhibition ratio (frequency during LED ON vs. before LED ON, mean ± SEM) in SPNs induced by 5 ms light pulse. Frequency decrease was significantly higher for SOM cell (purple) activation in DMS territory (p = 0.0258, n = 16 SOM cells and n = 16 PV cells), while this decrease was significantly higher for PV cells (green) activation in DLS territory (p = 0.0014, n = 14 SOM cells and n = 12 PV cells). d) Correlation of PV (green) and SOM (purple) cell inhibitory weight (discharge frequency ratio) and initial SPN discharge frequency. There is a significant correlation for SOM cells in both DMS (r^2 = 0.3481, Pearson’s correlation, p = 0.0437, n = 14) and DLS (r^2 = 0.4648, p = 0.0127, n = 14), and there was no significant correlation for PV cells (r^2 = 0.097 for DMS and r^2 = 0.1739 for DLS); *p < 0.05, **p < 0.01

Firing rate (p = 0.5973 for DMS and p = 0.4509 for DLS), whereas the effect of SOM cells was negatively correlated to the SPN initial firing rate (p = 0.0437 for DMS and p = 0.0127 for DLS) (Fig. 4d). These results show that PV cells have a constant inhibitory weight regardless of the level of SPN spiking activity, whereas feedforward inhibition exerted by SOM cells decreases with increasing SPN spiking activity.

**GABAergic microcircuits have distinct properties in DMS and DLS.** We next investigated whether the difference in local inhibitory weight of SOM and PV interneurons in DMS and DLS reflected anatomical and/or functional specificities of these microcircuits in both territories. Because enrichment of one population in a specific region could account for a stronger inhibitory weight on SPNs, we first examined the anatomical distribution of SOM and PV interneurons in DMS and DLS (Fig. 5a, b). Immunostaining for somatostatin and parvalbumin revealed a heterogeneous distribution of interneurons (p < 0.0001, F_{5, 24} = 22.79). SOM cells were equally distributed in DMS and DLS (179.9 ± 16.2 in DMS and 175.7 ± 14.8 in DLS, p = 0.8510, n = 9 mice) (Fig. 5a) and their density was maintained in adult animals as well (Supplementary Fig. 8). In contrast, PV cells were particularly enriched in the DLS when compared to the DMS (+160%, 296.0 ± 23.2 cells per 0.1 mm^3 in DLS vs. 187.9 ± 14.2 in DMS, p = 0.0018, n = 7 mice) (Fig. 5b). This heterogeneous distribution was observed in 1–2-month-old animals (Fig. 5a, b) and was maintained in adult animals as well (Supplementary Fig. 8).
**Fig. 5** Heterogeneous distribution, electrical properties and connectivity of SOM and PV cells in DMS and DLS. 

**a, b** Confluent microscopy images showing a representative overview of SOM (a) and PV (b) interneuron expression in DMS and DLS (scale bars: 50 µm), identified following immunostainings. On the right panels, quantification of SOM (a) and PV (b) interneurons in DMS and DLS is represented by the cell density (number of cells per 0.1 mm³) of SOM and PV interneurons in each territory (n = 9 mice for SOM interneurons and n = 7 mice for PV interneurons). 

**c** Representative responses of SOM cells to identical current steps (+20 pA) in DLS and DMS. 

**d** Membrane and electrical properties of SOM cells in DMS (orange, n = 20) and DLS (blue, n = 27) (mean ± SEM). 

**e** Representative responses of PV cells to identical current steps (+170 pA) in DLS and DMS. 

**f** Membrane and electrical properties of PV cells in DMS (orange, n = 17) and DLS (blue, n = 30). There is no significant difference in rheobase (p = 0.2761), RMP (p = 0.6030) and input resistance (p = 0.3260) of PV cells in DMS or in DLS. 

**g** Representative spontaneous activity of SOM cells in DMS and DLS. 

**h** Distribution and Gaussian fits of the discharge frequency in DLS (100 action potentials per cell, n = 8) and DMS (n = 6): the frequency is significantly higher in DMS (p < 0.0001). 

**i** Representative connections between PV-SPN and SOM-SPN connections in DMS and DLS in response to 20 Hz single AP trains evoked in PV and SOM cells with current injections. 

**j** Amplitude of unitary IPSPs in a SPN (single AP with paired patch-clamp recordings) in DMS and DLS. For PV-SPN connections the unitary IPSP was median (interquartile range (IQR)): 0.9 (0.6) mV (n = 5) in DMS and 1.3 (0.7) mV in DLS (n = 6) and for SOM-SPN connections 0.3 (0.8) mV (n = 6) in DMS and 0.4 (0.2) mV in DLS (n = 4). They were not statistically different in DMS (p = 0.0648) but much stronger from PV in DLS (p = 0.0094). Concerning light-induced IPSP amplitudes, SOM cell optoactivation induced significantly stronger IPSPs in DMS (p = 0.0158) and PV cells in DLS (p = 0.0019) (SOM-DMS: 2.3 (1.1) mV, n = 19, PV-DMS: 1.1 (0.5) mV, n = 16, SOM-DLS: 1.5 (1.1) mV, n = 14, and PV-DLS: 2.9 (3.2) mV, n = 15). *p < 0.05, **p < 0.01, ns not significant.
Because a heterogeneous anatomical distribution could not fully account for the functional dichotomy between DMS and DLS (the distribution of SOM interneurons is homogeneous, Fig. 5a), we next examined whether SOM and PV cells exhibited different electrophysiological properties in DMS and DLS. For this purpose, we performed whole-cell recordings of SOM and PV cells targeted thanks to the green fluorescent protein/yellow fluorescent protein (GFP/YFP) of the Arch/ChR2 constructs. All recorded fluorescent neurons exhibited typical basic properties and spiking activity of SOM or PV interneurons (Supplementary Table 1). We first cross-compared the electrophysiological properties of SOM and PV cells. We observed that, in both DMS and DLS, SOM interneurons display more depolarized resting membrane potential (RMP) ($p < 0.001$ for both DMS and DLS), lower input resistance ($R_{i}$) ($p < 0.001$ for both DMS and DLS) and lower rheobase ($p < 0.0001$ for both DMS and DLS) than PV cells, suggesting that SOM cells are more excitable than PV cells. We then compared the membrane properties of SOM and PV cells according to their location in DMS and DLS. We found no difference in RMP ($n = 30$ in DLS, $n = 18$ in DMS, $p = 0.6030$), $R_{i}$ ($p = 0.3260$) and rheobase ($p = 0.2761$) between PV-DMS and PV-DLS (Fig. 5e, f). In contrast, we observed a marked difference in the properties of SOM cells since those located in DMS exhibit a higher excitability than those located in DLS: lower rheobase ($n = 27$ in DLS and $n = 20$ in DMS, $p = 0.0011$), depolarized RMP ($p = 0.0059$) and higher $R_{i}$ ($p = 0.0049$) (Fig. 5c, d). Although PV cells were not spontaneously active (in vivo conditions), spontaneous activity was recorded in half of SOM cells in both territories (14/22 cells in DMS and DLS). We observed that the spontaneous firing frequency of active SOM cells was significantly higher in the DMS (DMS 11.7 ± 0.3 Hz, $n = 6$ vs. DLS 6.3 ± 0.1 Hz, $n = 8$, $p < 0.0001$) (Fig. 5g, h), which is in accordance with the higher excitability of SOM cells observed in DMS (Fig. 5c, d).

Another explanation could be that the unitary synaptic weight of PV-SHN and SOM-SHN connections are not the same in DMS and DLS. To go deeper in the origin of the differential effect of PV and SOM cells, we thus performed paired patch-clamp recordings to measure the unitary synaptic weight of PV-SHN and SOM-SHN connections. We observed that unitary inhibitory postsynaptic potential (IPSP) amplitudes induced with a single presynaptic action potential (AP) were not significantly different between PV and SOM in DMS but significantly higher for PV-SHN connections in DLS ($p = 0.0094$) (Fig. 5i, j). The comparison with light-induced IPSPs indicates that about 3 to 4 interneurons are recruited by the opto-activation in our conditions for SOM-DLS and PV-DLS, 1 to 2 interneurons for PV-DMS and 8 to 10 interneurons for PV-DMS. Therefore, when comparing PV and SOM cells in each territory, there is a higher amplitude for SOM cell opto-activation in DMS and PV cell opto-activation in DLS ($p = 0.0158$ for DMS and $p = 0.0019$ for DLS). These data show that, for each GABAergic population, there is no significant difference in the weight of their unitary connections in DMS and DLS. The differences of global inhibitory weight would thus result from the recruitment of more PV cells in DLS (due to their higher density in DLS) and more SOM cells in DMS (due to their higher excitability in DMS).

Together, these results show a marked heterogeneity in the GABAergic striatal microcircuits at the level of distribution, connectivity and electrophysiological properties, which could account for the territory functional specificity.

SOM and PV cells mediate a dual effect on SPNs in DMS and DLS. Due to their intrinsic properties, SPNs fire upon strong and correlated cortical activity while they remain mainly silent and operate in a large range of subthreshold activity in resting states. We therefore questioned whether SOM and PV cells could show selective modulation of subthreshold SPNs between DMS and DLS, as we found with spiking SPNs. Using SOM::ChR2 and PV::ChR2 mice, we established the current–voltage relationship of light-induced IPSCs in SPNs. We applied a brief stimulation (5 ms duration) to evoke single APs in SOM and PV interneurons (Supplementary Fig. 6). In active states (depolarized potentials, −40 mV), we observed stronger light-induced currents for SOM opto-activation in DMS ($p = 0.0354$, $n = 12$ PV cells and $n = 17$ SOM cells) and for PV opto-activation in DLS ($p = 0.0232$, $n = 8$ PV cells and $n = 11$ SOM cells) (Fig. 6a), which is consistent with the differential inhibitory effect we previously observed (Figs. 3, 4). In resting states (at hyperpolarized potentials, −80 mV), we observed larger IPSCs after opto-activation of SOM-DMS and PV-DLS cells compared to PV-DMS and SOM-DLS cells ($p = 0.0057$ for DMS, $n = 12$ PV cells and $n = 17$ SOM cells and $p = 0.0057$ for DLS, $n = 8$ PV cells and $n = 11$ SOM cells). Therefore, PV and SOM cells exert differential effect regardless of the SPN activity.

Interestingly, at hyperpolarized states, GABAergic interneuron activation resulted in depolarizing currents (Figs. 5i and 6a–c). This is explained by the fact that (i) SPN membrane fluctuations widely exceed $E_{\text{Cl}}$ threshold (physiological $E_{\text{Cl}}$ ~ −60 mV)25,26 and (ii) GABA is hyperpolarizing for $E_{\text{Cl}} > −60$ mV and depolarizing for $E_{\text{Cl}} < −60$ mV. To investigate whether the depolarizing GABA mediated by PV or SOM interneurons also displays differential effect in DMS and DLS as with inhibitory GABA, we applied brief opto-stimulations and measured light-induced IPSPs in resting SPNs (maintained at ~ −80 mV). In accordance with IPSC results (Fig. 6a), we observed significant differences between the different groups ($F_{3,50} = 7.187$, $p = 0.0003$, one-way analysis of variance (ANOVA)). In DMS, SOM-induced IPSP amplitude recorded in SPNs was larger than PV-induced IPSP (SOM activation 2.56 ± 0.28 mV, $n = 19$ and PV activation 1.48 ± 0.31 mV, $n = 16$; $p = 0.0022$). In contrast, in DLS, PV cells had a stronger effect than SOM cells (PV activation 3.48 ± 0.52 mV, $n = 15$ and SOM activation 1.54 ± 0.13 mV, $n = 14$; $p = 0.0043$) (Fig. 6d).

These differential effects were confirmed using trains of light-induced APs in GABAergic interneurons (at 10 or 50 Hz, Supplementary Fig. 6). Because temporal patterns of presynaptic activity strongly influence synaptic transmission, with specific short-term dynamics, we characterized the short-term dynamics recorded in SPNs induced by trains of opto-activation of either PV or SOM cells. PV cells displayed a marked short-term depression in SPNs in DLS ($p = 0.0018$ and $p < 0.0001$ respectively) but not in DMS ($p = 0.0842$ for 10 Hz and $p = 0.3706$ for 50 Hz) (Fig. 6e). Conversely, SOM cells led to short-term depression for 10 and 50 Hz stimulation in DMS ($p = 0.0290$ and $p < 0.0001$, respectively) and only for 50 Hz trains in DLS ($p = 0.1337$ and $p < 0.0001$ for 10 and 50 Hz). The observed short-term depression suggests that the probability of release of connections from SOM-DMS to SPN and from PV-DLS to SPN is higher than SOM-DLS to SPN and PV-DMS to SPN. This observation is in line with the larger responses in SPNs evoked by opto-stimulation of SOM-DMS cells and PV-DLS cells (Fig. 4).

Altogether, these results further confirm the selective role of SOM and PV interneurons in the control of subthreshold SPN activity in DMS and DLS. They also point out that when SPNs are at resting states, GABAergic microcircuits can induce a strong depolarizing effect, which is also mainly mediated by either PV or SOM cells depending on the striatal territory.

Differential shaping of subthreshold input integration and summation in DMS and DLS. The depolarizing properties of
GABA could have critical functional consequences on the integration of subthreshold inputs in SPNs at resting states. To assess whether SOM and PV interneurons could influence subthreshold integration in resting SPNs selectively in DMS and DLS, we investigated whether SOM and PV cells could shape cortically evoked EPSPs in SPNs in response to a single cortical stimulation (Fig. 7a). Opto-inhibition of SOM and PV cells significantly decreased EPSP decay time (Fig. 7b, c) and this effect was differentially regulated by SOM and PV interneurons in DMS and DLS, respectively: opto-inhibition of SOM cells decreased decay time in the DMS ($p = 0.001$, $n = 20$) but not in the DLS ($p = 0.1007$, $n = 11$), whereas opto-inhibition of PV cells decreased EPSP decay in the DLS ($p = 0.001$, $n = 19$) but not in the DMS ($p = 0.9564$, $n = 16$) (Fig. 7b, c). No effect could be observed on the rise time, slope and amplitude of EPSPs after interneuron opto-inhibition, neither in DMS nor in DLS (Supplementary Fig. 9).

This could be explained by the disynaptic nature of the feedforward inhibition onto SPNs (cortex-interneuron-SPN) that would delay the arrival of the GABA input until the decay phase of the monosynaptic cortico-SPN EPSP. These results show that SOM-DM and PV-DL efficiently shape single cortically evoked EPSPs in subthreshold SPNs by slowing down the EPSP decay phase and thus suggest they could affect the integration of subthreshold cortical inputs by SPNs.

We thus investigated whether the shaping of single EPSPs by SOM and PV interneurons could play a role in the summation of cortically induced subthreshold inputs in SPNs in DMS and DLS. To do so, different regimes of cortical activity inducing subthreshold activity in SPNs were mimicked with trains of cortical stimulations at various frequencies (5, 10, 20, 50 and 100 Hz). We compared the short-term dynamics of cortically evoked EPSPs in SPNs maintained at $E_{\text{m}}$ or in spiking SPN (APs are truncated for clarity of the figure). This was done by training SPNs at various frequencies (at 50 and 100 Hz) but not for medium frequencies (10 and 20 Hz). Synaptic depression could even be recorded for low-frequency activation (5 Hz) (both in DMS and DLS, Fig. 7f and Supplementary Fig. 10) or trains of light pulses (Fig. 6 and Supplementary Fig. 11) to viral expression of AAV-Flex-ArchT-tdtomato in SOM-Cre and PV-Cre mice (Supplementary Fig. 11). In control conditions (no light), temporal summation was efficient for high frequencies (at 50 and 100 Hz) but not for medium frequencies (10 and 20 Hz). Synaptic depression could even be recorded for low-frequency activation (5 Hz) (both in DMS and DLS, Fig. 7f and Supplementary Fig. 10). Selective opto-inhibition of SOM or PV cells decreased EPSP summation in DMS and DLS, respectively (Fig. 7d–g): opto-inhibition of SOM cells induced a decrease of temporal summation in DMS ($F_{1, 120} = 2.05, p < 0.0001, n = 25$ SPNs) but not in DLS ($F_{1, 80} = 0.11, p = 0.1536, n = 17$ SPNs), while opto-inhibition of PV cells following a 50 Hz train led to a decrease in the temporal summation of EPSP amplitude in the DLS ($F_{1, 120} = 2.10, p < 0.0001, n = 24$ SPNs) but not in DMS ($F_{1, 65} = 0.14, p = 0.1038, n = 14$SPNs) (Fig. 7d, e). Similar results were obtained for different frequencies (5, 10, 20 and 100 Hz) of cortical stimulations (Fig. 7f, g and Supplementary Fig. 10). In addition, similar results were observed with expression...
of Arch in SOM and PV cells restricted locally to DMS or DLS using viral strategy (Supplementary Fig. 11). Interestingly, silencing of SOM-DMS and PV-DLS cells had a stronger effect for medium frequencies (10, 20 and 50 Hz) and no or limited effect for low (5 Hz) and high frequencies (100 Hz) (Fig. 7), suggesting that the depolarizing effect of GABAergic microcircuits preferentially favors the subthreshold summation of such medium frequencies of cortical activity.

Finally, we asked whether the number of cortical cells activated and the resulting variation in EPSP amplitude in SPNs could influence the effect of interneuron silencing on summation. In control conditions, the amplitude of EPSP1 significantly
In vivo selective modulation of cortically evoked SNr activity was significant in control or with opto-inhibition of SOM or PV cells. d, e Top: Representatives 50 Hz trains of EPSPs recorded in SNr in control or with selective opto-inhibition of interneurons. Bottom: Temporal summations of EPSPs in SNRs after 50 Hz cortical electrical stimulation in control conditions (black) or with opto-inhibition of SOM (purple) or PV (green) cells. d In DMS, opto-inhibition of SOM or PV cells does not change this correlation regardless of the frequency (Fig. 7h–i). Thus, interneuron silencing would have the same effect for all tested amplitudes of the subthreshold EPSPs.

Altogether, these results show a physiological role for depolarizing GABA in the control of synaptic integration by SNRs that is selectively mediated by SOM and PV interneurons depending on the striatal region.

In vivo selective modulation of cortically evoked SNr activity by striatal GABAergic circuits. We finally investigated the outcome of the differential weight of SOM and PV interneurons in DMS and DLS, on the transfer of information between the (cortical) input and the (nigral) output of the striatum. In SOM: Arch3 and PV::Arch3 mice, we stimulated the cortical area (cingulate or somatosensory cortex) projecting to DMS and DLS, respectively, and recorded the evoked response of SNr units with and without opto-inhibition of SOM or PV cells in DMS or DLS (Fig. 8a–c). We observed a typical pattern of responses to cortical stimulation, consisting of three consecutive phases due to the activation of three cortico-SNr pathways27–29, (phase 1) an early excitation, corresponding to the activation of the “hyperdirect” non-trans-striatal pathway (cortico-STN-SNr), followed by (phase 2) an inhibition, corresponding to the “direct” trans-striatal pathway (cortico-striato-SNr), and (phase 3) a late excitation, reflecting the “indirect” trans-striatal pathway (cortico-striato-pallido-STN-SNr). This typical triphasic response was observed in SNr units, as well as other combinations of these three phases, with a similar proportion across conditions of the occurrence of “full” triphasic responses (37%, n = 27 units from 4 mice SOM-DMS; 48%, n = 31 from 4 mice PV-DMS; 35%, n = 20 from 4 mice SOM-DLS and 30%, n = 20 from 3 mice PV-DLS, p = 0.5918, Fisher’s exact test). Similar proportions of occurrence were also observed for each individual phase when considered independently (Supplementary Fig. 12). For each SNr unit, we calculated the deviation from baseline activity (50 ms before stimulation, in the absence or presence of light) caused by the cortical stimulation, and measured the area of each phase (when present). Interestingly, the area of the inhibition phase (phase 2) was significantly reduced only when opto-inhibiting PV interneurons in DLS (−859 ± 308%, p = 0.0237, n = 9 units, paired t-test), and not in any of the other conditions (PV-DMS: p = 0.2174, n = 19; SOM-DMS: p = 0.3714, n = 19; SOM-DLS: p = 0.6949, n = 13) (Fig. 8d, e). The duration of the inhibition phase and the peak of the late excitation phase displayed significant changes consistent with the area of each phase in SOM-DMS and PV-DLS conditions (Supplementary Fig. 12; Supplementary Table 2). In all conditions, the area of the early excitation (phase 1) was not affected by the opto-inhibition of either interneuron type in either territory (Supplementary Fig. 12; Supplementary Table 2) in line with the fact that this phase results from the activation of the non-trans-striatal pathway.

Altogether, these results indicate that the stronger effect of SOM interneurons in DMS and of PV interneurons in DLS translates into specific effect of these striatal interneurons/territory combinations on the trans-striatal transfer of information between the cortical input and the nigral output of the striatum.

Discussion
In the present study, we describe a marked specificity in GABAergic circuit properties and in the control of SPN activity and their downstream consequences on SNr activity depending on the functional striatal territory (DMS vs. DLS). These results demonstrate a strong heterogeneity in the composition and function of GABAergic microcircuits in DMS and DLS. We show that SOM cells regulate SPN activity more efficiently in the DMS, while PV cells have a stronger weight in the DLS. In addition, we show that GABAergic interneurons regulate SPN activity in a dual manner: hyperpolarizing for suprathreshold SPN activity and depolarizing for subthreshold SPN activity. The depolarizing effect efficiently controls the integration of cortical input in the subthreshold range of SPN activity. Because the expression of the opsins is in about half of the SOM and PV interneurons (both for transgenic and virally expressed), the observed effect might be even stronger when 100% of GABAergic microcircuits are recruited. In addition, similar results obtained in transgenic mice and virally expressed opsins show that the differential effect of SOM and PV cells is strictly due to local striatal microcircuits and not to external SOM and PV projections recently described from the cortex or the globus pallidus30–32.

In our study, we observed that the differential effect of PV cells might be due to their heterogeneous distribution throughout the striatum. The observed distribution in mice is consistent with previous studies showing a rostro-caudal gradient of PV cells in rats, monkeys and humans35–36. In addition, we observed here that PV cells have similar membrane and spiking properties in...
Fig. 8 Selective control of cortico-nigral information transfer by SOM and PV cells. a, c Experimental set up: in vivo multi-channel extracellular recordings of SNr unit activity using 4-shank 32-site silicon probe in response to stimulation in CG2 (a) or S2 (c) cortex, while PV or SOM interneurons are opto-inhibited using an optic fiber implanted in the DMS (a) or DLS (c). b Top: A cortical stimulation is applied in the absence (left) or presence (right) of light (300 ms, 10 mW, stimulation 100 ms after light onset, cycle repeated at least 100 times, 2-6 s between trials). Bottom: Raster plot of a representative SNr unit recorded in the PV-DLS condition (unit in e bottom), showing the response to the cortical stimulation in interleaved Ctrl (black ticks) and LED ON (red ticks) trials. d, e Left: Activity of a representative SNr unit displaying a triphasic response upon cortical stimulation of CG2 (d) or S2 (e), in the absence of (Ctrl) or during the opto-inhibition (LED ON) of SOM (top) or PV (bottom) interneurons in DMS (d) or DLS (e). The response is normalized to the baseline activity independently in each (Ctrl and LED ON) case. The measured areas of the trans-striatal phases (inhibition and late excitation) are illustrated in d top, and the measured differences in area (LED ON – Ctrl) are indicated for each representative unit. Right: Change in response areas corresponding to the trans-striatal inhibition (left), and late excitation (right), in all units displaying the corresponding phase. The cortico-nigral inhibition phase is significantly reduced only by the opto-inhibition of SOM striatal interneurons in the DMS condition (d, top, p = 0.0481, n = 17 units, paired t-test), while the cortico-nigral late excitation phase is significantly reduced only by the opto-inhibition of PV interneurons in the DLS (e, bottom, p = 0.0237, n = 9 units, paired t-test); *p < 0.05. For the 3D images in a and e, brain and brain structures are captured from the Allen Institute for Brain Science’s Mouse Brain Atlas74 (© 2004 Allen Institute for Brain Science, Allen Mouse Brain Atlas available from: mouse.brain-map.org/) using Brain Explorer*275

DMS and DLS; this observation being different from a recent study showing higher excitability of PV cells in DMS37 (probably due to different experimental conditions such as composition of extra- and intracellular solutions and slice orientation). In contrast, despite their homogeneous density throughout the striatum, SOM cells exhibit distinct electrophysiological properties in DMS and DLS, more excitable in DMS than DLS. Functionally, PV cells, or fast-spiking cells, have been the most extensively characterized in the striatum. PV cells are known to exert a strong inhibitory weight on SPNs since they delay or even stop the spiking activity in SPNs15,38,39. SOM cells (also expressing NPY and NOS) are also able to delay AP in SPNs15,38; they have been initially reported to have a lower connection probability with a weaker weight onto SPNs40 compared to PV cells, but high amplitude of evoked responses of SOM-SPN connections after SOM opto-activation was recently reported31,42, similar to what we observed in the present study. NPY expressing interneurons (overlapping with SOM cells) also strongly inhibit SPNs13. The differences in the characteristics of PV and SOM populations between DMS and DLS would give rise to the territory specificity. Indeed, even though the unitary connections from PV to SPN or from SOM to SPN are similar in both territories, their global action as populations is higher for PV cells in DMS due to their density and to SOM cells in DMS due to their intrinsic properties. In addition, cross-comparison shows that unitary PV-SPN connections are stronger than SOM-SPN connections in DLS. Altogether, we propose that these properties are underlying the functional dichotomy of PV and SOM cells in DMS and DLS.

The differential localization of the synapses from SOM and PV cells on the SPN dendrites could contribute to the specific modulation of cortical inputs. Nevertheless, even though PV cells contact SPN closer to the soma than SOM cells, their contacts are both located within the first 250 μm of the SPN dendritic arborization42, placing both of them in a strategic position to modulate the integration of glutamatergic inputs. In addition, we focused here on the two main subtypes of GABAergic interneurons but there are also other subtypes such as the electrophysiologically unidentified calretinin-expressing cells, and the
recently described tyrosine hydroxylase (TH)-expressing cells that could also play a role in the modulation of SPN activity. Furthermore, SPN collaterals exert a feedback inhibition though reported to be weaker than the feedforward inhibition. Here, the differential effect between SOM and PV cells cannot be explained by the involvement of the feedback inhibition since we also observed such differential effect under subthreshold activity regime of SPNs (Fig. 7), which implies that feedback inhibition was not engaged in these conditions.

Our in vivo experiments show that local modulation of striatal interneurons influence the basal ganglia output. Our recordings of SNr spontaneous activity show an activation of SNr upon opto-inhibition of both interneurons in both territories, meaning that the net effect of SOM and PV cells on SNr spontaneous activity is inhibitory. This suggests that interneurons modulate the balance between the direct (inhibitory on SNr) and indirect (disinhibitory) pathways, towards a relative activation of the direct pathway and/or inhibition of the indirect pathway, yet the striatal mechanism leading to this result could be multiple. Recent studies show that GABAergic interneurons control both direct (dSPNs) and indirect pathway SPNs (iSPNs) meaning that the local interneurons might control both SPN subtypes in a similar way. Since iSPNs are more excitable than dSPNs, their disinhibition would lead to a stronger increase in firing rate in iSPNs than dSPNs, thus explaining the observed overall increase of firing rate in SNr after silencing striatal interneurons. However, considering the dual depolarizing/hyperpolarizing effect of PV and SOM described here, the opposite hypothesis cannot be excluded: silencing striatal interneurons could lead to a decreased summation of spontaneous cortical inputs, which, if stronger in iSPNs, would also shift the net balance towards an activation of SNr. Other members of striatal microcircuits are also likely involved in this effect, and could contribute to either a decreased activity of the direct pathway or an increased activity of the indirect pathway. The stronger effect of silencing PV than SOM interneurons on SNr spontaneous activity in both territories could be due to a stronger spontaneous activity of PV cells, classically described as “fast-spiking” and identified in vivo by their higher firing rates. When stimulating cortical afferents, it is possible to visualize within the SNr different phases of response corresponding to hyperdirect, direct and indirect pathways. Similar to local striatal control, we observed a specific modulation of cortically evoked SNr activity from SOM-DMS and PV-DLS. Interestingly, SOM-DMS seems to modulate the inhibition resulting from the direct pathway and PV-DLS the late excitation related to indirect pathway recruitment. Though it would be tempting to jump to the conclusion that SOM/PV interneurons only affect each direct/indirect pathway, respectively, we think the interpretation should be more careful since the activation of the direct and indirect pathways are overlapping, and the phases that can be measured, while mainly corresponding to each pathway, are still the sum of this overlap. Nevertheless, iSPNs are more excitable and are recruited faster with stronger responses than dSPNs, which maintain a longer activation in response to cortical stimulation. The latency of interneuron activation is shorter for PV cells (Fig. 2 and coherent with kinetics described in studies, which would be likely to modulate more efficiently the first recruited iSPNs while SOM cells would control more efficiently dSPNs, activated later. In addition, our observation is also coherent with different organization of cortical inputs from cingulate cortex, which contact more dSPNs in DMS, and in DLS where iSPNs are more connected by somatosensory inputs. In vivo recordings also show that the interplay of inhibitory and excitatory effects of GABA is complex on the resulting effect on output structures such as SNr. Both SOM-DMS and PV-DLS opto-inhibition lead to a decrease of a striato-SNr pathway, meaning that the net effect of striatal interneurons would be excitatory locally on SPNs. Therefore, it could also play a role in the modulation of SPN activity. Furthermore, SPN collaterals exert a feedback inhibition though reported to be weaker than the feedforward inhibition. Here, the differential effect between SOM and PV cells cannot be explained by the involvement of the feedback inhibition since we also observed such differential effect under subthreshold activity regime of SPNs (Fig. 7), which implies that feedback inhibition was not engaged in these conditions.

Interestingly, we describe here that each interneuron subtype has a similar impact with a territory specificity, which translates into a differential effect on the downstream SNr. On cortically evoked activity, PV cells exhibit a strong weight in DLS while SOM cells more efficiently control DMS activity. We propose that potential underlying mechanisms to explain such specificity could come from the differences in the number of cells, their electrophysiological properties and the resulting local global connectivity. Therefore, the specificity stands in the fact that each GABAergic microcircuit, with its own intrinsic characteristics, has a specific role in DLS and DMS. The DLS is responsible for sensorimotor integration leading to habit formation. Sensory information requires to be quickly and reliably integrated and processed to produce a behavior adapted to the environmental stimuli. PV interneurons are reliably activated by the cortical activity, have fast-spiking characteristics and they modulate SPNs for any level of activity, which means that they tightly control sensory inputs of various amplitudes. Therefore, the intrinsic properties of PV cells and the fact that they are much more numerous in DLS with denser arborizations and their resulting action on SPNs are particularly adapted to the temporal precision needed to control sensorimotor information transmission. The DMS is involved in associative functions, receiving mainly inputs from the frontal parts of the cortex. Frontal cortex displays a lot of recurrent activity in the networks, particularly during working memory tasks. Fronto-corticostriatal inputs lead to recurrent activity in striatum that could be modulated by a more global inhibition, less precise in time but sufficient to modulate network activity level. SOM cells are less dependent on cortical inputs to discharge and are not time locked. In addition, they more efficiently control the first steps of build-up activity (for a small cortical activity) but then tend to lose their efficiency with increasing SPN activity. SOM cells would thus have the ability to drive the GABAergic modulation of associative integration. Therefore, the specificity of GABAergic microcircuits might be an active part of the different integration processes involved in the territory-specific striatal functions. We would like to point out that we focused here on feedforward inhibition mediated by cortical inputs but future studies should extend this work to another major striatal input coming from the thalamus.

A dual control of GABA on SPNs is functionally important because GABAergic interneurons would mediate in opposite direction sub- and suprathreshold events. The high amplitude of GABA currents we recorded for depolarized SPNs is coherent with a strong hyperpolarizing effect and a depolarizing effect of GABA has also been previously described in striatum. Of course, we cannot rule out that the depolarizing GABA could have a shunting effect and in that case decrease the efficiency of...
cortical inputs to activate SPNs. We describe here a functional role of the depolarizing GABA favoring the integration of cortical inputs in the subthreshold range of SPNs. This is in accordance with previous models predicting that some depolarizing inputs coming when SPNs are at rest with KIR activated should shift the inactivation of KIR, therefore reducing their availability and promoting summation of further inputs. The GABAergic system would thus counterbalance intrinsic properties of SPNs by favoring depolarization in SPNs at rest and slowing them down when they reach the AP threshold.

Membrane potential variations displayed by SPNs are dependent on the vigilance state of the animals. During slow-wave sleep or deep anesthesia, SPNs display large up and down fluctuations. The comparison of SPN fluctuations with various anesthetics and during wakefulness revealed that there are complex patterns of activity with depolarizing synaptic events of variable amplitude and in a goal-directed sensorimotor task, a successful trial can lead to either sub- or suprathreshold activity in SPNs. We also previously observed that subthreshold events can participate to Hebbian engram since they are involved in spike timing-dependent plasticity. These results illustrate that there is a large range of subthreshold activity which leads to subthreshold events in SPNs. Therefore, in addition to the modulation of the spiking activity, the role of PV and SOM cells in the modulation of subthreshold events in SPNs is functionally important. We show here that GABAergic interneurons strongly control integration of cortical activity in SPNs and are able to efficiently favor summation of cortical inputs. Given the properties of SPNs, the striatum might be a structure in which the depolarizing effect of GABA is particularly pronounced. Nevertheless, depolarizing GABA has also been described in other mature brain structures, such as cerebral cortex, hippocampus or amygdala. These observations are widening the impact of the dual role of GABA within the brain. It is now of importance to consider that the impact of GABAergic circuits is dynamic and will be determined by the context of the activity of the targeted neurons.

Methods

Animals.
All experiments were performed in accordance with the European Union (EU) guidelines (EU Directive 2010/63/EU) and local ethical committees. Animals were housed in temperature-controlled rooms with standard 12 h light/dark cycles and food and water were available ad libitum. Every precaution was taken to minimize stress and the number of animals used in each series of experiments. C57BL6 mice (Mus musculus adult (>3 months old) for in vivo experiments and 4 to 6 weeks old for ex vivo experiments of both sexes were used (there was no significant difference between males and females). The transgenic lines (SOC::Chrb2 and PV::Chrb2; SOM::Arch3 and PV::Arch3) were obtained by crossing homozygous SOM-IRESCre mice (Stock 01344, Jackson Laboratory, ME, USA) and PV-Cre mice (Stock 080069, Jackson Laboratory) with homozygous Chrb2(H134R)-eYFP mice (Stock 012569, Jackson Laboratory) or with homozygous Arch3-eGFP mice (Stock 012735, Jackson Laboratory). The resulting offspring selectively express a channelrhodopsin2-yellow fluorescent protein (Chrb2 (H134R)-eYFP) fusion protein or an archeorhodopsin3-green fluorescent protein (Arch3-eGFP) in either somatostatin- or parvalbumin-expressing neurons. In these transgenic mice, a specific channelrhodopsin2 or archeorhodopsin3 was observed in a given cell type, which allows reliable activation (Chrb2) or inhibition (Arch3) of PV or SOM interneurons (Supplementary Figs 1, 3, 5 and 6).

In vivo optogenetic and extracellular multi-channel recordings.
For surgery, general anesthesia was induced with isoflurane inhalation, and maintained with urethane injection (1.3 g/kg, intraperitoneal (i.p.) injection, supplemented to reach a surgical plane of anesthesia for each mouse up to 2 g/kg). Body temperature was kept constant with a heating pad. The skull was exposed and craniotomies were drilled over stereotaxic locations of the SNr, and a combination of DMS and/or DLS and/or cortex depending on the experiment. All implantations were performed on the same hemisphere, thanks to angled insertions to reduce cumber-someness (all coordinates are relative to bregma, except depth to brain surface). Two holes were drilled over the cerebellum to insert the ground and reference miniature screws. In the SNr (anterior-posterior (AP) –3.1 mm, medial-lateral (ML) 1.2–2.0), a multi-channel silicon probe (32 sites, 4 shanks with 200 μm spacing, high density bzsakai32 design, NeuroNexus) was inserted to 3.7 mm depth and slowly lowered to find typical SNr unit pattern of activity (regular spiking, >10 Hz; recording depths: 3.8–5.2 mm). In a first group of mice (n = 5; SOM::Arch3 and n = 6; PV::Arch3), 2 flat, 2 μm diameter cannulae (Thorlabs) were implanted in the DMS (AP +1.2 mm, ML 1.5 mm, depth 2.1 mm) and DLS (AP +0.5 mm with 10° angle towards the caudal pole, ML 2.85 mm, depth 2.3 mm). Two more groups of animals (n = 12 for DMS group and n = 9 for DLS group) were implanted with a bipolar concentric stimulation electrode in the SNr (AP –1.4 mm, ML 0.5 mm with 3° angle towards the midline and the caudal pole, depth = 1.6 mm) and the DMS for illumination (AP +1.2 mm, ML 1.5 mm with a 15° angle towards the midline). In the DLS group (n = 4 SOM::Arch3 and n = 5 PV::Arch3 mice), the cingulate cortex was targeted for stimulation (AP +1.2 mm, ML 0.5 mm with 3° angle towards the midline and the caudal pole, depth = 1.6 mm), and the DMS for illumination (AP +1.2 mm, ML 1.5 mm with a 15° angle towards the midline). A paraffin wax mixture was used to seal the craniotomies. Before implantation, silicon probes were painted with 2% Dil solution (Sigma), and optic fibers and stimulation electrodes were painted with 4'-diamidino-2-phenylindole (DAPI) solution (5 µg/mL, Molecular Probes) to facilitate the ex vivo localization confirmation.

For optogenetic and electrical stimulation, light from a 635 nm laser diode lightsource (FLS-635 nm–50 mW; DIPSS) collimated into a custom patch cord (105 μm core, 0.22 NA; Thorlabs) was connected to the brain-implanted optic fiber cannula. Light was delivered in square pulses with a light power of 10 mW at the tip of the fiber, adjusted before implantation in each experiment for both optic fibers using a photodiode power sensor coupled to a power meter (5120C and 91503A; HP, Agilent Technologies). Electrical stimulation was done with a 100 μA burst-pulse stimulator (Model 2100, A-M Systems) delivering bipolar biphasic pulses of 200 μA during 0.5 ms. Both light and electrical stimulations were driven by a Master-9 pulse stimulator (AMPI, Science Products). In mice with 2 optic fibers in DMS and DLS, only light stimulation was used (1 s at 0.2 Hz, 50 repetitions). In cortically stimulated mice, we alternated 3 types of trials: electrical stimulation only, electrical stimulation with light (300 ms light pulse, 100 ms delay between the onset of light) and light only (300 ms), each repeated at least 100 times with 2–6 s between any type of trial.

For electrophysiological recordings, extracellular signal was amplified, multiplexed and acquired continuously at 20 kHz using a multi-channel KJE-1001 system (Amplex) and stored for offline analysis.

Histological processing was used to confirm probe and optic fiber locations, the brain was removed at the end of the experiment, post-fixed overnight with 10% (vol/vol) formaline solution and cryoprotected in 30% (wt/vol in phosphate-buffered saline (PBSD)) sucrose solution. Coronal slices (80 µm thick) were cut using a freezing microtome (Microm), permeabilized with Triton X-100 (0.2%), counterstained with fluorescent Nissl staining (Neurotrace, Life Technologies) and mounted in Fluoromount (Southern Biotech). Probe and optic fiber tracks were visualized and photographed, aided by Dil and DAPI, on a DMRB (Leica) microscope. Six mice where the stimulation electrode touched the corpus callosum were excluded from the analysis of cortical stimulation responses, but were used for analysis of light-only trials.

For data analysis, data were visualized and processed using Neuroscope and NDManager (http://neurosuite.sourceforge.net). For unit detection, the signal was filtered, when a pass filter was used a high-pass filter was applied and single units were isolated using the semi-automated spike classifier KlustaKwik (http://sourceforge.net/projects/klustakwik) and further refined manually using the graphical spike sorting application Clusters (http://neurosuite.sourceforge.net). Only units of good quality (<1% spikes in the refractory period) and displaying typical SNr features (frequency >10 Hz, CV of interspike interval <1) were considered for analysis. Data were analyzed by built-in or custom-built procedures in MATLAB (MathWorks) and R environment (R version 3.4.3, RStudio 1.1.383). To quantify the effect of light on spontaneous activity, results from the first 50 light-only trials from electrically stimulated mice were pooled with results from fiber-only mice (analyzing only the 300 ms at the onset of the offset of the stimulus). To quantify the effect of light on cortical stimulation-evoked response, for each unit and type of trial (with or without light), we calculated a 1 ms-binned post-stimulus time histogram of the response, normalized by the baseline activity (mean of 50 ms before stimulation), and smoothed over 5 ms. Data within 2 ms of the stimulation were not considered in the analysis because the stimulation artifact affected spike detection in this time window (indicated with a gray shaded area on Fig. 8). On peristimulus time histogram (PSTH) shown in Fig. 8, cortico-SNr responses corresponding to typical phases of the “triphasic” response were identified as such if their responses were significantly above baseline and if 50% of the measurements were considered if the peak crossed ≥50% of baseline activity in either or both of Ctrl or LED ON trials, inhibition threshold was ~33%. (3) For area and duration measurements, onset and offset were defined as the bin where the PSTH crossed the baseline activity (0%), except for cases where two excitations were present without the inhibition, leading to two clearly separated peaks without crossing the baseline. For the case the two peaks was below the baseline of the early excitation (phase 1) and the onset of the late excitation (phase 3). If the onset of a component was masked by the stimulation artifact (since 2 ms on each side of the artifact are excluded from the analysis), it was taken as the first analyzable bin (i.e.,
The bin centered on 2.5 ms). (4) Response that were not clearly defined, such as excitations that could not be classified as early or late based on the peak latency or the presence of other identified phases, were not included in the analysis.

**Ex vivo optogenetic and multi-patch-clamp recordings.** For surgery for viral injections, stereotaxic intracranial injections were used to deliver adeno-associated viruses (AAVs) carrying opsins for ex vivo electrophysiological recordings in SOM-IRS-Cre and PV-Cre mice. Mice (P20-25) were anesthetized with isoflurane and placed in a stereotaxic frame. A small hole was drilled in the skull anterior to the 400 µL of AAVs (serotype 5/9) encoding either floxed Arch-T (tdTomato or floxed ChR2 (H134R)-mCherry (>10^12 genomic copies per mL, UPennCore) was injected viruses (AAVs) carrying opsins for ex vivo electrophysiological recordings in vivo and in vitro. For the long-term plasticity of IPSPs induced by each stimulation in the train and normalized it to the amplitude of the first one. For temporal summation, we measured the total amplitude of each IPSP/EPSP (from baseline to the peak of the response) and normalized it to the amplitude of the first IPSP/EPSP. For the effect of interneuron silencing on EPSP summation (Fig. 7f), the normalized amplitude corresponding to the amplitude of the third EPSP of the train compared to the first one. We compared the effect for each EPSP of the train and did not observe significant differences, and hence the third one was chosen as a representative. Data analysis was carried out in Igor-Pro 6.0.3 (Wavemetrics, Lake Oswego, USA) with the Neurevome package and Fitter (HEKA Elektronik, Germany).

**Statistical analysis.** Statistical analysis was performed using Prism 5.0 (GraphPad, San Diego, USA) and R environment (R version 3.4.3, RStudio 1.1.383). The sample size for the different sets of data is mentioned in the text or in the respective figure legends. Normality of each data set was checked using D’Agostino and Pearson’s test. Unless otherwise stated, all data sets are normal, reported as mean ± SEM, and statistical significance was assessed using Student’s t-test. Non-normal data were log transformed as median ± interquartile range. For each data set, we noted the median of the log transformed data (using a signed 2/3 root transform y = sign(x-median(x)) × abs (x-median(x))^2/3)) to maximize the normality of the model residuals. One-way ANOVA was used to compare all the effects together of PV and SOM cells in DMS and DLS. Pearson’s correlation was used for relationship between inhibitory effect rates in PV and SOM. For ANOVA following a post-hoc test was used to compare input/output curves, differences in short-term plasticity and to quantify the effect of silencing interneurons on temporal summation.

**Immunohistochemistry.** Quantification of interneurons and expression of Arch and ChR2 were done by immunohistochemistry targeting parvalbumin and somatostatin proteins and GFP/YFP or tdTomato/mCherry expressed together with the opsins. Mice were anesthetized and transcardially perfused with 4% paraformaldehyde for 16 h, extensively washed and sliced into 60 µm coronal sections using a vibratome (Microm HM 650V, ThermoScientific). Sections were immunostained with fluorescent-labeled secondary antibodies (Alexa 568 for PV and SOM and Alexa 488 for GFP). Sections were mounted on slides with Vectashield (Vector Labs). Images were acquired using an SP5 confocal microscope (Leica, Germany). Small (30–40 µm) Z-stacks with a 1 µm step size were acquired in DMS and DLS with a 20× objective (0.8 NA). For each animal, we acquired Z-stacks on 3 slices per territory (DMS and DLS) and in both hemispheres, so 6 Z-stacks per condition. We used ImageJ software for analysis. SVI (somatic volume index) was calculated as the ratio of the number of neurons measured by repeated current injections (−20 pA, 500 ms) and frequency was measured using the occurrence of a single action potential induced by a single electrical stimulation of the cerebellar cortex. For each cell, we repeated 6 to 8 control and LED ON interleaved trials to calculate the spike probability in each condition. To measure the effect of light on SPN spiking frequency, we triggered stable spike trains with 1 s current injection and measured either (1) the instantaneous frequency (Fig. 1b) by comparing the frequency between the spikes right before the 5 min LED pulse and at the time of the LED pulse or (2) the average frequency measured within 300 ms before, 300 ms during and 300 ms after the 300 ms LED pulse (Fig. 4c). We assessed short-term dynamics of either light-induced IPSPs or cortically evoked EPSPs with trains of stimuli (light pulses or electrical stimulations). We measured both short-term depression/potentiation and temporal summation. For the short-term plasticity of IPSPs induced by each stimulation in the train and normalized it to the amplitude of the first one. For temporal summation, we measured the total amplitude of each IPSP/EPSP (from baseline to the peak of the response) and normalized it to the amplitude of the first IPSP/EPSP. For the effect of interneuron silencing on EPSP summation (Fig. 7f), the normalized amplitude corresponding to the amplitude of the third EPSP of the train compared to the first one. We compared the effect for each EPSP of the train and did not observe significant differences, and hence the third one was chosen as a representative. Data analysis was carried out in Igor-Pro 6.0.3 (Wavemetrics, Lake Oswego, USA) with the Neuroview package and Fitter (HEKA Elektronik, Germany).

**Statistical analysis.** Statistical analysis was performed using Prism 5.0 (GraphPad, San Diego, USA) and R environment (R version 3.4.3, RStudio 1.1.383). The sample size for the different sets of data is mentioned in the text or in the respective figure legends. Normality of each data set was checked using D’Agostino and Pearson’s test. Unless otherwise stated, all data sets are normal, reported as mean ± SEM, and statistical significance was assessed using Student’s t-test. Non-normal data were log transformed as median ± interquartile range. For each data set, we noted the median of the log transformed data (using a signed 2/3 root transform y = sign(x-median(x)) × abs (x-median(x))^2/3)) to maximize the normality of the model residuals. One-way ANOVA was used to compare all the effects together of PV and SOM cells in DMS and DLS. Pearson’s correlation was used for relationship between inhibitory effect rates in PV and SOM. For ANOVA following a post-hoc test was used to compare input/output curves, differences in short-term plasticity and to quantify the effect of silencing interneurons on temporal summation.
corresponding to the ROIs. The sparseness of the striatal interneurons allowed us to easily count the number of cells in a given volume and to have unbiased analysis, the counting was performed by experimenters who were blind to the experimental conditions.

**Data availability** The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 12 April 2017 Accepted: 31 July 2018

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