DIFFERENTIAL CONNECTIONS IN THE BRAINS OF NEW WORLD AND OLD WORLD MONKEYS

By

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To mom and dad for never telling me I couldn't be a paleontologist, and supporting me when I realized I didn't want to dig up dinosaurs

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3a	Rostral Proprioceptive Area in Anterior Parietal Cortex
3b	Primary Somatosensory Area in Anterior Parietal Cortex (S1)
1	Caudal Cutaneous Somatosensory Area in Anterior Parietal Cortex
2	Caudal Proprioceptive Area in Anterior Parietal Cortex
5	Somatosensory area in Posterior Parietal Cortex
A	Anterodorsal Nucleus
APC	Anterior Parietal Cortex
AS	Arcuate Sulcus
BDA	Biotinylated Dextran Amine (tracer)
Cb	Calbindin
CC	Corpus Callosum
Cgc	Caudal Cingulate Region
CgS	Cingulate Sulcus
Cgr	Rostral Cingulate Region
CL	Central Lateral Nucleus
CM	Centre Médian Nucleus
CO	Cytochrome Oxidase
CS	Central Sulcus
CTB	Cholera Toxin Subunit B (tracer)
CTB-G	Cholera Toxin Subunit B Conjugated to Green Alexa Fluor-488 (tracer)
CTB-IR	Cholera Toxin Subunit B Immunoreactive
CTB-R	Cholera Toxin Subunit B conjugated to Red Alexa Fluor-594 (tracer)
DY	Diamidino Yellow (tracer)
F	Myelinated Fibers
FR	Fluororuby (tracer)
G	Gustatory Cortex
HFB	Hand-Face Border
HYPTH	Hypothalamus
ILS	Inferior Limiting Sulcus
INS	Insula
IOS	Inferior Pre-Central Sulcus
ipCS	Cytochrome oxidase
ipoCS	Inferior Post-Central Sulcus
IPS	Intraparietal Sulcus
LBLS	Lower Bank of the Lateral Sulcus
LD	Lateral Dorsal Nucleus
LP	Lateral Posterior Nucleus
LPC	Lateral Parietal Cortex
LPFC	Lateral Prefrontal Cortex
LS	Lateral Sulcus
LuS	Lunate Sulcus
M1	Primary Motor Cortex

MD	Medial Dorsal Nucleus
N	Nissil
OFC	Orbitofrontal Cortex
Pa	Anterior Pulvinar Nucleus
Pf	Parafascicular Nucleus
PFC	Prefrontal Cortex
Pl	Lateral Pulvinar Nucleus
Pm	Medial Pulvinar Nucleus
PMd	Dorsal Premotor Area
PMv	Ventral Premotor Area
PPC	Posterior Parietal Cortex
PR	Parietal Rostral Area
PrCS	Pre-Central Sulcus
Pre-SMA	Pre-Supplementary Motor Area
PS	Principal Sulcus
PV	Parietal Ventral Area
Pv	Parvalbumin
S1	Primary Somatosensory Area in Anterior Parietal Cortex
S2	Second Somatosensory Area
SC	Caudal Somatosensory Area in Anterior Parietal Cortex
SLS	Superior Limiting Sulcus
SMA	Supplementary Motor Area
SR	Rostral Somatosensory Area in Anterior Parietal Cortex
STS	Superior Temporal Sulcus
SSA	Supplementary Sensory Area
UBLS	Upper Bank of the Lateral Sulcus
VA	Ventral Anterior Nucleus
VGluT2	Vesicle Glutamate Transporter 2
VL	Ventral Lateral Nucleus
VPI	Ventroposterior Inferior Nucleus
VPL	Ventroposterior Lateral Sub-Nucleus
VPM	Ventroposterior Medial Sub-Nucleus
VPMpc	Ventroposterior Medial Parvicellular Nucleus
VPS	Ventroposterior Superior Nucleus
VS	Ventral Somatosensory Area
WGA-HRP	Wheat-germ Agglutinin Conjugated with Horseradish Peroxidase (Tracer)

CHAPTER I

INTRODUCTION

The number of cortical areas for processing tactile information varies across mammals. Primates have evolved more than the four or five fields devoted to somatosensory processing that are generally agreed to be common to all mammals (Qi, Preuss, & Kaas, 2008). Modifications to the basic somatosensory fields of the cortex and additional areas involved in tactile processing have been described in all primates. The expansion of somatosensory cortex along the primate line continued as they evolved, with differences in somatosensory organization arising between prosimian primates, like galagos and lemurs, and anthropoid primates as the somatosensory network was further elaborated in monkeys and apes (Kaas, 2007; Kaas, 2004a). Elaborations of the anterior parietal cortex (APC), the location of the primary somatosensory cortex (S1 or area 3b in primates), have been particularly well documented in anthropoid primates. For the most part, differences among extant non-primate mammalian, prosimian, and simian APC organization have been explained using phylogenetic relationships alone. Recently, a new model for interpreting APC organization among species based on both phylogenetic relationships and hand use has been proposed for primates (Krubitzer & Disbrow, 2008; Padberg et al., 2007). A difference in the number of APC areas reflects differences in network wiring that affect the function of the entire somatosensory network (Kaas, 2004b; Krubitzer & Kaas, 1990; Pons, Garraghty, Friedman, & Mishkin, 1987; Pons,

Garraghty, & Mishkin, 1992). Thus, it is important to resolve these two competing hypotheses to better understand the overall function of the somatosensory system.

In this chapter, we will provide a brief review of the implications of each of the models of APC organization and outline the aims of the experiments we used to test these hypotheses about the APC. First, we will describe the classic phylogeny-based model of APC organization and the modified model of APC organization that also takes the structure of and use of the hand into account. Second, we briefly discuss the role of the APC in the larger somatosensory processing network. Third, we will introduce our experimental aims. Finally, we will present the specific aims that are addressed in the following chapters.

Alternate models of anterior parietal expansion in primates

Traditionally, a cladistic approach (Hennig, 1996) has been used to relate differences in structure and function of areas in APC among species to phylogenetic relationships. The classic phylogeny-based model of APC organization (Fig. 1.1A) proposes that the modern mammalian APC is organized around three somatosensory areas, the primary somatosensory area (S1), a rostral somatosensory field (SR) and a caudal somatosensory area (SC) that appeared with the emergence of the early mammals (Kaas, 2004a). Variations in this organization occur, particularly in primates where the single SC of non-primate mammals appears to have diverged into two separate areas, areas 1 and 2. S1 (the non-primate homolog of primate area 3b) is the largest of the mammalian APC divisions and contains a systematic representation of the cutaneous

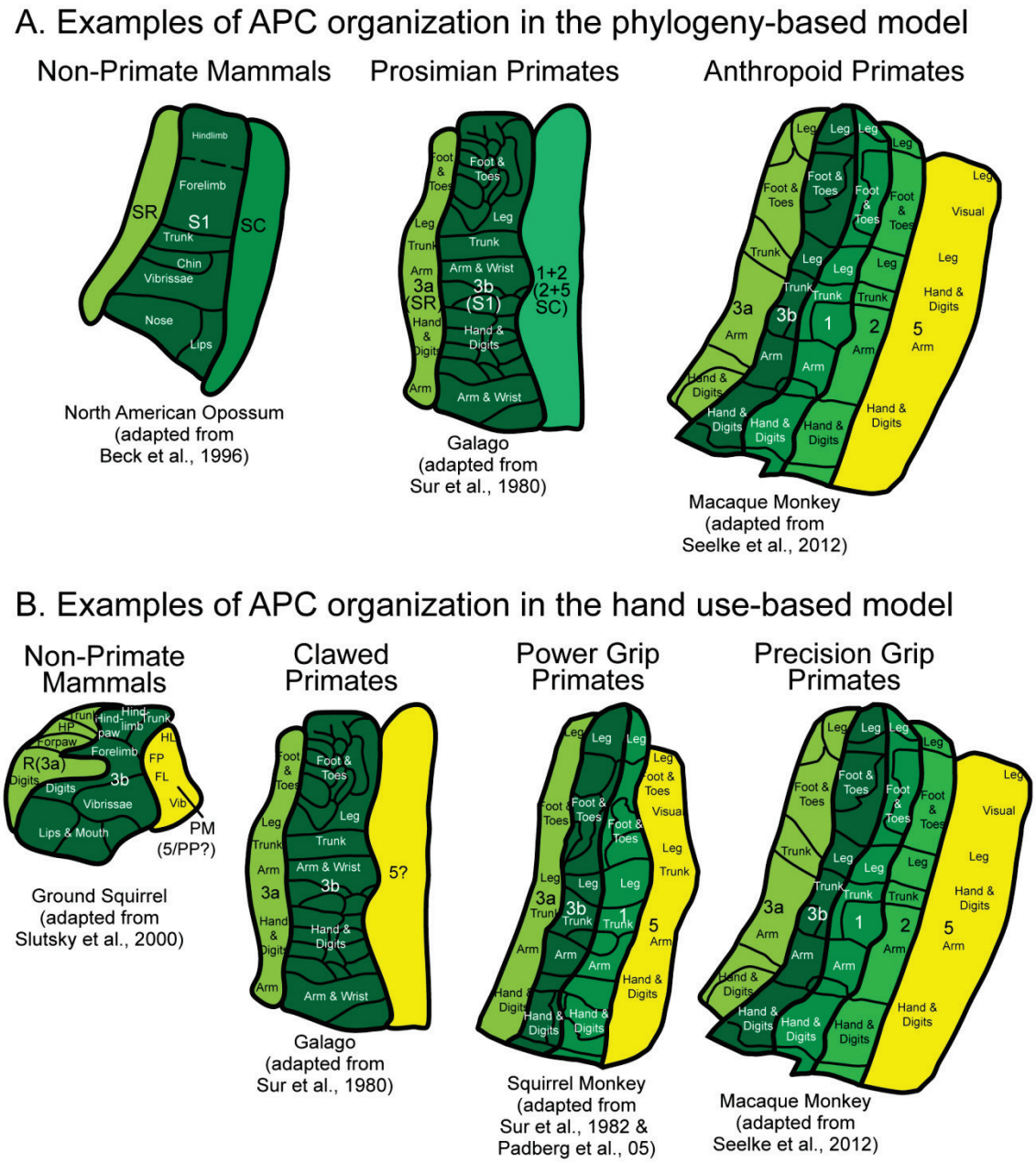

Fig 1.1. Alternate models of APC organization. A. Examples of APC organization from each category of mammals described in the phylogeny based model of APC organization. B. Examples of APC organization from each category of mammals described in the hand use-based model of APC organization.

receptors of the contralateral body from tongue to tail in a lateral-to-medial sequence (Kaas, 1983). SR is not strongly responsive to tactile stimulation under anesthesia,

however there is some evidence that SR responds to muscle spindle stimulation (Qi et al., 2008), suggesting it is homologous to primate area 3a where an organized map of the body's contralateral deep muscle and joint receptors has been described (Jones & Porter, 1980). The homology of SC has been more difficult to determine. Non-primate mammalian SC is unresponsive under anesthesia. By position, SC corresponds to the location of the unresponsive caudal somatosensory field that has been variably identified as area 1, area 1+2 (Wu & Kaas, 2003), or area 2+5 (Preuss & Goldman-Rakic, 1991) in prosimian galagos. The field just caudal to area 3b (S1 proper) in anthropoid New World and Old World monkeys, area 1, contains a second map of the cutaneous receptors of the contralateral body that mirrors the representation of the body in 3b. The cortex caudal to area 1 is variable in function in primates; however, a second map of the contralateral body's deep muscle and joint receptors mixed with cutaneous responses has been described in both New World cebus and Old World macaque monkeys (Felleman, Nelson, Sur, & Kaas, 1983; Pons, Garraghty, Cusick, & Kaas, 1985).

An modified model of APC organization has been proposed that takes into account not only phylogenetic relationships, but also the structure and use of the hand to reinterpret the evolutionary expansion of the APC and the functional organization of the APC in modern primates (Krubitzer & Disbrow, 2008; Padberg, Disbrow, & Krubitzer, 2005; Padberg et al., 2007). In the modified model, the organization of the primary somatosensory (S1 or 3b) and rostral somatosensory areas (SR or 3a) as processors of cutaneous and proprioceptive information, respectively, remain similar across all mammals. Rather, modifications were focused on the cortex caudal to primary somatosensory area, which is often unresponsive in anesthetized animals (Fig. 1.1B). In

non-primate mammals, the unresponsive SC was reinterpreted as homologous to primate area 5, a region of the posterior parietal cortex (PPC) that has been related to the integration of somatic inputs with the motor system (Padberg et al., 2005). A similar organization was proposed for primates who have clawed hands, such as prosimian galagos and New World marmoset and tamarin monkeys. In other anthropoid monkey species, area 1 has been defined along the caudal border of area 3b in this model. The identity of the unresponsive cortex caudal to the cutaneously responsive areas 3b and 1 in New World monkeys like owl and squirrel monkeys that rely on a power grip is interpreted differently than the same region in New World cebus and Old World macaque monkeys who regularly use their opposable thumbs to form a precision grip with the first and second digits. Padberg and colleagues (2005; 2007) related the function of the cortex caudal to area 1 to the evolution of monkeys with more dexterous hands and opposable thumbs. The lack of an opposable thumb limits use of the hand in most New World species where this region has been extensively studied electrophysiologically (owl, titi, and squirrel monkeys). Unable to form a precision grip with the first two digits, and with a smaller brain, these New World monkeys are proposed to lack an area 2. Rather, cutaneous area 1 is bordered caudally by PPC sensorimotor area 5 rather than APC proprioceptive area 2 in the modified model (Fig. 1.1B). In this alternate model, area 2 only appears in the larger brains of primates that need extra proprioceptive monitoring during skilled use of the hand, e.g. macaques. Arguing that increased use of a peripheral structure magnifies its representation in cortical areas (Krubitzer & Kaas, 2005) and that increasing the number of areas used to process information allows more parameters about the stimulus to be considered (Kaas, 1989), Padberg and associates (Krubitzer &

Disbrow, 2008; Padberg et al., 2005; Padberg et al., 2007) concluded that area 2 independently emerged in New World cebus and Old World macaque monkeys with the parallel evolution of an opposable thumb that allowed for precision hand use.

The differences in APC organization among non-primate mammals and prosimian and anthropoid primates are accompanied by differences in wiring among somatosensory thalamic nuclei and cortical areas that imply differences in the network for somatosensory processing as a whole (Fig. 1.2). When analyzing tactile information, the brain uses a combination of serial and parallel processing to build increasingly complex representations of the body with a great deal of flexibility (Pons et al., 1992; Toda & Taoka, 2001). The balance of serial and parallel processing is influenced by the

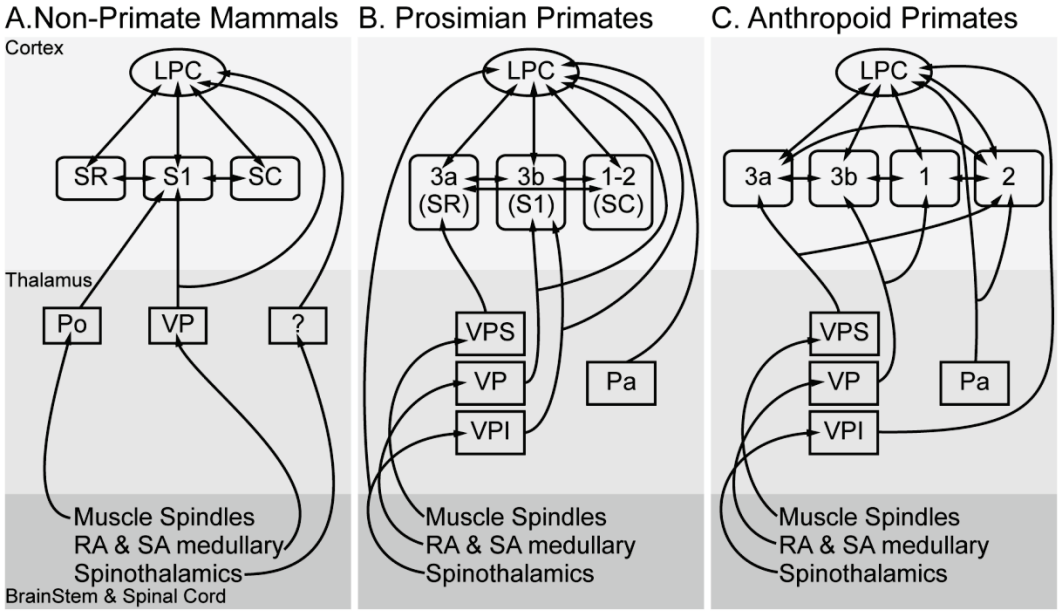

Figure 1.2. Schematics of the organization of somatosensory thalamic and APC connections of the somatosensory system. A-C. Depictions of somatosensory processing networks for (A) non-primate mammalian, (B) prosimian primate, and (C) anthropoid primates. Only the strongest known inputs are depicted. RA- rapidly adapting cutaneous afferents, SA- slowly adapting cutaneous afferents, VPS- ventroposterior superior nucleus, Po- posterior nucleus, Pa- anterior pulvinar nucleus. LPC- lateral parietal cortex.

organization of the APC. The differences in the number of and relationships between areas in the APC appear to reflect differences in how tactile information flows into and through the cortical somatosensory network.

In all mammals, somatosensory information from receptors in the skin, muscles, and joints is carried though the spinal cord and brain stem into the thalamus, and from there relayed to somatosensory-related cortical areas. Within the non-primate APC, serial processing, where information relayed from both cutaneous and deep receptor responsive nuclei is filtered through S1 before being sent to SR and SC, dominates. New inputs from the thalamus were added to the system as increasing thalamic specialization occurred and the APC expanded in primates. Each area of the primate APC receives parallel inputs from multiple thalamic nuclei (Padberg et al., 2009), however these appear to be largely modulatory as serial processing within the APC is still important and an intact area 3b (S1 proper) is required for proper function of other areas within the APC, including areas 3a, 1 and 2 (Ageranioti-Bélanger & Chapman, 1992; Garraghty, Florence, & Kaas, 1990; Randolph & Semmes, 1974). In non-primate mammals and prosimian galagos, the thalamic nucleus that processes cutaneous information, the ventroposterior nucleus (VP), and the region relaying information from the spinothalamic tracts, the ventroposterior inferior nucleus (VPI), each project to both the APC area S1 (3b in galagos) and the second somatosensory area (S2) in the lateral parietal cortex (LPC) (Burton & Carlson, 1986; Garraghty, Florence, Tenhula, & Kaas, 1991). Similar to non-primate mammals, ablations of galago area 3b failed to deactivate S2 (Garraghty et al., 1991). Thus, an arrangement of strong projections from VP and VPI to both S1 and S2 results in parallel activation of each of these regions and allows each to function somewhat independently

of the other. In anthropoid monkeys, dense inputs from VP are provided only to expanded APC areas, 3b in particular, (Coq, Qi, Collins, & Kaas, 2004; Friedman, Murray, O'Neill, & Mishkin, 1986; Krubitzer & Kaas, 1992; Qi, Lyon, & Kaas, 2002), and the projections from VPI to S2 alone do not appear to be enough to drive responses in the LPC (Garraghty, Florence, et al., 1990; Garraghty, Pons, & Kaas, 1990; Pons et al., 1987; however, Zhang et al., 1996; Zhang, Zachariah, Coleman, & Rowe, 2001).

Expansion of the APC in primates appears to have been accompanied by changes in the balance of parallel processing of somatosensory information across multiple regions of the parietal lobe. Within the APC, serial processing of information dominates in both non-primate mammals and primates, despite changes in thalamic input to the APC areas in primates. Changing thalamic inputs to the APC and somatosensory areas in the LPC also signaled a shift toward a more serialized scheme where the APC became vital to the proper processing of tactile inputs as it expanded. While this shift has been described, the functional advantage that provided as primates evolved has not been determined (Pons et al., 1992). With a clear picture of the organization of the APC in primates, we will be better able to understand the organization of the somatosensory system as a whole, as well as how tactile information is combined to create the normal perception of tactile stimuli.

Introduction of experimental aims

Since the phylogeny-based and hand use-based models of APC organization appear to reflect differences in the underlying network of connections among the

somatosensory thalamus and cortical areas, a better understanding of the pattern of projections to the different areas in the APC will help to distinguish which of the two APC organization models best reflects how information is processed in real brains. However, the projections to large regions of primary area 3b and much of the non-primary area 1 have not been thoroughly examined. Currently, there is only limited information available about the connections of the area 3b representation of the oral cavity in macaque monkeys. From evidence in New World monkeys (Iyengar, Qi, Jain, & Kaas, 2007), we expect that the 3b mouth region in macaques would reflect a pattern of connections with the rest of the somatosensory system that is similar to any other body part representation in area 3b, thus providing enough evidence to add the mouth representation, which has not been thoroughly described, into the models of APC organization. Furthermore, since the oral cavity representation includes a representation of a unique skin surface that contains receptors for both touch and taste in the tongue, the region of 3b devoted to the mouth may also receive set of projections not seen going to other body representations in 3b or other APC areas. Few studies have been focused on the connections of any part of the non-primary APC area 1, particularly in New World Monkeys. The projections of area 1 that have been described in macaques (Burton & Fabri, 1995) helped to identify each of the four APC areas (3a,3b, 1 and 2), thus studying the connections of this area in a New World species, squirrel monkeys, and comparing them to the results seen in macaques can resolve lasting questions about the organization of APC in New World monkey species that cannot be answered with physiological studies alone.

Primates have a large representation of the oral cavity, especially of the teeth and tongue, in the primary somatosensory cortex. Unlike the rest of the body, the oral cavity representation in area 3b contains a large bilateral representation of intra-oral structures in both cerebral hemispheres (Jain, Qi, Catania, & Kaas, 2001; Manger, Woods, & Jones, 1996; Qi & Kaas, 2004). Despite its large size and functional importance in evaluating and processing food and providing sensory feedback during movements of the jaw and tongue (Iyengar et al., 2007; JH Kaas, Qi, & Iyengar, 2006; Lin, Murray, & Sessle, 1993), little is known about the connections of the cortical representation of the oral cavity in macaques. This is due, in part, to the difficulties in reaching this most lateral part of area 3b during microelectrode mapping experiments and studies of connections based on cortical injections. We injected anatomical tracers into defined tongue, teeth, and face representations to reveal thalamic and cortical inputs to this region. This allowed us to compare the pattern of projections to and within the 3b oral-cavity representation to that seen in other representations within area 3b, particularly the large and well-studied representation of the hand. Because the tongue is a sensory surface with receptors for both touch and taste, many questions about how taste and touch are integrated to form the perception of flavor and evaluate the quality of food remain unanswered. For example, the presumptive taste nucleus of the thalamus has been shown to project to the tongue representation in area 3b in New World monkeys (Benjamin & Burton, 1968; Benjamin, Emmers, & Blomquist, 1968; Iyengar et al., 2007). However, the evidence in macaques has been contradictory (Pritchard, Hamilton, Morse, & Norgren, 1986; Roberts & Akert, 1963) and in some cases revised after results were reinterpreted by the author (Pritchard & Norgren, 2004). Our method of injecting tracer into electrophysiologically defined

representations of the tongue in area 3b and the subsequent study of the locations of the backfilled cells resulting from this injection provided new insight about the integration of touch and taste. The intrinsic connections within area 3b are also a region of interest. In light of the reorganization of the somatosensory cortex that occurs after peripheral damage and spinal cord injury (Fang, Jain, & Kaas, 2002; Merzenich et al., 1983; Merzenich et al., 1984), many questions have arisen about the extent of the connections between the representations of the face and hand (Fang et al., 2002). These experiments also allowed us to look for a pre-existing network that may underlie the changes resulting from injury. Our use of retrograde tracer injections in the 3b representations of intra-oral structures and the face provided new insight into both the extrinsic inputs to and intrinsic connections within the mouth and face region of area 3b, and to determine if information coming from the oral cavity and face is processed similarly to other that from other body part representations in primary somatosensory cortex. Furthermore, this new information about the role of cortex devoted to the inside of the mouth and face allowed us to add the oral cavity representation to models of APC organization, which currently do not take the intra-oral representation into account.

The pattern of projections to non-primary somatosensory areas in the APC has rarely been the focus of study, particularly in New World monkeys. New World squirrel monkeys are becoming a common model animal for studying the effects of spinal cord injuries (Bowes, Massey, Burish, Cerkevich, & Kaas, 2012; Chen, Qi, & Kaas, 2012; Qi, Chen, & Kaas, 2011). The organization of projections to squirrel monkey area 1, a non-primary area involved in tactile discrimination, has not been extensively studied. It has been proposed that the cortex immediately caudal to area 1 is equivalent to PPC area 5

rather than APC area 2 (Padberg et al., 2005; Padberg et al., 2007). We studied the projections from the thalamus and other areas of cortex to area 1 in depth by injecting anatomical tracers across the somatotopic body map in area 1. The use of multiple tracers revealed the internal organization of thalamic nuclei and difficult to access, understudied somatosensory and motor areas along the lateral sulcus and medial wall, providing insight into the organization of the squirrel monkey brain as a whole. Comparing our results to those previously published for macaque, owl, titi, and marmoset monkeys, as well as prosimian galagos allowed us to determine if the pattern of somatosensory projections suggests an APC organized with four areas, including an area 2, or one where PPC area 5 directly abuts the caudal border of area 1.

Specific aims

In summary, our experiments are designed to reveal connections of previously understudied regions of Old World monkey primary somatosensory cortex and a New World monkey non-primary somatosensory area. Thus, our experiments indicate changes in network organization that occurred over the course of primate somatosensory network expansion. Specifically, our aims are:

1. To determine the full complement of thalamocortical and corticocortical projections to the macaque monkey primary somatosensory representations of the face and oral cavity.

2. To demonstrate the projections to somatosensory area 1 in squirrel monkeys, with an emphasis on the organization of the anterior parietal cortex and the topographic organization of these projections

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CHAPTER II

THALAMIC INPUT TO THE 3B REPRESENTATIONS OF THE TEETH, TONGUE, AND FACE IN MACAQUE MONKEYS

Abstract

Representations of the parts of the oral cavity and face in somatosensory area 3b of macaque monkeys were identified with microelectrode recording 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 show that there is somatotopically-organized, fine-grained anatomical map in VPM, and that this nucleus sends organized projections to the part of 3b representing intra-oral structures and the face. Cells backfilled by cortical injections 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). No projections from VPMpc, the thalamic taste nucleus, were found after injections in the representation of the contralateral tongue, suggesting that taste and touch are processed separately at early

stages of their respective cortical networks in macaques. This result stands in contrast to those reported for New World monkeys.

Introduction

Texture is an important aspect of evaluating the quality of food (Shepherd, 2012). Somatosensory inputs from the oral cavity are also important in the guidance of movements of the tongue during complex oral behaviors (Lin, Murray, & Sessle, 1993). After a spinal cord injury, regions of primary somatosensory cortex (area 3b) normally representing the hand can be activated by stimulation of the face (Fang, Jain, & Kaas, 2002). While tactile sensation from the mouth is vital to an animal's survival in many ways, our understanding of the organization of the 3b mouth and face representations remains limited (Iyengar, Qi, Jain, & Kaas, 2007; Kaas, Qi, & Iyengar, 2006; Manger, Woods, & Jones, 1996). In macaque monkeys, in particular, the location of the intra-oral representation deep in the lateral extreme of the central sulcus has complicated and discouraged relevant research.

For the present research, our goal was to describe the complete complement of thalamic input to cortex representing the face and mouth in area 3b. However, we had a particular interest in determining the projections from the thalamus to the cortex representing the tongue. The current picture of thalamic inputs to the region of primary somatosensory (S1 proper or area 3b) cortex serving the tongue is unclear, with differences in species, techniques, and interpretations leading to a conflicted set of results. The oral cavity representation in area 3b is known to get some of its inputs from

the ventroposterior medial sub-nucleus (VPM) of the thalamus (Iyengar et al., 2007; Kaas et al., 2006; Nelson & Kaas, 1981). VPM has been shown to have distinct representations of the face and oral cavity that can be defined electrophysiologically (Jones & Friedman, 1982; Kaas, Nelson, Sur, Dykes, & Merzenich, 1984), correspond to architecturally distinct cytochrome oxidase subdivisions (Rausell & Jones, 1991a, 1991b), and project to specific parts of the representations of the teeth, tongue, and face in the cortex (Iyengar et al., 2007; Nelson & Kaas, 1981). Previous research on the organization of cortical oral cavity representation in New World monkeys has also revealed connections with the ventroposterior medial parvicellular (VPMpc) nucleus (Benjamin & Burton, 1968; Benjamin, Emmers, & Blomquist, 1968; Iyengar et al., 2007), the presumptive taste nucleus of the thalamus (Kaas et al., 2006; Pritchard, Hamilton, & Norgren, 1989; Pritchard & Norgren, 2004). VPMpc is distinct from VPM, not only in its' response characteristics, but also in its' connections, architecture, and histochemistry (Burton & Jones, 1976; Iyengar et al., 2007; Pritchard, Hamilton, Morse, & Norgren, 1986; Rausell & Jones, 1991a, 1991b; Roberts & Akert, 1963). It has been suggested that VPMpc also projects to the macaque area 3b representation of the mouth (Pritchard et al., 1986), though these results conflict with previous studies (Burton & Jones, 1976; Roberts & Akert, 1963) and have subsequently been reinterpreted to include projections from VPM with the possible exclusion of projections from VPMpc (Pritchard & 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 of the area 3b face and oral cavity representations.

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. All 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 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 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 the 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 lesions by using blood vessels and sulcal patterns as landmarks.

Multiunit mapping and injections

Neuroanatomical tracers were injected into physiologically defined representations of the tongue, teeth, gingiva, palate, buccal wall, lips, and chin. Maps of the 3b representations of the parts of the mouth and face were obtained by inserting low-impedance tungsten (1.0 mΩ at 1,000 Hz) 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 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 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 subsequently perfused though the heart with phosphate buffered saline 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.

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 (Angelucci, Clasca, & Sur, 1996; Bruce & Grofova, 1992) 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, Martonez-Garcia, & Lopez-Garcia, 1985) and stabilized in diamidinobenzidine (DAB). In all cases, series of sections were processed for cytochrome oxidase (CO; Wong-Riley, 1979) according to standard protocols. 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.

Calcium binding proteins: Calbindin and Parvalbumin. Our western blot analysis from macaque monkey cerebellar tissue shows that the mouse monoclonal anticalbindin antibody (Swant, Bellinozona, Switzerland, Catalog no. C98-49, Dilution factor 1:5,000, Isotope IgG, Calbindin D- 28K purified from chicken gut) labeled a band at 28 kDa weight, while the mouse monoclonal antiparvalbumin antibody (Sigma-Aldrich, St. Louis, Mo, Catalog no. P3088, Dilution factor 1:2,000, Isotope IgG1, Frog muscle parvalbumin clone PARV-19) 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; Qi, Gharbawie, Wong, & Kaas, 2011; Wong & Kaas, 2008). While staining for Cb reveals a different subset of GABA-immunoreactive interneurons from PV protein in the thalamus (Jones & Hendry, 1989; Rausell & Jones, 1991a, 1991b) and cortex (Van Brederode, Mulligan, & Hendrickson, 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 & 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 & Hendry, 1989; Rausell & Jones, 1991a, 1991b).

VGluT2. A mouse monoclonal anti-vesicular glutamate transporter 2 antibody clone, 8G9.2 (Chemicon now part of Millipore, Billerica, MA, Catalog no. MAB5504, Dilution factor 1:2,000, Isotope IgG1, Recombinat protein from rat VGLuT2), 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., submitted), confirming the manufacturer information. Previous reports indicate that VGluT2 immunostaining reveals the thalamocortical terminations in layer 4 of the cortex in rats (Fujiyama, Furuta, & Kaneko, 2001) and macaque monkeys (Hackett & de la Mothe, 2009), as well as the brainstem terminations in the thalamus of rats (Kaneko & Fujiyama, 2002), squirrels (Wong, Gharbawie, Luethke, & Kaas, 2008), galagos (Wong & 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 (Iyengar et al., 2007; Jain, Catania, & Kaas, 1998; Kaas et al., 2006; Qi & Kaas, 2004). In the face and mouth region of area 3b, myelin dense patches indicate anatomical representations of the different oral cavity and face structures (Iyengar et al., 2007; Jain, Qi, Catania, & Kaas, 2001; Kaas et al., 2006). 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 CO, VGluT2, PV, and Cb (See Fig. 2.1 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 collapsed representations of thalamic sections on which the locations of cells labeled by different injections are easily seen.

Results

Anatomy of VPM

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 for 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 & Jones, 1991a, 1991b). Staining for the mitochondrial enzyme CO (Fig. 2.1, column A) and the calcium binding protein 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, Cb (Fig. 2.1, column B), there was little expression in VPM, while VPMpc stained darkly.

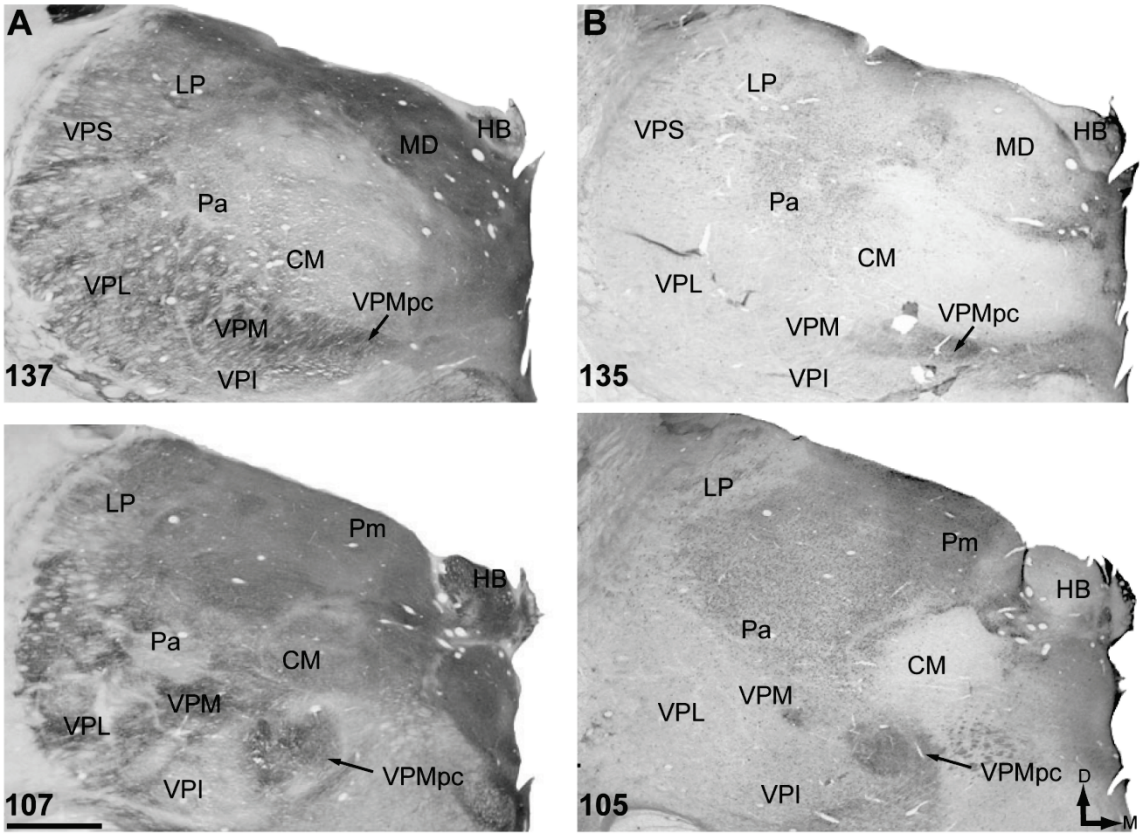

Figure 2.1. Histological characteristics of the somatosensory thalamus. A-B. Photomicrographs of coronal sections of thalamus stained for CO (column A) and Cb (column B) cut at 40 µm thickness. Representative sections were selected to show the borders of nuclei pertinent to cell labeling from the injections of tracers, including the somatosensory VPM and the gustatory VPMpc. There is approximately 1.16 mm between sections between rostral (CO 137 and Cb 135) and caudal (CO 107 and Cb 105) sections. Cb sections are the closet adjacent Cb to each CO stained section. Scale bar is 2 mm.

Our use of immunohistochemistry to reveal the distribution of 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 &

Fujiyama, 2002). Densely staining patches of VGluT2 expression were found in VPM (Fig. 2.2B). When aligned to adjacent CO stained sections (Fig. 2.2A) 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. 2.2C). Separate dense concentrations of CO in VPM have been shown to correspond to the representation of a single part of the face or mouth (Jones & Friedman, 1982; Rausell & Jones, 1991b). VGluT2 has been shown to provide a further refinement of the anatomical map revealed by CO staining in VPL, the lateral sub-nucleus of VP representing the body and limbs (Qi et al., 2011).Thus, each VGluT2 patch likely represents a smaller part of the thalamic face or oral cavity representation than the CO patch in which it sits.

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

Case 1. Thalamocortical connections were labeled by injecting tracers into the cortical representations of parts of the face and mouth in three monkeys. In case 1, one injection was placed into the tongue representation and another into the representation of the teeth (Fig. 2.3B). 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, the proximal chin, the anterior neck, and the hairs on the neck and

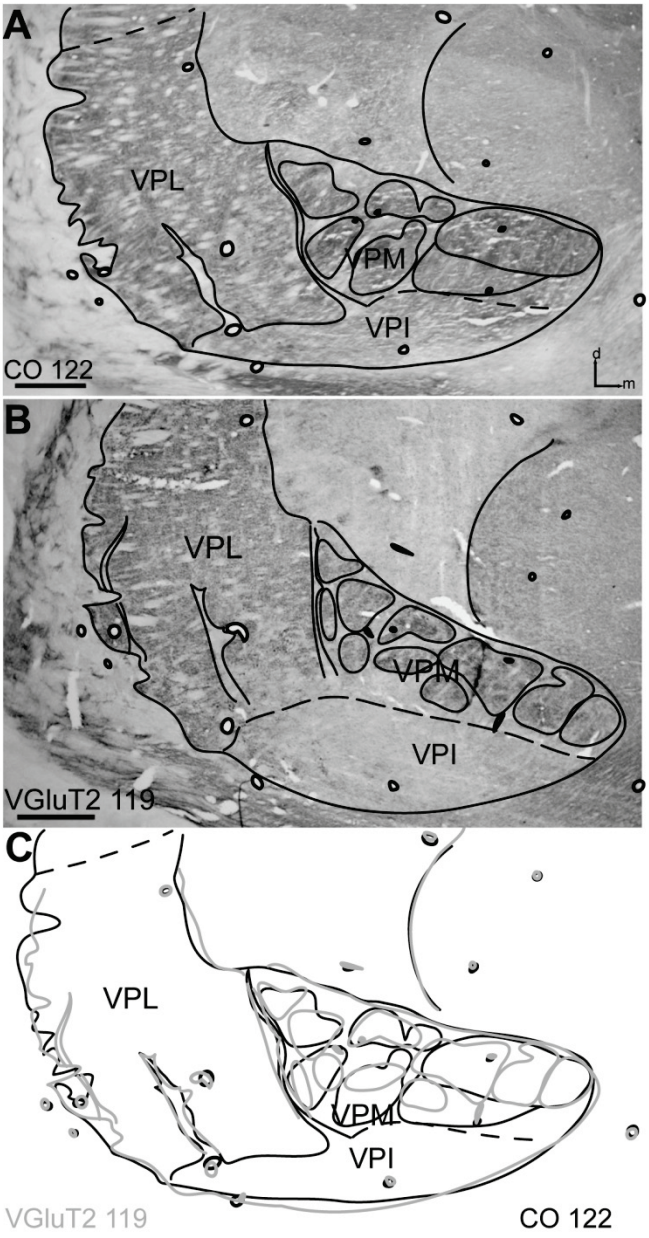

Figure 2.2. Comparison of CO and VGluT2 staining in the thalamus. A-B. Photomicrographs of thalamus sections stained for CO (A) and VGluT2 (B) cut at 40 μm. C. Drawings of aligned borders from CO and VGluT2 coronal sections. Thin lines are anatomical borders and CO or VGluT2 dense patches, respectively, within VPM. Thick lines are blood vessels and other landmarks. CO dense patches 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 (gray in C). These smaller VGluT2 dense subdivisions of CO patches likely represent an even more fine-grained anatomical representations of the parts face and mouth within VPM. While sections are best matched 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. Scale bar is 1mm.

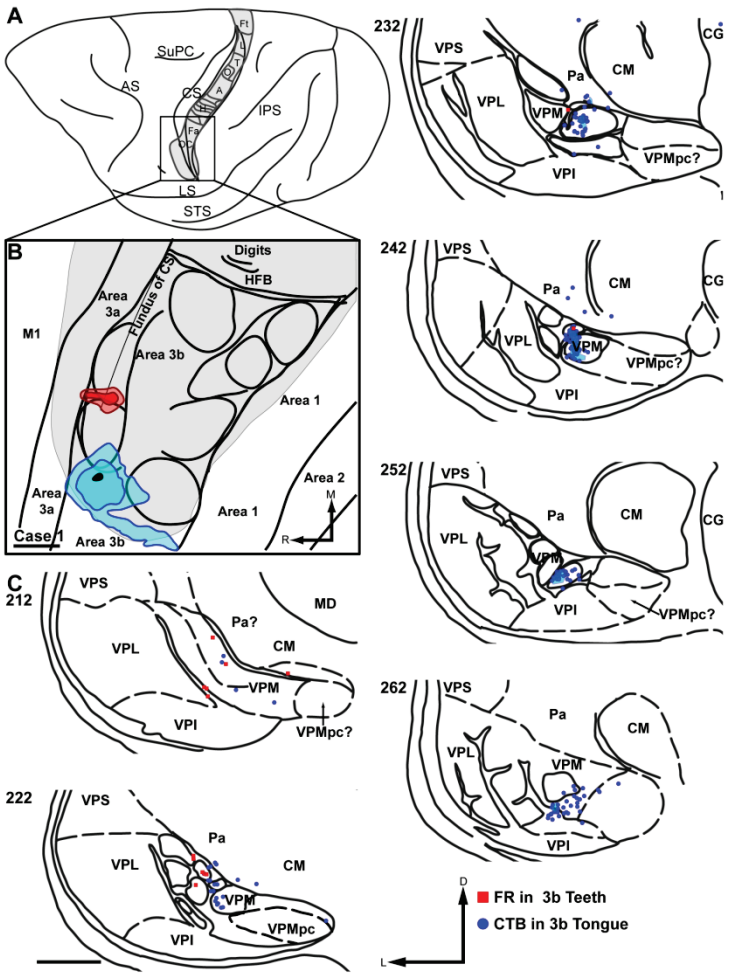

Figure 2.3. Thalamocortical projections in case 1. A. A lateral view of the brain with the central sulcus opened. Area 3b is shaded in gray with the somatotopic organization of area 3b outlined, adapted from Nelson et al., 1980 and Jain et al., 2008. Body representations: OC- oral cavity, Fa- face, H- hand, A- arm, O- occiput, neck, and shoulder, T- trunk, L- leg, Ft- foot. Rostral is left, medial is up. B. A closer 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 distribution 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 thalamic sections are numbered from rostral to caudal. Sections were cut at 40µm. Scale bar is 2 mm in both B and C. 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.

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.

The locations of cells labeled by these two injections related to the borders of thalamic nuclei are shown in part C of figure 2.3. 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 2.1), 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

Table 2.1. 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.

	Case1		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	—	—	—	—	—	—

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 2.1), 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. 2.4A 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. 2.4A green) was placed medial and slightly caudal to the first. The injection was

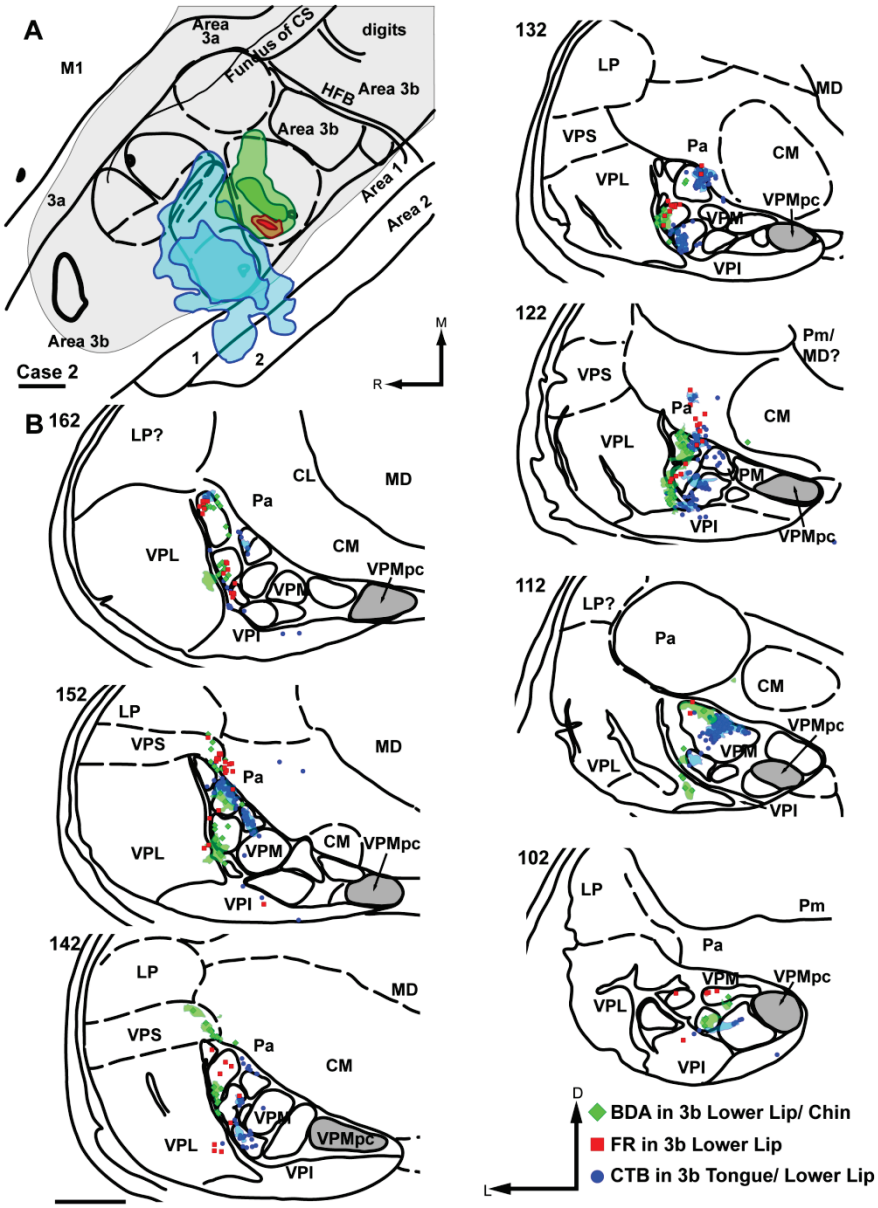

Figure 2.4. Thalamocortical projections in case 2. A. Outlines of the injection cores and diffusion zones in relation to the myeloarchitecture of the 3b mouth representation. The most medial of our 7 total injections was of BDA (green) into the representations of the lip and chin. A small injection of 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 its 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 distribution of labeled neurons in coronal sections from case 2. Alternate thalamic sections are numbered from caudal to rostral. Sections were cut at 40µm. All conventions as in figure 2.3. 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.

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. 2.4A 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. 2.4B). 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 the tongue/ lip representation were 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 (Fang et al., 2002; Krubitzer & Kaas, 1992). Filled cells from injections in the lip and chin representations were in the medial extreme of the ventroposterior superior nucleus (VPS) (Fig. 2.4, 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 2.1), 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 an 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. 2.5A). 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

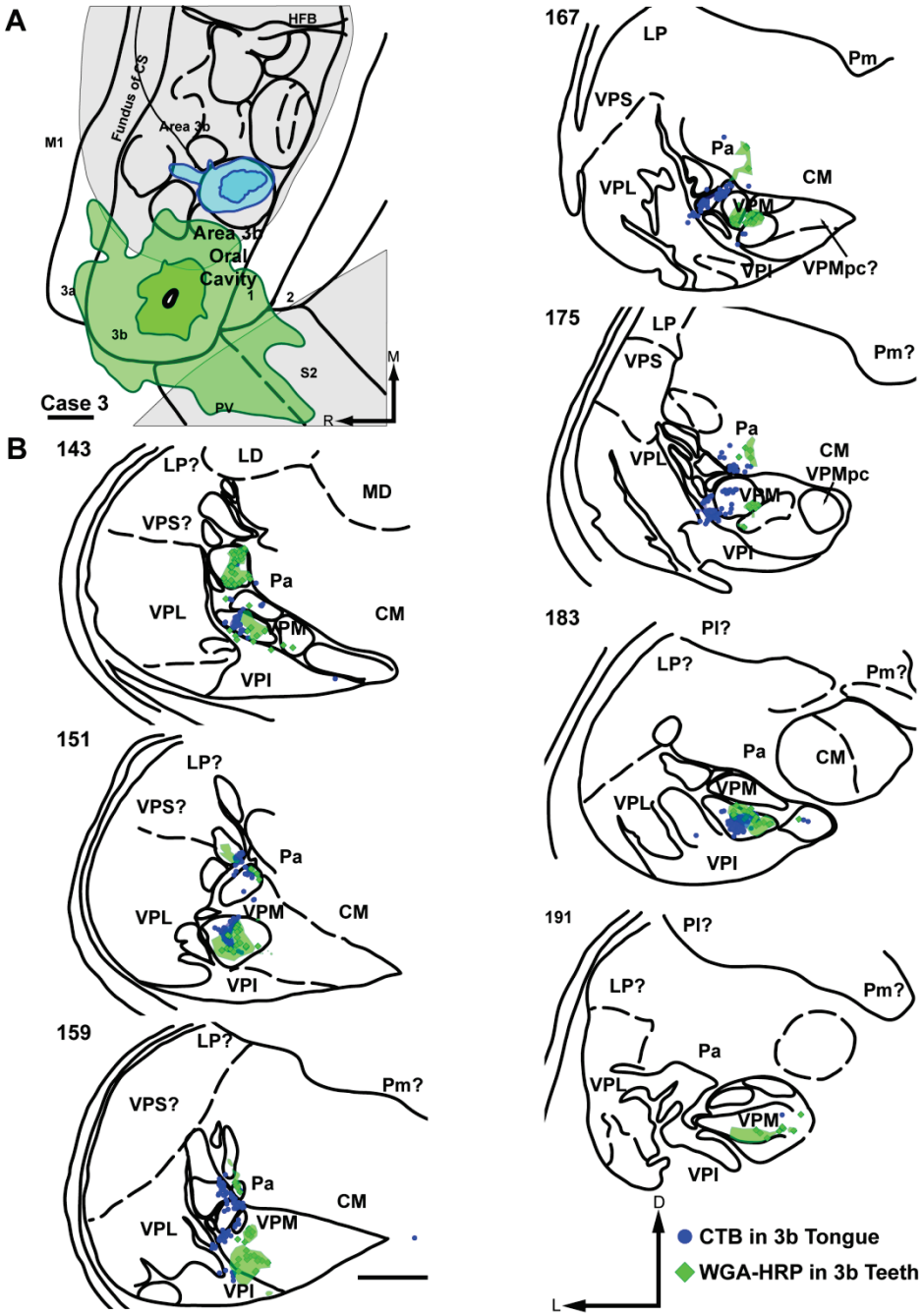

Figure 2.5. Thalamocortical projections in case 3. A. Outlines of the injection cores and diffusion zones in relation to the myeloarchitecture of the 3b mouth representation. CTB (blue) was injected into the representation of the tongue. WGA-HRP (green) was placed into a region of mixed ipsilateral and contralateral responses to stimulation of the teeth, gums, and palate. The CS and part of the LS are shaded in gray. B. The distribution of labeled neurons in coronal sections from case 3. Alternate thalamic sections are numbered from rostral to caudal. Sections were cut at 50µm. All conventions as in figure 2.3. 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.

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 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. 2.5, 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 (Fig. 2.5, 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 (not shown) 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 2.1), but were also found in Pa, VPI, VPS, VPL, CM, and VL. 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 serving 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 inside of the mouth received a wide range of thalamic inputs from somatosensory nuclei: VPM, Pa, VPI, VPS, and VPL; as well as the posterior parts of VL, CM, CL, and MD, 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 (Pritchard & Norgren, 2004; Scott & Plata-Salamán, 1999). In the thalamus, a similar separation is maintained with VPM processing touch and VPMpc responding to taste (Kaas et al., 2006; Pritchard & Norgren, 2004; Scott & Plata-Salamán, 1999). In the cortex, the two systems have been shown to interact in the orbitofrontal cortex (Kringelbach, 2004; Rolls, 2000; Rolls & Baylis, 1994; Scott & Plata-Salamán, 1999). However, if VPM and VPMpc provide inputs to the same area in cortex, then integration of touch and taste may begin earlier in a cortical network for flavor and appreciation of food than previously demonstrated. 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 related cortex) yielded damage in the taste nucleus of the thalamus. Thus, Roberts and Akert (1963) concluded

that VPM projected 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 & Burton, 1968; Benjamin et al., 1968). Rather, a large lesion including both cortical areas was required to cause complete loss in VPMpc, leaving the authors to conclude that the taste nucleus, VPMpc, supplied at least some input to both SI and gustatory cortex (Benjamin & Burton, 1968; Benjamin et al., 1968). Somewhat later, after injections of tritiated amino acids into the posterior thalamus of macaque and squirrel monkeys, Burton and Jones (1976) concluded 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, these injections would not have demonstrated it because 1) the focus 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 these results must be reconsidered in the face of 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 types 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 in macaques, while the area 3b tongue representation has a direct role in analyzing taste in New World monkeys. This finding merits further investigation into how taste is processed across primates 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 macaque 3b tongue representation (Ogawa, Ito, & Nomura, 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 in macaques (Cerkevich, Qi, & Kaas,

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 intra-oral tactile information into the perception of flavor occurs in other regions of the cortex (Kaas et al., 2006; Kringelbach, 2004; Rolls, 2000; Small et al., 1999).

Somatosensory modules in VPM

In macaques, VPM is subdivided into anatomical modules that respond to touch on different parts of the face and mouth (Jones & Friedman, 1982; Rausell & Jones, 1991a, 1991b). 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 (Ge et al., 2010; Graziano, Liu, Murray, & Jones, 2008) end on clusters of cells in VPM that are highly metabolically active and respond to stimulation to a circumscribed part 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, where the VGluT2 protein labeling provided more detail.

Here, 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. 2.3-2.5). The pattern of connections also provided evidence for the overall somatotopic organization in VPM. Responses to the face, lips and chin were 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. 2.6). Injections

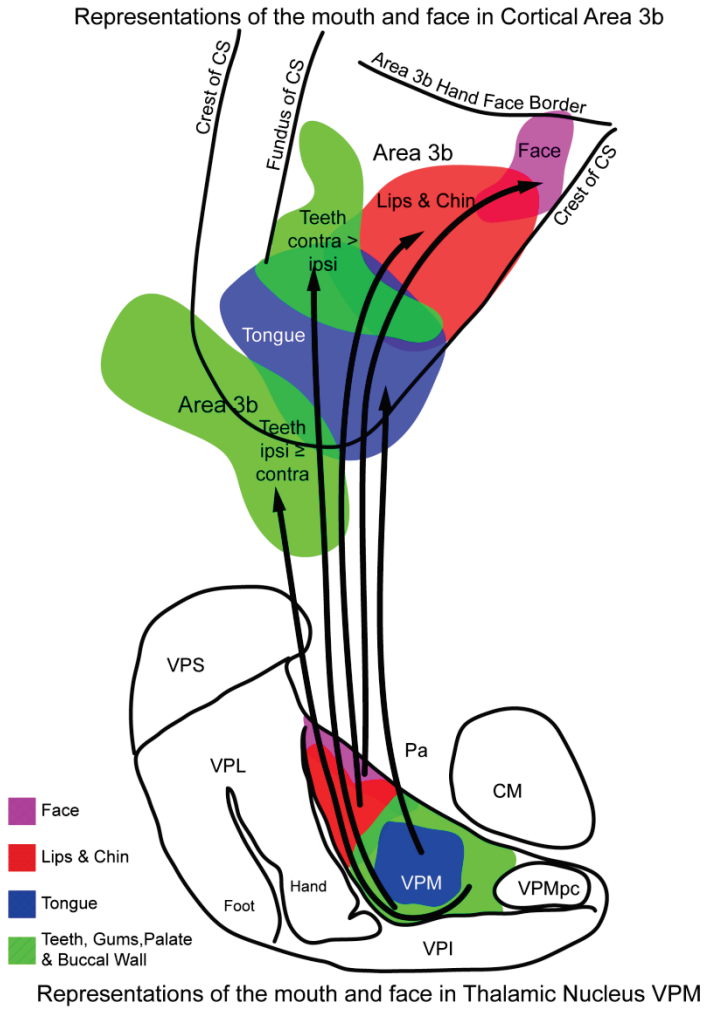

Figure 2.6. A summary of projections from representations of the face and oral cavity structures in thalamic VPM project to matching representations in area 3b. Electrophysiological mapping has shown organized representations of the face, lips, teeth, and tongue in both area 3b and in VPM (adapted from 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 of a face or mouth part in the thalamus projects to a matched representation in the cortex.

into this region yielded retrogradely labeled neurons in the lateral part of VPM (Fig.2.3 red; 2.4 red & green). Previous physiological mapping studies of VPM (Jones & Friedman, 1982; Rausell & Jones, 1991b) have indicated that this region of the thalamus is responsive to stimulation of the skin on the face and lips (Fig. 2.6). Our results indicated that the regions of lateral VPM representing the face and lips project to the regions of primary somatosensory cortex representing the face and lips (Fig. 2.6). Responses to the teeth in area 3b occur in two parts of the intra-oral representation (Iyengar et al., 2007; Jain et al., 2001; Kaas et al., 2006; Manger et al., 1996; Qi & Kaas, 2004). When an injection of tracer is placed in the more medial cluster of responses to stimulation of the teeth (generally dominated by responses to stimulation of contralateral teeth), resulting labeled cells in the thalamus were located lateral to those projecting from VPM to the 3b tongue region (Fig. 2.3). Physiological recordings also have identified a region of cells responsive to the contralateral teeth and surrounding structures near the lateral edge of the tongue representation in VPM (Rausell & 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. 2.5). This location seems to correspond to the region of VPM responsive to stimulation of the ipsilateral teeth and surrounding structures (Rausell &

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 2.6.

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 complete compliment of thalamic projections to the representations of the face and mouth in area 3b that has not been previously demonstrated in macaque monkeys. As in previous studies (Burton & Jones, 1976; Iyengar et al., 2007; Nelson & Kaas, 1981; Pritchard et al., 1986), VPM consistently projected to the oral cavity representation in area 3b. Other nuclei known to respond to both cutaneous and deep stimulation of the skin and joints (VPL, VPS, VPI, and Pa) also projected to area 3b. These converging inputs were relatively weaker in magnitude than those from VPM (Table 2.1), but they 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 area 3b's contribution to the coordination of smooth movements of the mouth and face during feeding and vocalization (Avivi-Arber, Martin, Lee, & Sessle, 2011; Itoh, Nishiura, Tabata, & Watanabe, 2002; Lin et al., 1993). Furthermore, these converging inputs may underlie the changes in the cortical representation of the oral cavity and face that occur after sensory loss (Jones, 2000; Kaas, 2000; Merzenich et al., 1983; Merzenich et al., 1984; Recanzone, Jenkins, Hradek, & Merzenich, 1992). Inputs from intralaminar nuclei CM and CL, as well as MD and possible visceral regions of the thalamus 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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CHAPTER III

CORTICOCORTICAL PROJECTIONS TO THE 3B REPRESENTATIONS OF THE TEETH, TONGUE, AND FACE IN MACAQUE MONKEYS

Abstract

We placed injections of anatomical tracers into distinct representations of the tongue, teeth, and face in the primary somatosensory cortex (area 3b) of macaque monkeys. Our injections revealed the intrinsic connections within the area 3b oral cavity and face region, as well as inputs from other cortical areas. There were strong projections to representations of the tongue and teeth from other parts of the oral cavity responsive region in 3b. The 3b face also provided input to the representations of the intra-oral structures. The primary representation of the face showed a pattern of intrinsic connections similar to that of the mouth. Little to no input from the area 3b hand was indicated to either the mouth or face representations. The mouth and face representations of area 3b received projections from the presumptive oral cavity and face regions of other somatosensory areas in the anterior parietal cortex and the upper bank of the lateral sulcus including areas 3a, 1, 2, the second somatosensory area (S2), the parietal ventral area (PV), and cortex that may include the parietal rostral (PR) and ventral somatosensory (VS) areas. Additional inputs came from primary motor (M1) and ventral premotor (PMv) areas. This pattern of projections is similar to the well-studied pattern revealed by tracer injections in regions of 3b representing the hand. The tongue representation appeared to be unique in area 3b in that it also received inputs from areas

in the anterior upper bank of the lateral sulcus and anterior insula that may include the primary gustatory area (area G) and other taste processing cortex, as well as a region of lateral prefrontal cortex (LPFC) lining the principal sulcus.

Introduction

Primates have a large representation of the oral cavity, especially of the teeth and tongue, in the primary somatosensory cortex (area 3b). Unlike the rest of the body representation in 3b this region contains a large bilateral representation of intra-oral structures in both cerebral hemispheres (Jain, Qi, Catania, & Kaas, 2001; Manger, Woods, & Jones, 1996; Qi & Kaas, 2004). Despite its large size, functional importance in evaluating and processing food (Shepherd, 2012), expansion after spinal cord injury (Fang, Jain, & Kaas, 2002; Jain, Catania, & Kaas, 1997; Jain, Florence, & Kaas, 1998; Jain, Qi, Collins, & Kaas, 2008; Pons et al., 1991), and role in coordinating movements of the tongue and jaw (Lin, Murray, & Sessle, 1993, 1994), little is known about the connections of the cortical representation of the oral cavity in macaques. This is due, in part, to the difficulties in reaching this most lateral part of S1 during microelectrode mapping experiments and studies of connections based on cortical injections.

Much like the hand, representations of different parts of the oral cavity and face have been found in multiple areas in somatosensory and motor cortex. Microelectrode mapping studies have shown, at least in part, orderly arrangements of the responses to stimulation of the oral cavity and face in somatosensory areas including 3b, 3a, 1, 2, the second somatosensory area (S2), and parietal ventral area (PV) in Old World macaques

(Krubitzer, Clarey, Tweedale, Elston, & Calford, 1995; Manger, Woods, & Jones, 1995; Manger et al., 1996; Nelson, Sur, Felleman, & Kaas, 1980; Qi & Kaas, 2004; Toda & Taoka, 2001, 2002a, 2002b, 2004) and New World monkeys (Iyengar et al., 2007; Jain, Qi, Catania, et al., 2001; Krubitzer & Kaas, 1990; Qi, Lyon, & Kaas, 2002). Representations of the face have been described in additional somatosensory areas in the lateral sulcus, the ventral somatosensory (VS) and parietal rostral (PR) areas, in New World titi monkeys (Coq, Qi, Collins, & Kaas, 2004). The existence of similar representations has been suggested in macaques (Krubitzer et al., 1995), though mapping in these regions has been incomplete. In the frontal lobe, intracortical stimulation (Ghosh & Gattera, 1995; Graziano, Taylor, & Moore, 2002; McGuinness, Sivertsen, & Allman, 1980), recording (Avivi-Arber, Martin, Lee, & Sessle, 2011; Hoffman & Luschei, 1980; Murray & Sessle, 1992), and cooling (Murray, Lin, Moustafa, & Sessle, 1991) have all indicated that regions of the lateral primary motor cortex (M1) and ventral premotor area (PMv) are involved in controlling movements of the face and tongue. Few studies have been done in macaques to show how these multiple mouth and face representations interconnect.

Most of the anatomical information about the representation of the oral cavity has come from studies in New World monkeys where it is most accessible, although a few studies have been done on restricted parts of the 3b mouth representation in macaques. The organization in area 3b defined during microelectrode mapping of responses to tactile stimulation of the mouth and face has been shown to overlie densely myelinated ovals lateral and anterior to the 3b hand representation in prosimian galagos, and anthropoid marmoset, owl, squirrel, and macaque monkeys (Iyengar et al., 2007; Jain,

Catania, & Kaas, 1998; Jain, Qi, Catania, et al., 2001; Kaas et al., 2006; Qi & Kaas, 2004). Projections from within area 3b and the presumptive face representations in areas 3a, 1, 2, and 5 to representations of the upper face and lip in area 3b were described as similar to those going to other parts of area 3b during a larger study of connections of areas in the anterior parietal cortex (Burton & Fabri, 1995). Iyengar and colleagues (2007) used physiological mapping to target representations of individual intra-oral structures, parts of the tongue and upper and lower teeth, in primary somatosensory cortex for injection of neuroanatomical tracers in three species of New World monkeys. In all three species, the 3b oral cavity representations received a pattern of projections from somatosensory and motor areas largely similar to any other region of 3b (Iyengar et al., 2007). Interestingly, injections into the representation of the tongue also labeled regions of cortex in the anterior upper bank of the lateral sulcus (UBLS) and insula that included the primary gustatory cortex (area G) (Iyengar et al., 2007; Sanides, 1968) and possibly other area involved in processing taste (Ogawa, Ito, & Nomura, 1989; Plata-Salamán & Scott, 1992; Pritchard & Norgren, 2004; Scott & Plata-Salamán, 1999). Little is known about the projections of taste areas to the 3b representation of the tongue in macaques.

In the present study, we injected anatomical tracers into physiologically defined tongue, teeth, and face representations in area 3b to reveal corticocortical inputs to this region. This allowed us to compare the pattern of projections to the 3b oral cavity and face representations to that seen going to other body part representations of area 3b, particularly the large and well-studied representation of the hand. Furthermore, these experiments allowed up to look for a pre-existing network that may underlie the resulting

reorganization after deafferenting spinal cord injuries (Fang et al., 2002; Jain et al., 1997; Jain, Florence, et al., 1998; Jain et al., 2008; Pons et al., 1991). Our injections of retrograde tracers allowed us to use the location of backfilled cells to determine the extent of mouth and face representations in projecting areas. Finally, our injections in the primary somatosensory tongue representation allowed us to provide some new insight into the integration of touch and taste by determining which areas involved in processing taste in the opercular and insular cortex (Ogawa et al., 1989; Pritchard & Norgren, 2004; Scott, Yaxley, Sienkiewicz, & Rolls, 1986; Scott & Plata-Salamán, 1999; Smith-Swintosky, Plata-Salaman, & Scott, 1991) provide input to area 3b.

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. All surgical procedures were performed under aseptic conditions.

Surgical procedure

The animals were anesthetized with an initial dose of ketamine hydrochloride (10-50 mg/kg, i.m.), and secured in a stereotaxic frame. A surgical level of anesthesia was maintained with an intravenous ketamine drip (4 mg/mL in sterile saline) and supplemental injections of xylazine (0.4 mg/Kg, i.m.). Urethane (1.6 mg/Kg, i.p.) was

also given to maintain a stable anesthetic plane 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 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. A head post was attached to a portion of the skull that did not overlie the region of interest in order to avoid interference from eye and mouth bars during mapping. A craniotomy was performed to expose the lateral half of the central sulcus. The bone around the opening was cauterized to prevent bleeding over the course of the mapping. The dura was removed, and the exposed cortex was kept moist with regular application of sterile saline until the injections were completed 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 lesions using blood vessels and sulcal patterns as landmarks.

Multiunit mapping and injections

Neuroanatomical tracers were injected into physiologically defined representations of the tongue, teeth, gingiva, palate, buccal wall, lip, and chin representations were obtained by inserting low-impedance tungsten (1.0 mΩ at 1,000 Hz) 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 wood or glass probes. While our goal was to orient the mapping electrode so that it was perpendicular to layer IV, this was sometimes impossible since much of the mouth representation is on the caudal bank

of the central sulcus. 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 marked on standard drawings of a macaque face and the inside of the mouth. After the initial mapping revealed sites of distinct representations of the tongue, teeth, and face, the electrode holder was removed, and a Hamilton syringe with a glass pipette tip attached was placed onto the stereotaxic arm. This ensured that the path of the syringe followed the same angle used for mapping on the way to the targeted location. Pressure injections of up to three of the following anatomical tracers were placed across the 3b representations of the mouth and face: 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 or Invitrogen). In three animals (cases 1, 3, and 4), recording continued for up to three days immediately after the injections to provide a more extensive map of the oral cavity representation and allow time for tracer transport. In case 2, due to the use of a different set of neuroanatomical tracers, injections were placed during a short initial mapping procedure. Following the injections, gel film was inserted to replace the opened dura, the craniotomy was closed with dental cement, the muscle was reattached, and skin was 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 for more extensive recording around the locations of the injections was performed as in the other cases. Once electrophysiological mapping was complete in all cases, the animals were given a lethal injection of sodium pentobarbital (80 mg/Kg) and subsequently perfused though the heart with phosphate buffered saline followed by 2-3% paraformaldehyde in buffered saline and 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 so that sections cut tangential to the surface could be mounted onto glass slides (Fig. 3.1). The main somatosensory blocks were cut along the arcuate sulcus (AS) from the lateral tip to the spur, across the central sulcus (CS) medial to the area 3b hand face border to the intraparietal sulcus (IPS), through the IPS and the caudal lateral sulcus (LS), along the fundus of the inferior limiting sulcus (ILS), and then back up to the lateral AS. These blocks included all of the anterior parietal and lateral sulcal somatosensory fields, the insula, much of the frontal operculum, and the entire lateral motor and premotor cortex. Blocks containing the rest of the ipsilateral area 3b and cingulate and the contralateral somatosensory cortex were also flattened. The prefrontal and orbitofrontal cortex was either flattened or kept whole to be cut coronally. Flattened blocks were held between two glass slides and stored for cryoprotection along with the unflattened blocks and thalamus from each case overnight in 30% sucrose at 4° C.

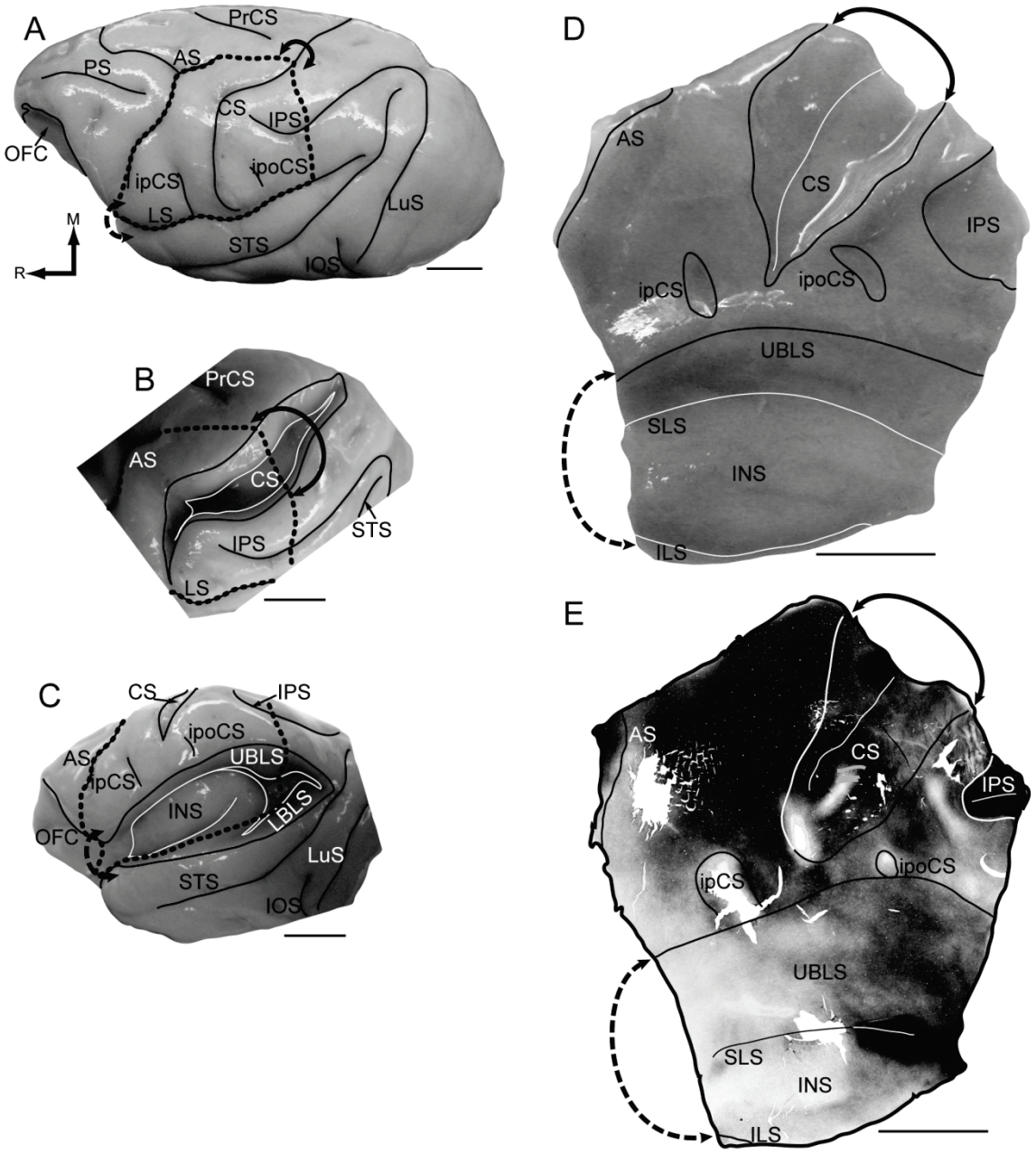

Figure 3.1. Cortical flattening procedure. A. Lateral view of the left hemisphere of case 2. B-C. Closer views of the opened CS (B) and LS (C). D. The flattened somatosensory block from the same hemisphere. E. A 40 µm thick section through this block stained to reveal myelinated fibers with sulci labeled. Heavy arrows indicate the opening of the CS (solid) and LS (dashed). Dotted lines indicate the region of the left hemisphere that was blocked to fit the size of the slides on which sections were later mounted. The block was cut to contain all of the area 3b oral cavity representation, the 3b hand face border and digit representations, as well as the lateral half of the areas 1, 2, 3a, M1 and PMv, as well as the cortex of the UBLS and insula. Abbreviations as in table 1. Rostral is left, medial is up. Scale bars are 1 cm.

Cortical histology

The flattened blocks were cut tangential to the surface while unflattened frontal blocks were cut coronally at a thickness of 40-50 μm on a freezing microtome. Series 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 (Angelucci, Clasca, & Sur, 1996; Bruce & Grofova, 1992) to visualize CTB labeled cells. When appropriate, BDA was visualized by an avidin biotin-peroxidase reaction (ABC-kit, Vectastain, Vector, Burlingame, CA; Veenman et al., 1992), and WGA-HRP series were processed with tetramethlybenzidine as chromogen and ammonium molybdate (Olucha, Martonez-Garcia, & Lopez-Garcia, 1985) and stabilized in diamidinobenzidine (DAB). In all cases, series of sections were processed for myelinated fibers with both modified silver (Gallyas, 1979) and black gold (Schmued & Slikker Jr, 1999) staining 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). The injection cores, the diffusion zones around them, and regions of anterograde labeling revealed by bidirectional tracers were outlined. Landmarks including blood vessels, electrode tracts, 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. Photomicrographs were then stitched together in Adobe Photoshop to create an image of a single whole section as seen in figure 3.1E.

Reconstruction

Cortical sections stained for myelinated fibers were used to determine the borders of cortical areas and confirm the location of injection cores and labeled neurons. While dense myelin staining characterizes all of area 3b (Qi & Kaas, 2004), the face and mouth region of primary somatosensory cortex is separated from the rest of 3b by a myelin poor septum known as the hand face border (Fang et al., 2002; Jain, Catania, et al., 1998; Qi & Kaas, 2004). Myelin dense ovals lateral to the hand face border have been shown to indicate anatomical modules containing physiological representations of the different oral cavity and face structures in prosimian galagos and New World monkeys, and similar modules have been suggested in macaque monkeys (Iyengar et al., 2007; Jain, Qi, Kaas, & Nicolelis, 2001; Kaas et al., 2006; Qi & Kaas, 2004). Because the lateral extreme of the LS is deep, curved, and often full of small folds that differ from animal to animal, complications of flattening and individual differences have made it difficult to understand the pattern of myeloarchitecture in this region of area 3b in macaques. However, we were able to find myelin dense ovals representing the parts of the face and intra-oral structures in each case (Fig. 3.2). Because the myelin ovals in macaques often appeared to be incomplete or disjointed due to imperfect flattening of layer 4 along the bank and base of

central sulcus, the myeloarchitecture within area 3b is best determined by reconstructing through the depth of cortex.

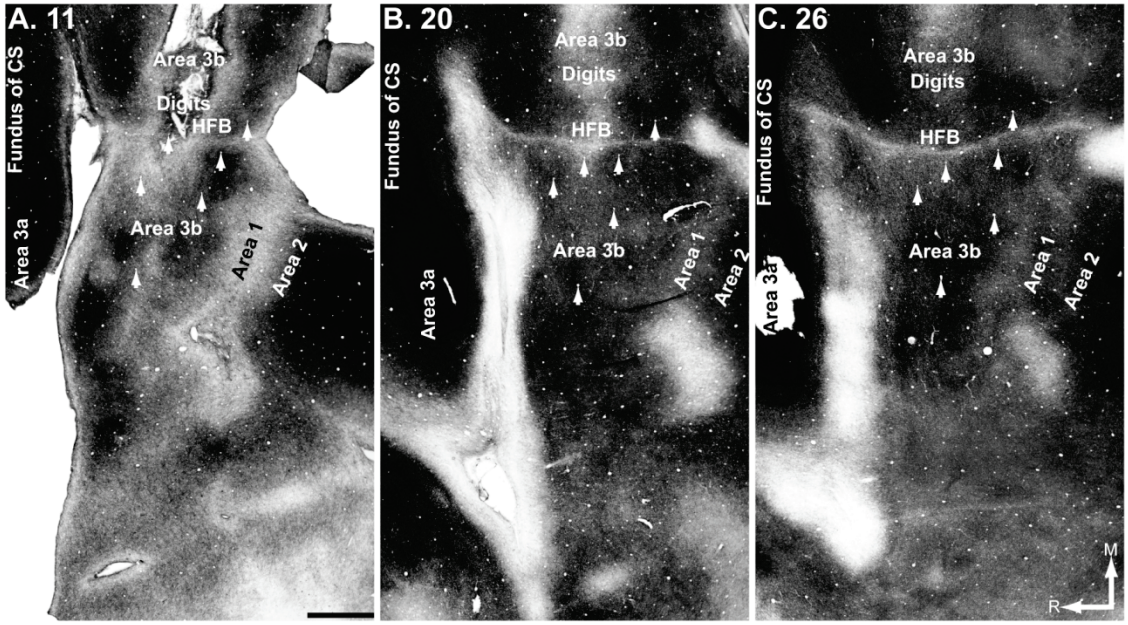

Figure 3.2. Myeloarchitecture of the 3b face and mouth region. A-C. Sections from case 4 stained to reveal myelinated fibers from most superficial (A) to deepest (C). Staining the cortex for myelin revealed dense patches that indicate the anatomical representation of the parts of the face and mouth through the depth of cortex in 3b oral cavity representation. These sections were used to draw a reconstruction of the areal boundaries throughout the depth of cortex by aligning landmarks including blood vessels and electrolytic lesions from each section. Arrows mark corresponding landmarks that were used to align sections during reconstruction of areal borders. Sections were cut at 40 µm, and there are 320 µm between sections 11 and 20, and 200 µm between sections 20 and 26. HFB- Hand face border. Rostral is left, medial is up. Scale bar is 1 mm.

To reconstruct the anatomical boundaries, the myeloarchitecture was drawn using a projection microscope. A single section at the approximate depth of layer 4 (for example, Fig. 3.2B) was used as the reference section. The myeloarchitecture in area 3b, other areal boundaries, indications of cortical sulci, and landmarks including: blood vessels, electrode tracks, electrolytic lesions, and injection sites were drawn from the

reference section. Boundaries of myelin ovals, areal boundaries, and sulcal outlines, were then completed by using landmarks to align sections superficial and deep to the reference section (Fig. 3.2A and 3.2c, respectively). Physiological mapping data was added to the anatomical reconstructions by aligning penetrations along the trajectory of the electrode tracks that could be seen in the stained fiber sections and matching the locations of injection sties and lesions (Fig. 3.3). Plotted cortex sections indicating the injection sites and distribution of backfilled cells were then aligned to the reconstructed borders and physiological maps using blood vessels, electrolytic lesions, and the injection cores as landmarks. This allowed us to characterize the complete receptive field represented by the region encompassed by each injection (Fig. 4). All reconstructions were created with Adobe Illustrator CS2 & CS5™ (Adobe Systems).

Results

Ipsilateral corticocortical connections

Case 1 Tongue representation. The CTB injection was placed near the lateral tip of the central sulcus (Fig. 3.4A), into the region representing the tongue. While it was centered on the representation of the tip of the tongue, the tracer diffused around the injection core to include all of the anterior tongue and much of the contralateral tongue. This injection may have spread into area 3a, though that could not be confirmed

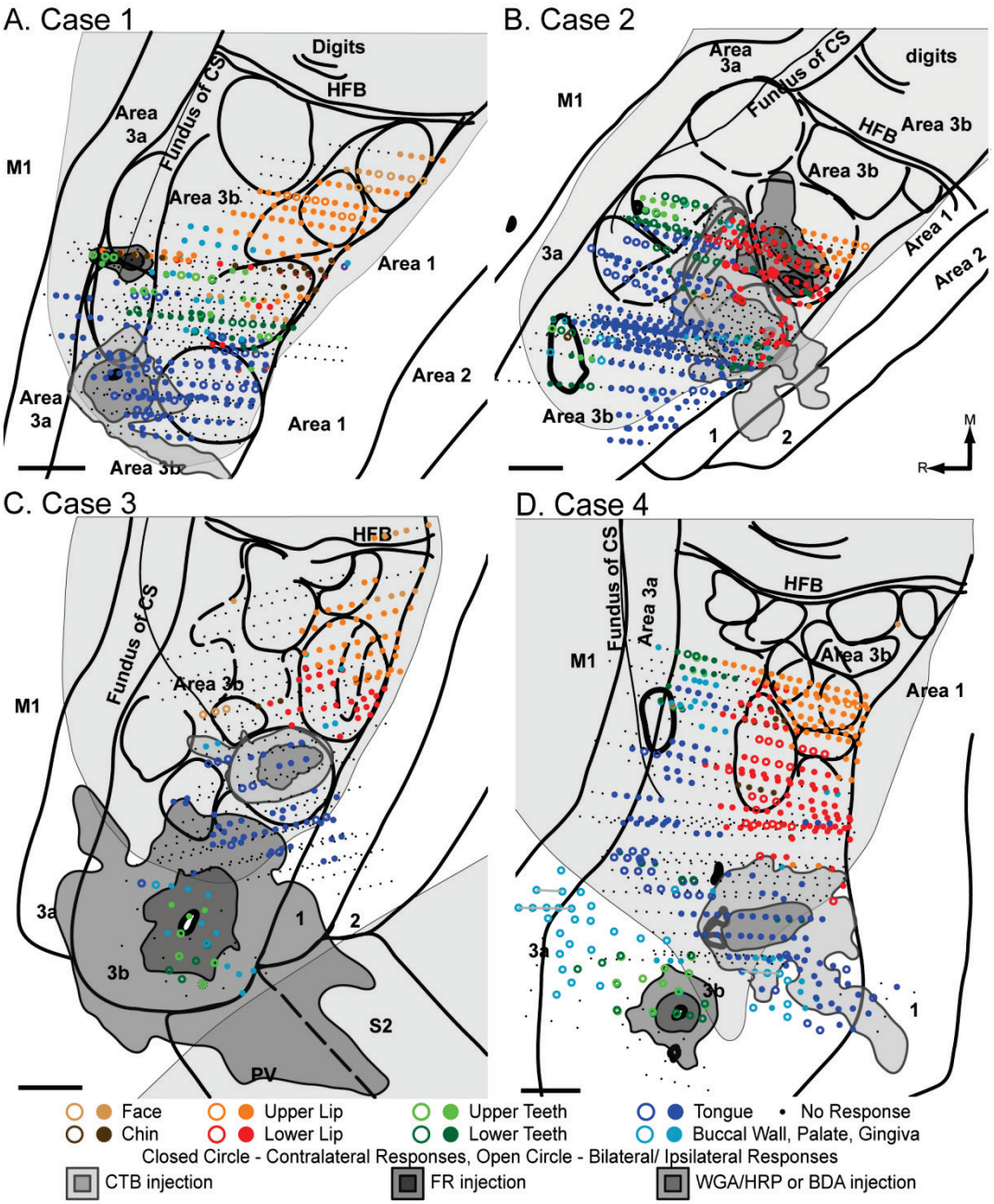

Figure 3.3. Physiological characterization of injection sites. A-D. Physiological maps of responses to tactile stimulation of the face and intra-oral structures during microelectrode mapping of area 3b aligned to reconstructions of myeloarchitecture and injection sites for each case, respectively. Thin black lines are the myeloarchitecture within area 3b and areal boundaries. Thick black lines are tears. The CS and LS are shaded in light gray. Circles are color coded to indicate the area of skin stimulated to elicit responses at that cortical location during mapping. Injections cores and surrounding diffusion zones are outlined and shaded in darker grays. The anatomical and electrophysiological maps were aligned on the basis of landmarks including tracer injection sites, lesions, and electrode tracts. Rostral is left, and medial is up. Scale bars are 2 mm.

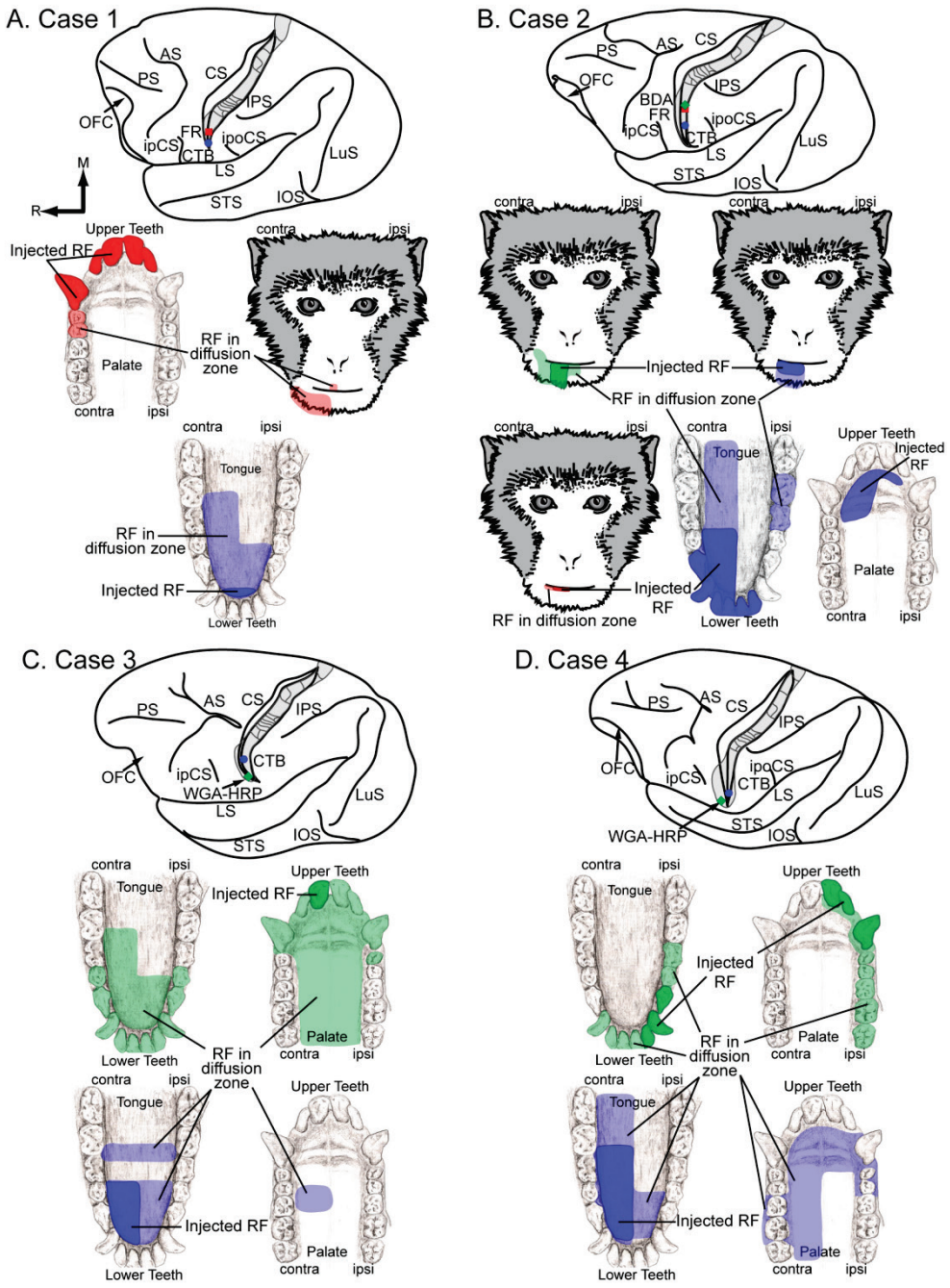

Figure 3.4. Locations of injections sites in the central sulcus and the injected representations. A-D. Markers in the open central sulcus indicate the center of each tracer injection in the representations drawn below. The darkly shaded region is the part of the body represented at the center of each 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. Blue- CTB, Red - FR, Green - WGA-HRP or BDA. 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. Rostral is left, and medial is up.

physiologically because we could not orient the electrode to reach layer 4 in this location (Fig. 3.3).

This injection revealed strong intrinsic connections from within area the 3b tongue representation, as well as the representations of the other intra-oral structures within area 3b including: the lower and upper teeth and tissue lining the inside of the cheek and lips (Fig.3. 5, Table 3.1). Overlapping representations of the lower lip and chin also contained cells labeled by the CTB injection into the region of 3b that responded to stimulation of the tongue. Filled neurons were also found in the 3b upper lip region. The 3b hand representation was almost completely devoid of labeled cells (Table 3.2), indicating that there was little input from the hand region to the part of 3b serving the tongue.

Outside of primary somatosensory cortex, there were projections to the 3b tongue representation from other somatosensory areas in the anterior parietal cortex and upper bank of the lateral sulcus (UBLS), cortex of the insula, and frontal cortex (Fig 3.5). Though unmapped, regions of lateral areas 3a 1, and 2 that likely contain representations of the inside of the mouth provided input to the 3b tongue. The field of labeled cells extended into lateral area 5 and the posterior parietal cortex (PPC). Backfilled neurons were found throughout second somatosensory (S2) and parietal ventral (PV) areas, though they tended to be concentrated along the medial border of both areas, a region known to be responsive to stimulation of the face and mouth (Krubitzer et al., 1995). Separate foci of labeled cells could also be found in the UBLS rostral to PV. While the locations of these foci were not distinct in the fiber staining, they presumably include face oral cavity representations in the parietal rostral (PR) and ventral somatosensory

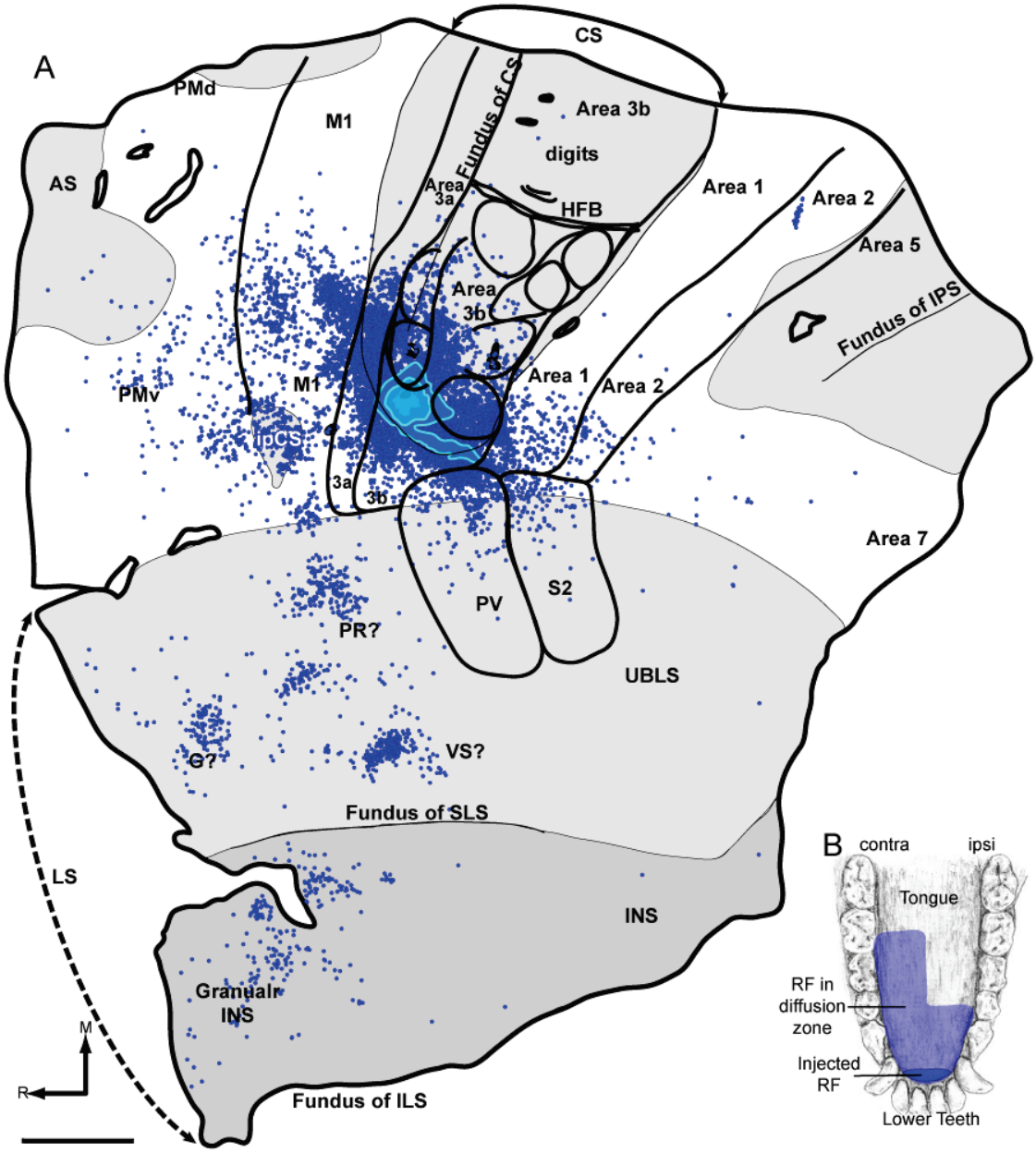

Figure 3.5. Case 1 CTB injection into 3b tongue. A. Distribution of CTB labeled cells through the somatosensory cortex of case 1. Each blue circle indicates the location of an individual CTB filled cell. The core, full extent of diffusion zone around the injection, and patches of anterogradely filled axon terminals are outlined and shaded in blue. Thin black lines are anatomical borders. Thick black lines are tears and the edges of the block. Large gray shaded regions are cortex in a sulcus; the fundus of each sulcus is marked by a fine black line. Areal boundaries were determined from adjacent sections stained for myelin, physiological mapping, and measurements based on previously published studies. Rostral is left, medial is up. Scale is 5mm. This CTB injection into the 3b tongue representation revealed strong local connections with other parts of the 3b mouth and face regions, as well as inputs from other somatosensory areas in the parietal cortex and UBLS, M1, PMv, and UBLS and insular cortex that may be involved in taste processing. B. Injected receptive field.

Table 3.1. Distributions of retrogradely labeled cells throughout the ipsilateral cortex 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. Cortical areas containing labeled cells are listed in the first column, and successive columns contain the percentage of cells labeled in each area. PPC includes all parietal areas caudal to area 2. Because they were often difficult to distinguish cells in area S2 and PV were counted together. UBLS includes all of the cells labeled in the UBLS that were not in any part of anterior parietal areas 3b, 3a, 1 and 2 that extended onto the lateral sulcus or in S2/PV.

	Case1		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
	18447	2758	34128	2125	7264	35322	8967	65416	5255
3b	62.54	84.52	54.32	96.05	96.83	61.69	35.51	63.18	86.41
3a	14.92	8.27	3.48	0.09	0.11	1.59	8.64	8.49	6.98
1	2.79	1.92	12.12	1.08	1.46	12.65	7.62	15.04	0.36
2	0.42	0.40	8.85	0.56	0.72	9.18	3.95	1.81	0.11
PPC	0.14	0.18	7.40	1.65	0.67	0.37	0.71	0.03	0.61
S2/ PV	3.72	0.69	2.65	0.05	0.00	5.00	7.05	2.22	0.88
UBLS	3.95	3.30	2.49	0.00	0.06	2.47	20.56	3.18	1.08
INS	1.27	0.36	0.56	0.00	0.15	0.03	0.67	1.13	1.01
M1	8.61	0.33	6.38	0.52	0.00	4.73	9.90	4.85	1.39
PM	1.6	0.04	1.74	0.00	0.00	2.28	5.27	0.06	3.06
LPFC	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.2. Distributions of cells labeled within area 3b. Conventions as in table 2. 3b mouth and face includes all cells in area 3b lateral to the dorsal edge of the hand face border.

	Case1		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
	11536	2331	18539	2041	7034	21789	3184	41327	4541
3b mouth & face	99.97	99.96	99.36	100	99.97	99.96	99.84	100	99.87
3b hand	0.03	0.04	0.64	0.00	0.03	0.04	0.16	0.00	0.13

(VS) areas (Coq et al., 2004; L. Krubitzer et al., 1995; Qi et al., 2002). Lateral motor (M1) and ventral premotor (PMv) each contained CTB labeled cells. Though no motor mapping was performed for this study, previous studies in motor and premotor cortex lateral to the arcuate sulcus have shown that microstimulation here can evoke movements of the face and mouth (Graziano et al., 2002; McGuinness et al., 1980), and that this region is important to the proper performance of tasks involving movement of the tongue and jaw (Hoffman & Luschei, 1980; Murray et al., 1991; Murray & Sessle, 1992). Thus, we feel that our results show that regions of motor and premotor cortex that are involved in moving the tongue project to the 3b tongue representation. The injected 3b tongue representation also received projections from more anterior parts of the UBLS and insula. Patches of filled neurons were found near the fundus of the superior limiting sulcus (SLS) in the presumptive location of the primary gustatory area (G) in the UBLS and other taste related areas of the anterior insula (Ogawa, 1994; Ogawa et al., 1989; Plata-Salamán & Scott, 1992; Pritchard & Norgren, 2004; Sanides, 1968; Smith-Swintosky et al., 1991). A few cells (Table 3.1) labeled by this injection were also found in the lateral prefrontal cortex (LPFC), near the fundus of the principal sulcus (not shown).

Case 1 Medial representation of the teeth. An injection of FR in case 1 was placed near the fundus of the CS (Fig. 3.4A) into a region that responded to touches on all of 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 this injection. Like the injection into the representation of the tongue in this case, this injection may have spread very slightly into area 3a.

Again, the majority of inputs to the injected representation of the teeth came from other parts of the 3b representation of the mouth (Tables 3.1 & 3.2), specifically the teeth, gums, and buccal wall (Fig. 3.6). A focus of labeled cells was also found in the rostrolateral part of area 3b that continued out of the CS and onto the surface of the brain. This region was not mapped in this case for technical reasons, but, given the mapping results in our other cases (Fig. 3.3) we feel that these cells likely lie in a second more lateral representation of the teeth and gums that includes more ipsi- and bilaterally responsive sites than the injected medial representation of the teeth. The adjacent tongue representation also provided inputs to the representation of the teeth and lip, though perhaps not from the center of the tongue region that was injected with CTB. The lip and chin representations in 3b projected to the adjacent medial representation of the teeth as well. There was almost no input from the 3b hand representation (Table 3.2).

Cells filled by the FR injection were scattered outside of area 3b, but show a pattern of projections largely similar to that revealed by the CTB injection into the tongue region in case 1 (Fig. 3.6). Labeled cells were in presumably matched representations of the teeth in areas 3a, 1, 2, 5, S2, and PV. A low number of backfilled neurons were found in the UBLS. M1 and PMv also contained labeled cells. Unlike the tongue representation injection, only a low number of neuron were labeled in regions of cortex that may process taste by our injection into the more medial representation of the teeth.

Case 2 Tongue and lip representation. In case 2, an injection of CTB was placed on the caudal bank of the CS into a region representing the contralateral anterior tongue, including the tip (Fig. 3.4B). A second mapping session revealed that the center of this injection included responses to touches on the whole of the contralateral dorsal surface of

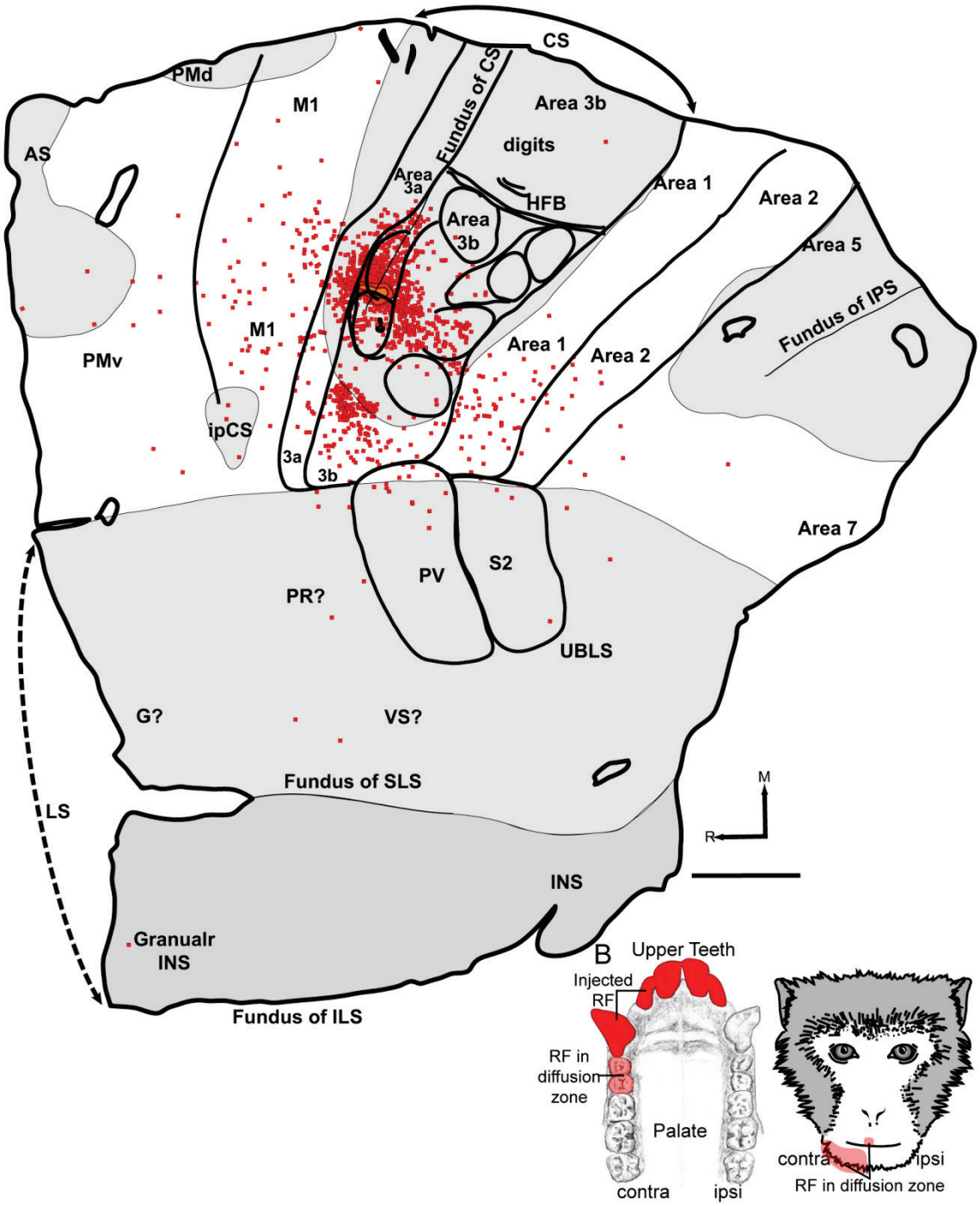

Figure 3.6. Case 1 FR injection into 3b teeth. A. Distribution of FR labeled cells through the somatosensory cortex of case 1. Each red square is one FR filled cell. The injection core and diffusion zone are outlined in dark red. All conventions as in figure 3.5. An injection of FR into a region responding to the upper front teeth, lip, and chin resulted in filled cells scattered throughout somatosensory and motor cortex in a pattern similar to the case 1 tongue representation injection. There was, however, little projection from possible taste cortex in the insula and UBLS to the representation of the teeth in this case. B. Injected receptive field.

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 (very weak responses to stimulation of these teeth were indicated). The diffusion zone around this CTB injection may also have included parts of adjacent areas 1 and 2 (Fig 3.3B), but strong uptake from the diffusion zone is unlikely.

The injection centered on the tongue and lip representations in case 2 revealed a pattern of projections similar to that seen in case 1 (Fig. 3.7, Table 3.1). Labeled cells were found in other parts of 3b responding to stimulation of the tongue, lips, and chin. Within 3b, inputs to the tongue/ lip representation also came from the more medial representation of the teeth, though a few labeled cells were found in the electrophysiological defined lateral representation of the teeth as well. There were labeled cells in an unmapped portion of area 3b between the anatomical hand face border and mapped lip and teeth representations that likely represents other parts of the face (Kaas et al., 2006; Qi & Kaas, 2004). Once again, the vast majority of filled neurons after the CTB injection into the 3b tongue/ lip representation were in the region area 3b devoted to the face and mouth (Table 3.1). Outside of area 3b, there were backfilled cells in other somatosensory areas including areas 3a, 1, 2, 5, S2, and PV. Labeled neurons were also found in the PPC and into the presumed locations of mouth and face representations in PR and VS. M1 and PMv also provided input to the injected tongue/ lip region. The anterior UBLS and insula contained CTB labeled neurons, suggesting that taste cortex in these regions provides some input to the 3b tongue representation.

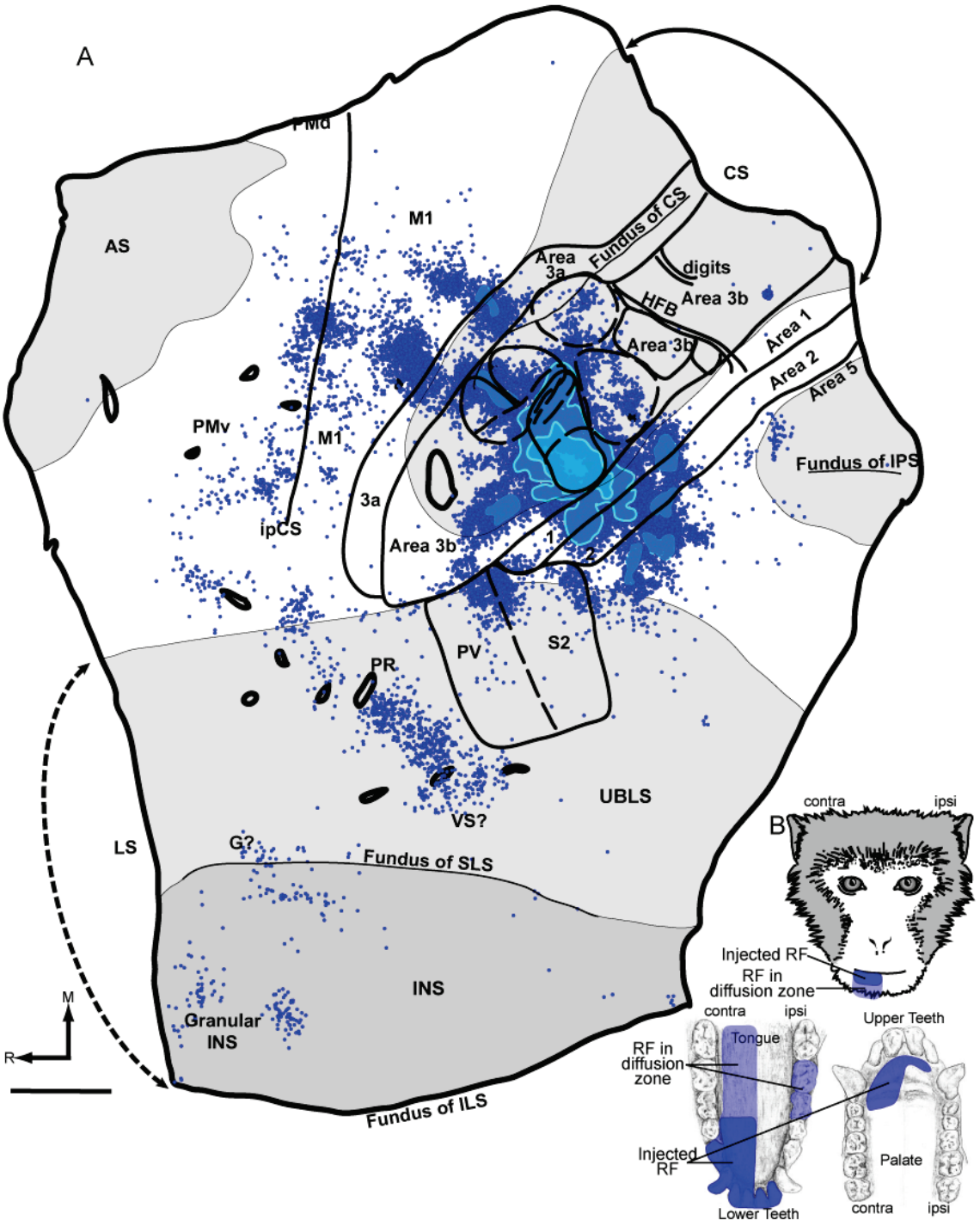

Figure 3.7. Case 2 CTB injection into 3b tongue/lip. A. Distribution of CTB labeled cells through the somatosensory cortex of case 2. All conventions as in figure 3.5. This injection of CTB includes parts of both the tongue and lip representations. Neurons labeled by this injection were found throughout the 3b face and mouth representation, in other anterior parietal and lateral sulcus somatosensory areas, motor and premotor cortex, and in likely taste responsive regions of the anterior UBLS and insula. B. Injected receptive field.

Case 2 Lip and chin representations. The case 2 BDA injection was our most medial injection (Fig. 3.3 and 3.4B). It was centered on a region 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 upper 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 stimulation on the contralateral tongue.

The BDA injection in case 2 revealed a pattern of label similar to that seen after the case 2 CTB injection in the tongue/ lip representation, but more restricted (Fig. 3.8, Table 3.1). Within area 3b inputs to the chin/ lip representation largely came from the rest of 3b representing the lips and chin. There were a large number of back filled cells in unmapped 3b cortex between the mapped representations of the chin and lips and the hand face border that is presumed to represent the rest of the face. Inputs from representations of the teeth came only from the nearby medial representation and not the more lateral part of 3b that was also responsive to touches on the teeth. A small, more medial part of the tongue representation also projected to the injected chin region. Because this the injection the closest to the 3b hand face border that we made, and the chin has been show to expand into hand cortex after a spinal cord lesion (Manger, Woods, Munoz, & Jones, 1997), this injection was the best candidate to label cells in the 3b hand representation. There was, however, little indication of cells projecting from the 3b hand cortex to the 3b face region (Table 3.2). A few cells within the septum between

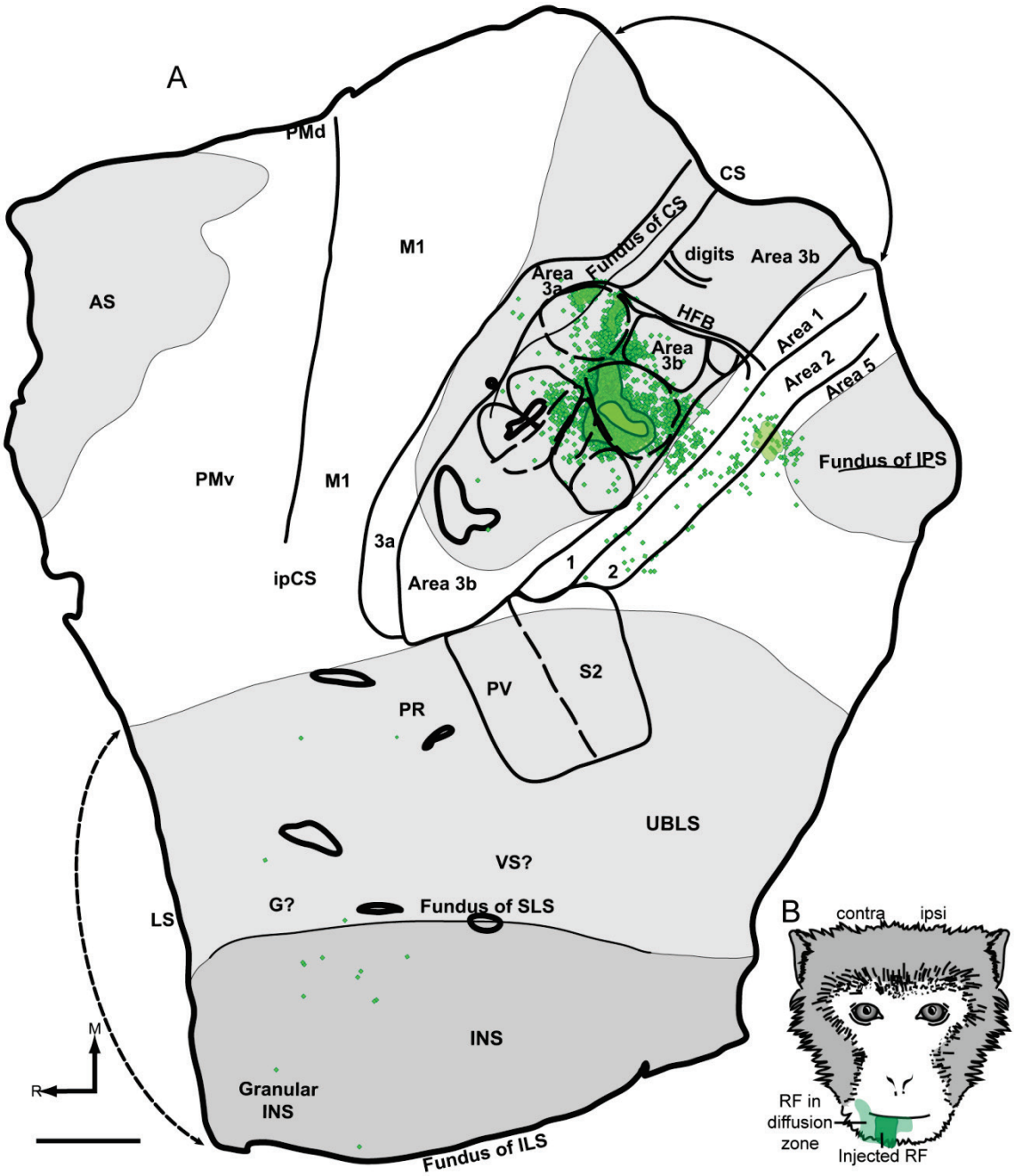

Figure 3.8. Case 2 BDA injection into 3b lip/chin. A. Distribution of BDA labeled cells through the somatosensory cortex of case 2. Each green diamond is one BDA filled cell. All conventions as in figure 3.5. The injection of BDA was centered on the representation of the medial lower lip and anterior chin, but spread to include representations of most of the lower lip and the rest of the chin. Like other case 2 injections, this one labeled cells in a pattern similar to injections in other parts of 3b, with strong local inputs coming from within the injected representation, more inputs from other nearby representations within 3b, and then inputs from other somatosensory areas. Although this was our most medial injection, no BDA backfilled cells were found medial to the hand face border. B. Injected receptive field.

these two representations were filled with BDA. Outside of area 3b, the pattern of label was similar to that of the case 2 tongue/ lip injection with inputs from all of the anterior parietal somatosensory areas indicated. Few labeled cells were found in M1 and the UBLS, perhaps due to poor tracer transport, after this injection of BDA into the lip/ chin representation in area 3b. The few scattered neurons labeled in the insular cortex may have been the result of BDA injection spread into the tongue region.

Case 2 Lip representation. A small injection of FR was placed into the representation of the contralateral middle and medial lower lip between cores of the tongue/ lip (CTB) and lip/chin (BDA) injections in case 2 (Fig. 3.4B). This FR injection included representations of the skin and hair along 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 the FR 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.

The 3b lower lip FR injection was small, but showed a pattern of projections like that revealed by the other case 2 injections (Fig. 3.9). Backfilled neurons were primarily in the representations of the lower lip and chin. Again, the presumptive upper face representation in the unmapped part of 3b just lateral to the hand face border contained labeled neurons. The most medial portion of cortex representing the tongue also provided inputs to the injected lip region. No label was found in 3b hand cortex (Table 3.2). Areas 3a, 1, 2, and 5 also provided inputs to the lip representation. Projections from motor and lateral sulcus somatosensory cortex were also indicated by FR filled cells. Unlike the CTB and BDA injections in case 2, no region of cortex responding to the tongue was

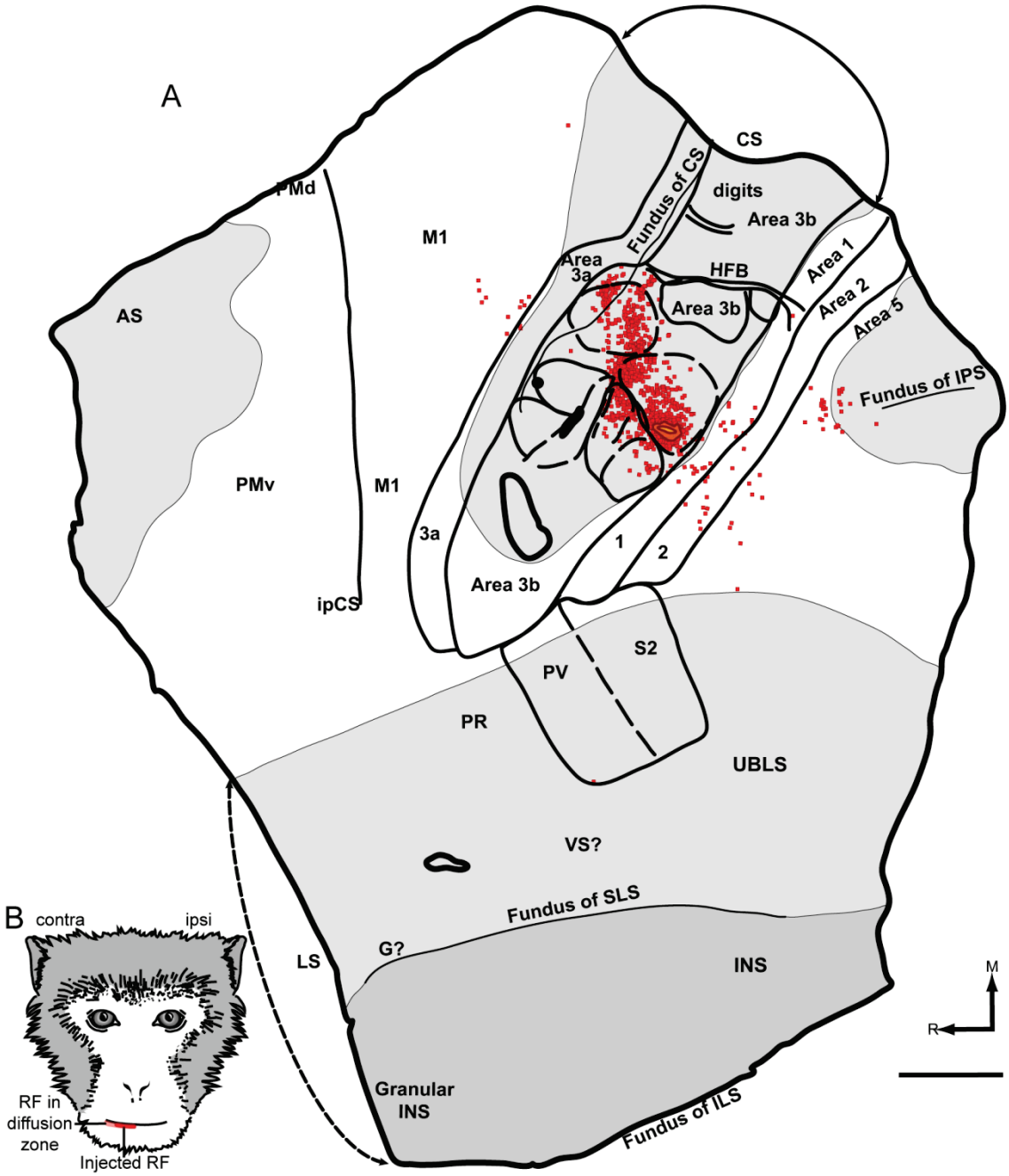

Figure 3.9. Case 2 FR injection into 3b lip. A. Distribution of FR labeled cells through the somatosensory cortex of case 2. All conventions as in figure 3.5. The injection of FR centered on the lower lip representation was small, but does show a pattern of cells projecting to the 3b lip region from within 3b face responsive cortex, areas 3a, 1, 2 and M1 that is similar to that revealed by injections into the tongue and lip representations. B. Injected receptive field**.**

involved in the FR injection, and there no neurons backfilled with FR were found in presumptive taste areas of the UBLS and insula.

Case 3 Tongue representation. CTB was injected in the CS into a region representing the much of the middle and anterior tongue, centered on a region of responses to touches on the contralateral anterior and tip of the tongue (Fig 3.4C). Part of the contralateral middle palate was also included in the case 3 CTB injection. The results of this CTB injection are complicated, somewhat by a second injection of CTB into the area 5 hand representation in the contralateral hemisphere for an unrelated study. We are confident that few if any of the labeled neurons in somatosensory regions representing the face were labeled by this second injection.

In case 3, CTB backfilled cells were distributed across much of cortex (Fig. 3.10). The lightly colored medial patch of filled neurons in more medial areas 1, 2, and 5 in figure 3.10 were the likely result of a contralateral CTB injection made during an unrelated experiment. Because it was more difficult to use location determine the likely origin of the CTB labeled cells in other, less well-mapped somatosensory areas in the UBLS, these filled neurons are shown in full. All backfilled cells were counted when we determined magnitude of projections to ensure that we did not miss any cells projecting to the 3b tongue representation (Table 3.1). There were strong projections to this region from within area 3b with the 3b mouth (the rest of the tongue, teeth, and palate) and face (chin, lips, and weaker from the face) representations contained labeled cells (Fig. 3.10). As with the other cases' tongue injections, there were few labeled cells in 3b hand cortex after the case 3 CTB injection into the tongue representation (Table 3.1). Again, there were projections to the 3b tongue representation from areas 3a, 1, 2, S2, PV and

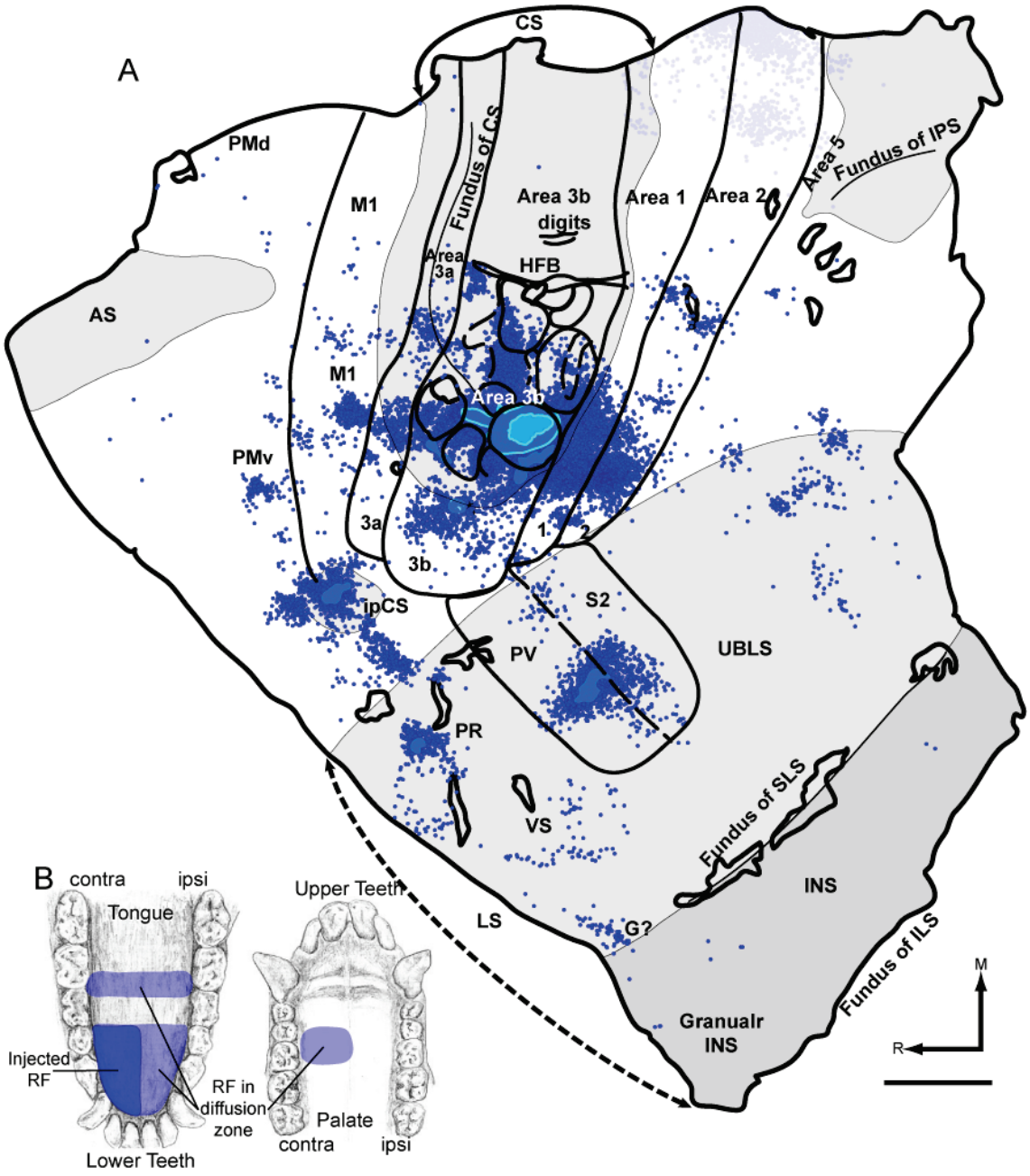

Figure 3.10. Case 3 CTB injection into 3b tongue. A. Distribution of CTB labeled cells through the somatosensory cortex of case 3. All conventions as in figure 3.5. In case 3, CTB was injected into a region including responses to the tongue and part of the contralateral palate. Projections to this tongue region were largely similar to those of other cases, with strong local connections, projections from other somatosensory and motor areas, and input coming from presumptive taste cortex. The medial patch of lightly shaded cells in the likely result of a contralateral injection placed for another study. B. Injected receptive field.

presumptive VS and PR. Labeled cells indicating inputs from motor and premotor cortex were concentrated laterally in M1 and PMv. Finally, there were CTB filled neurons near the SLS at the extreme anterior junction of the UBLS and insula may indicate a projection from gustatory cortex to the 3b tongue representation.

Case 3 Lateral representation of the teeth. The WGA-HRP injection in case 3 was a large injection in the most lateral portion of 3b on the surface of the brain beyond the tip of the CS (Fig. 3.4C). While centered on a representation of the contralateral upper first incisor, this WGA-HRP injection was placed into a region containing 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, the contralateral lower first premolar, the ipsilateral upper first premolar, all of the contralateral hard palate, and the bilateral anterior hard palate. All though we feel the zone of uptake from this WGA-HRP injection was restricted, the diffusion zone around the injection core was large 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. It also spread into unmapped regions of areas 1, PV and S2 that likely also represented the face (Fig. 3.3C).

Much of the ipsilateral cortex contained labeled neurons (Fig. 3.11, Table 3.1). There were strong inputs from the rest of the teeth, tongue, palate, lip, and face representations within area 3b. While the filled neurons outside of area 3b revealed by the case 3 WGA-HRP injection cover a wider region of cortex than the injection into the medial representation of the teeth in case 1, the pattern of these projections was similar to both the case 1 FR injection in the medial region of the teeth and that revealed by

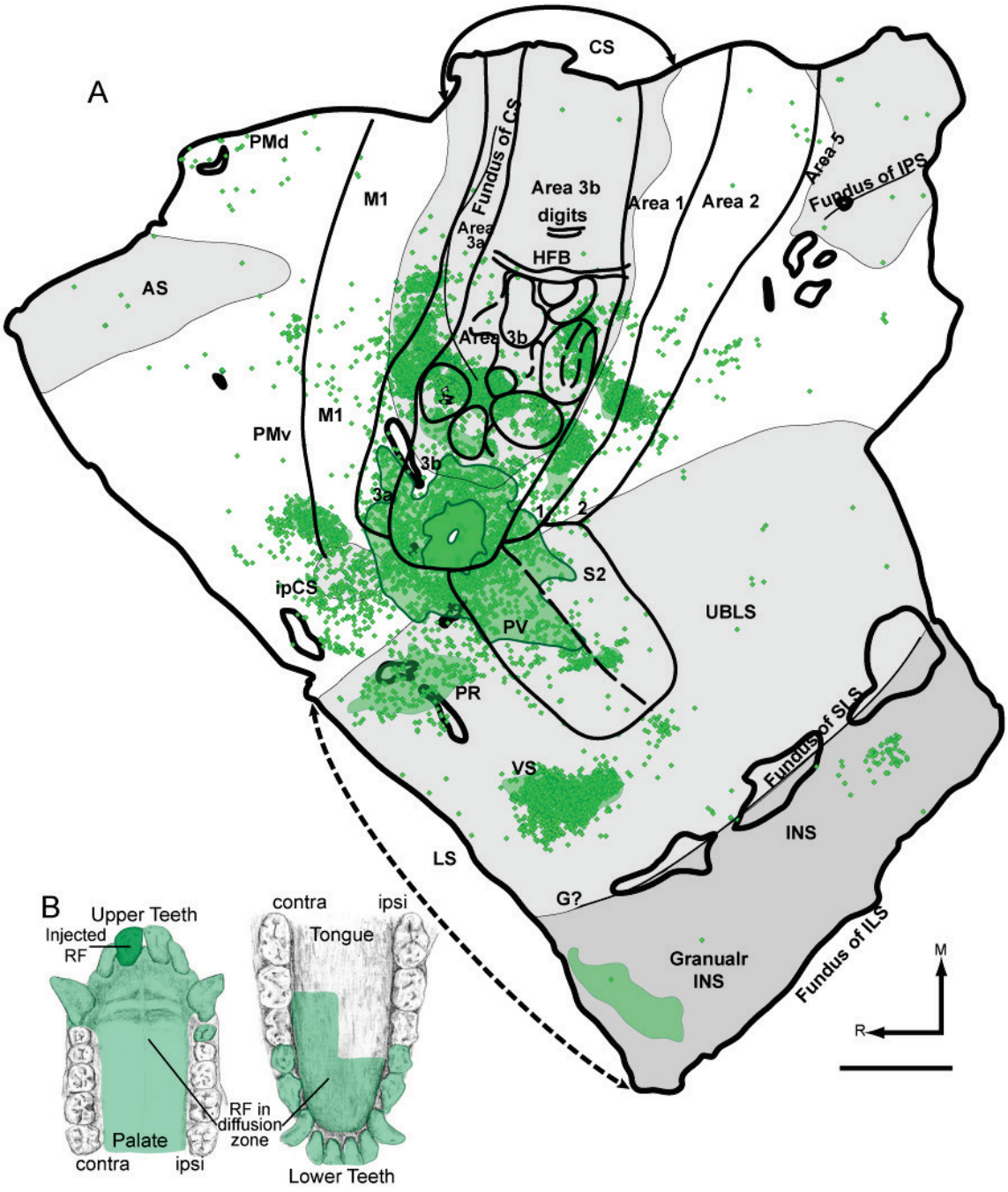

Figure 3.11. Case 3 WGA-HRP injection into lateral 3b teeth. A. Distribution of WGA-HRP labeled cells through the somatosensory cortex of case 3. Each green diamond is one WGA-HRP filled cell. All conventions as in figure 3.5. WGA-HRP injected into the representation of the teeth, gums, and palate in area 3b resulted in a pattern of inputs similar to that of the medial teeth representation injection in case 1. Label in the anterior UBLS and anterior insula after this injection was in contrast to that of the case 1 teeth representation injection; however, the diffusion zone of this injection did include parts of the tongue representation in 3b and possibly other areas. B. Injected receptive field.

injections into the 3b tongue cortex in all cases. As with the medial representation of the teeth, this injection centered on the lateral representation of the teeth failed to label cells in the region of the anterior UBLS and anterior insula that contained filled neurons after case 3's tongue injection.

Case 4 Tongue representation. The case 4 injection of CTB into the tongue representation was centered on a region responding to stimulation of the contralateral middle and anterior tongue without the tip (Fig 3.4D). This injection of CTB spread to include representations of the rest of the contralateral tongue, the anterior half of the ipsilateral tongue, and the tip of tongue; the gums around the contralateral lower premolars and first molar; the upper first and second molars, and the ipsilateral upper canine and premolars; and all of the anterior palate and the contralateral half of the rest of the palate. The diffusion zone included an adjacent part of area 1 (Fig. 3.3D).

Resulting CTB label revealed strong projections from other parts of the oral cavity in 3b as well as anterior parietal, UBLS, insular, and motor cortex (Fig. 3.12, Table 3.1). Intra-oral representations containing CTB filled neurons after the injection into the 3b tongue region included: other parts of the tongue representation, and representations of the palate, gingiva, teeth, and buccal wall. The inputs from the representations of the teeth and gums came only from the more lateral representation, and not the more medial part of cortex that responded to stimulation of the teeth and gums. The region of mixed lower lip and chin responses in 3b projected to the region of the 3b tongue representation injection. Fewer labeled cells were found in the upper lip and face representations in 3b. Almost no CTB filled neurons were found in the 3b hand cortex (Table 3.2). In addition to these local connections, there were projections from all of the

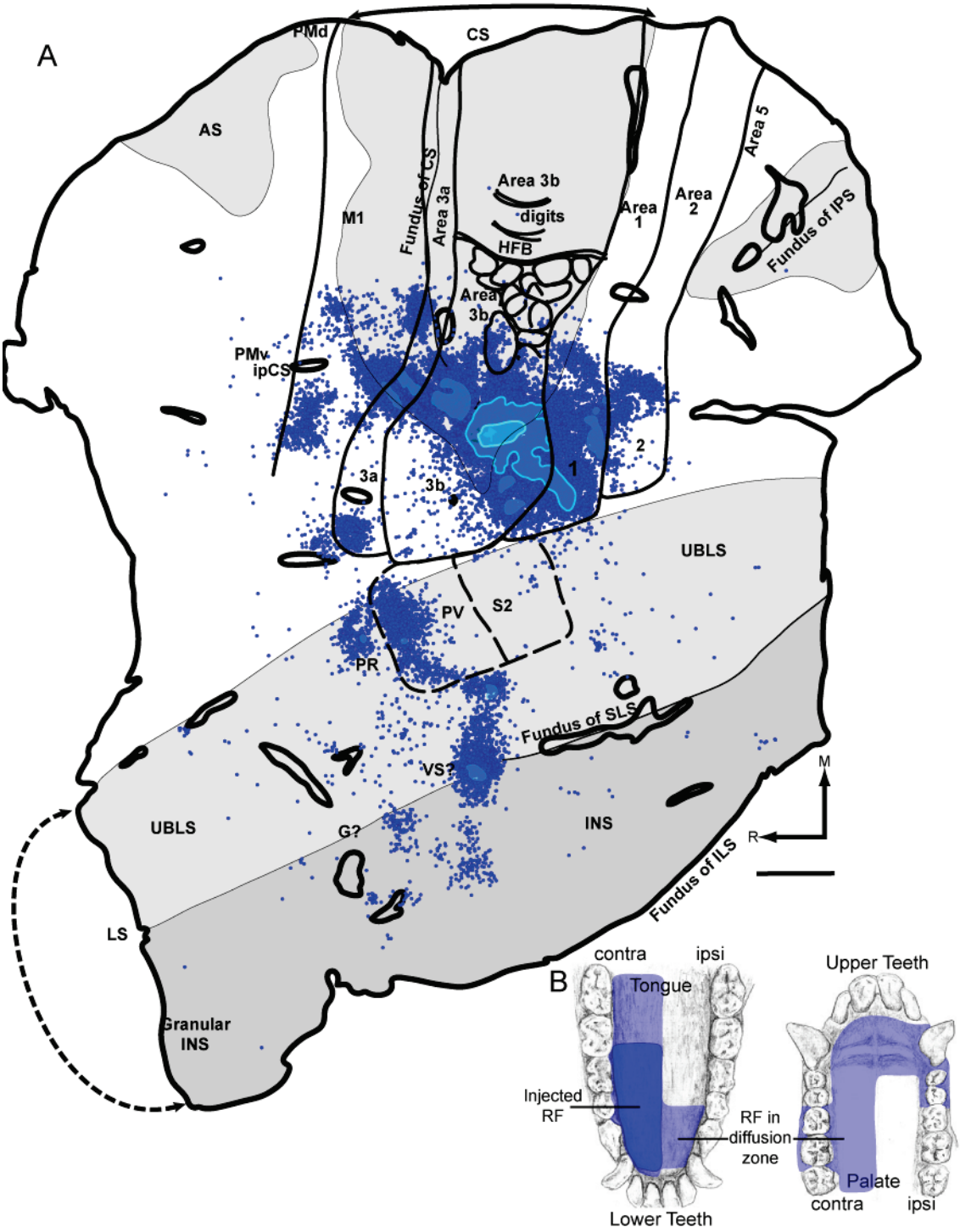

Figure 3.12. Case 4 CTB injection into 3b tongue. A. Distribution of CTB labeled cells through the somatosensory cortex of case 4. All conventions as in figure 3.5. A CTB injection into the tongue region in case 4 reveled a pattern of strong local connections, projections from somatosensory, motor, and possible taste cortex similar to that in other tongue injections. B. Injected receptive field.

anterior parietal and lateral sulcus somatosensory areas, and lateral M1 and PMv to the 3b tongue representation in case 4. A number of filled neurons were in the anterior UBLS and insula. These inputs indicated that cortex that may include area G and other taste regions does send projections to area 3b.

Case 4 Lateral representation of the teeth. Representations of the ipsilateral upper incisors and canine and the ipsilateral lower second incisor, canine and first premolar were at the center of Case 4's WGA-HRP injection in rostrolateral 3b on the surface of the brain (Fig. 3.4D). The injected region also included cortex with responses to stimulation on 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 gum surrounding the ipsilateral upper anterior teeth was also a part of this WGA-HRP injection.

Like the injections in our other cases, the case 3 WGA-HRP injection into the lateral representation of the teeth resulted in many backfilled cells in the other parts of the mouth representation in area 3b (Fig. 3.13, Table 3.1). There was a high concentration of labeled neurons within the lateral teeth/ gum representation, but none was found in the more medial representation of the teeth. Regions processing signals from the palate, check pouch, gums, and tongue also projected to the lateral representation of the teeth. While both the lower and upper lip representations contained labeled neurons, the connections coming from the lower lip appeared to be denser. Once more in case 4, almost all of the filled cells in area 3b were in the representations of the mouth and face (Table 3). Outside of primary somatosensory cortex, there were labeled cells indicating projections from other somatosensory areas in the anterior parietal cortex including 3a, 1,

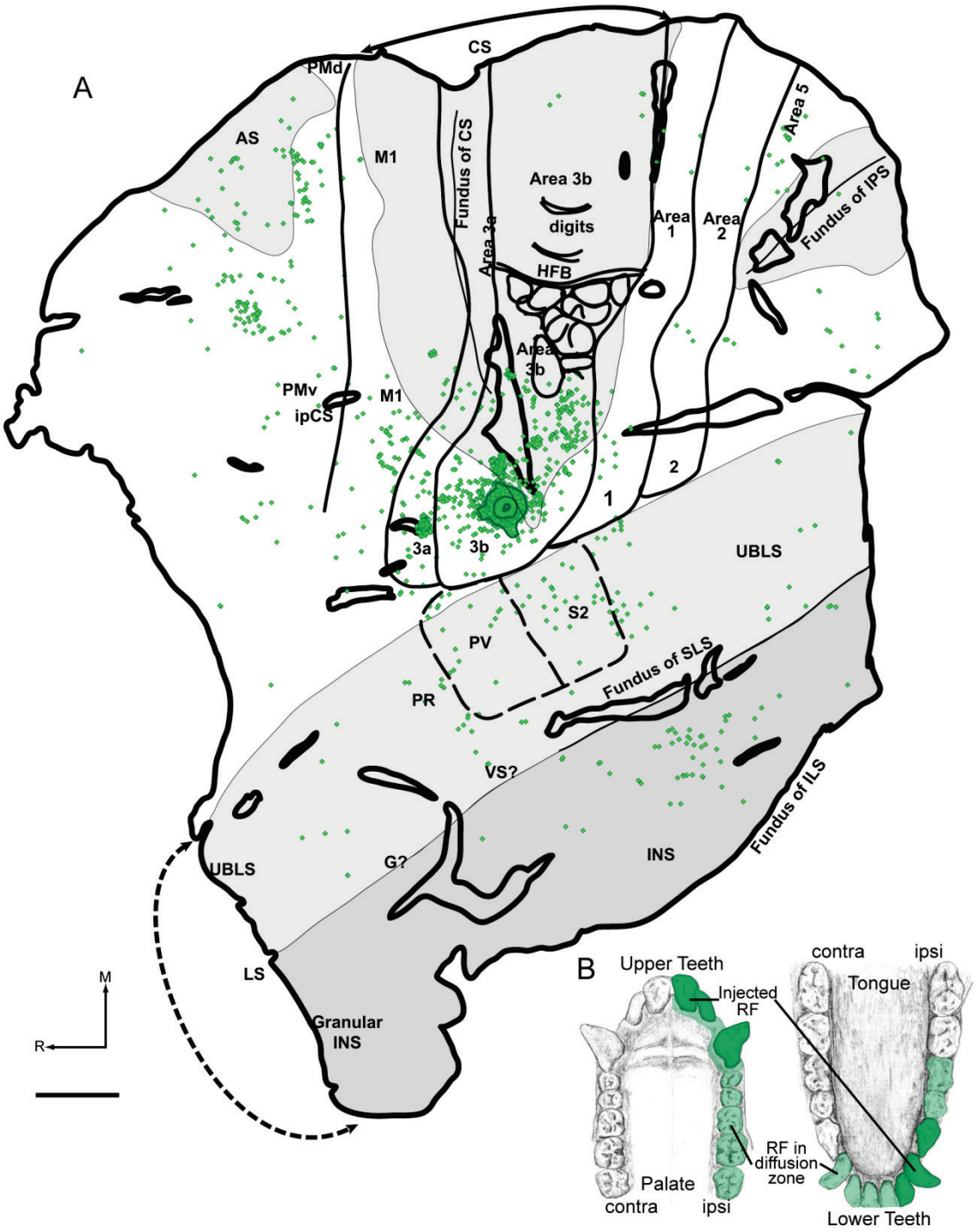

Figure 3.13. Case 4 WGA-HRP injection into lateral 3b teeth. A. Distribution of WGA-HRP labeled cells through the somatosensory cortex of case 4. All conventions as in figure 3.5. In case 4, WGA-HRP was injected into a representation mostly of the ipsilateral teeth. Local projections to this region were strong. Inputs from areas 3a, 1, 2, S2/PV, M1, and PMv were similar to those of other cases with. As with the other teeth representation injections, few labeled cells were found in presumptive taste cortex. B. Injected receptive field.

2, and 5. S2, PV, and presumptive PR and VS also projected to the injected location in area 3b. Filled cells were found in M1, PMv, and the field of labeled cells spread into the AS. There were fewer labeled neurons in the anterior UBLS and anterior insula than with the case 4 tongue representation injection.

Contralateral inputs to the 3b mouth and face representation

Our injections into the 3b tongue representation consistently resulted in labeled neurons in the contralateral hemisphere in all of our cases (Fig. 3.14). Based on their location in relation to the myeloarchitecture, CTB filled cells were found in the 3b tongue representation of the contralateral hemisphere. This indicates that the lateral part of area 3b in one hemisphere projects to the tongue representation on the other side of the brain. Many labeled neurons were anterior to area 3b in area 3a and primary motor cortex. A small number of projections from contralateral areas 1 and 2 and somatosensory areas in the UBLS were also indicated.

Discussion

Our results have revealed the cortical network for the processing of tactile information from the inside of the mouth in macaque monkeys for the first time. Injections of anatomical tracers into 3b representations of the tongue and teeth revealed projections to this region from across the cortex (Fig. 3.15). Oral cavity representations had strong intrinsic connections, with the rest of the 3b mouth and face region containing many filled neurons after all of our tracer injection. Outside of area 3b, inputs to the mouth region arose from area 3a, 1, 2,5, S2, PV, presumptive PR and VS, M1, and PMv.

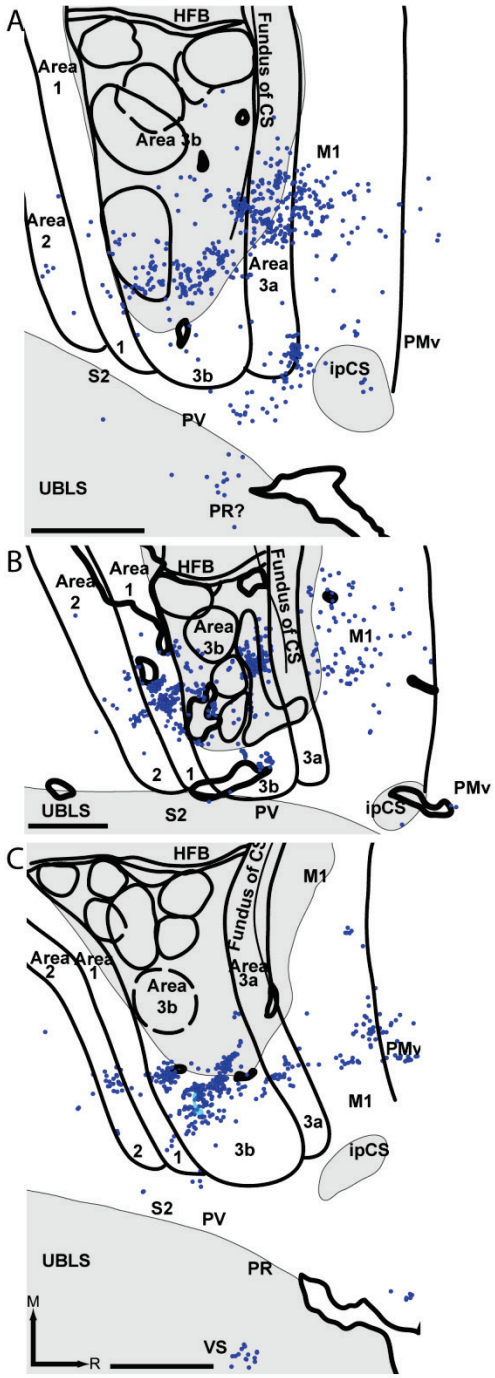

Figure 3.14. Contralateral projections to the 3b tongue representation. A-C. Distribution of CTB labeled cells through the contralateral somatosensory and motor cortex of cases 1 (A), 2 (B), and 4 (C). Rostral is righ, medial is up, all other conventions as in figure 3.5. In all cases, cells labeled after CTB injections into the area 3b tongue representation were concentrated in the lateral part of the contralateral are 3b, presumably the matching tongue representation. Backfilled cells were also found in contralateral area 3a, 1, 2, M1, and PMv in all cases. A few projections from other somatosensory areas (S2, PV, PR, and VS) were variably indicated.

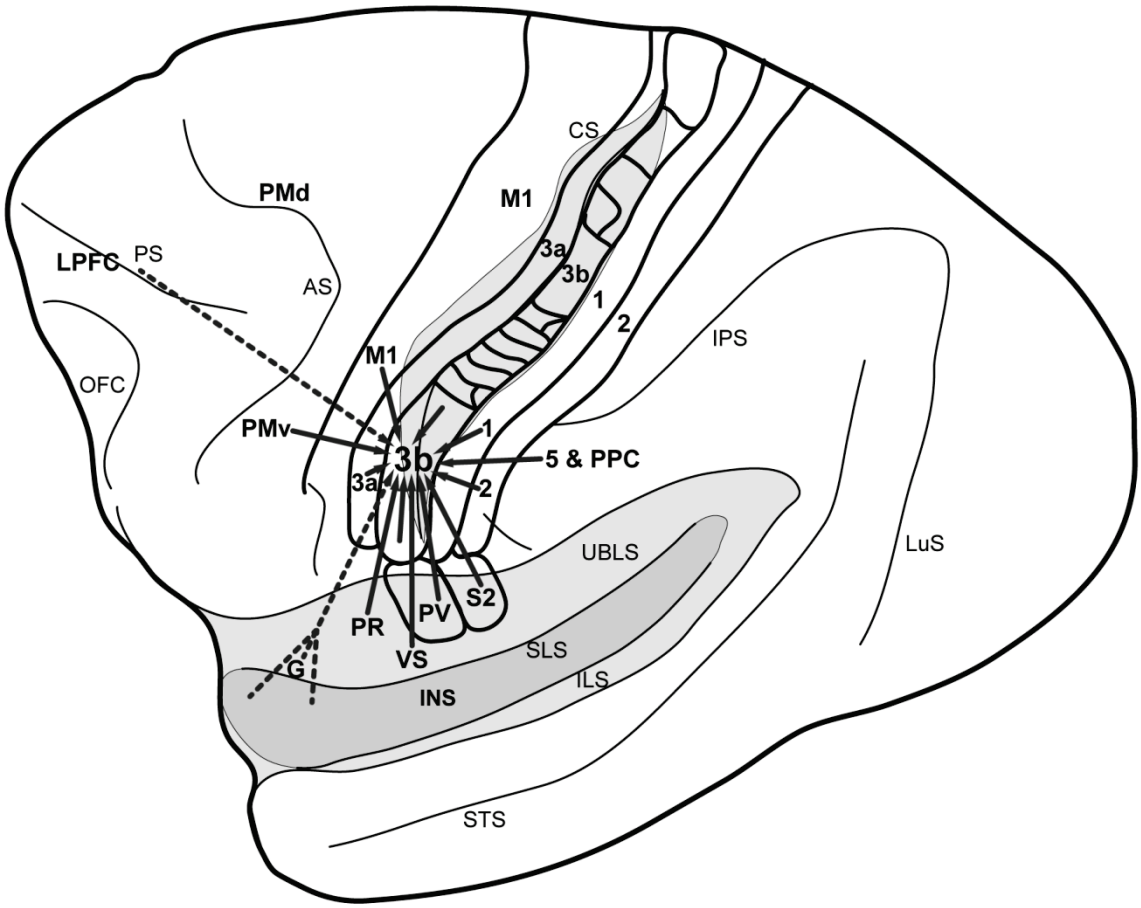

Figure 3.15. Summary of projections to the 3b representation of the oral cavity and face. The 3b representations of the oral cavity and face receive inputs from within mouth and face representations of 3b, the presumptive mouth and face representations in the other somatosensory areas of the anterior and posterior parietal cortex and in the lateral sulcus, and regions of motor and premotor cortex involved in controlling movements of the mouth and face (solid lines). Presumptive area G and regions of the insula implicated in processing taste, as well as cortex in the dorsal bank of the PS, were shown to project only to the 3b tongue representation (dotted lines).

Cortex in the anterior UBLS and insula and around the PS only contained neurons filled by injections into the representation of the tongue in area 3b. Additional tracer injections into representations of the lips and chin in primary somatosensory cortex revealed a similar pattern of projections to the 3b face cortex. The inputs from within area 3b to the face representation came largely from the rest of face and oral cavity cortex, with little to no input from other body part representations, i.e. the hand, in area 3b.

Network for processing somatosensory information from the oral cavity

The system for processing somatosensory information from the inside of the mouth and face is large and complex, similar to that for the hand. All somatosensory areas in the anterior parietal cortex (3b, 3a, 1, and 2) and the lateral sulcus (S2, PV, PR, and PV), as well as associated areas in the PPC contained filled cells after our injections into 3b face and mouth representations (Fig. 3.15, solid arrows). Additionally, motor and premotor areas also provided input to the representations of intra-oral structures and the face in area 3b. Similar patterns of inputs to the 3b oral cavity and face representations have been shown in New World owl, squirrel, and marmoset monkeys (Iyengar et al., 2007). Tracer injections into representations of other body parts in area 3b have demonstrated a similar pattern of projections across all primates (Burton & Fabri, 1995; Coq et al., 2004; Darian-Smith, Darian-Smith, Burman, & Ratcliffe, 1993; Kaas, 2004; Kaas & Pons, 1988; Krubitzer & Kaas, 1990; Pons & Kaas, 1985, 1986; Qi et al., 2002; Wu & Kaas, 2003). Thus, our results revealed a network for processing tactile information from the inside of the mouth that is similar to that for other body parts, and likely common to all primates.

Because each of our injections was placed into an electrophysiologically defined representation of the tongue, teeth, lip, or chin, the locations of labeled cells helped to identify poorly studied oral cavity and face representations in other somatosensory areas. Backfilled neurons were concentrated in the lateral parts of areas 3a, 1, and 2. Though, we did not map these regions in during our experiments, limited mapping in these regions in macaques and New World monkeys suggest that representations of the face and mouth lie in the lateral region of each of these areas (Huffman & Krubitzer, 2001; Iyengar et al.,

2007; Jain, Qi, Catania, et al., 2001; Manger et al., 1995; Manger et al., 1996; Manger et al., 1997; Nelson & Kaas, 1981; Pons, Garraghty, Cusick, & Kaas, 1985a, 1985b; Qi & Kaas, 2004; Seelke et al., 2012). Given that the representations any body part in each of the anterior parietal areas are preferentially connected to the matching body part representation in area 3b (Burton & Fabri, 1995), it is likely that the projections from areas 3a, 1, and 2 revealed by our injections indicate the oral cavity and face representations in each of these areas. Face and mouth representations have not been thoroughly described in area 5, however, lesions in the face region of the primary somatosensory face cortex resulted in degeneration in lateral area 5 (Pearson & Powell, 1985; Pearson & Powell, 1978). Our injections of retrograde tracers resulted in labeled cells in the cortex just caudal to the presumptive area 2 face representation. Projections from area 5 to area 3b are weak, though they have been described as arising from same approximate mediolateral level in area 5, thus the likely matching body part representation, as an injection in area 3b (Burton & Fabri, 1995).

There are multiple somatosensory areas in the cortex of the UBLS, and each one contained labeled neurons after our injections. While electrophysiological mapping of these areas in macaques has been limited by their difficult to access position on the UBLS, the organization of S2, PV, and parts of VS have been described in these large-brained monkeys (Krubitzer et al., 1995). The oral cavity was not completely mapped in S2, and PV, and the responses to stimulation of the face and intra-oral structures were often non-adjacent. Though the precise organization of the map of the face and mouth in these regions is unclear, the responses to stimulation on the face and mouth were concentrated near the crest of the LS closer to the junction with areas 3b, 1, and 2 than

the ventral border of S2 and PV deeper in the UBLS. Labeled cells in these S2 and PV tended to be concentrated medially, though the field of labeled neurons often extended toward the ventral border. Receptive fields for neurons in S2 and PV are often large, leaving the organization of the map in these areas is not as clear as that in area 3b where smaller receptive field dominate. Thus, it may be that representations of larger regions of the skin surface of the mouth and face in area S2 and PV project to the more circumscribed representations of individual oral cavity and facial structures in area 3b. Organized projections from S2 and PV to area 3b seen for other body parts in New World monkeys and prosimian galagos where accessing S2 and PV for study is easier and physiological maps are more complete (Coq et al., 2004; Iyengar et al., 2007; Krubitzer & Kaas, 1990; Qi et al., 2002; Wu & Kaas, 2003). The existence of a distinct representations of the inside of the mouth in area PR and VS has only been implied by limited mapping of the mouth and face (Coq et al., 2004; Krubitzer et al., 1995; Qi et al., 2002) and the results of previous tracer studies in New World monkeys (Iyengar et al., 2007). The existence of projections from these areas in our macaques further suggests the existence of face and oral cavity representations in PR and VS, though more extensive physiological exploration of these parts of the UBLS will be required to identify them with complete confidence.

Our injections into the 3b representations of the oral cavity and face also labeled neurons in lateral M1 and PMv. Movements of the face and mouth have been elicited by stimulation of these regions in both New World monkeys and macaques (Gharbawie, Stepniewska, & Kaas, 2011; Ghosh & Gattera, 1995; Graziano et al., 2002; McGuinness et al., 1980; Stepniewska, Preuss, & Kaas, 2006). Furthermore, cells in this region have

also been shown to be active during movements of the jaw and tongue (Hoffman & Luschei, 1980; Murray & Sessle, 1992). Furthermore, both lateral somatosensory and motor cortex must be functioning properly for a monkey to properly perform a tongue protrusion task (Lin et al., 1993; Murray et al., 1991).The homotopic projections from M1 and PMv to area 3b representations of structures in the mouth and face that we have demonstrated here may underlie a network for proper integration of sensory information to guide movements of the face and mouth during feeding and vocalization.

Projections unique to the 3b tongue representation

While most of the projections to the 3b representations of the face and mouth were similar to those seen going to any other body part representation in area 3b, the representation of the tongue appears to receive unique projections from several areas implicated in the system for processing taste (Fig. 3.15, dotted arrows). Primarily, these inputs arose in the cortex of the anterior UBLS and insula. The system for processing taste is not well understood. There is general agreement about the existence of a primary gustatory area, G, in all primates that is similar to that described by Sanides (1968) in squirrel monkeys (Cipolloni & Pandya, 1999; Ogawa, 1994; Ogawa, Ito, & Nomura, 1985; Ogawa et al., 1989; Scott & Plata-Salamán, 1999). G is often defined anatomically as a region of cortex in the UBLS that receives strong input from the ventroposterior medial parvicellular nucleus (VPMpc), the taste nucleus of the thalamus (Pritchard, Hamilton, Morse, & Norgren, 1986). Functionally, this area is difficult to define due to sparse responses to taste stimuli (Ogawa et al., 1989; Plata-Salamán & Scott, 1992; Pritchard & Norgren, 2004; Scott et al., 1986) and fairly high incidence of cells that

respond to touch (Ogawa et al., 1989; Scott et al., 1986). Nearby regions of the insula have also been implicated in the processing of taste (Kringelbach, 2004; Ogawa et al., 1989; Plata-Salamán & Scott, 1992; Pritchard & Norgren, 2004). It is likely that the cells backfilled by our injections into the tongue representations in our cases were in area G and possibly these other gustatory related areas. In, macaques, there is no projection from VPMpc to the 3b tongue representation (Chapter II). Thus, it appears that projections from taste related cortex to the macaque 3b tongue representation form the only pathway by which gustatory information can access the primary sensory cortex for the tongue. However, we cannot rule out the possibility that it is only the somatosensory responsive cells in these regions are that project to area 3b.

Both the orbitofrontal cortex (OFC) and the lateral prefrontal cortex (LPFC) have been implicated in the qualitative evaluation of taste stimuli (Carmichael & Price, 1995; Kringelbach, 2004; Kringelbach, de Araujo, & Rolls, 2004; Rolls, Sienkiewicz, & Yaxley, 1989; Rolls, 2000; Rolls & Baylis, 1994), and may be vital to integrating information from multiple sensory systems to create the rewarding response often associated with food (Shepherd, 2012). Our injections did not label cells in the OFC, but a few neurons in the LPFC were labeled by out injections into the tongue region. No connections between the LPFC and area 3b have been demonstrated before (Yeterian, Pandya, Tomaiuolo, & Petrides, 2012), and the projection we found was very weak, consisting of only 8 out of the 18,447 total CTB cells labeled by case 1's 3b tongue representation injection, making it difficult to interpret these results. However, in macaques, cells in the LPFC have been shown change activity during delay periods in tasks where food rewards were visible or associated but not seen (Hikosaka & Watanabe,

2000; Watanabe, 1996). Responses to taste (Kringelbach et al., 2004) and changes fatty acid concentrations in the blood after a meal (Tataranni et al., 1999) have been demonstrated in the dorsolateral prefrontal cortex in humans. These responses in humans appear to be in regions of cortex that correspond the location of our labeled cells in LPFC (Petrides, Tomaiuolo, Yeterian, & Pandya, 2012).

Face-hand interaction in area 3b

It has long been know that peripheral amputations (Merzenich et al., 1984) and deafferenting injuries of the peripheral nerves (Garraghty, Hanes, Florence, & Kaas, 1994; Garraghty & Kaas, 1991; Merzenich et al., 1983; Merzenich et al., 1983) and spinal cord (Jain et al., 1997; Jain, Florence, et al., 1998; Jain et al., 2008; Pons et al., 1991) can lead to changes in the responsiveness of the region of area 3b representing the hand. Reorganization in the somatosensory cortex after such injuries often results in hand cortex becoming responsive to stimulation on the face (Fang et al., 2002; Jain et al., 1997; Jain, Florence, et al., 1998; Jain et al., 2008; Manger et al., 1997; Merzenich et al., 1984; Pons et al., 1991). When small injections of distinguishable fluorescent tracers were placed into mapped representations of the 3b hand and face in fully intact owl, squirrel, and marmoset monkeys, no connections between the two regions were found (Fang et al., 2002). Intrinsic connections of the 3b hand have been shown to be confined to a mediolaterally restricted region of area 3b (Burton & Fabri, 1995; Lund, Yoshioka, & Levitt, 1993). Although the full extent of the hand representation was not mapped and the hand face border was not defined in these studies, few of these connections appeared to extend into the representation of the face that lies laterally in 3b compared to the hand

representation. Our injections into representations of the face and intra-oral structures resulted in very few, if any, labeled cells area 3b medial to the hand face border (Table 3.2). These combined results suggest there is no preexisting face-hand network in area 3b. Thus, the reorganization that occurs after deafferenting injuries must result from changes at all levels of the neuroaxis that begin in the spinal cord.

Conclusions

By injecting anatomical tracers into electrophysiologically defined representations of the tongue, teeth, and face in the primary somatosensory cortex, we were able to reveal the projections to the 3b oral cavity region in macaques. This is the first time the network for somatosensory processing from the oral cavity has been demonstrated in Old World monkeys. For the most part, the projections to area 3b's oral cavity representation from other somatosensory and motor areas are similar to those going to the better studied 3b hand. The tongue, however, received idiosyncratic inputs from taste related areas buried deep in the lateral sulcus, insula, and very weakly from the lateral prefrontal cortex. Finally, no network of connections that may underlie cortical reorganization after spinal cord injury between the 3b representations of the face and mouth and 3b hand was indicated.

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CHAPTER IV

PROJECTIONS TO CORTICAL AREA 1 REVEAL ORGANIZATION IN THE THALAMUS AND CORTEX

Abstract

The thalamocortical and corticocortical projections to area 1 in squirrel monkeys were determined by placing multiple injections of anatomical tracers into separate body part representations defined by multiunit microelectrode mapping in area 1. Multiple thalamic nuclei projected to area 1. Projections from both the lateral and medial subdivisions of the ventroposterior nucleus (VPL and VPM), the ventroposterior inferior nucleus (VPI), the ventroposterior superior nucleus (VPI), and anterior pulvinar nucleus (Pa) were topographically organized. The pattern of labeled cells in the cortex indicated that area 1 contained strong intrinsic connections within each body part representation. Area 1 received inputs from somatotopically matched regions of areas 3b, 3a, 2, and 5. Somatosensory areas in the lateral sulcus, including the second somatosensory area (S2), the parietal ventral area (PV), and the presumptive parietal rostral (PR) and ventral somatosensory (VS) areas also sent roughly organized projections to area 1. Organized projections to area 1 also came from the primary motor cortex (M1), the dorsal and ventral premotor areas (PMd and PMv), and the supplementary motor area (SMA). Labeled cells were also found in motor and sensory areas along the medial wall. The pattern of projections revealed by twelve injections of five different tracers across the squirrel monkey area 1 body map was similar to that seen to area 1 in Old World

macaque monkeys, suggesting a basic network of inputs to area 1 that is common across anthropoid primates.

Introduction

The network of connections among different somatosensory related regions of the spinal cord and brain provides the foundation for many of the changes seen after spinal cord injuries. Studies of the connections of non-primary cortical somatosensory areas have been limited even in monkeys with fully intact spinal cords. The connections of the non-primary, cutaneously responsive area 1 have not been specifically described in squirrel monkeys. A description of these inputs in a normal animal is important in understanding the effects of spinal cord injuries that have been described in squirrel monkeys (Bowes, Massey, Burish, Cerkevich, & Kaas, 2012; Chen, Qi, & Kaas, 2012; Qi, Chen, & Kaas, 2011).

Limited studies of the projections to area 1 in macaque monkeys (Burton & Fabri, 1995; Padberg et al., 2009; Pons & Kaas, 1985, 1986) and New World titi monkeys (Coq, Qi, Collins, & Kaas, 2004; Padberg, Disbrow, & Krubitzer, 2005) have indicated inputs from thalamic nuclei in the ventroposterior (VP) complex and pulvinar, somatosensory cortical areas in the anterior parietal cortex (APC) and along the upper bank of the lateral sulcus (UBLS), motor areas in the frontal lobe, and motor and sensory areas on the medial surface of the brain. Our goal was to determine which thalamic nuclei and cortical areas projected to area 1 in squirrel monkeys by placing multiple injections of anatomical tracers across the physiologically mapped representation of the contralateral body in

squirrel monkey area 1. Using distinguishable tracers injected into defined body part representations also allowed us to determine if any of these projections were organized, providing more insight into the somatotopic organization of difficult to access, understudied thalamic nuclei and cortical areas buried in the lateral sulcus and along the medial wall.

The APC consists of four somatosensory areas (3b, 3a, 1 and 2) that each respond to different types of somatosensory stimulation. The most well studied of these areas is area 3b, the primary somatosensory cortex (Kaas, 1983), which processes cutaneous information and must be intact for proper somatosensory processing to occur (Garraghty, Florence, & Kaas, 1990). Area 1 is a second cutaneously responsive field that lies just caudal to area 3b, that is important for tactile discrimination (Carlson, 1981; Randolph & Semmes, 1974). It has been described in several species of New World and Old World monkeys (Coq et al., 2004; Felleman, Nelson, Sur, & Kaas, 1983; L. A. Krubitzer & Kaas, 1990; Merzenich, Kaas, Sur, & Lin, 1978; Nelson, Sur, Felleman, & Kaas, 1980; Padberg et al., 2005; Sur, Nelson, & Kaas, 1982). Similar to Old World macaque monkeys (Nelson et al., 1980; Pons, Garraghty, Cusick, & Kaas, 1985b), squirrel monkey area 1 contains an organized map of the contralateral body surface that mirrors the somatotopic organization in area 3b (Jain, Qi, Catania, & Kaas, 2001; Sur et al., 1982). The existence of a more caudal APC field known as area 2, a region that has been linked to skilled hand use, has been questioned in New World monkeys (Padberg et al., 2005). A third complete map of the body in area 2 has been electrophysiologically defined in the cortex caudal to area 1 macaque monkeys (Pons, Garraghty, Cusick, & Kaas, 1985a; Pons et al., 1985b), but has been difficult to find with physiological methods alone in

New World monkeys. Padberg and colleagues (2005; 2007) proposed that area 2 is a largely proprioceptive area that is needed only in species that need fine position monitoring of the digits to form a precision grip. They concluded that the cortex immediately caudal to area 1in many New World monkeys is equivalent the sensorimotor area 5 of the posterior parietal cortex (PPC) rather than APC area 2. In light of the full body map and weak cutaneous responses that can be found in macaque area 2 (Pons et al., 1985a, 1985b; Seelke et al., 2012), and since the responsiveness of the region caudal to area 1 in New World monkeys seems heavily influenced by anesthesia in these much smaller animals, our secondary goal was to use the pattern of cortical projections revealed by anatomical tracer injections to area 1 to identify APC areas by comparing the resulting distribution of labeled cells in this region to that seen in macaques (Burton & Fabri, 1995; Pons & Kaas, 1986).

Methods

Three adult squirrel monkeys (*Saimiri*) 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. All surgical procedures were performed under aseptic conditions.

Like other primates, the map of the contralateral body in squirrel monkey area 1 is inverted, with the face and hand lying laterally and the leg and foot more medial (Jain et al., 2001; Sur et al., 1982). Similar to macaques, the body map in area 1 mirrors that in area 3b of squirrel monkeys (Nelson et al., 1980; Sur et al., 1982). Multiunit

microelectrode recording was used to guide the placement of injections of multiple, distinguishable tracers into distinct body part representations in area 1. Two multiunit microelectrode mapping sessions, one to determine targets for injections and a second to confirm the boundaries of the representation that was injected and determine the physiological borders between areas, were performed in each animal. During each session, the investigator delimiting the receptive field was blind to the location of the electrode in the brain.

Surgical procedures

The animals were anesthetized with an initial dose of ketamine hydrochloride (10-50 mg/kg, i.m.), secured in a stereotaxic frame, and maintained on 0.25-5% isoflurane during surgical 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. A craniotomy was performed to expose all of areas 1 and 3b from the midline to the lateral sulcus, and the dura was removed. Electrophysiological mapping was performed under intravenous ketamine anesthesia (20-40 mg/kg/h in sterile saline) delivered through either a leg or tail vein and supplemental injections of xylazine (0.2-0.4 mg/Kg i.m.). The exposed cortex was kept moist with regular application of sterile saline or covered with silicone oil. 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 lesions using blood vessels and sulcal

patterns as landmarks. Descriptions of receptive fields determined during physiological mapping were recorded and drawings of each receptive field were made on standard drawings of the squirrel monkey body.

Multiunit mapping and injections

The first mapping session was performed to target distinct body part representations in area 1 for tracer injections. Low-impedance tungsten microelectrodes (1.0 mΩ at 1,000 Hz) attached to a stereotaxic arm were inserted perpendicularly into the cortex at multiple sites in rows across areas 1 and 3b, while multiunit recording was used to map receptive fields based on activity driven by stimulation of the skin with glass or wooden probes. The mediolateral level of each injection site was chosen when distinct representations of different body parts were found, i.e. a row of responses to stimulation of the forearm versus a more medial row of sites with receptive fields on the leg. Areas 1 and 3b both responded to light touches on the skin. Area 1's rostral border with 3b was defined by a reversal of the progression of the receptive fields across the body. In the hand, for example, the digit tips are represented caudally in area 1 and the proximal palm along the rostral border (Chen, Friedman, & Roe, 2005; Chen, Friedman, & Roe, 2009; Sur, 1980). This mirrors area 3b, where the palm is represented along the caudal border with area 1 and the digit tips rostral near the area 3b/ area 3a boundary. Along the central sulcus where much of the arm, trunk, and leg are represented, it was impossible to insert the electrode perpendicular to layer 4. In these locations, the progression of receptive fields from those in area 1 to those in 3b was determined by moving the electrode in steps of 200-300 μm down the caudal bank of the central sulcus, parallel to layer IV. The

caudal border of area 1 with area 2 was determined by a loss of response or a change in responses from being good and elicited by cutaneous stimulation to weak and elicited by tapping on the skin, pressing or squeezing of a muscle, and moving the joints to stimulate deep receptors.

Once discrete representations of different parts of the body in area 1 were determined, the electrode was removed from the stereotaxic arm and replaced with a Hamilton syringe with a glass pipette tip attached to ensure that the injections were placed along the same angle at which the receptive fields were mapped. Pressure injections of 0.04-0.6 µl of up to five of each of following tracers were made: Cholera Toxin Subunit-B (1% CTB in distilled water, Sigma, St. Louis, MO), CTB conjugated to green alexa fluor-488 (1% CTB-G in distilled water, Invitrogen, Carlsbad, CA), CTB conjugated to red alexa fluor-594 (1% CTB-R in distilled water, Invitrogen), Diamidino Yellow (2% DY in 0.1 M phosphate buffer, Sigma, St. Louis, MO, or Dr. Illing Plastic GmbH, Breuberg, Germany), and Fluororuby (10% FR in distilled water, Invitrogen). Following the injections, gel film was inserted to replace the opened dura, the craniotomy was closed with dental cement and skin sutured shut. The animal was then recovered from anesthesia, and treated with prophylactic antibiotic and analgesics.

A second mapping session following the same procedures as the first was performed to determine the full extent of each body part representation in areas 1 and 3b and physiological boundaries of each area after 10-14 days of tracer transport. During this session electrolytic lesions were made by passing cathodal current (10 µA) at the approximate sites of mirror reversals between 3b and 1 body representations. These lesions were later used to relate the physiological maps to the cortical myeloarchitecture.

Once electrophysiological mapping was complete, the animals were given a lethal dose of sodium pentobarbital (80 mg/Kg), and perfused though the heart with phosphate buffered saline followed by 2% paraformaldehyde in buffered saline and 2% 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 (Fig. 4.1). The cortex ipsilateral to the injections was cut into two blocks. The main block contained all of the frontal and parietal lobes, including as much of the upper bank of the lateral sulcus and insula as possible. In some cases, parts of the upper bank of the lateral sulcus and insula stayed attached to a second block of cortex that included most of the temporal lobe and rostral parts of the occipital lobe. In case 10-19, the occipital lobe was retained and analyzed for projections to area 1. Since no labeled cells were found in the occipital lobe in case 10-19, the occipital lobe in other cases was removed and used in unrelated experiments. 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.

Tissue processing

The flattened cortical blocks were cut tangential to the surface and the thalamus was cut coronally at a thickness of 40 μm. 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 (Angelucci, Clasca, & Sur, 1996; Bruce & Grofova, 1992) to visualize CTB filled cells. CTB-immunoreactive (CTB-IR) cells included those labeled by injections of CTB and CTB

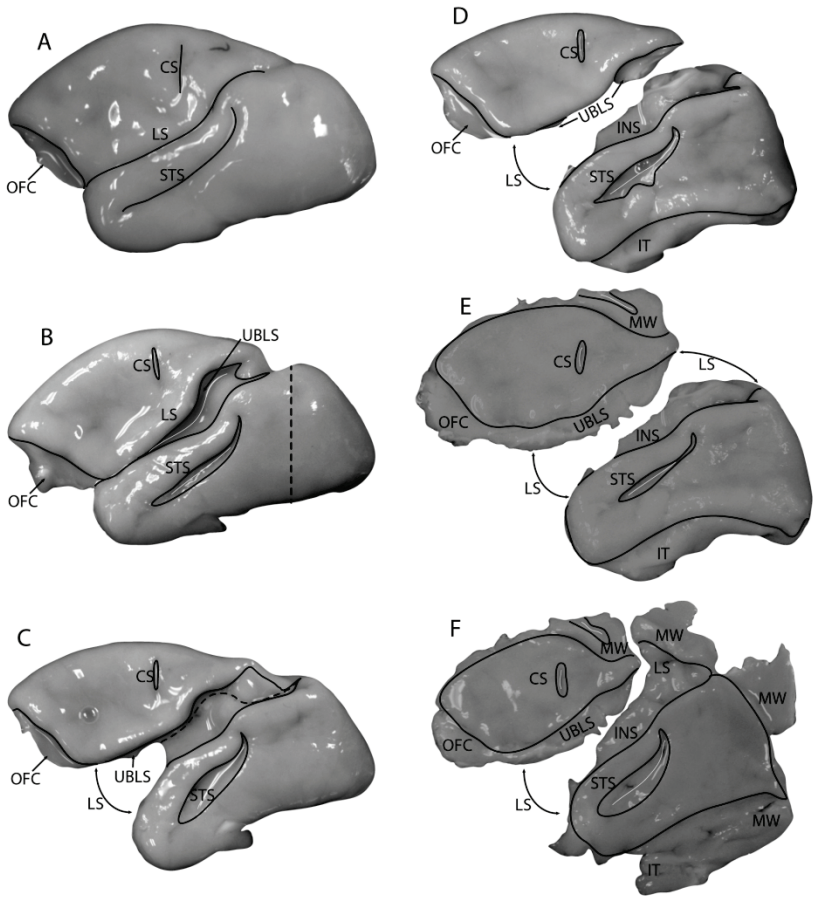

Figure 4.1. Cortical flattening procedure. A. Lateral view of the left hemisphere of case 10-19. B-F. Opening of sulci, blocking of the hemisphere, and subsequent unfolding of the cortex. Black lines are the crests of sulci. White lines are the sulcal fundi. The dotted line in panel B indicates the approximate location where the occipital lobe was dissected away in cases 11-33 and 12-40. The dotted line in C indicates when the frontal and parietal lobes were separated from the insula, temporal and occipital lobes in this case.

conjugated to fluorescent alexas. This allowed us the advantage of essentially filling area 1 with a single tracer to reveal all projections to area 1, while also allowing us to see if these projections were topographically organized by comparing the locations of the distinct CTB-G and CTB-R filled cells that can be seen with fluorescence microscopy to the locations of CTB-IR cells, anatomical boundaries, the results of our physiological mapping in the cortex, and published maps of the body in the cortex and thalamus (Kaas,

Nelson, Sur, Dykes, & Merzenich, 1984; Sur et al., 1982). Additional series of sections were processed to reveal anatomical boundaries. In the thalamus, series of sections were processed for cytochrome oxidase (CO; Wong-Riley, 1979) and vesicular glutamate transporter 2 (VGluT2; mouse monoclonal anti-VGluT2 antibody, Chemicon, now part of Millipore, Billerica, MA) according to standard protocols. The VGluT2 antibodies used in this study were previously characterized and confirmed with western blot analysis. A mouse monoclonal anti-vesicular glutamate transporter 2 antibody was used, clone 8G9.2 (Millipore, catalog number MAB5504, dilution factor 1:2,000, isotope IgG1, recombinant protein from rat VGluT2), to reveal the distribution of VGluT2 protein. This antibody stained a single band at 56 kDa molecular weight in western blots from macaque cerebellar tissue (P. Balaram, personal communication, September 4, 2012), confirming the manufacturer information. Previous reports have indicated that VGluT2 immunostaining reveals the brainstem terminations in the thalamus of rats (Kaneko & Fujiyama, 2002), squirrels (Wong, Gharbawie, Luethke, & Kaas, 2008), galagos (Wong & Kaas, 2010), and squirrel monkeys (H-X Qi, Gharbawie, Wong, & Kaas, 2011). This antibody showed a pattern of staining in that was similar to that demonstrated in VPL by Qi and colleagues (2011). A modified Gallyas (1979) method for silver staining of myelinated fibers was used to reveal boundaries of cortical areas and sulcal patterns.

Data analysis

Distributions of neurons filled by each tracer were plotted with a fluorescent/ brightfield Leitz microscope coupled to a computer running Neurolucida™ plotting software (MBF Bioscience, Williston, VT). The injection cores and the diffusion zones

around them were outlined. Landmarks including blood vessels, electrode tracts, 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. When multiple photomicrographs were required to image the whole section, they were stitched together in Adobe Photoshop to create an image of a single whole section.

Reconstruction

Borders of thalamic nuclei were determined from the differential distribution of CO and VGluT2. Architectural sections were projected onto corresponding plots of neurons filled as a result of each of the different injected tracers. Each architectural section was aligned to the closest adjacent the plot of a tracer stained section using blood vessels and other landmarks, and the boundaries and internal structures of the thalamic nuclei were drawn onto each plot. The borders and other landmarks were then used to align plots taken from series processed for different tracers to create collapsed representations of adjacent thalamic sections that allowed us to directly compare the locations of tracer labeled cells to each other and to previously published physiological maps of the body representation (Kaas et al., 1984) and topographic projections to area 3b (Cusick & Gould, 1990; Cusick, Steindler, & Kaas, 1985) of somatosensory related thalamic nuclei of squirrel monkeys.

Since a single section rarely revealed all areal boundaries, series of cortical sections stained for myelinated fibers were used to determine the borders of cortical areas and anatomically confirm the location of injection cores and labeled neurons. The outline of the myelin stained sections, areal borders, sulcal patterns, and landmarks, such as blood vessels, electrolytic lesions, and damage left after injections, were drawn. These landmarks were used to align architectonic boundaries throughout the depth of the cortex and create complete reconstructions of the cortical areas. Like other monkeys, a strip of dense myelin staining characterizes all of area 3b in squirrel monkeys (Jain, Catania, & Kaas, 1998; Jain et al., 2001; Krubitzer & Kaas, 1990; Qi & Kaas, 2004). Caudal to area 3b myelin staining revealed a strip of cortex that stained moderately for myelinated fibers. This moderately stained region matched the locations of area 1 responses during our physiological mapping, and a similar myelin staining pattern has been described for area 1 in other New World monkey species (Coq et al., 2004; L. A. Krubitzer & Kaas, 1990). Though often less distinct, staining of the cortex caudal to area 1 was used to identify area 2 (Seelke et al., 2012) and the border of this area was confirmed by measurements from previously published studies in squirrel monkeys (Gharbawie, Stepniewska, & Kaas, 2011). The extent of other cortical areas was similarly determined by the distribution of myelin. Physiological mapping data was added to the anatomical reconstructions by aligning penetrations along the trajectory of the electrode tracks that could be seen in the stained fiber sections and matching the locations of injection sties and lesions. Plotted cortex sections indicating the injection sites and distribution of backfilled cells were then aligned to the reconstructed borders and physiological maps using blood vessels, electrolytic lesions, and the injection cores as landmarks. The

density of neurons labeled by each tracer injection was analyzed determine if tracer labeled cells were evenly distributed, or grouped in any way that might suggest the locations of cortical areas or specific body part representations within them. To do this, each cortical reconstruction was overlaid with a 250 μm square grid. The labeled neurons within each grid square were counted using Adobe Illustrator's document info panel to determine the number of cells per bin. All reconstructions and density maps were created with Adobe Illustrator CS5™ (Adobe Systems).

Results

Twelve injections of distinguishable tracers across area 1 in three squirrel monkeys revealed organized projections from multiple thalamic nuclei and cortical areas. Not all of these injections were confined to area 1, and the results from the injections that spread into areas 3b and 2 will be described as well. Results are presented in three parts. First, descriptions of the locations of injection sites in discrete body part representations in areas 1 and 3b are given. Second, we present the thalamocortical projections to area 1. Finally, we describe the corticocortical projections revealed in the ipsilateral hemisphere.

Defining injection sites

Four injections were placed into representations of the contralateral body in area 1 in case 10-19. The first was an injection of CTB centered on the distal digit 3 representation in caudal area 1. The zone of tracer diffusion around the core of this injection encompassed sites responsive to digits 2 and 4, parts of the palmar pads, and

into the digit representation of presumptive area 2. The arm region in area 1 was the target for an injection of CTB-R that included representations of the forearm, upper arm, and armpit. A small injection of FR was placed rostrally in area 1 into representation of the dorsal and lateral middle trunk. The case 10-19 CTB-G injection was centered on representations of the leg, specifically the knee, upper leg, and hip. The core of this injection extended slightly into the adjacent representation of the nearby trunk. Immunohistochemical staining revealed a large diffusion zone around the CTB-G injection that spread across area 1 foot, leg and trunk representations, and slightly overlapped the FR injection in this case. The diffusion zone of the CTG-G injection extended into the 3b trunk representation.

Five injections were made in case 11-33 area 1. In the hand representation, CTB was injected into representations of distal digits 2, 3, and 4 and extended into adjacent representations of pads 3 and 4. The tracer diffused to include other parts of the digit and palm representations, and into the area 2 hand representation. A FR injection was contained entirely in the area 1 arm representation, and covered a zone that included responses to touch and stroking of hairs on the dorsal forearm and upper arm. An injection of CTB-G centered on representation of the occiput and dorsal trunk diffused to include the parts of the representations of the shoulder and neck. Mapping in this case was incomplete, so only responses at the center of the final two injections can be described. Representations of the knee and distal upper leg were at the center of a DY injection in case 11-33. Finally, CTB-R was injected into representations of the foot pads, insole of the foot, the fourth toe, and part of dorsal toe 1 along the inner edge of the foot.

In case 12-40, we placed injections of three tracers into area 1. We attempted to make an injection into the face representation in the lateral extreme of area 1; however, the center of this DY injection into a representation of the contralateral upper lip was on the border of areas 1 and 3b. The diffusion zone included representations of the nose and muzzle. In this case, the center of a CTB injection was in a region of caudal area 1 representing distal digits 2, 3, and 4. The diffusion zone in area 1 continued into the rest of the digit 3 representation, but also spread into rostral area 2, the presumed location of digit tip representations in area 2. FR was injected largely into the 3b representation of the dorsal forearm, though a small part of the core and diffusion zone did extend into area 1's representation of the dorsal forearm and hand.

Projections from thalamic nuclei

Case 10-19. Backfilled cells resulting from all four injections were found in several nuclei of the thalamus (Fig. 4.2). The primary somatosensory relay nucleus from the body, the lateral division of the ventroposterior nucleus (VPL), contained cells from all four injections. Additional somatosensory related nuclei including the ventroposterior inferior (VPI), ventroposterior superior (VPS), and anterior pulvinar (Pa) also projected to all parts of area 1 that were injected. A few labeled neurons were also found rostral to VPL in the ventral lateral nucleus (VL) as well as in the centre médian (CM) and mediodorsal nuclei (MD). This pattern is similar to that revealed by retrograde tracers injections in the macaque area 1 hand representation (Padberg et al., 2009).

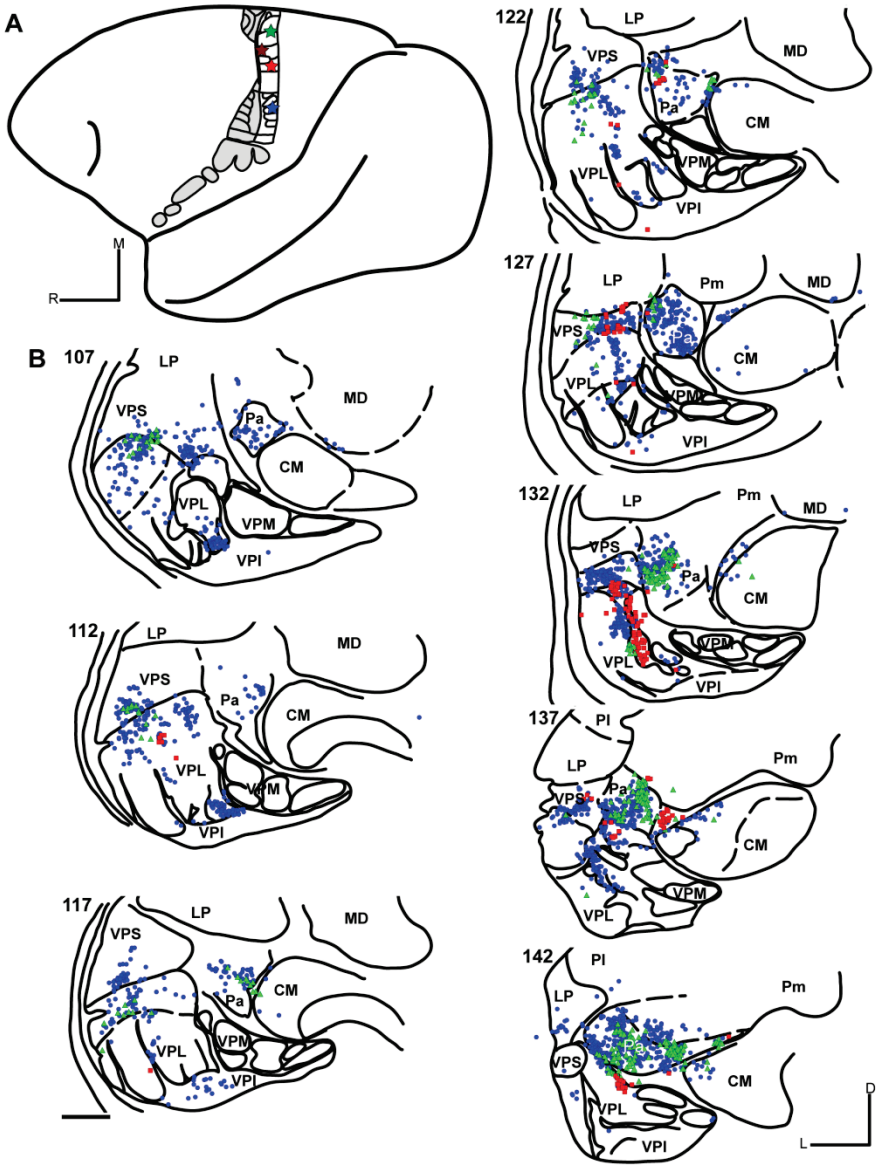

Figure 4.2. Distribution of labeled thalamic cells in case 10-19. A. The locations of injections sties on the somatotopic maps of the parts of areas 3b (gray) and 1 that can been seen on the brain's surface. Blue- CTB in distal D3, Dark Red- CTB-R in the ventral upper arm, Bright Red- FR in ventral middle trunk, Green- CTB-G in ankle and dorsal foot. Rostral is left, medial is up. B. The distribution of labeled neurons in the thalamus. All four injections resulted in labeled neurons in the thalamus. Each marker indicates a single labeled cell. Blue circle- CTB-IR, Green triangle- CTB-G, Red square- CTB-R and FR. CTB-IR cells include those labeled by the CTB, CTB-R, and CTB-G injection, thus any region where green triangles or red squares overlap blue circles indicates a region of projection to the arm or leg representations, respectively. Black lines indicate borders of thalamic nuclei identified from adjacent sections stained for CO and VGluT2. Serial thalamic sections are numbered from rostral to caudal. Abbreviations as in table 1. Sections were cut at 40µm. Lateral is left, dorsal is up. Scale bar is 1 mm. Backfilled cells resulting from all four injections were found in VPL, VPI, VPS, Pa, CM, and MD.

Topographic organization of projections to area 1 was suggested from several nuclei. Physiological maps of the body in VPL, VPI, and VPS (Kaas et al., 1984), and organized projections from these three nuclei to area 3b (Cusick & Gould, 1990) have been described in squirrel monkeys. CTB-IR neurons were found throughout VPL, though they were not evenly distributed. Aligning the CTB-immunoreacted sections with those containing CTB-G and CTB-R filled cells revealed under fluorescence microscopy shows that not all of the patches of CTB-IR cells project to the same region in area 1 (Fig. 4.3). In the dorsolateral corner of VPL, where the leg is represented, CTB-G filled cells overlapped CTB-IR neurons, while only CTB-IR cells could be found in the ventral hand representation. Cells backfilled by the CTB-R injection into the arm representation and the FR injection into the trunk region were indistinguishable, however some red labeled cells were found to overlap CTB-IR in the dorsomedial corner of VPL, while others in the more central part of dorsal VPL did not. When matched to a topographic map of the body of VPL derived from Kaas et al., (1984) in becomes apparent that the locations of foci of label from each injection matched previous physiological mapping of this nucleus (Fig. 4.3). Course maps of the body in VPS and VPI have been shown to roughly parallel the body representation in VPL, and again proper alignment of immunohistochemical and fluorescent sections revealed at least roughly organized projections from these nuclei. Pa has not been explored with physiological mapping in squirrel monkeys. In case 10-19, we found only CTB-IR cells in the more rostral part of Pa, suggesting that these cells were labeled as a result of the injection into the area 1 hand (Fig. 4.2, section 112). Moving caudally, a few CTB-R and FR filled neurons that resulted from the injections into the arm and trunk were found (Fig. 4.2, section 122).

These cells were displaced medially as CTB-IR and CTB-G filled cells began to appear in the lateral part of the nucleus (Fig 4.2, section 137). In the most caudal sections, for example section 142 in figure 4.2, CTB-IR and CTB-G filled cells were found, suggesting caudal Pa largely projects to the leg representation in area 1. This rostral to caudal progression from hand to trunk to leg, with cells projecting to the leg/ foot representation lying lateral and caudal to those projecting to the trunk, was also demonstrated in projections from Pa after injections of distinguishable tracers into different body part representations in area 3b (Cusick & Gould, 1990).

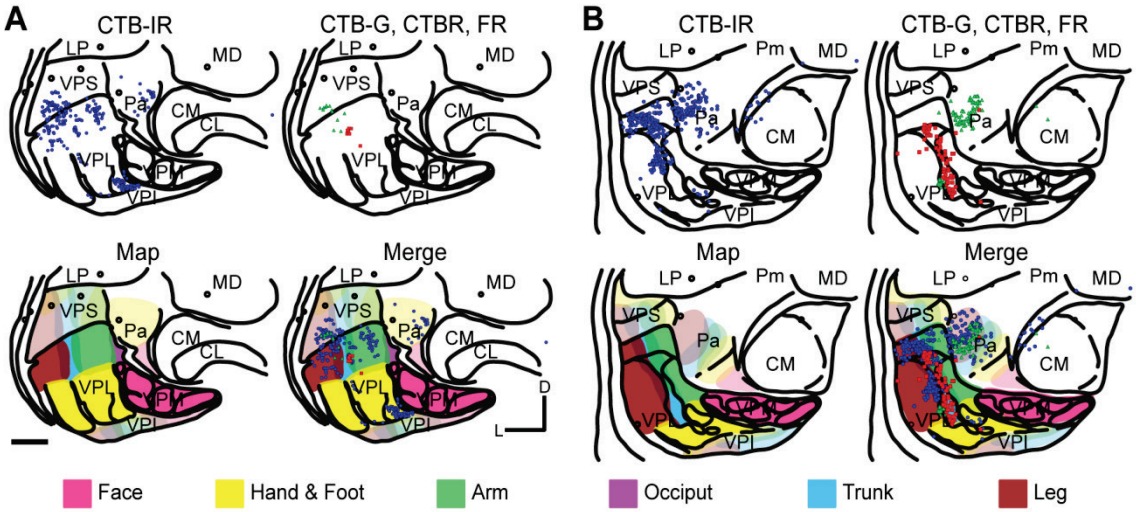

Figure 4.3. Topographic organization of projection from the thalamus to area 1 in case 10-19. A-B. The distribution of labeled cells compared to body maps of thalamic nuclei in more rostral section 112 (A) and caudal section 132 (B). In both A and B, CTB-IR neurons are shown separately from CTB-G, CTB-R, and FR along the top row. The estimated body maps derived from physiological mapping (Kaas et al., 1984) and projections to area 3b (Cusick and Gould, 1990) in VPL, VPI, VPS, and Pa are shown in the lower left corner. Because the somatotopy in VPS, VPI, and Pa have not been as well described as that in VPL, they are shown as lighter in color. Conventions as in figure 4.2. When the CTB-IR and fluorescent sections are aligned with the approximated body maps using blood vessels (black circles) and other landmarks, the topographic organization of projections from each of these nuclei is confirmed.

Case 11-33. Injections of five tracers across area 1 in case 11-33 resulted in distribution of labeled cells similar to that seen in case 10-19. Once again, VPL, VPI, VPS, Pa, VL, and CM all contained cells labeled by at least one of the injected tracers (Fig. 4.4). Cells filled by the DY injection into the area 1 leg representation were lateral and dorsal in VPL. CTB-IR neurons were found in two locations in VPL. One focus of CTB-IR cells was in a ventromedial position in the region of the digit representation, and is the likely result the CTB injection into D2. An injection of CTB-G was placed in the area 1 representation of the shoulder. Cells in the second focus of CTB-IR label in the dorsomedial corner of the VPL likely project to the location of the occiput/ upper trunk representation that was injected with CTB-G, however the CTB-G signal was weak under fluorescence microscopy and could not be used to confirm which CTB-IR cells result from the injection of CTB-G versus those labeled by the other CTB injections. A patch of cells labeled by the FR injection in to the area 1 arm representation was consistently found dorsally in VPL, between the region of dense DY labeling in the dorsolateral VPL and the more dorsomedial CTB-IR focus (Fig. 4.4). A few cells filled by one of our red tracer injections were found in the region of VPL that represents the foot. Transport and immunohistochemical staining of CTB-R was weak, but, given the location of this patch of cells, it is likely that they indicate a projection to the injected representation of the sole of the foot. Once again, VPS and PA sent organized projections to area 1.

Case 12-40. Unlike our other cases, case 12-40 had an injection placed into the face representations in areas 1 and 3b. The ventroposterior medial sub-nucleus (VPM), the primary somatosensory relay for information from the face, and adjacent part of VPI contained DY labeled cells (Fig. 4.5). It is unclear which of these cells project to the 3b

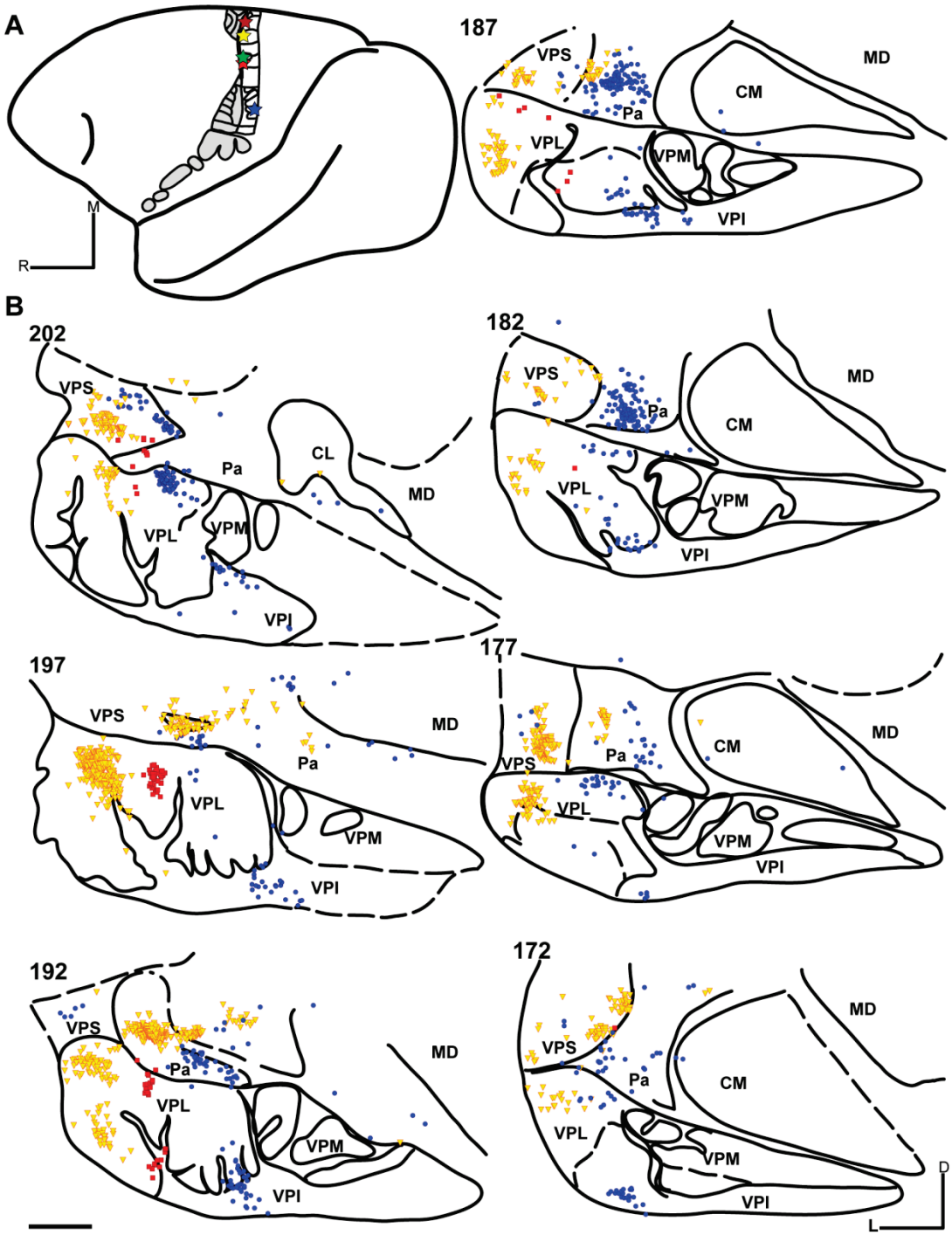

Figure 4.4. Distribution of labeled thalamic cells in case 11-33. A. The locations of injections across area 1. Blue- CTB in D2, Bright Red- FR in the dorsal upper arm and ventral shoulder, Green- CTB-G in the upper trunk, shoulder, and occiput, Yellow- DY in the knee an distal upper leg, Dark Red- CTB-R in pads of foot. Rostral is left, medial is up. B. The distribution of labeled neurons in the thalamus. Serial thalamic sections are numbered from rostral to caudal. Conventions as in figure 4.2. Labeled neurons were found in VPL, VPI, VPS, Pa, CM and MD. Cells appeared to lie in regions of VPL that matched the somatotopic location of each injection.

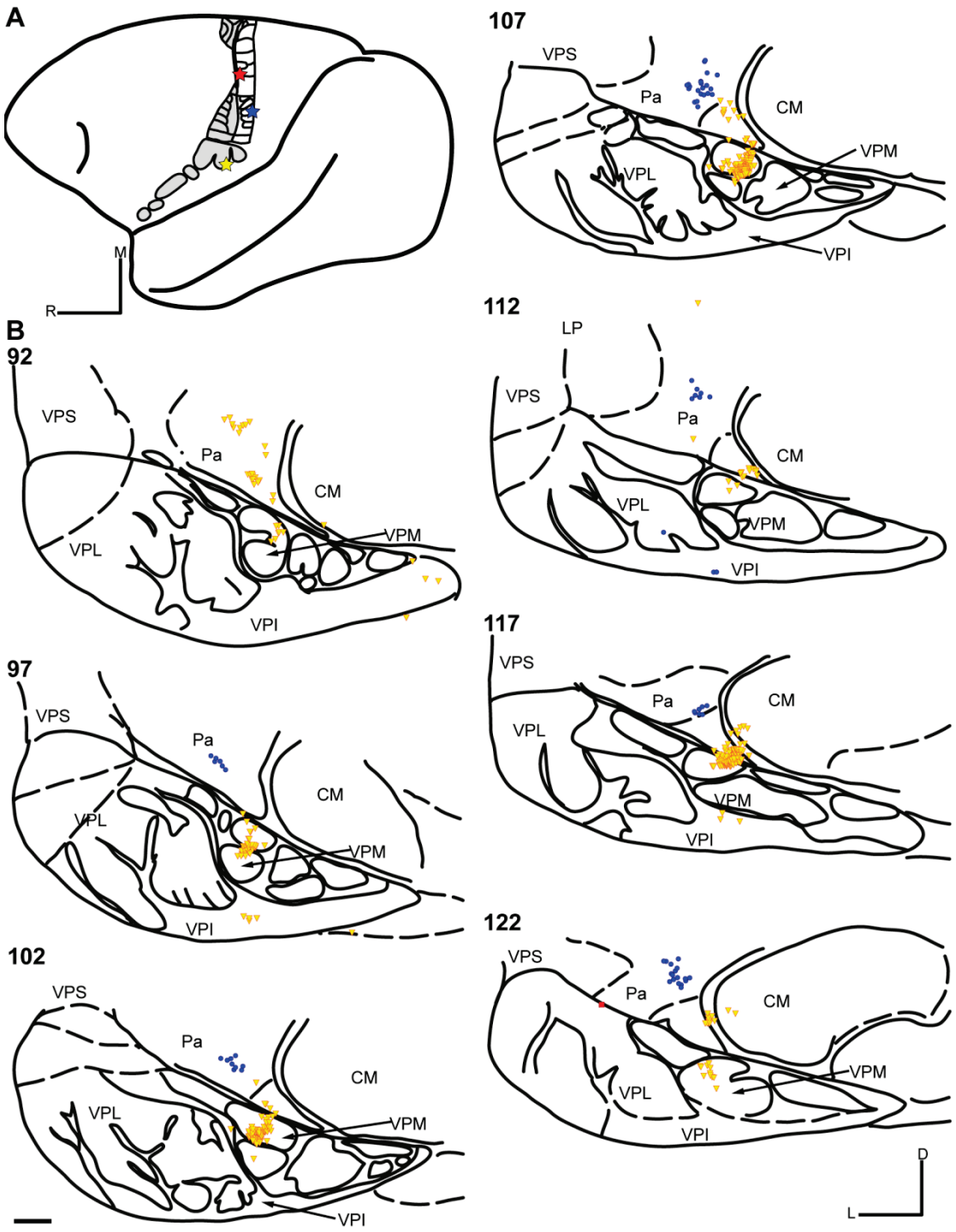

Figure 4.5. Distribution of labeled thalamic cells in case 12-40. The locations of injections across area 1. Yellow- DY in the area 3b/1 upper lip and nose, Blue- CTB in area 1 distal digits 3-4, Bright Red- FR in the area 3b dorsal forearm and dorsal hand. Rostral is left, medial is up. B. The distribution of labeled neurons in the thalamus. Serial thalamic sections are numbered from rostral to caudal. Conventions as in figure 4.2. Cells labeled by the DY injection into the face injection were found in corresponding face representations in VPM, VPI, and Pa.

face representation versus those projecting to the representation of the face in area 1 because this injection was on the 3b/1 border. Backfilled DY neurons in the rostral and medial Pa are consistent with projections to area 1. CTB-reactivity was low in the thalamic sections in this case; however the CTB-IR cells that were revealed in this case were in somatotopically appropriate parts of VPL and Pa. A single cell filled as a result of the FR injection that was placed largely into the area 3b arm representation was found in the approximate location of the arm representation in VPL.

Ipsilateral corticocortical projections

Case 10-19. The four tracers injected into area 1 in case 10-19 revealed a distinct pattern of projections from somatosensory areas within the anterior parietal cortex (APC), and parts of posterior parietal cortex (PPC). Immunohistochemical staining of the three CTB injections into area 1 showed that we essentially filled area 1, and revealed extensive intrinsic connections and projections from areas 3b, 3a, and 2 (Fig. 4.6). The distribution of fluorescent labeled cells in areas 1, 3b, 3a, and 2 was similar to, though more restricted than, that of the CTB-IR cells. Backfilled CTB-G neurons from an injection into the leg representation sat medial to those labeled by FR and CTB-R into the representations of the trunk and arm. No fluorescent tracer labeled cells were found lateral to the lateral tip of the central sulcus in any APC area. Labeled cells were also found in cortex caudal to area 2, a region presumed to include PPC area 5. The field of label continued into the PPC, though these projections were only clear in sections reacted to reveal CTB-IR neurons. Making individual markers that represent labeled cells large enough to be easily seen in our illustrations made it difficult to distinguish discrete

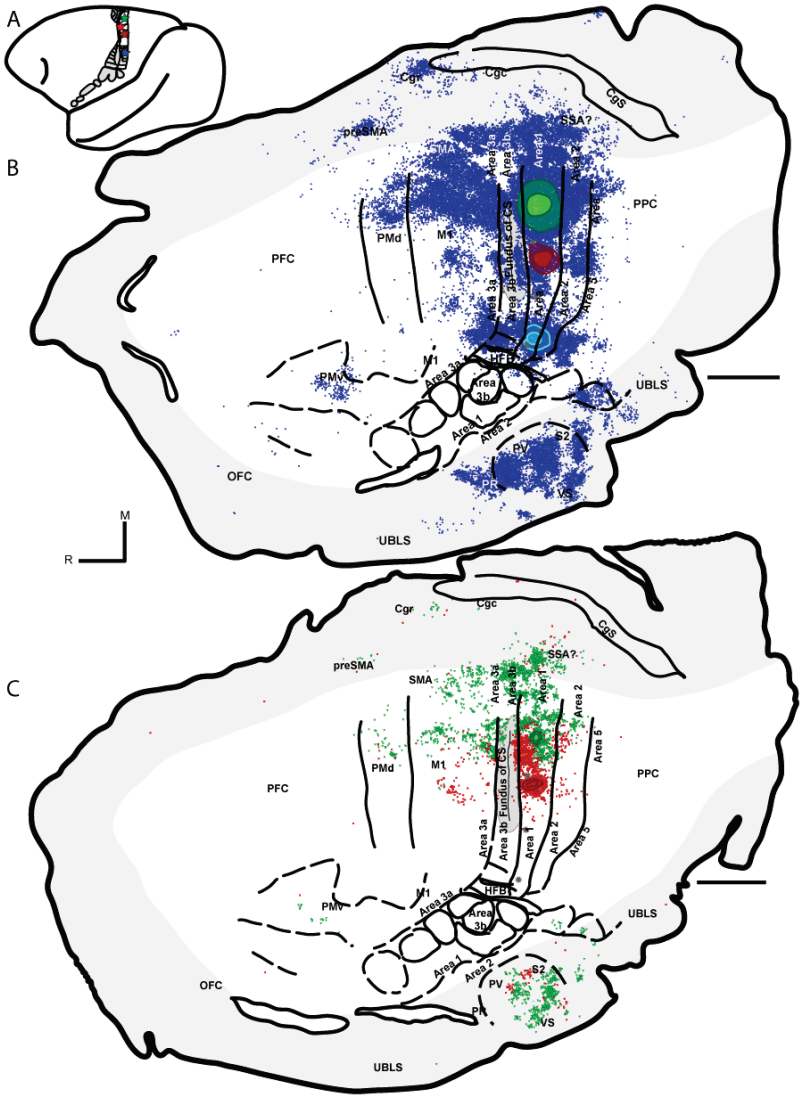

Figure 4.6. Distribution of cells labeled by CTB, CTB-R, FR, and CTB-G injections into area 1 digit, arm, trunk, and leg representations in case 10-19. A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of CTB-R and FR neurons. Each marker indicates a single labeled cell. Areal boundaries were determined using myeloarchitecture. Thin black lines are borders of areas. Thick black lines are tears and the edges of the block. Gray shaded regions are the central sulcus, UBLS, and medial wall. The CTB, CTB-R, and CTB-G injection sites are outlined and shaded in blue, red, and green, respectively. Rostral is left, and medial is up. Scale bar is 5 mm. Inputs to each injected representation were similar across area 1. There were strong intrinsic projections within area 1. Projections from APC arose from areas 3b, 3a and presumptive area 2. The field of label extended into PPC. There was extensive labeling in the somatosensory areas of the lateral sulcus including areas S2, PV, VS and PR. M1, PMd, PMv, SMA, pre-SMA and cingulate areas also contained back filled cells. Fluorescent labeled cells show that many of these inputs come from restricted regions of each cortical area, that match the expected location of representations that match injected representations in area 1.

groupings of filled neurons in each APC area, so an analysis of the density of the labeled cells was performed (Fig. 4.7). When depicted in this way, regions of high CTB-IR cell density could be seen at the approximate mediolateral level of each of the CTB injections in all four APC areas and area 5, suggesting the strongest projections come from the same mediolateral level as each injection. Since an individual body part tends to be represented at approximately the same mediolateral level in areas 3a, 3b, 1, 2, and 5, it is likely that dense projections labeled at three different mediolateral locations in each APC area indicate topographically organized projections form these somatosensory areas to area 1. Density analysis also suggested topographic organization of projections from within areas. The high density of CTB-IR cells near the digit 3 representation injections in lateral area 1 showed that the strongest intrinsic connections to the injected digit 3 representation arose in nearby area 1 where fingertips are represented. The density of projections is lower along border of areas 1 and 3b where the palm is represented, and increases again in rostral 3b where the distal digits are represented. Regions of dense labeling in area 3a along its caudal border with area 3b and in rostral area 2 also correspond to the expected locations of digit representations in these areas. Similar dense patches that match the somatotopic location of the area 1 injections are seen in areas 3b, 3a, and 2 at the levels of the CTB-R and CTB-G injections. Density analysis of the projections revealed by the fluorescent injections indicated a similar pattern of dense label in mirrored locations in APC areas after the CTB-R and FR injections into the arm and trunk representations and the CTB-G injection centered on the area 1 leg representation.

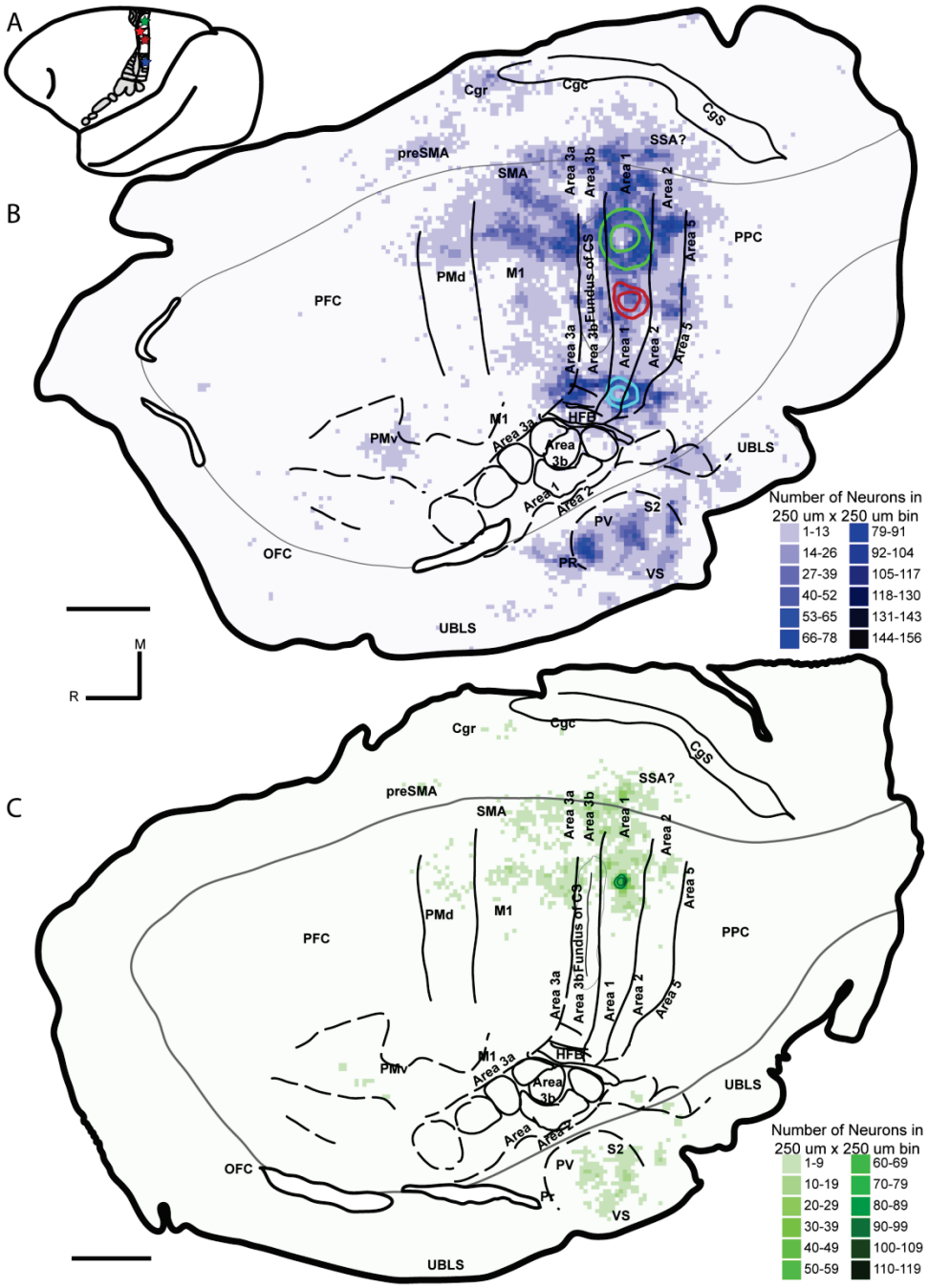

Figure 4.7. Density of projections labeled by CTB, CTB-R, FR, and CTB-G injections into area 1 digit, arm, trunk, and leg representations in case 10-19. A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The density of CTB-IR labeled cells. C. The density of CTB-G labeled cells. D. The density of CTB-R and FR labeled cells. Each colored square indicates the number of cells per 250 x 250 µm bin. Darker colors indicate a higher number of cells. Conventions as in figure 4.6. Density analysis reveals that the densest projections to a given injected region in area 1 arise in somatotopically matched representations in other areas, and allowed us to distinguish the locations separate medial wall areas containing labeled cells.

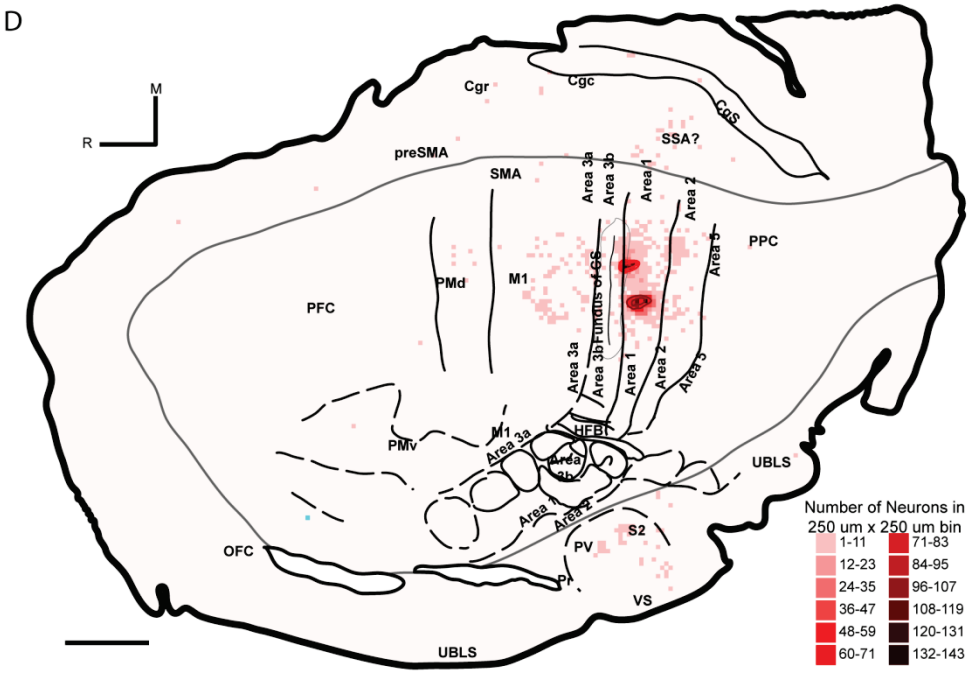

Figure 4.7, continued

Because CTB-IR neurons were unevenly distributed in each APC areas, and density analysis suggested inputs to area 1 come from matched representations in other areas, we aligned the body maps derived from our physiological mapping in areas 1 and 3b to confirm that these differences were result of topographically organized projections, and not uneven staining or weak signal under fluorescence microscopy (Fig. 4.8). Within area 1, the clusters of CTB-IR neurons appeared to be localized within the injected body part representation (Fig. 4.8C), e.g. there was a patch of CTB-IR cells centered on the digit representation that was separate from a patch that tended to be clustered in the arm representation. The CTB-G injection diffused across a wider region of area 1 and included parts of the leg, arm, and foot representations. A third, somewhat less distinct collection of CTB-IR cells was centered on the representation of the leg, but spread over a similar region of cortex. When plots of CTB-G, CTB-R and FR labeled neurons were

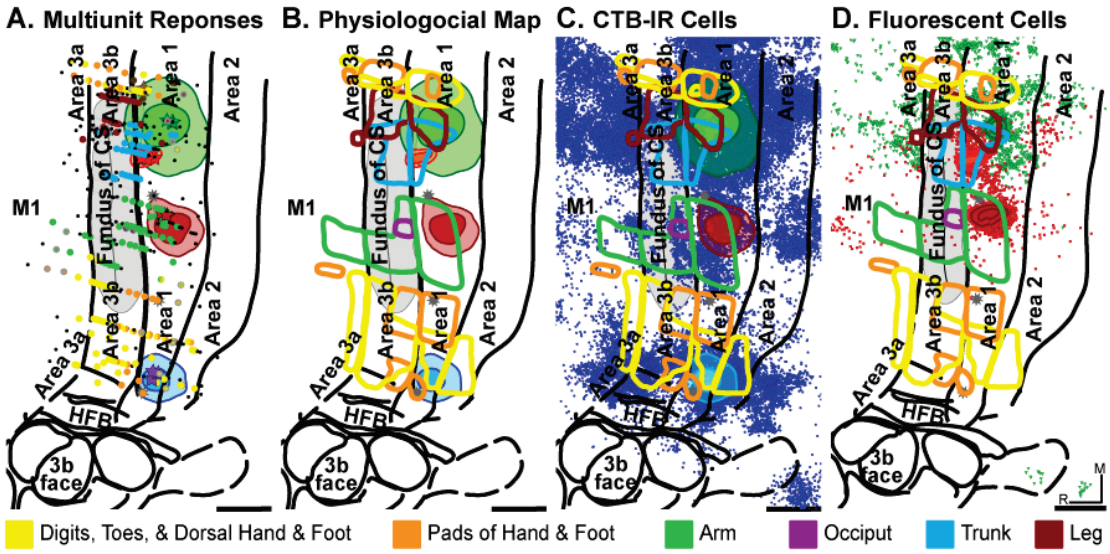

Figure 4.8. Topographic organization of projections in the anterior parietal cortex. A. The results of multiunit microelectrode mapping in anterior parietal areas. Circles are color coded to indicate the area of skin stimulated to elicit responses during mapping. Small black circles are unresponsive sites. B. Color coded physiological maps in areas 1, 3b, 3a, and 2. Sites responsive to the same body part were outlined in each area to create these color coded body maps. C. The distribution of CTB-IR cells related to the body maps in APC areas. D. The distribution of CTB-R, FR, and CTB-G filled cells related to body maps in APC areas. Black lines are the borders of areas determined from sections stained to reveal myeloarchitecture. The central sulcus is shaded in gray. Injection sites are marked with stars in according to the locations recorded during mapping sessions, and were outlined and filled in color in plotted sections. The darkest shaded region is the core of the injection. Blue- CTB, Dark red- CTB-R, Bright red- FR, Green-CTB-G. Rostral is left, and medial is up. Scale bars are 2 mm. Each injected body representation in area 1 received inputs from somatotopically matching representations and nearby representations of adjacent body parts in areas 1, 3b, 3a, and 2.

matched to the electrophysiological maps in area 1, a similar pattern of constellations of backfilled neurons centered in individual body part representations was revealed (Fig. 4.8D). CTB-R and FR filled neurons were found across the area 1 arm and trunk representations, while none were found in the region responding to stimulation on the hand and few passed beyond the trunk representation in to the representations of the more distal leg and foot. In contrast, after an injection of CTB-G into a region representing parts of the leg and trunk, labeled cells were found in representations of the leg, foot, and

trunk, but not the arm or hand. Responses to skin stimulation could be consistently evoked from area 3b, and revealed a mirrored body map in this area. Much like in area 1, patches of CTB-IR neurons found in area 3b tended to cluster in the representations of body that had been injected in area 1 (Fig. 4. 8). Matching the 3b body map to the pattern of fluorescent filled cells showed that inputs from area 3b to the parts of the body represented at the center of each of these injections tended to arise in matched body representations. No CTB-R, FR, or CTB-G cells were found in the 3b hand region, suggesting that the CTB-IR cells found in this region resulted only from the CTB injection in the area 1 hand. Area 3a was less consistently responsive to stimulation, however the few responses found here indicated another parallel, mirrored map of the body in area 3a as has been described for other primate species (Huffman & Krubitzer, 2001; L. A. Krubitzer & Kaas, 1990; Seelke et al., 2012; C. W. H. Wu & Kaas, 2003). Distinct patches of CTB-IR, FR, CTB-R, and CTB-G filled cells that roughly matched our incomplete map of the body in area 3a indicated topographically organized inputs from area 3a to area 1. Area 2 was largely unresponsive during mapping sessions, however a mirrored representation of the contralateral body has been described in this region in macaque monkeys (Pons et al., 1985a, 1985b), and the few responsive sites in area 2 in case 10-19 suggested a hand representation caudal to the hand representation in area 1 (Fig. 4. 8A). Groups of CTB-IR neurons in area 2 fell at the same mediolateral level as each of our CTB injections in case 10-19. CTB-R, FR, and CTB-G cells labeled as the result of injections in the arm, trunk, and leg regions were found in area 2, at the approximately matching mediolateral level of each injection (Fig. 4.8D). No fluorescent tracer filled neurons were found in the area 2 hand representation, while this region did

contain CTB-IR cells that were likely labeled by the CTB injection into the area 1 digits. No mapping was performed in area 5 in any of our squirrel monkeys, but partial body maps have been demonstrated here in other primate species (Pearson & Powell, 1978; Pons et al., 1985b; Seelke et al., 2012). Separate foci of CTB-IR label and the few CTB-G and FR filled neurons here suggest that projections from area 5 to area 1 may also be topographically organized. Taken together, our results show that projections from other APC somatosensory areas tended to arise in somatotopically matched representations of the body in area 3b, 3a, and 2, and possibly area 5.

Somatosensory areas of the lateral parietal cortex that lie in the upper bank of the lateral sulcus (UBLS) contained both CTB-IR and fluorescent tracer filled cells. There was extensive CTB-IR labeling in the second somatosensory area (S2), parietal ventral area (PV), and the presumptive parietal rostral (PR) and the ventral somatosensory (VS) areas. A region of slightly darker myelin staining did indicate the location of area S2 and PV; however no border between them could be determined from the fiber stained sections. Comparing CTB-IR pattern to the distribution of CTB-R, FR, and CTB-G cells (Fig. 4.6) in this region indicated the location of each area, and that the projections from at least areas PV and S2 to area 1 are topographically arranged. A dense patch of CTB-IR label was found in what we presume to be the SV and PV hand representations near the center of the myelin dense S2/ PV region. Separate foci of CTB-G filled cells resulting from the injection into area 1 leg representation helped to distinguish projections coming from PV and S2. One CTB-G focus was more caudal and ventral to the cells we presume were labeled by the CTB injection into the digit representation, and was densest along the ventrocaudal border of S2. The second focus of CTB-G filled neurons overlapped the

region of CTB-IR staining at the center of the S2/PV region, though it tended to cover only the ventral half of this density, a region that corresponds to the location of the leg and foot representations in the ventral half of PV (Coq et al., 2004). When compared to the inputs from the leg, foci of cells filled by the CTB-R and FR injections matched the location of the trunk and arm in relation to the leg in published body maps of S2 and PV (Coq et al., 2004; L. Krubitzer, Clarey, Tweedale, Elston, & Calford, 1995; Hui-Xin Qi, Lyon, & Kaas, 2002), thus we conclude that labeled neurons in the caudal half of the myelin dense region along the UBLS were in S2, while those that were more rostral were in PV. Cells filled by all injections were found in the presumptive VS, ventral to S2 and PV. Only CTB-IR neurons were found rostral to PV in the location of presumptive PR. Neither PR nor VS have been extensively explored physiologically and the fluorescent signal from our injections is weak in this region, so it remains unclear if projections from PR and VS to area 1 are organized.

Motor, premotor, and supplementary motor areas in the frontal lobe all contained neurons labeled by each of the four area 1 injections in case 10-19 (Fig. 4.6). Each of these areas has previously been demonstrated to have a mosaic somatotopic map of the body in squirrel monkeys and other primate species (Gharbawie, Stepniewska, Burish, & Kaas, 2010; Ghosh & Gattera, 1995; Gould, Cusick, Pons, & Kaas, 1986; Graziano, Taylor, & Moore, 2002; Stepniewska, Preuss, & Kaas, 1993; Stepniewska, Cerkevich, Fang, & Kaas, 2009; Wu, Bichot, & Kaas, 2000), and the distribution of labeled cells suggests organized projections from each area to area 1. Primary motor cortex (M1) contained CTB-IR, CTB-R, FR, and CTB-G filled cells. CTB-IR density analysis showed dense foci of CTB-IR cells at mediolateral levels that roughly corresponded to the level

of each CTB injection (Fig. 4.7). Neurons backfilled by the CTB-R and FR arm and trunk injections were restricted to the region of M1 that was at the same level as these representations in area 1 (Fig. 4.7). CTB-G labeled neurons were distributed in medial M1, at the same level as this injection into the leg and trunk representations (Fig. 4.6), and were often intermingled with the medial-most red labeled cells in M1. The regions containing cells labeled by our fluorescent tracers correspond to the mosaic somatotopic map of the body in M1, and indicate inputs from M1 to area 1 arise from at least roughly somatotopically matched regions. Dorsal and ventral premotor areas (PMd and PMv, respectively) both projected to area 1. The distribution of labeled cells in PMd suggests organized projections to area 1, with CTB-G, FR, and CTB-R labeled neurons overlapping CTB-IR cells in the medial half of the area, but not in the more lateral region devoted to movements of the hand. All injections labeled neurons in PMv with CTB-R, FR, and CTB-G backfilled neurons overlapping CTB-IR cells along only a small rostral portion of the CTB-IR grouping, suggesting a full body representation in PMv. The supplementary motor area (SMA) was medial to M1 and PMd and extend onto the medial wall contained labeled neurons. There was CTB-IR labeling in both regions. More restricted projections from SMA to area 1 were also indicated by the presence of fluorescent labeled cells. In SMA, the CTB-G cells did not extend as far rostral as the overall CTB-IR labeled field, indicating projections to the lower half of the body likely come from caudal SMA. The few red fluorescent cells labeled by the CTB-R and FR injections into the arm and trunk representations were mixed with the more rostral CTB-G filled neurons in the likely location of the arm and trunk regions of SMA. In the most rostral SMA, only CTB-IR cells were present indicating a projection from the SMA hand

representation to the hand region of area 1. Rostral to the defined motor areas of the frontal lobe, CTB-IR, CTB-R, FR, and DY were also found in the prefrontal cortex (PFC).

The presence of multiple foci of CTB-IR and fluorescent labeled cells along the medial wall suggest the location of motor and sensory areas that have not been explored in squirrel monkeys (Fig. 4.6-4.7). A number of CTB-IR and fluorescent labeled cells were found in presumptive pre-SMA. Deeper in the cingulate gyrus, labeled neurons from each of our injections were found in the caudal and rostral cingulate divisions (Cgc and Cgr, respectively) where cingulate motor areas have been described in other primates (Picard & Strick, 1996; Wu et al., 2000). Along the medial wall, the densest distribution of cells for all tracers was found in a region of the cingulate gyrus medial to the regions of area 1 and 3b that cross onto the upper part of the medial wall (Fig. 4.7). This appears to correspond to the location of the supplementary sensory area (SSA) that has been described in monkeys (Blomquist & Lorenzini, 1965; Murray & Coulter, 1982).

Case 11-33. Three injections of CTB and CTB conjugates, FR, and DY in case 11-33 revealed a pattern of corticocortical projections similar to that in case 10-19. CTB-IR cells were unevenly distributed throughout area 1 and clustered near each injection site in the area 1 and, occiput/ shoulder, and foot cortex (Fig. 4.9). These CTB-IR foci corresponded to regions of area 1 that contained little to no FR or DY label from injections placed between the CTB injections. Neurons labeled by the FR injection into the arm representation were in the general location of the arm representation, and the filled cells extended into the adjacent representation of the dorsal hand. For the most part, the backfilled DY cells resulting from the injection into the region representing the leg

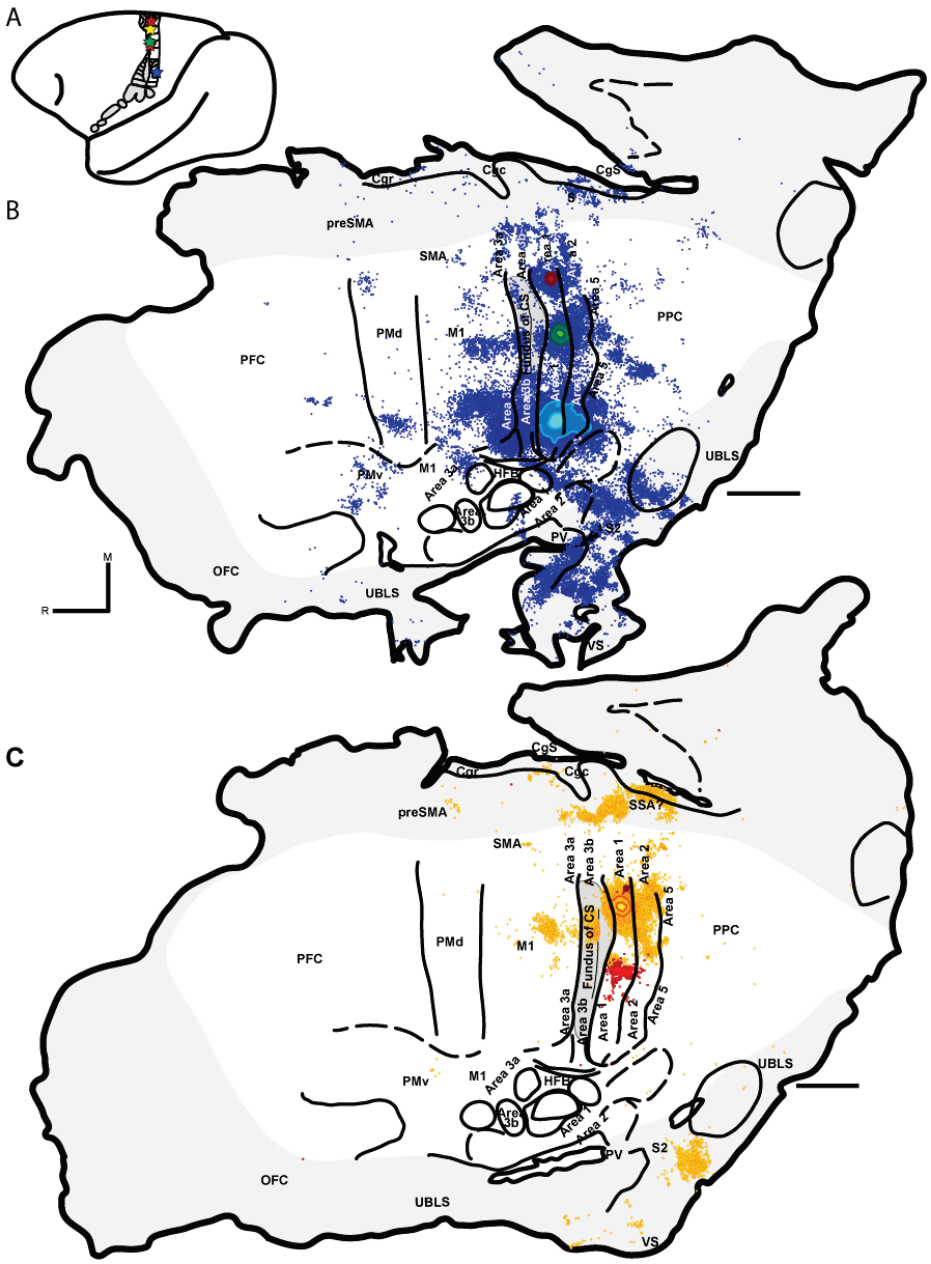

Figure 4.9. Distribution of cells labeled by CTB, FR, CTB-G, DY, and CTB-R injections into area 1 digit, arm, trunk, leg and foot representations in case 11-33. A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of DY and FR filled neurons. Conventions as in figure 4.6. Three injections of CTB across area 1 revealed strong intrinsic projections. Labeled neurons were also found in areas 3b, 3a, 2, 5, SV, PV, VP, M1, PMd, PMv, SMA, pre-SMA, PFC, and the cingulate gyrus. DY filled cells were found in the leg representations of the same areas labeled by CTB injections in this case. FR transport in this case was poor, however the intrinsic projections in area 1 and the inputs from area 2 revealed by this injection suggest that projections to this region were from matching arm representations. A few cells labeled by the CTB-R injection into the foot representation are visible in medial area 1.

were in the leg representation, but were also found in representations of the trunk, arm. There was a small focus of DY labeled cells in a region overlapping the FR cells in the occiput/ shoulder representation. Microelectrode mapping in this case was incomplete, and the DY injection may have spread into the expected representations trunk and foot. The diffusion of the injection beyond the boundaries of the leg representation may explain the expansion of DY labeled cells into seemingly mismatched body representations in area 1. Uptake of CTB-R in this case was low, and both the immunohistochemical and fluorescent signals were weak; however, a very low number of CTB-R filled neurons were found with fluorescence microscopy around the region of the CTB-R injection that corresponded to weakly stained CTB-IR cells found in the area 1 foot representation in the CTB-processed sections. Given that non-adjacent representations were not shown to project to each other in case 10-19, we feel that these foci of dense CTB-IR cells and cells labeled by FR and DY injections indicate strong intrinsic projections within each of the injected representations and from nearby representations of adjacent body parts. Areas 3b, 3a, and 2 consistently contained CTB-IR and DY filled cells that were consistently in matching representations. The pattern of dense patches of label in somatotopically matched representations indicated topographically arranged inputs from each of the other APC areas to area 1. CTB-IR and DY cells were found just caudal to the area 2 border in area 5 and in more caudal parts of PPC. The UBLS was damaged during the flattening procedure, however labeled cells were found in the S2/PV region. S2 was the most intact area in, and somatotopically organized projections were indicated when the locations of CTB-IR and DY filled cells were compared. Neurons labeled by the injection of DY into the leg representation were

concentrated caudally in S2, in the location of a break in the CTB-IR field of cells in the likely location of the S2 leg representation. The parts of PV and VS that were intact contained both CTB-IR and DY filled cells.

Labeled neurons were found in frontal motor areas and along the medial wall in case 11-33 (Fig. 4.9). M1 contained both CTB-IR and DY labeled cells. CTB-IR cells and DY collected in foci that paralleled the mediolateral level of injected area 1 representations. No DY filled cells were found in the expected location of the hand representation in motor cortex. Lateral M1 did, however, contain a high number of CTB-IR neurons, the likely result of the CTB injection into the area 1 hand representation. Thus, a rough somatotopic arrangement of projections from M1 to area 1 is again indicated. PMd contained CTB-IR cells with collections in the approximate locations of the hand and foot representations in area PMd. The few DY labeled cells in PMd were in the more medial part of PMd, and they were caudal and lateral to those CTB-IR neurons we presume were labeled by the CTB-R injection into the foot. Patches CTB-IR cells were found in PMv, as were neurons backfilled with DY. SMA and the PFC also contained CTB-IR and DY filled neurons. The CTB injections into the area 1 hand, occiput/shoulder, and foot representations and the DY injection into the area 1 leg also labeled cells along the medial wall. Backfilled neurons resulting from these injections were found in pre-SMA, Cgr, Cgc, and the presumptive SSA.

Case 12-40. Injections into area 3b/1 face, area 1 hand, and area 3b arm backfilled cells throughout the cortex. The injection of CTB caudal in area 1 into the digit representation revealed a pattern of projections similar to the area 1 hand injections in our other cases (Fig. 4.10). Only one CTB injection was placed in case 12-40, and CTB-IR

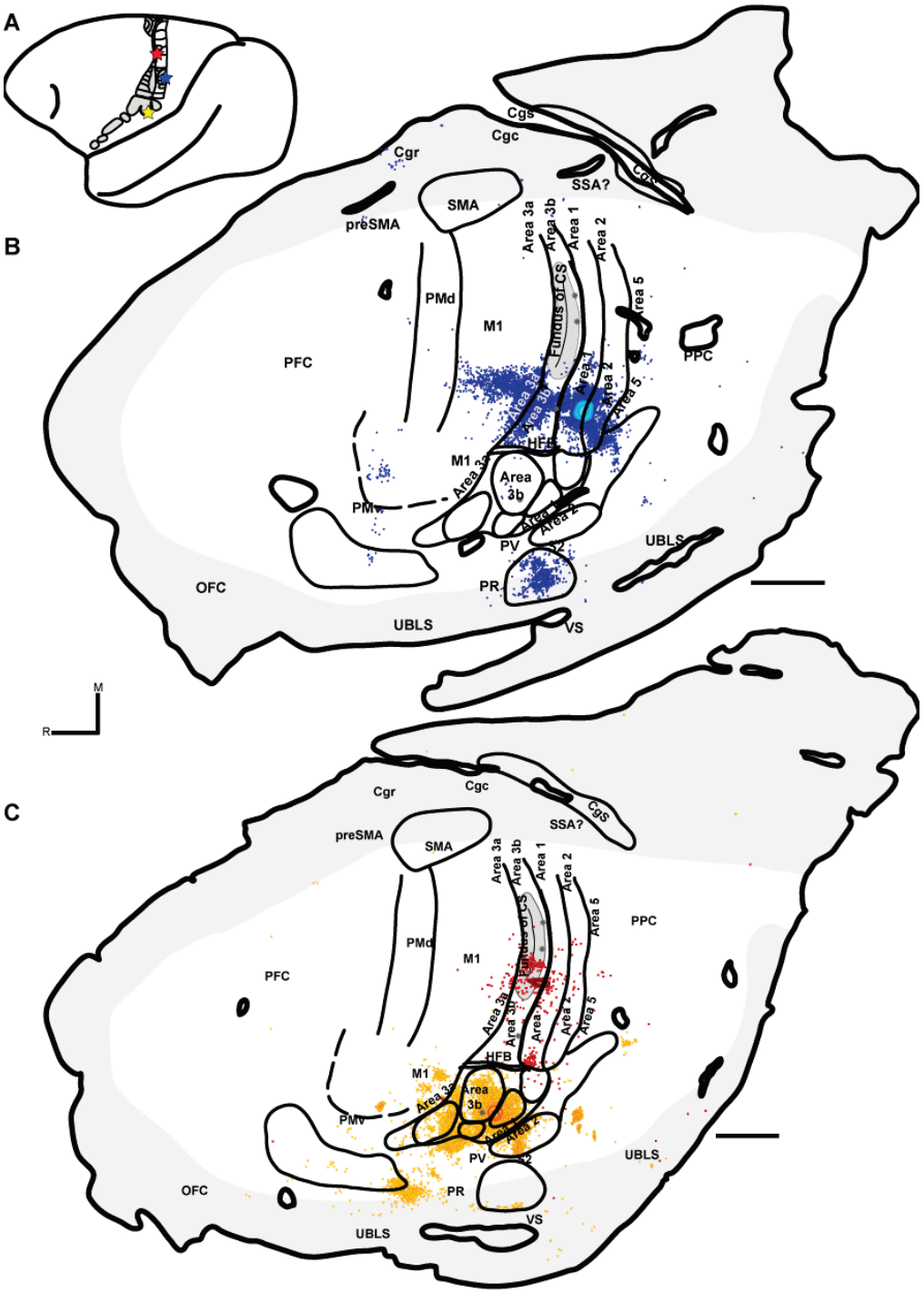

Figure 4.10. Distribution of cells labeled by CTB, DY, and FR injections into area 1 hand, area 1/3b face, and area 3b arm representations in case 12-40. A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of FR and DY filled neurons. Conventions as in figure 4.6. Neurons labeled by injections into the area 1 hand and the 3b/1 face representations of were distributed similarly in somatosensory and motor areas. The FR injection into the 3b arm resulted in distribution of labeled cells typical after injections into area 3b.

cells were found in presumptive hand representations in APC, UBLS, and motor areas. Fewer CTB-IR neurons were labeled in SMA, pre-SMA, and cingulate areas than in other cases. Since the DY injected was made on the border of areas 1 and 3b, cells filled with DY in this case may project to either the area 1 or 3b. The pattern of projections to the injected face representations in these areas was similar to that to other body parts. DY labeled neurons in areas 1 and 3b indicate intrinsic connections within both areas and connections between the two areas. Presumptive face cortex in both areas 3a and 2 also contained DY backfilled cells. Laterally, cells labeled with DY were in presumptive face regions of PV, S2, and VS, as well in PR. Lateral M1 and PMv projected to the 3b/1 face representations. Very few DY cells labeled by the injection into the 3b/1 face representation were found in PMd. A low number of neurons filled by the DY injection were scattered in PFC, SMA, and the cingulate gyrus. The FR injection into the representations of the forearm and dorsal hand in area 3b resulted in cells distributed in a pattern that was unlike our area 1 injections. FR labeled neurons were found in areas 3b, 1, 2, 5, and M1, with a few cells scatted in the UBLS and frontal cortex. A concentration of backfilled FR neurons in was found in lateral area 1, just medial to hand face border. Area 1 was not mapped this far lateral, but this region likely contains responses to D1 and the radial half of the dorsal hand.

Discussion

Overall, our injections of tracers revealed a pattern of thalamocortical and corticocortical projections to area 1 that is largely similar to that seen in other primate

species, particularly the larger-brained Old World macaque. Multiple thalamic nuclei projected to area 1 in squirrel monkeys. Because we made multiple injections across the body map in area 1, we were able to determine that the projections the ventroposterior nucleus (lateral and medial subdivisions, VPL/ VPM), the ventroposterior inferior nucleus (VPI), the ventroposterior superior nucleus (VPS), and the anterior pulvinar nucleus (Pa) were topographically arranged. Labeled cells in the cortex indicated that area 1 contained strong intrinsic connections that are generally restricted to the injected representation and the nearby representations of adjacent body parts. Area 1 received inputs from adjacent parts of area 3b, mostly from matched body part representations. Weaker, but still organized, projections from area 3a were also present. The cortex caudal to area 1, including presumptive area 2 and parts of area 5 and the rest of the posterior parietal cortex, also provided organized inputs to area 1. Somatosensory areas in the lateral sulcus, including the second somatosensory (S2), parietal ventral (PV), parietal rostral (PR), and ventral somatosensory (VS) areas sent topographically organized projections to area 1 as well. Primary motor (M1), dorsal and ventral premotor (PMd and PMv), supplementary motor (SMA), pre-SMA, and motor and sensory areas in cingulate cortex also projected area 1, often in an organized fashion.

Multiple nuclei in the thalamus send organized projections to area 1

Several nuclei in the thalamus projected to area 1 in squirrel monkeys (Fig. 4.11). The main thalamic input to area 1 arose in somatotopically matched regions of VPL/VPM. While VP relays cutaneous information to area 1, these inputs alone are not

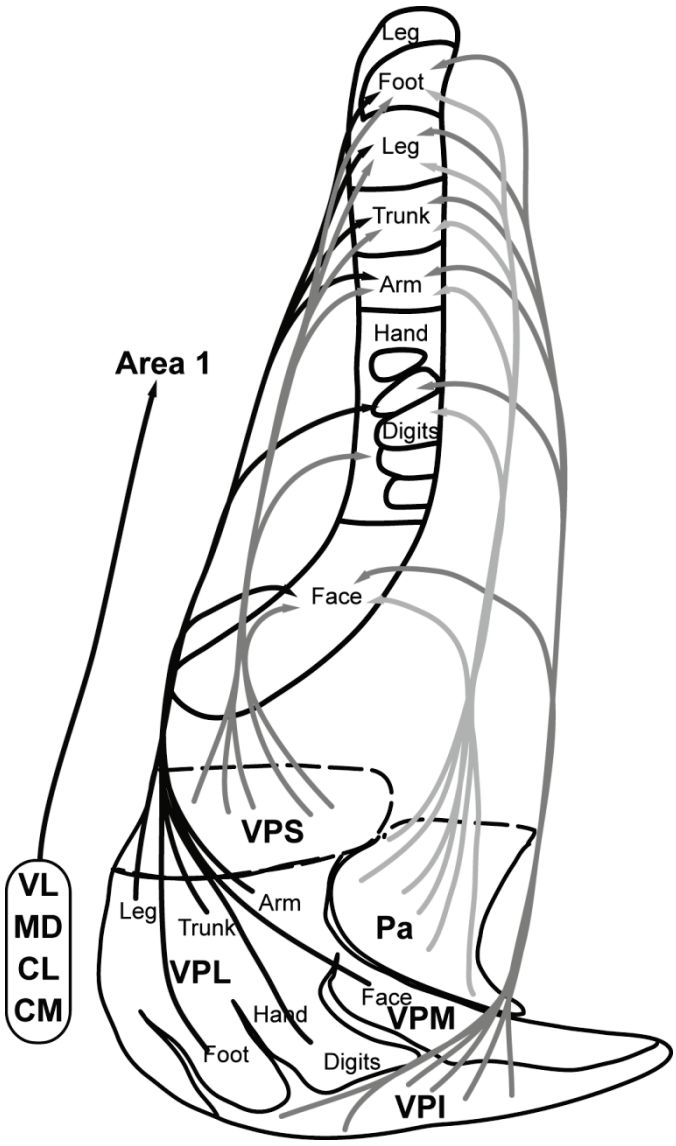

Figure 4.11. Summary of thalamocortical projections to area 1. Area 1 receives inputs from multiple thalamic nuclei. The darkest arrows are projections from VPL/VPM that come from well mapped, matched body representations of VPL/VPM. Physiological mapping in VPI and VPS has indicated maps of the body that roughly parallel that in VP. The more roughly organized projections from VPS and VPS are indicated in gray. The lightest gray arrows indicate the projection from Pa appear to be roughly topographically organized, but have not been confirmed by physiological mapping. Weaker projections from VL, MD, CL, and CM were also indicated.

enough drive area 1as an intact area 3b is required for area 1 to function properly

(Garraghty et al., 1990; Randolph & Semmes, 1974). VPI, the thalamic nucleus that has

been shown to receive spinothalamic and spinal trigeminal inputs (Kaas, 2008), also sends at least loosely organized projections to area 1. Organized projections to area 1 also arise in VPS, which contains another map of the body's deep receptors in joints and muscles that is roughly parallel to that seen in VPL/VPM (Kaas et al., 1984). Little work has been done to study the function of Pa, however, it is known to receive inputs from multiple cortical somatosensory areas (Cusick & Gould, 1990; Pons et al., 1985b). Our injections of distinguishable tracers into area 1 face, hand, arm, trunk, and leg representations, suggested that not only does Pa project to area 1, but that these projections are organized along a roughly rostral-caudal progression. A similar gradient from projections to area 3b, with the face inputs arising rostrally in Pa and those to lower body representations caudally, has also been demonstrated in squirrel monkeys (Cusick & Gould, 1990). It has been hypothesized that Pa coordinates activity across cortical somatosensory areas (Sherman & Guillery, 2006), and our results suggest some degree of somatotopic organization to this process. Weaker projections from the ventral lateral nucleus (VL) and the central lateral (CL) and centre médian (CM) intralaminar nuclei were also indicated, but it is unclear if these projections to area 1 are organized somatotopically. In total, this pattern is similar to macaque monkeys (Nelson & Kaas, 1981; Padberg et al., 2009; Pons & Kaas, 1985) with a distinct set of projections to area 1.

Projections from other cortical somatosensory areas to area 1 tend to arise in matched representations

Projections revealed by our injection into area 1 suggested organization within the anterior parietal cortex indicative of four somatotopically organized areas (Fig. 4.12).

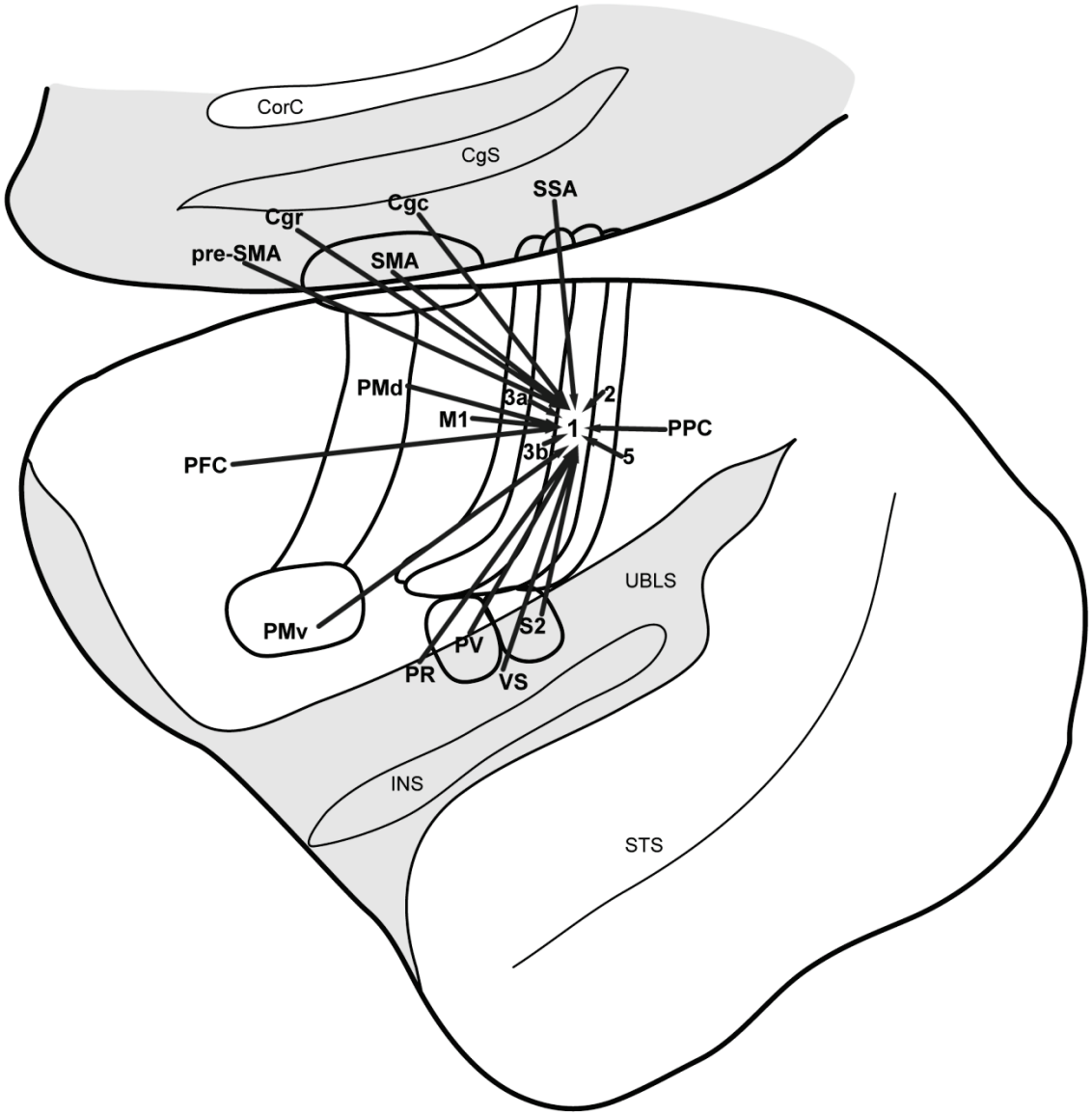

Figure 4.12. Summary of corticocortical projections to area 1. Area 1 receives inputs from cortical somatosensory areas in the anterior parietal cortex and along the upper bank of the lateral sulcus, from multiple motor and premotor areas, and from the location of presumptive motor and sensory fields on the medial wall.

Intrinsic connections in area 1 tended to arise from within individual body part representations, with some input to a given representation coming from nearby representations of adjacent regions of the skin. Non-adjacent representations of non-adjacent body parts did not appear to have strong connections with each other within area

1. The primary somatosensory area, 3b, contained some of the densest projections to area 1, and these appeared to come largely from matched body part representations. Similarly, when small amounts of anterograde anatomical tracers were injected into the digit representations in area 3b, terminal label was largely confined to the matching finger representation in area 1 (Négyessy et al., 2013). Rostral to area 3b, proprioceptive area 3a also provided organized inputs to area 1. In the disputed cortex caudal to area 1, distinct foci of retrogradely labeled cells were found in the predicted locations of the injected body part representations in mirrored body map in area 2. A map of the body that mirrors that in area 1 has been described in area 2 in macaque monkeys (Pons et al., 1985a, 1985b), and our results suggest a similar arrangement in squirrel monkeys. Projections of APC areas to area 1 in macaques (Burton & Fabri, 1995) were similar to those described here for squirrel monkeys. Given the pattern of projections revealed by our injections and the similar physiological organization and APC connections in squirrel monkeys and macaques, we conclude that squirrel monkeys, and likely all New World monkeys, do have an area 2. This is in contrast to APC organization scheme proposed by Padberg and associates (2005; 2007), where the cutaneously responsive area 1 is immediately abutted by the sensorimotor area 5 of the posterior parietal cortex.

Somatosensory areas along the upper bank of the lateral sulcus (UBLS) are difficult to access for microelectrode mapping, and contain cells with large receptive fields that make interpreting the somatotopic organization in these areas difficult. Body maps in PV and S2 have been described in macaque (Krubitzer et al., 1995), marmoset (Qi et al., 2002), and titi monkeys (Coq et al., 2004). The locations in PV and S2 of cells backfilled by our injections across area 1 suggested body maps in PV and S2 that are

similar to previously described in other monkey species. We found cells labeled by our area 1 injections in the presumptive location of both PR and VS; however these areas have not been extensively mapped, and the projections from PR and VS to area 1 we revealed were too weak to determine if they were topographically organized.

Mosaic body maps in motor areas provide organized inputs to area 1

We found projections to area 1 from M1, PMd, PMv, and SMA (Fig. 4.12). Somatotopically organized, mosaic maps of the body have been described in each of these areas in several primate species (Gharbawie et al., 2011; Gould et al., 1986; Picard & Strick, 1996; Wu et al., 2000). M1, PMd, PMv, and SMA all contained cells labeled by each of our injections across the body map in area 1. Cells projecting to different body part representations in area 1 were in the expected location of the matching representation in each of the motor, premotor, and supplementary motor areas. Our results also suggested a full body map in PMv. A large representation of orofacial and forelimb movements has been described in PMv, with variable reports about the existence of other body part representations (see C. Wu et al., 2000 for comparison among primates). Cells in PMv were generally labeled by each our injections into area 1, including those in the trunk and leg representations, suggesting a representation of the lower half of the body in PMv. Organized inputs from multiple motor areas to area 1 may provide area 1 with information about the planning and execution of movements during tactile discrimination tasks.

The locations of motor and sensory areas along the medial wall are suggested by projections to area 1

Distinct foci of cells retrogradely labeled by our injections into area 1 were found in multiple locations along the medial wall in our squirrel monkeys (Fig. 4.12). The most rostral focus of cells is in the location of the pre-SMA. No clear organization of inputs from pre-SMA was suggested by the pattern of projections to area 1. The pre-SMA, lacking responses to somatosensory stimuli and any clear somatotopic organization, has been implicated in motor preparation (Picard & Strick, 1996). Our injections of tracers into area 1 resulted in two collections of labeled neurons in the cingulate cortex. These locations may correspond to the rostral cingulate motor area and one or more of the subdivisions of the caudal cingulate motor area that have been described in macaques (Picard & Strick, 1996). This part of the cingulate has not been extensively explored in the squirrel monkey, thus we describe cells projecting to area 1 as simply in the rostral and caudal cingulate divisions (Cgr and Cgc) until further evidence in squirrel monkeys confirms homology with the cingulate motor areas of other species. Finally, we found cells labeled by our area 1 injections in a region medial to areas 3b and 1. Responses to somatosensory stimulation have described in this location in squirrel monkeys (Blomquist & Lorenzini, 1965), and it has been named the supplementary somatosensory area (SSA) (Blomquist & Lorenzini, 1965; Murray & Coulter, 1982). We have tentatively followed this naming scheme in our squirrel monkeys, and our connections to area 1 may also match the rostral and caudal locations of the upper and lower body representations that have been described in the SSA.

Conclusions

Taken together, the pattern of projections from thalamic nuclei and other somatosensory cortical areas to area 1 in squirrel monkeys suggests a pattern of anterior parietal organization similar to macaque monkeys, including from the disputed area 2, suggesting a network common across anthropoid primates. Many of these projections are topographically organized. Additional organized inputs to area 1 arose in frontal lobe motor areas. Projections labeled by retrograde tracer injections into area 1 indicated the locations of multiple areas on the medial wall. Little is known about these areas in squirrel monkeys, and further study is required to understand how they relate to cingulate motor and sensory areas described in other primate species.

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CHAPTER V

DISCUSSION

The brain's network for somatosensory processing in primates is greatly expanded compared to other mammals, consisting of multiple thalamic nuclei and cortical areas. In an effort to better understand the changes that arose during this expansion, we studied primate somatosensory organization by looking at the connections that underlie the processing of tactile information. We placed injections of neuroanatomical tracers into different body part representations in primary and non-primary somatosensory cortical areas to reveal the network of projections to cutaneously responsive areas in the brains of Old World and New World monkeys. Thus, we were able to reveal aspects of the organization of the somatosensory network in both Old World and New World monkeys. Our results provide support for a model of anterior parietal cortex (APC) organization where phylogenetic relationships explain differences in APC organization among non-primate mammals, prosimian primates, and anthropoid primates, rather than a model built on hand-use that adds a fourth category of APC organization that is unique to certain New World monkey species. Variations within the basic APC pattern that are seen in species in each of the non-primate, prosimian, and simian categories of APC organization reflect specializations related to structure and use of the peripheral body.

The role of the oral cavity representation in the APC

Our first experimental aim was to determine the thalamocortical and corticocortical projections to representations of the oral cavity and face in the primary somatosensory cortex (area 3b) of Old World macaque monkeys, an APC region that has not been fully described and is rarely discussed in any model of APC organization. Tracer injections into representations of different oral cavity structures in macaque area 3b resulted in labeled neurons in several thalamic nuclei (Chapter II). Labeled cells were consistently found in the somatosensory ventroposterior medial sub-nucleus (VPM), the primary nucleus for the relay of cutaneous information from the face and mouth to area 3b. Populations of cells projecting to the face, teeth, or tongue representations in cortex were from matching representations in VPM. No labeled neurons were found in the presumptive thalamic taste nucleus, the ventroposterior medial parvicellular nucleus (VPMpc). Much like the 3b hand representation (Padberg et al., 2009), the mouth region in macaque 3b also received inputs from the ventroposterior inferior (VPI), ventroposterior (VPS), anterior pulvinar (Pa), ventral lateral (VL), medial dorsal (MD), centre médian (CM), and central lateral (CL) nuclei of the thalamus. Thalamic projections revealed by our injections into macaque area 3b oral cavity representations revealed similar connectivity as the rest of the body's representation in macaque primary somatosensory cortex. A difference between Old World macaques and New World monkeys was observed, in that the thalamic taste nucleus, VPMpc, does provide input to area 3b in New World species (Iyengar, Qi, Jain, & Kaas, 2007), but not in macaques. Since the majority of thalamic input to the primary representation of the oral cavity

comes from a similar compliment of somatosensory related nuclei in all anthropoid primate species that have been tested, we conclude that the thalamic inputs implicate a role for the oral cavity representation in the primary somatosensory cortex that is largely similar in all anthropoid primates.

Our injections into the representations of the oral cavity and face in macaque area 3b revealed both intrinsic connections within the area 3b mouth and face representations and inputs from presumptive oral cavity representations in other cortical areas (Chapter III). Representations of the face and different parts of the mouth in area 3b have strong intrinsic connections within the 3b oral cavity and face representations. The mouth and face representations of area 3b received projections from other somatosensory areas in the APC and the lateral parietal cortex (LPC) including areas 3a, 1, 2, the second somatosensory area (S2), the parietal ventral area (PV), and cortex that may include the oral cavity representations in the parietal rostral (PR) and ventral somatosensory (VS) areas, as well as the presumptive oral cavity representation of posterior parietal area 5. There were also incoming projections from the primary motor (M1) and ventral premotor (PMv) areas. This pattern of projections is similar to the pattern revealed by tracer injections in regions of 3b representing other body parts. Unlike other parts of 3b, the tongue representation also received inputs from areas in the anterior upper bank of the lateral sulcus and anterior insula that may include gustatory cortex (area G) and other cortical regions involved in processing taste. Thus, we conclude that the position of the area 3b oral cavity representation in macaque monkeys and other anthropoid primates to the overall function of the APC is similar to that of any other body part. However, the 3b

representation of the tongue appears to have specialized interactions with cortical regions related to taste processing that is not seen for other regions of the anthropoid APC.

The characteristics of area 1 projections in relation to APC models

The goal of our second experimental aim was to reveal the projections from the thalamus and the rest of cortex to APC area 1 in New World squirrel monkeys (Chapter IV). We also compared our results to those known for more dexterous, Old World macaque monkeys that Padberg and colleagues (2005; 2007) predicted to have an APC area, area 2, that squirrel monkeys lack. In the APC organization model based on phylogenetic relationships, squirrel and macaque monkeys have similar APC organization, as they are both anthropoid primates. In contrast, the hand-use modified APC organization model of Padberg and associates (2005; 2007) suggests that squirrel monkeys, with no ability to form a precision grip, should fall into a separate New World monkey category whose members, unlike monkeys with an opposable thumb (e.g. macaques), lack an APC area 2 that provides for additional processing of proprioceptive information. The results of our retrograde tracer injection into squirrel monkey area 1 revealed that multiple thalamic nuclei, including: VP, VPI, VPS, Pa, VL, MD, CL, and CM projected to area 1 in squirrel monkeys, often in a topographically organized manner. This pattern was similar to the results of tracer injections into area 1 in macaque monkeys with an APC that includes areas 3b, 3a, 1, and 2 (Padberg et al., 2009). In the cortex, cells labeled by injections into area 1 indicated that area 1 contained strong intrinsic connections. For the most part, these intrinsic connections were restricted to the injected

representation and immediately adjacent body part representations. Area 1 received inputs from other APC areas 3b, 3a, and 2 that arose mostly in body part representations that matched those that were injected in area 1. APC area 2 and posterior parietal area 5 were distinguished from each other by separate projections from each area to area 1. LPC somatosensory areas along the upper bank of the lateral sulcus, including S2, PV, and presumptive PR and VS, sent topographically organized projections to area 1 as well. Motor areas of the frontal lobe and cortical areas along the medial wall also projected to area 1. Taken together, the pattern of projections from other somatosensory cortical areas and thalamic nuclei to area 1 in squirrel monkeys suggests that they have an APC organization more similar to macaque monkeys than non-primate mammals and prosimian primates with a single caudal somatosensory field. Thus, we conclude that there is a common plan of APC organization in all anthropoid primates, supporting the phylogeny-based model of APC organization rather than the hand use-based model where most New World monkeys are separated from other anthropoid primates (Fig. 5.1A).

Other evidence supporting the phylogeny-based model of APC organization

The areas of the anthropoid APC (3b, 3a, 1, and 2) were defined on the basis of structure (Broadmann, 1909) before separate maps of receptors in the contralateral body confined to each area were defined with electrophysiological mapping (Kaas, Nelson, Sur, Lin, & Merzenich, 1979; Merzenich, Kaas, Sur, & Lin, 1978). Unique patterns of connections in each of these areas can also be used to help distinguish their boundaries (Jones, Coulter, & Hendry, 1978; Pons & Kaas, 1985, 1986). The primary somatosensory

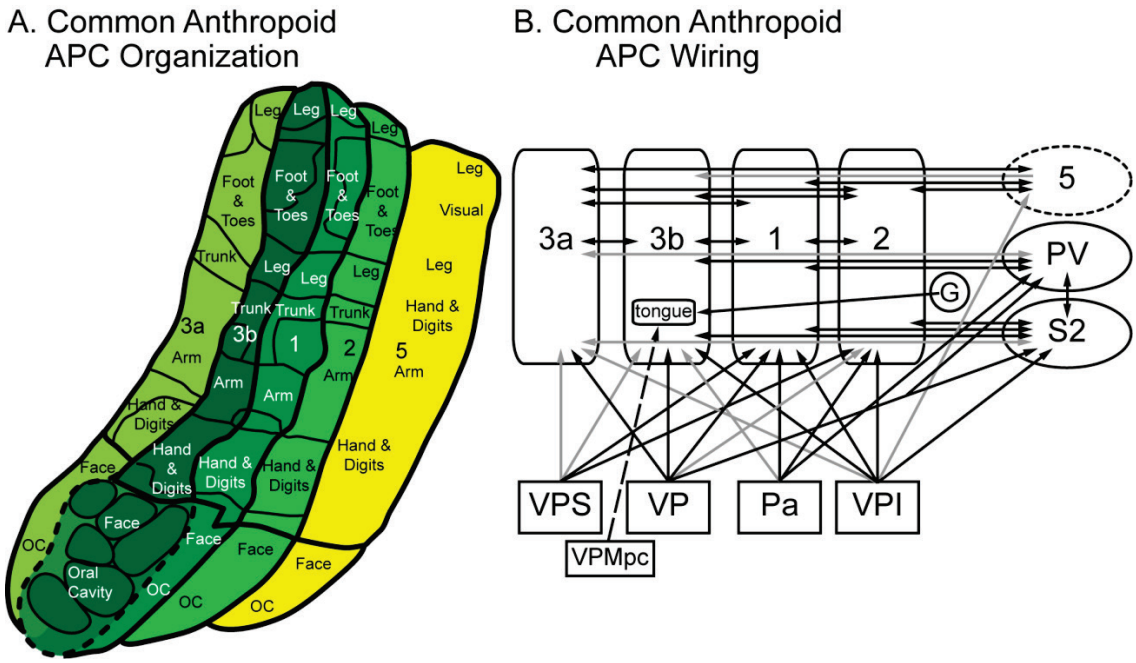

Figure 5.1. A summary of the common anthropoid primate ACP organization. A. The proposed of the arrangement of the four ACP areas common to all anthropoid primates. Face and oral cavity representations of areas 1 and 3a may infiltrate between anatomical modules for different face representations in area 3b, thus we have indicated the 3b border in oral cavity region with a dashed line. B. A schematic of thalamic and cortical connections within the somatosensory system. Multiple nuclei in the thalamus (squares) provide input to each of the APC somatosensory areas (rounded rectangles). The APC areas are highly connected and interdependent. The APC areas are connected to the PPC area 5 (dotted circle) and provide driving inputs to multiple areas in the LPC (ovals) including S2 and PV. Weaker connections are shown in gray. The 3b representation of the tongue receive a unique set of inputs from the thalamic taste nucleus, VPMpc, in New World monkeys and gustatory area G in both New World and Old World species.

area (S1 or area 3b) is similar in all mammals (Qi, Preuss, & Kaas, 2008). S1 (3b) is generally characterized by dense packing of cells in layer IV, and high myelin density and expression of the mitochondrial enzyme cytochrome oxidase (CO).

Anatomical modules representing specific body parts are often apparent in surface views of S1 (3b) stained for myelin or CO, for example the barrel field of rats (Jain, Diener, Coq, & Kaas, 2003) and face ovals of primates (Jain, Qi, Catania, & Kaas, 2001). In both non-primate mammals and primates, S1 (3b) receives strong inputs from the thalamic VP

nucleus, and contains an organized representation of cutaneous receptors of the contralateral body. S1 of non-primate mammals and prosimian primates distributes cutaneous information to both rostral and caudal somatosensory areas in the APC, specifically SR (the likely homolog of primate area 3a) and SC. Information flow through area 3b is similar in anthropoid primates, however, 3b appears to provide driving inputs not only to other APC areas (Garraghty, Florence, & Kaas, 1990), but also to the second somatosensory area (S2) in the LPC (Pons, Garraghty, & Mishkin, 1992). In non-primates and prosimians, S2 receives its own set of projections from VP and functions largely independently of S1 (Garraghty, Florence, Tenhula, & Kaas, 1991). Our results showed that the representation of the oral cavity in area 3b of macaques was architectonically much like that described in New World monkeys (Jain et al., 2001), and had a similar pattern of strong inputs from the division of VP devoted to processing information from the face and mouth, VPM (Iyengar et al., 2007).

The rostral somatosensory field (SR or area 3a), is also largely similar across mammalian species (Qi et al., 2008). It is architectonically less distinct than S1, with a less prominent layer IV and increasing number of large pyramidal cells in layer V. It receives inputs from regions of the thalamus devoted to processing information from receptors deep in the muscles and joints, as well as from S1 (Jones & Porter, 1980). Though we did not focus on area 3a specifically, the projections to the macaque 3b face representation and squirrel monkey area 1 that our injections revealed were similar to those described for other body parts and primate species (Burton & Fabri, 1995; Coq, Qi, Collins, & Kaas, 2004; Wu & Kaas, 2003), suggesting a similar role for area 3a as a

proprioceptive area in the APC in all anthropoid species that is largely homologous to that of SR in non-primate mammals.

The cortex caudal to the S1, SC and areas 1 and 2, is more variable across mammalian species. In non-primate mammals, this region is architectonically indistinct and usually physiologically unresponsive. Little input has been demonstrated from the thalamus to SC in non-primate mammals, and SC appears to receive most of its inputs from S1 (Kaas, 2004). Similar indistinct architecture and S1 inputs have been demonstrated in the region of cortex caudal to 3b in prosimian galagos. Unlike non-primate mammals, the caudal region of somatosensory cortex in galagos does receive some thalamic input leading it to be alternately identified as area 1 (Kaas, 2004), area 1+2 (Wu & Kaas, 2003), and area 2+5 (Preuss & Goldman-Rakic, 1991). In monkeys, area 1 is characterized by less densely packed cells and lower expression of calcium binding protein parvalbumin, especially in layer III, when compared to area 3b (Qi et al., 2008). Area 1 has been demonstrated to contain a complete map of cutaneous responses to stimulation of the contralateral body in both New World and Old World monkey species (Merzenich et al., 1978; Nelson, Sur, Felleman, & Kaas, 1980; Sur, Nelson, & Kaas, 1982). Notably, the cortex in the region of area 1 was not responsive in anesthetized tamarin or marmoset monkeys (Carlson, Huerta, Cusick, & Kaas, 1986; Krubitzer & Kaas, 1990). Area 2 is often difficult to distinguish architectonically, having similar characteristics to both area 1 and, particularly, area 5 in many monkey species (Qi et al., 2008). Physiologically, area 2 has been shown to contain a map of the contralateral body's deep receptors, though with some regions of cutaneous response, in larger macaque and cebus monkeys (Felleman, Nelson, Sur, & Kaas, 1983; Pons, Garraghty,

Cusick, & Kaas, 1985; Seelke et al., 2012), but cortex in the predicted location of area 2 is usually unresponsive in smaller anesthetized New World monkeys. Area 2 is often best distinguished from surrounding cortex by looking at its pattern of inputs, which come largely from the thalamic VPS and cortical area 1 (Padberg et al., 2009; Pons & Kaas, 1985, 1986)

The architectonic indistinctiveness and lack of electrophysiological responsiveness in the cortex caudal to cutaneously responsive APC areas in small New World monkeys who do not have an opposable thumb led Padberg and associates (2005; 2007) to conclude that non-cebus New World monkeys have a unique organization of areas in the APC. Clawed primates (prosimian galagos and marmosets and tamarin monkeys) were described as having an APC organized similarly to non-primate mammals where the primary somatosensory cortex was caudally abutted by PPC sensorimotor area 5. Most other New World monkeys, including owl, squirrel, and titi monkeys, were placed in a non-skilled hand use group that had an area 1, but like clawed primates, the cutaneously responsive cortex was bordered by sensorimotor area 5. Monkeys with the ability to form a precision grip, cebus and macaque monkeys, were placed in a separate skilled hand use group where both areas 1 and 2 are present in the caudal half of the APC. While this region is quite variable in anthropoid primates, accumulating anatomical and physiological evidence supports the existence of two areas, 1 and 2, caudal to area 3b in both New World and Old World monkeys. Maps of the full body, not just the hand, have been found in area 2 (Felleman et al., 1983; Pons et al., 1985), suggesting that the proprioceptive processing that occurs in area 2 is not limited to the hand. Furthermore, in cases where anesthesia was light, physiological responses could be evoked from the

predicted region of the hand in normal squirrel monkeys (Chapter IV), and reorganized representations of the hand and arm were indicated in areas 1 and 2/5 of marmosets with long-term spinal cord lesions (Bowes, Burish, Cerkevich, & Kaas, In Prep), suggesting that the lack of a proven body representation in area 2 in most New World monkeys may be related more to anesthetic plane than differences in APC organization. The projections the presumptive oral cavity regions of areas 1 and 2 to the oral cavity representation in area 3b in macaque monkeys revealed by our tracer injections provided more evidence for a complete map of the body, including the mouth, in area 2 (Chapter III). Furthermore, the distinct inputs from areas 2 and 5 in squirrel monkeys that we reveled in the experiments discussed in chapter IV further supported the phylogeny-based model of APC where areas 1 and 2 occur in all anthropoid primates. Finally, an APC that consists of four distinct areas has been suggested for apes and humans as well (Qi et al., 2008). Putting the evidence from studies of physiology, anatomy, and connections of the caudal half of the APC, we conclude that there are four cortical areas (3a, 3b, 1, and 2) in all anthropoid primates.

Functional consequences of APC organization

Different patterns of organization in the APC tend to reflect different levels of serial and parallel processing in the network for the analysis of somatosensory information. In general, the inputs of the thalamus to the APC are restricted to S1 in non-primate mammals. As primates emerged, and the organization of the APC began to change in this line, new thalamic inputs to SC began to appear, in part contributing to the

identification of the cortex immediately caudal to 3b in prosimian galagos as area 1+2 or 2+5 (Kaas, 2004). With the emergence of four distinct APC fields of anthropoid primates, there was an increase in parallel thalamic input to area 3b, 3a, 1 and 2 (Fig 5.1B). However, the basic serial processing of information within the APC was maintained, particularly for cutaneous information (Garraghty, Florence, et al., 1990; Randolph & Semmes, 1974), suggesting that the expanded parallel projections from the thalamus have a mostly modulatory influence on the APC. These changing patterns of connections also influenced the relationships of somatosensory areas in the APC and LPC. For the most part, non-primate mammals and prosimian primates have been demonstrated to have APC primary (S1) and LPC second somatosensory (S2) areas that can function independently (Garraghty et al., 1991). This parallel function may be based in parallel thalamic input from VP to both S1 and S2. However, in anthropoid primates, S2 does not receive strong inputs from VP (Krubitzer & Kaas, 1990), and normal function in S2 relies on intact inputs from all four of the areas of the APC (Garraghty, Pons, & Kaas, 1990; Pons, Garraghty, Friedman, & Mishkin, 1987; Pons et al., 1992). A shift from faster, more redundant parallel processing of somatosensory information in non-primate mammals to serial processing in anthropoid primate suggests that primate somatosensory cortex evolved toward increasingly complex analysis of somatosensory stimuli.

Conclusion

The organization of the somatosensory system in extant mammals is informed by the organization of the somatosensory network in the mammalian common ancestor.

Phylogenetic relationships among non-primate mammals, prosimian primates, and anthropoid primates explain basic differences in overall APC organization. Differences in organization of the APC among mammalian species reflects wiring, and therefore functional, differences in the somatosensory network that appear to have developed since the divergence of anthropoids from prosimian primates and other mammalian species. Variation within the APC that does occur is best reflected in specializations within areas, for example the region for the tongue, rather than the differences in overall APC organization that the hand use-based model of APC organization predicts.

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