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Neuronal Circuits with Whisker-Related Patterns

Keisuke Sehara · Hiroshi Kawasaki

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Abstract Neuronal circuits with whisker-related patterns, such as those observed in the rodent somatosensory barrel cortex, have been widely used as a model system for investigating the anatomical organization, development and physiological roles of functional neuronal circuits. Whisker-related patterns exist not only in the barrel cortex but also in subcortical structures along the trigeminal neuraxis from the brainstem to the cortex. Here, we briefly summarize the organization, formation, and function of each neuronal circuit with whisker-related patterns, including the novel axonal trajectories that we recently found with the aid of *in utero* electroporation. We also discuss their biological implications as model systems for the studies of functional neuronal circuits.

Keywords Whisker-related pattern · Barrel · Somatosensory · Development · *In utero* electroporation

Introduction

To understand the function of neuronal circuits, it is important to uncover the precise organization and formation of the neuronal connections within the circuitry. The rodent somatosensory barrel cortex has been widely used for investigating the anatomical organization, formation, and function of neuronal circuits [1–7]. Layer 4 of the barrel cortex contains an anatomical map of cell clusters, called "barrels," which receive thalamocortical axons (TCAs) derived from the dorsomedial portion of the ventroposteromedial nucleus (VPMdm) in the thalamus (Figs. 1 and 2) [8, 9]. Between barrels are septa, which are associated with distinct thalamocortical circuits involving the medial division of the posterior nucleus (POm) (Figs. 1 and 2) [8, 9]. It has been proposed that barrels and septa represent two separate streams of vibrissa information processing (Fig. 1) [10–13].

The spatial distribution pattern of barrels is called a "whisker-related" pattern because each barrel has one-to-one relationship with the corresponding whisker on the contralateral snout of the animal, as indicated by the following evidence. First, when perinatal lesions of whisker follicles are performed, the corresponding barrels shrink [14]. Second, animals with aberrant patterns of whiskers possess the corresponding aberrant distribution patterns of barrels [15, 16]. Finally, neurons in each barrel are activated best by the corresponding whisker on the contralateral snout [17–19]. Because of this precise projection pattern, together with the fact that barrels can easily be visualized using basic histological techniques, whisker-related patterns in the barrel cortex have been serving as an excellent model system for investigating the anatomical structures, functional information processing, and development of neuronal circuits.

Interestingly, in addition to being found within the barrel cortex, whisker-related patterns are also found along the somatosensory pathway from whiskers to the barrel cortex in both barrel- and septum-related circuits (Fig. 1). Here, we briefly review the anatomical organizations of the neuronal circuits with whisker-related patterns and the time courses of their formation during development. We also describe a novel intracortical circuit with whisker-related patterns that we recently found. Finally, we discuss their potential physiological importance and their roles as a

K. Sehara · H. Kawasaki (✉)
Department of Molecular and Systems Neurobiology,
Graduate School of Medicine, The University of Tokyo,
Hongo 7-3-1,
Bunkyo-ku, Tokyo 113–0033, Japan
e-mail: kawasaki@m.u-tokyo.ac.jp

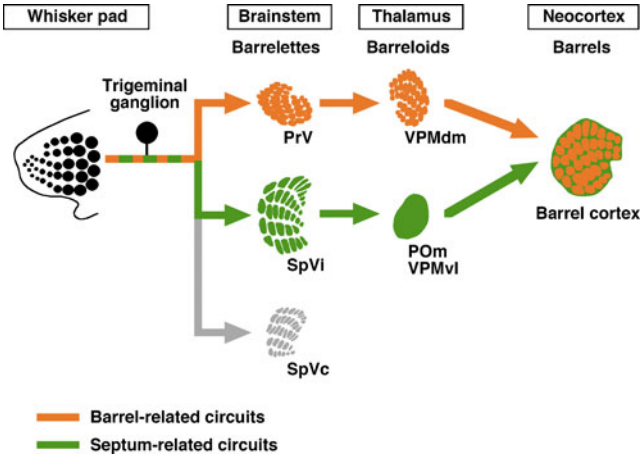


Fig. 1 Overview of neuronal circuits with whisker-related patterns. In rodents, neural circuits with whisker-related patterns are found at every level along the somatosensory pathway from whiskers to the neocortex. The structures corresponding to whiskers are called barrelettes in the brainstem, barreloids in the thalamus, and barrels in the cerebral cortex. The somatosensory pathway contains neuronal circuits termed either barrel-related circuits (*orange*) or septum-related circuits (*green*). Barrel-related circuits involve the principal trigeminal nucleus in the brainstem (*PrV*) and the dorsomedial portion of the thalamic ventroposteromedial nucleus (*VPMdm*), whereas septum-related circuits comprise the interpolar division of the spinal trigeminal complex (*SpVi*), the posteromedial thalamic nucleus (*POm*), and the ventrolateral portion of VPM (*VPMvl*). Of these neuronal circuits, whisker-related patterns are found in *PrV*, *SpVi*, *VPMdm*, and the barrel cortex. Whisker-related patterns are also found in the caudal division of the spinal trigeminal complex (*SpVc*). Neurons in *SpVc* rarely project to the thalamus and are thought to be mainly involved in lower sensorimotor feedback loops [46, 78], which is beyond the scope of this review

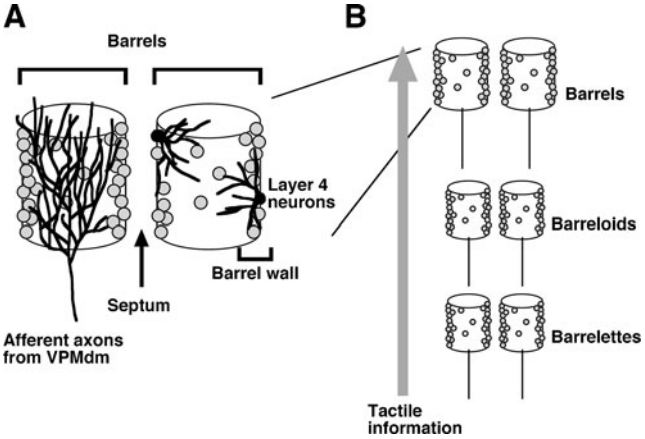


Fig. 2 The anatomical architecture of whisker-related neuronal circuits. **a** The architecture of barrels in the barrel cortex. Afferent TCAs from VPMdm form axonal clusters at the center of each barrel. Layer 4 neurons surround these axonal clusters as barrel walls and send their dendrites toward the center, or the hollow, of the barrel. The cell-sparse region that is found between barrels is called the septum. **b** Neuronal architecture along the somatosensory pathway from whiskers to barrels. Barrels, barreloids, and barrelettes share the characteristic distribution patterns of neurons described in a

general model system for the study of functional neuronal circuits.

Barrel-Related Circuits

In the barrel cortex, each barrel is comprised of discrete pre- and postsynaptic components (Fig. 2a). As the presynaptic component, TCAs that are derived from VPMdm of the thalamus form discrete axonal clusters within each barrel [9, 20, 21], which has a one-to-one relationship with the whiskers. These axonal clusters occupy the center, or the hollow, of each barrel. Neurons in layer 4 of the barrel cortex tend to be sparse within the barrel hollow and dense in the barrel wall [8], surrounding the axonal clusters. The layer 4 neurons in the barrel wall send their dendrites toward the barrel hollow [22, 23]. Collectively, these structures show the whisker-related pattern of barrels.

Similar whisker-related patterns are also found at each level along the somatosensory pathway from whiskers to barrels (Figs. 1 and 2b). Tactile information from whiskers is first sent to the principal trigeminal nucleus (PrV) of the brainstem, next to VPMdm in the thalamus, and then to the barrel cortex. The barreloids in VPMdm [24] and the barrelettes in PrV [25] correspond to the barrels in the cortex, and have similar whisker-related patterns as described below. Afferent fibers form axonal clusters, each of which has one-to-one relationship with the corresponding whisker. The neurons that receive inputs from the axonal clusters surround the axonal clusters. Furthermore, these neurons preferentially send their dendrites toward the axonal clusters (however, in the case of barreloid neurons in mice, Brown et al. (1995) observed that initially asymmetrically projecting dendrites upon barreloid formation turn out to be symmetrical in the adult [26]). In summary, barrel-related circuits, from whiskers to barrels, have similar whisker-related patterns, regardless of the location along the neuraxis.

It is well established that barrel-related circuits convey tactile information detected by whiskers [7], and that tactile information derived from each single whisker is well segregated from that derived from other whiskers [6]. When neurons in barrel-related circuits are activated by the deflection of whiskers, the direction, velocity, and amplitude of the deflection are encoded in the responses of neurons in barrel-related circuits [27–29]. Furthermore, recent physiological studies revealed a direction preference map within each barrel column in the barrel cortex of the rat [30, 31]. Collectively, these results suggest barrel-related circuits are important for characterization and identification of external objects using tactile information detected by whiskers [6, 32].

Septum-Related Circuits

The neuronal circuits related to septa arise from the interpolar division of the spinal trigeminal complex (SpVi), pass mainly through POm in the thalamus, and reach the septal regions in the barrel cortex (see Fig. 1) [12]. The septum-restricted innervation of TCAs derived from POm results in another whisker-related pattern in the barrel cortex that is complementary to the distribution pattern of TCAs from VPMdm [9].

One notable feature of septum-related circuits is that, in contrast to those in barrel-related circuits, neurons in septum-related circuits are often activated by multiple whiskers. This feature may result from the fact that, in the rat trigeminal nucleus, single barrelette neurons in SpVi send their dendrites across multiple barrelettes [33, 34]. Consistently, neurons in SpVi, POm, and the septum are activated by multiple whiskers in rats [35–37].

Another distinct feature of septum-related circuits is the sensory modality they correspond to. Septal neurons in the barrel cortex respond much less to tactile stimulation than barrel neurons do [35, 38]. Rather, they respond well to rhythmic whisker movements that mimic the exploratory whisking of rats [39]. It has been proposed that septum-related circuits monitor active whisker movements [32, 40].

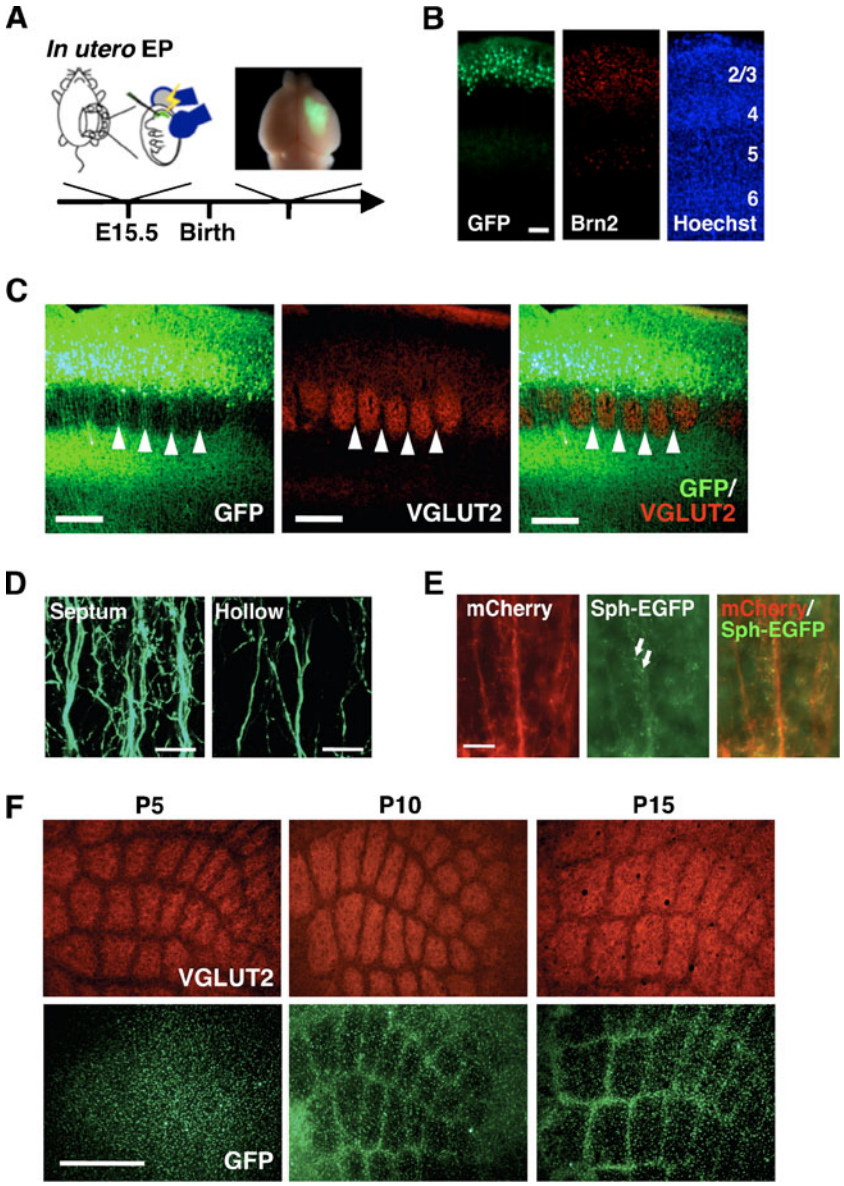
The septal region of the barrel cortex, at least in the rat, contains an additional neuronal circuit. This circuit involves SpVi, the ventrolateral part of VPM (VPMvl), and the septal region of the barrel cortex (Fig. 1) [41]. Although both VPMvl and POm in the thalamus receive inputs from SpVi and send information to the septum in the barrel cortex, these two constitute distinct circuits anatomically and functionally [39, 41]. Specifically, SpVi contains two types of trigeminothalamic neurons, the large and small neurons [42], which project to POm and VPMvl, respectively [41]. Electrophysiological recordings from individual thalamic nuclei revealed that there is a fundamental difference between the response properties of thalamic neurons in POm and those in VPMvl [39]. POm neurons respond to both whisking events and whisker deflections [39, 43], while neurons in VPMvl selectively respond to physical contacts with objects, irrespective of the occurrence of whisking events [39]. Collectively, these observations indicate that POm- and VPMvl-associated circuits are separate circuits with distinct response properties [32, 39]. It would be intriguing to examine how POm-associated circuits, VPMvl-associated circuits, and barrel-related circuits contribute to information processing in the barrel cortex.

Intracortical Circuitry with Whisker-Related Patterns

Although afferent neuronal circuits between whiskers and the barrel cortex have been extensively investigated, the organization of intracortical circuitry within the barrel cortex is not fully understood. A large amount of the existing knowledge about intracortical circuitry was derived from neuroanatomical studies using neuronal tracers. In contrast to the rather diffuse horizontal connections within layers 2/3 and 5, it has been suggested that layer 4 of the rat barrel cortex contains intracortical circuitry with distinct whisker-related patterns [44, 45]. When neuronal tracers were focally injected into the septal region of the barrel cortex, the labeled fibers in layer 4 preferentially distributed in a manner complementary to the whisker-related patterns of TCAs [44, 45]. Such intracortical connections are proposed to be intercolumnar circuits connecting layer 4 septal neurons across the barrel cortex [46]. However, it is not clear whether layer 4 neurons are actually responsible for the observed intracortical circuit. This is, at least partially, because it is often difficult to define the precise identities of the neurons sending the labeled axons, especially when using neuronal tracers. Neuronal tracers are often taken up not only by dendrites but also by axon terminals and fibers-of-passage, and therefore the somata of the labeled neurons are not necessarily located around injection sites [47–51]. In other words, neurons whose dendrites and/or axons extend into injection sites might be labeled with neuronal tracers even though the locations of neuronal somata are far from injection sites.

In order to understand intracortical circuitry correctly, it is important to determine the precise identities of the neurons contributing to the observed intracortical connections. Therefore, in a recent study, we selectively visualized the axonal trajectories of layer 2/3 neurons in the mouse barrel cortex using *in utero* electroporation [52]. *In utero* electroporation is a simple and efficient method to express genes of interest in a selected population of cortical neurons (Fig. 3a, b) [53–55]. Making use of this advantage, we found that the axons of layer 2/3 neurons in layer 4 were preferentially located in the septal region of the barrel cortex (that is, similar to the pattern exhibited by the axons from POm) and showed whisker-related patterns (Fig. 3c, f). Because high magnification confocal images showed green fluorescent protein (GFP)-positive axons with mesh-like distributions in the septum as if they surrounded each barrel (Fig. 3d), we named this novel whisker-related axonal organization "barrel nets." In addition, by labeling presynaptic structures using GFP-tagged synaptophysin, it was suggested that the axons of layer 2/3 neurons form synapses in barrel nets (Fig. 3e) [52]. In summary, barrel nets are novel intracortical circuit components with whisker-related patterns.

It would be intriguing to explore the identities of neurons postsynaptic to the axon terminals located in barrel nets. One possibility is that the axons of barrel nets make synapses onto the apical dendrites of layer 5 pyramidal



neurons in layer 4. This seems plausible because the apical dendrites of layer 5 pyramidal neurons also locate preferentially in the septal region in layer 4 of the barrel cortex in mice and rats [56–59]. The other possibility is that axons in barrel nets form synapses with layer 4 septal neurons. Visualizing synapses between specific types of neurons using GRASP [60] or BLINC [61] may help in examining this point. Investigating postsynaptic neurons which receive inputs at barrel nets would contribute to the understanding of the physiological functions of barrel nets.

Although the physiological role of barrel nets is currently unclear, there are several possibilities. One possibility is that barrel nets integrate information from barrel-related and septum-related circuits. To test this possibility, it would be interesting to examine whether the axons of layer 2/3 neurons within single barrel columns send their axons to the septum. If this is the case, it seems reasonable to speculate that information from barrel-related circuits and that from septum-related circuits converge into barrel nets. Related to this point, Kim and Ebner (1999) showed that, in rats, layer 2/3 neurons in barrel columns appeared to give off their axons to the surrounding septa in layer 4 [10]. A detailed examination, in relation to barrel nets, of axons derived from layer 2/3 neurons within single barrel and/or septal columns might help in understanding the physiological roles of barrel nets.

Although we identified barrel nets in the barrel cortex ipsilateral to GFP-positive neuronal somata, axonal projection patterns complementary to whisker-related patterns of TCAs were originally reported to be contralateral to neuronal somata in the barrel cortex [62]. Earlier studies using neuronal tracers have shown that callosal projections

◀ **Fig. 3** Whisker-related patterns of barrel nets in the mouse somatosensory cortex. **a**, **b** Selective labeling of layer 2/3 neurons using *in utero* electroporation. *In utero* electroporation was performed at E15.5, and GFP-positive postnatal brains were collected (**a**). Coronal sections of 50-μm thickness containing the barrel cortex were prepared from P10 brains and were stained using anti-Brn2 antibody, which predominantly labels neurons in layers 2/3 and 5 (**b**). To examine the location of GFP-positive somata, which were brighter than GFP-positive axons, images were taken with short exposure times. By performing *in utero* electroporation at E15.5, neurons in layer 2/3 can be selectively labeled (in this case, with GFP). *Scale bar*, 100 μm. **c** Organization of barrel nets. Layer 2/3 neurons were selectively labeled with GFP using *in utero* electroporation at E15.5, and coronal sections of 50-μm thickness were prepared at P9. The sections were immunostained with anti-VGLUT2 antibody, in order to reveal the distribution of thalamocortical axons with whisker-related patterns. Although a small number of cortical neurons also express some amount of VGLUT2 at early postnatal ages, whisker-related patterns of thalamocortical axons can be clearly visualized with anti-VGLUT2 antibody [79] presumably because of the dense axonal projections from VPMdm. The images of GFP were taken with long exposure times in order to clearly visualize GFP-positive axons. Axons from layer 2/3 neurons are preferentially located in the septal region in layer 4 of the barrel cortex (*arrowhead*). Because layer 2/3 neurons also send their axons into layer 5, layer 5 was also labeled with GFP. *Scale bar*, 200 μm. **d** High magnification confocal images of GFP-positive axons in layer 4. Layer 2/3 neurons were selectively labeled with GFP using *in utero* electroporation at E15.5, and coronal sections of 50-μm thickness were prepared at P15. High magnification confocal microscopic images were three-dimensionally reconstructed and are shown as maximal projection images stacked along the *z*-axis. Note that, in addition to the radially running GFP-positive axons, a number of fine GFP-positive axonal branches run in oblique directions in the septum (*left*), while fine branches are not as evident in the barrel hollow (*right*). *Scale bars*, 10 μm. **e** Presynaptic structures in barrel nets. mCherry and a presynaptic marker protein, GFP-tagged synaptophysin (*Sph-EGFP*), were co-expressed selectively in layer 2/3 neurons in the mouse barrel cortex using *in utero* electroporation at E15.5. Coronal sections of 50-μm thickness were prepared at P15. mCherry-positive axons that comprise barrel nets are shown. GFP-positive puncta (*arrows*), presumably presynaptic sites, are observed on the axons of barrel nets. GFP-positive puncta were also found in layer 5, where layer 2/3 neurons send their axons (data not shown). *Scale bar*, 20 μm. **f** The developmental time course of barrel net formation. Layer 2/3 neurons were labeled with GFP using *in utero* electroporation at E15.5, and tangential sections of 50-μm thickness were prepared at the indicated time points. Sections that contained layer 4 of the barrel cortex were stained using anti-VGLUT2 antibody, in order to reveal the distribution of thalamocortical axons with whisker-related patterns. While whisker-related patterns of barrels already exist at P5 (*upper panels*), whisker-related patterns of barrel nets are visible only after P10 (*lower panels*). *Scale bar*, 500 μm. (Adapted from Sehara et al. [52] with kind permission of the Society for Neuroscience.)

have predominant axonal arborizations between barrel hollows in layer 4 of the contralateral hemisphere in rats [62–64]. These pioneering observations raised the possibility that the septal region is a special passage site in layer 4. This possibility was reinforced by other previous studies demonstrating that apical dendrites of layer 5 pyramidal neurons also locate preferentially to the septal region in mice and rats [56–59] and that thalamocortical axons from POm are predominantly found in the septal region of the rat cerebral cortex [9]. Together with barrel nets, these intracortical circuits with whisker-related patterns may contribute to vibrissa information processing in the barrel cortex.

Development of Whisker-Related Patterns

It has been noted that, during development, circuits with whisker-related patterns maturate sequentially from periphery to center (Fig. 4) [3]. Developmental processes, such as neurogenesis, whisker-related pattern formation, and critical period plasticity induced by follicle cauterization, all appear to progress firstly in the brainstem (barrelettes), then in the thalamus (barreloids), and finally in the cortex (barrels), with largely overlapping but strictly ordered time periods [65]. In this developmental time course, the whisker-related patterns are sequentially transmitted from the periphery to the center [3].

Consistently, the developmental time course of barrel net formation suggested that whisker-related patterns of local intracortical circuitry develop after the initial appearance of whisker-related patterns of TCAs (Fig. 3f). Interestingly, when whisker-related patterns of barrels were altered by cauterization of whisker follicles, those of barrel nets were also affected accordingly. Together, these results suggest that whisker-related patterns of barrel nets are determined by those of barrels [52]. These observations may also reinforce the idea that circuits with whisker-related patterns maturate sequentially from periphery to center (see Fig. 4).

Our results suggested that the axonal trajectories of layer 2/3 neurons are determined by whisker-related patterns of barrels [52]. It is plausible that TCAs and/or layer 4 barrel neurons have instructive roles in determining the distribution pattern of the axons of layer 2/3 neurons. If this is the case, it will be important to investigate which are responsible, TCAs and/or layer 4 barrel neurons. Cortex-

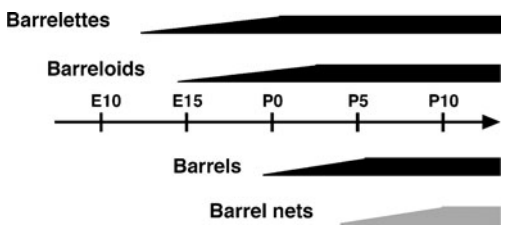


Fig. 4 Ordered developmental time courses of whisker-related pattern formation in the somatosensory pathway. Whisker-related pattern formation proceeds from periphery to center. The *line* beside the name of each structure shows the maturational time course of the structure, with its *full thickness* meaning that the whisker-related pattern of the indicated structure is formed by this time point. Barrelettes are formed first, followed by barreloids, then barrels, and finally barrel nets. Each point on the scale along the *arrow* roughly indicates the corresponding age of mice. *E*, days embryonic; *P*, days postnatal

restricted NMDAR1 knockout mice would be useful to address this point because the formation of cytoarchitectonic barrels is blocked in cortex-restricted NMDAR1 knockout mice, whereas whisker-related patterns of TCAs are relatively preserved [66]. In summary, investigating the mechanisms of barrel net formation should reveal fundamental principles underlying the pattern formation of intracortical circuitry. In addition, comparisons between barrels and barrel nets would reveal similarities and differences in the formation of thalamocortical and intracortical circuits in the barrel cortex.

Compared with that of barrel-related circuits, the developmental time course of septum-related circuits is less understood. Recently, Kichula and Huntley (2008) followed the developmental time course of the intracortical innervation of POm neurons in the barrel cortex in mice and rats. It was clearly shown that the timing of septum-specific axonal branching occurs well after the initial appearance of axonal clusters formed by TCAs derived from VPMdm [67]. The results imply that the development of barrel-related and septum-related circuits is differentially regulated in the barrel cortex.

Future Perspectives

Since the first description of barrels by Woolsey and Van der Loos (1970), a number of neuronal circuits with whisker-related patterns have been reported along the trigeminal neuraxis of rodents. It would be intriguing to examine if the insights obtained from the studies using whisker-related patterns in the somatosensory pathway are also applicable to other circuits with columnar organization. For example, axons from layer 2/3 neurons in the visual or motor cortices could also have net-like structures that are related to the columnar organization of the neocortex. For addressing this point, higher mammals such as carnivores and primates might be appropriate because previous studies reported that rats do not have column-like structures in the visual cortex [68].

Currently, the molecules responsible for barrel net formation are totally unclear. *In utero* electroporation, combined with conditional gene knockout techniques, should help in uncovering the molecular mechanisms underlying whisker-related pattern formation of barrel nets. Once the molecular mechanisms are understood, we might be able to fabricate mice without barrel nets. These mice should be useful for addressing the physiological roles of barrel nets in information processing at the levels of neuronal circuits and behavior.

In order to visualize and investigate circuits with specific patterns, *in utero* electroporation seems to be a powerful tool. Using this method, one can efficiently introduce genes of interest into selected populations of neurons [53, 54, 69–71]. Although several neuronal circuits with whisker-related patterns have been uncovered with the aid of neuronal tracers or transgenic reporter mice, the identities of the neurons responsible for the observed patterns are often in question. Using *in utero* electroporation, one can easily visualize the morphology of the selected neuronal population and subcellular structures such as synapses [52].

Recently, local microcircuits within the cerebral cortex have been extensively investigated using new physiological techniques such as multiple simultaneous patch recordings, laser scanning photostimulation and channelrhodopsin-2-assisted circuit mapping [72–77]. Combining these techniques with neuroanatomical analysis using *in utero* electroporation would contribute toward an understanding of the structure and function of neuronal circuits with whisker-related patterns.

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