Thalamic POM projections to the dorsolateral striatum of rats: potential pathway for mediating stimulus–response associations for sensorimotor habits

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Smith JB, Mowery TM, Alloway KD. Thalamic POM projections to the dorsolateral striatum of rats: potential pathway for mediating stimulus–response associations for sensorimotor habits. J Neurophysiol 108: 160–174, 2012. First published April 11, 2012; doi:10.1152/jn.00142.2012.—The dorsolateral part of the striatum (DLS) represents the initial stage for processing sensorimotor information in the basal ganglia. Although the DLS receives much of its input from the primary somatosensory (SI) cortex, peripheral somesthetic stimulation activates the DLS at latencies that are shorter than the response latencies recorded in the SI cortex. To identify the subcortical regions that transmit somesthetic information directly to the DLS, we deposited small quantities of retrograde tracers at DLS sites that displayed consistent time-locked responses to controlled whisker stimulation. The neurons that were retrogradely labeled by these injections were located mainly in the sensorimotor cortex and, to a lesser degree, in the amygdala and thalamus. Quantitative analysis of neuronal labeling in the thalamus indicated that the strongest thalamic input to the whisker-sensitive part of the DLS originates from the medial posterior nucleus (POM), a somesthetic-related region that receives inputs from the spinal trigeminal nucleus. Anterograde tracer injections in POM confirmed that this thalamic region projects to the DLS neuronal. In subsequent experiments, simultaneous recordings from POM and the DLS during whisker stimulation showed that POM consistently responds before the DLS. These results suggest that POM could transmit somesthetic information to the DLS, and this modality-specific thalamostriatal pathway may cooperate with the thalamostrital projections that originate from the intralaminar nuclei of the basal ganglia; neuronal tracing; somatosensory; tactile stimulation; thalamostrital; thalamus.

SUBSTANTIAL EVIDENCE INDICATES that the striatum is involved in regulating the selection and execution of specific motor behaviors (McHaffie et al. 2005; Redgrave et al. 2010; Smith et al. 2011). Furthermore, although the dorsomedial striatum is important for mediating goal-directed behaviors, the dorsolateral striatum (DLS) is necessary for executing well-learned sensorimotor habits (Aldridge and Berridge 1998; Balleine and Smith 2011). Furthermore, although the dorsomedial striatum is important for mediating goal-directed behaviors, the dorsolateral striatum (DLS) is necessary for executing well-learned sensorimotor habits. As such, it has the hallmarks of a species-specific motor habit that does not depend on rewarded outcomes. As such, it has the hallmarks of a species-specific motor habit that is executed, in part, by neural mechanisms in the DLS (Gao et al. 2001; Welker 1964). Consistent with this view, unexpected whisker contacts with external stimuli evoke stereotyped patterns of whisker movements that seem to reflect the formation of a stimulus–response association (Mitchinson et al. 2007; Sachdev et al. 2003). The importance of somesthetic information in regulating whisking behavior and other well-learned motor habits is underscored by the fact that the DLS receives dense, overlapping projections from the primary somatosensory (SI) barrel cortex and other somatosensory cortical areas (Alloway et al. 2000, 2006; Brown 1998; Hoffer and Alloway 2001).

These dense corticostriatal projections have prompted recent comparisons of cortical and DLS responses during controlled whisker stimulation (Mowery et al. 2011; Pidoux et al. 2011; Syed et al. 2011). Findings from our laboratory indicate that repetitive whisker deflections evoke consistent responses in the DLS that are qualitatively different from the responses recorded simultaneously in the SI barrel cortex (Mowery et al. 2011). Whereas SI cortical responses decline in magnitude as the frequency of whisker stimulation increases (Aliassar et al. 2001; Chakrabarti and Alloway 2009; Khatri et al. 2004; Melzer et al. 2006), the responses of the DLS neurons remain relatively constant. Furthermore, analysis of the response latencies shows that DLS neurons respond either before or at the same time as the neurons in the SI barrel cortex. These findings strongly suggest that subcortical regions must be involved in transmitting somesthetic information to the DLS.

Tracing studies have identified many subcortical regions that project to the rodent striatum, including the amygdala, the substantia nigra pars compacta (SNpc), and several intralaminar and modality-specific thalamic nuclei (Alloway et al. 2006; Cheatwood et al. 2005; Erro et al. 2001, 2002; Kelley et al. 1982; Pan et al. 2010; Redgrave and Gurney 2006). No study, however, has quantified the relative contributions of these subcortical inputs to the whisker-sensitive regions in the DLS. Furthermore, although the DLS and other parts of the striatum receive thalamic projections from the centromedial and parafascicular nuclei (Castle et al. 2005; Deschenes et al. 1996; Smith et al. 2009), there are conflicting data regarding whether the DLS receives somesthetic-related projections from the medial posterior (POM) and ventral posteromedial (VPM) thalamic nuclei (Alloway et al. 2006; Deschenes et al. 1995; Erro et al. 2001, 2002). To establish whether thalamostriatal projections could transmit somesthetic information directly to DLS, we injected retrograde and anterograde tracers into whisker-responsive regions of the DLS and thalamus, respectively. Our results demonstrate that POM, but not VPM, projects to whisker-sensitive parts of the DLS. Furthermore, simultaneous recordings of whisker-sensitive neurons in POM and the DLS indicate that POM responds to whisker deflections immediately before the DLS is activated.

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MATERIALS AND METHODS

Animals. Experiments were performed on male Sprague–Dawley rats weighing 250–700 (465 ± 18) g. All surgeries and other procedures complied with National Institutes of Health (NIH) guidelines and were approved by the Institutional Animal Care and Use Committee.

Rat surgery. Rats were sedated with an intramuscular (IM) injection of ketamine (40 mg/kg) and xylazine (12 mg/kg). Atipamezole methyl nitrate (0.05 mg/kg), dexamethasone sodium phosphate (5 mg/kg), and chloramphenicol (50 mg/kg) were administered IM, and all rats were orally intubated before being placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Heart rate, blood oxygen saturation, and end-tidal CO₂ were monitored continuously, and body temperature was maintained at 37°C by a heated water pad and a homeothermic heating blanket. The scalp was infiltrated with lidocaine, and a midline incision was made to expose the cranial surface. Flat machine screws were inserted over the left frontal cortex for electrocorticography (ECoG), and over the cerebellum to provide a ground lead for neuronal recordings and iontophoretic tracer injections. A 1-mm² craniotomy was made over the DLS or thalamus to enable neuronal recordings and tracer injections.

In all experiments, a data acquisition system (SciWorks, ver. 6.0; DataWave Technologies, Broomfield, CO) provided on-line ECoG displays to indicate the anesthetic state of the rat (Friedberg 1999). Activity recorded from the dorsal surface of the frontal cortex was amplified, filtered (0.3–300 Hz), and sampled at 256 Hz by an analog to digital board (DT2839; Data Translation, Marlboro, MA). A color-coded fast Fourier transform of ECoG activity was displayed and updated once per second to visualize changes in cortical frequencies. Power spectra dominated by 1–2 Hz were observed during deep anesthesia when the rat was unresponsive to noxious stimuli. As ketamine and xylazine were metabolized, the power spectra shifted to frequencies of 5–7 Hz, which indicates a lightly anesthetized state (Friedberg et al. 1999). In this state, whisker stimulation evoked frequencies of 5–7 Hz, which indicates a lightly anesthetized state with preserved neuronal discharges in the DLS. Low levels of isoflurane (0.50 –1.0%) (Friedberg et al. 1999). In this state, whisker stimulation evoked frequencies of 5–7 Hz, which indicates a lightly anesthetized state with preserved neuronal discharges in the DLS.

Activity recorded from the dorsal surface of the frontal cortex was amplified, filtered, and sampled at 256 Hz by an analog-to-digital board (DT2839; Data Translation, Marlboro, MA). A color-coded fast Fourier transform of ECoG activity was displayed and updated once per second to visualize changes in cortical frequencies. Power spectra dominated by 1–2 Hz were observed during deep anesthesia when the rat was unresponsive to noxious stimuli. As ketamine and xylazine were metabolized, the power spectra shifted to frequencies of 5–7 Hz, which indicates a lightly anesthetized state (Friedberg et al. 1999). In this state, whisker stimulation evoked frequencies of 5–7 Hz, which indicates a lightly anesthetized state with preserved neuronal discharges in the DLS.

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regions, we plotted all sections through the thalamus to obtain an accurate count of labeled neurons in all of the thalamic nuclei. The normalized proportion of labeled neurons in each thalamic nucleus was calculated as described earlier.

For anterograde tracing experiments, sections through the DLS were examined for the presence of BDA-labeled beaded varicosities that represent en passant synapses (Kincaid and Wilson 1996; Meng et al. 2004; Voight et al. 1993). Labeling of thalamic projections to the DLS and cortex were photographed, and digital reconstructions of the BDA-labeled varicosities were plotted relative to anatomical landmarks as described previously (Alloway et al. 2009).

Neurophysiology recordings. Neuronal discharges were recorded from whisker-sensitive neurons in all rats. In the tracing experiments, tracer-filled pipettes were used to record neuronal discharges from whisker-sensitive regions in the thalamus or DLS before the tracer was injected. In the remaining experiments, whisker-sensitive neurons in the DLS and POm were recorded simultaneously with two high-impedance (1–2 megohms) tungsten electrodes to compare the onset of well-isolated neuronal responses in these regions during controlled whisker stimulation.

Prior to simultaneous recordings from the DLS and POm, an acrylic headstage was constructed over the occipital ridge while the rat’s head was held by stereotaxic ear bars. After exposing the cranium, holes were drilled for electrode penetrations into the DLS and POm. After applying dental acrylic (Hygenic, Akron, OH) over small screws in the occipital ridge, two small bolts were placed head down in the acrylic, approximately 10 mm apart. After the acrylic cured for 10 min, a gooseneck manipulator (Flexbar Machine Corp., Islandia, NY) was fastened to each bolt with a nut. Subsequently, the stereotaxic ear bars were withdrawn to remove nociceptive inputs originating from the external auditory meatus. Consequently, low concentrations of isoflurane (0.5–1.0%) were sufficient to maintain each rat in a stable, lightly anesthetized plane that facilitated detection of whisker-evoked neuronal responses in the DLS and POm. Although isoflurane produces a dose-dependent suppression of thalamocortical transmission, the concentration of isoflurane used for simultaneous recordings from the POm and DLS was minimized and was well below levels that produce significant suppression of thalamic responses (Detrick et al. 1999, 2002; Masamoto et al. 2009).

Craniotomies for simultaneous recordings in the DLS and POm were made at the same coordinates used to inject tracers into these regions (see TRACER INJECTIONS). A single electrode penetration was made at the same coordinates used to inject tracers into these regions (Kincaid and Wilson 1996; Meng et al. 2004; Voight et al. 1993). Labeling of thalamic projections to the DLS and cortex were photographed, and digital reconstructions of the BDA-labeled varicosities were plotted relative to anatomical landmarks as described previously (Alloway et al. 2009).

Whisker stimulation. Multiple vibrissae (rows A–E, arcs 1–5) were stimulated in tandem by a series of computer-controlled movements. As described previously (Mowery et al. 2011), a small screen attached to a galvanometer was positioned near the whisker pad (~10 mm away) so that the whiskers protruded through the screen openings. A waveform generator (ArbStudio; LeCroy, Chestnut Ridge, NY) controlled the movements of the galvanometer. In the initial experiments, each trial consisted of three groups of four 50-ms triangular waves presented at frequencies of 2, 5, and 8 Hz. In later experiments, a sequence of four 50-ms triangular stimuli were presented in which the interstimulus intervals decreased so that successive stimuli on each trial were presented at intervals of 500 ms (2 Hz), 200 ms (5 Hz), and 125 ms (8 Hz). In both sets of recording experiments, the first stimulus in each block of four stimuli was classified as a 1-Hz stimulus because it was preceded by an interval of 1 s or longer in which no stimuli were administered. Each stimulus moved the whiskers in the caudal direction (1.5 mm) during the first 25 ms and then allowed them to return to the original resting position over the next 25-ms period.

RESULTS

Results were obtained from a total of 24 rats. As shown in Fig. 1, whisker-sensitive sites in the DLS of 7 rats received focal deposits of FG to reveal retrogradely labeled neurons in all brain regions that project to this part of the DLS. Subsequently, in a second group of rats (n = 10), an anterograde tracer was placed in somesthetic-specific nuclei of the thalamus to confirm the retrograde tracing results. In the last set of experiments (n = 7), neuronal discharges were recorded simultaneously in POm and DLS during controlled whisker stimulation.

Retrograde tracer injections in DLS. An example of FG deposit at a whisker-sensitive site in the DLS is illustrated in Fig. 2. The neuronal response at this injection site displayed clear responses to repetitive whisker stimulation at 2.5, or 8 Hz and showed minimal adaptation at these frequencies (Fig. 2, A and B). Although on-line neuronal isolation was often difficult to achieve with tracer-filled pipettes, off-line waveform sorting helped isolate responses that were consistent with our previous results (Mowery et al. 2011). After recording neuronal responses to controlled whisker stimulation, the tracer was iontophoretically deposited (Fig. 2D) from the same pipette while it was positioned at the recording site. Occasionally, the recording-deposit site was marked by a small amount of necrosis (Fig. 2D).

In the seven cases used to analyze retrograde labeling patterns, tracer leakage did not appear along the pipette trajectory. The absence of tracer leakage rules out the possibility that any labeled neurons represent projections to the tissue surrounding the electrode penetration. For all seven cases, tracer injections were made 1.4 to 2.0 mm caudal to bregma and were located entirely within the DLS without any diffusion into the external capsule. Tracer deposits were located in the neuropil...
and appeared to avoid the fiber fascicles that contain corticothalamic and thalamocortical projections.

Retrograde labeling throughout the CNS. Neuronal cell bodies labeled by FG injections in the DLS were plotted in both hemispheres for all brain regions. Differences in the spatial extent of the tracer injections in the DLS led to differences in the total number of labeled neurons. Among the seven rats that received FG deposits in the DLS, we plotted an average of 10,012 \pm 3,619 (mean \pm SE) neurons throughout the brain. Although the number of FG-labeled neurons varied with the size of the tracer injections, virtually the same brain regions contained labeled neurons in each case. Brain regions that consistently contained neuronal labeling included sensorimotor cortex, globus pallidus (GP), amygdala, substantia nigra pars compacta (SNpc), the dorsal raphe, and several thalamic nuclei. In cases that received the largest DLS injections, some neuronal labeling appeared in limited parts of cortex that were interpreted to be the insular, auditory, and visual cortical regions.

The densest retrograde labeling in the cortex appeared in areas that are associated with sensorimotor functions (Paxinos and Watson 2005). In these regions, cortical labeling was most prominent in layer Va (Fig. 3, A, B, B’, and C). This labeling was distributed bilaterally, but most of the labeled neurons were in the ipsilateral hemisphere, which is consistent with our previous results (Alloway et al. 2006).

In contrast to that previous study, in which tangential cortical sections were processed for cytochrome oxidase, the boundaries of many cortical areas were not clearly demarcated in the coronal sections obtained in the present study. Nonetheless, cortical labeling patterns in the two studies appeared similar. As in our previous report (Alloway et al. 2006),
approximately 40% of the cortical neuronal labeling appeared in regions identified as motor cortex (Paxinos and Watson 2005), and slightly more than half of the cortical labeling was in somatosensory regions directly above the DLS. This densely labeled region probably represented the combination of the SI and SII cortical areas. The remaining cortical labeling was more scattered and appeared laterally in regions that represent the parietal ventral and perirhinal regions.

Compared with labeling in the presumed SI and SII regions, which was predominantly on the ipsilateral side, neuronal labeling in MI was more evenly distributed across both hemispheres. This finding is consistent with the presumed roles of MI cortex and the striatum in coordinating the bilateral, synchronous movements of the whiskers during behavioral exploration (Alloway et al. 2009). As shown in Fig. 3, FG-labeled neurons appeared in the amygdala of both hemispheres but were predominantly on the ipsilateral side. On each side, neuronal labeling in the amygdala was restricted to the magnocellular and intermediate portions of the basal nucleus; the central, lateral, medial, and cortical nuclei did not contain any labeled neurons.

The ipsilateral SNpc contained many large, brightly labeled neurons with oblong soma and intermingled dendritic processes that were predominantly oriented in the mediolateral dimension (Fig. 3, F, F′, G, and H). In all rats, labeled neurons in the SNpc appeared in two separate clusters that resided in the dorsal and lateral subnuclei. Based on their projections to the DLS, labeled neurons in the SNpc were presumed to be dopaminergic, but this could not be confirmed because sections were not processed for the presence of tyrosine hydroxylase.

Although we occasionally observed FG-labeled neurons in the SNpc located contralateral to the DLS injection sites, the number of midline-crossing nigrostriatal projections accounted for <1% of all labeled neurons in the SNpc. This result is consistent with reports indicating that few dopaminergic neurons in the SNpc project to the striatum in the contralateral hemisphere (Consolazione et al. 1985; Pritzel et al. 1983).

Additional retrograde labeling was observed in the GP and raphe nuclei. In agreement with other studies (Pan et al. 2010), only a small number of labeled neurons appeared in the midline dorsal raphe. Although more labeled neurons appeared in the GP than in the dorsal raphe, the combined sum of labeled neurons in both regions accounted for only 1% of the total number of labeled neurons plotted in each rat.

Retrograde labeling in the thalamus. Retrogradely labeled neurons were observed in several thalamic nuclei of each rat. Consistent with the known topography of thalamostriatal projections (Berendse and Groenewegen 1990), labeled neurons were located in the parafascicular (Pf), centromedian (CM), and ethmoid nuclei. In addition to these intralaminar regions, many labeled neurons also appeared in modality-specific regions, including the ventrolateral (VL), ventral posterolateral (VPL), ventral posteromedial (VPM), lateral posterior (LP), and POM nuclei.
As shown by the photomicrographs and plotted reconstructions in Figs. 4 and 5, neuronal labeling was most prominent in the POm, Pf, and CM nuclei. Coronal sections that contained the Pf and POm nuclei usually contained labeled neurons in both of these regions. In the Pf nucleus, scattered clusters of neurons with small, brightly lit cell bodies appeared just lateral to the fasciculus retroflexus (Fig. 4, C’ and D’). More laterally, larger numbers of FG-labeled neurons were present throughout the nucleus POm, with the densest clusters located near the VPM border (Fig. 4, E’ and F).

**Anterograde tracer injections in the thalamus.** Both POm and VPM process somesthetic information received from the trigeminal nuclei (Chiaia et al. 1991; Peschanski 1984; Timofeeva et al. 2004; Veinante et al. 2000a). Consequently, either of these thalamic nuclei could transmit whisker-related information directly to the DLS. To rule out the possibility that the retrogradely labeled neurons in these thalamic nuclei might represent tracer uptake by fibers of passage, we performed some anterograde tracing experiments. The anterograde tracer BDA or a mixture of BDA and FG was injected into POm, VPM, or VPL of 10 rats (see Table 1). Mixing FG with BDA enhanced the retrograde transport of BDA, thereby enabling BDA transport in both the anterograde and retrograde directions.

**Thalamostriatal projections from POm.** As indicated in Table 1, a total of four rats received tracer deposits entirely within the POm nucleus. Figure 6 illustrates a case that received the largest injection of BDA in the POm. Consistent with other reports (Diamond et al. 1992b), neurons recorded at this and other POm injection sites displayed responses to multiple whiskers. As indicated by Fig. 6A, POm neurons occasionally contained more than one response for each stimulus when whiskers were deflected at 2 Hz. In view of evidence that POm receives both descending projections from the SI cortex and ascending projections from the trigeminal nuclei (Ahissar 1998; Alloway 2008), these dual responses could reflect corticothalamic feedback that is asynchronous with respect to the ascending sensory input. Regardless of the exact mechanism that mediates this temporal pattern, our histology confirmed that BDA was injected into the POm and that it diffused throughout much of the nucleus (Fig. 6, C and C’). Consistent with a previous report (Deschenes et al. 1998), inspection of the thalamus revealed large numbers of BDA-
labeled fibers originating from POM that traversed the VPM en route to the DLS and cortex. In addition, we also observed retrogradely labeled neurons in the interopolaris and other spinal trigeminal nuclei, which is consistent with tracer injections in the POM (Chiaia et al. 1991; Peschanski 1984).

The mixture of FG and BDA revealed both anterograde and retrograde labeling in SI barrel cortex (see Fig. 6, D, E, and E’). Compared with adjacent sections processed for cytochrome oxidase, the BDA-labeled terminals were densest in layer Va, and this pattern replicates previous anterograde tracing data (Wimmer et al. 2010). Like other reports that analyzed corticothalamic projections to the POM (Killackey and Sherman 2003; Veinante et al. 2000b), retrogradely labeled neurons were predominantly in layer Vb and VIb of the SI cortex, with additional neuronal labeling in layer VIa of the septal-aligned columns. Terminal labeling from the POM injections was also observed in motor cortical areas that contain the whisker representations (Alloway et al. 2009).

Injections of BDA into the POM revealed dense bundles of axons and their terminal arbors throughout the posterior DLS (Fig. 7). High-power photomicrographs revealed labeled terminals in the DLS neuropil that displayed small (<1 μm) beaded varicosities representing en passant synaptic contacts (Fig. 7C). Reconstructions of these axonal enlargements indicate that thalamostriatal projections from POM terminate most densely along the edge of the DLS that adjoins the external capsule. Furthermore, in every case that received a BDA deposit in the POM (n = 5), small puffs of BDA-labeled terminal arbors were apparent in the DLS neuropil as described previously (Deschenes et al. 1995). These data validate the retrograde tracing results showing that POM projects to the whisker-sensitive parts of the DLS.

Absence of thalamostriatal projections from VPM. As indicated in Table 1, a total of three rats received tracer deposits that were located entirely within the VPM. Figure 8 illustrates a tracer deposit at a VPM site that responded primarily to deflections of a single whisker, and similar receptive field properties were recorded in other VPM cases. As indicated by the PSTH obtained at the injection site (see Fig. 8A), the neuronal response to repetitive whisker stimulation was robust and displayed little adaptation to progressive increases in stimulus frequency. In all cases, tracer deposits in the VPM produced dense thalamocortical terminal labeling in the layer IV barrels and labeled many corticothalamic neurons whose soma were located in layer VIa (see Fig. 9). These results corroborate previous reports that characterized VPM connections with the SI barrel cortex (Killackey and Sherman 2003; Veinante et al. 2000b; Wimmer et al. 2010).

Microscopic inspection of the DLS revealed bundles of labeled fibers en route to the overlying cortex, but did not reveal any axonal or beaded varicosities in the DLS neuropil. Similar results were obtained in all cases in which tracers were injected into the VPM or VPL nuclei. These findings support previous data indicating that VPM neurons send their axonal projections through the striatum en route to the SI cortex, but do not innervate the DLS neuropil (Deschenes et al. 1996). Thus, retrogradely labeled VPM neurons produced by tracer injections in the DLS are probably due to tracer uptake by fibers of passage.

Quantitative analysis of thalamic labeling. Based on our retrograde and anterograde tracing results, we performed several statistical analyses to determine the relative contributions of brain regions that project to the whisker-sensitive part of the DLS. Figure 10A, for example, depicts the normalized distri-

Table 1. Summary of thalamic tracer injections

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<thead>
<tr>
<th>Case</th>
<th>Tracer&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Nucleus&lt;sup&gt;2&lt;/sup&gt;</th>
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<tr>
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<td>LP/POM</td>
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<sup>1</sup>BDA, biotinylated dextran amine; FG, Fluoro-Gold. <sup>2</sup>POM, posteromedial; VPM, ventroposteromedial; VPL, ventroposterolateral; LP, lateral posterior.
bution of retrogradely labeled neurons after placing FG in the DLS. As the bar graphs in that figure indicate, the cerebral cortex contained approximately two thirds of the labeled neurons, and the vast majority of these were located in SI, MI, and other sensorimotor cortical regions. By comparison, the amygdala and thalamus contained the second (13.6%) and third (8.8%) largest proportion of labeled neurons, respectively. Consistent with these regional variations, a two-way ANOVA indicated significant differences in neuronal labeling across brain regions ($F_{12,11005} = 19.50$, $P_{11021} = 0.0001$) and indicated that labeling was significantly higher in the ipsilateral than in the contralateral hemisphere ($F_{1,11005} = 31.75$, $P_{11021} = 0.0001$). In fact, paired $t$-tests confirmed that neuronal labeling was higher on the ipsilateral side for both cortex ($t_{11005} = 22.31$, $P_{11021} = 0.0001$) and the amygdala ($t_{131} = 4.50$, $P_{155} = 0.001$).

Variations in thalamic labeling were apparent because of differences in the size and locations of the DLS tracer injections (see Fig. 1), but the normalized distributions were highly similar in each rat. As indicated by Fig. 10B, approximately a third of all labeled neurons in the thalamus were in the nucleus POM, and this proportion was nearly twice that observed in the Pf (17.3%) or Cm (16.5%) nuclei. These values, however, reflect the inclusion of VPM and VPL in the overall distribution of thalamic labeling.

In view of our anterograde tracing results, we reanalyzed the distribution of thalamic labeling after removing all counts of labeled neurons that appeared in VPM and VPL. As shown in Fig. 10B, making this correction revealed that 40% of the thalamostriatal projections originated from POM, and that the PF and CM nuclei each contributed slightly >20% of the thalamic projections to the DLS injection sites. A one-way ANOVA revealed significant differences in the regional distribution of labeled neurons in the thalamus ($F_{3,25} = 25.5$, $P_{21} = 0.0001$). A paired $t$-test confirmed that the proportion of labeled neurons was significantly higher in POM than in the CM ($t = 3.69$, $P < 0.05$) or Pf ($t = 3.25$, $P < 0.05$) nuclei.

Simultaneous recordings in POM and DLS. To determine whether POM could be responsible for the rapid transmission of somesthetic information to the DLS, neuronal responses to computer-controlled whisker deflections were simultaneously recorded in POM and the DLS. Results from one of these experiments are illustrated in Fig. 11. As the PSTHs in Fig. 11...
indicate, whisker-sensitive neurons in POM and the DLS respond to repetitive stimulation with minimal amounts of sensory adaptation (see Fig. 11, B and D). Importantly, comparison of the latencies of these neuronal responses indicate that activation of the DLS neuron was preceded by a response from the POM neuron (Fig. 11E).

Simultaneous neuronal recordings from the DLS and POM of seven rats generated whisker-evoked responses from 18
POm and 20 DLS neurons. In this sample, the latency of stimulus-evoked responses in POm ranged from 9 to 20 ms, whereas the corresponding values in the DLS ranged from 10 to 22 ms. As indicated by Fig. 11D, mean response latencies were at least 1.7 ms shorter in the POm than in the DLS for each of the stimulus frequencies tested. These differences in latency were significant when whiskers were deflected at 1 Hz, 2 Hz, or 5 Hz ($t > 1.71 P < 0.05$, one-tailed $t$-test), but failed to reach statistical significance at 8 Hz.

DISCUSSION

In contrast to studies that relied only on stereotaxic coordinates to locate tracer injection sites in the striatum, we injected retrograde tracers only at those DLS locations in which neurons responded to whisker stimulation. This procedure revealed that two thirds of all neurons projecting to the whiskersensitive DLS are located in the cerebral cortex, and that the vast majority of these are in the sensorimotor regions of the ipsilateral hemisphere. Neurons in the amygdala accounted for approximately 15% of all labeled neurons, making it the second largest fraction of inputs to the whisker-sensitive DLS.

These results indicate that the cortex and amygdala must influence striatal processing, but these structures are several synapses away from the periphery and are unlikely to represent the fastest route for transmitting sensory information to the DLS.

The thalamus contained the next highest proportion of labeled neurons, and several pieces of evidence suggest that the POm nucleus is a major source of the short-latency responses that we recorded in the DLS during whisker stimulation. First, our retrograde tracing results revealed that POm contained more labeled neurons than any other thalamic nucleus. Subsequent experiments with anterograde tracers confirmed that whisker-sensitive sites in POm project to the DLS. Finally, our physiology experiments indicate that POm responds to whisker deflections at latencies that precede the responses of whisker-sensitive neurons in the DLS. Collectively, these results strongly suggest that stimulus-induced somesthetic inputs are conveyed directly to the DLS by projections from the POm nucleus.

Although our data indicate that the whisker-sensitive DLS region receives more inputs from POm than from any other

Fig. 9. Cortical and striatal labeling produced by the BDA injection shown in Fig. 8. A and B': coprocessed section showing layer IV barrel field and BDA labeling in SI cortex. C: magnified view of colabeled barrels from the rectangle in B. C': adjacent section shows BDA terminal labeling in layer IV barrels (D) and retrogradely labeled neurons in layer Vla (E). F–H: inspection of the DLS reveals BDA-labeled fiber bundles but no labeled varicosities in the DLS. Scale bars: 1 mm (A); 500 μm (B,F); 250 μm (C,G); 100 μm (D,H).
thalamic nucleus, other thalamic nuclei are more dominant in other parts of the striatum. Thalamostriatal projections are topographically organized (Groenewegen and Witter 2004; Smith et al. 2004), and our BDA injections in POm indicate that this thalamic nucleus projects mainly to the DLS. By contrast, the midline and intralaminar nuclei innervate more widespread parts of the striatum (Berendse and Groenewegen 1990; Groenewegen and Witter 2004; Smith et al. 2004), many of which receive few if any projections from POm.

**Sensory responsiveness in the POm.** Substantial controversy surrounds the functional role of the POm in processing somesthetic information. Much of this controversy stems from reports indicating that POm responses are weaker and have latencies that are longer and more variable than the responses recorded from VPM neurons (Ahissar et al. 2000; Diamond et al. 1992b; Masri et al. 2008; Sosnik et al. 2001). In addition, POm neurons become less responsive if the SI cortex is inactivated (Diamond et al. 1992a).

Several facts can account for the apparent discrepancy between the strong POm responses that we observed and the weak POm responses that others have reported. First, we recorded POm and DLS responses from rats in a very lightly anesthetized state, as indicated by ECoG activity that was dominated by frequencies of 5–7 Hz (Friedberg et al. 1999). The anesthetic state has a significant impact on DLS responsiveness, and stimulus-evoked neuronal discharges are rarely recorded in the DLS of more deeply anesthetized rats (Pidoux et al. 2011; West 1998). Indeed, we found that increasing the concentration of isoflurane >1% suppressed neuronal responses in both POm and the DLS to a similar extent (data not shown); this further supports the view that DLS responses to somesthetic stimuli depend, in part, on inputs from POm.

In contrast to studies that relied on air-jet stimulation to evoke whisker-related responses in POm (Masri et al. 2008; Sosnik et al. 2001), we used direct mechanical contact to simultaneously deflect multiple whiskers. Direct contact with the whiskers removes variations in the onset of whisker movement and, by moving multiple whiskers at the same time, simultaneously activates convergent inputs to the POm. This is important because POm neurons have large receptive fields that extend across multiple whiskers (Diamond et al. 1992b). Although corticothalamic feedback contributes to POm responsiveness (Ahissar 1998; Diamond et al. 1992a), strong sensory activation of convergent projections from the spinal trigeminal nuclei should evoke rapid responses in POm. Neurons in the POm have bushy radiating dendrites and extensive axonal arbors that terminate in well-defined bands in the DLS (Deschenes et al. 1995, 1998). These structural features should promote synchronous activation of clusters of POm neurons, thereby providing effective excitatory drive to neuronal targets in the DLS.

**Thalamostriatal projections.** Most discussions of thalamostriatal projections have focused on projections from the intralaminar nuclei, especially the centromedian and parafascicular (CM/Pf) nuclear complex (McHaffie et al. 2005; Smith et al. 2009). Consistent with this focus, the CM/Pf complex contains a large fraction of the thalamic neurons that project to the whisker-sensitive part of the DLS. The function of the thalamostriatal projections from the CM/Pf complex is poorly understood, however, because very few studies have characterized neuronal response properties in the intralaminar nuclei. In monkeys, some PF neurons display short-latency responses to multiple modalities, including visual, auditory, and somatosensory inputs (Minamimoto and Kimura 2002). In rats, PF neurons and other intralaminar nuclei respond to whisker deflections, but response latencies to peripheral stimulation have not been measured (Krauthamer et al. 1992). Importantly, superior colliculus lesions reduce the detectability of somesthetic responses in PF, whereas direct electrical stimulation of the superior colliculus effectively activates Pf and other intralaminar regions (Grunwerg and Kauthamer 1992). These and other findings have prompted the hypothesis that the superior colliculus transmits highly salient, multimodal sensory inputs to midline and intralaminar thalamic nuclei that are involved in regulating the striatal-based “decisions” that underlie behavioral selection (McHaffie et al. 2005; Smith et al. 2009).

Relevant to this discussion, the striatum also receives inputs from higher-order thalamic nuclei that process sensory-specific information. The pulvinar and lateral posterior nuclei, for example, receive visual information directly from the retina (Boire et al. 2004; Cowey et al. 1994; Itaya and van Hoesen 1983) and from the upper layers of the superior colliculus (Abrahamson and Chalupa 1988; Harting et al. 2001a). In turn, these extrageniculate thalamic nuclei project to the striatum.
presumably to convey vision-related information (Day-Brown et al. 2010; Harting et al. 2001b; Lin et al. 1984; Takada et al. 1985).

These findings suggest that transmission of multimodal sensory information through the multisynaptic tecto-thalamo-striatal circuits could be augmented by parallel sets of thalamostriatal pathways that originate in modality-specific higher-order thalamic nuclei. Thus, projections from POm to the DLS could represent a higher-order thalamostriatal pathway that augments the tecto-thalamo-striatal circuit connections. Whereas thalamostriatal projections from intralaminar nuclei are thought to be important for conveying unexpected sensory signals needed to redirect attention, we propose that sensory-specific thalamostriatal projections from higher-order nuclei (e.g., pulvinar or POm) cooperate with the thalamostriatal projections from the intralaminar nuclei. Such cooperation could increase both the salience of a peripheral stimulus and, in turn, the likelihood of activating postsynaptic targets in the DLS. In addition, these thalamostriatal projections could rapidly provide the modality-specific information needed to select and initiate a specific sensory-guided response to an external stimulus.

Corticostriatal projections. Our quantitative tracing data, both here and in a previous report (Alloway et al. 2006), indicate that sensorimotor cortex sends the most inputs to the DLS. These findings, however, could easily be misinterpreted as indicating that the sensorimotor cortex is the most influential source of somesthetic inputs to the DLS. This is not necessarily correct because only a small fraction of the sensorimotor cortical population is likely to be synchronized at any given time during different sensorimotor behaviors. By contrast, dense clusters of POm neurons with widespread overlapping dendrites should become synchronized in response to stimuli that simultaneously deflect multiple whiskers, and this could effectively activate whisker-sensitive neurons in the DLS (Deschenes et al. 1995).

The precise role of corticostrial projections from SI and other sensorimotor regions during peripheral somatosensory stimulation remains unclear. The exact nature and importance of corticostrial contributions to DLS responses evoked by peripheral stimulation require additional research. For example, determining the contribution of thalamostriatal inputs on DLS responsiveness could be achieved by recording stimulus-

Fig. 11. Comparison of response latencies recorded simultaneously in the whisker-sensitive parts of POm and the DLS. A: microlesion at a whisker-sensitive recording site in POm (circle) and the PSTH showing the corresponding neuronal response recorded prior to making the lesion. B: microlesion (circle) made at a site in DLS where a whisker-sensitive medium spiny neuron was recorded simultaneously (PSTH) with the POm response shown in A. C: PSTHs showing the mean response to the 2-Hz stimulus presentations. Arrows indicate the latency of the responses as defined by the first bin to exceed the 99% confidence limits (dashed line). D: response latencies at each stimulus frequency for the pair of POm and DLS responses displayed in A and B. E: mean response latencies for the sample of neurons recorded in POm and the DLS. Error bars represent SE. Asterisks indicate significant differences in latencies for POm and DLS neurons (*P < 0.05).
evoked DLS responses before and after selective inactivation of SI and surrounding somatosensory cortical areas.

Amygdalostratial projections. Consistent with our previous work (Alloway et al. 2006), the present study indicates that the whisker-sensitive DLS receives bilateral inputs from the amygdala. This agrees with previous data showing that the amygdala projects bilaterally to the posterior DLS (Kelly et al. 1982). Although labeling was substantially greater in the ipsilateral hemisphere, each amygdala contained neuronal labeling in the magnocellular and, to a lesser extent, in the intermediate portion of the basal nucleus.

Most studies examining amygdalostratial projections in the rat have emphasized the strong amygdaloïd projections to the ventral striatum while also noting the relative lack of projections to the rostral portion of the DLS (Kelly et al. 1982; Russchen and Price 1984). Yet these studies also indicate that amygdalostratial topography is complex. In fact, careful inspection of the labeling patterns in these reports indicates that the basolateral nuclei in the amygdala send dense projections to posterior parts of the DLS that correspond to the whisker-sensitive regions that we injected in the present study.

Several lesion-behavioral studies indicate that distinct parts of the amygdaloid complex are differentially involved in goal-directed and habitual motor behaviors (Balleine et al. 2003; Corbit and Balleine 2005; Lingawi and Balleine 2012). Although the basolateral amygdaloid has been implicated in goal-directed behaviors that depend on the dorsomedial striatum, the anterior portion of the central amygdaloid nucleus appears to be involved with the acquisition of behavioral habits that depend on DLS processing. In view of these findings and the presumption that whisking behavior is a well-learned sensorimotor habit, it is surprising that the whisker-sensitive part of the DLS receives projections from the basal (or basolateral) amygdala but not from the central amygdaloid nucleus. Evidence indicating the association between the central amygdala and the DLS has prompted the view that the central nucleus exerts an indirect influence on the DLS by virtue of its connections with the nigrostriatal projection system (Lingawi and Balleine 2012). In fact, the central nucleus represents a major source of amygdaloïd projections to other brain regions, and intraamygdala connections enable all its nuclear components, including the basolateral nuclei, to influence the central nucleus (Pitkanen et al. 1997; Sah et al. 2003). In this context, it is noteworthy that whisking behavior is tightly coordinated with sniffing (Kepecs et al. 2006; Welker 1964), and this prompts speculation that the whisker-sensitive part of the DLS might receive direct inputs from the amygdala to enable coordination of these sensorimotor and limbic-related behaviors.

Interpretative limitations. Together with our anatomic findings, our preliminary physiologic results suggest that the POM could transmit somesthetic information directly to the DLS. Our results do not, however, address the potential impact of other thalamostriatal pathways in transmitting somatosensory information to the DLS. Determining whether POM cooperates with the CM/Pf complex in activating the DLS requires systematic characterization of peripherally evoked responses in these thalamic nuclei and in the DLS simultaneously. Such an analysis, along with manipulations that suppress the influence of corticostriatal projections, will indicate more completely the functional roles and relative contributions of the different thalamostriatal pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.B.S., T.M.M., and K.D.A. conception and design of research; J.B.S. and T.M.M. performed experiments; J.B.S., T.M.M., and K.D.A. analyzed data; J.B.S. and K.D.A. interpreted results of experiments; J.B.S., T.M.M., and K.D.A. prepared figures; J.B.S. drafted the manuscript; J.B.S., T.M.M., and K.D.A. edited and revised the manuscript; J.B.S., T.M.M., and K.D.A. approved the final version of the manuscript.

REFERENCES


Castle M, Aymerich MS, Senchez-Escobar C, Gonzalez N, Obeso JA, Lanciego JL. Thalamic innervation of the direct and indirect basal ganglia.


