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Title

Quantitative Analyses of the Projection of individual Neurons from the Midline Thalamic Nuclei to the Striosome and Matrix Compartments of the Rat Striatum

Tomo Unzai^{1,2,3}, Eriko Kuramoto^{2,4}, Takeshi Kaneko², and Fumino Fujiyama^{1,3*}

¹Laboratory of Neural Circuitry, Graduate School of Brain Science, Doshisha University, Kyoto 619-0225, Japan

²Department of Morphological Brain Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

³Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo 102-0076, Japan

⁴Department of Oral Anatomy and Cell Biology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan

***Correspondence to:** Prof. Fumino Fujiyama

Laboratory of Neural Circuitry

Graduate School of Brain Science, Doshisha University

Kyoto 619-0394, JAPAN

Tel. 81(Japan)-774-65-7887, Fax. 81-774-73-1931

e-mail: ffujiyam@mail.doshisha.ac.jp

Running title: Single thalamic neuron and striatal compartments.

Abstracts

A fundamental organizing principle of the striatum is the striosome/matrix system that is defined by inputs/outputs and neurochemical markers. The thalamostriatal projection is highly heterogeneous originating in many subnuclei of the thalamus including the midline (ML) and intralaminar (IL) nuclei. We examined the dendritic morphology and axonal trajectory of 15 ML and 11 IL neurons by single-neuron labeling with viral vectors in combination with mu-opioid receptor immunostaining in rat brains. Dendritic and axonal morphology defined ML neurons as type II cells consisting of at least two subclasses according to the presence or absence of striatal axon collaterals. In the striatum, ML neurons preferentially innervated striosomes, whereas parafascicular neurons preferentially innervated the matrix. Almost all single thalamostriatal neurons favoring striosome or matrix compartments also innervated the cerebral cortical areas that supplied cortical input to the same striatal compartment. We thus revealed that thalamostriatal projections are highly organized 1) by the similarity in morphological characteristics and 2) their preference for the striatal compartments and cortical areas. These findings demonstrate that the functional properties of striatal compartments are influenced by both their cortical and thalamic afferents presumably with a different time latency and support selective dynamics for the striosome and matrix compartments.

Keywords: anterograde tracer, basal ganglia, opioid receptor, single neuron tracing, thalamostriatal

Introduction

Midline (ML) and intralaminar thalamic nuclei (IL) send excitatory projections to striatal regions in mammalian brains (for review, cf. Bentivoglio et al. 1991; Groenewegen and Berendse 1994; Mengual et al. 1999; Van der Werf et al. 2002). The principal striatal target of ML neurons is the nucleus accumbens (NAc) and the olfactory tubercle (OT), whereas for IL neurons it is the caudate-putamen (CPu, dorsal striatum). In rodents, the IL consists of the central medial (CeM), central lateral (CL), paracentral (Pc), and parafascicular nuclei (Pf); the former three nuclei are collectively named “the rostral group of the IL (ILr)” in the present study. The ML is composed of the paraventricular (Pv), rhomboid (Rh), reuniens (Re), intermediodorsal (IMD), and paratenial nuclei (Pt), although the Rh and Pv are sometimes included in the IL and epithalamus, respectively (cf. Jones 2007).

The CPu has a mosaic organization consisting of matrix and striosome compartments (striosome compartment were often described as patch compartment in rodents; cf. Graybiel et al. 1981), distinguishable from each other by cortical input and striatofugal output organization (for review, cf. Gerfen 2004; Crittenden and Graybiel 2011). From previous studies at the level of thalamic nuclei, the situation appears similar for thalamostriatal afferents; IL neurons have been reported to send axons preferentially to the matrix compartment (Herkenham and Pert 1981; Sadikot et al. 1990, 1992; Ragsdale and Graybiel 1991). Furthermore, the striosome compartment in the cat has been reported to receive input from ML neurons located at the Pv and Rh (Ragsdale and Graybiel 1991), and the striosome-like compartment of rat NAc is innervated by the Pv (Berendse et al. 1988). Other reports have shown difference in synaptic organization of thalamostriatal neurons between the striosome and matrix compartments (Fujiyama et al. 2006; Raju et al. 2006) and differences in dendritic and axonal morphologies between CL and Pf neurons (Pinault 1996; Lacey et al. 2007). These suggest the subnuclei of the thalamus that give rise to thalamostriatal projections are heterogeneous with respect to the striosome and matrix organization; however, how single neurons relate to this organization is lacking.

To precisely understand how the striatal compartments are driven by the thalamostriatal projections, it is necessary to know whether the information of a single

thalamic neuron is transferred to the matrix, to the striosome or to both compartments. However, only a few morphological studies of individual thalamostriatal axons using a small injection of anterograde tracer into the IL have been reported (Deschênes, Bourassa and Parent 1996; Deschênes, Bourassa and Doan et al. 1996; Parent and Parent 2005). Furthermore, in these studies, the axonal distribution was investigated without reference to the matrix and striosome compartments. Recently, we developed viral vectors expressing membrane-targeted fluorescent proteins, and applied them to single-neuron tracing (Kuramoto et al. 2009, 2015; Matsuda et al. 2009; Fujiyama et al. 2011; Ohno et al. 2012; Koshimizu et al. 2013). In the present study, the thalamostriatal projections of single ML neurons and single IL neurons were examined, particularly with respect to the striosome/matrix organization identified by immunoreactivity for mu-opioid receptor (MOR) (Arvidsson et al. 1995; Kaneko et al. 1995) and similarly for the striosome-like compartment in the NAc (Furuta et al. 2002; Zhou et al. 2004). Additionally, we investigated how the single thalamostriatal neurons innervated the cortical areas as well as the subcortical regions.

Materials and Methods

Animals

Adult male Wistar rats weighing 250–350 g were used in the present study. The experiments were conducted in accordance with the guidelines for animal care outlined by the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University that complied with the Society for Neuroscience policy on the use of animals in medical research. All efforts were made to minimize both the suffering and the number of animals used.

Injection of Sindbis Viral Vector and Fixation

Ninety-three rats were anesthetized by intraperitoneal injection of chloral hydrate (35 mg/100 g body weight). For single-neuron labeling, either non-proliferative Sindbis viral vectors expressing palmitoylation site-attached green fluorescent protein (palGFP; Furuta et al. 2001) or palmitoylation site-attached monomeric red fluorescent protein (pal-mRFP; Nishino et al. 2008) was used. A mixture of 200–400 infectious units of palGFP and pal-mRFP viral vectors in 0.2

μL of 5 mM sodium phosphate (pH 7.4)-buffered 0.9% saline (PBS) containing 0.5% bovine serum albumin (BSA) was injected bilaterally into the thalamic nuclei by pressure through a glass micropipette attached to a Picospritzer III (General Valve Corporation). The coordinates were as follows: Pv (2.8–4.0 mm posterior to the bregma, 0.3–0.5 mm lateral to the midline, and 5.2–5.8 mm deep from the brain surface), Rh (2.4–3.3 mm posterior, 0.3 mm lateral and 6.2–6.5 mm deep), Pt (1.6–2.6 mm posterior, 0.4–0.5 mm lateral and 4.8 mm deep), ILr (2.8–3.8 mm posterior, 0.3–0.5 mm lateral and 5.8–6.8 mm deep), or Pf of the thalamus (3.7–4.3 mm posterior, 1.2–1.4 mm lateral and 5.0–6.0 mm deep). After survival for 36–48 h, the rats were anesthetized with chloral hydrate (70 mg/100 g body weight) and perfused transcardially with 200 mL of PBS, followed by 200 mL of 4% formaldehyde and 75%-saturated picric acid in 0.1 M Na₂HPO₄ (adjusted using NaOH to pH 7.0). The brains were then removed, cut at the midline into 2 hemispheric blocks, and postfixed for 4 h at 4°C with the same fixative. After cryoprotection with 30% sucrose in PBS, the blocks were cut into 40-μm-thick parasagittal sections on a freezing microtome collected serially in PBS.

Characterization of palGFP- or pal-mRFP-Expressing Thalamic Neurons

The sections, including the injection site, were examined under an epifluorescent microscope, Axiophot (Zeiss), to identify the infected thalamic neurons. The following incubations were all performed at room temperature and followed by a rinse with PBS containing 0.3% Triton X-100 and 0.02% merthiolate (PBS-X). The sections containing palGFP-positive thalamic neurons were incubated for 1 h with 2 μg/mL of propidium iodide in PBS-X, and the sections containing pal-mRFP-positive thalamic neurons were incubated for 1 h with a 1:200-dilution of NeuroTrace 500/525 green fluorescent Nissl stain (Invitrogen) in PBS-X. Under the epifluorescent microscope, the location of the labeled neurons was determined in fluorescent Nissl-like cytoarchitecture with reference to the atlas of Paxinos and Watson (Paxinos and Watson 2007).

Double Immunoperoxidase Staining for either GFP or mRFP and MOR

All the serial sections in the hemisphere of Sindbis virus-injected rats that contained 4 or less

palGFP- or pal-mRFP neurons were incubated overnight with 0.5 µg/mL affinity-purified rabbit antibody against GFP or mRFP (see details in Tamamaki et al. 2000; Hioki et al. 2010) in PBS-X containing 0.12% lambda-carrageenan, 0.02% sodium azide, and 1% donkey serum (PBS-XCD). After a rinse with PBS-X, the sections were incubated overnight with 10 µg/mL biotinylated goat anti-rabbit IgG antibody (Vector) in PBS-XCD and then for 1 h in PBS-X with an avidin-biotin-peroxidase complex (ABC Elite, Vector). After a rinse with PBS, the bound peroxidase was developed to black reaction product by a 10–30 min incubation in 0.02% (w/v) diaminobenzidine-4HCl, 10 mM nickel-ammonium sulfate, and 0.0002% (v/v) H₂O₂ in 50 mM Tris-HCl, pH 7.6. The reaction was terminated by a rinse with PBS containing 2% (w/v) sodium azide, followed by 2 rinses in PBS. Subsequently, the sections were again incubated for an overnight period, with 0.5 µg/mL affinity-purified guinea pig antibody to MORs (Kaneko et al. 1995) in PBS-XCD. After a rinse with PBS-X, the sections were incubated for 1 h in PBS-XCD with 10 µg/ml of biotinylated goat anti-guinea pig IgG antibody (Vector) and then for 1 h in PBS-X with the avidin-biotin-peroxidase complex. Finally, the second bound peroxidase was revealed by a violet-red reaction product by incubation for 10–30 min with 0.01% (w/v) Tris-aminophenol methane (TAPM; Nacalai tesque), 0.07% (v/v) *p*-cresol, and 0.002% (v/v) H₂O₂ in 50 mM Tris-HCl, pH 7.6 (Kaneko et al. 1994). All the above incubations and reactions were performed at room temperature. The stained sections were serially mounted onto the gelatinized glass slides, dried, washed in running water, dried again, cleared in xylene, and finally coverslipped.

Reconstruction and Morphological Analysis of Single ML, ILr, and Pf neurons

The cell body, dendrites, and projecting axons of stained ML, ILr, and Pf neurons were reconstructed under a microscope with a camera lucida apparatus attached. After the reconstructed figures were captured by a digital scanner, they were retraced and digitized on a computer using the software CANVAS X (ACD Systems International, Inc.). The borders between the CPu and the core and shell portions of the NAc were determined by comparing the stained sections with a complete series of control parasagittal sections stained for Nissl with cresyl violet (Kuramoto et al., 2009) with reference to the atlas of Paxinos and Watson (2007).

The axons were also reconstructed section by section onto a parasagittal plane, and then superimposed in the computer. The fine morphological indices, such as the axon varicosities, were observed with a 40× objective lens (Plan; numerical aperture = 0.65; Nikon), which was attached to an Optiphot light microscope (Nikon).

Statistical Analysis

For the Steel-Dwass test (two-way analysis of variance), the software R (R Development Core Team) was used. For the Wilcoxon signed-rank test (one-way analysis of variance and Bonferroni multiple comparison test), the software GraphPad Prism (Graphpad Software, Inc.) was used.

Results

Technical Considerations

The palGFP- and pal-mRFP-Sindbis viral vectors were considered to have little morphological effect on the thalamic dendrites and axons. The survival time of the viral-injected rats was suitable for neuronal labeling and showed no degenerative changes (Furuta et al. 2001). In recent studies of single neuron tracing using the viral tracers, it was shown that neither filopodia-like nor growth cone-like structures were observed on the axon fibers and that there was little effect on the dendritic morphology (Kuramoto et al. 2009; Matsuda et al. 2009). No differences in the labeling produced by the pal-mRFP-Sindbis and the palGFP-Sindbis viral vectors were observed.

Selection of palGFP- or pal-mRFP-Labeled Neurons for Single-Neuron Tracing

A mixture of appropriately diluted palGFP- and pal-mRFP-expressing Sindbis viral vectors was injected bilaterally into the ML or IL of 93 rat brains (186 hemispheres). Neurons marked by fluorescence were found in 129 of the 186 hemispheres, of which 89 hemispheres contained <5 GFP-positive or mRFP-positive neurons in their thalamic nuclei. The parasagittal sections of the 89 hemispheres were stained with propidium iodide or NeuroTrace Green to reveal the location of the infected neurons in the cytoarchitecture of the thalamic nuclei (Fig. 1A–D).

Although we tried to inject viral vectors principally into the Pv and Rh of the ML, a few infected neurons were also found in the Re and IMD. We also injected into the Pt because this region has been shown to project to the ventral striatum in rats (Berendse and Groenewegen 1990).

After excluding the hemispheres that contained no ML or IL neurons, 34, 15, and 17 hemispheres containing ML, ILr, and Pf neurons, respectively, were immunostained for GFP or mRFP with the ABC method using the DAB/nickel peroxidase reaction. We further immunostained the sections for MOR using a TAPM/*p*-cresol peroxidase reaction (Fig. 1I–Q), and then mounted them serially. When >1 thalamostriatal projection neurons was labeled, the striatal sections were examined carefully for the intermingling of their axons. Excluding the cases with intermingled striatal axons or with incomplete axonal labeling, we isolated and reconstructed 15 ML, 6 ILr, and 5 Pf neurons in 13, 5, and 5 hemispheres, respectively (Table 1). The labeling of axons was considered incomplete when it faded away from the axon fibers in more distal portions, possibly due to delayed infection and insufficient production of membrane-targeted fluorescent protein. Axonal staining was judged complete when the ends of the axons in the striatum were clearly observed to have small terminal varicosities. The locations of the cell bodies of the reconstructed neurons were plotted onto the nearest parasagittal plane (Figure 1E–H).

Morphological Characteristics of Thalamostriatal Axons

Each axon fiber of the reconstructed neurons was clearly marked in black, and all the axon collaterals in the striatum were varicose (Fig. 1I–N). In previous studies (Deschênes, Bourassa and Parent 1995; Deschênes, Bourassa and Parent 1996; Lacey et al. 2007), IL neurons have been classified into at least 2 types according to the morphological differences of their dendrites and axons: 1) Type I cells in the CL had bushy dendrites and their thalamostriatal axons arborized loosely as long collaterals with many varicosities of the en passant type; 2) Type II cells in the Pf possessed long reticular-like dendrites and formed dense clusters/plexuses of axon varicosities containing many terminal boutons in the CPu. In the present study, axon varicosities or boutons were defined as localized swellings of axon fibers

with a diameter 1.5 times larger than the thickness of the inter-varicose axon fibers; and the intrastriatal axon collaterals of type I and type II thalamostriatal cells were named type I and type II axon fibers, respectively (Table 1). Intrastriatal axons of single ML and ILr neurons were primarily classified as type I axons (Fig. 1L, M), giving rise to many en passant boutons (black arrowheads in Fig. 1O, P), whereas the intrastriatal axons of many Pf neurons were classified as type II axons (Fig. 1N), forming multiple clusters of dense terminal boutons (bouton terminaux; white arrowheads in Fig. 1Q). According to the CPu results, type II axons of Pf neurons were found mostly in the matrix compartment, where MOR immunoreactivity was negative or very weak (designated as MOR(–) in the present study; Fig. 1K, N). This distribution and axon fiber type of the Pf neuron thalamostriatal projections in the matrix compartment are supported by previous reports of rat (Herkenham and Pert 1981; Deschênes, Bourassa and Doan et al. 1996; Lacey et al. 2007), cat (Ragsdale and Graybiel 1991), and monkey brains (Sadikot et al. 1990, 1992; Parent and Parent 2005). In contrast, type I axon fibers were distributed not only in the matrix but also in the striosome compartment, which was moderately to strongly immunopositive for MOR (MOR(+); Fig. 1 I, J, L, M).

Single-Neuron Tracing of ML Neurons

The thalamic neurons that were isolated and reconstructed are listed in Table 1. None of the neurons possessed axon collaterals within the thalamus except for the thalamic reticular nucleus (Rt) in which each neuron (except neuron 26) gave rise to a collateral with a few tens of varicosities. The thick non-varicose axons of the 15 ML neurons heading toward the forebrain passed through the internal capsule, frequently emitting axon collaterals to the striatum (12/15 neurons), and subsequently entered various cortical areas, including the medial prefrontal, orbitofrontal, and insular areas. Of the 15 ML neurons, 7 neurons gave rise to a large number of axon collaterals in the CPu and NAc (Table 1). Three gave rise to dense projections to the CPu (neurons 2, 6, and 12), 3 to the NAc (neurons 3, 13, and 15) and 1 to both regions (neuron 1) (Table 1).

Of the 5 Pv neurons, 2 gave rise to multiple collaterals innervating both the CPu and NAc, forming many varicosities of the en passant type (Type I; Fig. 2A–D). The other 3

neurons projected mainly to the NAc (Fig. 2E–H). Two of the Pv neurons gave rise to many collaterals in the multiform layer (layer III) of the OT (Fig. 2 C–F). One of the Pv neurons that gave rise to the fewest number of varicosities exclusively innervated the NAc and formed clusters/plexuses of axon varicosities containing many terminal boutons (Type II). The intrastriatal axon varicosities were distributed in both the MOR(+) striosome and MOR(–) matrix compartments of the CPu, or in both the MOR(+) and MOR(–) regions of the NAc (Fig. 2). The quantitative analysis of these distributions is described below.

Of the 5 labeled Rh neurons only one projected widely and en-masse to the CPu (neuron 6 in Table 1; Fig. 3A, B) and another neuron (neuron 8) gave rise to a few collaterals in CPu (Fig. 3C, D). The arborizations were of type I. One Re neuron (neuron 11) gave rise to a few collaterals in the CPu (Fig. 3E, F) whereas one of the IMD neurons (neuron 12) gave rise to a considerable number of type I collaterals widely distributed in matrix compartment of the CPu (Fig. 3G, H) to a greater extent than those of Rh neuron 6 (Fig. 3B). Three of the labeled Pt neurons gave rise to type I axonal arborizations mainly in the NAc and distributed in both the MOR(+) and MOR(–) regions (Fig. 4B, D, F). Axons of Pt neurons that innervated the CPu, they were located close to the border between the CPu and NAc (Fig. 4B, D, F). One Pt neuron (neuron 13) innervated the multiform layer of the OT (black circles in Fig. 4B).

Almost all the single ML neurons innervated the cerebral cortex together with the striatum. In most of the neurons, a thick main axon coursed within the internal capsule and targeted the cerebral cortex after emitting collaterals to the striatum (Table 1). Of notable exception, the corticopetal axon collaterals of Pv neuron 1 emerged from the intrastriatal axonal bush as a fine caliber axons (Fig. 2B). Considered together, the ML neurons appeared to direct their axons to the limbic cortical areas, particularly the prelimbic cortex. More explicitly, the majority of the main axons of the Pv and Pt neurons targeted the prelimbic, infralimbic, anterior cingulate, and insular areas (Table 1), whereas those of the Rh neurons targeted the orbital or insular areas. These thalamocortical fibers were mainly distributed in the middle to deep layers rather than in the superficial layers of the cerebral cortex (not shown). In addition to the striatum and cortical areas, many ML neurons (except the Rh neurons) projected to the basal forebrain and amygdaloid nuclear complex. With respect to the

subcortical regions, 4 neurons (neurons 2, 3, 12, and 14) projected to the ventral pallidum and 4 (neurons 2, 5, 13, and 14) to the sublenticular portion of the substantia innominata. All the Pv neurons (neurons 1–5; Table 1) projected to the interstitial nucleus of the posterior limb of the anterior commissure. Six neurons (1, 2, 4, 12, 13, and 15) sent axon fibers to the amygdaloid complex, 4 (neurons 4, 8–10) projected to the claustrum, and 3 (neurons 1, 5 and 10) innervated the endopiriform nucleus. One of the Pv neurons (neuron 5) projected heavily to the periaqueductal grey in the mesencephalon but not the cerebral cortex. These findings indicate that the target regions of the ML neurons are more diverse than those of the specific thalamic relay neurons, such as the neurons in the ventral lateral, ventral anterior, ventral medial, and posterior thalamic nuclei; the projections of which were found to be mostly limited to the cerebral cortex, striatum and thalamic reticular nucleus in previous single-neuron labeling studies (Kuramoto et al. 2009, 2015; Ohno et al. 2012). Our results revealed that the single ML neurons favoring the striosome compartment preferentially projected to the limbic cortex.

Single-Neuron Tracing of IL Neurons

The intrastriatal axon collaterals of 6 ILr and 5 Pf neurons were analysed at the single neuron level (Table 1). All except one IL neuron emitted axon collaterals in the striatum and innervated cortical areas, including motor, somatosensory, cingulate, retrosplenial, orbital, and/or insular areas.

The thalamostriatal axonal arborizations of 6 ILr neurons consisted of 1 CeM, 1 CL, and 4 Pc neurons. All emitted collaterals to the Rt and a variable number to the CPu before entering the cerebral cortex (Table 1). Three ILr neurons (neurons 16–18) sent a moderate to large number of axon collaterals widely to the CPu (Fig. 5), although the other three (Pc neurons 19–21) did not innervate the striatum or gave rise to a very small innervation. All the axon collaterals of the ILr neurons were of type I. The axonal arborization of CL neuron 17 (Fig. 5C, D) was denser than that of CeM neuron 16 (Fig. 5A, B) and Pc neuron 18 (Fig. 5E, F), but noticeably less dense than that of the Pf neurons (Fig. 6). The axon varicosities were distributed in both the compartments showing no preference for one or the other (Fig. 5B, D,

F). ILr neurons directed axon collaterals to the middle and deep layers of the motor, somatosensory, orbital, and insular areas in the cerebral cortex, particularly in the middle layer of the primary motor cortex. Interestingly, no ILr neurons projected to the ventral striatum, and 2 of 6 neurons innervated the ventral pallidum or amygdaloid complex.

All 5 of the Pf neurons projected to the CPu, and 4 of them (neurons 22–25) gave rise to clear type II collaterals (Fig. 6B, C, E, G). The axonal clusters were restricted to focal regions of the CPu, suggesting that they have selective excitatory effects within these focal regions. Furthermore, these type II axon fibers were distributed preferentially in the matrix compartment (Fig. 6), as reported previously (Herkenham and Pert 1981; Deschênes, Bourassa and Doan et al. 1996). One of the Pf neurons (neuron 26) differed from the others in that it had characteristics of type I cells of Deschênes and colleagues with bushy dendrites (Fig. 8) and a type I intrastriatal arborization (Table 1). It lacked axon collaterals in the Rt (Table 1) and innervated the ventral striatum as well as to the CPu. This neuron was located medially (Fig. 1F) and is thus consistent with previous findings that the ventral striatum often receives afferents from the medial portion of the Pf (Berendse and Groenewegen 1990; Otake and Nakamura 1998). The reconstructed Pf neurons projected to the middle and deep layers of the motor, somatosensory, insular, cingulate, prelimbic, and orbital areas. In addition, Pf neurons preferred to other basal ganglia regions, including the external segment of the globus pallidus, entopeduncular nucleus, subthalamic nucleus and substantia nigra, as reported in the previous single-neuron tracing study (Deschênes, Bourassa and Doan et al. 1996).

Quantitative Comparison of the Axon Varicosities of ML, ILr, and Pf neurons

The axon collaterals and varicosities of ML neurons showed a greater preference for the striosome compartment than ILr and Pf neurons (Figures 2–6; Table 2). Two-way ANOVA revealed a significant interaction between the thalamic nuclei (ML, ILr, or Pf) and striatal compartments ($p = 0.0013$). The striosome rate of each reconstructed neuron was defined by dividing the number of varicosities in the striosome compartment by the total number of varicosities in the CPu. The striosome rate of the ML neurons ranged from 7.3 to 73.3% with a mean $\pm$ SD of $47.9 \pm 21.3\%$ (Fig. 7A, B). Since the striosome compartment, including the

MOR(+) subcallosal streak, has been reported to occupy 11–15% of total striatal volume in the rat (relative striosome volume in Fig. 7B, C; Lança et al. 1986; Johnston et al. 1990; Desban et al. 1993; Wang et al. 2007), indicating that many ML neurons (other than Re and IMD neurons), preferentially innervate the striosomes. Furthermore, the striosome rate of the Pv and Rh neurons ranged from 46.1 to 73.3% (Mean $\pm$ SD, $58.0 \pm 10.6\%$), indicating a preferential innervation of striosomes as has been suggested in cats (Ragsdale and Graybiel 1991).

In contrast to the ML neurons, the striosome rate of ILr and Pf neurons was much smaller, 1.8–22.4% and 1.4–7.5% (Table 2) with a mean $\pm$ S.D. = $9.1 \pm 8.1\%$ and $4.8 \pm 2.3\%$, respectively, and the difference was statistically significant (Steel-Dwass test; Fig. 7B). Analysis of thalamostriatal neurons that gave rise to >500 axon varicosities in the dorsal striatum revealed that the mean striosome rate $\pm$ S.D. of the ML, ILr, or Pf neurons was $44.2 \pm 17.4\%$ ($n = 7$), $13.3 \pm 8.0\%$ ($n = 3$), or $4.8 \pm 2.3\%$ ($n = 5$), respectively. The difference between the ML and Pf neurons still remained statistically significant ($p = 0.02$). Finally, to evaluate the striosomal rate of the ML neurons as a neuron group, we calculated the cumulative striosome rate by dividing the summed number of intrastriosomal axon varicosities by the total number of intrastriatal varicosities of each neuron group (Fig. 7C). The cumulative striosome rate of the ML neurons was 35.4% and far beyond the range of the relative striosome volume (11–15%), whereas that of the Pf neurons was 4.5%, clearly below the range. Furthermore, for ILr neurons, 12.9% of their axon varicosities were located in the striosomes, which is within the range of the relative striosome volume, suggesting that they, as a group, had no preference for the matrix or striosome compartment.

Pv neurons 1–5 and Pt neurons 13–15 in the ML and Pf neuron 26 in the IL projected to the NAc. The NAc has striosome-like compartments that were moderately to intensely immunoreactive for MOR (detailed distribution is depicted in Figs. 8, 10, and 11 of Zhou et al. 2004). The MOR(+) region rates (equivalent to the ‘striosome rate’ for the dorsal striatum) of the 8 ML were 16.1–65.5% (mean $\pm$ SD, $37.9 \pm 15.7\%$) and that of the Pf neuron was 5.4%, similar to the striosome rate in the ML and Pf neurons. In addition, there was a positive correlation between the striosome rate and the MOR(+) region rate in the 7 ML and single Pf

neurons that projected to both the CPu and NAc (Wilcoxon signed-rank test, $r = 0.571$, $p = 0.0078$; Pearson's correlation coefficient $r = 0.803$), showing that the preference of a neuron for MOR-expressing region was similar for thalamo-CPu and thalamo-NAc projections.

The numbers of axon varicosities was further compared between the core and shell portions of the NAc (Table 3). Axon varicosities of 7 ML neurons were observed in the core portion and of which MOR(+) region rates ranged between 41.9 and 70.4%, whereas those of 8 ML and 1 Pf neurons were located in the shell portions with MOR(+) region rates of 5.4–63.4%. For the 7 ML neurons that projected to both the core and shell portions, the MOR(+) region rate of the axon varicosities ($53.4 \pm 15.9\%$) in the core region was significantly greater than that in the shell region ($30.3 \pm 17.2\%$; $p = 0.016$ by Wilcoxon signed-rank test). However, because the relative volume of the MOR(+) region was larger in the core than in the shell (Figs. 8, 10, and 11 of Zhou et al. 2004), this difference might just reflect the relative volume of the MOR(+) region across the core and shell of the NAc.

Dendrites and Cell Bodies of the ML, ILr, and Pf Neurons

All the reconstructed ML, ILr, and Pf neurons were multipolar neurons with many dendrites (Fig. 8). ILr neurons 16–21 showed many relatively short radiating dendrites, the field of which extended to ~ 300 – 400 μm in diameter. In contrast, Pf neurons 22–25 had fewer, though much more widely extended dendritic branches (≥ 500 μm) than those of the ILr neurons. The former ILr neurons were classified as “bushy” relay neurons, whereas the latter Pf neurons were the large “reticular-like” neurons, as reported previously (Deschênes, Bourassa and Parent 1995; Deschênes, Bourassa and Doan et al. 1996; Lacey et al 2007). It is important to note that Pf neuron 26 had bushy dendrites with larger numbers of arborization than the other Pf neurons, and projected type I axon fibers to the striatum (see also Beatty et al. 2009). The presence of a bushy Pf neuron in the present samples was consistent with a recent finding that about one third of neurons in the lateral Pf had bushy dendrites and emitted type I axons in the CPu, and the remaining two-thirds had reticular-like dendrites and projected type II axons (Beatty et al. 2009).

The dendrites of the ML neurons were somewhat less bushy and more widely spread

than those of the ILr neurons but were clearly not as “reticular-like” as Pf neurons 22–25. This observation was confirmed by the Sholl analysis (right lower corner of Fig. 8). The Re and Pt neurons (neurons 11, 13–15) tended to have wider dendritic arborization than the other ML neurons. Although Pv neuron 5 sent a small number of type II axons to the striatum, the dendrites were relatively short and different from Pf neurons 22–25. Thus, this neuron was not classified as either the type I or type II cell that described by Deschênes et al. No significant difference in the size of cell bodies was noticed among the ML (cross-sectional area = $262 \pm 87 \mu\text{m}^2$), ILr ($236 \pm 58 \mu\text{m}^2$), and Pf neuron groups ($285 \pm 97 \mu\text{m}^2$).

Discussion

The main finding of the present study is that the dendritic architecture of thalamostriatal neurons varies according to their location in agreement with previous findings. Our quantitative analyses reveal that *individual* ML thalamostriatal neurons project preferentially to the striosome compartment, whereas *individual* Pf thalamostriatal neurons project preferentially to the matrix compartment. The findings thus demonstrate that the functional properties of the striosome and matrix compartments are not only dependent on the neurochemical profiles of the two compartments, their input from different regions of the cortex and their efferent targets but also on their innervation by subnuclei of the ML and IL (Fig.9).

Thalamostriatal Projection of ML Neurons

The striatal target of ML neurons is often considered to be the ventral striatum, consisting of NAc and OT, rather than the CPu (for review, see Bentivoglio et al. 1991; Groenewegen and Berendse 1994; Van der Werf et al. 2002). However, in the present study, 11 of the 15 reconstructed ML neurons sent axon collaterals to the CPu, and 7 of them projected to the ventral striatum. Most of the reconstructed Pv and Pt neurons sent collaterals to the CPu as well as to the ventral striatum. Furthermore, no Rh, Re, or IMD neuron examined in the present study projected to the ventral striatum, although these nuclei were reported to contain neurons projecting to the ventral striatum (Groenewegen and Berendse 1994; Van der Werf et

al. 2002). In the sense of innervating patterns in the cortex and ventral striatum, ML could be divided into 2 groups. One group consisting Pv and Pt mainly projected to the limbic cortex and ventral striatum, whereas another group consisting of Rh, Re and IMD gave the projections to the orbital and motor cortex additionally but no projection to the ventral striatum (Table 1). We suppose that the proportion of the neurons projecting to the ventral striatum may be lower in the Rh, Re, and IMD than Pv and Pt. Therefore, no Rh, Re, and IMD neurons projecting to the ventral striatum were observed by the single-neuron tracing study using the viral vector.

The ML is divided into the dorsal and ventral groups; the dorsal group is composed of the Pv, Pt, and IMD, whereas the ventral group is composed of the Re and Rh (for review, see Groenewegen and Berendse 1994). In rats, the dorsal group is the main source of thalamostriatal projection of ML (Berendse and Groenewegen 1990; Su and Bentivoglio 1990; Pinto et al. 2003; Parsons et al. 2007), which is confirmed by the present finding in that practically all the neurons in the dorsal group sent axon collaterals to striatal regions. On the other hand, the ventral group reportedly emits only a few axons to the striatum, and projects mainly to the hippocampus and amygdala (Su and Bentivoglio 1990), but in the ventral group, however, Rh neurons send more axon fibers to the striatum than Re neurons (Ohtake and Yamada 1989; Vertes et al. 2006). Consistent with these findings, we found that only 3 of the 6 Rh and Re neurons projected to the striatum, and only one Rh neuron sent a large number of axon varicosities (≥ 500) to the striatum. The major targets of the other 5 Rh and Re neurons were cortical areas, particularly the limbic cortical areas (Table 1). Considered as a whole nuclei group, the role of ML is to innervate the striatal striosome compartment and limbic cortical areas. In the ML, the dorsal group preferentially projected to the striosome compartment, whereas the ventral group favored the limbic cortical areas.

Two Types of Thalamostriatal Neurons

Deschênes et al. described 2 different types of thalamostriatal neurons in the rat brain (Deschênes, Bourassa and Parent 1995; Deschênes, Bourassa and Parent 1996; Deschênes,

Bourassa and Doan et al. 1996): Type I cells are bushy relay cells found in the CL and associative thalamic nuclei, and their axons arborize sparsely in the CPu, with long varicose axon collaterals; Type II cells are large, reticular-like, relay neurons that are located in the Pf and ethmoid nucleus, with axon fibers forming dense clusters of terminations in the CPu. In addition to these morphological differences, these 2 types of neurons have been reported to have different electrophysiological properties, different synaptic connections as well as differences in the functional properties of their striatal synapses (Lacey et al. 2007; Ellender et al. 2013).

In the present study, all the neurons in the ILr were bushy relay cells and, except for neuron 21, all had relatively sparse arborizations of long varicose thalamostriatal fibers. In contrast, 4 of the 5 Pf neurons were type II cells, emitting long reticular-like dendrites and forming dense clusters of axonal terminations in the CPu. One of our Pf neurons (neuron 26) was classified as a type I cell; this is still consistent with previous reports as Beatty et al. described the presence of type I cells in the Pf (Beatty et al. 2009).

In contrast to the cytological heterogeneity of IL neurons, the ML neurons reconstructed in the present study were mostly classified as type I cells because they all had bushy dendrites, and except Pv neuron 5, also emitted type I axon collaterals in the CPu. ML neurons are thus rather homogeneous in terms of dendritic morphology and axon fiber types, although they can be divided into at least 2 subclasses according to the presence or absence of striatal axon collaterals.

Axonal Arborizations in Relation to the Striosome and Matrix Compartments

One of the main findings of the present study relates to the degree of innervation the striosome and matrix compartments by individual thalamostriatal neurons. We found that Pv neurons, and to a lesser extent, Rh and Pt neurons sent axon varicosities to the CPu with a clear preference for the striosome compartment. This is consistent with a study of the cat brain, where anterograde labeling from Pv and Rh led to preferential innervation of the striosome compartment (Ragsdale and Graybiel 1991). Furthermore, Pv, Rh, and Pt neurons preferentially innervated MOR(+) regions in the NAc. In contrast to the “striosome-

preferring” projections of Pv, Rh, and Pt neurons, we observed that the Re and IMD neurons did not show a preference for the striosome compartment, with a striosome rate for the Re or IMD neurons of 12.0 and 7.3%, respectively, that is, within or below the range of the relative striosome volume.

Neurons in cat and monkey center median nucleus-Pf complex (CM-Pf) almost selectively innervate the acetylcholinesterase-rich matrix compartment (Sadikot et al 1990, 1992; Ragsdale and Graybiel 1991). The CM-Pf mostly corresponds to the Pf in rat thalamic nuclei (for review, cf. Jones 2007), and rat Pf neurons have been revealed to project mainly to the acetylcholinesterase-rich and opiate binding-poor matrix compartment (Herkenham and Pert 1981). Thus, our findings of the axonal arborization of *individual* Pf neurons are consistent with the previous findings from anterograde labeling studies of rats, cats, and monkeys. We further show that intrastriatal axon collaterals of ILr neurons are not biased toward the matrix or striosome compartment (Fig. 7B, C). These observations are also consistent with a previous anterograde tracing study in the cat (Ragsdale and Graybiel 1991). It is thus concluded that the rat neostriatal compartments were innervated differentially by the ML and IL neurons: 1) The striosome compartment mainly receives thalamic input from Pv, Rh, and Pt neurons; 2) the matrix compartment is heavily innervated by Pf neurons; and 3) both the compartments receive relatively unbiased projections from ILr neurons (Fig. 9). These findings demonstrate that the striosome and matrix compartments of the CPu are distinguishable by not only the cortical input, their outputs and various molecular markers, but also the basis of their inputs from thalamostriatal neurons.

Functional Considerations

The rat ML is known to receive information from many subcortical brain regions, such as the hypothalamic nuclei, periaqueductal grey, deep mesencephalic reticular formation, parabrachial nuclei, and nucleus of the solitary tract (Cornwall and Phillipson 1988; Krout and Loewy 2000a, b; Krout et al. 2002). The Pv subdivision of the ML is characterized by abundant input from a variety of hypothalamic nuclei (for review, see Risold et al. 1997), suggesting that Pv neurons are associated with the autonomic or visceral functions. Because

our findings revealed that the thalamostriatal neurons in ML directed axons to the limbic cortical areas and the striosome compartment that received afferents from frontal and limbic areas in rats (Gerfen 1984, 1989; Donoghue and Herkenham 1986; Kincaid and Wilson 1996), the striosome compartment integrates the information from the neurons in both the limbic cortex and ML with a time latency.

It has been established that striosome, but not matrix neurons, in the CPu direct projections to the substantia nigra pars compacta (Gerfen 1985; Jiménez-Castellanos and Graybiel 1989; Tokuno et al. 2002; Fujiyama et al. 2011) and innervate dopamine neurons directly (Watabe-Uchida et al., 2012). Mesencephalic dopamine neurons show complex reward-related responses and are thought to play an important role in the reinforcement learning hypothesis of the basal ganglia (Schultz et al. 1998; Schultz 2007a, b). The findings in the present study that the striosome compartment is preferentially innervated by ML neurons, and in particular by Pv neurons, that transmit a variety of subcortical inputs, including hypothalamic information, leads to the suggestion that striosome neurons may integrate both the cortical cognitive/affective information and the hypothalamic autonomic/visceral information, and also control the activity of the mesencephalic dopamine neurons.

The Pf-derived thalamostriatal axon terminals, which are located mainly in the matrix compartment (Fig. 6; Table 2; Herkenham and Pert 1981; Sadikot et al. 1990, 1992; Ragsdale and Graybiel 1991), predominantly make asymmetric synapses on the dendritic shafts of medium-sized spiny projection neurons (MSNs) as well as dendritic shafts of cholinergic and parvalbumin-positive GABAergic interneurons (Xu et al. 1989; Lapper and Bolam 1992; Smith et al. 1998; Rudkin and Sadikot 1999; Sidibé and Smith 1999; Raju et al. 2006; Lacey et al. 2007). The innervation of cholinergic interneurons seems to convey significant sensory signals for monitoring and switching (in primate, Matsumoto et al. 2001). In contrast, 84% of VGluT2-immunopositive thalamostriatal axon terminals in the striosome compartment form asymmetric axospinous synapses (Fujiyama et al. 2006), suggesting that the target of most thalamostriosomal synapses originating from ML is the MSNs. Since axospinous synapses are generally considered to show plasticity as revealed in corticostriatal synapses (Calabresi et al. 1999, 2000), it is conceivable that thalamostriosomal synapses, a

part of which may be formed with the axon terminals of Pv neurons, are plastic in order to learn the integration of cognitive/affective and autonomic/visceral lines of information (see also Ellender et al. 2013).

In conclusion, the findings of the present study demonstrate that striosome and matrix compartments are defined not only on their cortical input, their output, and various neurochemical markers but also on their innervation by thalamic neurons. Although these conclusions are predicted by previous studies at the level of thalamic nuclei, the present study has some important points that require single-neuron tracing such as 1) the dendritic morphologies of neurons projecting to specific targets and 2) whether and how single neurons project to multiple targets. Concerning the first point, most ML neurons classified as type I cells in terms of dendritic morphology and axon fiber types preferentially projected to the striosome compartment and limbic cortical areas. With respect to the second point, our single-neuron studies already showed that the thalamostriatal projections arise from thalamocortical axon collaterals, although subsets of thalamocortical neurons extend their axons across the striatum without giving rise to collaterals within the striatum (Kuramoto et al. 2009; Ohno et al. 2012; for review, Clascá et al. 2012). In addition, the present study revealed that the single thalamic neurons favoring striosome or matrix compartments also innervated the cerebral cortical area that supplied cortical input to the same striatal compartment. Therefore, the functional properties of the networks of the subcompartments of the striatal complex are influenced by both their cortical and thalamic afferents presumably with a different time latency. Thus, this demonstrates the selective dynamics for the striosome and matrix compartments.

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Table 1. Thalamic neurons processed for single neuron tracing in the present study.

Neuron	Thalamic nucleus	Axonal arborization				Cortical regions	Other regions
		Rt	CPu	NAc	OT		
ML							
1	Pv	+	I, +++	I, +++	–	PrL ^a (VI+)	BL (+), CeA (+), IPAC, End
2 ^b	Pv	+	I, +++	I, ++	I, ++	[AI (+), PrL (III-VI+)]	BL (+), VP, IPAC, Sisl
3	Pv	+	I, ++	I, +++	I, +++	PrL (VI+), TT (+), IL (VI+)	VP, IPAC
4	Pv	+	I, +	I, ++	–	TT (+)	CeA (++), LA (++), LS (+), IPAC, Cl LPAG (++), End (++), IPAC (+), Sisl (+)
5	Pv	+	–	II, +	–	–	
6 ^c	Rh	+	I, +++	–	–	AI (+), M1 (V, VI+) LO (++), Pir (+), VO (+),	GPe
7 ^c	Rh	+	–	–	–	M2(I) [Cg(++), RSD(++), S1(VI++), V2(VI+), V1(VI+), PA(+), VO(+), M2(VI+), PrL(II-VI+),	–
8 ^d	Rh	+	I, +	–	–	LO(+), AI] [Cg(++), RSD(++), S1(VI++), V2(VI+), V1(VI+), PA(+), VO(+), M2(VI+), PrL(II-VI+),	[Cl]
9 ^d	Rh	+	–	–	–	LO(+), AI] VO (++), PrL (II-VI++), Cg (+), M2 (II-VI+), MO (+),	[Cl]
10	Rh	+	–	–	–	LO (+)	End, Cl
11 ^e	Re	+	I, +	–	–	[M2, V1, V2, RSD, RSG, VO, LO, PrL, IL, Cg, FA, PA, Ent, DTr, PrS, PoS]	–
12	IMD	+	I, +++	–	–	PrL (III, IV++), AI (++) PrL (II-III+++), AI (+),	BL (+), VP
13	Pt	+	I, ++	I, +++	I, ++	Cg (+)	Sib, MA, LPO, Sisl
14	Pt	+	I, +	I, ++	–	PrL (++) IL (II-III++), Cg (+), PrL (II-	LH, ZI, Sisl, Sib, VP
15	Pt	+	I, ++	I, +++	–	III+)	CoA (++), LS (+), AON (+)
ILr							
16	CeM	+	I, ++	–	–	DI (++), AI (++), LO	VP (+)
17	CL	+	I, +++	–	–	M1 (VI+), M2 (VI+), S1(VI) AI (++), LO (++), DI (++),	–
18	Pc	+	I, +++	–	–	S1 (VI+)	BL (+)
19 ^f	Pc	+	I, +	–	–	[M1(IV+++)]	–
20 ^f	Pc	+	I, +	–	–	[M1(IV+++)]	–
21	Pc	+	–	–	–	S1 (VI+++), S2 (VI++)	–

Pf							
22	Pf	+	II, +++	–	–	M1(VI+), S1(I-III+)	CeA SNc(++), GPe(+), STh(+),
23	Pf	+	II, +++	–	–	M1(VI+), S1(VI) M2(++), AI(+), DI(+),	EP(+)
24	Pf	+	II, +++	–	–	S1(+), GI(+)	GPe(++), SIsI(+)
25	Pf	+	II, +++	–	–	M1(VI+), Cg(+), PrL(VI+)	SNc(+), GPe(+)
26	Pf	–	I, +++	I, ++	I, +	AI(+), VO, RSG, LO	Cl(+), GPe, IcA

Note: AI, agranular insular cortex; AON, Anterior olfactory nucleus; BL, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; Cg, cingulate cortex; Cl, claustrum; CL, central lateral thalamic nucleus; CeM, central medial thalamic nucleus; CoA, cortical amygdaloid nucleus; CPu, caudate putamen; DI, dysgranular insular cortex; End, endopiriform nucleus; EP, Entopeduncular nucleus; FA, frontal association cortex; GI, granular insular cortex; GPe, external globus pallidus; IcA, intercalated nuclei of the amygdala; IL, infralimbic cortex; IPAC, interstriatal nucleus of the posterior limb of the anterior commissure; LA, lateral amygdaloid nucleus; LH, lateral hypothalamus; LO, lateral orbital cortex; LPAG, lateral periaqueductal gray; LS, lateral septal nucleus; M1, primary motor cortex; M2, secondary motor cortex; MO, medial orbital cortex; NAc, nucleus accumbens; OT, olfactory tubercle; Pc, paracentral thalamic nucleus; Pf, parafascicular thalamic nucleus; Pir, piriform cortex; PrL, prelimbic cortex; PrS, presubiculum; PoS, postsubiculum; PA, parietal association cortex; Pv, paraventricular thalamic nucleus; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; RSD, retrosplenial dysgranular cortex; RSG, retrosplenial granular cortex; Rt, thalamic reticular nucleus; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SIb, substantia innominata, basal component; SIsI, substantia innominata, sublenticular component; SNc, substantia nigra, pars compacta; STh, subthalamic nucleus; TT, tenia tecta; V1, primary visual cortex; V2, secondary visual cortex; VO, ventral orbital cortex; VP, ventral pallidum; ZI, zona incerta.

I, type I; II, type II. –, none; +, 1–500; ++, 501–3000; +++, ≥3000.

^aRegions were arranged in order of decreasing number of branching points. Boldfaced regions had over five branching points.

^bThis neuron was found in a hemisphere containing another neuron of mediodorsal thalamic nucleus. Except for the cerebral cortex, axons of these 2 neurons were separated from each other. The data shown in parentheses may contain data relating to other neurons.

^cThese 2 neurons were found in the same hemisphere, but their axonal arborization were separated from each other.

^dThese 2 neurons were observed in the same hemisphere, and separated from each other in the CPu and Rt, but inseparable in other areas.

^eThis neuron was found in a hemisphere containing 5 other neurons of the thalamus and separated from the other neurons in the CPu and Rt, but inseparable in the other areas. The 5 neurons were located in the AM and Rh.

^fThe same as c.

Table 2. The number of axon varicosities of thalamostriatal neurons in the striatal regions.

Neuron number	Thalamic nucleus	CPu				NAc			OT	
		total	striosome	matrix	striosome	total	MOR(+)	MOR(-)	MOR(+)	
		[x]	[y]		rate [y/x*100]	[x]	region [y]	region	region rate [y/x*100]	
1	Pv	4400	2187	2213	49.7%	4181	1664	2517	39.8%	0
2	Pv	5551	2845	2706	51.3%	2132	344	1788	16.1%	817
3	Pv	590	372	218	63.1%	7511	2419	5092	32.2%	4280
4	Pv	75	55	20	73.3%	891	584	307	65.5%	0
5	Pv	0	0	0	—	216	47	169	21.8%	0
6	Rh	6679	3080	3599	46.1%	0	0	0	—	0
8	Rh	54	35	19	64.8%	0	0	0	—	0
11	Re	383	46	337	12.0%	0	0	0	—	0
12	IMD	9305	680	8625	7.3%	0	0	0	—	0
13	Pt	1210	592	618	48.9%	3321	1204	2117	36.3%	1183
14	Pt	139	94	45	67.6%	2752	1412	1340	51.3%	0
15	Pt	877	376	501	42.9%	7750	3113	4637	40.2%	0
16	CeM	2397	235	2162	9.8%	0	0	0	—	0
17	CL	15158	1158	14000	7.6%	0	0	0	—	0
18	Pc	9540	2140	7400	22.4%	0	0	0	—	0
19	Pc	358	14	344	3.9%	0	0	0	—	0
20	Pc	113	2	111	1.8%	0	0	0	—	0
22	Pf	6401	277	6124	4.3%	0	0	0	—	0
23	Pf	16029	223	15806	1.4%	0	0	0	—	0
24	Pf	10917	821	10096	7.5%	0	0	0	—	0
25	Pf	18413	911	17502	4.9%	0	0	0	—	0
26	Pf	5252	318	4934	6.1%	1531	82	1449	5.4%	184

Table 3. The number of axon varicosities of thalamostriatal neurons in the core and shell portions of the NAc.

Neuron number	Thalamic nucleus	Core portion of NAc				Shell portion of NAc			
		total	MOR(+)	MOR(–)	Percentage in	total	MOR(+)	MOR(–)	MOR(+)
			region	region	striosomes		region	region	region
		[x]	[y]		[y/x*100]	[x]	[y]		rate [y/x*100]
1	Pv	2090	1093	997	52.3%	2091	571	1520	27.3%
2	Pv	503	144	359	28.6%	1629	200	1429	12.3%
3	Pv	1933	875	1058	45.3%	5578	1544	4134	27.7%
4	Pv	274	193	81	70.4%	617	391	226	63.4%
5	Pv	0	0	0	–	216	47	169	21.8%
13	Pt	1417	937	478	66.3%	1904	265	1639	13.9%
14	Pt	1517	1046	471	69.0%	1235	366	869	29.6%
15	Pt	4167	1746	2421	41.9%	3583	1367	2216	38.2%
26	Pf	0	0	0	–	1531	82	1449	5.4%

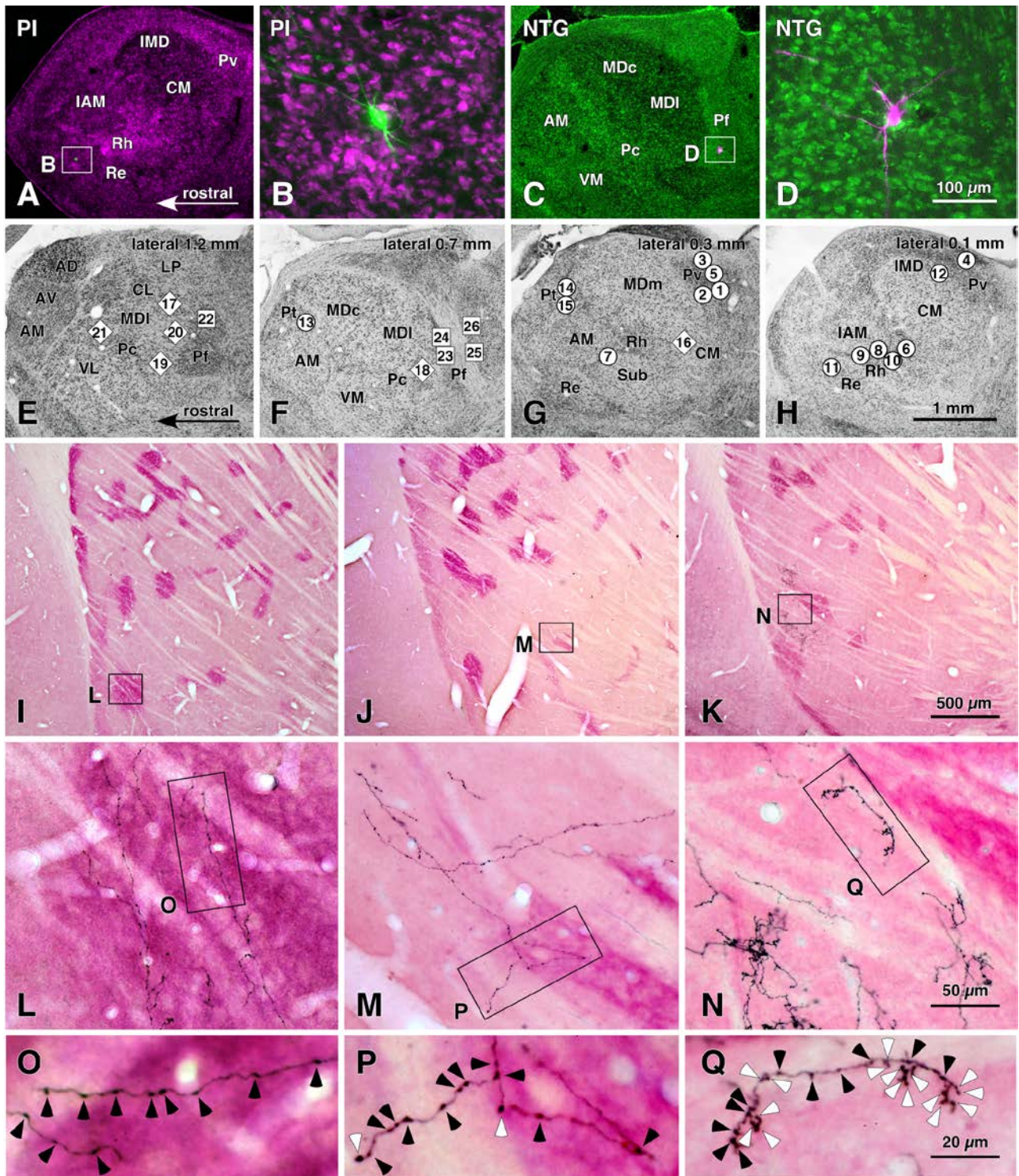


Figure 1. Single cell labeling with Sindbis viral vector. **A, B:** Parasagittal section stained with propidium iodide (PI) containing palGFP-labeled neuronal cell body. Higher magnification of the boxed area in **A** is shown in **B**. **C, D:** Section stained with NeuroTrace Green 500/525 (NTG) containing a pal-mRFP-labeled neuronal cell body. Higher magnification of the boxed area in **C** is shown in **D**. **E–H:** The locations of the thalamic neurons analyzed in the present study projected onto parasagittal Nissl-stained control sections.

Neurons 1–15 were located in the ML, neurons 16–21 in the ILr, and neurons 22–26 in the Pf. **I–Q**: Axon collaterals and varicosities of the thalamic neurons in the striatum. The axon collaterals of thalamostriatal afferents were stained black with DAB/nickel, and MOR immunoreactivity was visualized red with TAPM/*p*-cresol. Black arrowheads in **O–Q** indicate en passant boutons, and white arrowheads indicate terminal boutons. Axons of ML neurons (**I**, **L**, **O**) and ILr neurons (**J**, **M**, **P**) are forming many en passant boutons. In contrast, axons of Pf neurons (**K**, **N**, **Q**) are forming clusters/plexuses containing many terminal boutons. AD, anterodorsal nucleus; AM, anteromedial nucleus; AV, anteroventral nucleus; IAM, interanteromedial nucleus; LP, lateral posterior nucleus; MDc, central part of the mediodorsal nucleus (MD); MDl, lateral part of the MD; MDm, medial part of the MD; Sub, submedial nucleus; VL, ventral lateral nucleus; VM, ventral medial nucleus. Scale bar in **D** also applies to **B**; scale bar in **H** also applies to **A**, **C**, **E–G**; scale bar in **K** also applies to **I**, **J**; scale bar in **N** also applies to **L**, **M**; and scale bar in **Q** also applies to **O**, **P**.

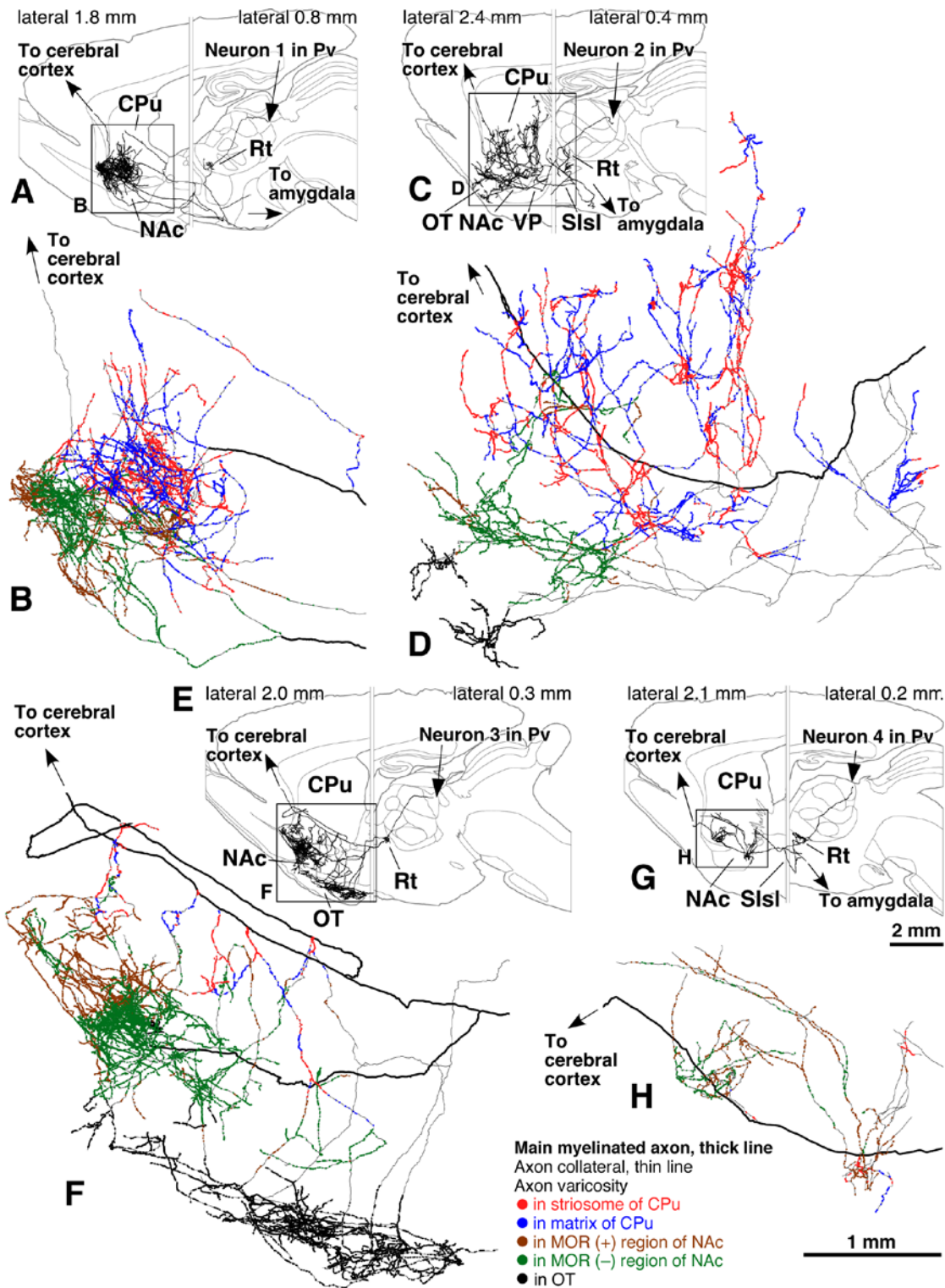


Figure 2. Camera lucida reconstructions of the thalamostriatal axons of 4 ML neurons located in the Pv. **A, C, E, G:** Neurons 1–4 showed widespread distributions of axon collaterals in the CPu and NAc. **B, D, F, H:** Higher magnification images of the striatal collaterals of the same neurons. Individual varicosities were indicated by small circles, the colors of which indicate the location in which they occurred. Axon varicosities of neurons 1–4 were located preferentially in the striosome compartments of the CPu (small red circles). For abbreviations,

see the text and the legend of Table 1. Scale bar in **G** also applies to **A, C, E**, and bar in **H** also applies to **B, D, F**.

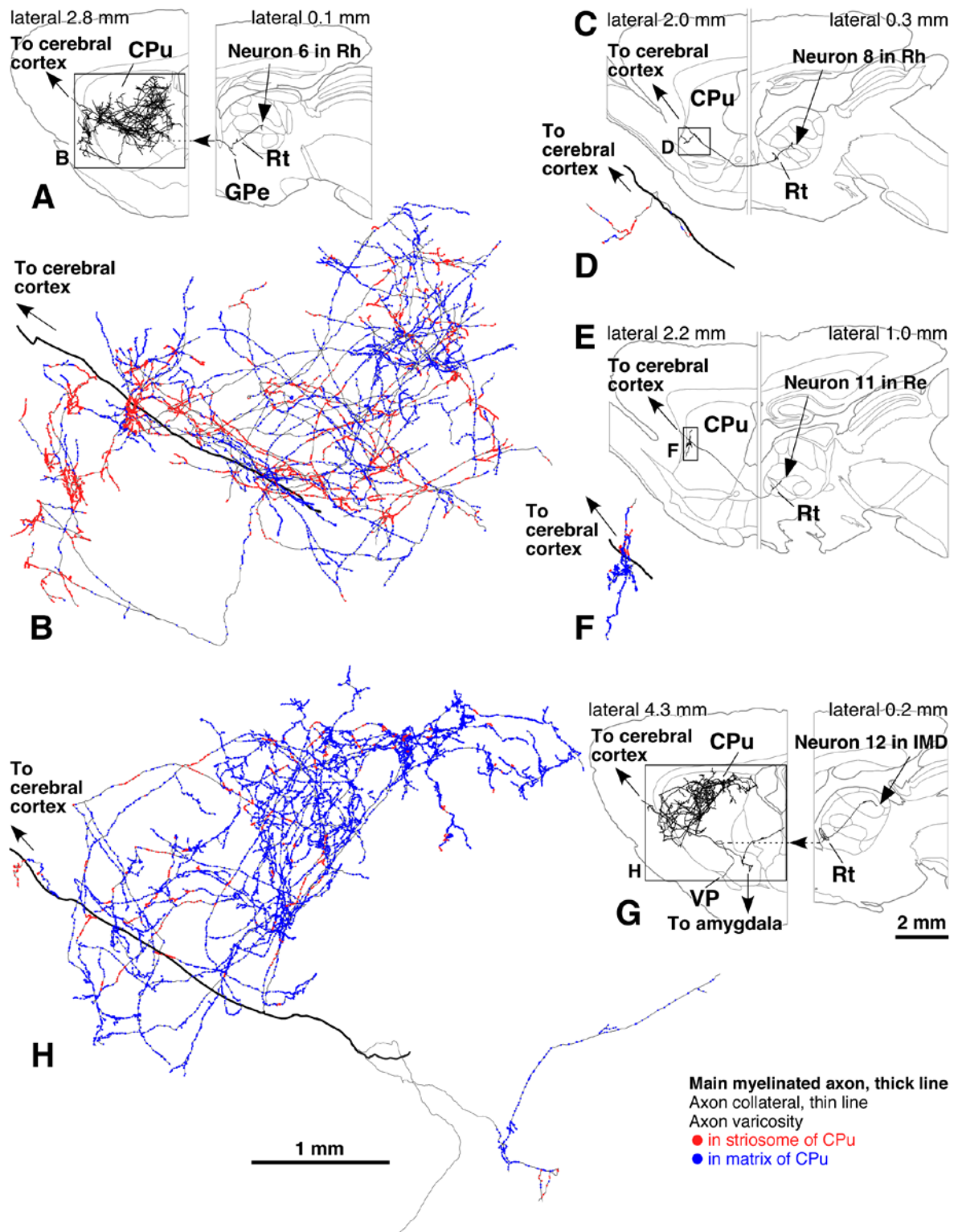


Figure 3. Reconstruction of thalamostriatal axons of ML neurons located in the Rh, Re, and IMD. Rh neuron 6 and IMD neuron 12 emitted widespread type I axon collaterals in the CPu (A, G). In contrast, Rh neuron 8 and Re neuron 11 gave rise to only very minor axon collaterals in the CPu (C, E). In the high magnification reconstructions of the striatal axons, the axon varicosities of Rh neurons 6 and 8 were distributed preferentially in the striosome

compartment (red varicosities) (**B**, **D**), whereas those of Re neuron 11 and IMD neuron 12 were located mainly in the matrix compartment (blue varicosities) (**F**, **H**). For abbreviations, see the text and the legend of Table 1. Scale bar in **G** also applies to **A**, **C**, **E**, and bar in **H** also applies to **B**, **D**, **F**.

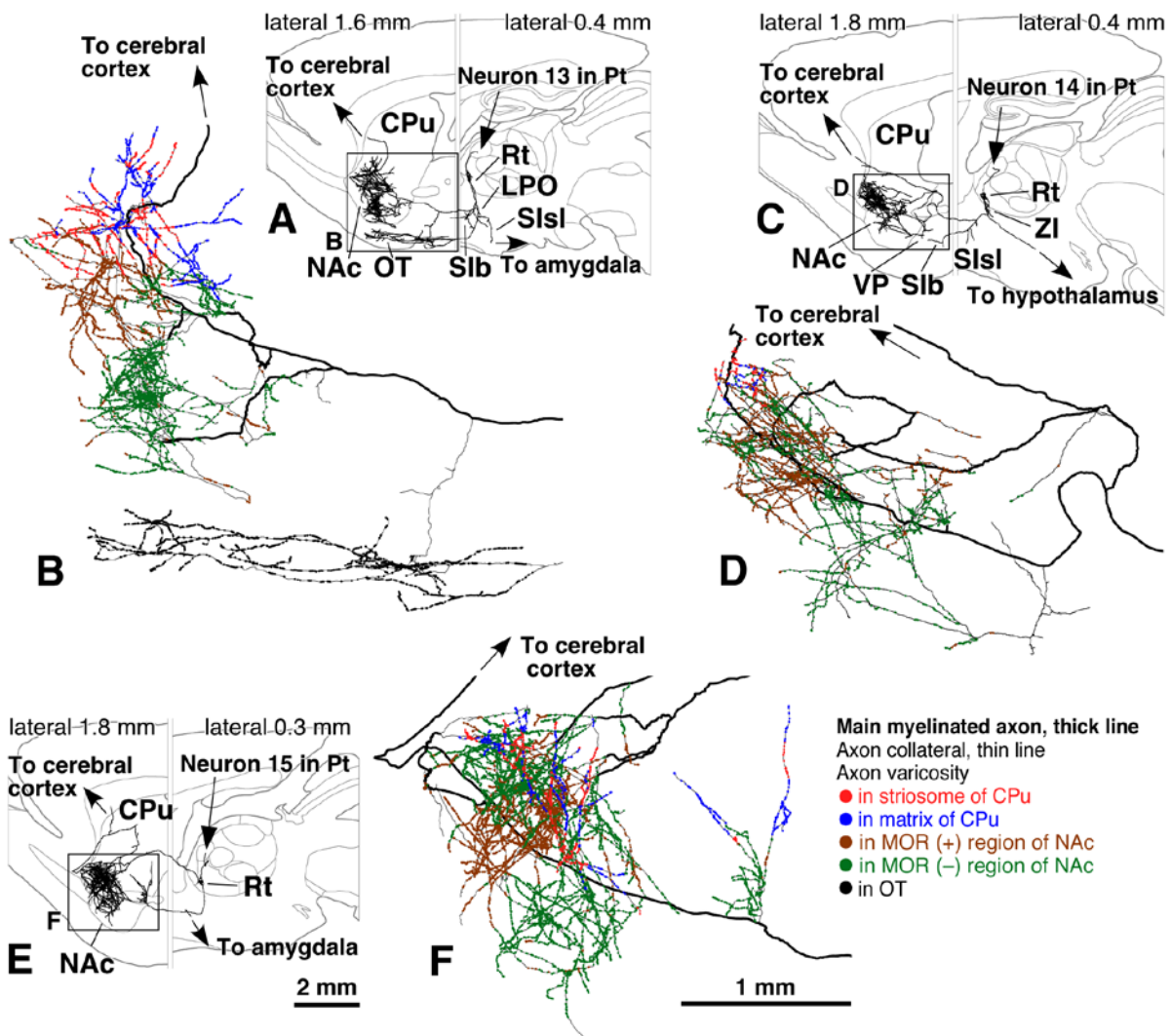


Figure 4. Reconstruction of thalamostriatal axons of 3 Pt neurons (neurons 13–15). Each neuron emitted widespread type I axon collaterals in the striatal complex, particularly the ventral striatum, the NAc, and OT (A, C, E). The high magnification reconstructions in B, D, F show that axonal varicosities were distributed in both the MOR(+) and MOR(-) compartments of the NAc (brown and green circles, respectively). For abbreviations, see the text and the legend of Table 1. Scale bar in E also applies to A, C, and bar in F also applies to B, D.

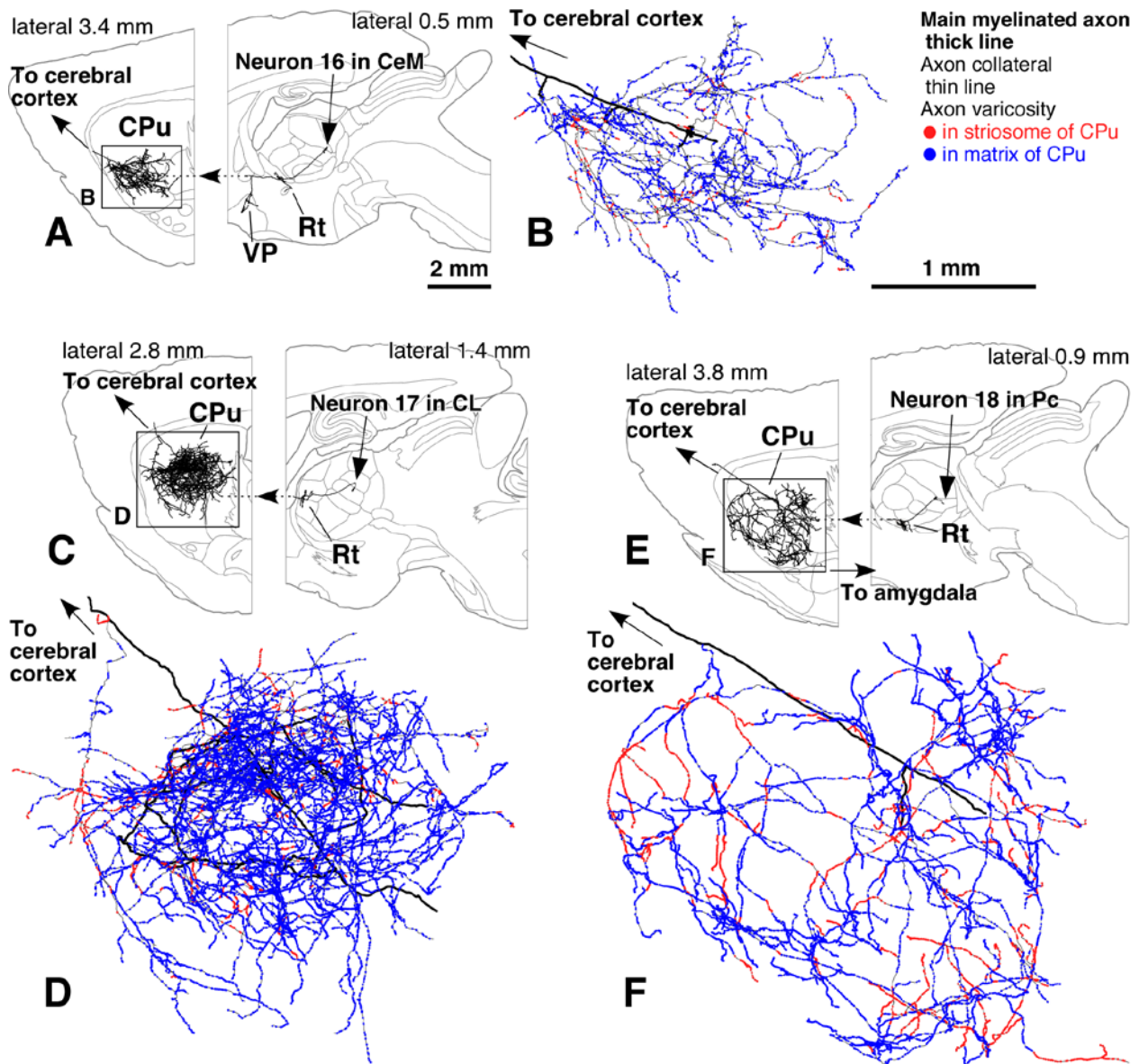


Figure 5. Reconstruction of the thalamostriatal axons of 3 ILr neurons (neurons 16–18). ILr neurons gave rise to a considerable number of type I axon collaterals widely spread in the CPu (A, C, E). The high-magnification reconstructions in B, D, F show that axonal varicosities were distributed non-selectively in both the striatal compartments. Of the 5 ILr neurons examined in the present study, Pc neuron 18 emitted axon varicosities most preferentially to the striosome compartment (see the striosome rate in Table 2). For abbreviations, see the text and the legend of Table 1. Scale bar in A also applies to C, E, and that in B also applies to D, F.

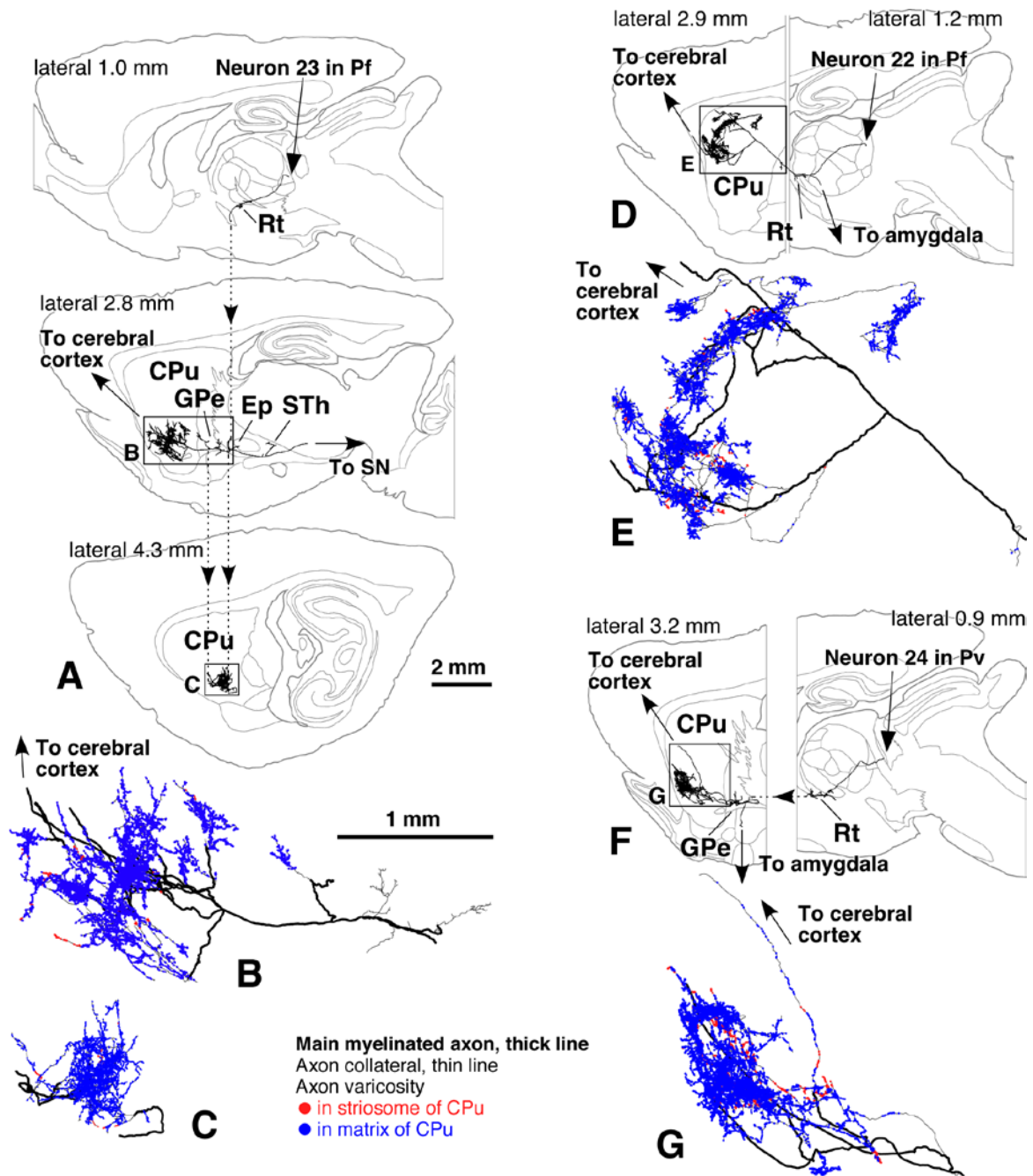


Figure 6. Reconstruction of the thalamostriatal axons of 3 Pf neurons. Neurons 22-25 emitted type II axon fibers mostly to the matrix compartment of the CPu. The axon fibers were often clustered locally, and some neurons formed multiple clusters of axon varicosities (**A**, **D**, **F**). In the high magnification reconstructions of the striatal axons (**B**, **C**, **E**, **G**), the clustered axons were equipped not only with en passant boutons but also with many terminal boutons as shown in Figure 1Q. Furthermore, the axon varicosities were mostly distributed in the matrix compartment (small blue circles in **B**, **C**, **E**, **G**). For abbreviations, see the text and the legend of Table 1. Scale bar in **A** also applies to **D**, **F**, and that in **B** also applies to **C**, **E**, **G**.

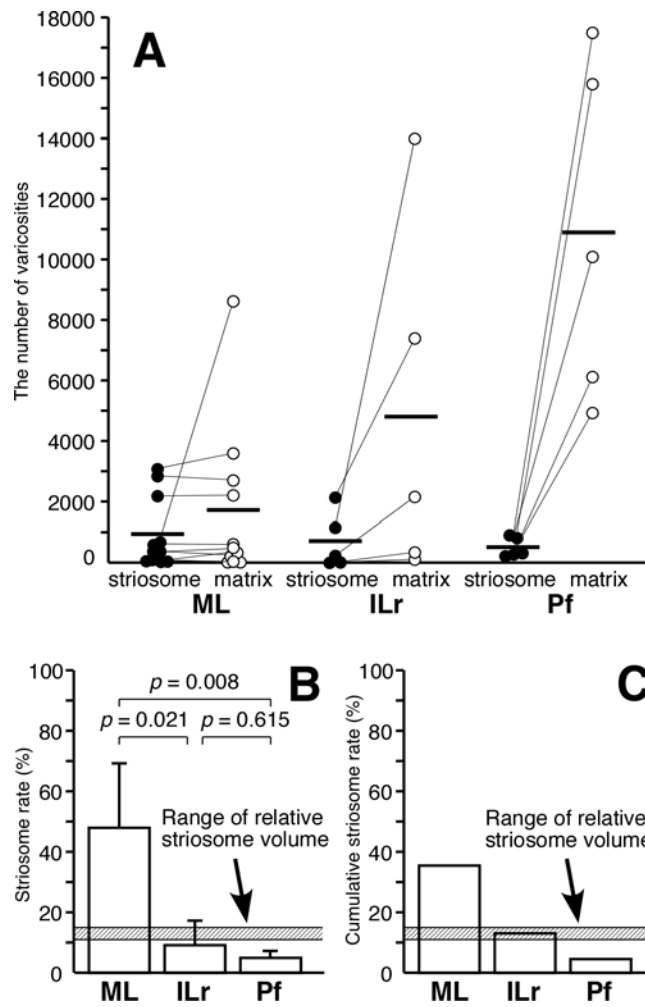


Figure 7. Quantitative analysis of axonal varicosities in the striosome and matrix compartments of the CPu. **A:** The number of varicosities in the striosome and matrix compartments plotted to compare thalamostriatal projections of the 3 groups of thalamic neuron; ML, ILr, and Pf neurons. The open and filled circles connected with a straight line indicate the data for the striosome and matrix compartments of a single neuron. The thick horizontal lines indicate the mean number of axon varicosities. **B:** The striosome rate for the 3 neuron groups. The shaded area indicates the range of relative striosome volume reported in the CPu (11–15%; Lança et al. 1986; Johnston et al. 1990; Desban et al. 1993; Wang et al. 2007). The striosome rate of the ML neuron group was significantly greater than that of the ILr or Pf neuron group (Steel-Dwass test). **C:** Cumulative striosome rate of the 3 neuron groups. The total number of intrastriosomal varicosities of the reconstructed neurons in each neuron group was divided by the total number of intrastriatal varicosities of the neuron group in order to calculate the cumulative striosome rate.

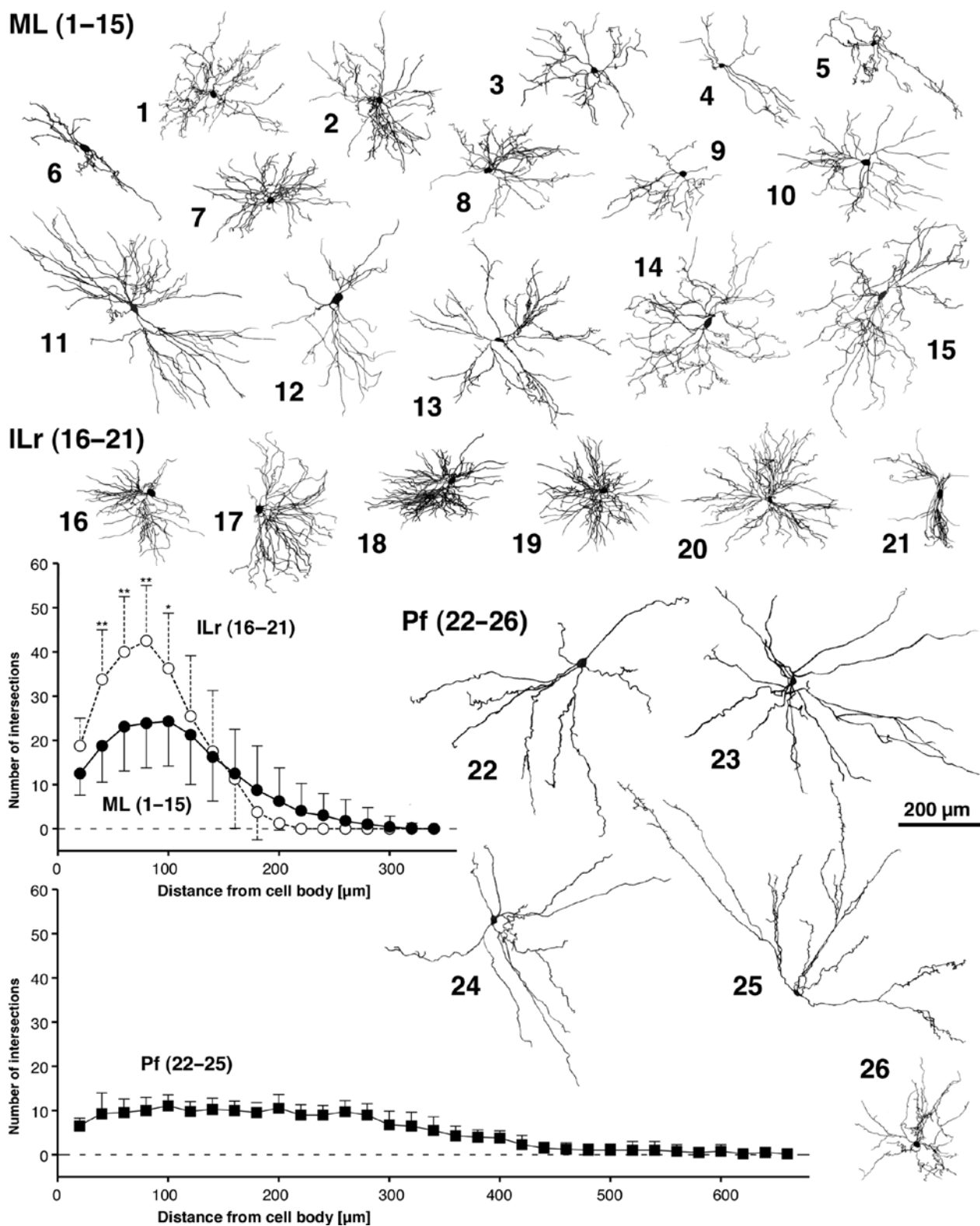


Figure 8. Camera lucida reconstructions of the dendrites and cell bodies of neurons analyzed in the present study. As reported previously in CL neurons, all ILr neurons had bushy dendrites, whereas all Pf neurons except neuron 26 possessed long reticular-like dendrites (Deschênes, Bourassa and Parent 1995; Deschênes, Bourassa and Doan et al. 1996). The dendrites of ML neurons were to be classified as the bushy type, even though they are

somewhat less bushy than the dendrites of ILr neurons. In the upper graph, the dendrites of ML neurons were compared with those of ILr neurons by the Sholl analysis (Sholl 1953; Circles and bars indicate mean and SD, respectively; ** $p < 0.01$ and * $p < 0.05$ by Bonferroni multiple comparison test). In the lower graph, we showed the dendrites of Pf neurons to compare with those of ML and ILr neurons.

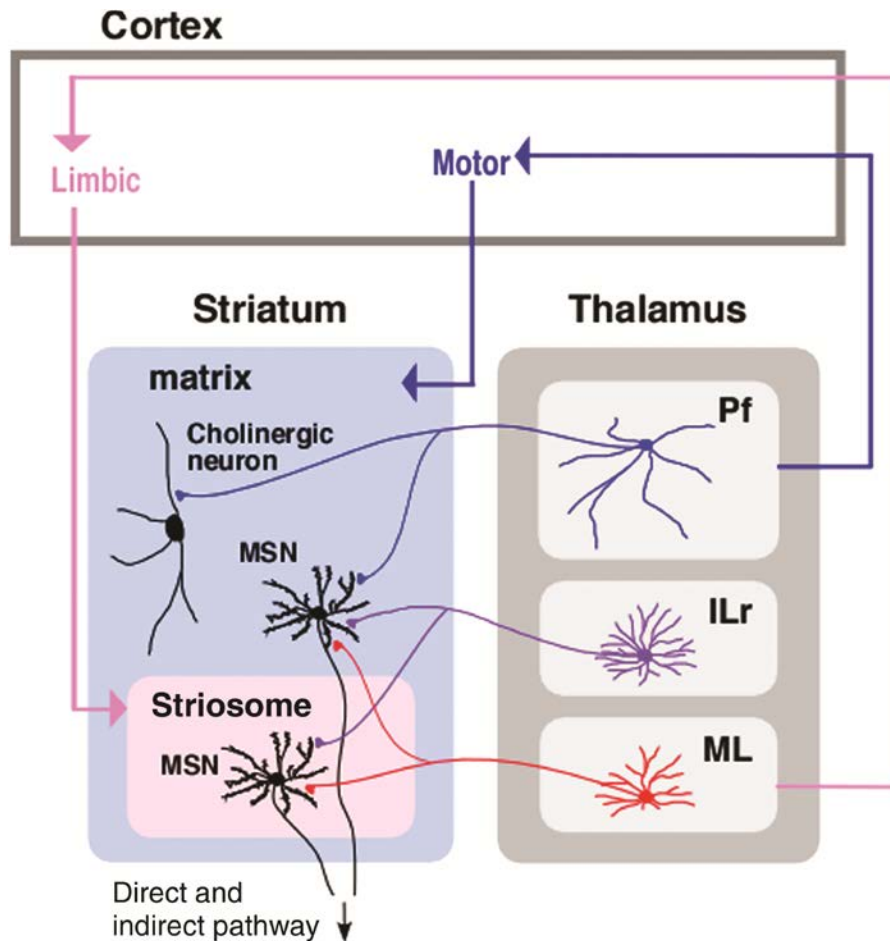


Figure 9. Summary figure of the present study. ML thalamostriatal neurons project preferentially to the striosome compartment, whereas Pf thalamostriatal neurons project preferentially to the matrix compartment. ILr thalamostriatal neurons projects to the both compartments non-selectively. Almost all single thalamostriatal neurons favoring striosome or matrix compartments also innervated the cerebral cortical area that supplied cortical input to the same striatal compartment.