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Thalamic input to representations of the teeth, tongue, and face in somatosensory area 3b of macaque monkeys

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Abstract

Representations of the parts of the oral cavity and face in somatosensory area 3b of macaque monkeys were identified with microelectrode recordings and injected with different neuroanatomical tracers to reveal patterns of thalamic projections to tongue, teeth, and other representations in primary somatosensory cortex. The locations of injection sites and resulting labeled neurons were further determined by relating sections processed to reveal tracers to those processed for myeloarchitecture in the cortex and multiple architectural stains in the thalamus. The ventroposterior medial sub-nucleus (VPM) for touch was identified as separate from the ventroposterior medial parvicellular nucleus (VPMpc) for taste by differential expression of several types of proteins. Our results revealed somatotopically matched projections from VPM to the part of 3b representing intra-oral structures and the face. Retrogradely labeled cells resulting from injections in area 3b were also found in other thalamic nuclei including: anterior pulvinar (Pa), ventroposterior inferior (VPI), ventroposterior superior (VPS), ventroposterior lateral (VPL), ventral lateral (VL), centre médian (CM), central lateral (CL), and medial dorsal (MD). None of our injections, including those into the representation of the tongue, labeled neurons in VPMpc, the thalamic taste nucleus. Thus, area 3b does not appear to be involved in processing taste information from the thalamus. This result stands in contrast to those reported for New World monkeys.

Keywords

Somatosensory Cortex; Ventroposterior Thalamus; Gustatory Thalamus

Introduction

In the present study, we determined the thalamic connections of parts of the primary somatosensory cortex (area 3b) of macaque monkeys that represent the tongue and teeth. The connections of other parts of the body representations in area 3b of macaque monkeys, especially the hand representation, have been extensively studied (Darian-Smith et al., 1990; Qi et al., 2011), but the lateral location of the head and mouth representations in macaques have made this part of area 3b difficult to study, and little is known. Yet, the cortical processing of information from the tongue and teeth is obviously important for evaluating the quality of food, the chewing and processing of food while protecting the teeth from

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damage, and the guidance of tongue and mouth movements during complex oral behaviors such as during vocalizations (Lin et al., 1993; Shepherd, 2012). As expected from having such important sensory functions, the representations of the tongue and teeth occupy a large portion of area 3b in primates (Manger et al., 1995; Jain et al., 2001; Iyengar et al., 2007), including Old World macaque monkeys (Manger et al., 1996).

The sources of thalamic inputs to the representation of the tongue in area 3b were of special interest in the present study because of the possibility that this representation is not only involved in processing tactile information, but also taste information. Previous research on the thalamic connections of the cortical representation of the tongue in New World owl, squirrel, and marmoset monkeys provided evidence for inputs, not only from the medial division of the ventroposterior nucleus (VPM), but also for the architectonically distinct ventroposterior medial parvicellular nucleus (VPMpc), the taste nucleus of the thalamus (Benjamin and Burton, 1968; Benjamin et al., 1968; Iyengar et al., 2007; also known as the ventral medial basal nucleus, VMb, according to Jones, 2007). The results of one study suggested that VPMpc also projects to the tongue representation in area 3b of macaque monkeys (Pritchard et al., 1986), although these results are inconsistent with those of previous studies (Roberts and Akert, 1963; Burton and Jones, 1976). More recently, the results of Pritchard and colleagues (1986) have been reconsidered to allow for the likelihood that labeled projections to the somatosensory cortex from VPMpc were actually the result of a slight involvement of VPM (Pritchard and Norgren, 2004).

The present study was designed to determine the thalamic connections of the parts of primary somatosensory cortex representing the oral cavity and face, while also learning more about the organizations of VPM and VPMpc in macaque monkeys. We set out to accomplish this by using microelectrode recordings to map the face and intra-oral representations in 3b, placing injections of neuroanatomical tracers in different parts of these electrophysiologically defined representations, and characterizing the full extent and organization of thalamic inputs to the different parts area 3b face and oral cavity representations. As both area 3b and VPM are characterized by architectonically distinct modules or subdivisions related to the representations of different body parts (Rausell and Jones, 1991b; a; Iyengar et al., 2007; Qi et al., 2011), we processed cortical and thalamic tissue to reveal these subdivisions, and related connection patterns to them, and to other architectonically defined thalamic nuclei. Our results indicate that VPM provides the vast majority of inputs to the tongue and teeth representations in area 3b, although other nuclei also contribute, and that the area 3b tongue representation does not receive direct taste information from VPMpc.

Methods

Four adult macaque monkeys (one *Macaca radiata*, case 1, and three *Macaca mulatta*) were used in this study. The experimental procedures were approved by the Vanderbilt University Animal Care and Use Committee and adhered to National Institutes of Health guidelines, and surgical procedures were performed under aseptic conditions.

Surgical procedure

In preparation for surgery, the monkeys were anesthetized with an initial dose of ketamine hydrochloride (10-50 mg/kg, i.m.), and the head secured in a stereotaxic frame. A surgical level of anesthesia was maintained with an intravenous ketamine drip (4 mg/ml in sterile saline), supplemented with injections of xylazine (0.4 mg/Kg, i.m.) and urethane (1.6 mg/Kg, i.p.) during terminal procedures. Heart rate, blood oxygen levels, and temperature were monitored throughout the mapping procedure. Each monkey was placed on a heating pad or under a heating lamp to maintain the body temperature at 37°C. A local anesthetic, lidocaine

hydrochloride, was applied to the ears and subcutaneous skin before the skin was incised to expose the skull. To avoid interference from eye and mouth bars during mapping, a head post was attached to a portion of the skull that did not overlie the region of interest. A craniotomy was performed to expose the lateral half of the central sulcus. The dura was removed to allow for electrophysiological mapping. The exposed cortex was kept moist with regular application of sterile saline until the injections of tracers into cortex were completed, and then covered with sterile silicone oil during the subsequent mapping. Photographs of the surface of the brain were used to mark the placement of microelectrodes, responses at different sites, and relative locations of tracer injections and electrolytic lesion locations by using blood vessels and sulcal patterns as landmarks.

Multiunit mapping & tracer injections

Neuroanatomical tracers were injected into physiologically defined representations of the tongue, teeth, gingiva, palate, buccal wall, lips, and chin in area 3b. Maps of the 3b representations of the parts of the mouth and face were obtained by inserting low-impedance tungsten or stainless steel (1.0 mΩ at 1,000 Hz) microelectrodes into the cortex to record activity while the intra-oral structures and face were stimulated with thin wood or glass probes. While our goal was to orient the mapping electrode so that it was perpendicular to layer IV, other orientations where the electrode was parallel to layer IV were used since much of the mouth representation was on the caudal bank of the central sulcus. Thus, the electrode was advanced in 200-300 μm steps with a hydraulic Microdrive (David Kopf Instruments, Tujunga, CA) starting at a depth of 600-700 μm and continuing until responses were lost, sometimes as deep as 7300 μm. Electrode penetrations were marked on high resolution digital photographs of the brain. Receptive fields were outlined on standard drawings of a macaque face and the inside of the mouth. The initial mapping was used to identify regions of area 3b representing parts of the tongue, teeth, and face for injections. Based on the initial mapping, pressure injections of up to three of the following anatomical tracers were placed into different parts of the oral cavity and face representation using glass pipettes attached to a Hamilton syringe: 0.25-0.6 μl cholera toxin subunit B (1% CTB in distilled water, Sigma, St. Louis, MO or Molecular Probes, Carlsbad, CA), 0.25-0.3 μl Fluororuby (10% FR in distilled water, Molecular Probes or Invitrogen, Carlsbad, CA), 0.01-0.04 μl wheat-germ agglutinin conjugated with horseradish peroxidase (0.2% WGA-HRP in distilled water, Sigma), and 0.4 μl biotinylated dextran amine (10% BDA in phosphate buffer, Molecular Probes, Eugene, OR or Invitrogen). In three animals (cases 1, 3, and 4), recording continued for up to three days immediately after injection to provide a more extensive map of the oral cavity representation and allow time for tracer transport. In case 3, due to the use of a different set of neuroanatomical tracers, injections were placed in a short initial mapping procedure. Gel film was then inserted to replace the opened dura, the craniotomy closed with a cap of dental cement, and skin opening sutured shut. The animal was then recovered from anesthesia, and treated with prophylactic antibiotic and analgesics. After two weeks, the optimal amount of time for tracer transport, the cortex was again exposed, and more extensive recording around the locations of the injections was performed. Once electrophysiological mapping was complete, the monkeys were given a lethal injection of sodium pentobarbital (80 mg/Kg), and, when areflexive, perfused though the heart with phosphate buffered saline (pH 7.4) followed by 2-3% paraformaldehyde in buffered saline, and then 2-3% paraformaldehyde with 10% sucrose.

The cortex was separated from the rest of the brain. The sulci were opened, and each hemisphere was flattened and blocked. Flattened blocks were held between two glass slides and stored for cryoprotection along with the thalamus from each case overnight in 30% sucrose at 4°C. For detailed flattening procedures, see Gharbawie et al., 2011.

Thalamic Histology

The thalamus was cut at a thickness of 40-50 μm in either the coronal (cases 1-3) or sagittal (case 4) plane. Subsets of sections were mounted unstained for fluorescence microscopy. Alternate series were appropriately processed to reveal tracers. In all cases, one series was reacted for CTB immunohistochemistry (Bruce and Grofova, 1992; Angelucci et al., 1996) to visualize CTB labeled cells. BDA was visualized by an avidin biotin-peroxidase reaction (ABC-kit, Vectastain, Vector, Burlingame, CA; Veenman et al., 1992). WGA-HRP series were processed with tetramethlybenzidine as chromogen and ammonium molybdate (Olucha et al., 1985) and stabilized in diamidinobenzidine (DAB). In all cases, series of sections were processed for cytochrome oxidase (CO; Wong-Riley, 1979). Additional series of sections in case 4 were processed to reveal parvalbumin (PV; mouse monoclonal antibody, Sigma-Aldrich, St. Louis, MO), calbindin (Cb; mouse monoclonal antibody, Swant, Bellinzona, Switzerland) and vesicular glutamate transporter 2 (VGluT2; mouse monoclonal anti-VGluT2 antibody, Chemicon, now part of Millipore, Billerica, MA).

Antibody Characterization

All antibodies used in this study were previously characterized and subjected to a western blot analysis within the laboratory. Details are summarized in Table 2.

Calcium binding proteins: Calbindin and Parvalbumin. Our western blot analysis from macaque monkey cerebellar tissue shows that the mouse monoclonal anticalbindin antibody labeled a band at 28 kDa weight, while the mouse monoclonal antiparvalbumin antibody linearizes on a 2D gel with the same level of staining as other antibodies (P. Balaram, personal communication, September 4, 2012). These analyses confirmed the manufacture's technical information that these antibodies specifically recognize the calcium binding sites of Cb (MW = 28 kDa) and PV (MW = 12 kDa) (P. Balaram, personal communication, September 4, 2012 Wong and Kaas, 2008; Qi et al., 2011). While staining for Cb reveals a different subset of GABA-immunoreactive interneurons from PV protein in the thalamus (Jones and Hendry, 1989; Rausell and Jones, 1991b; a) and cortex (Van Brederode et al., 1990), these calcium binding proteins have also been used to distinguish thalamic nuclei with VP (medially VPM) expressing high levels of PV, and VPMpc expressing high levels of Cb (Rausell and Jones, 1991a). Immunohistochemistry in the present study shows a pattern of cellular staining for Cb and PV and distribution in VPM that is identical to previous descriptions (Jones and Hendry, 1989; Rausell and Jones, 1991b; a).

VGluT2. A mouse monoclonal anti-vesicular glutamate transporter 2 antibody clone, 8G9.2 Millipore, MAB5504, was used, to reveal the distribution of VGluT2 protein in VPM. This antibody stained a single band of 56 kDa molecular weight in western blots from macaque cerebellar tissue (Balaram et al., 2013), confirming the manufacturer information. Previous reports indicate that VGluT2 immunostaining reveals the thalamocortical terminations in layer 4 of the cortex in rats (Fujiyama et al., 2001) and macaque monkeys (Hackett and de la Mothe, 2009), as well as the brainstem terminations in the thalamus of rats (Kaneko and Fujiyama, 2002), squirrels (Wong et al., 2008), galagos (Wong and Kaas, 2010), and macaques (Qi et al., 2011). This antibody showed a pattern of staining in macaque VPM that was similar to that demonstrated in VPL by Qi and colleagues (2011).

Cortical Histology

The cortex was cut tangential to the surface at a thickness of 40-50 μm on a freezing microtome. Alternate series of sections were mounted unstained to identify injection sites of fluorescent tracers, processed to visualize CTB, BDA, and WGA-HRP injection cores, or processed for myelinated fibers (Gallyas, 1979) to reveal cortical myeloarchitecture.

Data analysis

Distributions of neurons filled with CTB, FR, WGA-HRP, and BDA were plotted with a fluorescent/brightfield Leitz microscope coupled to a computer running Neurolucida™ plotting software (MBF Bioscience, Williston, VT). Landmarks including blood vessels and lesions were also marked for use during reconstruction. Digital photomicrographs of sections stained to reveal architecture were taken using a Nikon DXM1200 camera mounted on a Nikon E800 microscope (Nikon, Melville, NY). These digital images were then adjusted for contrast, saturation, lightness, and curves with Adobe Photoshop CS2 (Adobe Systems, San Jose, CA), but were not otherwise altered.

Reconstruction

Cortical sections stained for myelinated fibers were used to determine the borders of cortical areas and localize injection cores and labeled neurons within architectonic fields. Area 3b is characterized by dense myelin staining (Jain et al., 1998; Qi and Kaas, 2004; Kaas et al., 2006; Iyengar et al., 2007). In the face and mouth region of area 3b, myelin dense patches indicate anatomical representations of the different oral cavity and face structures (Jain et al., 2001; Kaas et al., 2006; Iyengar et al., 2007). A reconstruction of cortical areal boundaries in each case was created by using a projection microscope to draw the myeloarchitecture through the depth of cortex by aligning landmarks including blood vessels, electrolytic lesions, and injection cores across sections within the case. Plotted cortex sections indicating the injection sites were then aligned to these reconstructed borders using the same landmarks. All reconstructions were created with Adobe Illustrator CS2 & CS5™ (Adobe Systems).

Borders of thalamic nuclei were determined from the differential distribution of several proteins, including the metabolic enzyme CO, VGluT2, PV, and Cb (See Fig. 1 for representative CO and Cb sections). Architectural sections were projected onto corresponding plots of the distributions of neurons labeled by each of the different injected tracers. Architectural sections and the plots of labeled neurons were aligned using blood vessels and other landmarks, and the boundaries and internal structures of the thalamic nuclei were drawn onto each plot. The borders of nuclei, structure within nuclei, and other landmarks were then used to align plots of different tracers and create the collapsed representations of thalamic sections seen in figures 4-6.

Results

Anatomy of VPM and VPMpc

Because evidence of how thalamic nuclei project to cortex representing the tongue is conflicting, we needed to properly identify the ventroposterior medial division (VPM) of the ventroposterior complex, the relay of touch signals from the oral cavity and face, and ventroposterior medial parvicellular nucleus (VPMpc), the thalamic taste relay, in order to determine if only one or both of these nuclei project to area 3b. Our procedures used to identify and distinguish VPM and VPMpc are consistent with those of previous studies on the histochemistry of these nuclei (Rausell and Jones, 1991b; a). Staining for the mitochondrial enzyme cytochrome oxidase (CO) (Fig. 1, column A) and the calcium binding protein parvalbumin (PV) in adjacent sections resulted in large, overlapping patches of dark staining in VPM, and less dense staining in the more medial VPMpc. In sections processed for a second calcium binding protein, Calbindin (Cb) (Fig. 1, column B), there was little expression in VPM, while VPMpc stained darkly.

Our use of immunohistochemistry to reveal the distribution of vesicular glutamate transporter 2 (VGluT2) demonstrated a new aspect of VPM's architecture. VGluT2 is a

transporter that accumulates in the feedforward terminals of axons in sensory systems (Kaneko and Fujiyama, 2002). Densely staining patches of VGluT2 expression were found in VPM (Fig. 2B). When aligned to adjacent CO stained sections (Fig. 2A) on the basis of blood vessels and other landmarks, these VGluT2 densities overlapped CO dense patches. Furthermore, the patches of VGluT2 subdivided regions of dense CO staining (Fig. 2C). Each dense patch of CO in VPM corresponds to the representation of a single part of the face or mouth (Jones and Friedman, 1982; Rausell and Jones, 1991b). VGluT2 has been shown to provide a further refinement of the anatomical map in ventroposterior lateral sub-nucleus of VP (VPL) that represents the body and limbs (Qi et al., 2011), thus it is likely that VGluT2 staining reveals more anatomical subdivisions of the functional map in VPL than CO staining. Similarly, each VGluT2 patch in VPM likely represents a smaller part of the thalamic face or oral cavity representation than the CO density in which it sits.

Projections of the thalamus to the 3b oral cavity and face representations

Thalamocortical connections were labeled by injecting tracers into the cortical representations of parts of the face and mouth in four monkeys. In case 1, one injection was placed into the tongue representation and another into the representation of the teeth (Fig. 3A). The CTB injection (blue) was centered on the representation of the tip of the tongue, but included parts of the tongue representation with receptive fields on the dorsal-ventral margin of the tongue tip on both sides of the midline, on the dorsal surface of the anterior tongue and on the contralateral half of the middle tongue. An injection of FR (red) was placed into a cortical region medial to the tongue representation that responded to touches on the upper front teeth and contralateral premolars, the skin of the medial upper lip, proximal chin, and anterior neck, and the hairs on the neck and jaw. The contralateral upper canine and all four upper incisors were represented at the center of the injection site. Both of these injections may have spread slightly into area 3a (Fig. 4B).

The locations of cells labeled by these two injections related to the borders of thalamic nuclei are shown in part C of figure 4. The CTB injection into the tongue representation labeled cells through the rostrocaudal extent of the thalamus. The majority of backfilled cells were in VPM (75.22%, Table 3), and the CTB filled neurons lay largely medial and inferior to cells labeled by the injection of FR (sections 222-242). Cells labeled with CTB were also found in ventroposterior inferior nucleus (VPI). The anterior pulvinar (Pa) nucleus, a somatosensory nucleus associated with the pulvinar complex, contained slightly fewer CTB filled neurons, indicating a weaker magnitude of projection of area 3b in this case. CTB positive cells were also in the medial dorsal (MD) and centre médian (CM), and other regions of the thalamus that may include parts of the posterior complex. One neuron filled as a result of the tongue representation injection was on the dorsal border of VPMpc, but not in the nucleus. The CTB injection into the tongue representation also resulted in a regions of terminal label that overlapped the retrogradely labeled neurons in VPM. While fewer cells were labeled by the FR injection (Table 3), the pattern of projections revealed by this injection was similar to that revealed by the CTB injection. The majority of FR labeled neurons were in VPM, but a few FR positive neurons appeared in VPI and Pa. This injection into a region medial to the 3b tongue representation revealed that the representation of the contralateral teeth, lips, and chin in area 3b received a projection from a region of VPM lateral to that devoted to the tongue.

Case 2—In case 2, an injection of CTB (Fig. 3B blue) was placed into a region representing the contralateral anterior tongue, including the tip. A second mapping session revealed that the central core of this injection included cortex responsive to touch over the whole of the contralateral dorsal surface of the tongue, the lower contralateral anterior teeth and ipsilateral incisors, part of the anterior palate, and the skin and hairs of the contralateral

lower lip and chin. This injection spread to include 3b representations of the gums around the contralateral anterior lower teeth, and possibly the ipsilateral first and second premolars. The diffusion zone of this injection may also include part of adjacent area 1, but strong uptake from area 1 was unlikely. A second injection of BDA (Fig. 3B green) was placed medial and slightly caudal to the first. The injection was centered on a region of cortex responding to both the skin and hairs of the contralateral middle and medial lower lip and anterior chin. This injection also involved a region responsive to all of the contralateral lower lip including corner of the mouth and the surrounding cheek, and the rest of the chin. Some cortex representing the ipsilateral medial lower lip and contralateral anterior chin was included in this injection. The BDA and CTB injections overlapped slightly, with the BDA diffusion zone crossing into a region that included very weak responses to the contralateral tongue. A small injection of FR (Fig. 3B red) was placed into the representation of the contralateral middle and medial lower lip between the tongue/lip (CTB) and lip/chin (BDA) injections of case 2. This injection included representations of the skin and hair on the rest of the contralateral lower lip. A region of cortex with weak responses to the contralateral lower incisors may also have been involved in this injection, but these penetrations were on the edge of the injection site, and it is unclear how much tracer uptake occurred in this representation of the teeth.

While the fields of labeled neurons resulting from these injections did overlap, perhaps due to the overlapping injection cores and diffusion zones, a general topography of the thalamic projections from VPM was apparent in case 2 (Fig. 5B). Cells labeled by the chin/lip representation injection (BDA, green) were located more laterally, cells labeled by the tongue/lip injection (CTB, blue) more medially, and cells labeled by the lip injection (FR, red) in between. Projections from ventral Pa to the 3b mouth and face region were also indicated by each of the three tracers. All three injections also resulted in backfilled cells in VPI. Neurons backfilled by the tracer placed into tongue/lip representation occurred just ventral to the field of CTB label in VPM. The cells indicated by the lip/chin and lip only injections in 3b were more lateral and dorsal compared to those of the tongue/lip, with many of these cells found in the septum that separates VPM from VPL (Krubitzer and Kaas, 1992; Fang et al., 2002). Filled cells from injections in the lip and chin representations were in the medial extreme of the ventroposterior superior nucleus (VPS) (Fig. 5, sections 152 & 142). A single cell backfilled by the injection in the tongue representation was also found in VPS. VPL contained a small number of neurons labeled by each injection (Table 3), although none of the injections were close to the hand representation in area 3b. CM, in this case, projected to both the chin (BDA) and tongue (CTB) representations in 3b. The tongue representation injection was the largest injection in this case and it resulted in unique pattern of label in case 2. CTB filled cells were found not only in the main somatosensory nuclei, but also in motor and intralaminar nuclei including: the ventral lateral (VL), CL, and MD. Additionally, a few CTB backfilled cells were found posterior to VPM in a region presumed to respond to spinothalamic or visceral stimulation (not shown). Finally, neurons labeled as a result of the injection into the tongue representation were also found in the hypothalamus (not shown).

Case 3—Case 3 had two injections of tracer (Fig. 3C). CTB (blue) was introduced into a region representing much of the middle and anterior tongue, centered on a region of contralateral anterior and tip of the tongue responses. Part of the representation of the contralateral middle palate was also included in this injection. The WGA-HRP injection (green) in case 3 was centered rostral and lateral to the tongue injection on a representation of the contralateral upper first incisor that was on the crest of the central sulcus. This injection, however, was placed into a region with responses to all of the anterior teeth as well as parts of the gums on both sides of the mouth including: the bilateral upper and lower anterior teeth and surrounding gum, contralateral lower first premolar, ipsilateral upper first

premolar, all of the contralateral hard palate, and bilateral anterior hard palate. Although the core zone of uptake from this WGA-HRP injection was restricted, the diffusion zone around the injection core was large (Fig. 6A) and included representations of the ipsilateral upper second incisor and canine, the bilateral lower incisors, canines, and premolars, and the bilateral anterior half of the tongue.

There was overlap in the populations of cells labeled by the two tracers in the anterior VPM (Fig. 6, sections 143- 159), likely caused by spread of the WGA-HRP injection in the lateral representation of the teeth into part of the adjacent 3b tongue representation. In more posterior sections (167-183), the two injections labeled neurons in separate foci in VPM. The WGA-HRP injection was into the representation of the teeth that is rostrolateral to the tongue representation, and it includes more responses to the ipsilateral teeth than the more medial representation of the teeth that was injected in case 1. Labeled neurons were located in a region of VPM medial and ventral to the focus of cells labeled after the CTB injection into the 3b tongue representation. This is unlike the case 1 FR injection into the representation of the teeth medial to the 3b tongue representation, which received inputs from a region lateral to the cells projecting to the tongue representation. Both injections in case 3 also labeled neurons in extreme medial VPM, caudal and lateral to VPMpc. The injections also revealed projections from other somatosensory nuclei: Pa, VPI, VPL and VPS, intralaminar nucleus CL, and the motor nucleus VL.

Case 4—The case 4 injection of CTB into the tongue representation (Fig. 3D) was centered on a region responding to stimulation of the contralateral middle and anterior tongue without the tip. This injection spread to include representations of the rest of the contralateral tongue, the anterior half of the ipsilateral tongue, the tip of tongue, the gums around the contralateral lower premolars and first molar, upper first and second molars, the ipsilateral upper canine and premolars, all of the anterior palate, and the contralateral half of the rest of the palate. Representations of the ipsilateral upper incisors and canine and the ipsilateral lower second incisor, canine and first premolar lateral to the tongue representation were at the center of the WGA-HRP injection. As in case 3, the injected region was just on the surface of the brain at the crest of the central sulcus and included cells with responses to the rest of the ipsilateral upper teeth, the ipsilateral lower first molar, second premolar and first incisor, and the contralateral lower anterior teeth. The representation of the gums surrounding the ipsilateral upper anterior teeth was also within this injection core.

Cells labeled as a result of the both injections were mainly in VPM (Table 3), but were also found in Pa, VPI, VPS, VPL, CM, and VL (not shown). Laterally in the thalamus, cells labeled by the CTB injection into the tongue representation dominated VPM, with WGA-HRP filled neurons resulting from an injection into the representation of the ipsilateral teeth generally more caudal and ventral in VPM. In more medial sections, the number of neurons filled by the injection into the lateral representation of the teeth increased relative to those filled by the tongue representation injection. WGA-HRP labeled cells in these more medial sections filled the rostrocaudal extent of VPM, but generally sat in the more ventral part VPM just above the border with VPI. Again, labeled cells were in medial VPS and ventral Pa.

Discussion

We injected multiple anatomical tracers into electrophysiologically defined parts of area 3b representing the oral cavity and face in two species of macaque monkeys (*Macaca radiata* and *Macaca mulatta*). The region of primary somatosensory cortex processing information from the face and mouth received a wide range of thalamic inputs from somatosensory nuclei including the: ventroposterior medial (VPM), anterior pulvinar (Pa), ventroposterior

inferior (VPI), ventroposterior superior (VPS), and ventroposterior lateral (VPL) nuclei. Additional inputs arose from the posterior parts of the ventral lateral nucleus (VL); the centre médian (CM), central lateral (CL), and medial dorsal (MD) nuclei; and the region of thalamus caudal to VP. Furthermore, we have shown that the somatotopically organized VPM sends organized projections to area 3b. Regions of VPM projecting to the 3b representation of the teeth in the central sulcus medial to the 3b tongue representation were lateral to those VPM neurons projecting to the 3b tongue representation. Meanwhile, cells in VPM projecting to the portion of 3b responsive to teeth on the crest of the central sulcus and surface of the brain lateral to the 3b tongue representation were located medial in VPM compared to those projecting to the 3b tongue representation. Labeled neurons were concentrated in the cell-dense patches in VPM that are CO dense. These patches were even more effectively revealed by the dense presence of VGluT2 protein. Finally, we found no projections from the thalamic taste nucleus, VPMpc, to the 3b tongue representation in macaque monkeys.

Projections of the thalamic taste nucleus to primary somatosensory cortex

Since the evaluation of food items relies in part on integrating information from the tongue about both taste and texture (Shepherd, 2012), it is important to determine where these two sensory pathways intersect. At the level of the brainstem, cutaneous touch information from the inside of the mouth and taste (as well as some touch and temperature) information from the tongue are processed separately (Scott and Plata-Salamán, 1999; Pritchard and Norgren, 2004). In the thalamus, a similar separation is maintained with VPM processing touch and largely VPMpc responding to taste stimuli (Scott and Plata-Salamán, 1999; Pritchard and Norgren, 2004; Kaas et al., 2006). In the cortex, the two systems have been shown to interact in the orbitofrontal cortex (Rolls and Baylis, 1994; Scott and Plata-Salamán, 1999; Rolls, 2000; Kringelbach, 2004); however, if both VPM and VPMpc provide inputs to the same area in cortex, then integration of touch and taste may occur earlier in area 3b. Whether or not the cortical projection zones of these two thalamic nuclei overlap in macaques has been unclear.

The results of previous studies of the projection pattern of the thalamus in monkeys are complicated by the use of different species of primates, changes in techniques used to reveal these projections, and changing interpretations of results. The earliest work on this subject used cortical lesions in macaques to reveal connections. Roberts and Akert (1963) found that lesions of the primary somatosensory cortex representing the mouth resulted in degeneration only in VPM. Only lesions that included the frontal operculum and insula (the presumptive location of taste cortex) yielded degeneration in the taste nucleus of the thalamus. Thus, Roberts and Akert (1963) concluded that VPM projects to somatosensory cortex and VPMpc to opercular and insular cortex. Subsequently, it was demonstrated in squirrel monkeys that a lesion of either the tongue representation in primary somatosensory cortex, as defined by evoked potentials from stimulation of tongue nerves, or of the presumptive gustatory cortex, also defined by evoked potentials, alone resulted in incomplete degeneration in VPMpc (Benjamin and Burton, 1968; Benjamin et al., 1968). Rather, a large lesion including both cortical areas was required to cause a complete loss of neurons in VPMpc, leaving the authors to conclude that the taste nucleus, VPMpc, supplied at least some input to both SI (area 3b) and gustatory cortex (Benjamin and Burton, 1968; Benjamin et al., 1968). Somewhat later, Burton and Jones (1976) used evidence from injections of tritiated amino acids into the thalamus of macaque and squirrel monkeys, to conclude that VPM provided inputs to cytoarchitectonically defined SI and VPMpc projected to gustatory cortex in both species. If there was a difference between the two species, however, their injections would not have demonstrated it because 1) the core of the amino acid injections in the macaques did not include VPMpc and 2) the injections in the squirrel monkey cases

filled much of the thalamus and involved both VPM and VPMpc such that the idiosyncratic projections of each nucleus could not be distinguished. Another study with amino acids injected into VPMpc led to the conclusion that the taste nucleus did indeed project to SI in macaques (Pritchard et al., 1986), but this conclusion was reconsidered in a more recent reevaluation of the injection cores. Pritchard and Norgren (2004) revisited these anterograde tracer injections, and concluded that that the injections that resulted in labeled axon terminals in both somatosensory and gustatory cortex included not only VPMpc but also VPM.

The combined results of our present study and previous results from our laboratory provide help to clarify the conflicting interpretations of the projections of VPMpc to somatosensory cortex. The use of retrograde tracers allowed us to inject specific representations in area 3b that were identified by electrophysiological mapping. After more extensive mapping results in a terminal session, we were able to fully specify the locations of our injections within aligned physiological and architectural maps obtained from area 3b. The anatomical tracer injections placed in the tongue representations of two species of macaques (rhesus and bonnet), failed to reveal projections from VPMpc. In contrast, similar injections in three species of New World monkeys (owl, squirrel and marmoset monkeys) did result in backfilled neurons in VPMpc (Iyengar et al., 2007). The combined evidence suggests that the area 3b tongue representation of macaques processes tactile information from the tongue, but is not directly involved in processing of taste, while the area 3b tongue representation has a direct role in analyzing taste in New World monkeys. The combined results suggest that area 3b of New World monkeys does have direct taste inputs, while Old World macaques do not. Humans and apes are expected to resemble macaques in this regard, while prosimian primates may resemble New World monkeys. Overall, our findings justify further investigations into how taste is processed across primate and non-primate species with differing evolutionary relationships, brain sizes, and feeding behaviors.

As a further complication, a sparse distribution of neurons responding to gustatory stimuli has been reported in the somatosensory area 3b tongue representation in macaques (Ogawa et al., 1985; 1989). If the gustatory input is not coming from the thalamic taste nucleus, how might it arrive? In a preliminary report of cortical connections from the present cases, we have shown that the tongue representation in area 3b of macaques receives inputs from parts of the anterior upper bank of the lateral sulcus and anterior insula that likely include the primary gustatory area (G) in macaques (Cerkevich et al., 2011). For these Old World species, at least, this set of cortical connections may underlie the few neurons with responses to taste that have been found in the primary somatosensory area, while the integration of tactile information from the tongue and mouth into the perception of flavor occurs in other regions of the cortex (Small et al., 1999; Rolls, 2000; Kringelbach, 2004; Kaas et al., 2006; Shepherd, 2012).

Somatosensory modules in VPM

In macaques, VPM is subdivided into CO-dark modules that respond to touch on different parts of the face and mouth (Jones and Friedman, 1982; Rausell and Jones, 1991a; b). Here, we found a finer-grained anatomical map in VPM by using immunohistochemistry to reveal the distribution of the protein VGluT2. These smaller patches of VGluT2 suggest an arrangement where incoming axons from the principal sensory nucleus of the trigeminal nerve (Graziano et al., 2008; Ge et al., 2010) end on clusters of cells in VPM that are highly metabolically active and respond to stimulation to a circumscribed part of the of the oral cavity or face. These patches of cells and incoming VGluT2 positive inputs are separated from each other by narrow zones with few feedforward inputs. The VGluT2 label seems to be more restricted than CO as a marker, so that more subdivisions of VPM, perhaps with narrow separating septa, are revealed. Thus, both stains for the metabolic enzyme CO and

the vesicle packing protein VGluT2 reveal aspects of the modular organization of VPM, with the VGluT2 protein labeling provided more detail.

We also found that populations of cells projecting to the face, teeth, or tongue representations in cortex were related to distinct CO and VGluT2 modules in VPM (Fig. 4-6). The pattern of connections also provided evidence for the overall somatotopic organization in VPM. Neural responses to stimulation of the face, lips and chin were clustered in the most medial part of the 3b physiological map of the face and mouth, closest to the septum that indicates the border between the hand and face representations in 3b (Fig. 7). Injections into this region yielded retrogradely labeled neurons in the lateral part of VPM (Fig. 4 red; 5 red & green). Previous physiological mapping studies of VPM (Jones and Friedman, 1982; Rausell and Jones, 1991b) have indicated that the lateral region of VPM is responsive to stimulation of the skin on the face and lips (Fig. 7). Our results indicated that this region of lateral VPM representing the face and lips projects to the regions of primary somatosensory cortex representing the face and lips (Fig. 7). Neurons responding to stimulation of the teeth are located in two parts of the intra-oral representation in area 3b (Manger et al., 1996; Jain et al., 2001; Qi and Kaas, 2004; Kaas et al., 2006; Iyengar et al., 2007). When an injection of tracer was placed in the more medial region of cortex with responses to stimulation of the teeth (generally dominated by responses to stimulation of contralateral teeth), labeled cells in the thalamus were located lateral to those projecting from VPM to the 3b tongue region (Fig. 4). Consistent with this result, physiological recordings have identified a region with cells responsive to stimulation of the contralateral teeth and surrounding structures near the lateral edge of the tongue representation in VPM (Rausell and Jones, 1991b). A second representation of the teeth in cortical 3b occurred on the crest of the central sulcus and extended out onto the surface of the brain lateral to the 3b representation of the tongue in our cases. This representation tended to have a higher incidence of sites responsive to ipsilateral and bilateral stimulation of the teeth than the medial representation of the teeth. When the more lateral 3b representation of the teeth was injected, labeled VPM neurons were more ventral and medial compared to those labeled by the injection into the tongue representation (Fig. 6). This location seems to correspond to the region of VPM previously found to be responsive to stimulation of the ipsilateral teeth and surrounding structures (Rausell and Jones, 1991b). The overall pattern of thalamocortical projections of VPM to area 3b, including those of the two representations of the teeth is summarized in figure 7.

Many thalamic nuclei project to the 3b representations of the mouth and face

An important contribution of the present study is a description of the complement of thalamic projections to the representations of the face and mouth in area 3b, including those that that have not been previously demonstrated in macaque monkeys. In previous studies (Burton and Jones, 1976; Nelson and Kaas, 1981; Pritchard et al., 1986; Iyengar et al., 2007), VPM has been consistently found to project to the oral cavity representation in area 3b. Other nuclei known to respond to cutaneous or deep receptors of the skin, muscles, and joints (VPL, VPS, VPI, and Pa) were also known to project to area 3b. Here, we further determine that these converging inputs are relatively weaker in magnitude than those from VPM (Table 3). These nuclei likely contribute to the functions of area 3b in detecting and identifying stimuli on the tongue, teeth, and face (Padberg et al., 2009). This information, as well as that coming from the motor nucleus VL, is likely vital to the contribution of area 3b to the coordination of smooth movements of the mouth and face during feeding and vocalization (Lin et al., 1993; Itoh et al., 2002; Avivi-Arber et al., 2011). Furthermore, these converging inputs may contribute to the changes in the cortical representation of the oral cavity and face that occur after sensory loss (Merzenich et al., 1983; Merzenich et al., 1984; Recanzone et al., 1992; Jones, 2000; Kaas, 2000). Inputs from intralaminar nuclei CM and

CL, as well as MD provide less organized inputs to area 3b that may modulate the processing of information from the intra-oral structures and face (Jones, 2007).

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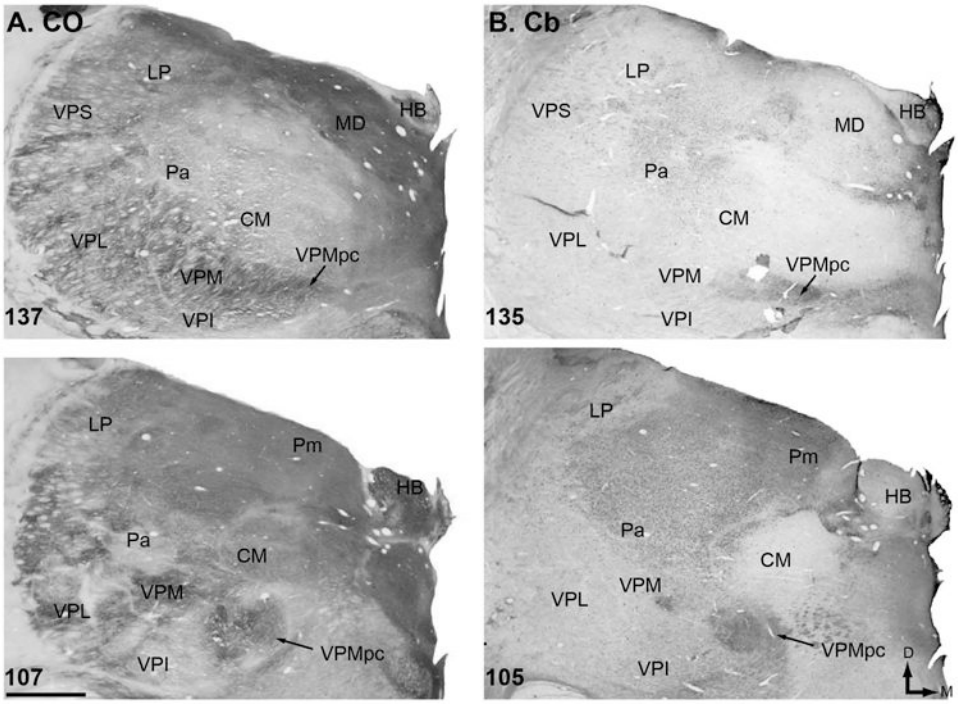

Figure 1. Histological characteristics of the somatosensory thalamus

Photomicrographs of coronal sections of thalamus stained for CO (column A) and Cb (column B) cut at 40 μm thickness. Representative sections were selected to show the borders of nuclei pertinent to cell labeling from the injections of tracers, including the somatosensory VPM and the gustatory VPMpc. There is approximately 1.16 mm between rostral (CO 137 and Cb 135) and caudal (CO 107 and Cb 105) sections. Cb sections are the closet to the CO sections. Abbreviations as in table 1. Scale bar 2 mm.

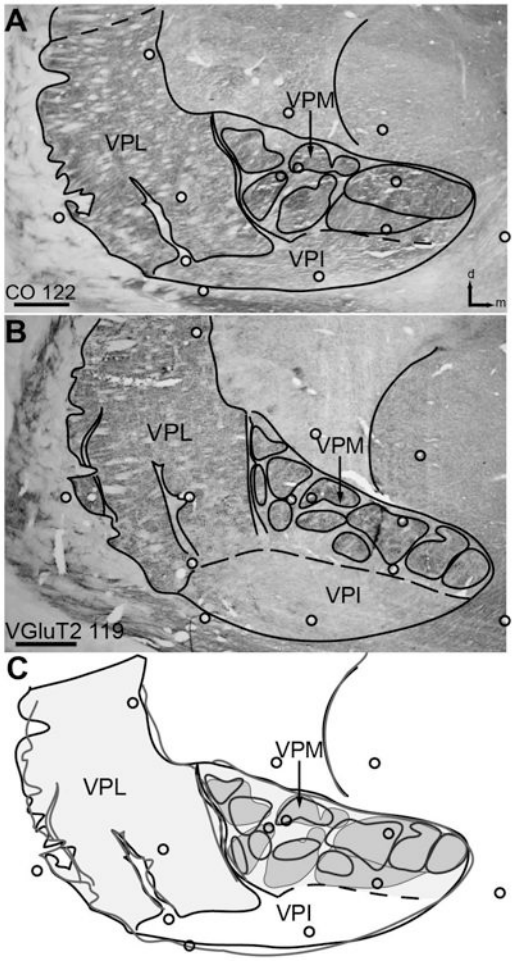

Figure 2. Comparison of CO and VGluT2 staining in the thalamus

(A-B) Photomicrographs of coronal thalamus sections stained for CO (A) or VGluT2 (B). Black lines mark anatomical borders and CO or VGluT2 dense patches within VPM. Circles indicate blood vessels and other landmarks. (C) Drawings of aligned borders from these CO and VGluT2 sections. Black lines mark borders of thalamic nuclei revealed by CO staining. CO densities in VPM are shaded in gray. Gray lines indicate borders of thalamic nuclei revealed by VGluT2 expression. VGluT2 patches in VPM are outlined in dark gray. CO densities have been shown to represent different parts of the face and mouth (Rausell & Jones, 1991a). When aligned according to landmarks, VGluT2 dense patches in VPM often subdivided larger CO dense patches in the structure. These smaller VGluT2 dense subdivisions of CO patches likely represent an even more fine-grained anatomical representation of the parts face and mouth within VPM. There is a slight mismatch between dense patches of CO and VGluT2 expression because the two sections are not immediately adjacent, with 80 μm between them. Intervening sections were used for staining to reveal tracers and different immunohistochemical stains. Coronal sections are 40 μm thick. Abbreviations as in table 1. Scale bar is 1mm.

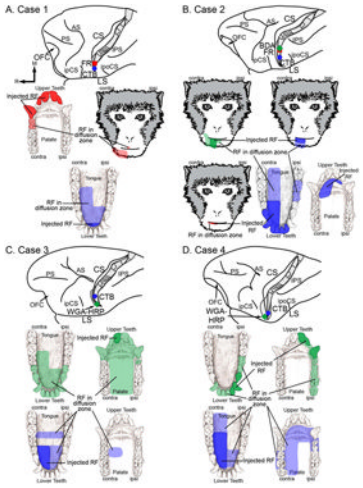

Figure 3. Locations of injection sites in the central sulcus and the injected representations

A-D. Markers in the open central sulcus indicate the center of each tracer injection in the representations indicated below. Area 3b is shaded in gray. The somatotopic organization of area 3b from mouth (lateral) to foot (medial) is outlined, adapted from our mapping of the face and maps of the body by Nelson et al., 1980 and Jain et al., 2008. Sulci: AS- arcuate sulcus, CS- central sulcus, IOS- inferior occipital sulcus, ipCS- inferior pre-central sulcus, ipoCS- inferior post-central sulcus, IPS- intraparietal sulcus, LS- lateral sulcus, LuS- lunate sulcus, OFC- orbitofrontal cortex, PS- principal sulcus, STS- superior temporal sulcus, SuPC- superior pre-central sulcus; Rostral is left, and medial is up. The representations encompassed by each injection are outlined below the brain in each case. The darkly shaded region is the part of the body represented at the center of each injection. The lightly shaded region is an estimation of all receptive fields included in the full extent of the tracer diffusion zone around each injection.

Abbreviations: RF-receptive field. Blue- CTB, Red - FR, Green - WGA-HRP or BDA. Other abbreviations as in table 1.

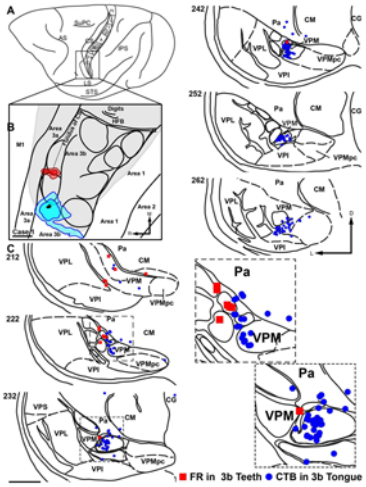

Figure 4. Thalamocortical projections in case 1

(A) A lateral view of the brain with the central sulcus opened. Area 3b is shaded in gray with the somatotopic organization of area 3b indicated (adapted from Nelson et al., 1980 and Jain et al., 2008). Sulci: AS- arcuate sulcus, CS- central sulcus, IPS- intraparietal sulcus, LS- lateral sulcus, STS- superior temporal sulcus, SuPC- superior pre-central sulcus; Body representations: OC- oral cavity, Fa- face, H- hand, A- arm, O- occiput, neck, and shoulder, T- trunk, L- leg, Ft- foot. Rostral is left, medial is up. (B) A magnified view of the 3b representation of the face and oral cavity in area 3b in the tip of the CS. Outlines of the case 1 injection cores and diffusion zones are indicated in relation to the myeloarchitecture of the 3b mouth representation. The FR injection (red) was centered on a representation of the upper anterior teeth, and CTB (blue) on the tip of the tongue representation. Both injections may have spread slightly in to area 3a. Black lines indicate the borders between areas and the architecture within area 3b. The central sulcus is shaded in gray. HFB- 3b hand-face border. Rostral is left, and medial is up. (C) The distributions of labeled neurons in coronal sections from case 1. Borders of thalamic nuclei were identified from adjacent sections stained for CO. Each marker indicates one cell. Shaded regions indicate patches of axon terminal filled with the respective tracer. Serial, 40μm coronal sections through the thalamus are numbered from rostral to caudal. Most of the cells labeled by these injections were in VPM. Other nuclei known to process somatic information, Pa and VPI, also contained neurons projecting to the 3b tongue and teeth representations. Additionally, CM and MD had backfilled neurons.

Abbreviations as in table 1. Scale bar is 2 mm in both B and C.

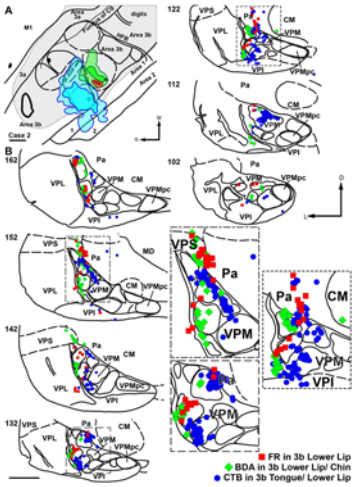

Figure 5. Thalamocortical projections in case 2

(A) Outlines of the injection cores and diffusion zones in relation to the myeloarchitecture of the 3b mouth representation. The most medial of our 7 total injections was of BDA (green) into the representations of the lip and chin. A small injection of FR (red) was placed into the 3b representation of the medial part of the lower lip, just lateral to the core of the BDA injection and within the BDA diffusion zone. Finally, CTB (Blue) was injected into the regions responding to much of the mouth including the tongue, teeth, and lower lip. (B) The distributions of labeled neurons in coronal sections from case 2. Alternate, 40μm coronal sections through the thalamus are numbered from caudal to rostral. All three injections filled neurons in VPM. Injections into representations of the tongue, lip, and chin filled cells mainly in VPM, with the cells projecting to the 3b tongue representation lying most medial and chin closest to the lateral edge of VPM. Projections from VPI, VPS, and Pa were also indicated. Several other thalamic nuclei also contained filled cells including: VL, MD, CM, and CL. Abbreviations as in table 1. Conventions as in figure 4.

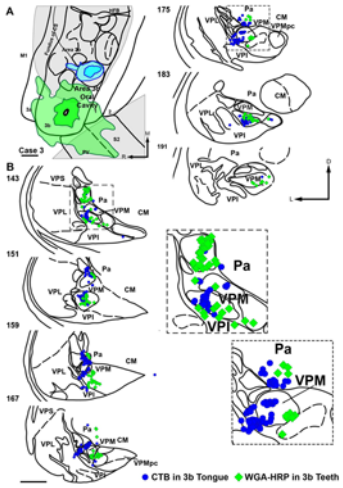

Figure 6. Thalamocortical projections in case 3

(A) Outlines of the injection cores and diffusion zones in relation to the myeloarchitecture of the 3b mouth representation. CTB (blue) was injected into the representation of the tongue. WGA-HRP (green) was placed into a region of mixed ipsilateral and contralateral responses to stimulation of the teeth, gums, and palate. The CS and part of the LS are shaded in gray. (B) The distributions of labeled neurons in coronal sections from case 3. Alternate, 50μm coronal sections through the thalamus are numbered from rostral to caudal. Cells labeled by both injections were concentrated in VPM. Like in other cases, backfilled cells in the thalamus resulting from both injections in case 3 lay mostly in VPM, though VPI, Pa, VPL, VL, CM, and CL also contained labeled neurons. Abbreviations as in table 1. Conventions as in figure 4.

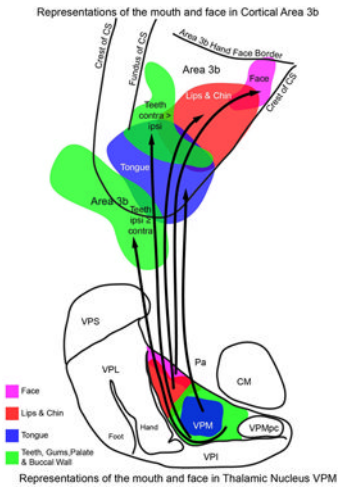

Figure 7. A summary of projections from representations of the face and oral cavity structures in thalamic VPM project to matching representations in area 3b

Electrophysiological mapping has shown organized representations of the face, lips, teeth, and tongue in both area 3b and in VPM (adapted from Rausell & Jones, 1991b) of macaque monkeys. Our injections of retrograde neuroanatomical tracers into these electrophysiologically defined representations of the face and intra-oral structures in area 3b revealed organized projections from VPM, such that the representation parts of the face or mouth in the thalamus project to matching representations in the cortex. Abbreviations as in table 1.

Table 1

Abbreviations

Anatomical Tracers	
Biotinylated dextran amine	BDA
Cholera Toxin Subunit	CTB
Fluororuby	FR
Wheat-germ Agglutinin conjugated with Horseradish Peroxidase	WGA-HRP
Histological Stains	
Calbindin	Cb
Cytochrome Oxidase	CO
Myelinated Fibers	F
Nissil	N
Parvalbumin	PV
Vesicle Glutamate Transporter 2	VGluT2
Thalamic nuclei	
Anterodorsal Nucleus	A
Anterior Pulvinar Nucleus	Pa
Central Lateral Nucleus	CL
Centre Médian Nucleus	CM
Lateral Dorsal Nucleus	LD
Lateral Posterior Nucleus	LP
Lateral Pulvinar Nucleus	Pl
Medial Dorsal Nucleus	MD
Medial Pulvinar Nucleus	Pm
Parafascicular Nucleus	Pf
Ventral Anterior Nucleus	VA
Ventral Lateral Nucleus Posterior Division	VLp
Ventroposterior Inferior Nucleus	VPI
Ventroposterior Lateral Sub-Nucleus	VPL
Ventroposterior Medial Parvicellular Nucleus	VPMpc
Ventroposterior Medial Sub-Nucleus	VPM
Ventroposterior Superior Nucleus	VPS

Table 2

Antibody characterization

Antibody	Host (type)	Source	Catalog no.	Dilution factor	Isotope	Immunogen
Pv	Mouse (monoclonal)	Sigma-Aldrich, St. Louis, Mo	P3088	1:2,000	IgG1	Frog muscle parvalbumin (Clone PARV-19)
Cb	Mouse (monoclonal)	Swant, Bellinozona, Switzerland	C98-48	1:5,000	IgG	Calbindin D-28K purified from chicken gut
VGluT2	Mouse (monoclonal)	Chemicon now part of Millipore, Billerica, MA	MAB5504	1:2,000	IgG1	Recombinant protein from rat VGluT2

Table 3

Distributions of retrogradely labeled cells in thalamic nuclei after injections into 3b representations of the oral cavity and face

Rows sequentially list case numbers, injected representations, injected tracers, and total number of cells labeled by each injection. Thalamic nuclei containing labeled cells are listed in the first column, and successive columns contain the percentage of cells labeled in each nucleus.

	Case 1		Case 2			Case 3		Case 4	
	Tongue	Teeth	Tongue/Lip	Lip	Lip/Chin	Tongue	Teeth	Tongue	Teeth
	CTB	FR	CTB	FR	BDA	CTB	WGA-HRP	CTB	WGA-HRP
	230	16	979	121	108	789	311	1148	436
VPM	75.22%	81.25%	78.55%	57.85%	59.26%	87.45%	83.28%	75.26%	92.43%
VPI	6.09%	12.50%	3.47%	7.44%	9.26%	2.79%	6.11%	11.06%	1.15%
VPL	—	—	2.55%	4.13%	13.89%	1.01%	—	0.17%	0.46%
VPS	—	—	0.31%	14.88%	13.89%	0.51%	—	3.92%	0.92%
VPMpc	—	—	—	—	—	—	—	—	—
Pa	5.65%	—	6.74%	14.88%	2.78%	4.94%	9.65%	6.01%	3.90%
VL	—	—	2.86%	—	—	1.65%	0.96%	0.61%	0.46%
CM	1.30%	6.25%	0.51%	—	0.93%	—	—	2.26%	0.46%
CL	—	—	0.41%	—	—	0.63%	—	—	—
MD	1.74%	—	0.31%	—	—	—	—	—	—
OTHER	10.00%	—	3.37%	0.83%	—	1.01%	—	0.70%	0.23%
HYPTH	—	—	0.92%	—	—	—	—	—	—