Somatotopic organization of rat thalamocortical slices

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Abstract

The thalamocortical slice is widely employed for in vitro studies of cortical circuits. This preparation was developed in order to preserve anatomical and functional connectivity between the ventrobasal thalamus and somatosensory (whisker/barrel) cortex of young mice, and thalamocortical slice experiments have contributed significantly to our understanding of the thalamocortical synapse. Cortical somatotopy within thalamocortical slices, however, has not been characterized, and this greatly limits their use in studies that require identification of cortical areas associated with particular regions of the sensory periphery. To address this shortcoming we used electrophysiological recording and neuroanatomical labeling techniques in rats to mark the position of functionally defined whisker barrels, in vivo. We subsequently processed the brains in a plane appropriate for TC slices and characterized the location of somatotopically identified barrels in relation to other aspects of slice topology. We found that barrels associated with the large mobile whiskers occupy a particular location in TC slices, but that there are certain constraints to studying this portion of the barrelfield in vitro.

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1. Introduction

The somatosensory cortex (S1) of rodents contains distinct clusters of neurons in layer 4 that are related in a one-to-one manner to individual sensory organs on the contralateral body surface (Woolsey and Van der Loos, 1970). These neuronal clusters, termed barrels, are a visible manifestation of functional cortical columns that process information from the thalamus (see Simons, 1995 for review). Signals from the sensory periphery influence the establishment of the system’s distinctive anatomical organization during development and regulate the functional properties of cortical neurons throughout the animal’s life (Van der Loos and Woolsey, 1973; Simons and Land, 1987; Diamond et al., 1993). Consequently, the barrel cortex has become an important model for studies aimed at understanding cortical development and function.

The barrelfield consists of approximately 200 barrels and barrel-like structures (Woolsey and Van der Loos, 1970), most of which are associated with small hairs (microvibrissae; Brecht et al., 1997) of the upper and lower lips, rostral snout and nares. Barrels associated with small hairs constitute the anterolateral barrel subfield (ALBSF; Woolsey and Van der Loos, 1970). In contrast, there are only about 30 barrels associated with the large mobile whiskers that are used for active tactile exploration (macrovibrissae; Carvell and Simons, 1990; Brecht et al., 1997). These larger barrels comprise the posteromedial barrel subfield (PMBSF; Woolsey and Van der Loos, 1970). The unique anatomical, physiological and behavioral characteristics of the whisker-to-PMBSF barrel system have provided the framework for nearly all in vivo studies of barrel function (Armstrong-James, 1975; Simons and Woolsey, 1979; Carvell and Simons, 1990; Armstrong-James et al., 1991; Land et al., 1995; Brecht et al., 1997; White et al., 1997). Further, PMBSF barrels have been the focus of every investigation of experience-dependent plasticity in the whisker-barrel system (Simons and Land, 1987; Akhtar and Land, 1991; Diamond et al., 1993; Carvell and Simons, 1996; Finnerty et al., 1999; Keller and
Carlson, 1999; Land and Akhtar, 1999; Lendvai et al., 2000).

Several years ago Agmon and Connors developed a novel in vitro slice preparation of the infant mouse somatosensory cortex, termed the thalamocortical (TC) slice, that was a major advancement for studies of synaptic circuitry and plasticity in the barrel cortex (Agmon and Connors, 1991). In TC slices, sections are prepared in an unconventional plane that maintains viable connectivity between ventrobasal (VB) thalamus and S1 cortex, thereby permitting selective activation of the principal ascending cortical input in vitro. It is possible in TC slices to visualize VB and numerous layer 4 barrels, and consequently one can characterize the physiology, pharmacology, and development of thalamocortical synapses onto individual cortical neurons (Agmon and Connors, 1991; Crair and Malenka, 1995; Feldman et al., 1998; Egger et al., 1999; Kidd and Isaac, 1999).

To date there has been no description of cortical somatotopy in TC slices, so there remains a high degree of uncertainty about whether recordings are made from neurons in ALBSF barrels or PMBSF barrels. Such information, however, is important for at least two reasons. First, as noted above, whiskers associated with different barrel subfields have unique behavioral roles; consequently the underlying barrel circuitry is likely to differ. Second, formation of barrels in these two regions appears to be governed by different mechanisms. For example, disruption of serotonergic signaling (Cases et al., 1996; Persico et al., 2001) or N-methyl-D-aspartate (NMDA) receptor functioning (Iwasato et al., 2000) during development differentially affect barrels in the PMBSF versus the ALBSF. Thus, knowledge about the relative location of PMBSF and ALBSF in TC slices is necessary to correctly interpret results obtained from this preparation.

To overcome this shortcoming we combined in vivo and in vitro approaches to characterize topographic relationships of forebrain structures in TC slices. A principal aim was to establish the location of PMBSF barrels representing the large mobile vibrissae and to determine the functional integrity of thalamic inputs onto these barrels in TC slices. We first used electrophysiological recording and neuroanatomical labeling techniques in rats to mark the position of functionally defined barrels, in vivo. We then processed the brains in a plane appropriate for TC slices and visualized the location of somatotopically identified barrels in relation to other aspects of slice topology. We found that PMBSF barrels can be localized in a TC slice by their topographic relationship to the dorsal hippocampus and fornix. Importantly, only the central-most rows of PMBSF barrels (i.e. rows B, C and D) are present in TC slices that also contain the VB nucleus. We also noted in parallel in vitro experiments that slight modification of the slice plane from that appropriate for mice was necessary to preserve functional thalamocortical connections in rats, a species in which a majority of functional and behavioral studies have been performed.

2. Materials and methods

Studies were performed on Sprague–Dawley rat pups (born in our breeding colony) ranging in age from postnatal day (P) 14 to P-28. The care and handling of animals was approved by the University of Pittsburgh Institutional Animal Care and Use Committee and conformed to NIH guidelines.

2.1. In vivo experiments

Our aim was to establish the topographical relationships between functionally identified locations in S1 (e.g. barrels associated with particular whiskers) with other anatomical landmarks that can be observed in living TC slices. To do this we used extracellular recordings and whisker stimulation in vivo to map the position of selected barrels in the PMBSF that represent large, caudal whiskers (i.e. macrovibrissae). We then marked their locations with small iontophoretic deposits of horseradish peroxidase (HRP; Boehringer-Mannheim, grade I) and processed the brains in the TC-slice plane for HRP and for cytochrome oxidase (CO).

2.1.1. Electrophysiological mapping and iontophoresis

Rats were anesthetized with tribromoethanol (232 mg/kg, i.p.) and placed in a small animal headholder. Skin was incised over the dorsum of the skull and a craniectomy was made over right parietal cortex. A tungsten microelectrode (FHC, Bowdoinham, ME) was oriented perpendicular to the pial surface and advanced through the dura, to a depth of 400–800 μm. The contralateral whiskers and body surface were probed by hand with a small glass rod or orange stick while we monitored extracellular multunit responses.

The electrode was repositioned until we encountered units responding to deflection of a whisker in a lower-ordered arc of one of the rows of large whiskers (e.g. A1-3, B1-3, etc.), thus identifying elements of the PMBSF (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). The tungsten electrode then was replaced with a glass micropipette (tip diameter 5–10 μm) containing HRP (10% solution in 0.05 M Tris–HCl, pH 8.6). The HRP pipette was advanced through the dural opening created by the recording electrode to a depth of 700 μm below the pial surface (i.e. layer 4) and a deposit of HRP was made by passing 0.6–0.8 μA intermittent current (electrode positive) through the micropipette for 10 min. Following injection the scalp
was sutured, closed and the rat was returned to the litter where it was observed until it recovered from anesthesia.

2.1.2. Histology

Eighteen to 24 h after HRP injection rats were deeply anesthetized with tribromoethanol (280 mg/kg, i.p.) and perfused transcardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Brains were removed, postfixed for 8–12 h, and transferred to 30% sucrose in phosphate buffer until they sank. Brains were frozen and sectioned with a sledge microtome at 50 μm in a plane similar to that described by Agmon and Connors (1991) with slight modification (see below). Briefly, brains were placed with the ventral surface down onto a 10° ramp with the anterior aspect oriented downhill (Agmon and Connors, 1991). A razor blade was used to make a vertical, blocking cut at 50° from the mid-sagittal plane (vs. 55° that is appropriate for mice; see below). Sections were rinsed in 0.1 M phosphate buffer, incubated in dianinobenzidine and H2O2 to reveal HRP deposits (Adams, 1977), rinsed for 30 min in several changes of phosphate buffer and then stained to reveal CO activity (Wong-Riley, 1979; Land and Simons, 1985). Histochromically treated sections were mounted onto chrome alum gelatinized slides, dehydrated and coverslipped. Numerous brains prepared in a variety of section planes in prior studies were available for comparison (Land and Simons, 1985; Land et al., 1995).

2.2. In vitro experiments

We used in vitro electrophysiological recording and stimulation to test the functional integrity of thalamocortical axons in living TC slices. Anatomical connectivity between thalamus and cortex was verified in some slices using anterograde labeling of thalamocortical axons.

2.2.1. Stimulation and recording in TC slices

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and decapitated. Brains were rapidly removed and placed into chilled artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 1.3 MgSO4, 3.1 CaCl2, 5 KCl, 1.25 KH2PO4, 26 NaHCO3, and 10 dextrose, bubbled with 95% O2/5% CO2. Brains were blocked as described by Agmon and Connors (1991) (see above), except that in preliminary experiments we varied the angle of the final vertical cut between 45 and 55°. The cut surface of the brain was glued with cyanoacrylate to a vibratome stage (DTK-1500E, Ted Pella, Redding, CA), and 400 μm TC slices were obtained and transferred to an interface chamber for at least 2 h before recording. For recording, individual slices were transferred into a submerged-type recording chamber where they were continuously superfused with oxygenated ACSF at room temperature (3–5 ml/min).

Bipolar stimulation electrodes (two tungsten micro-electrodes [FHC, Bowdoinham, ME, USA] at 100–150 μm distance) were positioned into VB and field potential recordings (patch pipette filled with ACSF) were used to identify responding barrels (Agmon and Connors, 1991). The barrel exhibiting the largest VB-evoked field potentials was chosen for single cell recordings. Whole-cell patch clamp recordings were made from visually identified barrel neurons using infrared illumination and a 40× water immersion objective on an upright microscope (Olympus BX50WI) equipped with a gradient contrast system (Luigs and Neumann, Ratingen, Germany). Patch electrodes were filled with internal solution containing (in mM) 100 K-gluconate, 1 MgCl2, 10 HEPES, 11 EGTA, 1 CaCl2, 10 KCl, 5 ATP, and 0.3 GTP. For intracellular staining and verification of recording sites and neuron types, 0.5% biocytin (Sigma, St. Louis, MO) was included in the pipette solution.

2.2.2. Neuronal labeling in TC slices

In some slices we placed small crystals of biocytin into the VB nucleus in order to label the extent of thalamocortical axons. Such slices were maintained in vitro for 6–8 h. These slices, and those containing intracellularly stained barrel neurons were fixed overnight in 4% paraformaldehyde. Slices were rinsed in phosphate buffer, equilibrated in 30% sucrose and resectioned at 80 μm. Sections subsequently were stained for biocytin using Vectastain elite reagents according to the manufacturer’s instructions (Vector Labs, Burlingame, CA), mounted onto subbed slides and coverslipped as above.

3. Results

3.1. Topography of TC slices

Although an unconventional section plane is used to prepare TC slices, it nevertheless is possible to recognize many key landmarks within them. As noted by Agmon and Connors (1991) (see also Bernardo and Woolsey, 1987) one can visualize numerous forebrain structures, and in particular components of the thalamocortical system, even in unstained TC slices of mouse brain. This is also true for rats. Fig. 1 shows sections prepared in the TC slice plane that were counterstained for CO to better demonstrate slice topography. Centers of whisker-related barrels in parietal cortex (darkly reactive in the CO stain) occupy a large expanse of neocortex (e.g. black arrowheads). This cortical region overlies the dorsal hippocampus (Hip) and corpus striatum (Str). In some (though not all) slices that contain barrels it is also possible to identify portions of the VB thalamic nucleus.
and fibers in the internal capsule (IC) coursing between thalamus and cortex.

3.2. Somatotopy within TC slices

The somatotopic identity of particular barrels in TC slices is not apparent upon simple visual inspection. To establish the general orientation of the cortical somatotopic map within a slice we used extracellular recordings and whisker stimulation in vivo to map the position of selected whisker-related barrels. We then marked their locations with small iontophoretic deposits of HRP and processed the brains in the TC-slice plane for HRP and for CO. Fig. 1A through D, respectively, show sections from different brains in which we deposited HRP in the vicinity of barrel neurons responding to the E1, the D1, the C1 or the A2 facial whiskers. Note that the labeled barrels—which are among the most posterior barrels in the PMBSF—lie radially above the dorsal hippocampus, thus marking the caudal margin of the PMBSF.

Other anatomical features of TC slices permit us to further deduce the cortical somatotopy within them. Thus, in addition to labeled barrel D1 the section shown in Fig. 1B fortuitously included an electrode tract, created during the mapping process, where neurons responded to whisker C3. Note that barrels in this arc overlie the fornix and lateral ventricle. Barrel C3 in this slice is followed laterally by a barrel-free area (white arrowheads). Similarly, Fig. 1C shows several large barrels (black arrowheads) lateral to labeled barrel C1, which are followed by a stretch of small barrel-like structures overlying the striatum (indicated by white arrowheads). Taken together, such observations indicate that sections in the TC slice plane cross the barrel field as shown by the black lines in Fig. 1E, which shows a

Fig. 1. Cortical somatotopy in rat TC slices. A through D show HRP/CO double-stained TC sections from juvenile rats in which we mapped cortical somatotopy in vivo and labeled identified barrels. (A) Section from a P-18 rat in which HRP (black reaction product) was deposited near barrel neurons responding to deflection of whisker E1 (indicated by arrow labeled E1). Darkly reactive barrel centers (e.g. black arrowheads) can be seen in the middle cortical depths throughout this section. The ventral lateral thalamic nucleus (VL) is contained in this section. (B) HRP/CO double-stained section from a P-21 rat in which HRP was deposited near barrel D1 (labeled arrow). Arrow labeled C3 indicates microelectrode penetration (i.e. red blood cells in electrode track) where barrel neurons responded to whisker C3 during cortical mapping. Note absence of barrels in large expanse of cortex (white arrowheads) lateral to barrel C3. Fx = fornix, Hip = hippocampus, IC = internal capsule, Str = corpus striatum, VB = ventrobasal thalamic nucleus. (C) HRP/CO double-stained section from a P-20 rat in which HRP was deposited near barrel C1 (labeled arrow). Several other large barrels (black arrowheads) are visible to the right of (i.e. lateral to) C1. Lateral to the large barrels is a series of small barrels (white arrowheads). (D) HRP/CO double-stained section from a P-21 rat in which HRP was deposited near barrel A2 (labeled arrow). A single additional barrel is visible medial to the A2 (arrowhead); no barrels are visible laterally. Note the lateral (LG) and medial geniculate nuclei (MG) present in this section. (E) CO-stained tangential section through layer 4 of SI cortex showing somatotopic distribution of barrels and barrel-like structures. The five rows of barrels comprising the PMBSF is indicated in arc one of each row (e.g. A1, B1, etc.); some additional barrels in higher-ordered arcs are indicated by Arabic numbers. Black lines labeled ‘A’ through ‘D’ indicate orientation of TC slice-plane with respect to the cortical somatotopic map as deduced from the experiments illustrated in A through D. Representations of other body regions are indicated by lower case letters: fp = forepaw, hp = hindpaw, ll = lower lip, n = nares, t = trunk, ul = upper lip. Arrows labeled r and m point rostrally and medially, respectively. Bar in D = 500 μm (applies also to A–C); bar in E = 1 mm.
tangential section through layer IV of S1. For example, the plane labeled ‘A’ (i.e. deduced from Fig. 1A) intersects barrel E1 then extends obliquely across numerous row E and D barrels. The plane labeled ‘B’ intersects barrel D1 and C3 and then passes through a portion of dysgranular cortex separating barrel row C from the small barrel-like structures denoting the nares representation (n). Plane ‘C’ in turn traverses barrels C1, C2, B3 and B4 before intersecting directly the nares barrels, while plane ‘D’ (i.e. see Fig. 1D) traverses labeled barrel A2 and a medially located barrel in a lower ordered arc (e.g. barrel A1 or B1; black arrowhead). Importantly, it is evident from these analyses that PMBSF barrels associated with the large mobile vibrissae can be confidently identified in a region of the slice that overlies the lateral ventricle, fornix, and hippocampus. Furthermore, barrels associated with caudally placed whiskers (e.g. C1) are located medially in the slice. These are followed laterally by barrels corresponding to progressively more rostral and dorsal whiskers.

The above analyses also revealed two important and unexpected findings. First, TC slices pass obliquely across barrel rows. This makes it impossible—without prior labeling—to be certain of the somatotopic identity of individual barrels in a slice. Second, the ventrobasal thalamic nucleus (VB), which provides the principal ascending input to cortical barrels, is only present within the central-most slices that contain barrels (e.g. compare Fig. 1A and D with B and C). Thus, to investigate TC connections for barrels corresponding to dorsally placed (i.e. row A) or ventrally placed (row E) whiskers will require further modification of slice parameters.

### 3.3. Functional anatomy in TC slices

We next examined what section plane optimally conserves thalamocortical connectivity. In these experiments we noted that to preserve viable TC connections in ‘juvenile’ rats (e.g. between P-14 and P-28) it was necessary to modify the slice plane from that originally described for mice. Specifically, a vertical cut made at an angle of 50° from the mid-sagittal plane was essential for preserving thalamocortical connectivity (Fig. 2, see Section 2; see also Feldman et al., 1998; Feldmeyer et al., 1999). Slightly increasing or decreasing the angle by 5° (to 45 or 55°) never produced TC slices that had intact thalamocortical connections with PMBSF barrels as examined by biocytin tracing or electrophysiological recordings. In contrast, functional thalamocortical connectivity is well preserved in slices cut at 50° (Fig. 2A). Fig. 2A illustrates a section from a P-16 TC slice that was maintained in vitro for 8 h, during which time we implanted a crystal of biocytin into the VB thalamus (white asterisk) in order to label TC axons. Numerous fascicles of labeled axons can be seen coursing from the thalamus through the internal capsule and subcortical white matter. Individual axons rise through the deep cortical layers and arborize within layer 4 (inset). Thus, 50° TC slices of juvenile rats preserve the anatomical integrity of thalamocortical axons.

To test functional thalamocortical connectivity in 50° TC slices, patch-clamp recordings were made from barrel spiny stellate neurons while stimulating the VB thalamic nucleus. We targeted our recordings to PMBSF barrels dorsal to the hippocampus, since previous studies have not documented whether functionally intact thalamocortical connections are preserved in this location. An example of a recorded neuron that was labeled intracellularly with biocytin and recovered from a P-18 TC slice is shown in Fig. 2B. This neuron exhibits the typical morphological features of a barrel spiny cell such as small diameter soma and several radiating, spine-covered dendrites. Upon intracellular current injection, the neuron fired with an adapting spike train (Fig. 2C), thus identifying this neuron as a regular-spiking cell as is typical for layer 4 spiny stellate cells (e.g. Feldmeyer et al., 1999). Electrical stimulation of VB elicited postsynaptic potentials (Fig. 2D) with an average onset latency of 5.44 ms ± 0.1 S.D. (5.2–5.6 ms, n = 18, recording at room temperature). The mean peak amplitude was 3.7 mV ± 0.7 S.D. (2.3–4.8 mV, n = 18). The small variations in latencies indicate that responses were most likely the result of stimulating a monosynaptic connection. These observations are consistent with those of previous in vitro studies following activation of thalamocortical inputs to mouse barrel cortex (Agmon and Connors, 1991).

### 4. Discussion

Our observations establish the relationship of the basic cortical somatotopic map in S1 with major landmarks that are visible in TC slices from juvenile rats. The data further indicate that functional TC connections in slices cut at 50° are only conserved for the middle rows of large barrels (i.e. rows B, C and D) but are absent for the flanking rows.

#### 4.1. Topography and somatotopy in TC slices

The general orientation of the cortical barreelfield in TC slices has not been formally described until now. This is a minor issue for experiments focusing on the most basic characteristics of neural transmission between the VB thalamus and neurons in cortical layer 4. It presents a more serious problem, however, for studies seeking to investigate functional interactions among thalamic and cortical neurons related to particular tactile organs (e.g. macrovibrissae vs. microvibrissae). For example, to study the effects of tactile deprivation on dynamics of cortical synapses, Finnerty et al. (1999)
developed a brain slice preparation wherein they could identify unambiguously the 30 or so PMBSF barrels corresponding to the five rows of macrovibrissae (see also Land and Simons, 1985). However, by virtue of its orientation that slice plane severs thalamocortical axons below the white matter, making it impossible to directly investigate thalamocortical synaptic transmission. Our experiments demonstrate that those barrels associated with the large mobile whiskers (i.e. the PMBSF) can be confidently identified in TC slices in a cortical region underlying the dorsal hippocampus, fornix and lateral ventricle. The remaining 170 or so smaller barrels are located more rostrally and laterally, many of them over the striatum. It should be noted, however, that because TC slices pass obliquely across barrel rows, they do not permit unequivocal recognition of specific barrels (e.g. C2 vs. D2). That level of specificity in principle could be achieved by in vivo identification and labeling prior to slice preparation. The spatial distribution of barrels we observed in rats is somewhat different than that postulated previously for mice (Agmon et al., 1991). This may represent a species difference, but it is also likely to reflect the fact that individual barrels were functionally identified and then labeled in the present study.

4.2. Preservation of thalamocortical connectivity

Our data also indicate a slight modification of slice plane from 55 to 50° from the mid-sagittal plane substantially improves preservation of functional thalamocortical connectivity in juvenile rats. Indeed this small but essential variation in technique may partly explain the scarcity of studies employing TC slices to investigate thalamocortical transmission in rats (Feldman et al., 1998; see also Feldmeyer et al., 1999).

Original development of the TC slice preparation exploited the unique topology of the infant mouse brain in order to preserve anatomical and functional connectivity between thalamus and S1 cortex. While there are many superficial similarities in the brains of rats and mice, there are numerous differences. Overall brain dimensions obviously are dissimilar. More importantly, the size, shape and orientation of the barreelfield within the telencephalon vary substantially in the two species (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). The present findings indicate that it is possible to circumvent such variations in forebrain geometry in mice and rats, thereby expanding the feasibility of TC slice studies to the latter species.

Our studies also highlight an additional important issue. Specifically, from a set of TC slices that contain barrels only, the central-most slices will also contain the VB thalamic nucleus. Thus, thalamocortical inputs to barrels representing dorsal (i.e. row A) or ventral whiskers (row E) are unlikely to be preserved in TC slices. This finding indicates that functional studies targeted to large barrels, in combination with TC slices, must be limited in rats to barrel rows B, C and D.
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