

Sparse innervation and local heterogeneity in the vibrissal corticostriatal projection

Reviewed Preprint

v2 • August 13, 2025

Revised by authors

Reviewed Preprint

v1 • May 7, 2025

Kenza Amroune, Lorenzo Fontolan, Agnès Baude, David Robbe, Ingrid Bureau 

Aix-Marseille Université, INSERM, INMED, Marseille, France • Aix-Marseille Université, INSERM, INMED, Turing Centre for Living Systems, Marseille, France

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eLife Assessment

This revised manuscript provides **fundamental** findings on how the mouse barrel cortex connects to the dorsolateral striatum, uncovering that inputs from discrete whisker cortical columns are convergent and SPN-specific, but topographically organized at the population level. The evidence supporting this claim is **compelling**, demonstrating that SPNs uniquely integrate sparse input from variable stretches across the barrel cortex. The study would be of interest to basal ganglia and sensory-motor integration researchers.

<https://doi.org/10.7554/eLife.106621.2.sa4>

Abstract

The density and overlap of cortical axons in the dorsolateral striatum (DLS) have suggested that striatal neurons integrate widespread information from cortical regions that are functionally related. However, *in vivo*, DLS neuronal responses to sensory stimuli have shown unexpectedly high selectivity, raising questions about the actual degree of input convergence of functional corticostriatal projection on individual striatal cells. Here, we investigated this question by focusing on the projections from different whisker cortical columns, as they overlap in the striatum and are co-active during behavior. Using *ex vivo* patch-clamp recordings in the DLS and glutamate uncaging for focal stimulations in the barrel cortex, we were able to map the location of presynaptic neurons to individual striatal projection neurons (SPNs). We found that each SPN was innervated by cells located in a small number of whisker cortical columns scattered across the barrel field in the slice. Connectivity of single SPNs with cortical neurons was thus highly discontinuous horizontally, despite the presence of more potential connections. Moreover, connectivity patterns were specific to each cell, with neighboring SPNs sharing few common clusters of presynaptic cells in the cortex. Despite this sparse and distinct innervation of individual SPNs, the projection was topographically organized at the population level. Finally, we found similar innervation patterns for D1- and D2-type SPNs, but observed differences in synaptic strength in their connections with certain cortical layers, notably the associative layer 2/3. Our results suggest that the high convergence of somatosensory inputs to the striatum, enabled by diffuse and

overlapping cortical innervation, is accomplished through sparse yet complementary connectivity to individual SPNs.

Introduction

The dorsolateral striatum receives dense glutamatergic innervation from all sensory cortical areas. Its presumed function is to monitor the animal's ongoing sensory information, contributing to the modulation of sensory-guided behaviors and the learning of voluntary tasks (Pennartz et al., 2009; Znamenskiy and Zador, 2013; Sippy et al., 2015; Robbe, 2018). The observations that cortical neurons are overabundant compared to striatal projection neurons (SPN) (Oorschot 1996), that axonal projections originating from functionally linked cortical regions overlap in the striatum (Flaherty and Graybiel, 1991; Alloway et al., 1999; Wright et al., 1999; Hooks et al., 2018) and that synaptic glutamatergic inputs must overcome strong feedforward inhibition (Mallet et al., 2005; Pidoux et al., 2011), have led to the idea that striatal neurons integrate dense and broad cortical inputs. In fact, studies examining the density of axonal boutons relative to the single cell dendritic spines have predicted that several thousand cortical neurons could potentially connect every SPN if synapses were formed promiscuously (Kincaid et al., 1998). However if synapses were formed in a selective manner, this would reduce the degree of convergence of cortical input onto individual SPNs and potentially increase the heterogeneity of inputs between SPNs (Kincaid et al., 1998), a scenario with important implications for how cortical information is integrated in the striatum. These alternative connectivity models schematized in **Fig. 1** remained to be tested functionally.

Here, we investigated this question at the level of the projections originating from the barrel field in the mouse primary somatosensory cortex (S1) and targeting the SPNs. Vibrissal information is transmitted to the cortical columns of S1 through parallel sensory channels, each corresponding to an individual whisker (reviewed in Adibi 2019). In S1, layer 4 contains optically dense, barrel-shaped structures whose organization mirrors the spatial arrangement of whiskers on the snout. This feature provides a framework for studying neuronal connectivity in relation to the whisker cortical columns *ex vivo*. However, this model has not yet been used to examine connectivity within the functional vibrissal corticostriatal projections, leaving the degree of input convergence in this pathway unclear. On the one hand, axons of neurons located in different whisker cortical columns overlap heavily in the dorsal striatum, suggesting that SPNs could receive dense and broad vibrissal cortical input (Wright et al., 1999; Alloway et al., 2000). On the other hand, cell activity evoked by whisker deflection showed notable heterogeneity in the DLS: neurons were either tuned to multiple whiskers, selective to a single whisker, or yet non-responsive (Carelli and West, 1991; Pidoux et al., 2011). These observations prompted us to investigate whether the vibrissal innervation of SPNs violated Peters' rule stating that connectivity is proportional to the overlap of axons and dendrites (Peters, 1979; Braitenberg and Schüz, 1998).

SPNs comprise two main populations, defined by their projection targets and expression of dopamine receptors: D1 receptors in the direct pathway and D2 receptors in the indirect pathway (Smith et al., 1998; Kravitz et al., 2010) (neurons are hereafter referred to as D1 and D2 SPNs). Electrophysiological studies have found that inputs received by D1 SPNs were stronger than those received by D2 SPNs, both *in vivo* and *ex vivo* (Reig and Silberberg, 2014; Filipović et al., 2019; Kress et al., 2013; Parker et al., 2016). These observations may indicate that inputs converge to different degrees to D1 and to D2 SPNs.

To investigate the connectivity and input convergence on SPNs, we used a functional mapping technique that combines patch-clamp recordings and laser scanning photostimulation (LSPS) with glutamate uncaging on a novel slice preparation, which preserved the organization of the vibrissal corticostriatal projections, from the superficial layers of the barrel cortex to the DLS. LSPS enabled precise mapping of corticostriatal functional connectivity by identifying cortical sites where

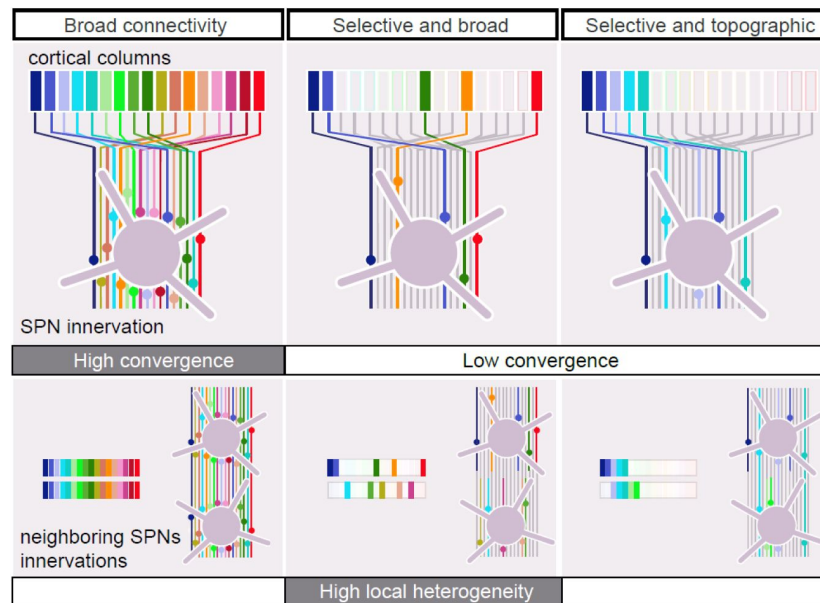


Figure 1.

Potential connectivity patterns within the corticostriatal projection.

A rich and overlapping cortical innervation of the striatum (lines) permits different connectivity patterns: Left, synapses are formed promiscuously. In this case convergence is high as each SPN receives broad cortical input, from many cortical origins (colored bars on top). Middle and right, only a fraction of the potential connections are actually formed and, in these cases, convergence is lower. The selected presynaptic neurons are either scattered (middle) or topographically (right) positioned in the cortex. Increasing the selectivity of the corticostriatal connections while keeping their broad origin (middle) could generate maximal heterogeneity in the patterns of input for SPNs sharing the same striatal volume, due to a large number of combinations of cortical origins. In the case of a topographic innervation (right), neighboring SPNs form synapses with presynaptic neurons located in the same cortical region, reducing the local heterogeneity.

stimulation evoked synaptic currents in the recorded SPNs, thereby localizing the cell bodies of their presynaptic neurons. This approach allowed us to determine both the cortical column and layer of origin within the barrel field in the slice for each SPN input. We found overall discrete innervations, as presynaptic neurons were thinly-scattered across the barrel field, or present in a single cortical column. SPNs located nearby in the same slice had distinct patterns of innervation, suggesting that connections on each SPN were fewer than the local potential connections. No difference was found between the innervation patterns of D1 and D2 SPNs, but the strength of their connections with certain layers differed.

Results

A novel preparation for investigating functionally the organization of projections from the barrel cortex to individual SPNs in the DLS

To investigate the spatial organization of sensory projections to SPNs, we have developed a parasagittal somatosensory corticostriatal slice, which contained ~ 8 whisker barrels (Methods; [Fig. 2A](#) and Supplemental Fig. 1A). Biocytin-labeled projections extended from the top of the barrel cortex to the DLS ([Fig. 2A,B](#)). To further validate the angles used in the preparation, we virtually re-sectioned a brain from the Allen Mouse Connectivity Atlas in which pyramidal cells of the barrel cortex had been labeled *in vivo* (i.e., prior to slicing; Supplemental Fig. 1B). Cortical axons extended into the striatum in a similar manner in both the reconstituted and acute slices (compare [Fig. 2A](#) and Supplemental Fig. 1B), supporting the idea that projections from the present cortical columns targeting the recorded striatal region were largely preserved in the acute slice. Animals were *Drd1a*-tdTomato hemizygous mice in which D1 receptor expressing neurons were labeled. D1⁺ and D1⁻ (D2⁺) SPNs were recorded in the whole-cell voltage-clamp configuration ($n = 101$ cells, $N = 54$ mice). Simultaneously, an ultraviolet laser beam was directed at every site of a 29×16 pixel grid (2.1×1.1 mm, $75 \mu\text{m}$ spacing) to uncage glutamate over the barrel cortex, excite cortical neurons, and thereby reveal cells presynaptic to the recorded SPN ([Fig. 2C,D](#)). When glutamate was uncaged on an excitatory cortical neuron innervating the recorded SPN, it elicited short-lived excitatory postsynaptic currents (EPSCs; < 50 ms duration) ([Fig. 2D](#)). We and others have shown that only subthreshold synaptic events are elicited with this method, implying that the evoked EPSCs are mono-synaptic and that feedforward inhibition is prevented ([Shepherd et al., 2003](#); [Bureau et al., 2008](#)). In fact, glutamate uncaging excited cortical neurons above their firing threshold at a maximal distance of $75 \mu\text{m}$ from their cell body ([Fig. 1E](#); Supplemental Fig. 2A), on 2.8 ± 0.3 sites of the LSPS grid (mean $\pm$ sem; L5 pyramidal cells, $n = 40$; $75 \mu\text{m}$ spacing grid; see Methods), consistent with a direct (i.e., non synaptic) and nearly somatic excitation (data per cell type in the Methods and Supplemental Fig. 2). Thus, the sites where LSPS evoked EPSCs in SPNs contained or were adjacent to the cell bodies of cortical neurons that were directly connected to the recorded cell. These sites are hereafter referred to as “connected sites”. EPSCs had small amplitudes, 40 ± 1 pA ($n = 550$). Based on known amplitudes of spontaneous and miniature EPSCs in SPNs (10–20 pA on average; [Kreitzer and Malenka, 2007](#); [Cepeda et al., 2008](#); [Dehorter et al., 2011](#); [Peixoto et al., 2016](#)), this finding is consistent with the presence of only one or a few presynaptic cells (≤ 5) at each connected site of the map.

For each SPN, an input map was assembled from the excitatory response evoked at the uncaging sites of the LSPS grid ([Fig. 2D](#), top). From this input map, a binary map was derived which reported the presence or lack of evoked EPSC, referred to as “connectivity map”. We first analyzed the ensemble of connectivity maps to characterize the global organization of the projections from the barrel field in the slice and checked that their properties were consistent with those described by anatomical studies ([Fig. 2F](#)). When all SPN connectivity maps were superimposed and overlaid on the barrel cortex (see Methods; [Fig. 2F](#)), the connected sites principally highlighted the layer (L) 5a. Indeed, $\sim 65\%$ of all SPNs received input from this layer ([Fig. 2G](#)). Responses were elicited less frequently when stimulations were in L4, L5b, L2/3 and in L6. We found that 50

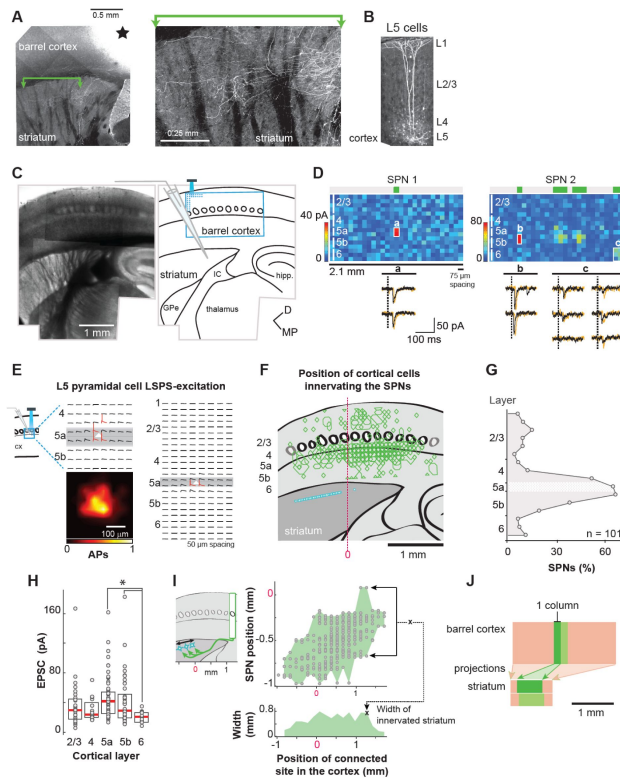


Figure 2.

A novel slice preparation for investigating the spatial organization of the somatosensory projections to the DLS neurons.

A. A somatosensory corticostriatal slice in which axons from L5 neurons were labeled with biocytin iontophoresis. Black star, the electrode position in L5a. Right, focus on a region in the striatum.

B. Right, Example dendritic arbors of L5 cortical cells labeled with biocytin in the slice.

C. Montage of a corticostriatal slice (left) and layout of the experiment (right). A SPN was recorded in the dorsolateral striatum while cortical neurons were photostimulated with LSPS. The grid of LSPS (blue) was positioned on the barrel cortex. GPe, globus pallidus, external segment; IC, internal capsule; hipp., hippocampus.

D. Top, examples of synaptic input maps for individual SPNs showing one or four clusters of input (SPN 1 and 2, respectively). In the color maps, each pixel of color indicates the peak amplitude of EPSCs detected within a 50 ms window after the stimulus onset. The different cortical layers are represented by solid white vertical lines on the left side of the map. The green boxes at the top of the maps, the clusters of sites in the connectivity maps collapsed in the vertical axis whose stimulation evokes EPSCs. Bottom, EPSC traces evoked at the sites indicated by letters in the maps above. Two repetitions are superimposed (black and orange). Vertical dashed lines, the stimulus onsets (2 ms stimulus).

E. LSPS evoked excitation of two L5a pyramidal neurons recorded in current-clamp mode. LSPS was a 8 x 8 (left) or 24 x 8 (right) 50 μ m spacing grid. Cortical neurons are positioned at the center (white triangles). Traces with an action potential are in red. Bottom, Average number of action potentials evoked at every site of the LSPS grid.

F. Overlay of the sites in the barrel cortex (green polygons) where stimulations evoked EPSCs in SPNs (cyan symbols; $n = 101$ cells, $N = 54$ mice). The red dashed vertical line is the reference (Ref_{hor} in the Methods) used for aligning slices across experiments horizontally, on the junction of the striatum, GPe and IC.

G. Contribution of each cortical layer to the SPN innervations. The 16 rows of the grid correspond to different cortical layers (Layer 2/3: 1-6; L4: 7-9; L5a: 10; L5b: 11-13; L6: 14-16). The horizontal white band is L5a.

H. Amplitude of SPN EPSCs as a function of their laminar origin. Median (red) and 25-75th percentiles (boxes). * Kruskal-Wallis $p = 0.000125$, Dunn-Sidak posthoc tests, $p = 0.0001$ and 0.03415 .

I. Top, Positions of SPNs in the striatum for each position of connected sites on the horizontal axis of the LSPS grid. Bottom, maximal distance between SPNs for every connected site position in the barrel cortex on the horizontal axis, binned every 150 μ m, in other words the width of the projection zone of one cortical column within the striatum

J. Schematic of the slice with the size of the cortical and striatal regions that are connected by projections to SPNs (pink) and the size of the striatal domain with functional projections from a single cortical column (shades of green).

% of L5a cells fired action potentials when stimulations were at the bottom of L4, indicating that a fraction of the relatively high connectivity rate seen at the bottom of L4 was in fact with nearby L5a pyramidal cells (**Fig. 2E** [↗](#); Supplemental Fig. 2A). In contrast, photo-stimulations in L2/3 never elicited the firing of L5a cells ($n = 11$; **Fig. 2E** [↗](#)), indicating that all EPSCs evoked by stimulations in these superficial layers arose from direct synaptic connections between L2/3 pyramidal cells and SPNs. EPSCs evoked with stimulations in L2/3 to L5b had similar amplitudes (**Fig. 2H** [↗](#)), suggesting that L5a dominance over these other layers is primarily due to a higher likelihood of SPNs being connected to it, rather than to stronger synaptic inputs. However, L6-EPSCs were smaller compared to L5a and L5b-EPSCs (Kruskal-Wallis, $H(4) = 22.8$, $p = 0.0001$; Dunn post-hoc test and Šidák correction, $p = 0.0001$ and 0.034 ; **Fig. 2H** [↗](#)). These observations are aligned with the anatomical studies which have identified L5a as the primary, although not exclusive, source of cortical innervation in the DLS (Wise and Jones, 1977 [↗](#); Cowan and Wilson, 1994 [↗](#); Wall et al., 2013 [↗](#)).

In the superimposed connectivity maps, the ensemble of connected sites observed across recordings formed a band in the barrel cortex that was larger than the region in the DLS containing the recorded SPNs (2.5 mm vs. 1 mm, **Fig. 2F** [↗](#)). An opposite asymmetry was observed when analyzing the striatal region connected to individual cortical columns: sites within a single cortical column ($\sim 150 \mu\text{m}$ in this preparation) were connected to SPNs distributed across a larger striatal region, spanning about $600 \mu\text{m}$ (**Fig. 2I** [↗](#)). These two asymmetric relationships (converging vs. diverging), summarized in **Fig. 2J** [↗](#), implied that functional projections from adjacent cortical columns overlapped in the DLS, which is consistent with their known anatomy (Wright et al., 1999 [↗](#); Alloway et al., 2000 [↗](#); Hooks et al., 2018 [↗](#)). Thus, projections from the cortical columns in the slice overlapped in the striatum, which allowed us to investigate the degree of convergence of whisker cortical inputs onto individual SPNs.

Low convergence of projections from the barrel cortex to individual SPNs in the DLS

To investigate the number and position of whisker cortical columns from which individual SPNs received synaptic inputs, each connectivity map was collapsed along its vertical axis and the horizontal distribution of connected sites was characterized using the following metrics (**Fig. 3A** [↗](#)): 1-the input field, or the overall region within the barrel field in the slice from which an SPN receives inputs. Its width is the distance between the SPN most distant connected sites.; 2-the cluster, a region in the collapsed connectivity map constituted of adjacent connected sites; 3-the cortical column, or its proxy: 1 to 2 adjacent connected sites in the collapsed connectivity map (i.e. $75\text{--}150 \mu\text{m}$ width). The connected sites were organized in 1 to 6 clusters, 1.9 ± 0.1 on average in the input fields (**Fig. 3B** [↗](#)). Typically, the width of clusters was about one cortical column ($171 \pm 7 \mu\text{m}$) and it rarely exceeded two adjacent columns (**Fig. 3C** [↗](#)). Finally, SPNs were innervated by cells distributed in close to 3 columns (2.7 ± 0.2), though some SPNs with several clusters (54 %) were innervated by as many as eight columns (**Fig. 3D** [↗](#)). The distance between clusters was 75 to $900 \mu\text{m}$, $265 \pm 28 \mu\text{m}$ on average ($n = 55$; **Fig. 3C** [↗](#)). Thus, SPNs could receive inputs from clusters of cortical cells separated by substantial connectivity gaps, often spanning several columns. Heterogeneous spacing and number of clusters contributed to the variability of the input fields width, which ranged between 0.075 and 1.6 mm (average, $535 \pm 42 \mu\text{m}$; **Fig. 3E** [↗](#)). Strikingly, regions lacking evoked synaptic responses (i.e., connectivity gaps) made up an average of 45 % of the length of input fields with multiple clusters (maps collapsed along the vertical axis; **Fig. 3F** [↗](#), bottom). Moreover, the number of clusters was not increased proportionally in very large input fields, $> 1.4 \text{ mm}$, in which connectivity gaps amounted to more than 50 % of space in the horizontal axis (**Fig. 3F** [↗](#), bottom). Altogether, these findings suggest a connectivity pattern characterized by a few small patches of presynaptic cortical cells scattered across the axis of barrels.

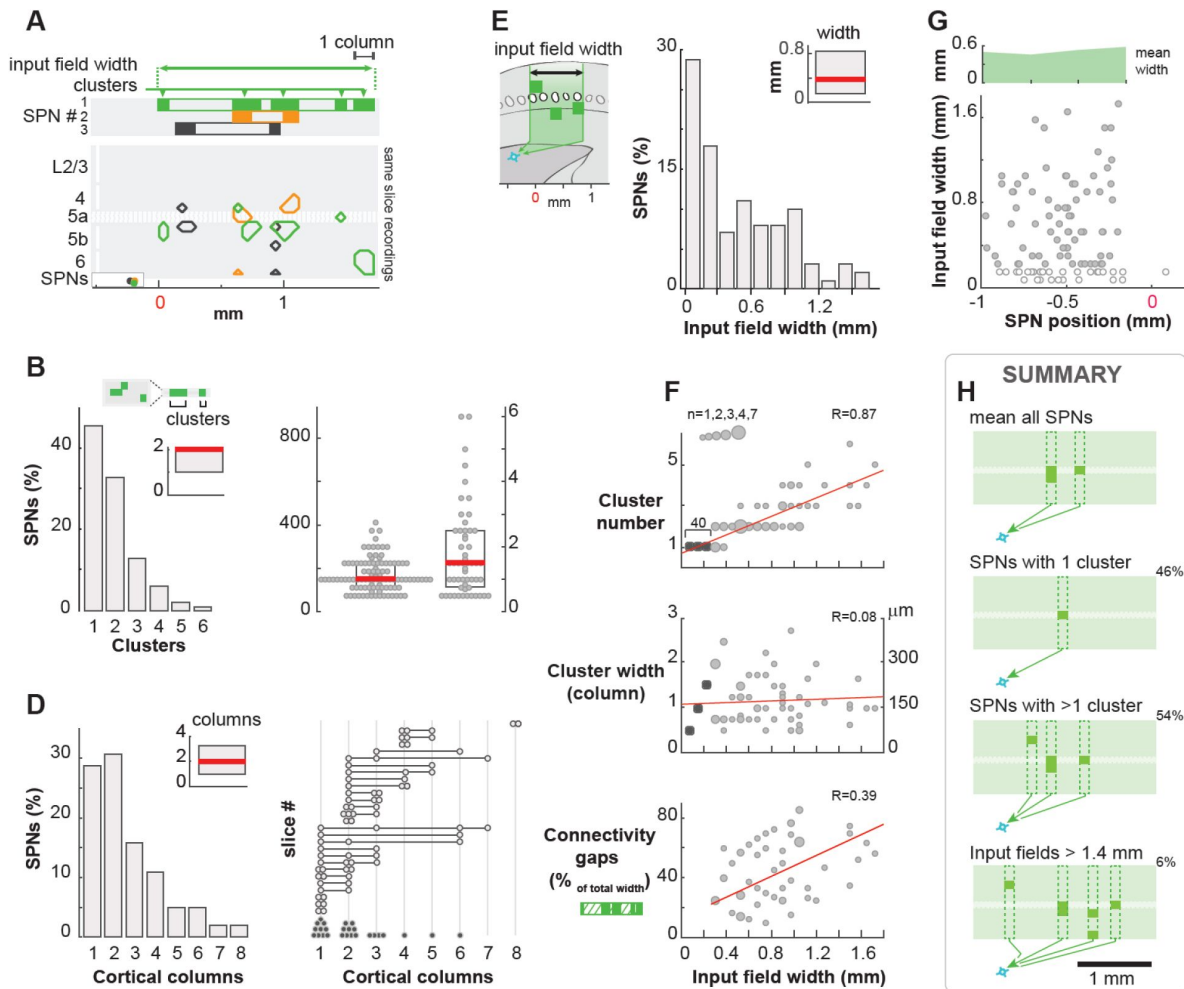


Figure 3.

Sparse functional projections from the barrel cortex to individual SPNs in the DLS.

A. Example slice with 3 recordings. The hatched horizontal band is L5a, the polygons are the stimulation sites evoking EPSCs, the solid boxes on top are the connectivity clusters and the open rectangle the input field in the collapsed connectivity map. The circles at the bottom mark the positions of SPNs on the horizontal axis. Recordings are color coded.

B. Fraction of SPNs receiving inputs from 1 to 6 clusters of projections in the barrel cortex. In inset, median (red) and 25-75th percentiles (box). Clusters are defined as the ensemble of contiguous sites in the connectivity map collapsed in the vertical axis, whose stimulation evokes EPSCs (see examples in A, boxes at the top). $n = 101$ cells, $N = 54$ mice.

C. Median (red) and 25-75th percentiles (box) of cluster width and spacing, in μm (left Y axis) and in number of cortical columns (right Y axis).

D. Left, Fraction of cells receiving inputs from 1 to 8 cortical columns in the barrel area. In inset, median (red) and 25-75th percentiles (box). Right, Number of cortical columns innervating individual SPNs. Symbols are cells, lines are slices. Light gray symbols, slices with ≥ 2 recordings. Dark symbols, one recording per slice.

E. Fraction of SPNs with input fields from 0.075 to 1.6 mm.

F. Top to bottom, for every input field width, the number of clusters, the cluster's width (in number of cortical columns and in μm) and the percentage of the region without connected site in the input fields collapsed along the vertical axis. Gray symbols, cells with ≥ 2 connectivity clusters. Other symbols, cells with 1 cluster. Top to bottom, $p < 0.0001$, $p = 0.43$ and $p = 0.0031$ for the correlations.

G. Bottom, width of input fields in the barrel cortex for different positions of SPNs in the striatum. SPNs with an input field the size of a single or of several cortical columns are shown (open and solid symbols, respectively). Top, The average width of input fields as a function of SPN positions binned every 250 μm .

H. Schematized average and principal connectivity patterns. The % of SPNs is indicated for each pattern.

It could be that small input fields that contained a single cortical column (75-150 μm) were obtained when recordings were performed on the edges of the striatal region with cortical innervation in the slice. Contrary to this hypothesis, we found that SPNs with small input fields and those with broader ones were intermingled along the horizontal axis in the striatum (open and solid symbols in **Fig. 3G**, respectively). In fact, the width of the input fields remained relatively constant across SPN positions in the slice (**Fig. 3G**, top; Spearman correlation coefficient, $R = 0.05$, $p = 0.61$). This suggests that the position of our recordings did not impact the SPN input fields. Furthermore, SPNs receiving input from a single column were often located in slices where other cells received input from multiple ones (**Fig. 3D**), reinforcing that the low functional connectivity with barrel columns in the slice was genuine in these cases.

Next, we investigated these connectivity patterns along their vertical axis. Individual SPNs had connected sites distributed in 2.4 ± 0.1 cortical layers on average (2.9 ± 0.1 for SPNs with several clusters). But these sites were not necessarily vertically aligned within a cortical column (examples in **Fig. 2D** and **3A**). In fact, in 55 % of the cases, a column of the barrel field innervated an SPN through projections from a single cortical layer. This suggests that neurons projecting to an SPN are dispersed in the barrel cortex in such a way that the transmitted inputs may originate from distinct layers as well as from different whisker cortical columns. Our findings are summarized in **Fig. 3H**, with schematics of the average and principal connectivity patterns.

Individual SPNs have unique innervation

The above analysis has revealed great heterogeneity between SPN innervations. We then investigated whether innervations were more similar when SPNs were close to each other. To address this question, we compared the connectivity patterns of SPNs recorded in the same slice (**Fig. 4A**). First, we observed that the majority of SPN pairs had their input fields overlapping in the barrel cortex ($n = 70$; **Fig. 4B**). The ratio was the highest, 77 %, for SPNs that were neighbors in the striatum ($< 100 \mu\text{m}$, $n = 33$), but remained above 50 % for cell distances of 400-500 μm . We next examined to what extent the connected sites were shared or vertically aligned between the connectivity maps. In contrast to input fields, alignment or overlap of connected sites was only observed when SPNs were less than 300 μm apart (**Fig. 4B,C**). Moreover, this concerned only a small fraction of the pair's connected sites: when SPNs were less than 100 μm apart, only 18 ± 2 % of the connected sites in the two connectivity maps were vertically aligned (**Fig. 4C**) and 7.7 ± 1.6 % of them overlapped (**Fig. 4D**). Overall, these results indicate that DLS SPNs could receive inputs from the same domain in the barrel cortex and yet have patterns of cortical innervation without or little redundancy. They support a connectivity model in which synaptic connections on each SPNs are sparser than the potential connections, and with significant heterogeneity between SPNs in terms of their whisker-related cortical inputs.

Topographic organization of SPN functional vibrissal innervation

The sparse innervation patterns, with limited similarity among neighboring SPNs, may seem inconsistent with the known topographic organization of somatosensory corticostriatal projections. To examine the global organization of functional projections in our preparation, we superimposed the connectivity maps of SPNs and labeled the connected sites based on the SPN's positions in the striatum (**Fig. 5A**). SPNs located laterally/medially in the dorsal striatum had an input field whose connectivity center of mass (CM, see Methods) was in the lateral/medial part of the barrel cortex (**Fig. 5A,B**). Thus, the functional projections from the barrel cortex were topographically organized in the DLS, consistent with anatomy (Flaherty and Graybiel, 1991; Alloway et al., 1999; Wright et al., 1999; Hooks et al., 2018). Given the orientation of the slice (Supplemental Fig. 1A), a shift of the connectivity CM on the horizontal axis of the barrel field corresponded principally to a shift between whisker arcs (e.g. E1 to E8 barrel). The connectivity CMs of two adjacent SPNs could be 1 mm apart in the barrel cortex (**Fig. 5B**), indicating that the topographic organization was not as precise as for intracortical projections (Shepherd and

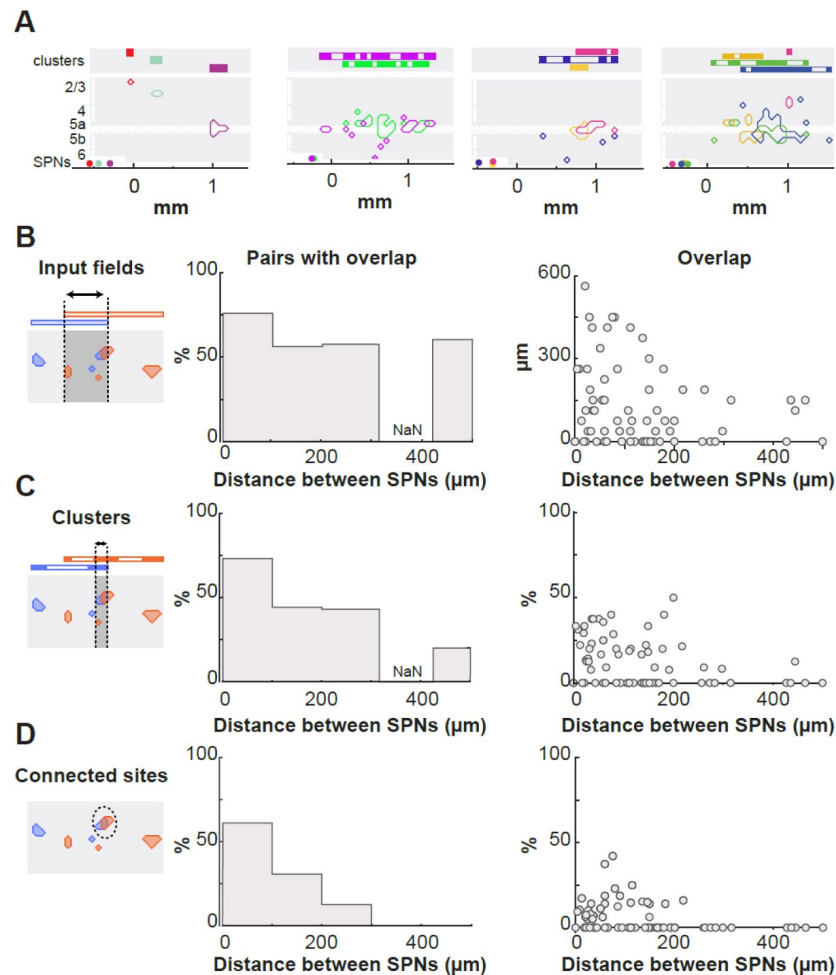


Figure 4.

Individual SPNs have unique cortical innervation.

A. Four example slices with 2 to 4 recordings. Same as in [Fig. 3A](#).

B. Left, fraction of pairs with overlap in their input field. Right, width of overlap as a function of the horizontal distance separating cells. $n = 70$ pairs. NaN indicates the lack of data.

C. Same as in B for the fraction of vertical alignment between SPN connectivity maps.

D. Same as in B for the fraction of overlap between SPN connectivity maps.

Svoboda, 2005 [↗](#); Erlandson et al., 2015 [↗](#)). This finding, together with previous analyses, supports a connectivity model that falls between the two illustrated in the middle and right panels of **Fig. 1** [↗](#) (selective, broad and loosely topographic).

The EPSC amplitudes were heterogeneous within each input map (**Fig. 5C** [↗](#); ex **Fig. 2D** [↗](#)). To test the hypothesis of a topographic organization influencing synaptic strength, we examined whether strong connections were associated with presynaptic cells located close to the connectivity CM. If such an organization of synaptic strengths existed, we would expect it to improve the correlation between the CM of synaptic input maps and SPN positions. However, we found nearly identical correlations whether taking the connectivity CM or the synaptic input CM, which is based on the connectivity pattern weighted by EPSC amplitudes (see Methods; $R = 0.60$ vs. 0.62 ; $F(1) = 115.08$, $p = 0.98$, ANCOVA; **Fig. 5D** [↗](#)). While residuals from the correlation curve tended to be smaller using synaptic input CMs, the difference was not statistically significant ($-9 \pm 7 \mu\text{m}$, $p = 0.084$, Wilcoxon, SPNs with >1 cluster). This was because synaptic input CMs were only slightly shifted from the connectivity CMs, by $45 \pm 5 \mu\text{m}$ for SPNs with two or more clusters (**Fig. 5D** [↗](#)). These findings indicate that EPSC amplitudes showed no consistent pattern relative to the connectivity CM of the input field. This confirms the connectivity patterns as principal determinants of the topographic organization of somatosensory corticostriatal projections.

Finally, given that previous studies have revealed that responses of some striatal neurons exhibited strong selectivity for specific whiskers *in vivo* (Carelli and West, 1991 [↗](#); Pidoux et al., 2011 [↗](#)), we investigated whether, in cases where SPNs were innervated by multiple clusters, one cluster might dominate the others. Comparing the sum of EPSCs corresponding to clusters, we found substantial variability, with the strongest cluster providing input that was 1.12 to 55 times greater than other clusters within the input field ($\times 6.8 \pm 1.4$ on average; **Fig. 5E** [↗](#)). Thus, connectivity patterns with multiple clusters may still permit some SPNs to have a preference for a particular whisker cortical column, due to the heterogeneity between clusters.

D1 and D2 SPNs receive similar input from the barrel cortex

Finally, we investigated whether innervation patterns differed between the two populations of SPNs, expressing D1 or D2 receptors. Based on previous studies, unlabeled neurons in *Drd1a*-tdTomato hemizygous mice were principally SPNs expressing the D2 receptor (Bertran-Gonzalez et al., 2008 [↗](#); Ade et al., 2011 [↗](#); Enoksson et al., 2012 [↗](#); Thibault et al., 2013 [↗](#); Cao et al., 2018 [↗](#)). As previously reported (Wall et al., 2013 [↗](#)), projections to D1 and D2 SPNs were spatially intermingled along the axis of barrels (**Fig. 6A,B** [↗](#); $n = 47$ and 54 , cells respectively). We found that D1 and D2 SPNs had similar input fields: first, their widths were not different (**Fig. 6C** [↗](#)). In addition, they received input from a similar number of cortical columns, which were organized in clusters of similar widths (**Fig. 6D,E** [↗](#)). Paired analysis of D1 and D2 SPNs recorded in the same slice also showed that their input fields did not significantly differ in width, number of cortical columns or cluster width ($p = 0.44 - 0.50 - 0.26$, Wilcoxon; **Fig. 6C-E** [↗](#)). None of these parameters exhibited a clear relationship between subtypes ($R = 0.13 - 0.30 - 0.003$; $p = 0.43 - 0.058 - 0.99$, Pearson; **Fig. 6C-E** [↗](#)). Finally, both types of SPNs received input from each cortical layer in similar proportions (**Fig. 6F** [↗](#)). Thus, the heterogeneity in patterns of innervation is not due to a difference between SPN subtypes. Overall, there was no difference in the total inputs received by D1 and D2 SPNs (EPSC sum or EPSC^T; **Fig. 6G** [↗](#)). In addition, the principal cluster was similarly dominant over the other clusters (**Fig. 6H** [↗](#)). However, differences were observed in a layer-specific manner (**Fig. 6I** [↗](#)). Whereas L5-EPSC^T was similar in D1 and D2 cells, differences were found in L2/3 and L6: L2/3-EPSC^T was larger in D2 SPNs (132 ± 36 vs. 53 ± 22 pA, $p = 0.005$, Mann-Whitney) but L6-EPSC^T was larger in D1 SPNs (37 ± 10 vs. 16 ± 2 pA, $p = 0.038$). Further analysis suggested a difference in the strength of synapses as principal mechanism. Indeed, L2/3-EPSCs evoked at single sites were larger in D2 SPNs (48 ± 11 pA vs. 27 ± 5 pA; $p = 0.043$) whereas L6-EPSCs were larger in D1 SPNs (24 ± 2 pA vs. 16 ± 2 pA; $p = 0.038$).

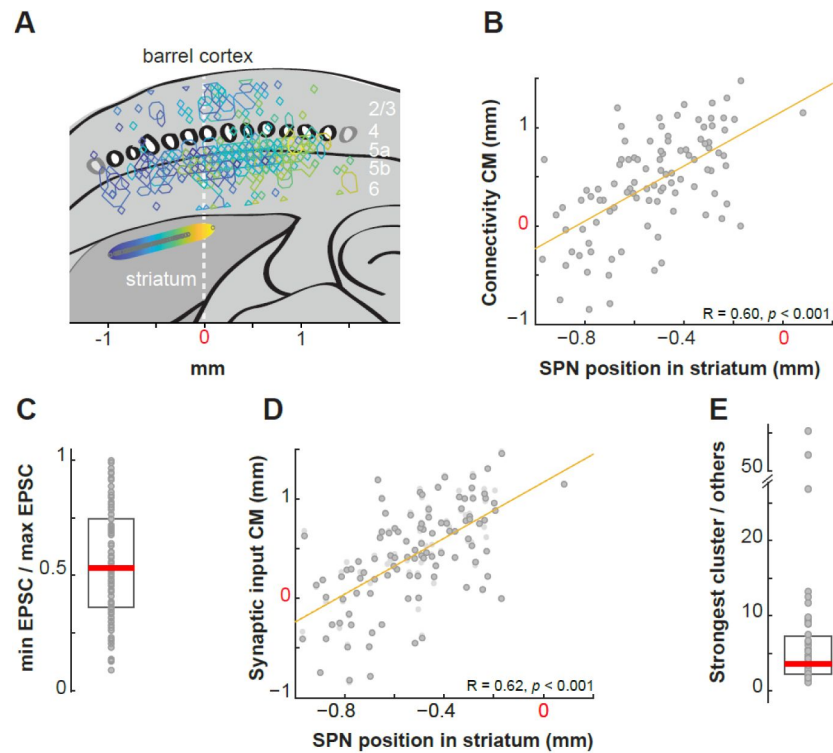


Figure 5.

Topographic organization of the functional vibrissal innervation of SPNs in the DLS.

- A.** Overlay of the sites in the barrel cortex where stimulations evoked EPSCs in SPNs. The colors, yellow to blue, indicate the position of the SPNs (gray circles) along the horizontal axis in the striatum (axis at the bottom, 0 is Ref_{hor} in the Methods; $n = 101$, $N = 54$). Blue shades are for lateral SPNs.
- B.** Position of the connectivity center of mass (CM) as a function of the SPN position in dorsal striatum. Zero on the x and y axis is the position of the vertical dashed line shown in A.
- C.** Ratio of the largest EPSC over the smallest EPSC for each recording. Median (red) and 25-75th percentiles (box).
- D.** Position of the synaptic input CM as a function of the SPN position in dorsal striatum. In light gray, the connectivity CM.
- E.** EPSC sum obtained in the strongest connectivity cluster relative to others sum. Cells with ≥ 2 clusters, $n = 55$.

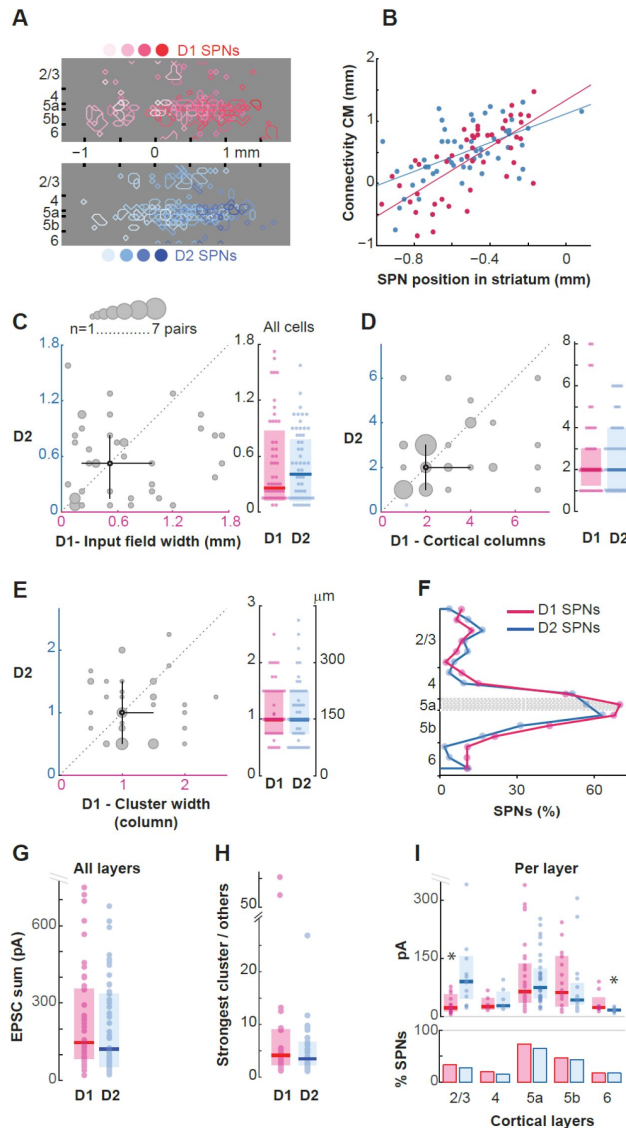


Figure 6.

D1 and D2 SPNs have similar patterns of innervation from the barrel cortex.

A. Overlay of the sites in the barrel cortex where stimulations evoked EPSCs in D1 (blue) or D2 SPNs (red). The shades, dark to light, indicate the position of the SPNs (bottom circles) along the medio-lateral axis in the dorsal striatum (axis at the bottom). D1 cells, $n = 47$, $N = 36$; D2 cells, $n = 54$, $N = 37$.

B. Position of the connectivity center of mass (CM) as a function of the SPN position in the dorsal striatum (D1, red; D2, blue). $R = 0.70$ (D1, $n = 47$, $N = 36$); $R = 0.57$ (D2, $n = 54$, $N = 37$).

C. Left, D2 SPN input field width as a function of the D1 SPN input field width, in the same slice. The black symbol and lines are the median value and 25-75th percentiles. Large symbols indicate $n = 2$ to 3 pairs. $n = 42$ pairs. Right, medians (thick lines) and 25-75th percentiles (boxes) of D1 and D2 input field widths, across all cells. $n = 101$.

D. Same as in C for the number of cortical columns.

E. Same as in C for the width of connectivity clusters.

F. Contribution of each cortical layer to the SPN innervations. The horizontal hatched band is L5a.

G. EPSC sum for D1 and D2 SPNs. Outliers were not shown for clarity (1 D1, 2 D2, 1.2-1.5 nA)

H. EPSC sum obtained from the strongest cluster relative to others. Cells with ≥ 2 clusters. D1, $n = 24$; D2, $n = 30$.

I. Top, D1 and D2 SPN EPSC sum as a function of laminar origin. For each layer, only cells with inputs are included. Outliers are not shown for clarity (3 D1, 2 D2, 350-700 pA). * indicates a significant difference ($p = 0.005$ and $p = 0.008$; Mann-Whitney). Bottom, fraction of cells with input.

Discussion

We investigated the organization of functional projections originating in the barrel cortex and targeting projection neurons in the dorsal striatum in a novel preparation. We found overall little input convergence as cortical neurons innervating an SPN were either located in a single cortical column or thinly scattered across the barrel field. Each SPN had a distinct pattern of innervation from the barrel cortex, albeit with occasional overlap for SPNs closely positioned in the DLS. D1 and D2 presented similar patterns of innervations.

Sparse sensory innervation of single SPNs

As previously revealed by tracings of corticostriatal projections, the primary source of innervation of SPNs in this study is the upper part of L5 in the barrel cortex, L5a, although, SPNs are occasionally innervated by cells located in deeper and superficial layers (Wise and Jones, 1977 [↗](#); Reiner et al., 2003 [↗](#); Wall et al., 2013 [↗](#); Guo et al., 2017 [↗](#); Yamashita et al., 2018 [↗](#); Bertero et al., 2022 [↗](#)). Given the abundance of cortical axons in the striatum, it has been assumed that a large number of cortical cells innervate each SPN, potentially up to few thousands if synaptic connections were made promiscuously (Kincaid et al., 1998 [↗](#)). On the other hand, it has also been reported that neurons in the DLS fire in response to the stimulation of a single body-part in monkeys and rodents *in vivo*, even of a single whisker in some cases (Carelli and West, 1991 [↗](#); Jaeger et al., 1995 [↗](#); Cho and West, 1997 [↗](#)). This finding suggested that each striatal neuron was in fact innervated by one small subregion of S1, although feedforward inhibition could have masked a broader selectivity (Pidoux et al., 2011 [↗](#)). Here, the LSPS connectivity maps indicate that convergence from the barrel cortex to individual SPNs is indeed low as 60 % of SPNs responded to the stimulation of only one or two whisker cortical columns present in the preparation. The LSPS combined with glutamate uncaging mapped projections contained in the slice, intact from the presynaptic cell bodies to the SPN dendrites. Some cortical inputs targeting distal SPN dendrites may have gone undetected, either due to attenuation of synaptic events recorded at the soma or because distal dendritic branches were lost during slice preparation. Indeed, about 80 % of S1 synaptic contacts are distributed along dendrites (Sanabria et al., 2024 [↗](#)). However, synapses located distally are proportionally rare (Sanabria et al., 2024 [↗](#)), and our estimates suggest that the loss of S1 input was minimal (see Methods). More significantly, our mapping only included projections from neuronal somata located within the S1 barrel field in the slice: projections from cortical columns outside the slice were not stimulated. For this reason, our study characterized connectivity patterns rather than the full extent of connectivity with the barrel cortex. A number of critical findings argue that innervation on individual SPNs is genuinely sparse: i) The cortical columns present in the slice corresponded to different whisker arcs, an axis associated with the greatest overlap of cortical axons in the striatum (Alloway et al., 1999 [↗](#)), and therefore where input convergence could have been high. This organization was visible in the global SPN LSPS connectivity map (Fig. 2J [↗](#)). ii) The peak of connectivity was with L5a as previously documented, not with a layer closer to striatum, as this would be the case if the native organization of the projection was altered in the preparation. iii) The connected sites were scattered along broad horizontal and vertical axes, implying that projections in between were preserved. Critically, the fact that adjacent SPNs had different connectivity patterns in the same slice further supported the presence of a pool of potential connections larger than the number of actual connections made on each SPN. iv) Connectivity clusters were sparser in larger input fields, suggesting that the number of presynaptic cells to an SPN was regulated and reached a plateau. v) EPSCs were small, consistent with one or a few presynaptic cells at each connected site. Altogether, our results support the model in which, within a loosely topographical organization of projections, each SPN integrates limited and heterogeneous rather than exhaustive inputs transmitted by the

barrel cortex (**Fig. 7**). The speckled connectivity pattern of individual SPNs, arising from the abundant and diffuse cortical innervation in the DLS, suggests that somatosensory corticostriatal synapses are established through a selective and/or competitive process.

It is important to determine whether this sparse innervation of SPNs by S1 is a characteristic shared with other projections. In particular, it will be interesting to test this hypothesis on the auditory projections targeting the posterior striatum, where neurons exhibit clear tone frequency selectivity (Guo et al., 2018).

Specific cortical input to individual SPNs

A peculiar feature of the corticostriatal innervation is the imbalance between the number of cortical cells projecting to the striatum and the number of striatal cells, thought to be at a ratio of 10 to 1 (Oorschot, 1996). Based on this ratio and the ultrastructure of the corticostriatal connection, the density of axonal boutons and spines, C. Wilson's group concluded that the probability of an SPN being contacted by one given axon entering the domain of its dendritic arborization was low, 0.04 - 1.4 %, depending on the model. Hence, the chance that two SPNs were innervated by the same cortical axon was even lower according to this model. Consistent with it, here, we found that overlaps between the connectivity maps of SPNs were rare and, when present, involved only a small fraction of the connected sites. This indicates that neighboring SPNs predominantly integrated distinct inputs from the barrel cortex, although it is possible that overlapping inputs received in distal dendrites were not all detected. Overlaps occurred only for SPNs located within 250 μm of each other. In contrast, input fields continued to intersect significantly beyond 250 μm and up to 500 μm of separation between SPNs. This means that relatively distant SPNs could share a general region of connectivity in the barrel cortex without any of their presynaptic partners residing within the same cortical column. Interestingly, the observation that two SPNs must be less than 250 μm apart to share some of their inputs was not predicted by the known ultrastructure of corticostriatal connections. In fact, it predicted the opposite: that two SPNs would more likely to be innervated by the same cortical cell if their dendrites extended into different striatal subregions, as this would increase the likelihood of each SPN contacting boutons of this cortical cell, as these are sparsely distributed in the striatum (Kincaid et al., 1998). One possible explanation for this discrepancy could be that, in our experiments, pairs of SPNs with shared connectivity were innervated by different neurons that were co-localized in the barrel cortex (< 75 μm apart). Such events could also be at the origin of the striatal clusters described *in vivo*, in which neurons had similar, yet non-identical, sensory selectivity (Carelli and West, 1991; Jaeger et al., 1995).

Similarities and specificities in the patterns of projection to SPNs of the direct and indirect pathways

Electrophysiological studies have shown that D1 SPNs exhibit larger responses than D2 SPNs to whisker deflections (Reig and Silberberg, 2014; Filipović et al., 2019). Also, the optostimulation of cortical efferents in brain slices showed a bias towards D1 SPNs (Kress et al., 2013; Parker et al., 2016). In our study, we had contrasting results. There was no difference in the innervation of D1 and D2 SPNs, neither in the rate at which cells were connected by layers, nor in the organization of the projection. The principal layer innervating SPNs, L5a, delivered inputs of similar strengths to D1 and D2 SPNs. However, layers with lower incidence in the pattern of innervation showed bias in the amplitude of responses: stimulations in L2/3 induced stronger responses in D2 cells whereas stimulations in L6 activated D1 cells more strongly. We have shown that stimulation in L2/3 activates the L2/3 projection only. Similarly, the L6-evoked EPSCs are consistent with the axon collaterals that L6 corticofugal projection neurons have in the striatum (Guo et al., 2017; Bertero et al., 2022). The larger L2/3 inputs received by the D2 SPNs is intriguing because of the role of this layer in higher-order integration processes, such as those activated during operant sensory discrimination tasks (Kwon et al., 2016; Han and Helmchen, 2024; Oryshchuk et al., 2024). Moreover, the activity of L2/3 cells during mouse displacement

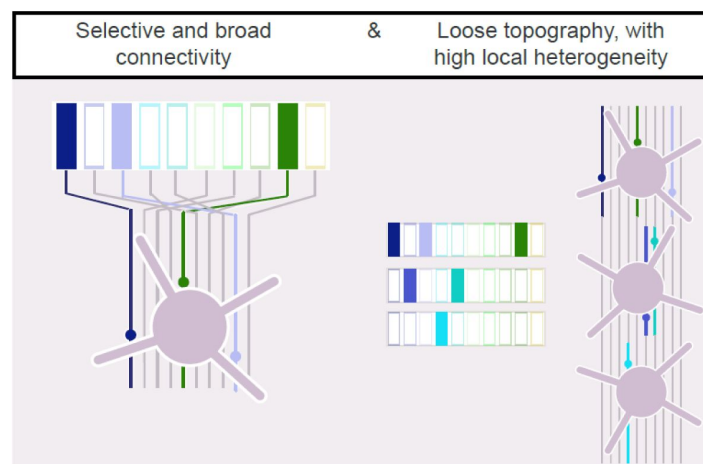


Figure 7.

Connectivity pattern within the somatosensory corticostriatal projection to SPNs.

Left, Our results support the model in which each SPN integrates limited and heterogeneous rather than exhaustive inputs transmitted by the barrel cortex, intermediate between the middle and right panels in [Fig. 1](#). Right, Each SPN representation of the whisker cortical columns complements the representations of its neighbors.

is distinctive, exhibiting a sustained response to ongoing whisker-wall contacts, as opposed to the transient response observed in L5 (Ayaz et al., 2019 [DOI](#)). In L6, the population of corticofugal projection neurons is diverse, with some responding to whisking and sensory stimuli, while others are active during transitions into quiet periods of behavior (Dash et al., 2022 [DOI](#); Vélez-Fort et al., 2014 [DOI](#)). Further investigation is needed to specifically examine projections from the upper and lower cortical layers and how they influence the dynamics of sensory integration in the direct and indirect pathways of the DLS. However, these data illustrate the diversity of paths of sensory integration involving D1 and D2 SPNs.

In conclusion, we found a low and sparse connectivity within the cortical vibrissal projections to individual SPNs. While projections respected a loose topography, the innervation patterns of individual SPNs displayed substantial connectivity gaps and heterogeneity. Since the inputs to a single SPN represent only a limited subset of whisker columns, a complete representation of whiskers could emerge at the population level, with each SPN's representation complementing those of its neighbors (Fig. 7 [DOI](#)). These observations raise the hypothesis of a selective or competitive process underlying the formation of corticostriatal synapses. The degree of input convergence onto SPNs could be modulated by plasticity, potentially enabling experience-driven reconfiguration of S1 corticostriatal coupling.

Materials and Methods

Animals and ethics

Hemizygous male and female B6.Cg-Tg(Drd1a-tdTomato)6Calak/J mice (JAX stock #016204; Ade et al., 2011 [DOI](#)) were used on postnatal day 22-43, in accordance with institutional guidelines and the French Ministry of Research (APAFIS#27242).

Brain slices preparation and electrophysiology

Mice were deeply anesthetized with isoflurane (4 %) prior to cervical dislocation and decapitation. We prepared corticostriatal slices (350 μ m thick) from the brain left hemisphere, based on stereotaxic coordinates placing the striatal cells 1-2 mm anteriorly to the projection neurons in the barrel cortex (Aronoff et al., 2010 [DOI](#); de la Torre-Martinez et al., 2023 [DOI](#)). Parasagittal slices were cut with a 60° angle from the midline and a 10° angle in the dorso-ventral axis (Supplemental Fig. 1A) in a chilled cutting solution containing (in mM): 110 choline chloride, 25 NaHCO₃, 25 D-glucose, 11.6 sodium ascorbate, 7 MgCl₂, 3.1 sodium pyruvate, 2.5 KCl, 1.25 NaH₂PO₄, and 0.5 CaCl₂ (Sigma Aldrich). Slices were then transferred to artificial cerebrospinal fluid (ACSF) containing (in mM): 127 NaCl, 25 NaHCO₃, 25 D-glucose, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, and 1.25 NaH₂PO₄, aerated with 95% O₂/5% CO₂. Slices were first incubated at 34 °C for 30 min and then maintained at room temperature for 20 min prior to use. Slices used for LSPS (1-2 per animal) contained barrels in the L4 of cortex, the globus pallidus, its external segment (GPe), the internal capsule, the ventral posteromedial nucleus of thalamus and the anterior hippocampus. ~ 8 barrels (5-13) were visible in the slice. 2 rows of barrels may have been superimposed. At the end of each experiment, a picture of the slice was saved in order to superimpose it digitally to other slices, according to visual landmarks (Adobe Photoshop; Adobe Inc.). SPNs were visualized under infrared and fluorescent lights in a BX61WI microscope (Olympus) and patched with borosilicate electrodes (3–6 M Ω) and recorded in the voltage-clamp whole-cell configuration using a Multiclamp 700A amplifier (Axon Instrument, Molecular Devices). The holding membrane potential was – 80 mV. The intracellular solution contained (in mM) 128 Cs-methylsulfate, 4 MgCl₂, 10 HEPES, 1 EGTA, 4 Na₂ATP, 0.4 Na₂GTP, 10 Na-phosphocreatine, 3 ascorbic acid; pH 7.25; 290-300 mOsm. Cells in L5 and L2/3 of the barrel cortex were recorded in the current-clamp mode, with an intracellular solution in which Cs-methylsulfate was replaced by K-methylsulfate. All experiments were performed at room temperature (21°C).

LSPS with glutamate uncaging

LSPS was performed as described previously (Bureau et al., 2006). Recirculating (2 mL/min) ACSF solution contained (in mM): 0.2 MNI-caged glutamate (Tocris), 0.005 CPP [(0-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid)], 4 CaCl₂, and 4 MgCl₂. Focal photolysis of caged glutamate was accomplished with a 2 ms 20 mW pulse of a pulsed UV (355 nm) laser (DPSS Lasers Inc.) through a 0.16 NA 4 × objective (Olympus). 25 mW laser pulses were used for stimulating cortical neurons in mice older than P30 to maintain their excitation at a similar level than in younger mice (Supplemental Fig. 2B). The stimulus pattern for mapping the corticostriatal projections was 464 positions spaced by 75 μm on a 29 × 16 grid (2.1 × 1.1 mm) over barrel cortex. The corticostriatal slice and the LSPS grid were oriented in such a way that layer 5a was laid out horizontally. UV stimuli were applied every 700 ms and their successive positions on the LSPS grid were such as to maximize the time between stimulations of neighboring sites. Electrophysiological traces consisted of 100 ms baseline, a 450 ms window followed by a -5 mV 100 ms test pulse. A minimum of 2 and up to 4 stimulations were performed at each site at several minutes intervals. Custom software for instrument control and acquisition (Suter et al. 2010) was written in Matlab (Mathworks). Excitation profiles of pyramidal neurons were generated under similar conditions except that cells were recorded in current-clamp mode and glutamate was uncaged on a smaller 8 × 8 grid covering their soma and dendrites (50 μm spacing; 350 × 350 μm). In a subset of L5a cell recordings, a 8 × 24 grid was used to stimulate up in L1 (50 μm spacing).

Analysis of LSPS data

Synaptic input maps of neurons were constructed by taking the peak amplitude of EPSCs detected in a 50 ms time window starting at stimulation onset for each position in the LSPS grid. Measures were averaged across repetitions of stimulations (2-4). The threshold for EPSC detection was 3 standard deviations from baseline, or 9.2 ± 0.4 pA. To disambiguate evoked responses from spontaneous activity, synaptic responses occurring less than 2 times across repetitions of maps were set to zero. Averaged maps were superimposed taking L5a as reference in the vertical axis and the junction of the GPe, dorsal striatum and internal capsule as reference in the horizontal axis (Ref_{hor}). In order to detect connectivity clusters and center of mass in the LSPS map, we used the binary version of the map ('connectivity map') reporting the location of connected and non-connected sites (i.e., yielding EPSCs or none in the recorded SPN). To detect clusters, the binary map was collapsed along its vertical axis. A cluster comprised 1 or more consecutive connected sites that was framed by 1 or more non-connected sites. Thus, a cluster here may include connected sites that were not adjacent on the vertical axis in the original map, and may combine synaptic inputs from different layers. The number of cortical columns was estimated based on the number of consecutive connected sites (ConsSites) in the collapsed map and the mean width of a barrel in the slice, 150 μm, which is equal to 2 connected sites in the map.

Hence, the number of cortical columns = $\Sigma (\text{ceiling}(\text{ConsSites}/2))$. The connectivity center of mass was computed based on the uncollapsed map as follows : $\Sigma (\Sigma_{\text{vert}} \text{ connected sites} \times \text{lateral distance from Ref}_{\text{hor}}) / \Sigma (\Sigma_{\text{vert}} \text{ connected sites})$. The synaptic input center of mass was computed based on the original map, after thresholding, as follows: $\Sigma (\text{mean}_{\text{vert}} \text{ EPSC} \times \text{lateral distance from Ref}_{\text{hor}}) / \Sigma (\text{mean}_{\text{vert}} \text{ EPSC})$. Traces from current clamp recordings were analyzed to count the number of action potentials (APs) elicited upon glutamate uncaging. In the 8 × 8 grid, 50 μm spacing, total number of spikes was 2.2 ± 0.2 for L2/3 pyramidal cells (n = 26), 5.4 ± 0.6 for regular spiking (RS) pyramidal cells in L5a (n = 30) and 9.9 ± 1.6 for non-RS pyramidal cells in L5b (n = 10). The number of spikes elicited by uncaging at single sites was 1 or 2 and on average: 1.04 ± 0.04 for L2/3 cells, 1.03 ± 0.02 for L5 RS cells and 1.04 ± 0.02 for L5 non-RS cells. As spacing in the stimulation grid was 50 μm in these particular experiments, we used the following equation to compute the number of sites with AP (sAP) in a stimulation grid with a spacing of 75 μm (i.e. as in connectivity maps): $\text{sAP}_{75} = \text{sAP}_{50} \times (75/50)^2$. To estimate the loss of S1 synaptic contacts caused by slice preparation, we modeled the SPN dendritic field as a sphere centered on the soma. S1 synapses were at 80 %

distributed radially along dendrites, according to the specific distribution described by Sanabria et al. (2024) [\[1\]](#). The simulation also incorporated the known distribution of SPN dendritic length as a function of distance from the soma (Gertler et al., 2008 [\[2\]](#)). Finally, it assumed that synapse placement was isotropic, with equal probability in all directions from the soma. Truncation was simulated by removing a spherical cap at one pole of the sphere, reflecting the depth of our recordings (beyond 80 μm). Based on this simulation, the loss of S1 inputs was $< 10\%$.

Labeling of cortical cells

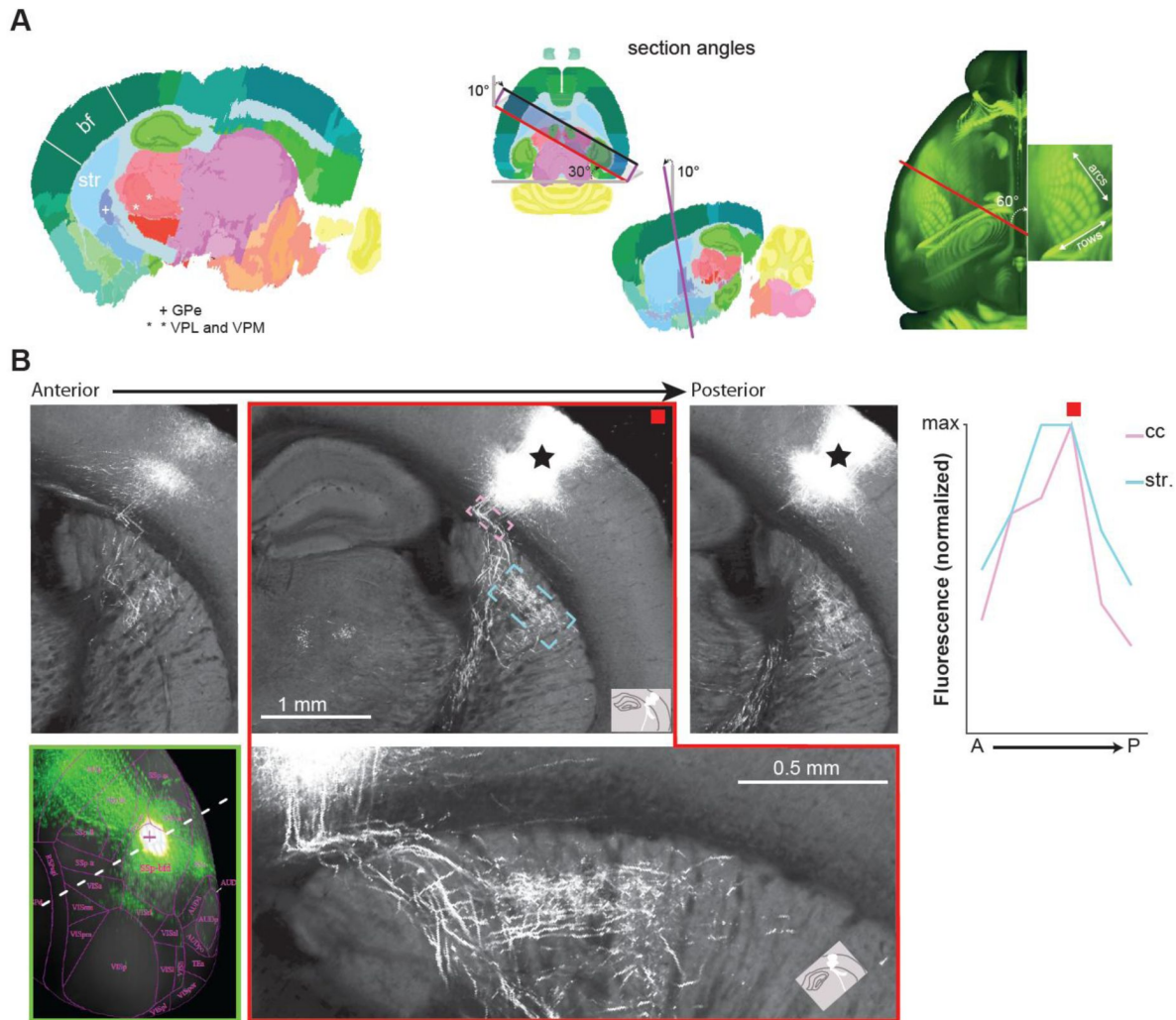
In a somatosensory corticostriatal slice prepared as described above, biocytin (2 %) was injected by iontophoresis in the L5a through a patch electrode (200 ms, 2.5 Hz) for 2 x 10 minutes (Chang, LoTurco and Nisenbaum, 2000 [\[3\]](#)). The slice was incubated for 4 hours at room temperature in ACSF and transferred to 4 % paraformaldehyde at 4°C overnight. It was rinsed in PBS containing 0.3 % Triton X-100 (PBS-T), incubated overnight at room temperature in streptavidin-AlexaFluor488 (1:1000 in PBS-T) and mounted using Vectashield mount medium. Image acquisition was performed using a LSM 800 confocal Zeiss microscope.

Statistical analysis

All data are expressed as mean $\pm$ SEM in the text. Median and 25-75th percentiles are shown in Figures. N and n are the numbers of animals and neurons, respectively. Unless stated otherwise, paired or unpaired non parametric tests, and Kruskal-Wallis test for repeated measures followed by Dunn post-hoc test and Šidák correction for multiple comparisons were used. $p < 0.05$ is considered as statistically significant.

Acknowledgements

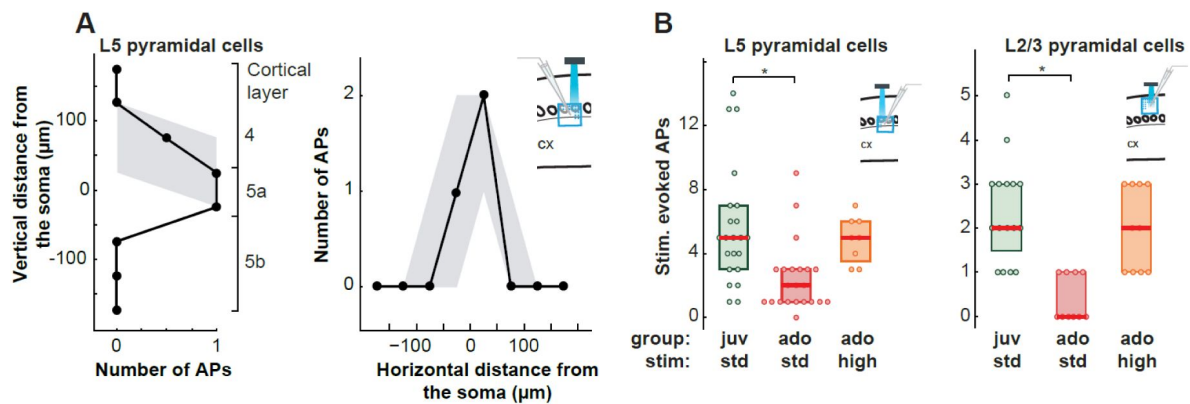
This work was supported by funding from the Institut National de la Santé et de la Recherche Médicale and grants from the Agence Nationale de la Recherche (Corticostriatal, ANR-20-CE16-0002; STEP, ANR-20-CE17-0025-01). K. Amroune is supported by fellowships from the Ministère de l'Enseignement Supérieur et de la Recherche, from the governmental "France 2030" program via A*Midex (Initiative d'Excellence d'Aix-Marseille Université, AMX-19-IET-004) and by ANR funding (ANR-17-EURE-0029). L. Fontolan is supported by funding from Excellence Initiative of Aix Marseille Université - A*MIDEX (Turing Centre for Living Systems). We thank the staff of the animal and genotyping facilities, the histology and imaging platforms of INMED, the members of the CBGB group and Antoine Depaulis for their help and support. We thank Elodie Fino, Rosa Cossart, Ede Rancz, Roustem Khazipov, Thomas Morvan, Jean-Luc Gaiarsa, Corinne Beurrier and Lydia Kerkerian Le Goff for their critical reading of the manuscript.



Supplemental Figure 1.

A. Left, the corticostriatal slice generated from the Allen mouse brain reference atlas (CutNII custom-angle slice visualization tool; G. Csucs). bf, barrel field. str, striatum. VPL, ventral posterolateral thalamic nucleus. VPM, ventral posteromedial thalamic nucleus. Center, the section angles illustrated on horizontal and longitudinal sections of the brain. Right, the section angle with respect to the orientations of the arcs and rows in the barrel field of the left hemisphere (Allen mouse brain atlas).

B. A simulated somatosensory corticostriatal slice generated using data from the Allen mouse connectivity atlas (https://connectivity.brain-map.org/projection/experiment/cortical_mp/293728197). eGFP was expressed in L2/3 and L5 pyramidal cells principally, in 3-4 arcs of the D and E rows of whisker columns (whole brain visualization in the green box at the bottom left; the dashed line indicates the slice orientation). Acquisitions of serial two-photon tomography were stacked in Fiji and the reconstituted brain was sectioned using the angles to produce the acute somatosensory corticostriatal slices (BigDataViewer plugging). The red box identifies the "experimental slice" with intact projections from the barrel cortex to the striatum. Fluorescence in the striatum (blue) and in the corpus callosum (cc, pink) was quantified on consecutive max projections (300 μ m thick) obtained in the anterior-posterior axis (A-P). Note that slices, being from the opposite hemisphere to our experiments, have the reverse orientation in the medio-lateral axis compared to **Fig. 2A,C**.



Supplemental Figure 2.

A. Vertical (left) and horizontal (right) excitation profile of L5 pyramidal cells recorded in current clamp, in the juveniles and adolescents combined (20 and 25 mW stimulation, respectively). Evoked action potentials (APs) were summed along each column (left) or line (right) of the LSPS grid (50 μm spacing). Solid line, median. In gray, 25-75th percentiles. $n = 30$, $N = 14$.

B. Total number of APs evoked in the stimulation grid for L5 (left) and L2/3 (right) pyramidal cells in juvenile (juv, P22-30) and adolescent (ado, P31-41) mice. A higher stimulation intensity was used for adolescent mice (25 mW instead of the standard -std -intensity, 20 mW), so that the total number of APs matched between the two age groups. L5, juv, $n = 22$, $N = 9$; ado std, $n = 22$, $N = 13$; ado high: $n = 8$, $N = 5$; L2/3, juv: $n = 16$, $N = 3$; ado std and high $n = 10$, $N = 2$; * $p < 0.001$, Mann-Whitney.

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Author information

Kenza Amroune

Aix-Marseille Université, INSERM, INMED, Marseille, France
ORCID iD: [0009-0004-4483-4741](#)

Lorenzo Fontolan

Aix-Marseille Université, INSERM, INMED, Turing Centre for Living Systems, Marseille, France
ORCID iD: [0000-0002-1566-6636](#)

Agnès Baude

Aix-Marseille Université, INSERM, INMED, Marseille, France
ORCID iD: [0000-0002-7025-364X](#)

David Robbe

Aix-Marseille Université, INSERM, INMED, Turing Centre for Living Systems, Marseille, France
ORCID iD: [0000-0002-9450-0553](#)

Ingrid Bureau

Aix-Marseille Université, INSERM, INMED, Marseille, France
ORCID iD: [0000-0001-9166-2250](https://orcid.org/0000-0001-9166-2250)

For correspondence: ingrid.bureau@inserm.fr

Editors

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Reviewer #1 (Public review):

Summary:

By applying the laser scanning photostimulation (LSPS) approach to a novel slice preparation, the authors aimed to study the degree of convergence and divergence of cortical inputs to individual striatal projection neurons (SPNs).

Strengths:

The experiments were well-designed and conducted, and data analysis was thorough. The manuscript was well written and related work in the literature was properly discussed. This work has the potential to advance our understanding of how sensory inputs are integrated into the striatal circuits.

<https://doi.org/10.7554/eLife.106621.2.sa3>

Reviewer #2 (Public review):

Summary:

How corticostriatal synaptic connectivity gives rise to SPN encoding of sensory information is an important and currently unanswered question. The authors utilize a clever slice preparation in combination with electrophysiology and glutamate uncaging to dissect the synaptic connectivity between barrel cortex and individual striatal SPNs. In addition to mapping connectivity across major anatomical axes and cortical layers, the authors provide data showing that SPNs uniquely integrate sparse input from variable stretches across barrel cortex.

Strengths:

The methodology shows impressive rigor and the data robustly support the authors conclusions. Overall, the manuscript addresses its core question, provides valuable insights into corticostriatal architecture, and is a welcomed addition to the field.

<https://doi.org/10.7554/eLife.106621.2.sa2>

Reviewer #3 (Public review):

Summary:

The authors explored how individual dorsolateral striatum (DLS) spiny projection neurons (SPNs) receive functional input from whisker-related cortical columns. The authors developed and validated a novel slice preparation and method to which they applied rigorous functional mapping and thorough analysis. They found that individual SPNs were driven by sparse, scattered cortical clusters. Interestingly, while the cortical input fields of nearby SPNs had some degree of overlap, connectivity per SPN was largely distinct. Despite sparse, heterogeneous connectivity, topographical organization was identified. The authors lastly compared direct (D1) vs. indirect (D2) pathway cells, concluding that overall connectivity patterns were the same, but D1 cells received stronger input from L6 and D2 cells from L2/3. The paper thoughtfully addresses the question of whether barrel cortex broadly or selectively innervates SPNs. Their results indicate selective input that is loosely topographic. Their work deepens the understanding of how whisker-related somatosensory signals can drive striatal neurons.

Strengths:

Overall this is a carefully conducted study, and the major claims are well-supported. The use of a novel ex vivo slice prep that keeps relevant corticostriatal projections intact allows for careful mapping of the barrel cortex to dorsolateral striatum SPNs. Careful reporting of both columnar and layer position, as well as postsynaptic SPN type (D1 or D2) allows the authors to uncover novel details about how the dorsolateral striatum represents whisker-related sensory information.

Weaknesses:

Most technical weaknesses have now been addressed in the text.

<https://doi.org/10.7554/eLife.106621.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Reviewer #1 (Public review):

This work focuses on the connection strength of the corticostriatal projections, without considering the involvement of synaptic plasticity in sensory integration.

Thank you for raising this point. Indeed, sensory integration is a complex process with a multitude of factors beyond connectivity patterns and synaptic strength. In addition, it is true that both connectivity levels and synaptic strength can be modified by plasticity.

We modified our conclusion as follows, line 354:

“Since the inputs to a single SPN represent only a limited subset of whisker columns, a complete representation of whiskers could emerge at the population level, with each SPN's representation complementing those of its neighbors (Fig. 7). These observations raise the hypothesis of a selective or competitive process underlying the formation of corticostriatal synapses. The degree of input convergence onto SPNs could be modulated by plasticity, potentially enabling experience-driven reconfiguration of S1 corticostriatal coupling. “

Reviewer #2 (Public review):

A few minor changes to the figures and text could be made to improve clarity.

We thank you for having taken the time to indicate where changes could benefit the paper. We followed your recommendations.

Reviewer #3 (Public review):

(1) Several factors may contribute to an underestimation of barrel cortex inputs to SPNs (and thus an overestimate of the input heterogeneity among SPNs). First, by virtue of the experiments being performed in an acute slice prep, it is probable that portions of recorded SPN dendritic trees have been dissected (in an operationally consistent anatomical orientation). If afferents happen to systematically target the rostral/caudal projections of SPN dendritic fields, these inputs could be missed. Similarly, the dendritic locations of presynaptic cortical inputs remain unknown (e.g., do some inputs preferentially target distal vs proximal dendritic positions?). As synaptic connectivity was inferred from somatic recordings, it's likely that inputs targeting the proximal dendritic arbor are the ones most efficiently detected. Mapping the dendritic organization of synapses is beyond the scope of this work, but these points could be broached in the text.

Thank you for this analysis. The positions of S1 spines have been mapped on the SPN dendritic arbor by the group of Margolis (B.D. Sanabria et al., ENeuro 2024,10.1523/ENEURO.0503-23.2023). They observed that S1 spines were at 80 % on dendrites but with a specific distribution, on average rather close to the soma. In this study, S1 spines did not exhibit a specific distribution that would systematically hinder their detection in a slice. But, it remains that the position in the dendritic arbor where an S1 input is received does indeed impact its detection in somatic recordings. We modified the discussion as follows, line 275:

“The LSPS combined with glutamate uncaging mapped projections contained in the slice, intact from the presynaptic cell bodies to the SPN dendrites. Some cortical inputs targeting distal SPN dendrites may have gone undetected, either due to attenuation of synaptic events recorded at the soma or because distal dendritic branches were lost during slice preparation. Indeed, about 80 % of S1 synaptic contacts are distributed along dendrites (Sanabria et al., 2024). However, synapses located distally are proportionally rare (Sanabria et al., 2024), and our estimates suggest that the loss of S1 input was minimal (see Methods). More significantly, our mapping only included projections from neuronal somata located within the S1 barrel field in the slice: projections from cortical columns outside the slice were not stimulated. For this reason, our study characterized connectivity patterns rather than the full extent of connectivity with the barrel cortex.”

We explain our estimation of truncated S1 contacts in the Methods, line 434:

“To estimate the loss of S1 synaptic contacts caused by slice preparation, we modeled the SPN dendritic field as a sphere centered on the soma. S1 synapses were at 80 % distributed radially along dendrites, according to the specific distribution described by Sanabria et al. (2024). The simulation also incorporated the known distribution of SPN dendritic length as a function of distance from the soma (Gertler et al., 2008). Finally, it assumed that synapse placement was isotropic, with equal probability in all directions from the soma. Truncation was simulated by removing a spherical cap at one pole of the sphere, reflecting the depth of our recordings (beyond 80 μ m). Based on this simulation, the loss of S1 inputs was < 10 %.”

(2) In general, how specific (or generalizable) is the observed SPN-specific convergence of cortical barrel cortex projections in the dorsolateral striatum? In other words, does a

similar cortical stimulation protocol targeted to a non-barrel sensory (or motor) cortex region produce similar SPN-specific innervation patterns in the dorsolateral striatum?

This is an interesting question that could be addressed using the LSPS approach in areas for which ex vivo preparations have been designed to maintain the integrity of the corticostriatal projections, such as A1, M1 and S2.

We included this point in the discussion, line 299:

” The speckled connectivity pattern of individual SPNs, arising from the abundant and diffuse cortical innervation in the DLS, suggests that somatosensory corticostriatal synapses are established through a selective and/or competitive process. It is important to determine whether this sparse innervation of SPNs by S1 is a characteristic shared with other projections. In particular, it will be interesting to test this hypothesis on the auditory projections targeting the posterior striatum, where neurons exhibit clear tone frequency selectivity (Guo et al., 2018).”

(3) In general, some of the figure legends are extremely brief, making many details difficult to infer. Similarly, some statistical analyses were either not carried out or not consistently reported.

We thank you for having taken the time to indicate where changes could benefit the paper. We have followed your recommendations.

Reviewer #1 (Recommendations for the authors):

A few limitations should be discussed in the manuscript:

(1) The manuscript should mention that most corticostriatal synapses are formed at the dendritic spines of the SPNs, not their cell bodies. This is particularly important regarding the analysis and interpretation of the data in Figure 4.

Thank you for this comment. This characteristic is important with regards to a limitation of electrophysiological recordings. This is now discussed:

Line 275:

“The LSPS combined with glutamate uncaging mapped projections contained in the slice, intact from the presynaptic cell bodies to the SPN dendrites. Some cortical inputs targeting distal SPN dendrites may have gone undetected, either due to attenuation of synaptic events recorded at the soma or because distal dendritic branches were lost during slice preparation. Indeed, about 80 % of S1 synaptic contacts are distributed along dendrites (Sanabria et al., 2024). However, synapses located distally are proportionally rare (Sanabria et al., 2024), and our estimates suggest that the loss of S1 input was minimal (see Methods).“

Line 313:

[...], we found that overlaps between the connectivity maps of SPNs were rare and, when present, involved only a small fraction of the connected sites. This indicates that neighboring SPNs predominantly integrated distinct inputs from the barrel cortex, although it is possible that overlapping inputs received in distal dendrites were not all detected”

(1) SPNs show up- and down-states in vivo, which were not mimicked by the present study since all cells were held at - 80 mV (Line 364) and recorded at room temperature (Line 368). It should be discussed how the conclusion of the present work may be affected by the up/down states of SPNs in vivo.

Thank you for raising this point. Indeed, our experimental conditions were not designed to capture the effects of network oscillatory activity. Instead, LSPS conditions were optimized to reveal monosynaptic connectivity between neurons in S1 and their postsynaptic targets. These optimizations include the use of a high concentration of extracellular divalents (4 mM Ca^{2+} and Mg^{2+}) to generate robust yet moderate and spatially-restricted stimulations of cortical cells and reliable neurotransmitter release (Shepherd, Pologruto and Svoboda, *Neuron* 2003; 10.1016/s0896-6273(03)00152-1; in our study, see Fig. 1D and Suppl Fig. 2). Investigating the pre- and postsynaptic modulations of the corticostriatal coupling by up- and down-states would require specific conditions.

The conclusion now acknowledges that functional connectivity is subject to plasticity in general, line 358:

“The degree of input convergence onto SPNs could be modulated by plasticity, potentially enabling experience-driven reconfiguration of S1 corticostriatal coupling.”

(2) In addition to population-level integration (Line 337), sensory integration is likely to involve synaptic plasticity (like via NMDARs), which was not studied in the present work

Thank you for raising this point. Indeed, we agree that sensory integration is a complex process with a multitude of factors beyond connectivity patterns and synaptic strength. We also agree that both connectivity levels and synaptic strength can be modified by plasticity.

We modified our conclusion as follows, line 354:

“Since the inputs to a single SPN represent only a limited subset of whisker columns, a complete representation of whiskers could emerge at the population level, with each SPN’s representation complementing those of its neighbors (Fig. 7). These observations raise the hypothesis of a selective or competitive process underlying the formation of corticostriatal synapses. The degree of input convergence onto SPNs could be modulated by plasticity, potentially enabling experience-driven reconfiguration of S1 corticostriatal coupling. “

(3) The potential corticostriatal connectivity may be underestimated due to loss of axonal branches during slice resection, and this might contribute to the conclusion of "sparse connectivity". Whether the author has considered performing LSPS studies within the striatum (i.e., stimulating ChR2-expressing cortical axon terminals) and whether this experiment may consolidate the conclusion of the present work.

We appreciate the suggestion to employ Subcellular Channelrhodopsin-2-Assisted Circuit Mapping (sCRACM) to study the density of S1 spines on SPNs dendritic arbor. If ChR2 is broadly expressed in S1, this approach would likely increase spine detection, as spines contacted by presynaptic neurons located inside and outside the slice would now be activated. If ChR2 expression could be restricted to the whisker columns present in our preparation, enhanced detection could still occur, but in this case, it would reflect the activation of spines contacted by specific ChR2^{+} axonal branches that exit and re-enter the slice to form synapses on the recorded SPN. The anatomy of corticostriatal axonal arbors suggest convoluted axonal trajectories could be relatively rare (T. Zheng and C.J. Wilson, *J Neurophysiol.* 2001; 10.1152/jn.00519.2001; M. Lévesque et al., *Brain Res.* 1996; 10.1016/0006-8993(95)01333-4).

Moreover, it is important to remember that sCRACM does not generate connectivity maps between 2 structures, but maps of spines on dendritic arbors (Petreanu L.T. et al., *Nature* 2009; 10.1038/nature07709.). Precise localization of presynaptic cell bodies was key for the present study, as it enabled distinguishing between different connectivity patterns and between different degrees of convergence of inputs from adjacent S1 cortical columns

present in the slice (schematized in Fig. 1). Distinguishing these inputs using the stimulation of axon terminals would require the possibility to express one distinct opsin in each whisker column (or each cortical layer, depending on the axis of investigation). This is an exciting perspective but the technology is not yet available to our knowledge.

To emphasize our reasons for using LSPS, we revised the final paragraph of the Introduction, line 69:

“LSPS enabled precise mapping of corticostriatal functional connectivity by identifying cortical sites where stimulation evoked synaptic currents in the recorded SPNs, thereby localizing the cell bodies of their presynaptic neurons. This approach allowed us to determine both the cortical column and layer of origin within the barrel field in the slice for each SPN input.”

Reviewer #2 (Recommendations for the authors):

(1) Figure 2F: SPN and cortical regions - both are shown in green. The distinction between the two would be clearer if SPNs were made a different color.

Done

(2) Figure 2H: Based on their data, the authors conclude that since EPSCs in SPNs had small amplitudes (~40pA), only one or a few presynaptic cortical neurons (< 5) were activated by uncaging. It is not clear how this number was estimated. Either this statement should be qualified with data or citations provided to support it.

We thank you for noticing it. We modified this part as follows, line 105:

“Based on known amplitudes of spontaneous and miniature EPSCs in SPNs (10-20 pA on average; Kreitzer and Malenka, 2007; Cepeda et al., 2008; Dehorter et al., 2011; Peixoto et al., 2016), this finding is consistent with the presence of only one or a few presynaptic cells (≤ 5) at each connected site of the map.”

(3) Figure 2I: The top graph is difficult to understand without already seeing the lower plot. Moving it below or to the side would help the reader follow the data more easily.

done

(4) Figure 3D: In Line 162, the authors state, " Furthermore, SPNs receiving input from a single column were often located near others receiving input from multiple ones (Figure 3D), reinforcing that the low functional connectivity with barrel columns in the slice was genuine in these cases." However, Figure 3D does not show spatial information about SPNs relative to each other. This data should be added or the statement adjusted to reflect what is shown in the panel.

Corrected as follows, line 167:

“Furthermore, SPNs receiving input from a single column were often located in slices where other cells received input from multiple ones (Fig. 3D), reinforcing that the low functional connectivity with barrel columns in the slice was genuine in these cases.”

(5) Figure 3F: Are the authors attempting to show how cluster number, cluster width, and connectivity gaps contribute to input field width? If so, this could be clarified by flipping the x- and y-axes so that the input field width is the y-axis in each case. Additionally, the difference between black and white points should be stated (or, if there is no difference,

made to be the same). The significance of the dotted red line vs. the solid red lines should also be stated in the figure legend.

These plots illustrate how cluster number, cluster width, and ratio of connectivity gaps over total length vary as a function of input field width. As expected, wider input fields contain more clusters (top). However, the overall density of connected sites does not increase with input field width, as indicated by a higher ratio of connectivity gaps over total length (bottom).

This suggests the presence of a mechanism that regulates the connectivity level of individual SPNs (mentioned in the discussion). We prefer this orientation because the flipped one makes a cluttered panel due to different X axis labels. Symbols and lines were corrected. The correlation coefficients and statistics are now indicated in the panels and in the legend.

(6) Figure 3H: The schematic is very useful for highlighting the core conclusions and is greatly appreciated. The pie charts are a bit hard to see and could be replaced with the percentages stated simply as text within the figure. It would also help to label the panel as "Summary," so readers can quickly identify its purpose.

Done

(7) Figures 4B-D: To clarify the overall percentage, the maximum for the y-axis should be set to 100% in each panel.

Done

Reviewer #3 (Recommendations for the authors):

(1) Though mostly minor, several sentences/statements in the manuscript are confusing or overstated. For example:

a. Lines 62-63: "Studies have found that inputs received by D1 SPNs were stronger than those received by D2 SPNs" is a broad statement that should be qualified.

We changed this sentence for:

“Electrophysiological studies have found that inputs received by D1 SPNs were stronger than those received by D2 SPNs, both in vivo and ex vivo (Reig and Silberberg, 2014 ; Filipović et al., 2019 ; Kress et al., 2013 ; Parker et al., 2016).”

b. Lines 118-119: "EPSCs evoked with stimulations in L2/3 to L5b had similar amplitudes (Figure 2H), suggesting that L5a dominated these other layers thanks to a greater connectivity with SPNs principally." Here, the word "connectivity" is vague and could easily be misunderstood. Connectivity could refer to the amplitude of corticostriatal EPSCs, which the authors stated are not different between L2/3-L5b. Presumably, connectivity here refers to % of connected SPNs, but for the sake of clarity, the authors should be more explicit, e.g., "...L5a dominated the other layers because a larger fraction of SPNs received connections from L5a, rather than because L5a synapses were stronger."

We changed the sentence for (line 122):

“EPSCs evoked with stimulations in L2/3 to L5b had similar amplitudes (Fig. 2H), suggesting that L5a dominance over these other layers is primarily due to a higher likelihood of SPNs being connected to it, rather than to stronger synaptic inputs.”

c. In the Figure 4 legend, (A) says "Four example slices with 2 to 4 recordings. Same as in Figure 2A." Did the authors mean Figure 3A?

Done

d. Line 184: Should Figure 4B, C actually be Figure 4D?

Done

(2) Line 32: typo in Sippy et al. reference.

Done

(3) In Figure 2I, the label "dSPN" is confusing, as in the literature, dSPN often refers to the direct pathway SPN.

Done

(4) The y-axes in Figure 3C should be better labeled/explained.

Fig.3C. Median (red) and 25-75th percentiles (box) of cluster width and spacing, expressed in μm (left Y axis) and number of cortical columns (right Y axis). Labels have been changed in the figure.

(5) Lines 150-152: "...45 % of the input fields with several clusters produced no synaptic response upon stimulation." This wording is confusing. It can be inferred that the authors mean "no synaptic response in the gaps between clusters." However, their phrasing omits this crucial detail and reads as though those input fields produce no response at all.

We changed this sentence for (line 154):

"Strikingly, regions lacking evoked synaptic responses (i.e., connectivity gaps) made up an average of 45 % of the length of input fields with multiple clusters (maps collapsed along the vertical axis; Fig. 3F, bottom). "

(6) Lines 184-186: "DLS SPNs could receive inputs from the same domain in the barrel cortex and yet have patterns of cortical innervation without or little redundancy." This should be rephrased to "with little to no redundancy."

Done

(7) Lines 186-187: "They support a connectivity model in which synaptic connections on each SPNs..." should be revised to "connections to each SPN..."

Done

<https://doi.org/10.7554/eLife.106621.2.sa0>