Thalamostriatal Projections from the Medial Posterior and Parafascicular Nuclei Have Distinct Topographic and Physiologic Properties

Abbreviated Title: Thalamostriatal Projections from POm and Pf

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Acknowledgements: This work was supported by NIH grant NS37532.
ABSTRACT

The dorsolateral striatum (DLS) is critical for executing sensorimotor behaviors that depend on stimulus-response (S-R) associations. In rats, the DLS receives its densest inputs from primary somatosensory (SI) cortex, but it also receives substantial input from the thalamus. Much of rat DLS is devoted to processing whisker-related information, and thalamic projections to these whisker-responsive DLS regions originate from the parafascicular (Pf) and medial posterior (POm) nuclei. To determine which thalamic nucleus is better suited for mediating S-R associations in the DLS, we compared their input-output connections and neuronal responses to repetitive whisker stimulation.

Tracing experiments demonstrate that POm projects specifically to the DLS, but the Pf innervates both dorsolateral and dorsomedial parts of the striatum. The Pf nucleus is innervated by whisker-sensitive sites in the superior colliculus, and these sites also send dense projections to the zona incerta, a thalamic region that sends inhibitory projections to the POm. These data suggest that projections from POm to the DLS are suppressed by incertal inputs when the superior colliculus is activated by unexpected sensory stimuli.

Simultaneous recordings with two electrodes indicate that POm neurons are more responsive and habituate significantly less than Pf neurons during repetitive whisker stimulation. Response latencies are also shorter in POm than in Pf, which is consistent with the fact that Pf receives its whisker information via synaptic relays in the superior colliculus. Compared to the Pf nucleus, these findings indicate that POm transmits somatosensory information to the DLS with a higher degree of sensory fidelity.
The dorsolateral striatum (DLS) is critical for the expression of behaviors that do not depend on rewarded outcomes but are emitted automatically in response to specific stimuli (Balleine and O’Doherty 2010; Graybiel, 2008; Yin and Knowlton, 2006; Yin et al., 2006). In addition to having a role in acquiring and executing well-learned sensorimotor habits mediated by S-R associations, the DLS is involved in the expression of highly-repetitive, innate behaviors such as grooming (Aldridge et al., 2004; Cromwell and Berridge, 1998).

In this context, substantial evidence indicates that the DLS mediates some aspects of exploratory whisking, which is an innate behavior in rats and other rodents. The DLS receives dense inputs from SI barrel cortex (Alloway et al., 1999, 2006; Brown et al., 1998), and DLS neurons discharge rhythmically during exploratory whisking (Carelli and West, 1991). Like other DLS-linked behaviors, whisking does not depend on a rewarded outcome, but occurs automatically (Moreno et al., 2010; Welker, 1964). Free-air whisking is characterized by frequency-specific epochs of whisking sequences that are replaced by stereotyped patterns of whisker movements when a rat encounters a novel object (Mitchinson et al., 2007; Sachdev et al., 2003; Towal and Hartmann, 2006). Furthermore, even though whisking is an innate behavior, it can be modified by instrumental conditioning procedures that have been used to establish S-R associations in the DLS (Gao et al., 2003; Mermajo et al., 1996).

The whisker region in DLS receives its densest innervation from sensorimotor cortex (Smith et al., 2012), but recent data indicate that DLS responses to whisker deflections do not depend entirely on cortical inputs (Mowery et al., 2011). Thus, DLS neurons discharge before SI neurons when the whiskers are deflected at frequencies of 5 Hz or more, and SI neurons adapt quickly to repetitive whisker stimulation, whereas DLS neurons do not. These results suggest that whisker-evoked responses are transmitted to the DLS by subcortical projections. Many thalamic nuclei innervate the striatum (Castle et al., 2005; Deschenes et al., 1995, 1996; Smith et al., 2004; Van der Werf et al., 2002), and whisker-sensitive regions in DLS receive most of their thalamic inputs from the posterior POm and Pf nuclei (Smith et al., 2012).
The Pf nucleus projects to the striatum, where it innervates cholinergic interneurons (Berendse and Groenewegen, 1991; Lapper and Bolam, 1992). When activated by thalamostriatal inputs from Pf and other intralaminar nuclei, the cholinergic neurons initiate a pause in the activity of other striatal neurons (Ding et al., 2010). Presumably, this mechanism enables Pf and other intralaminar nuclei to interrupt ongoing striatal processing so that another behavior can be selected (Matsumoto et al., 2001; Thorn and Graybiel, 2011; Van der Werf et al., 2002).

Consistent with this view, the Pf receives multimodal sensory inputs from the superior colliculus (Grunwerg and Krauthamer, 1992; Krout et al., 2001; Schultz et al., 2009), a brain region instrumental in directing attention to unexpected stimuli (Yamasaki and Krauthamer, 1990). Although these collicular projections are known, whisker-evoked responses have never been recorded in the Pf nucleus, and the full extent of the thalamic targets of whisker-sensitive sites in the superior colliculus are not well-established.

The POm complex receives ascending inputs from the trigeminal nuclei and it responds to whisker stimulation (Chiaia et al., 1991; Masri et al., 2008; Sosnik et al., 2001; Veinante et al., 2000). While POm is known for innervating sensorimotor cortex (Lu and Lin, 1993; Ohno et al., 2011; Smith et al., 2012; Viaene et al., 2011; Wimmer et al., 2010), neurons in the posterior POm also send collateral projections to the striatum (Deschenes et al., 1995, 1998; Ohno et al., 2012; Smith et al., 2012). The POm could be important for controlling striatal processing, but no study has characterized the topographic specificity of its projections to the striatum to determine how it compares with thalamostriatal projections from Pf.

Here we used anterograde and retrograde tracing techniques to characterize the input-output connections of the posterior POm and Pf nuclei as they relate to striatal processing of sensory information from the whiskers. In addition to comparing the topography of their projections to the striatum, we also compared their neuronal responses during repetitive whisker stimulation to determine which of these regions might be involved in mediating S-R associations in the DLS.
MATERIALS AND METHODS

Animals. This study used male Sprague-Dawley rats ranging from 300 to 500 g. All procedures followed NIH guidelines and were approved by Penn State’s Institutional Animal Care and Use Committee (IACUC).

Surgeries. Each rat was injected intramuscularly (IM) with ketamine (40 mg/kg) and xylazine (12 mg/kg). Subsequently, IM injections of atropine methyl nitrate (0.05 mg/kg), dexamethasone sodium phosphate (5 mg/kg), and enrofloxacin (2.5 mg/kg) were administered prior to intubating the rat through the oral cavity and placing it in a stereotaxic instrument. Body temperature was maintained at 37°C by a homeothermic heating blanket, and vital signs (i.e., heart rate, blood oxygen levels, and end-tidal CO₂) were monitored continuously. After infiltrating the scalp with multiple injections of bupivacaine, a midline incision exposed the cranial surface. A machine screw inserted in the cranium over the cerebellum served as a ground lead, and a second screw inserted over the left frontal cortex enabled electrocorticographic (ECoG) recordings. A craniotomy over the posterior thalamus and midbrain provided access to the Pf, POm, or superior colliculus for neuronal recordings and tracer injections. Stereotaxic coordinates for the craniotomies were measured with respect to bregma: 1.2 mm lateral and 4.1 mm caudal for Pf, 2.6 mm lateral and 3.8 mm caudal for the posterior POm, and 2.0 mm lateral and 6.5 mm caudal for the superior colliculus.

Dental cement was applied over the screws inserted in the occipital ridge and two inverted bolts were inserted into the cement. After the cement cured, a goose-neck manipulator was fastened to each bolt. Stereotaxic ear bars were then removed to eliminate nociceptive inputs from the soft tissue in the auditory meatus.

A SciWorks data acquisition system (ver. 6.0, DataWave Technologies, Broomfield, CO) displayed ECoG recordings continuously to indicate the anesthetic state of the rat (Friedberg et al., 1999). Electrical potentials at the dural surface of the frontal cortex were amplified, filtered (0.3 - 300 Hz), and sampled at 256 Hz by an A/D board (DT2839, Data Translation, Marlboro,
A color-coded fast Fourier transform of the ECoG activity was displayed and updated once per second to visualize changes in cortical frequencies. Power spectra dominated by 1-2 Hz occurred during deep anesthesia when rats were unresponsive to noxious stimuli, but as the ketamine and xylazine were metabolized, the power spectra gradually shifted to 5-6 Hz, which indicates a lightly anesthetized state (Friedberg et al., 1999). To prevent wakefulness, the rat was maintained in an unconscious state by ventilating it with isoflurane through the endotracheal tube. Isoflurane concentration was gradually increased over 2-3 hours until it reached a concentration of 0.75 - 1.0%, which was sufficient to maintain the rat in a constant anesthetic plane in which ECoG activity was dominated by frequencies of 5-6 Hz for the remainder of the experiment. Isoflurane produces dose-dependent suppression of thalamocortical transmission, but the concentrations used in this study were below the levels that prevent stimulus-induced responses in the thalamus (Detsch et al. 1999, 2002).

**Tracer injections.** All tracers were iontophoretically injected from solutions in glass pipettes with tip diameters of 20-25 μm. A 2% solution of Fluoro-Gold (FG) in saline was used to produce retrograde labeling; a 15% solution of biotynylated dextran amine (BDA) in 0.01M phosphate buffered saline (PBS) was used to produce anterograde labeling. In some cases, we injected a combined solution of FG and BDA to reveal anterograde and retrograde connections from the same injection site. All tracer injections were made by vertically-oriented pipettes at the following depths below the cortical surface: 5.0-5.4 mm ventral for Pf, 4.5-5.6 mm ventral for POM, and 4.5-5.5 mm ventral for the superior colliculus. Prior to reaching the target depth, a retention current (-5 μA) was applied to the tracer solution to prevent tracer leakage.

After reaching the target, the retention current was turned off. The silver wire used to conduct the retention current was then connected to the head stage of an extracellular amplifier (Dagan 2200, Minneapolis, MN) to record neuronal discharges in response to whisker deflections (see Neurophysiology Recordings). This technique verified that tracers were injected at whisker-sensitive sites in Pf, POM, and the superior colliculus. The tracer-filled pipette was
slowly advanced while the contralateral whiskers were repetitively deflected by a computer-controlled stimulator (see Whisker Stimulation). When whisker-sensitive neurons were encountered, their responses were recorded during computer-controlled whisker stimulation. We also used a hand-held light-emitting diode to test whether visual stimulation evoked neuronal responses in Pf or POm. Subsequently, the tracer was iontophoretically ejected by applying positive current pulses in 7-s on-off intervals. For FG deposits, current (2 μA) pulses were applied for 30 minutes; for BDA deposits, current (2-5 μA) pulses were applied for 20-40 minutes. When both tracers were in the pipette solution, current was applied as 3 μA pulses for 20-30 minutes.

Histochemistry. After a transport period of 7-10 days, injected rats were deeply anesthetized with ketamine (60 mg/kg) and xylazine (18 mg/kg), and were transcardially perfused with physiological saline containing 1% heparin, followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 6.9), and 4% paraformaldehyde containing 10% sucrose. After the brain was removed from each animal’s head, the olfactory bulbs, cerebellum, and caudal brainstem were sliced off and the rest of the brain was stored in 4% paraformaldehyde with 30% sucrose. A shallow slit in the ventral surface of the left hemisphere provided a fiduciary mark for mounting sections in the correct left-right orientation. A freezing microtome was used to slice coronal sections at a thickness of 60 μm.

For brains that received FG deposits, alternate serially-ordered sections were mounted on gel-coated glass slides and dried overnight. Cytoarchitecture was revealed in one series either by staining the sections with thionin or by processing them for the presence of cytochrome oxidase (Land and Simons, 1985; Wong-Riley, 1979). The other series was processed for FG labeling by dehydrating the sections in ethanol, defatting them in xylene, and coverslipping them with Cytoseal.

For brains that received BDA deposits, brain sections were divided into three series. One was stained with thionin or processed for cytochrome oxidase to visualize neural structures. The
second series was processed for BDA as described before (Alloway et al, 1998; Kincaid and Wilson, 1996; Smith et al., 2012), and the third series was either processed for FG or served as a backup for BDA if necessary. To visualize BDA labeling, sections were agitated in 0.3% H$_2$O$_2$ to reduce background enzymes and then placed in 0.1M PBS with 0.3% Triton X-100 (pH = 7.4) before incubating them for 2-4 hours in an avidin-biotin horse-radish peroxidase solution (Vector Novocastra Laboratories, Burlingame, CA). After rinsing the sections with PBS, the tracer was visualized with 0.05% diamino-benzidine, 0.005% H$_2$O$_2$, 0.04% NiCl$_2$, and 0.04% CoCl$_2$ in 0.1M Tris buffer (pH = 7.2) for 9-12 min. The reaction was stopped by subsequent washes in PBS, and the processed sections were mounted on gel-coated slides.

**Anatomical analysis.** The BDA injections and BDA-labeled terminals were examined with an Olympus BH-2 microscope. Locations of BDA-labeled axon varicosities, which represent en passant synapses (Kincaid and Wilson, 1996; Meng et al., 2004; Voight et al., 1993), were plotted and digitally reconstructed with respect to neighboring anatomical landmarks by using an Accustage microscopic reconstruction system (St. Paul, MN) as described previously (Alloway et al., 2009). Sections that contained FG-labeled neurons were visualized by means of a UV filter (110000v2, Chroma Technology, Bellows Fall, VT). Only FG-labeled neurons that displayed one or more dendrites were plotted and reconstructed with respect to surrounding landmarks. Photomicrographs of tracer injections were acquired by a Retiga EX CCD digital camera (Q-imaging, Surrey, British Columbia, Canada) mounted on the microscope.

The tissue volumes infiltrated by BDA injections were estimated using the Accustage reconstruction system. Outlines of the tracer injections were drawn on the plotted reconstructions, and an Accustage module was used to measure the area enclosed within the outline. Because BDA injections appeared only in sections processed for BDA, tracer diffusion in the intervening sections was estimated by interpolating the areas measured on adjacent sections that had been processed for BDA. Section areas of all tracer injections, both measured
and estimated by interpolation, were summed and multiplied by section thickness to estimate the
volume of thalamic tissue that was infiltrated by the BDA tracer injection.

The volume of the striatal neuropil innervated by labeled projections was calculated using a
procedure similar to the one for estimating tracer injection volumes in the thalamus. Each
reconstructed section of the plotted BDA-labeled varicosities was subdivided into 25-μm square
bins. The number of square bins containing two or more plotted varicosities was summed and
multiplied by 25 μm$^2$ to estimate the area of terminal labeling in each coronal section.

Interpolation was used to estimate the area of terminal BDA-labeling in the intervening sections
that were not processed for BDA. For each rat, sectional areas of the striatal labeling, either
measured or estimated by interpolation, were summed and multiplied by section thickness to
estimate the total striatal volume that was innervated by the injection site in Pf or POm.

The density of plotted varicosities in the striatum was calculated from the reconstructed
sections. The total number of 25-μm square bins containing plotted varicosities was summed
across all sections reconstructed for each animal, and then this area was divided by the total
number of varicosites that had been plotted for that case. Density was then expressed as the
number of plotted varicosites per square millimeter.

Neurophysiology recordings. Tracer-filled pipettes having impedances of 0.5 MegOhms
were used to record whisker-evoked responses in the thalamus and superior colliculus prior to
tracer injection. Extracellular discharges were visualized on a digital oscilloscope (Tektronix
DPO4034; Tektronix, Beaverton, OR) while listening to the neuronal responses on an acoustic
speaker. Trial-based responses were stored on hard disk and were replayed off-line to sort the
waveforms according to their amplitude, width, and other characteristics. Biphasic discharges
were plainly evident in waveforms recorded by tracer-filled pipettes, but the discharges were
usually not as well-isolated as waveforms recorded by higher impedance electrodes.

The depth of anesthesia was generally greater in the tracer injection experiments, and given
that neuronal isolation was not optimal in tracer-filled pipettes, comparisons of response
properties in Pf and POm were analyzed in separate experiments using saline-filled pipettes that had impedances of 1-1.5 MegOhms and recorded well-isolated discharges. In some experiments, only one electrode was inserted into the thalamus to record whisker-sensitive neurons in Pf or POm. In most experiments two electrodes were inserted so that POm and Pf were recorded simultaneously by electrodes that could be moved independently. In these latter cases, the POm was recorded by an electrode that entered the brain 5.2 mm lateral and 3.6 mm caudal to bregma, at 33° from vertical. Discharges with a signal-to-noise ratio of at least 3:1 were time-stamped at a resolution of 0.1 ms and were displayed as PSTHs. After measuring the mean rate of spontaneous activity, 99% confidence limits were displayed on each PSTH, and whisker-evoked responses were considered significant if they exceeded the confidence limits for two or more contiguous bins. Based on PSTHs constructed with 1-ms bins, response latencies were measured from the time of stimulus onset to the time of two contiguous bins in which both exceeded the 99% confidence limit. Current was passed through the recording pipettes (5 mA for >30 s) to produce electrolytic lesions that marked the recording sites in the histological sections.

**Whisker stimulation.** Multiple vibrissae (rows B-E, arcs 1-5) were stimulated in tandem by computer-controlled movements of a Galvanometer obtained from a Grass polygraph. As done previously (Mowery et al, 2011; Smith et al., 2012; Zhang et al., 2004), a small piece of window screen was attached to the tip of the Galvanometer’s ink pen and this was positioned so that the whiskers, clipped to a length of 20 mm, protruded through the screen openings by 8-12 mm. Timed output from a digital waveform generator (ArbStudio, LeCroy, Chestnut Ridge, NY) was used to control Galvanometer movements. Each trial contained a sequence of twelve 50-ms triangular stimuli so that blocks of four stimuli were presented at a rate of 2, 5, and 8 Hz. The first stimulus in each block of four was classified as ≤1-Hz (ie., less than or equal to 1 Hz) because it was preceded by an interval of 1 second or more in which no stimulus was administered. For each 50-ms stimulus, the whiskers were initially deflected in the caudal
direction (~1.5 mm) during the first 25-ms, and then they returned to the original resting position over the next 25-ms period.

**RESULTS**

Anatomical and physiological results were obtained from a total of 38 rats. Tracing data came from 17 rats in which tracers were placed at whisker-sensitive sites in the superior colliculus (n = 6), the Pf nucleus (n = 6), or the posterior POm nucleus (n = 5) of rats that were deeply anesthetized. Tracing data from three of these POm cases were obtained from a previous study (Smith et al., 2012); all other tracing data were obtained from new unreported experiments. The remaining 21 rats were used exclusively for recording whisker-induced responses from Pf and the posterior POm in lightly-anesthetized rats.

**Tracer injections in superior colliculus.** Previous reports indicate that the superior colliculus responds to both visual and somatosensory stimulation (Cohen et al., 2008; Comoli et al., 2003), and that it projects to the Pf nucleus (Coizet et al., 2007; Grunwerg and Krauthamer, 1992; Yamasaki et al., 1986). Antidromic stimulation indicates that whisker-sensitive sites in superior colliculus project to the intralaminar nuclei (Grunwerg and Krauthamer, 1990), but these previous studies did not identify the exact PF regions that receive these whisker-related inputs. To clarify this issue, we injected a mixture of retrograde and anterograde tracers at collicular sites that respond to whisker stimulation.

An example of a combined deposit of BDA and FG in the superior colliculus is illustrated in Figure 1. Using a glass pipette filled with both tracers, we were able to isolate a neuron that discharged both spontaneously and in response to repetitive whisker deflections (see Fig. 1A). The neuron, located in the ventrolateral part of the superior colliculus (Fig. 1B), responded to whisker deflections administered at ≤1, 2, 5, and 8-Hz. The response latency for this neuron was 8 ms during 1, 2, and 8-Hz stimulation, but increased to 10 ms when the whiskers were deflected at a frequency of 8-Hz. When neural responses were recorded from tracer-filled pipettes, the
neuronal discharges were not always well-isolated and multiunit responses were recorded in some cases. Nonetheless, whisker-induced responses recorded at tracer injection sites in the superior colliculus during ≤1-Hz stimulation had a mean latency of 10.66 ± 1.6 ms (mean ± SEM).

As seen in Figure 1, iontophoretic ejection of BDA and FG at a whisker-sensitive site in the superior colliculus produced a large deposit in which both tracers diffused at least 500 μm in all directions from the recording site. Coronal sections through the thalamus of this case, which are illustrated in Figure 2, revealed dense BDA-labeled axonal terminals in the ventral part of the lateral Pf nucleus. This labeling pattern revealed the ventral contours of the Pf nucleus, which were also defined by cytochrome oxidase labeling in an adjacent coronal section (compare panels A and A’ in Fig. 2). Some labeled terminals also appeared in the medial Pf nucleus along the edge of the fasciculus retroflexus, but this labeling was less dense and extensive than the BDA labeling in the lateral Pf. Examination of multiple sections indicated that labeling in the lateral Pf nucleus extended rostrocaudally for at least 420 μm.

Projections from the superior colliculus also terminate densely in the zona incerta, which sends inhibitory projections to the POm nucleus (Bartho et al., 2002). Collicular projections to the zona incerta have rarely been reported (Roger and Cadusseau, 1985; Mitrofanis, 1985), but our data indicate that these projections extend throughout widespread parts of the zona incerta and are as dense as the collicular projections to the Pf nucleus. For example, the superior colliculus injection depicted in Figure 1 produced terminal labeling in the zona incerta that extended rostrocaudally for at least 1 mm. As seen in Figure 2A’, collicular projections terminated in medial parts of both the ventral and dorsal zona incerta, but other labeled projections from the same collicular injection site innervated more lateral parts of the zona incerta as seen in more rostral sections of this case (see Figure 3B’). Vision-related collicular projections to the subthalamic nucleus have been reported (Coizet et al., 2009), but we did not
observe any terminal labeling in the subthalamic nucleus following tracer injections into whisker-related regions in the ventrolateral superior colliculus.

Inspection of coronal sections through the spinal trigeminal nuclei confirmed the presence of retrogradely-labeled neurons in the interpolaris part of the spinal trigeminal nucleus (see Fig. 2C and C'). Hundreds of labeled neurons in the spinal trigeminal nucleus appeared across several coronal sections extending rostrocaudally over a distance of 780 μm, thereby confirming robust projections from the trigeminal nuclei to whisker-sensitive sites in the superior colliculus (Huerta et al., 1983). These findings indicate that the superior colliculus receives whisker-related information from the trigeminal system and can convey it to the lateral Pf nucleus.

Tracer injections in the Pf nucleus. To determine if whisker information could be conveyed from the Pf to the striatum, BDA-filled pipettes were used to search for whisker-sensitive sites in Pf. In addition to using computer-controlled whisker stimulation to detect Pf neurons, we used hand-held light-emitting diodes to shine light in the eyes, and we routinely found that whisker-sensitive Pf neurons also respond to visual stimulation.

As shown in Figure 4, whisker-sensitive neurons were recorded in the lateral Pf, which corresponds to the region that receives the densest projections from whisker-responsive sites in the superior colliculus (see Figs. 1 and 2). Neuronal responses in the lateral Pf were consistently evoked by whisker deflections at frequencies of ≤1 or 2-Hz, but these responses usually habituated when whisker stimulation increased to 5 or 8-Hz. The Pf responses depicted in Figure 3A, for example, had mean latencies of 22 and 35 ms during ≤1 and 5-Hz stimulation, respectively. Although discharges recorded by tracer-filled pipettes were not always well-isolated, neuronal responses at the Pf injection sites during ≤ 1-Hz stimulation had a mean latency of 19.75 ± 1.9 ms. Statistical analysis indicated that response latencies at the Pf injection sites were significantly longer than the latencies recorded at the superior colliculus injection sites (one-tailed t = 3.27, p < 0.01).
Because the Pf nucleus is relatively compact, care was taken to make small tracer deposits that did not diffuse into the surrounding thalamic regions. Low iontophoretic current levels (1.5-3.0 μA) were administered so that BDA, once expelled, would not be forced far from the pipette tip. The BDA tracer deposit shown in Figure 4, for example, is only 300-400 μm in diameter and was confined entirely within the Pf nucleus. While the size and location of BDA deposits varied across different cases, the injection site always appeared in the neuropil lateral to the fasciculus retroflexus. Furthermore, examination of the superior colliculus in this case revealed small numbers of BDA-labeled neurons scattered in its ventrolateral layers even though BDA is transported mainly in the anterograde direction (data not shown).

In two rats, BDA and FG were injected simultaneously into the Pf nucleus (data not shown). Inspection of the superior colliculus confirmed the presence of retrogradely-labeled neurons in its ventrolateral layers. In other sections through the brainstem, large retrogradely labeled neurons were observed in the dorsal part of the gigantocellularis nucleus of the reticular formation. We inspected the trigeminal nuclei and other brainstem nuclei, but no other retrogradely labeled neurons were observed in the brainstem of these two cases.

Despite the compactness of the BDA injection shown in Figure 4, projections from this Pf site innervated widespread parts of the striatum. As indicated by Figure 5, terminal labeling in the striatum was densest in rostral parts of the striatum and extended from its rostral tip to sites located 800 μm caudal to bregma. Dense clusters of BDA-labeled axon terminals were often seen in separate parts of the neuropil and were apparent throughout both the dorsomedial and dorsolateral parts of the striatum.

This profuse, divergent pattern of thalamostriatal innervation was observed in all cases in which BDA was placed in the Pf nucleus. Different Pf tracer injections produced variations in thalamostriatal topography, but the main difference concerned the amount of thalamostriatal labeling. As expected, the largest and densest Pf injections were characterized by more extensive and denser terminal labeling throughout the dorsomedial and dorsolateral striatum.
Tracer injections in Pf also revealed axonal projections to other brain regions. In the cases with the largest Pf injections, moderately dense collections of terminals and their beaded varicosities were observed in the neuropil of the globus pallidus as reported previously (Deschenes et al., 1996; Yasukawa et al., 2004). Some terminal labeling also appeared in the entopeduncular and thalamic reticular nuclei along with a few retrogradely-labeled neurons in these regions (Deschenes et al., 1996; Lacey et al., 2007). Sparse terminal labeling also appeared in motor cortex (Marini et al, 1996), especially in its deeper layers, although a few labeled fibers coursed more dorsally. Labeled projections from Pf also appeared in the deep layers of somatosensory cortex, but the density of this labeling was substantially less than in motor cortex. Finally, small clusters of labeled projections from Pf were present at multiple rostrocaudal levels of the claustrum.

**Tracer injections in the posterior POM nucleus.** Thalamostriatal projections from the posterior POM were labeled by using BDA-filled pipettes to record whisker-sensitive POM responses and then ejecting the tracer at the recording site. The POM nucleus is located just dorsal to the ventroposteromedial (VPM) nucleus, which represents the thalamic target for the lemniscal pathway. Although both POM and VPM receive whisker-related trigeminal inputs (Chiaia et al., 1991; Veinante et al., 2000; Williams et al., 1994), VPM neurons respond mainly to a single principal whisker, whereas whisker-sensitive neurons in the posterior POM have much larger, multi-whisker receptive fields (Diamond, 1995; Ito, 1988). Therefore, the recording pipette was lowered to stereotaxic coordinates that marked the dorsal POM. Subsequently, the pipette was very slowly advanced during computer-controlled whisker stimulation until whisker-sensitive neurons were encountered. Multi-whisker receptive fields were confirmed at all POM sites where tracer was placed. In addition, we periodically tested the effects of hand-held light stimulation in the eyes during POM recordings, but this did not evoke neuronal responses in POM.
As shown in Figure 6, whisker-sensitive neurons in the posterior POm were located away from the VPM border, which is consistent with projections to POm from SI barrel cortex (Alloway et al., 2003). Neurons in this part of POm were reliably activated when multiple whiskers were simultaneously deflected in tandem. Even when POm neurons were spontaneously active, responses to whisker deflections were apparent at frequencies up to 8-Hz. During ≤ 1-Hz whisker stimulation, the response latencies recorded at tracer injection sites in POm had a mean latency of 15.0 ± 3.0 ms.

As illustrated by Figure 6C’, tracer deposits in POm were larger than the tracer deposits in Pf. We usually injected BDA near the dorsal part of POm, and we used iontophoretic current levels (3-5 μA) that allowed the tracer to infiltrate a large area in POm without diffusing into VPM. Although BDA tracer deposits were larger in POm than in Pf, the labeled projections from POm innervated a smaller part of the striatum. As shown by Figure 7, labeled projections from whisker-sensitive sites in POm produced terminal labeling in the DLS but not in other parts of the striatum. In contrast to thalamostriatal projections from Pf, which terminated most densely in the striatal regions located rostral to bregma, most of the labeled projections from whisker-sensitive regions in the posterior POm were observed in DLS regions located caudal to bregma. This pattern was observed in all rats that received tracer injections in POm.

Labeled projections from POm terminated densely in somatosensory cortex. Consistent with previous reports (Lu and Lin, 1993; Wimmer et al., 2010), projections from POm innervated layer Va and the septal regions of layer IV. We also observed POm projections to the reticular nucleus of the thalamus and in motor cortex as described previously (Deschenes et al., 1995; Ohno et al., 2012; Smith et al., 2012).

Consistent with previous reports (Bartho et al., 2002), tracer injections in POm also revealed retrogradely-labeled neurons in the zona incerta. These labeling patterns were of special interest because inhibitory projections from zona incerta play a critical role in gating POm responses to trigeminal inputs (Lavallee et al., 2005). Figure 3D’ depicts the retrograde labeling observed in
the ventral part of the zona incerta after placing BDA in whisker-sensitive POM regions (case TS16 from Smith et al., 2012). The labeling pattern in this example indicates that incertal projections to whisker-sensitive sites in POM originate from the same region in the zona incerta that receives inputs from whisker-responsive sites in the superior colliculus (compare panels B’ and D’ in Figure 3).

Comparison of POM and Pf thalamostriatal projections. To emphasize the topographic differences in thalamostriatal projections from Pf and the posterior POM, we superimposed labeling patterns of the three cases that contained the most striatal labeling produced by tracer injections in these thalamic nuclei. For this procedure, striatal sections were selected at regular intervals within the rostrocaudal range in which striatal labeling overlapped.

As shown by Figure 8, tracer deposits in the Pf cases produced striatal labeling that extended 3.5 mm rostrocaudally, ranging from the most rostral tip of the striatum to nearly 1.5 mm caudal to bregma. In each case, striatal labeling in this group was densest in rostral sections located anterior to bregma. Although some cases displayed terminal labeling up to 2 mm caudal to bregma, the labeling was relatively sparse in sections caudal to those shown in Figure 8. Superimposition of the labeling patterns revealed labeled terminals across the mediolateral extent of the striatum; only the ventral portion and the extreme medial edge of the striatum were devoid of labeled projections from Pf.

By comparison, labeled projections from POM were less extensive both rostrocaudally and mediolaterally. Tracer deposits in POM produced striatal labeling that extended 2.5 mm rostrocaudally and was densest in DLS regions located 0.5 to 2.0 mm caudal to bregma. Despite inherent variations in the size and location of the POM tracer deposits in these three cases, labeled thalamostriatal projections from POM always terminated in the DLS.

Quantitative differences in the spatial extent of thalamostriatal projections from POM and Pf are illustrated in Figure 9. As Figure 9A indicates, the mean size of tracer injections in POM (0.116 ± 0.0346 mm³) was larger than the average tracer deposit in Pf (0.030 ± 0.015 mm³).
Despite this difference, the mean striatal volume that contained terminal labeling was greater after injecting Pf (0.316 ± 0.158 mm$^3$) than after injecting POm (0.125 ± 0.090 mm$^3$). For the POm cases, the volume of DLS labeling matched the volume of the POm tracer deposit as shown in Figure 9B. However, for cases in which tracer was injected into Pf, the ratio of striatal-to-thalamic volume was nearly 20 times greater (one-tailed $t = 2.102, p < 0.05$).

The density of labeled terminals in the striatum was virtually identical following tracer injections in either POm or Pf. After summing the total areal extent of striatal labeling produced by tracer injections in Pf or POm (see METHODS), our quantitative analysis revealed a mean density of 2,630 plotted varicosites/mm$^2$ in the POm cases and 2,808 plotted varicosities/mm$^2$ in the Pf cases. Statistical analysis indicated that these results were not significantly different ($t = 0.09, p > 0.05$).

**Comparison of POm and Pf neuronal responses.** Recordings with tracer-filled pipettes suggest that POm neurons are more responsive to repetitive whisker stimulation than neurons in Pf (compare Figures 4 and 6). In these cases, however, POm and Pf neurons were recorded in different groups of deeply-anesthetized rats, and the depth of anesthesia may have altered the whisker sensitivity of the neurons in one or both brain regions. Therefore, to verify that POm is more responsive than Pf, we recorded neuronal responses from both of these regions simultaneously.

A total of 43 POm and 31 Pf neurons were recorded that responded to whisker deflections. Within this overall sample, a total of 25 pairs of neurons were recorded simultaneously in POm and Pf of 12 rats. Examples of neuronal responses recorded simultaneously in POm and Pf are shown in Figure 10. As this figure indicates, the POm neuron showed similar responses to increasing frequencies of whisker stimulation, but the Pf neuron displayed substantial variation. The Pf neuron responded to the first whisker deflection in each block of frequencies, but failed to respond when the stimulus rate reached 8-Hz.
To compare stimulus-induced latencies in POm and Pf, neuronal responses were inspected at high temporal resolution as shown by the representative PSTHs in Figure 10C. As this figure indicates, the POm neuron responded at a similar latency for each of the tested frequencies. The Pf neuron, however, responded when the whiskers were stimulated at either ≤1 or 2 Hz, but the responses to 5 or 8-Hz did not satisfy the criteria for measuring latency (i.e., two contiguous bins that exceed the 99% confidence limits of the PSTH).

The cumulative distributions of latencies recorded from all POm (n = 43) and Pf (n = 31) neurons during ≤1 Hz stimulation are displayed in Figure 11A. As this figure indicates, the mean response latency in POm (15.0 ± 0.6 ms) was significantly less than the mean latency (20.4 ± 8.0 ms) recorded in Pf (t = 4.82, p < 0.001). When the analysis of response latencies was restricted to pairs of neurons (n = 25) recorded simultaneously in POm and Pf during 1 Hz stimulation (see Figure 11B), the mean latencies in POm (15.6 ± 0.8 ms) and Pf (21.1 ± 1.1 ms) confirmed that POm neurons responded before the neurons in Pf (paired one-tailed t = 5.23, p < 0.0001).

Among the 25 pairs of neurons recorded simultaneously in POm and Pf, most POm neurons responded to 2 Hz (n = 25), 5 Hz (n = 21), and 8 Hz (n = 14) stimulation, but fewer neurons responded in Pf during 2 Hz (n = 17), 5 Hz (n = 10), or 8 Hz (n = 2). Among the 17 pairs of responses recorded in POm and Pf simultaneously, the mean response latency in POm (16.6 ± 1.0 ms) was shorter than the mean latency in Pf (23.3 ± 1.0 ms) (paired one-tailed t = 4.88, p < 0.001). During 5 Hz stimulation, the 10 responsive pairs recorded simultaneously in POm and Pf indicated that mean response latency was shorter in POm (21.3 ± 1.5 ms) than in Pf (28.5 ± 1.0 ms) (paired one-tailed t = 3.63, p < 0.01). Because only two Pf neurons responded during 8 Hz stimulation, a meaningful matched-sample analysis could not be performed at this frequency.

To compare stimulus-induced habituation in POm and Pf, we counted the number of neuronal discharges during each stimulus period that exceeded the mean rate of spontaneous activity during the prestimulus period (see Methods). Results of this analysis, which are shown...
in Figure 12, indicate that neurons in both POM and Pf show some habituation as the rate of whisker stimulation increases to 8 Hz. An analysis of variance of stimulus-evoked response magnitudes in POM and Pf indicate that stimulus frequency has an effect on responsiveness (F = 9.04, p < 0.001), and that POM neurons are more responsive than Pf neurons (F = 54.86, p < 0.0001).

Because of clear differences between the raw response magnitudes of POM and Pf neurons, we also analyzed normalized values expressed as a ratio of the responses obtained at ≤1 Hz (see right bargraph in Fig. 12A). This analysis confirmed that the frequency of whisker stimulation had a significant effect on POM and Pf responsiveness (F = 17.11, p < 0.001), and that POM neurons habituate less than Pf neurons (F = 38.11, p < 0.0001).

Similar results were also obtained when the same analyses were conducted on neuron pairs in which responses from both thalamic nuclei were recorded simultaneously. As shown in Figure 12B, increases in stimulus frequency caused a significant amount of habituation (F ≥ 6.98, p < 0.001), and the amount of habituation was greatest for neurons in Pf (F ≥ 7.11, p < 0.01), regardless of whether we analyzed the raw or the normalized data.

In both brain regions we also analyzed whether stimulus order within each frequency block produced an effect on response latency or magnitude. An analysis of variance failed to detect any effect of stimulus order on latency or magnitude of responses at any frequency in either the Pf or POM (F ≤ 1.08, p ≥ 0.33) in all cases.

**DISCUSSION**

Our results demonstrate that whisker-sensitive sites in POM project to the DLS, but not to other striatal regions. This is significant because DLS is involved in the execution of highly-repetitive behaviors that do not depend on rewarded outcomes (Balleine and O’Doherty, 2010; Cromwell and Berridge, 1996; Yin et al., 2004, 2006). Whisking behavior is consistent with this
classification scheme, and the DLS receives dense inputs from the whisker representations in SI and MI cortex (Alloway et al., 2006; Hoffer and Alloway, 2001).

By comparison, the lateral Pf projects to both the DLS and the dorsomedial striatum. This is significant because the dorsomedial striatum mediates goal-directed behaviors (Yin et al., 2005a,b). Hence, when compared to the relatively focused thalamostriatal projections from POm, the projections from Pf should have a more general function.

Whisker-sensitive neurons in POm and Pf have distinct response properties that complement their differences in projection topography. While both thalamic regions display declines in responsiveness during repetitive whisker stimulation, neurons in POm habituate much less than neurons in Pf. Most neurons in POm responded to all the frequencies that we tested, but most Pf neurons responded only to 1 or 2 Hz. In addition, the latency of responses to whisker stimulation was much shorter in POm than in Pf. These facts indicate that POm conveys somesthetic information to the DLS with a higher degree of sensory fidelity. By comparison, the response properties and projection topography of the Pf nucleus indicates that this thalamic structure is not concerned principally with transmitting somesthetic-specific information, but is involved in more general aspects of striatal processing that affect the behavioral functions of both the dorsomedial and dorsolateral striatum. This is consistent with our observation that Pf neurons respond to both somesthetic and visual stimulation.

Rostrocaudal extent of POm and Pf projections. We found that thalamostriatal projections from Pf and the posterior POm differed in their rostrocaudal extent. Although projections from both thalamic regions overlapped substantially in DLS, the projections from the whisker-sensitive parts of POm were concentrated in the caudal DLS. By comparison, projections from Pf had a more extensive rostrocaudal distribution and were densest in striatal regions rostral to bregma.

The distribution of projections to the DLS from whisker-sensitive regions in POm matches the topography of corticostriatal projections from SI cortex. While SI barrel cortex innervates
the caudal DLS, the SI forelimb region innervates more rostral parts of the DLS (Hoffer and Alloway, 2001; Hoover et al., 2003). This topography is consistent with the fact that lesions in the rostral DLS interfere with behaviors that involve forelimb movements such as habitual bar-pressing and grooming behaviors (Cromwell and Berridge, 1996; Yin et al., 2004, 2006). Given that the POm whisker area projects to caudal DLS regions that receive inputs from SI barrel cortex, it is parsimonious to expect that POm forelimb sites probably project to more rostral parts of DLS that receive inputs from the SI forelimb area (Hoover et al., 2003).

**Functional role of Pf thalamostriatal projections.** Our tracing experiments demonstrate that whisker-sensitive regions in superior colliculus project to the Pf nucleus. Whisker-sensitive regions in Pf innervate widespread parts of the striatum, but respond only to low rates of whisker stimulation. These findings are consistent with the prevailing view that the Pf conveys unexpected sensory signals to the striatum (Galvan and Smith, 2011; Van der Werf et al., 2002). In primates, for example, the intralaminar nuclei respond to unexpected sensory stimuli but habituate when the same stimulus is repeated (Matsumoto et al., 2001).

Axon collaterals of individual Pf neurons form dense plexuses of terminals in local parts of the striatal neuropil (Deschenes et al., 1995; Lacey et al., 2007). Our bulk tracer injections in Pf extend these findings by revealing an extensive network of Pf projections throughout the dorsomedial and dorsolateral striatum, which are associated with the execution of goal-directed and stimulus-controlled behaviors, respectively.

Ultrastructural studies have shown that cholinergic interneurons in the striatum are innervated by the intralaminar thalamic nuclei, but not by corticostriatal projections (Meredith and Wouterlood, 1990; Lapper and Bolam, 1992). Activation of the intralaminar thalamus evokes a pause among tonically-active cholinergic neurons that resembles the behavioral pause evoked by salient stimuli in awake, behaving monkeys (Apicella, 2007; Ding et al., 2010). Synchronous activation of cholinergic interneurons facilitates the indirect, but not the direct, striatal pathways (Ding et al., 2010; Threlfell et al., 2012), and this suggests that Pf activation
could interrupt an ongoing behavior so that a new behavior can be selected that is more appropriate in the context of an unexpected stimulus (Thorn and Graybiel, 2010).

**Functional role of POm thalamostriatal projections.** The posterior POm nucleus receives whisker-related information from the trigeminal nuclei and projects to both the DLS and SI barrel cortex (Deschenes et al., 1995; Smith et al., 2012). Furthermore, whisker-sensitive regions in DLS receive projections from SI barrel cortex (Alloway et al., 1999, 2006; Hoffer and Alloway, 2001; Mowery et al., 2011). In fact, most corticostriatal projections to the DLS originate from layer Va (Alloway et al., 2006; Reiner et al., 2003), which is the main laminar target of thalamocortical projections from POm (Lu and Lin, 1993; Smith et al., 2012; Wimmer et al., 2010). Hence, POm innervates both DLS and the cortical neurons that project to the DLS, thereby forming a tightly-connected circuit that could monitor the somesthetic inputs that accompany an ongoing behavioral activity.

Our previous work indicates that most thalamic inputs to whisker-sensitive regions in the DLS originate from the posterior POm and, to a lesser extent, from Pf (Smith et al., 2012). We also showed that neurons in the caudal DLS respond to whisker movements and display little habituation when the whiskers are repetitively deflected at frequencies that are prominent during exploratory whisking behavior (Mowery et al., 2011). Related to these results, the present study indicates that POm responds before Pf and shows less habituation than Pf during repetitive whisker stimulation. This indicates that POm transmits somesthetic information with a higher degree of fidelity than the Pf nucleus.

These findings are significant because the DLS is involved in the expression of sensorimotor habits mediated by S-R associations that are often chained together to form a stereotyped behavioral pattern (Balleine and O’Doherty, 2010; Graybiel, 2008). Sensorimotor behaviors such as grooming and exploratory whisking are characterized by a series of highly-repetitive movements in which high-fidelity somesthetic feedback for each successive motor element could serve as a sensory stimulus for evoking the next motor response.
Based on this reasoning, somesthetic inputs from POm could play a critical role in the sequential execution of repetitive movements that characterize sensorimotor habits or other stereotyped behavioral patterns mediated by the DLS. We hypothesize that POm projections to the DLS transmit “expected” somesthetic signals that accompany the motor elements that are kinematically-linked during the execution of highly-repetitive behaviors such as grooming, whisking, or other fixed action patterns. In contrast to thalamostriatal projections from Pf, which probably interrupt ongoing behavior, the projections from POm cooperate with corticostriatal signals from SI and MI to facilitate and maintain the automatic sequence of movements that comprise an ongoing, highly-repetitive sensorimotor activity.

When an unexpected highly-salient stimulus occurs, we hypothesize that activation of the superior colliculus should excite both the Pf nucleus and the zona incerta. Given that the zona incerta sends inhibitory projections to the POm nucleus (Bartho et al., 2002), simultaneous activation of the Pf and ZI should have complementary effects on behavior. While Pf activation represents one mechanism for pausing striatal processing, collicular activation of the zona incerta represents a mechanism for inhibiting POm output, thereby disengaging thalamostriatal projections to the DLS that help maintain an ongoing sensorimotor behavior.

**Technical Considerations.** Few studies have recorded neuronal responses in the POm of intact rats during controlled whisker stimulation (Masri et al., 2008; Sosnik et al., 2001; Smith et al., 2012). The POm is difficult to activate because it receives inhibitory inputs from the zona incerta (Bartho et al., 2002), which is activated by some of the same neural circuits that project to the POm (Lavallee et al., 2005). In fact, lesions of the zona incerta increase POm responsiveness to whisker stimulation and allow POm to display short-latency responses to whisker deflections even when somatosensory cortex is inactivated (see Fig. 4 in Lavallee et al., 2005). The inhibitory output of the zona incerta is suppressed during excitation of the cholinergic brainstem activation system (Tragesar et al., 2006), which suggests that POm responsiveness is state-dependent. Consistent with this view, POm does not respond well during anesthesia, and POm
responses in the anesthetized state deteriorate even further if excitatory feedback from
somatosensory cortex is blocked (Diamond et al., 1992). Given that anesthesia reduces POm
responsiveness, and that the DLS is a postsynaptic target of the POm, it is not surprising that
DLS neurons do not respond to somatosensory stimulation during deep anesthesia (West, 1998).

For these reasons, we developed a lightly-anesthetized rat preparation so that we could evoke
responses in POm and DLS. In our recent studies (Mowery et al., 2011; Smith et al., 2012), we
found that POm and DLS neurons respond to repetitive whisker stimulation with little
adaptation. By comparison, regular spiking neurons in SI barrel cortex show much more
habituation when the whiskers are deflected repetitively at frequencies of 5 Hz or more (Mowery
et al., 2012).

Nonetheless, POm and DLS responses display variability in our studies. As indicated by
Figure 13A, when whiskers were stimulated at \( \leq 1 \) Hz, mean response latency in POm was 6.7% longer in the present study than in our previous report (Smith et al., 2012). In addition, Figure 13A shows that DLS response latencies varied by 28% in the two studies in which we compared DLS responses with those recorded in SI or POm (Mowery et al., 2011; Smith et al., 2012).

Variations in the latencies of POm responses to whisker deflections have been reported by
other labs as well. While some report that POm response latencies are correlated with the
frequency of whisker stimulation (Sosnik et al., 2001), others suggest that response latencies in
POm are not affected by the frequency of whisker movements (Masri et al., 2008). These
discrepancies have prompted a serious discussion about the methods that are most appropriate
for analyzing the coding mechanisms performed by POm (Ahissar et al., 2008).

A comparison of POm responses in the present study with our earlier work (Smith et al.,
2012) indicates that increasing the frequency of whisker stimulation produces an increase in the
latency of the POm responses (see POm responses in panels B and C in Figure 13).
Furthermore, the frequency-induced change in latency varies noticeably across our two studies.
Several factors could account for the variations observed across different studies, including those from our own lab. Our studies were performed by different sets of individuals, and small differences in the placement of the whisker stimulator, especially its proximity to the base of the whiskers, could easily change the latency of POm and DLS responses. This is possible because stimulus velocity was relatively slow (~ 60 mm/s), and slight changes in the distance between the stimulus and the base of the whisker (usually ~10 mm) would alter the angle of the whisker deflection and its impact on the afferent fibers that innervate the whiskers.

As indicated previously, anesthetic state has a critical effect on POm responsiveness. Although ECoG recordings were used to indicate a lightly-anesthetized state, variations in ECoG frequencies are inherent across different experiments, and this could contribute to differences in the neuronal responses that we recorded.

Because of these factors, it is critical that any analysis of regional differences in neuronal responses is conducted on pairs of neurons that are recorded simultaneously. As indicated by Figure 13, we have used this approach in our analysis of corticostriatal and thalamostriatal circuits because it allows comparisons of neuronal responses in separate brain regions when all other aspects of the experimental protocol are identical.

The present study demonstrates that POm neurons respond to whisker deflections before neurons in Pf. Similarly, as indicated by Figure 13B, POm neurons discharge before neurons in the DLS when both regions are recorded simultaneously (Smith et al., 2012). In addition, the DLS responds at the same time as SI when the whiskers are deflected at low rates, but responds before SI when rates increase to 5 or 8 Hz (see Figure 10 in Mowery et al., 2011). These facts strongly suggest that DLS neurons can be activated by thalamostriatal projections from POm.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Gabrielle M. Brennan for helping with the histology.
GRANTS
This work was supported by the National Institute of Neurological Disorders and Stroke Grant NS-37532 awarded to K.D. Alloway.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.
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FIGURE LEGENDS

Figure 1. Tracer deposit in the superior colliculus. A: Peristimulus time histogram (PSTH) showing whisker-sensitive responses in the superior colliculus during whisker deflections at ≤1 (filled circle), 2, 5, and 8 Hz frequencies; binwidths: 10 ms. Inset: mean neuronal waveform, scale: 50 μV, 1 ms, dots: first stimulus in each stimulus block is classified as ≤1 Hz. B, B’: Adjacent coronal sections show the recording location (arrow) and tracer deposit using the same tracer-filled pipette that recorded the response in panel A. Rectangle indicates the region shown in panel C. C: Overlapping deposits of Fluoro-Gold (FG) and biotinylated dextran amine (BDA) in the intermediate layers of the superior colliculus. Scale bars: 500 μm in B; 250 μm in C.

Figure 2. Afferent and efferent connections of whisker-sensitive region in the superior colliculus. A: Coronal section processed for cytochrome oxidase shows the fasciculus retroflexus (fr) and parafascicular (Pf) nucleus. A’, B: Adjacent section showing BDA-labeled terminals in Pf and the zona incerta (ZI) produced by the tracer deposit in Figure 1. C: Coronal section showing location of the spinal trigeminal nucleus, pars interpolaris (SpT-I). C’, D: FG-labeled neurons in SpT-I. Abbreviations: dsc, dorsal spinocerebellar tract; spt, spinal trigeminal tract; ml, medial lemniscus. Scale bars: 500 μm in A; 250 μm in B, C; 100 μm in D.

Figure 3. Whisker-responsive regions in superior colliculus project to regions in zona incerta (ZI) that project to whisker-responsive sites in POm. A, B: Low and high power views of the dorsal and ventral ZI in a CO-labeled coronal section from the injection case depicted in Figure 1, in which BDA was placed at a whisker-responsive site in the superior colliculus. B’: Adjacent section illustrating dense BDA-labeled terminals in the ventral ZI, which is located directly above the subthalamic nucleus (STN). This section is located 180 μm rostral to the section shown in Figure 2A’. C, D: Low and high power views of the ZI in a rat in which BDA was placed in a whisker-responsive region in POm (case TS16 from Smith et al., 2012). D’: Adjacent section illustrating BDA-labeled neurons in the ventral ZI region located above the
STN. Scale bars: 500 μm for panels A, C; 250 μm for panels B, B’, D, D’. Abbreviations: cp, cerebral peduncle; fr, fasciculus retroflexus; ml, medial lemniscus; mt, mammillothalamic tract; Pf, Parafascicular nucleus. Arrow heads indicate the same blood vessels.

**Figure 4.** Tracer deposit in the lateral parafascicular (Pf) nucleus. A: Pf response recorded from a BDA-filled pipette during repetitive whisker stimulation; binwidths, 10 ms. Inset: mean neuronal waveform; scales: 100 μV, 1 ms. B: Coronal section depicting the Pf nucleus and surrounding region; rectangle indicates the region in C. C: Recording site in Pf marked by necrosis (arrow). C’: Adjacent section illustrating the BDA deposit at the Pf recording site. Scale bars: 1.0 mm in B; 500 μm in C.

**Figure 5.** Reconstructions of the striatal innervation pattern produced by the Pf tracer deposit in Figure 4. Numbers indicate distance (in mm) from bregma. Scale, 1.0 mm.

**Figure 6.** Tracer deposit in POm. A: The PSTH illustrates the POm response recorded from the tracer-filled pipette during repetitive whisker stimulation; binwidths, 10 ms. Inset: mean neuronal waveform; scales: 200 μV, 1 ms. B: Coronal section through POm nucleus and surrounding thalamic regions. Rectangle indicates the region depicted in panel C. C: Recording site in POm marked by necrosis (arrow). C’: Adjacent section showing the BDA deposit at the POm recording site. Scale bar: 1.0 mm in B; 500 μm in C. VPM, ventroposteromedial nucleus.

**Figure 7.** Reconstructions of the striatal innervation pattern produced by the POm tracer deposit in Figure 6. Numbers indicate distance (in mm) from bregma. Scale, 1.0 mm.

**Figure 8.** Rostrocaudal series of striatal innervation patterns produced by tracer deposits in Pf (top) and POm (bottom). Each series was produced by superimposing plotted reconstructions obtained from three rats in each set of injections (ie., Pf and POm). Abbreviations: A, amygdala; GP, globus pallidus; ic, internal capsule; lv, lateral ventricle.

**Figure 9.** Quantitative comparisons of thalamostriatal projections from Pf and POm. A: Bargraphs showing the relative volumes of tracer injections in POm and Pf compared to the relative volumes of the striatal regions innervated by labeled terminals. B: Same data in panel A.
expressed as the ratio of striatal labeling to the size of the tracer deposits in POM and Pf. Brackets represent SEM; number of injected rats in each group indicated by legend.

**Figure 10.** Simultaneous neuronal responses in POM and Pf during whisker deflections at \( \leq 1, 2, 5, \) and 8 Hz. **A:** Coronal sections showing location of recording sites in POM (top) and Pf (bottom) indicated by arrowheads pointing to necrosis and electrode tracks. Scales, 500 \( \mu \)m. **B:** PSTHs display neuronal responses to repetitive whisker stimulation in POM (top) and Pf (bottom); binwidths, 10 ms. Insets show mean neuronal waveforms: scales, 200 \( \mu \)V, 1 ms. **C:** Mean frequency-specific PSTHs display the stimulus-induced responses in POM and Pf; binwidths, 1 ms. Horizontal dashed lines indicate 99% confidence limits; vertical dashed lines indicate the latency of the POM response at each stimulus frequency.

**Figure 11.** Comparison of response latencies in POM and Pf during \( \leq 1 \)-Hz whisker deflections. **A:** Cumulative distributions comparing the response latencies of all neurons recorded from POM (n = 43) and Pf (n = 31). **B:** Scatter plot illustrating latency differences for neuron pairs (n = 25) recorded in POM and Pf simultaneously. Large dot indicates where data from two pairs are superimposed.

**Figure 12.** Comparison of response adaptation in POM and Pf during whisker stimulation at different frequencies. **A:** Habituation among all neurons recorded in POM and Pf. Left bargraph shows change in mean responsiveness of POM (n = 43) and Pf (n = 31) neurons as a function of increasing frequency of whisker stimulation. Right bargraph shows same data normalized with respect to the mean response obtained at 1 Hz. **B:** Habituation among matched pairs of POM and Pf neurons recorded simultaneously. Left bargraph shows change in mean responsiveness of pairs of POM and Pf neurons (n = 25) recorded simultaneously. Right bargraph shows same data normalized with respect to the mean response obtained during 1 Hz.

**Figure 13.** Variations in response latencies **A:** Comparison of mean latencies in which pairs of neurons were recorded simultaneously in Pf and POM (25 pairs, present study), in POM and DLS (14 pairs, Smith et al., 2012), and in DLS and SI (24 pairs, Mowery et al., 2011) during \( \leq 1 \)-
Hz whisker stimulation. **B:** Comparison of response latencies in POM and DLS as a function of stimulus frequency; 14 neuron pairs from Smith et al., 2012. **C:** Effect of increases in stimulus frequency on POM responses recorded in the present study; 25 neurons at ≤ 1-Hz, 25 neurons at 2-Hz, 21 neurons at 5-Hz, 14 neurons at 8-Hz. Dashed lines indicate identical data displayed twice.