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Connectivity of Somatosensory Cortical Area 1 Forms an Anatomical Substrate for the Emergence of Multifinger Receptive Fields and Complex Feature Selectivity in the Squirrel Monkey (*Saimiri sciureus*)

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Abstract

Converging evidence shows that interaction of digit-specific input, which is required to form global tactile percepts, begins as early as area 3b in the primary somatosensory cortex with the involvement of intrinsic lateral connections. How tactile processing is further elaborated in area 1, the next stage of the somatosensory cortical hierarchy, is less understood. This question was investigated by studying the tangential distribution of intrinsic and interareal connections of finger representations of area 1. Retrogradely labeled cell densities and anterogradely labeled fibers and terminal patches were plotted and quantified with respect to the hand representation by combining tract tracing with electrophysiological mapping and intrinsic signal optical imaging in somatosensory areas. Intrinsic connections of distal finger pad representations of area 1 spanned the representation of multiple digits indicating strong cross-digit connectivity. Area 1 distal finger pad regions also established high-density connections with homotopic regions of areas 3b and 2.

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Although similar to area 3b, connections of area 1 distributed more widely and covered a larger somatotopic representation including more proximal parts of the finger representations. The lateral connectivity pattern of area 1 is a suitable anatomical substrate of the emergence of multifinger receptive fields, complex feature selectivity, and invariant stimulus properties of the neurons.

Indexing Terms

cortical hierarchy; optical imaging; touch; tract tracing; primate

Somatosensory cortical areas 3b and 1 represent the initial stages of tactile information processing in primates (Sur et al., 1980; Iwamura, 1998). Both areas are characterized by an orderly, somatotopic representation of the body surface with a disproportionately enlarged hand representation, indicating the importance of this region in tactile perception. Despite this common basic somatotopy, areas 3b and 1 exhibit distinct structural and functional organizational features. In contrast to the small receptive fields of area 3b neurons, which are confined to single digit tips, the classical receptive fields of area 1 neurons typically span more than one digit (Mountcastle and Powell, 1959; Hyvarinen and Poranen, 1978; Costanzo and Gardner, 1980; Sur et al., 1980, 1982, 1985; Iwamura et al., 1983a, 1993; Sripati et al., 2006; Hsiao, 2008). Compared with area 3b, the larger size also represents evolving complexities of the receptive field properties, as indicated by the greater intra- and interdigit integration, larger motion and direction selectivity, and invariant response properties (Iwamura et al., 1983b; Sinclair and Burton, 1991; Iwamura, 1998; Sripati et al., 2006; Bensmaia et al., 2008; Pei et al., 2010). Lesion of area 3b silences area 1 to peripheral stimulation; conversely, area 3b remains responsive for cutaneous stimulation following area 1 lesions (Garrahy et al., 1990). Anatomical studies show that, compared with area 3b, thalamic input to area 1 is weaker and the majority of input to area 1 originates in area 3b (Jones et al., 1978; Vogt and Pandya, 1978; Shanks et al., 1985). All these findings suggest that response properties of area 1 neurons are mostly determined by converging input from area 3b.

The anatomical circuitry underlying the emergent response characteristics of area 1 is unknown. Some possible sources include convergent inputs from either the thalamus (Padberg et al., 2009) or within area 3b (Chen et al., 2003; Reed et al., 2010, 2012; Thakur et al., 2012), and cross-digit interactions within area 1 (Friedman et al., 2008). However, studies of local circuitry within somatosensory cortex are few (Juliano et al., 1990; Burton and Fabri, 1995). Using anatomical tracers, Burton and Fabri (1995) reported intrinsic connectivity between neighboring finger representations in area 1 in the macaque monkey. However, they used relatively large injections, which may not have been confined to single-digit representations. Moreover, the distribution of transported tracer label relative to digit representations was not mapped functionally. Anterograde and retrograde labeling distributions were constructed based on computational unfolding of the cross sections; although the work was carefully conducted, distortions introduced by such unfolding procedures are possible, which could lead to significant interpretation errors given interdigit representation distances of 1 mm or less. Because of the paucity of data, additional studies of local intrinsic connections in area 1 are needed.

The goal of the present study was to explore the fine-scale organization of intrinsic and interareal (with areas 3b, 2, and 3a) connectivity of area 1 and to do so with respect to functionally mapped somatotopy. As the fingers and especially the fingertips play a prominent role in active touch and are a defining aspect of primate behavior, we have focused on the digit representation region of somatosensory cortex of the squirrel monkey. The cortex in these monkeys is lissencephalic, negating the need to reconstruct tracer label from unfolded cortex and thereby reducing error. Moreover, several areas can be simultaneously mapped with optical imaging and electrophysiological techniques (Chen et al., 2001, 2003; Friedman et al., 2004; Négyessy et al., 2013). These maps are then used to guide focal injections of tracer into identified single-digit tip representations that are precisely aligned with transported label, permitting accurate reconstruction of label respect to these maps. Using these methods, we report distinct inter- and intra-areal axes of information flow in somatosensory cortex: a prominent cross-digit axis within area 1 and a largely intradigit axis between area 1 and area 3b. Comparisons with labeling patterns from digit injections in area 3b (Négyessy et al., 2013) are discussed. Preliminary findings of this study have been published in abstract form (Ashaber et al., 2011).

Materials and Methods

Animal preparation

Two males (Mo, P) and one female (M) adult squirrel monkey (*Saimiri sciureus*; weighing 600-800 gm, 2-9 years old) served as experimental subjects. Animal care and surgeries were performed according to National Institutes of Health regulations and were in compliance with and approved by the Institutional Animal Care and Use Committee of Vanderbilt University.

Each animal was sedated with ketamine hydrochloride (10 mg/kg), given atropine (0.05 mg/kg), intubated, placed in a stereotaxic frame, and mechanically ventilated with isoflurane anesthesia (0.9-1.3%). Vital signs (blood oxygen saturation [SpO₂], heart rate, electrocardiogram, electroencephalogram, end-tidal carbon dioxide respiratory pattern, temperature) were monitored throughout the experiment. Body temperature was maintained between 37.0 and 38.5°C via a circulating water blanket (Gaymar Industries, Orchard Park, NY), and for hydration, lactated Ringer's solution (with 2.5% dextrose) was infused intravenously (2 ml/kg/hr). After a craniotomy (centered at AP 6 mm and ML 15 mm) and durotomy, the somatosensory cortex including area 3b and area 1 was exposed and identified by blood vessel landmarks and functional mapping (see below). After tracer injection, to provide time for transport, exposed cortex was covered with artificial dura, the craniotomy was closed by securing the bone flap with bone cement, the skin was sutured, and the animal awakened from anesthesia with postoperative pain management. For these studies, repeated craniotomies and durotomies over the same area of cortex were separated by at least 10 days.

Functional mapping

Demarcation of areal borders and digit representations were made as described in Négyessy et al. (2013). Following electrophysiological mapping of the hand and the finger pad

representations of areas 3b and 1, intrinsic signal optical imaging (IOS) was used to map the distal finger pad representations of fingers D2-D4. Injections were then made into defined distal finger pad regions of area 1. A short description of functional mapping is given below.

Microelectrode electrophysiological mapping—Using the end of the central sulcus as an anatomical landmark, tungsten microelectrodes were inserted into superficial cortical layers to map the somatosensory cortical areas studied. The number of penetrations used for mapping was 46 in case M, 20 in case Mo, and 40 in case P. The responsive skin area of the unit activity was identified by palpating areas on the contralateral hand while listening to an audio amplifier for spike activity. As generally observed in squirrel monkeys, area 3b was found slightly anterior to the central sulcus. Area 3b was identified by single and multiunit response properties having small receptive fields (restricted to a single finger) and brisk responsiveness to light touch. Area 1 was identified based on location, as it is found posterior to area 3b, whereas area 3a is located anterior to area 3b (Sur et al., 1982; Chen et al., 2001; Friedman et al., 2004, 2008). Putative area 2 was identified as the region caudal to distal finger pad representation of area 1, exhibiting the sensitivity characteristic of deep receptors and tip-to-tip somatotopic organization with area 1.

Finger stimulation—For topographical mapping with intrinsic optical imaging digit-specific cortical activity was mapped by stimulating the glabrous skin of the distal, middle, and proximal finger pads with vibrotactile indentations (see detailed descriptions in Chen et al., 2001, 2003; Friedman et al., 2004). Fingers were secured in plasticine, leaving the glabrous surfaces available for tactile stimulation. Finger pads (D2-D4) were stimulated with a Teflon probe (2-mm diameter) mounted on a piezoceramic actuator (Noliac, Kvistgaard, Denmark) that was driven by a Grass 88 stimulator (Grass-Telefactor, West Warwick, RI). The tactile stimulus consisted of a 3-second train of 8-Hz square wave taps (pulse duration of 30 ms; indentation amplitude of 0.48 mm). During rest or the blank condition, a probe was lightly touching the skin.

Image acquisition—Images with 630-nm wavelength illumination were acquired through an optical chamber with a CCD camera using either a Redshirt Imaging System running CortiPlex software (Redshirt Imaging, Decatur, GA) or an Imager 3001 system (Optical Imaging, Germantown, NY). With a macroscopic tandem lens combination, the majority of the reflected light is derived from the upper 500 μ m of cortex (Ratzlaff and Grinvald, 1991). A bloodvessel map, used for landmark purposes, was collected with 570-nm illumination. Tactile stimuli were presented in a randomly interleaved manner in blocks typically consisting of four conditions (1–4) targeting either A) the 1. proximal, 2. middle and 3. distal sites on a single digit and 4. a blank, no-stimulus condition or B) the distal finger pads of 1. D2, 2. D3, 3. D4 and 4. blank. For each condition, 30–50 trials were collected. Intrinsic image maps were collected at 5 image frames per second for 4 seconds starting 200 msec prior to stimulus onset. Interstimulus intervals were between 8 and 10 seconds. Estimates of borders between areas 3a/3b were localized to the rostral edge of the digit tip activations in area 3b, and those between area 3b/1 were roughly centered between the digit activations in area 3b and area 1. These estimates were also guided by electrophysiological maps (see below).

Image analysis—As the intrinsic signal time course peaks at around 2–3 seconds post stimulus onset, for each stimulus condition, image frames 6–15 were summed and compared with the prestimulus (first frame); this helps to maximize the signal-to-noise ratio. To reduce blood-vessel artifact, we used blank subtraction (where the “blank” reference was a 3-second image acquired during no-stimulus presentation—Blank condition). To delineate regions of strongest activation, images were low-passed-filtered with a 10-pixel (150- μ m-radius) Gaussian spatial filter. To confirm reliability and consistency of the signal, we evaluated the frame-to-frame temporal development of optical images and compared the similarity of images obtained by summing different blocks of trials (Chen et al., 2001; Friedman et al., 2004).

Tracer injection and perfusion

To study connections of the distal finger pads, we injected both anterograde and retrograde tracers in selected digit tip locations. Distal finger pad representations of area 1 localized by IOS and electrophysiology were injected with a 1:1 mixture of 10% biotinylated dextran amine (BDA) 10K (preferentially anterograde) and 10% BDA 3K (preferentially retrograde) (final cc 5% each) (Molecular Probes, Eugene, OR) in 0.01 M phosphate buffer (PB; pH 7.4). Injections were made via iontophoresis (3 μ A, 5 seconds on/off cycle) for 2–3–10 minutes at two depths, 750 and 350 μ m below the cortical surface. Injections in area 1 were made into the representation of the glabrous skin of the tip of digit 2 (D2t; M, P) and digit 4 (D4t; Mo) exhibiting slowly adapting responses to pressure stimuli. Table 1 summarizes the parameters of the injections for each case, including the physical parameters of the glass micropipettes.

After a 10–16-day survival period (M: 14 days, Mo: 16 days, P: 10 days), animals were deeply anesthetized before transcardial perfusion with 300 ml of 0.9% NaCl in distilled water and 1,000 ml fixative consisting of 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.1 M PB (pH 7.3). Brains were immediately removed and the region of interest was then cut from the cortex, flattened, and postfixated overnight.

Tissue processing

Series of 50- μ m-thick horizontal sections were cut with a Vibratome and collected at 160 μ m (M, Mo) and 150 μ m (P) spacing after cutting 3 $\times$ 100- μ m-thick sections from the very top of the flattened cortex. Among these three topmost sections, the first and second were cut from the arachnoidal tissue, and the third included the very surface of neural tissue where the pattern of blood vessels running parallel to the cortical surface were similar in appearance to the previous two sections. These thick sections were used for alignment (see below).

The standard ABC protocol (Elite kit, Vector, Burlingame, CA) was used to visualize BDA labeling with nickel-intensified diaminobenzidine (NiDAB; Sigma-Aldrich, Budapest, Hungary) as the chromogen (for more details about the procedure, see Négyessy et al., 2000, 2013). In short, sections were incubated with the avidin/biotinylated enzyme complex (ABC, 1:200 in PB, 0.1 M, pH 7.4) overnight at 4°C after freeze-thawing of the cryoprotected sections (30% sucrose in PB). Unbound aldehydes were reduced by

borohydride (1% NaBH₄ in PB, 30 minutes), and intrinsic peroxidase activity was blocked by 1% H₂O₂ in PB (30 minutes) before the ABC step. Then the NiDAB reaction sections were osmicated (1% OsO₄ [Electron Microscopy Sciences, Hatfield, PA] with 5% sucrose in PB, pH 7.4 for 60 minutes) and flat-embedded in resin (Durcupan, Sigma-Aldrich).

Data analysis

Data were analyzed in the same way as described in Négyessy et al. (2013). Mapping of the BDA labeling was made with Neurolucida (MicroBrightField Europe, E.K. Magdeburg, Germany) using an Olympus research microscope equipped with a motorized stage (MultiControl 2000, Märzhäuser Wetzlar, Wetzlar, Germany). Retrograde labeling was mapped at 10× and 20× (objective magnification) whereas axonal fibers (anterograde labeling) were examined and reconstructed with 20× and 40× objectives. Terminal patches were examined at 10× and 20× and outlined individually on each section. The size of the patches was measured by Neurolucida Explorer using the Feret maximum and Feret minimum functions (Négyessy and Goldman-Rakic, 2005; Négyessy et al., 2013). The average of the Feret maximum and minimum was calculated for comparative analysis. Neurolucida drawings were edited in Neurolucida Explorer (MicroBrightField Europe). Photomicrographs were taken by a digital camera and edited in Photoshop (Adobe Systems, San Jose, CA) together with the images exported from the Neurolucida Explorer.

Density plots of the retrograde labeling were generated by way of Voronoi tessellation of two-dimensional maps. For a set of points (labeled neurons) this procedure divides a region into polygons where each point in the polygon is closer to the seed point (labeled neuron) than any other seed; neuronal cell densities are then calculated based on the areas of these polygons. The distance between the serial sections was ignored and the reconstructed labeling was projected into a single plane (for more details, see Négyessy et al., 2013). The logarithms of Voronoi areas were averaged and the standard deviation (SD) computed to represent deviation from the mean. Target specificity of the injected cortical loci was examined by localizing terminal arborizations of the anterograde labeling. Also, the core of the injection site of 250–300-μm diameter was omitted from the analyses.

Densities of axon terminal-like structures were sampled with a 100× objective, using Neurolucida in the full depth of each section of the series in three neighboring 50-μm² frames centered in three defined regions that included: 1) terminal patches (P); 2) tracks of long-range axons (F); and 3) regions lacking retrograde labeling outside of the previous two areas (CF). Selected regions were localized to areas including areas 3b, 3a, 1, and/or 2. Altogether, in the three monkeys 3,712 bouton-like structures were counted in 37 regions (19 terminal patches, 14 fibers of passage, 4 without retrograde labeling outside of the previous two). The average and SD computed from the three sampling frames across cases were used to compare bouton densities in the different regions and cortical areas.

Alignment

Sections were scanned with in-house written software with a 4× objective on the microscope used for the Neurolucida reconstruction. In Photoshop, the three topmost sections were aligned with an image of the cortical surface vasculature obtained during optical imaging.

The scanned images of the consecutive sections of the 50- μ m-thick series were then aligned with the topmost sections and with each other by using blood-vessel patterns and cut edges of the sections (see Figs. 2, 5). Reconstruction of the BDA-labeled structures was made using the serial section reconstruction function of NeuroLucida, which results in a completely aligned series of the drawings. Finally, the NeuroLucida drawings were aligned with their corresponding scanned sections, which resulted in alignment of the serial reconstruction with the cortical surface. For more details see Négyessy et al. (2013).

Results

In the first part we describe the mapping of the hand representation, the size and localization of the BDA injections, and the nature and overall distribution of the labeling. In the second part the pattern of horizontal connectivity is defined on the basis of the somatotopic maps within area 1 and in neighboring areas 3b, 2, and 3a.

Functional mapping and BDA injections

Electrophysiological and IOS maps—Digit representations of areas 3b and 1 were mapped by IOS and electrophysiological recording in each case as described previously (Négyessy et al., 2013). Extracellular recording of neuronal responses evoked by mechanical stimulation was used to identify the gross topography of the hand representations of areas 3b and 1 (Fig. 1A). Based on known organization of the somatotopic maps (Sur et al. 1982; Kaas, 1983), this procedure was also used to define the borders between the areas of the somatosensory cortex via mapping the rostrocaudal shift of cortical activations accompanying distal-to-proximal digit stimulations (Fig. 1A). The lateral-to-medial cortical representation of the fingers was also determined by IOS following vibrotactile stimulations of the distal finger pads (Fig. 1B–D). The IOS and the electrophysiological maps were in good alignment (Figs. 1, 2). Areal identity was defined on the basis of the response selectivity: receptive fields in areas 3b and 1 exhibited clear topographic progression and had brisk sustained or phasic responses to tactile stimulation.

A primary difference between areas 3b and 1 was receptive field size: receptive fields of units recorded in area 3b were small and confined to single digits whereas those in area 1 were larger, at times spanning two or three digits (cf. Friedman et al., 2008). The border region between areas 3b and 1 comprised a greater number of sites with palmar receptive fields, whereas those at the area 3b/3a border were largely located on digit tips (D2t, D3t, and D4t; Figs. 1A, 2A–C). Units in area 3a were difficult to drive with tactile stimulation and were often activated only by sustained, deep stimulation and joint movement, consistent with the deep receptor activation previously described for area 3a (Huffman and Krubitzer, 2001; Krubitzer et al., 2004). Some units in area 3a were also activated by thermal stimulation, as previously described (Tommerdahl et al., 1996). The border between area 1 and area 2 was often difficult to place with confidence. We typically found that units in area 2 had large receptive fields (e.g., some spanning D2 base/join, D3 palm/deep, D2 palm cutaneous), were noticeably more difficult to drive, had more mixed responses (e.g., some heat responses, some deep responses), and were characterized by palmar receptive fields. Thus, we were reasonably confident about the borders between areas 3a and 3b as well as

between areas 3b and 1, and less certain about the borders between area 1 and area 2 and between areas 3a and presumed Brodmann area 4 (M1). Injections were then placed iontophoretically at selected sites in area 1 (Figs. 1, 2A–C).

Injection site—The core of the BDA injection sites exhibited dense and diffuse NiDAB labeling, spread through multiple layers and had a diameter of 300–400 μm (Table 1, Fig. 2D–F). A halo of labeling surrounding the core was characterized by a dense plexus of neuronal processes, and was clearly visible in Mo (Fig. 2E). In cases M and P, the injection site was more homogenous, with a less distinct halo around the core and was considered as the core of the BDA injection (Fig. 2D,F). Within the core, BDA labeling of the perikarya can result from direct uptake of the tracer without retrograde transport; therefore these labeled cell bodies were omitted from the analyses. Based on the electrophysiological recordings, the injection was localized within the distal finger pad representation of D4 in case Mo (Fig. 2B). In cases M and P, the injections apparently included the representations of the neighboring D1 tip and D2 middle phalange, respectively, in addition to the targeted D2 tip region (Fig. 2A,C). Although in Mo and P the injections were on the edge or offset relative to the IOS maps of the stimulated skin location, the size of the injection was similar or smaller than the size of the activated cortical area in all three cases (Fig. 2G–I). This was clearly shown in case M, in which the BDA injection targeted just a subregion of the stimulated distal finger pad (Fig. 2A,G). The vertical spread of the core of the injections is shown in Table 1 (range of core depth values in Table 1). The core of the injections included the supragranular layers in all three cases and infragranular layers in cases Mo and P.

Characterization of the BDA labeling

Retrograde and anterograde labeling—Input and output connections of the injected locus of area 1 were labeled by retrograde and anterograde BDA transport, respectively, in each case. Both retrograde and anterograde labeling revealed the fine details of the neuronal elements (Figs. 3, 4). The majority of labeled neurons were identified as pyramidal cells bearing frequently branching dendrites decorated by numerous spines and the occasional appearance of the apical dendrite (Fig. 3). Although a few smooth dendritic neurons were found near the injection site, these neurons were not specifically considered for further analyses.

Anterograde axonal labeling also revealed the fine branching pattern and the presence of axon terminallike structures including bouton-like structures with stalk and en passant enlargements (varicosities) (Fig. 4C). The anterogradely labeled fibers could be divided into three types: 1) terminal-like patches formed by a dense network of thin fibers with several bouton-like enlargements in a circumscribed cortical localization (Fig. 4A–C); 2) relatively thick, horizontally oriented long fibers decorated by bouton-like structures (Fig. 4D); and 3) smooth, long-range fibers lacking bouton-like structures (Fig. 4D).

Terminal axon arborizations—Patches formed by terminal axon arborizations are likely to be localized at preferred target areas of the injected cortical sites. After outlining we quantified the patch size (see Materials and Methods) and compared the density of bouton-like structures within and outside the patches (Fig. 5). Average patch diameters were about

350 μm (Fig. 5D). Average patch size did not significantly differ between cases and areas, as shown by two-way repeated measures analysis of variance (ANOVA).

If terminal patches are regions of synaptic integration, then we predicted there would be a greater percentage of boutons within patch zones than outside patch zones. We therefore quantified the density of axon terminal-like structures in three types of locations: regions containing patches of terminal-like axon arborizations (P), regions containing fiber tracts (F), and regions devoid of BDA cell bodies and labeled axonal tracts (CF) (Fig. 5A–C). Figure 5E shows the mean of the ratio and SD of bouton-like structures in each of these three regions after the data from the individual cases were combined. Regions containing patches of terminal-like axon arborizations (P) had a several-fold higher density of bouton-like structures than regions containing only fiber tracts (F) or devoid of labeled fiber bundles or cell bodies (CF). Accordingly, comparison of ratios of the bouton-like structures of the three regions (P, F, CF) using a one-way ANOVA indicated significant differences ($P = 0.01$). Post hoc comparison showed significant differences between P compared with F and CF (Fisher LSD test, $P = 0.03$ and 0.004 , respectively). The density of bouton-like structures between F and CF was not statistically different. This result supports the notion that terminal arbors are likely to be regions of greater synaptic integration.

Distribution of retrograde and anterograde labeling

In this section we first describe the patterns of retrograde and anterograde labeling resulting from area 1 digit tip injections. Then we discuss the relationship to mapped digit topography.

Retrograde labeling

The Voronoi diagram affords a measure of the retrograde labeling density at the neuronal level; therefore binning was not necessary. By avoiding binning, the Voronoi density map provides an undistorted, topographically appropriate, and high-resolution density distribution. Because the size of the Voronoi polygons is determined by the distance of the retrogradely labeled neurons from the neighbors or seeds, the smaller polygon indicates higher densities and vice versa. As the size of the polygons is a largely varying, continuous variable, a simple color coding became necessary to illustrate major connectional density gradients. For this purpose the deviation of the size of the area of the Voronoi polygons from the mean was computed and colored by dividing the deviations into ranges of unit sizes of standard deviation.

We observed two major patterns of retrograde labeling within and across cortical areas in the two-dimensional reconstructions (Fig. 6A–C). Within area 1, in all three cases, the highest density of labeling appeared around the injection sites, and the density of labeling decreased and became scattered further away. The size of this strongly labeled region varied; it was similar in cases M and Mo and exhibited the largest extent in case P (Fig. 6A–C). Computation of the density maps compensated for individual differences and made the pattern of retrograde labeling more comparable across cases (Fig. 7): note within area 1 the extent of the color-coded regions marked by yellow and green on the SD maps (Fig. 7). Around the injection sites, the yellow region representing a density 1 SD above the average

occupied a region of roughly 1 mm in diameter in the three cases within area 1 (Fig. 7). The density maps also show the gradual decrease of the retrograde labeling away from the injection sites in area 1 (Fig. 7).

Following single-digit tip injections in area 1, patterns of labeled cell clusters were observed in neighboring somatosensory cortical areas (Figs. 6A–C, 7). In each animal high-density retrograde labeling was found within a relatively small, circumscribed region of area 3b (Fig. 7). This region is very clearly outlined on the density maps in cases M and Mo (yellow and green areas on Fig. 7A,B). In case P, the clustering of the retrograde labeling was more apparent in the raw data (Fig. 6C) because the average density in area 3b did not reach above +1 SD (yellow), probably because of the very high-density labeling near the injection site (Fig. 7). Instead, closely spaced fragments of near average density regions (green regions representing –1 SD) were found (Fig. 7C). In general, the area of high-density labeling was smaller in area 3b than in area 1 in all the cases (Fig. 7). In addition, in area 2 another cluster of retrogradely labeled neurons emerged in case P and to a somewhat lesser extent in case M (Figs. 6A,C, 7A,C). Notably, the density analyses showed that labeling in area 2 also tended to be clustered in case Mo (Fig. 7B). The pattern of clustering in area 2 was less obvious than in area 3b and 1 perhaps because only part of this area was preserved. The relatively high-density labeling across the border between area 1 and 2 could represent the tip-to-tip organization of the hand representation of these two areas (Fig. 7).

Anterograde labeling

A massive bundle of anterogradely labeled, long-range fibers were observed rostral from the injection site entering area 3b and passing even further to area 3a and probably to motor cortical area 4 (M1) in Mo and P (marked by blue on Fig. 6E,F). Similarly, a dense, BDA-labeled axonal pathway was directed caudally from the injection into area 2 of Mo and P (shown by green on Fig. 6E,F). This caudal plexus of the BDA-labeled axons was apparently shorter than the one moving into the rostral direction. An additional course of fibers was oriented mediolaterally and restricted mostly to area 1, therefore representing mostly long-range intrinsic connections (red lines in Fig. 6E,F). In case M, only short segments of the horizontally running fibers could be reconstructed because of the oblique cut of the sections (Fig. 6D).

Terminal patches of axon arborizations were widely distributed with biases in the mediolateral orientation within area 1, whereas in area 3b a more focused distributional pattern was found (black contours on Fig. 6D–F). Case P was an exception by having a similarly widespread mediolateral distribution of the terminal patches in both areas 1 and 3b (Fig. 6F). Within area 1, patches were grouped closer to the caudal border (Fig. 6D–F). Although in area 3b the terminal patches apparently distributed more evenly in the rostrocaudal direction, a higher overlap tended to be localized closer to the anterior border of the area in the three cases (Fig. 6D–F). Terminal patches also accumulated in areas 3a and caudal to area 1, most probably within area 2, where the distribution of patches was spatially restricted (Fig. 6D–F). Case P was an exception: patches spread rostrocaudally in presumed area 2 (Fig. 6F). Laterally placed patches were also observed in presumed area 2 of case Mo

(Fig. 6E). In all cases terminal axon arborizations were distributed along the BDA-labeled fiber bundles (Fig. 6D–F).

Reciprocity

It is important to note that reciprocal connectivity was studied between somatotopically identified cortical regions on the two-dimensional maps. It is possible that although anterograde and retrograde labeling overlapped on the horizontal maps, i.e., identified as reciprocal, their laminar distribution could have been distinct. Laminar distribution was not examined in this study. Also, our data cannot reveal direct reciprocal connections between neuronal cells.

The overlapping distribution of retrograde and anterograde labeling indicated a reciprocal organization of area 1 connectivity (Figs. 6, 7). Reciprocity appeared as an overlap of the terminal patches of axon arborizations with the regions exhibiting local peaks of density of the retrogradely labeled neurons (Fig. 7A–C). The findings suggest that connections of the distal finger pad regions of area 1 are organized preferentially in a reciprocal way at the regional level. One major exception was area 3a, where dense terminal axon arborization colocalized with a low-density retrograde labeling in Mo and P (Fig. 7B,C). Another exception, which was especially prominent in case Mo (Fig. 7B), appeared laterally in presumed area 2 near the area 1 border, where terminal patches did not align with peaks of retrograde labeling densities. Although unreciprocated connections could be a result of section sampling, further studies are needed on a complete series of sections to unequivocally resolve the question of regional reciprocity of the input and target connections of area 1.

Input and target specificity of the distal finger pad representations of area 1

Input preference: retrograde labeling—With respect to the intrinsic cortical circuitry of area 1, the highest density of retrograde labeling occupied either fully (case M) or in a large part (cases Mo and P) the injected distal finger pad representation, based on the combined electrophysiological and IOS maps (yellow and orange on Fig. 8A–C). In all three cases the high-density region marked by the extent of the green area was elongated rostrally, suggesting considerable input from the representation of the proximal finger to the injected digit tip representation (Fig. 8A–C). This localization pattern was evident on the detailed electrophysiological maps (Fig. 7). In case M, e.g., the high-density input region colocalized with the representation of the proximal part of D2 (yellow in d2p on Figs. 7A, 8A). Similarly, in P the stimulation of the midphalanx of D2 evoked neuronal activity in a region with high-density retrograde labeling (d2m on Fig. 7C). High-density input to the injected digit representation also originated from neighboring finger representations. Accordingly, injection of the D4t representation resulted in high-density labeling in the D3t region in case Mo (green and yellow in Fig. 8B). However, labeling density dropped to a low level in the non-neighboring finger representations in this case (blue and pink on Fig. 8B).

Similar observations were made in case M, in which injection of the D2t representation resulted in low-density retrograde labeling in the D4t region (blue in Fig. 8A), whereas the neighboring D3t and also the D1t representations were densely labeled (yellow and green on

Fig. 8A). In case P, injection of the D2t representation resulted in lateral high-density labeling in the D1t representation (Fig. 7C). Medially, the D3t region identified electrophysiologically included high-density retrograde labeling, whereas further medially within the D3t region identified by IOS labeling density dropped to a low level (Fig. 8C). This case also exhibited widespread connections originating more from the proximal finger representations to the injected fingertip location (Figs. 7C, 8C).

Interareal connections with area 3b were characterized by the appearance of a high-density cluster indicating that the major input to the injected fingertip region of area 1 originated from the homotopic finger representation region (Fig. 8A–C). This pattern was clearly seen in cases M and Mo (overlap of yellow and orange with the D2t and D4t representations, respectively on Fig. 8A,B). In case P, the highest density retrograde labeling of area 3b was localized not only in the homotopic D2t region but also laterally within the D1t representation (green areas on Fig. 8C). Similarly, a relatively high-density spot appeared within the presumed D1 representation in the vicinity of the d1m responsive area of case M (green region at d1m in Fig. 8A). Interestingly, in cases M and P medial to the D2t region, retrograde labeling density decreased to a low level in the D3t representation (blue and pink on Figs. 7A,C, 8A,C). An asymmetric, laterally skewed distribution also appeared in case Mo, in which following the injection of the D4t region of area 1, a relatively high-density retrograde labeling was found in the D3t region (blue on Fig. 8B), whereas labeling density substantially decreased medially to the D4t region representing presumably D5 in area 3b (pink on Figs. 7B, 8B). Notably, a relatively high-density retrograde labeling spread caudally in area 3b into regions representing presumably the proximal parts of finger D2 in cases M and P and of D4 in case Mo in area 3b (Figs. 7, 8A–C).

High-density labeling was also localized caudally in presumed area 2 next to the injected finger pad representation, which suggests a similar homotopic topography of the interareal input from this region to area 1 (rostrocaudal spread of the green and blue region in area 3b on Fig. 7).

Target preference: anterograde labeling—Within area 1 the D2t representation gave birth to terminal axon arborizations localized near the injection site within the D2t region (Fig. 8D,F). Additional terminal patches were localized anterior to the injection targeting presumably the representation of the more proximal parts of D2 or the neighboring fingers (Fig. 8D,F). Considerable lateral spread of terminal axon arborizations was found in case P, in which patches were localized laterally in a region representing presumably D1, based on the localization of D1 in area 3b of this case (Fig. 8F). Similar to the retrograde labeling, case P also exhibited a large divergence of labeled afferents with terminal arborizations located in regions presumably representing proximal parts of the different fingers (Fig. 8F). In case Mo, injection of the D4t representation resulted in the labeling of terminal axon arborizations lateral to the injection site within the representations of D3t and D2t (Fig. 8E).

In area 3b terminal patches densely accumulated in the homotopic distal finger pad representations, i.e., within the D2t region of cases M and P and in D4t region of case Mo (Fig. 8D–F). In cases Mo and P, terminal patches were also found laterally in the neighboring D3t and D1t regions, respectively (Fig. 8E,F). Case P also included the labeling

of terminal patches in areas medial to the D2t representation, which could represent neighboring D3 and/or D4 (Fig. 8F).

Other interareal target sites included area 2 where numerous terminal patches close to the estimated area 1/2 border in the vicinity of the injected distal finger pad representations suggested similar homotopic topography of patch distribution as in the case of the retrograde labeling (Fig. 8D–F). Some terminal patches also appeared laterally (all three cases) and also medially (cases Mo and P) from the above-mentioned region, which suggests some divergence of the afferents of the distal finger pad regions within the cortical area caudal to area 1 (Fig. 8D–F). In case Mo, dense accumulation of terminal patches was localized caudolateral to the D4t injection, which might be the region of area 2 representing neighboring D3t on the basis of the topography found in area 1 (Fig. 8E). Within area 3a in cases Mo and P, terminal patches were clustered within a circumscribed region in the vicinity of the homotopic finger representation of area 3b, suggesting a similar homotopic topography of terminal patch distribution in area 3a as well (Fig. 8E,F).

Discussion

Our combined anatomical, optical imaging, and electrophysiological studies demonstrate that intrinsic connections of area 1 connect neighboring distal finger pad representations in the non-human primate, clearly extending beyond single-digit somatotopic activations (Chen et al., 2009). In contrast, interareal connections of distal finger pad regions of area 1 establish high-density connections largely with homotopic regions of areas 3b and 2. Furthermore, both intrinsic and interareal connections of area 1 distal finger pad regions spread into more proximal representation of the fingers.

General considerations

Consistent with what has been described in macaque area 1 (Burton and Fabri, 1995), horizontal cortical connections exhibit a lateral spread beyond the distal finger pad representations of area 1 of the squirrel monkey. Here, we have identified the extent of the lateral spread of intrinsic and interareal connectivity with respect to correlated functional maps. The highest densities of labeling were restricted to the representations of the injected finger and its immediate neighbors, similar to that found after similar injections in area 3b (Négyessy et al., 2013). Compared with the pattern observed in area 3b (Négyessy et al., 2013), the fingertip regions of area 1 are connected more strongly with the proximal parts of the injected finger representations, which is consistent with the physiological observations (Iwamura et al., 1983b). Our observations also show that connections of area 1 finger representations are largely reciprocal on the two-dimensional somatotopic maps (but not at the laminar or cellular levels, which were not studied here). One exception may lie in the connections with area 3a: whereas area 1 receives only a low-density connection from area 3a, it provides a relatively high-density projection to this area. This finding is in agreement with the results of degeneration studies showing that microlesions in area 1 result in anterograde but not retrograde degeneration in area 3a (Shanks et al., 1985).

A notable difference found after the area 1 injections in the present study compared with that of area 3b injections by Négyessy et al. (2013) is that on the retrograde density maps the

peak densities were surrounded by a larger spread of medium-to-low-density regions, which together occupied an apparently larger cortical territory than the activated region on the optical imaging maps in both areas 3b and 1. This pattern of cortical connectivity implies that within area 1, high-density, digit-matched inputs from area 3b form the anatomical basis of receptive field hotspots and the lower density flanking regions could provide convergent input from neighboring fingertip representations to form the multifinger receptive fields. Conversely, as shown by the distribution of the terminal axon arborizations, area 1 afferents originating from the distal finger pad could modulate not only the homologous but also the neighboring fingertip representations as well as more proximal representations of the injected fingers in areas 3b. This observation is consistent with a recent study showing that injecting multiple finger representations of area 3b results in overlapping labeling of the different finger representations of area 1 (Liao et al., 2013).

Technical considerations

The major findings were observed in all three cases used in this study. Some differences observed between the cases are discussed below. In case P, BDA injection resulted in a larger amount of retrograde and anterograde labeling and a more widespread distribution of the intrinsic connectivity than in the other two cases. The large mediolateral extent of the labeling in the rostral part of area 1 of case P suggests that intrinsic connections could include proximal parts of the finger representations throughout area 1. In P the injection was offset to the optical imaging map representing the center of the injected finger pad and could have been included in a region representing a more proximal part of the finger, as indicated by the closeness of the middle phalange on the electrophysiological map (Fig. 2C). Similarly, in case Mo, the injection targeted the periphery of the center of the distal finger pad representation on the IOS map, which could result in a slight shift of the labeling density relative to the IOS activation pattern in area 3b as well. However, the electrophysiological map indicated that the injection site was localized within the distal finger pad representation of area 1 in Mo. Possible pitfalls of bidirectional tracing have been discussed in Négyessy et al. (2013).

Functional speculations

Recent electrophysiological studies have revealed complex spatiotemporal receptive field structures in areas 3b and 1 (Sripati et al., 2006; Reed et al., 2010, 2012; Thakur et al., 2012). These findings are in agreement with the distribution of intrinsic and interareal connections of distal finger pad representation of area 1 and area 3b (Négyessy et al., 2013). Accordingly, intrinsic connections were found between neighboring distal fingertip regions, and a heavy interareal connection was localized to the homotopic representations. However, compared with area 3b, Sripati et al. (2006) observed larger nonlinearities of the area 1 receptive field structure and proposed that such processes could, in part, be responsible for features such as more complex spatial and less velocity selectivity. Compared with area 3b, the larger spread of intrinsic and interareal connections of area 1 distal fingertip regions could be an anatomical substrate of such nonlinearity. Specifically, surround inhibition, which is increased in area 1 compared with that in area 3b (Sripati et al., 2006), is thought to be mediated, at least in part, by long-range intracortical interactions (Angelucci and Bressloff, 2006; Shushruth et al., 2009). A further possible functional role for intrinsic

horizontal connectivity is suggested by Reynaud et al. (2012), who showed that in visual cortex V1 lateral cortical interactions compute local input normalization, an important step in contextual modulation. In area 1 such lateral cortical interactions could play an important role in processing invariant response properties observed in cases of velocity, texture, form, speed, and intensity (Sinclair and Burton, 1991; Pei et al., 2010). In contrast to the intrinsic connections, feedforward input from area 3b may be integrated more linearly in area 1. As shown by Pei et al. (2011), direction selectivity of area 1 neurons arises as vector averages of local motion detectors of area 3b.

These findings together raise the question of whether selective stimulus properties of area 1 neurons are formed by linear integration of convergent feed-forward afferents of area 3b, whereas invariant feature properties are computed by lateral intrinsic and possibly feedback connections from higher order somatosensory areas in area 1. More studies on different feature-selective properties are needed to answer this question. Although the present finding that connections of area 1 exhibit somewhat larger spatial spread than that of area 3b is consistent with the physiological observations and supports the idea of an increasing convergence of intrinsic and inter areal connections of area 1 (Jones, 1975; Iwamura, 1998), more studies at even higher spatial resolution will be necessary to map the circuitries responsible for the functional characteristics of area 1.

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Figure 1.

Mapping the glabrous part of digit representations in the somatosensory cortex with electrophysiology and optical imaging (case M is shown). **A:** Colored dots indicate electrophysiological recording sites. White ellipsoids encapsulate functional regions of similar somatotopic response preference shown also by the same color of the dots. d1–d5, digits 1–5; d1t–d5t, digit tips of fingers 1–5; d2m–d4m, middle phalanges of fingers 2–4; d2p, proximal part of finger 2; p, palm. The localization of BDA injection is indicated by the asterisk in the d2t region of area 1. **B:** Optical image of the somatosensory cortex without stimulations. **C,D:** Optical images of the response of somatosensory cortex to stimulation of the D2 (C) and D4 (D) distal finger pads. Red arrows show corresponding vessel landmarks on the different images. Note the medial shift of D4 activation relative to the D2 activation sites in areas 3b and 1. Dashed lines, extrapolated borders between the areas. R, rostral; m, medial. Scale bar = 1 mm in A and D (applies to B–D).

Figure 2.

Localization and size of the BDA injection are shown on maps that include the electrophysiological and intrinsic optical signal (IOS) functional maps of somatosensory cortex in the three cases (M, Mo, P). **A–C:** Overview of the cortical surface mapped by electrophysiology and IOS. Black outlines encircle the representation of the distal finger pad regions localized by IOS for the three cases. Dotted lines represent areal borders. Electrophysiological recording sites are marked by different symbols shown in the inset. **D–F:** Brightfield photomicrographs of the injection sites taken from the supragranular layers at a depth of 320 μm (M, Mo) and 450 μm (P) from the cortical surface. Note the core and halo organization of the BDA injection sites, which is best seen in Mo. The inner white spotted circle indicates the extent of the core, and the external circle shows the margin of the halo organization of the BDA injection. Core and halo regions could not be clearly delineated in M and P, in which the highest density regions were encircled. The black holes represent cross sections of descending blood vessels (asterisk). Arrows point to BDA-labeled neuronal cell bodies. **G–I:** Higher magnification shows the size of the BDA injection relative to the size of the cortical area activated in the IOS experiments in the three cases. Darkfield-like views, in which image brightness was inverted, are shown to enhance visibility. Small circle with white core, BDA injection site. Scale bar = 1 mm in A (applies to A–C); 250 μm in D (applies to D–F) and G (applies to G–I).

Figure 3.

Retrogradely labeled neurons. **A:** Pyramidal cells as identified by the appearance of the apical dendrites down the focal depth. White arrows indicate cross sections of the apical dendrites. Arrowhead, a horizontal branch originating from the apical dendrite with a protrusion. **B:** A pyramidal neuron can be seen in the vicinity of the injection site (dark area in the bottom). Asterisk indicates an electrode track made during the electrophysiological mapping. Framed region is enlarged in the inset showing dendritic spines (arrowhead). Note thin, varicose axonal fiber bending around a blood vessel cut across (white “hole”). **C:** Pyramidal cell with widely branching basal dendrites in the supragranular layer. Spiny dendritic spines (black arrowheads) and protrusions (white arrowheads) appear on higher magnification of the framed region in the inset. Scale bars = 100 μm in A; 50 μm in B,C; 25 μm in the inset.

Figure 4.

Anterograde labeling. **A:** Pale contour outlines a terminal patch of axonal arborizations. Note the low number of retrogradely labeled neurons (appearing as black dots) within the patch. The diagonal dark stripe is a chatter of the Vibratome sectioning. **B:** Dotted line shows the border between the patch (lower left) and the neighboring region with low density BDA labeling. **C:** Higher magnification reveals the presence of numerous thin fibers decorated by bouton-like structures. Arrowheads show terminal-like ending with stalk, and arrow indicates varicosity. Asterisks mark unlabeled neuronal cell bodies partially surrounded by thin axons. **D:** Long-range horizontal axonal fibers with (black arrows) and without (white arrows) bouton-like varicosities (black and white arrows, respectively). Note the thick and smooth appearance of the fiber shown by the white arrow. Scale bars = 250 μ m in A; 50 μ m in B,D; 25 μ m in C.

Figure 5.

Size of terminal patches and density of bouton-like structures. **A–C:** Overall distribution of the regions sampled for bouton density analysis on the two-dimensional reconstructions of the retrograde and anterograde labeling in M, Mo, and P. Fibers are indicated by light gray curves, and patches are marked by circular outlines. Black dots, retrogradely labeled neurons. CF, cell-free region; F, fiber tracts; P, terminal patches. **D:** Average patch size in the different cortical areas (1, 3b, 3a, and 2). **E:** The ratio (relative to the total counts in each case) of the average number of bouton-like structures is shown within the three different sampled regions (CF, F, P). Asterisk marks significant difference both from CF and F regions. Error bars indicate standard deviations. Scale bar = 1 mm in C (applies to A–C).

Figure 6.

Retrograde and anterograde labeling in the three cases (M, Mo, and P). **A–C:** Distribution of retrograde labeling. Green triangles, retrogradely labeled neurons. Injection sites are marked by red dots. **D–F:** Distributions of the anterograde labeling. Black outlines show terminal axon arborizations. Red lines, intrinsic connections; green lines, connections with area 2; blue lines, connections with areas 3b and 3a and perhaps M1. Red dotted lines represent areal borders. CS, central sulcus; r, rostral; m, medial. Scale bar = 1 mm in C (applies to A–C) and F (applies to D–F).

Figure 7.

Alignment of the distribution of the terminal axon arborizations, the retrograde density plots, and the functional electrophysiological maps. Color code for retrograde labeling density is given in units of standard deviation (SD) above and below average (for further details, see the Data analysis subsection). Dashed lines represent extrapolated borders between the areas. Colored dots indicate electrophysiological recording sites. White ellipsoids encapsulate functional regions of similar somatotopic response preference. d1–d5, digits 1–5; d1t–d5t, digit tips of fingers 1–5; d2m–d4m, middle phalanges of fingers 1–5; p, palm. Large white dots with black outline, injection site. White region around the injection site shows the area omitted from the analyses (250–300- μ m diameter). r, rostral; m, medial. Scale bar = 1 mm in C (applies to A–C).

Figure 8.

Alignments of the structural and functional maps in the three cases. **A–C:** Input preference is shown by projecting the electrophysiological (pale outlines) and intrinsic optical signal (black outlines) maps on the two-dimensional density plots of the retrograde labeling. **D–F:** Target preference is defined by aligning the terminal patches (red outlines) with the functional maps. Conventions are the same as in Figure 7. Scale bar = 1 mm in F (applies to A–F).

Table 1

Characteristics of the BDA Injections in the Three Cases, M, Mo, and P¹

Case	Tip diameter	Injection depth	Core diameter	Core depth	No. of slides	Depth of granular layer
M	30	350, 750	313	320	9	480
Mo	10	350, 750	295	160–800	16	640
P	10	350, 750	403	160–800	10	650

¹ Values are given in μm . Size of the glass capillary measured after the injection is shown. Tip diameter represents the inner diameter of the glass capillary used for the biotinylated dextran amine (BDA) injection. Injection depth was measured from the pial surface by a micromanipulator. Core diameter was measured in the series of the sections where it exhibited the densest and largest extent. Core depth: approximate depth of the densest and largest core diameter in the section series. Difference between the injection and core depths could result from pulsation and swelling of the brain during the injection. In Mo, core depth exhibited a bilaminar pattern as the core diameter was smaller at 640 μm than on the neighboring upper and lower sections in the series at 320 and 800 μm , respectively.