HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2016 January 05.

Published in final edited form as:

Ann N Y Acad Sci. 2011 April ; 1225: 119–129. doi:10.1111/j.1749-6632.2011.06024.x.

How do barrels form in somatosensory cortex?

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Abstract

The somatosensory cortex of many rodents, lagomorphs, and marsupials contain distinct cytoarchitectonic features named “barrels” that reflect the pattern of large facial whiskers on the snout. Barrels are composed of clustered thalamocortical afferents relaying sensory information from one whisker surrounded by cell dense walls or “barrels” in layer 4 of the cortex. In many ways, barrels are a simple and relatively accessible canonical cortical column, making them a common model system for the examination of cortical development and function. Despite their experimental accessibility and popularity, we still lack a basic understanding of how and why barrels form in the first place. In this review, we will examine what is known about mechanisms of barrel development, focusing specifically on the recent literature utilizing the molecular–genetic power of mice as a model system for examining brain development.

Keywords

neural development; somatosensory system; barrels

Introduction

The brain is an anatomically and functionally regionalized structure. Different areas of the neocortex, for instance, serve distinct sensory or motor functions, and have regionally specialized anatomical structures. In the somatosensory cortex, a dense “granular” cell layer (layer 4) receives input from the sensory periphery relayed via the thalamus, while the motor cortex lacks thalamic input and a granular layer but projects densely to the spinal cord through pyramidal neurons in an expanded layer 5. Within the somatosensory cortex, a diverse assortment of animals with large facial whiskers have specialized modules or “barrels” in layer 4 that contain neurons that respond selectively to stimulation of one whisker on the contralateral snout. The spatial arrangement of barrel modules in the somatosensory cortex recapitulates the arrangement of whiskers on the face. First, described by Woolsey and Van der Loos[1], a typical barrel in mouse cortex is oval shaped with cell-dense sides that surround a relatively cell-sparse hollow that contains neuropil, connecting thalamocortical afferents and dendrites of layer 4 neurons. Detailed barrel morphology differs slightly between species[2, 3], but they can be visualized with a variety of histology methods including cytochrome oxidase (CO), Nissl stain (Fig. 1) or markers for thalamocortical afferent terminals, such as VGlut1 or SERT.

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Because of its unique and distinct neuroanatomy, barrel cortex has been widely used in the last several decades as a model system to study cortex development, neuronal plasticity, and sensory motor integration. Despite this substantial attention, we still do not know the basic mechanisms responsible for the initial formation of the barrel pattern, and a substantial debate persists about the relative importance of intrinsic and extrinsic cues in barrel development. The application of modern molecular–genetic techniques in mice has yielded substantial clues, however, and barrel phenotypes have been described in a wide variety of mutant mice. In this review, we summarize our current understanding of the mechanisms responsible for the development of somatosensory barrel maps, highlight recent advances, and discuss fundamental remaining gaps in our understanding of the development of this canonical cortical sensory map. We will first examine how somatosensory cortex develops and how thalamocortical axons (TCAs) find their way to somatosensory cortex. We will then examine in some detail how the barrel pattern gets constructed using clues from genetically modified mice. We will end with a brief overview of how barrel-related patterns are assembled in the brain stem (barrelettes) and thalamus (barrelloids).

Somatosensory cortex (SI) specification and thalamocortical axon growth

Somatosensory cortex area specification

How is the area that is to become somatosensory cortex specified? Although still under debate, it is generally accepted that a combination of intrinsic genetic programs within cortex and extrinsic information relayed by thalamocortical afferents specifies the gross cytoarchitectural areas of cortex and establishes clear boundaries between brain areas[4]. Evidence in favor of an intrinsic genetic program comes from analysis of Gbx2 and Mash1 knockout mice in which thalamic axons fail to innervate the cortex, but the graded expression pattern of three classes of transcription factors (Lhx2, SCIP, and Emx1) and cadherins (Cad6, Cad8, and Cad 11) remains unchanged[4–6]. Further examination of intrinsic mechanisms in cortical arealization reveals a complex hierarchy of genetic regulatory mechanisms involving morphogens and transcriptional factors. Mophogens and signal molecules secreted by cortical patterning centers, such as fibroblast growth factors (FGFs) from the commissural plate, bone morphogenetic proteins (BMPs), and vertebrate orthologs of Drosophila wingless (WNTs) from the cortical hem[4] define the initial anterior–posterior and medial–lateral cortical axis. FGF8, in particular, is a key morphogen that determines the anterior-posterior position of somatosensory cortex. FGF8 is normally expressed in the anterior part of the forebrain, but introducing an exogenous posterior source of FGF8 produces a partial area duplication as evidenced by the presence of a second “ectopic” somatosensory barrel field[7]. FGF8 controls frontal/motor cortical area fate by regulating the expression of Emx2 and COUP-TFI and other transcription factors within cortical cell progenitors[4]. In summary, intrinsic genetic programs determine cortical arealization early in development, before thalamocortical axons (TCA) reach and innervate the cortical plate. Once TCAs reach their specific cortical target, the functional specification of primary sensory areas depends heavily on TCA input.

Communication between thalamocortical afferents and the developing cortical plate is essential in area pattering during later (postnatal) development. Various manipulations of the

sensory periphery induce plastic changes not only in the size of the sensory cortical area but also in cortical neuron cytoarchitecture and the thalamocortical projections[8]. A fundamental role for extrinsic signals in influencing cortical area patterning was demonstrated by several heterotopic transplant experiments. For example, transplants of embryonic visual cortex into the barrel field of parietal (S1) cortex leads to the emergence in the transplanted cortex of characteristics of barrels and patterned expression markers that are unique to S1 barrel field[4,19]. However, other transplant experiments support the dominant role of an intrinsic program in determining arealization. These studies use a transgenic enhancer trap mouse line, H2Z1, a mouse somatosensory cortex specific marker, driving lacZ expression[6, 10, 11]. When grafting prospective somatosensory cortex into other brain regions of the cortex, lacZ expression is maintained[11]. Neonatal thalamic lesions in these mice later in development demonstrate that the initial expression of barrel-specific cortical markers are independent of TCAs, but the maintenance of this expression after birth depends on intact thalamic innervation[12]. Thalamocortical afferents display area specific behavior even very early in development, before the functional differentiation of cortical neurons. The molecular mechanisms controlling area specific thalamocortical axon targeting are still not well known, but intracortical and subcortical axon guidance cues likely play a major role, as described in the next section.

Thalamocortical axon path finding

Thalamocortical axon path finding relies on axon guidance factors expressed in intermediate targets and the developing cortex. Limbic associated membrane protein (LAMP), Coup-TFI, cad6, cad8, cad11 as well as ephrin/Eph receptors, which are expressed in different brain regions, assist in the navigation of thalamocortical axons to their cortical targets[13]. Most of these proteins are multifunctional during development, coordinating cortical patterning and specific thalamocortical axon targeting. Thalamic axons in mice with a second barrel field induced by an ectopic source of FGF8[7] (described above) faithfully track changes in area position and innervate the duplicated somatosensory barrel field, indicating that thalamic axons use cortical area identity information to regulate their ingrowth[9]. During embryonic development (about E13.5), thalamic axons leave the dorsal thalamus, descend among ventral thalamic cells, and cross the boundary between the diencephalon and telencephalon. TCAs then extend through the medial and lateral ganglionic eminences (MGE and LGE, respectively), and continue to grow tangentially toward their cortical targets[6, 13]. A recent report[14] indicates that thalamocortical axon path finding depends on tangential migration of neurons from the LGE to MGE. Cells generated in the LGE function as corridor cells expressing membrane bound isoforms of Nrg1 (CRD-Nrg1or type III NRG1) provide a permissive corridor for TCA growth cones in a non-permissive environment. Netrin-1 is a well-known and intensively studied axon guidance factor that is expressed in the ventral telencephalon when thalamocortical axons are navigating through this region on their way to cortex. *In vitro* assays demonstrate that netrin-1 acts as a chemoattractant for TCAs and promotes thalamocortical axon growth through the ventral telecephalon[15]. This suggests that the topographic organization of thalamocortical axons in ventral telecephalon is controlled by netrin1. Due to the differential expression of chemoattractant and chemorepellent receptors for netrin-1 in rostral and caudal thalamocortical axons, topographic sorting of thalamocortical axons occurs immediately after they leave the dorsal thalamus[16]. Slit is also

implicated in the guidance of thalamocortical axons towards the cortex, as slits are chemorepellent for TCAs, endogenously expressed in the hypothalamus, and appear to prevent TCAs from entering the region of the hypothalamus and crossing the midline[17, 18].

Once TCAs reach the cortex, they interact with subplate (SP) neurons, which are among the first born cortical neurons and reside at the white matter/cortical plate boundary[20]. SP neurons serve as early "pioneers" in rodent sensory map formation, projecting subcortically to meet TCAs in the primitive striatum and superficially to transiently innervate layer 4[20–22]. Subplate neuron defects lead to failures in thalamocortical axon guidance and innervation of the cortex, suggesting that SP neurons coordinate TCA targeting and patterning in the cortex[20, 23]. As TCAs enter the cortex, they appear jumbled, but become arrayed in orderly bundles as they course radially into layer 4 and form whisker-related barrel patterns[24, 25]. The first synaptic contacts formed by thalamocortical axons within cortex are in subplate[13, 20]. The initial communication between subplate neurons and TCAs might be important for correct axon targeting and topographic map formation. When blocking neuronal activity with tetrodotoxin (TTX) at the same time as the arrival of thalamocortical axons to the subplate, only a few thalamic fibers are able to enter layer 4 of visual cortex[13, 26]. This result suggests that thalamocortical axon targeting depends on early neuronal activity.

Mechanism of cortical barrel formation

Barrels form once thalamocortical axons and cortical sensory areas settle into place in the first week after birth. In mice, a barrel is composed of presynaptic TCA clusters within a cell sparse barrel hollow surrounded by postsynaptic layer 4 neurons organized into a ring-like barrel wall that selectively orient their dendrites into the barrel hollow to form synapses with TCAs. The exact barrel form, assayed with Nissl histochemistry, varies between species[2, 3, 33]. Barrels can be hollow, solid, or somewhat indistinct. Barrels in mice are typically described as hollow, with cell dense walls separated by a cell-sparse "septa" or gap between neighboring barrels. Although rat barrels are composed of a solid, cell dense cluster of layer 4 neurons, which is somewhat different from the mouse, the overall pattern of the cortical barrel array in rat is quite similar to the mouse[33]. Indistinct barrels are found in some species, such as the squirrel and rabbit, and individual barrels are difficult to distinguish from each other in these animals[33]. Here we will discuss mechanisms of barrel formation in the mouse, as detailed mechanistic studies are now largely carried out in this species because of the ready availability of molecular genetic manipulations.

The role of peripheral input activity in barrel formation

Intact facial whiskers are clearly necessary for barrel formation. Cutting the infraorbital branch of the trigeminal nerve at birth completely disrupts the barrel pattern and results in reduced TCA arborization and broad terminal arbors[28]. When individual vibrissae are carefully injured at birth, the corresponding barrels are missing later in development[27], and mice with "supernumary" whiskers have extra barrels in the appropriate topographic location in the cortex[29]. Thalamic axons from somatosensory thalamus (VPM) that innervate embryonic visual cortex that is transplanted into presumptive somatosensory cortex express appropriate area-specific molecules and also produce barrels[19]. These experiments show that

input from the sensory periphery has an important influence on the structure of the developing cortex, and suggest that peripheral neuronal activity is required for barrel formation. However, other experiments argue against an essential role for neuronal activity in the formation of barrels.

When blocking peripheral neuronal activity using a polymer that slowly releases tetrodotoxin (TTX), a voltage-dependent sodium channel antagonist that prevents action potential propagation, barrel patterns assayed with CO histochemistry in the trigeminal brainstem complex, thalamus, and barrel cortex are normal[30]. A similar approach used to block cortical activity postnatally with TTX[31] or the NMDA receptor antagonist APV[32] also had no effect on the vibrissa-related barrel pattern in somatosensory cortex. Although these pharmacological manipulations suggest that barrel formation does not depend on neuronal activity, they do not explain how peripheral nerve injury and embryonic transplants (described above) have the observed affect on barrel development. To clarify these questions requires precise models that can eliminate peripheral neuronal activity or other peripherally derived factors[74] efficiently and specifically. Recent advances in molecular–genetic manipulations, described in the next section, may enable us to gain a deeper understanding into the mechanisms of barrel formation.

Molecular genetic studies for barrel formation

Adenylyl cyclase type 1 (*Adcy1 or AC1*) was the first gene identified to be critical for barrel map formation. The spontaneous mutant line, *barrelless* (*brl*), which has no AC1 expression, was discovered serendipitously[34]. Adcy1 is a calcium activated adenylyl cyclase that catalyzes the formation of cAMP, a promiscuous second messenger whose primary target is cAMP-dependent protein kinase (PKA) [35]. *Brl* mice lack spatially segregated clusters of thalamic afferents into individual barrels in the cortex, but display normal gross topographic organization[36]. The size of individual whisker representations in *brl* mutant mice, as judged by 2-deoxyglucose uptake, is similar to that of wild-type mice[34]. However, *in vivo* single-unit recordings in *brl* mice reveal abnormal short latency responses to stimulation of surround whiskers, which is consistent with an altered and enlarged cortical representation of the sensory periphery[34]. These results indicate that the development of coarse topographic order in the somatosensory cortex can and does occur without the segregation of thalamic inputs into clusters, but the formation of barrels do appear to be required for the discrete spatiotemporal relay of sensory information to the cortex. AC1 is expressed throughout the trigeminal pathway, but experiments on cortex-specific AC1 knock-out mice indicate barrels in cortex are grossly normal, though thalamocortical synapse function and plasticity are degraded[42]. These results suggest that AC1 in thalamocortical neurons, but not in the cortex, plays a critical role in cortical barrel formation, but exactly how AC1 regulates barrel formation remains a mystery.

Following the discovery of *brl* mice, somatosensory cortex barrel map defects have been observed in a number of additional lines of mutant mice. Many of these experiments have used a conditional knock-out approach to generate further detail about the role of specific genes in barrel map formation. Knock-out mice that lack functional NMDA receptors only in cortical excitatory neurons (Cx-NR1KO) have significant cortical barrel map defects[38].

The layer 4 neuron cellular aggregates that normally form barrels in somatosensory cortex do not develop in Cx-NR1KO mice, though thalamocortical afferent clustering is largely normal, as are barrelettes in the brain stem and barrelloids in the thalamus[38]. Mice that lack the type IIβ regulatory subunit of PKA (PKARIIβ KO mice) have similar postsynaptic barrel map defects as Cx-NR1KO mice[38, 39], and PKARIIβ is expressed postsynaptically at thalamocortical synapses. TCA clustering in both PKARIIβ KO mice and Cx-NR1KO is normal (Fig. 2), suggesting that proper layer 4 barrel organization can be disrupted independent of a gross defect in TCA patterning[6]. PKA is a principal downstream signaling molecule for AC1, and AC1 is activated by elevated intracellular Ca^{2+}, which may be provided by NMDA receptors at thalamocortical synapses. NMDA-AC1-PKA signaling may be a central pathway in the regulation of barrel formation. It is interesting to note that this same signaling pathway is also a key regulator of synaptic plasticity[75]. Although barrel map development and plasticity involve many of the same signaling pathways, we lack conclusive evidence that these events are linked[37, 40, 41].

Serotonin (5-HT) signaling has also been strongly implicated in barrel development. 5-HT in the cortex originates from axon terminals of raphe nucleus neurons in the brain stem. At thalamocortical synapses, 5-HT is taken up by TCAs from the extracellular space via 5-HT transporters (5-HTT)[6,43, 44]. 5-HT is then packaged into synaptic vesicles via the vesicular monoamine transporter, VMAT2[6,44,45]. 5-HT not packed into TCA terminals is degraded by an enzyme, monoamine oxidase A (MAOA). This residual 5-HT left in the thalamocortical synaptic cleft can activate the G-protein coupled 5-HT1B receptor expressed on TCAs. 5-HT1B, 5-HTT, and VMAT2 are all expressed during the first three weeks postnatally in thalamocortical axon terminals, suggesting they may play a significant role in thalamocortical synapse development[6,43, 44, 46]. A comprehensive analysis of mice with mutations in genes coding for one or more of these proteins provides interesting results. Barrels do not form in single or double knockouts of 5-HT and MAOA, but barrels can be rescued by knocking out 5HT1B in 5-HTT and MAOA single or double mutants. This indicates that 5-HT1B is essential for the disruption of barrels in 5-HT signaling mutants. However, 5HT1B mutant mice themselves have normal barrel maps, suggesting that 5-HT signaling is not normally necessary for barrel formation. In addition, pharmacological hyper-activation of 5-HT1B receptors *in vivo* produces abnormal barrel patterns. These experiments indicate excessive 5-HT in the synaptic cleft, acting through 5-HT1B receptors on TCAs, produces barrel map defects in these mutant mice[47]. Moreover, 5-HT may directly modulate thalamocortical synaptic transmission[48, 49], or it may act through AC1, since presynaptic 5-HT1B negatively regulates the activity of AC1 *in vitro*[50, 51]. Thus, 5-HT signaling in barrel map formation may converge to the AC1-cAMP-PKA signaling pathway (see Fig. 3) to modulate barrel map development.

mGluR5 and PLCβ mutant mice have similar postsynaptic barrel map defects as Cx-NR1KO and PKARIIβ KO mice [53, 54] (Figure 2) and are also thought to act in a common signaling pathway[52]. Recent reports examining thalamocortical synaptic transmission *in vitro* in mGluR5 mutant mice show that presynaptic thalamocortical and AMPA receptor-mediated functions are normal, but the kinetics of NMDA receptor dependent currents is faster in the mGluR5 mutant mice[54, 55]. Moreover, intracortical inhibitory synaptic development appears

to be disturbed in mGluR5 mutant mice[55]. The interaction between mGluR5 and cAMP-PKA signaling has been demonstrated by several studies[56–58]. During thalamocortical synapse development, mGluR5 may be upstream of the AC1- cAMP-PKA signaling pathway, or mGluR5-PLCβ signaling may operate in parallel to the NMDA-cAMP-PKA signaling pathway in the regulation of barrel formation.

Another classical downstream pathway for PLCβ mediated G protein coupled signaling is the Ras-GTPase-MAPK/Erk pathway (Fig. 3). Several recent reports examining mouse mutants in this classical signaling pathway further implicate it in barrel map formation. Mutating the neurofibromatosis type 1 (*NF1*) gene in a majority of cortical neurons and astrocytes prevents the formation of cortical barrels in the somatosensory cortex, but the segregation of thalamic axons into barrel clusters within the somatosensory cortex appears unaffected[59]. The NF1 gene encodes a large protein that contains a Ras GTPase-activating (GAP) domain that negatively regulates Ras activity. Germline mutant mice for SynGAP, another Ras-GAP-containing protein, also lack cortical barrels[60]. These studies suggest that Ras signaling plays an essential role in barrel formation. Despite the similar barrel phenotypes in NF1 mutants with the other postsynaptic proteins described above (NR1, AC1, etc.), there is no difference in expression levels of NR1, PLCβ, SynGAP, or the PKA-RIIβ proteins in NF1 conditional knockout mice. Instead, levels of phospho-Erk are significantly increased[59], indicating that the Ras–mitogen-activated protein kinase (MAPK) pathway is constitutively activated in NF1 mutant mice, which may be the cause of their barrel phenotypes. This is quite distinct from the AC1-cAMP-PKA mutants, which argues against crosstalk between these two signaling pathways, though this needs to be carefully elucidated.

Proteins that are involved in barrel map formation based on the presence of barrel defects in mutant mice, but for whom specific mechanisms remain uncertain, are GAP-43, NeuroD2 and LMO4 [6, 61–64]. GAP-43 is a membrane bound phosphoprotein whose detailed cellular function of the protein is still not very clear, though it has been implicated in neurite outgrowth and synaptic transmission. Indeed, GAP-43 mice show reduced thalamic innervation of barrel cortex and decreased paired-pulse ratio and increased evoked AMPAR miniature currents in GAP-43 KO thalamocortical synapses indicating GAP-43 regulates neurotransmission release[62]. NeuroD2 and LMO4 are the only two transcription factors identified thus far that regulate barrel map formation. Both are activity-regulated and respond to increases in intracellular Ca^{2+} [63, 64]. In NeuroD2 null mice, thalamocortical axon terminals fail to segregate in the somatosensory cortex, and postsynaptic barrel organization is also disrupted. NeuroD2 may act through GAP-43, as the GAP-43 promoter has a binding site for NeuroD2 and expression of GAP-43 in NeuroD2 knock out mice is reduced [63]. Similarly, thalamocortical afferents in cortex-conditional LMO4 null mice fail to segregate into distinct clusters and the cortical layer 4 neuron barrel pattern is disrupted. LMO4's activity is regulated by CaMKIV and the MAPK pathway, but the exact mechanism for how LMO4 regulates barrel map formation is not known.

Mechanisms of barrelloid and barrelette formation

The organization of the whisker representation within the neocortex is dependent on intact subcortical barrel patterning in the brain stem (barrelettes) and thalamus (barrelloids). To date, molecular mechanisms involved in barrelette formation include neurotrophic factors, glutamatergic receptors, and transcription factors[72, 73]. Transcription factor lmx1b, and its down stream factor, Drg11, have been studied in detail about their role in barrel formation in brain stem. Barrel pattern in principal sensory nucleus (PrV) failed to form in knock out studies of either gene[65–67]. There is massive cell death of PrV neurons in Drg11 knock out mice. Interestingly, it does not account for malformation of barrelettes[67]. These mice have disrupted barrel patterns in PrV, VPM, and barrel cortex, but intact barrel pattern in SpV, [65] probably because Drg11 is expressed in PrV but not in the barrelette-forming components of SpV interpolaris and caudalis [65, 67, 68]. Although both SpV and PrV receive trigeminal ganglion neurons inputs, mechanisms regulating their patterns formation could be different. Other transcription factor that has been found important for barrelettes formation is Hoxa2, an isoform of Hox transcription factor family. Selective inactivation Hoxa2 in rhombomere 3 where whisker-related maps present in the brain stem results in lack of barrelettes formation[69]. Furthermore, sharing the same concept with barrel cortex, glutamatergic receptors are also important for barrel patterning in brain stem and thalamus. NMDA receptors are predominant glutamatergic receptors at the time of barrelettes forming. Barrelettes do not form in the absence of NR1 and NR2B[70, 71]. Molecular mechanisms for barrrelloids have not been determined specifically, however, similar rules from barrellets and barrel cortex studies could apply to mechanistic study of barrelloids formation.

Conclusions and perspectives

Molecular mechanisms known to underlie barrel map formation in somatosensory cortex can be summarized into two major cellular signaling pathways (Fig. 3): The cAMP-PKA pathway and the Ras-GTPase-MAPK pathway. The activation of NMDA receptors, AC1, and 5HT signals converge onto the cAMP-PKA pathway, whereas mGluR5-PLC1β, NF1/SynGAP, and LMO4 appear to converge onto the Ras-GTPase-MAPK pathway. These two major signaling pathways have some crosstalk, as G-protein receptors can regulate AC1-cAMP levels, and the activity of LMO4 is sensitive to Ca^{2+} concentration, which is influenced by NMDA receptor activation. Both pathways may eventually regulate the transcription and function of a common series of proteins that are responsible for the regulation of axon clustering, layer 4 neuron aggregation into barrel walls, and the tendency of developing layer 4 neuron dendrites to orient toward barrel hollows.

The molecules and genes known to be involved in barrel map formation continue to expand, and many questions remain unanswered. What is the interplay between molecular mechanisms involved in barrel development and neuronal activity? The bundling of thalamic axons into barrel-like patterns appears to be necessary for cortical neurons to reposition their cell bodies into barrel walls and orient their dendrites into barrel hollows. This suggests that communication between the presynaptic thalamocortical afferent and postsynaptic layer 4 neuron is required for barrel formation. In what form does this communication take place? It is reasonable to argue that neuronal activity itself provides this

link and is playing an instructive role for barrel formation, although it is conceivable that some other molecule, trophic factor, or even transcription factor plays this role[74]. Moreover, despite decades of intense experimentation, we have not identified any specific functional role for barrels, nor attributed specific functional lapses to their absence *in vivo*.

To answer these questions will require integrating genetic, molecular, anatomical, and *in vivo* functional and behavioral studies in the mouse. The highly ordered representation of facial whiskers in rodents is impressive; the anatomical organization of the somatosensory system in the mouse rivals that of any sensory system in higher animals, including human. It is reasonable to expect that a fundamental understanding of the development and function of barrels in mouse somatosensory cortex will also provide important insights into the mechanisms underlying the development, structure, and function of the human neocortex.

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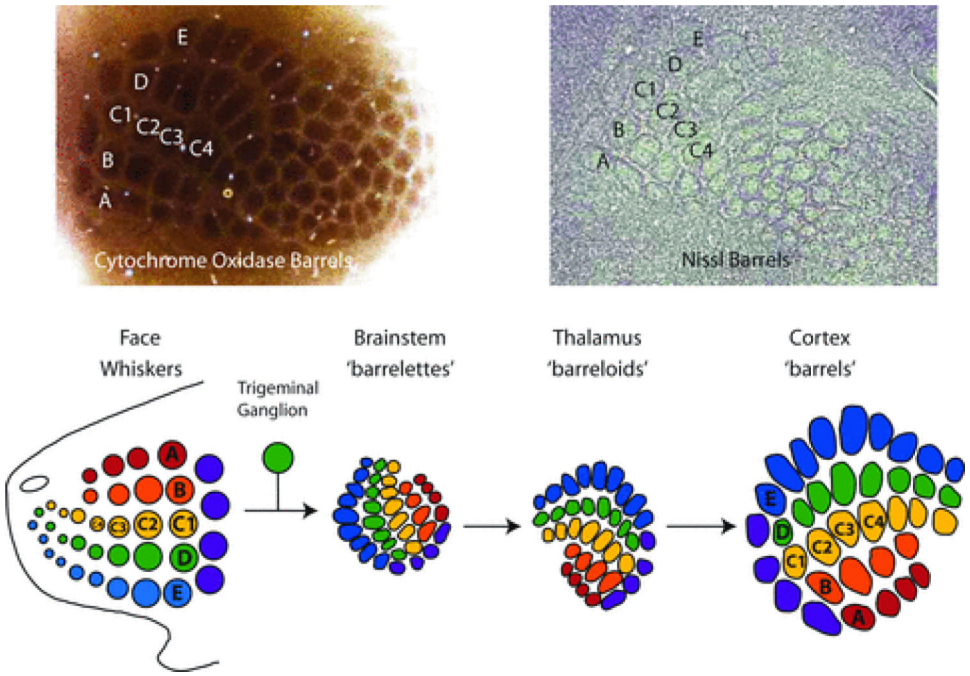

Figure 1.

Barrels can be visualized by cytochrome oxidase (CO) and Nissl histochemistry. (A) CO staining in flattened tangential section through layer 4 showing the barrel field. (B) Nissl histochemistry showing postsynaptic layer 4 neurons segregate into barrels with cell-dense walls and cell-sparse hollows. (C) The spatial arrangement of whiskers on the face are recapitulated in the brain stem as “barrelettes,” in the thalamus as “barreloids,” and in the somatosensory cortex as “barrels.”

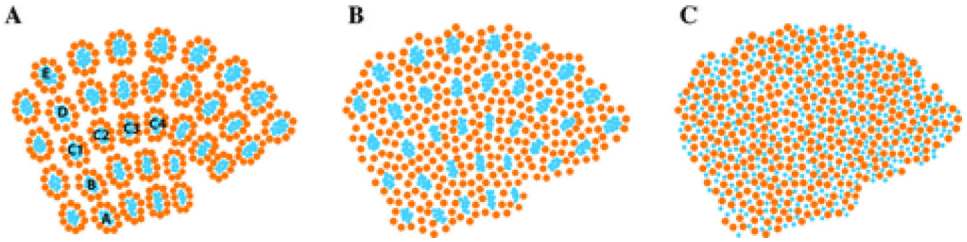

Figure 2.

Schematic barrel phenotypes in various mutant mice. (A) Barrel pattern in wildtype (WT) mice. Blue is thalamocortical (TC) axons clustered in the center of barrels (similar to the CO pattern). Orange is layer 4 cortical neurons arranged into barrels, which ring the TC afferent clusters. (B) In a group of barrel mutants, such as Cx-NR1 knock out (KO), mGluR5 KO, PLC-β KO, and PKARIIβ KO mice, TC axon clustering into a barrel pattern is intact (blue), but layer 4 barrel walls are missing (orange). (C) In a group of severe barrel mutants, all barrel features appear to be missing, including TC afferent clustering and layer 4 barrel walls. Mutants of this type include AC1 KO, 5-HTT KO, MAO KO, GAP-43 KO, NF1 KO, NeuroD2 KO, and Cx LMO4 KO mice. Note that no known mutant has intact layer 4 (Nissl) barrels but absent TC afferent (CO) barrels.

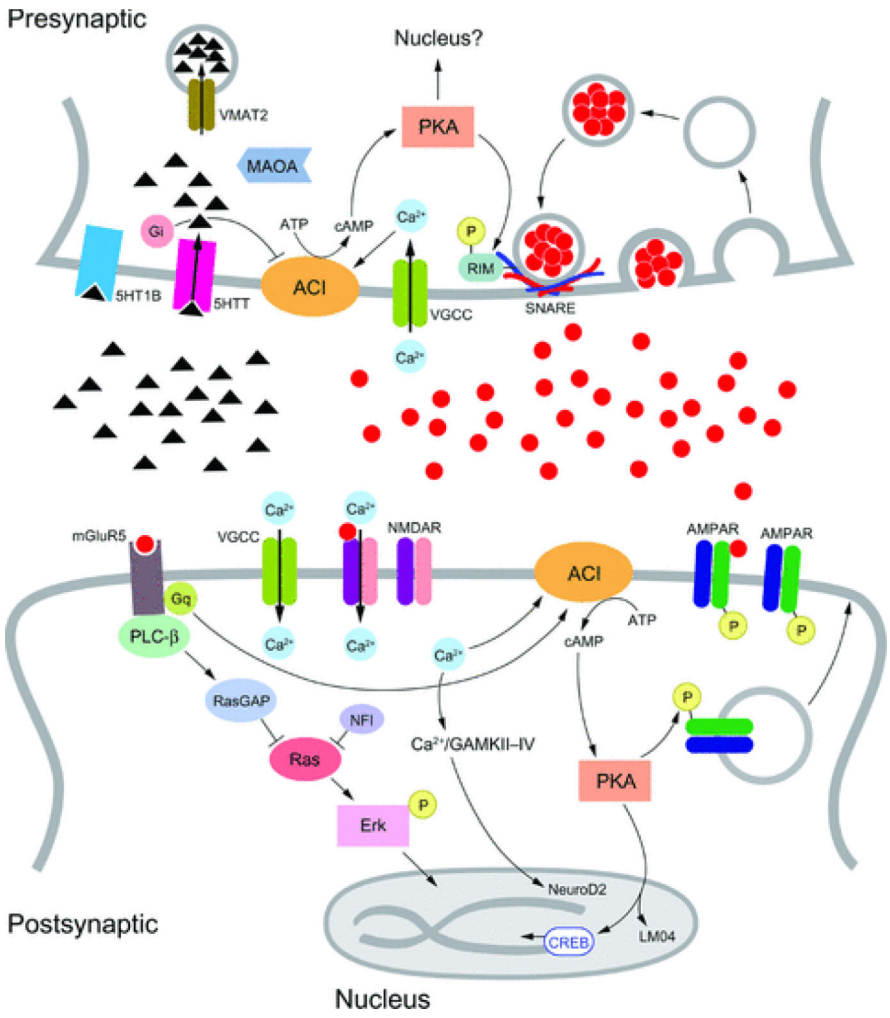

Figure 3.

Pre- and postsynaptic mechanisms of barrel map formation at thalamocortical synapses. Presynaptic: action potentials activate voltage-gated Ca2+ channels (VGCC). The increase in Ca2+ concentration at axon terminals triggers glutamate neurotransmitter release. Intracellular Ca2+ also activates adenylyl cylase 1 (AC1), which catalyzes the formation of cAMP. cAMP, in turn, activates PKA. PKA regulates neurotransmitter release directly via the phosphorylation of RIM1α.61 In addition, PKA may regulate thalamocortical axon clustering into a barrel pattern via changes in the expression of a number of proteins through modulation of nuclear transduction. 5-HT at the synaptic cleft is released by nerve terminals originating in the raphe nuleus. 5-HT can be transported into the axon terminal by 5-HTT and packaged into vesicles by VMAT2. Excess intracellular 5-HT is degraded by MAOA. The presence of 5-HT1B receptors on thalamocortical axon terminals, when activated by binding to extracellular 5-HT, may negatively regulate AC1 activity via G-proteins. Postsynaptic: two major signal pathways regulating barrel map formation occur in layer 4 postsynaptic neurons: AC1–cAMP–PKA and Ras–GAP–MAPK. Glutamate released from presynaptic terminals activates glutamate receptors, including mGluRs, AMPARs, and NMDARs. The simultaneous binding of glutamate and postsynaptic depolarization activates

NMDARs, allowing Ca2+ to flow through NMDARs and VGCCs. The increased Ca2+ concentration thereby activates postsynaptic AC1 and PKA. PKA phosphorylates AMPARs, facilitating AMPAR traffic to the membrane. mGluR5 activates PLCβ (phospholipase C-β), which acts through the Ras–GAP–Erk(MAPK) pathway. mGluR5s may interact with the AC1–cAMP–PKA signal pathway via Gq proteins. Neurofibromatosis type 1 (NF1) is one of a family of Ras–GAP proteins that negatively regulates Ras–Erk (MAPK). Together with NeuroD2 and LMO4, signals converge into the nucleus, influencing the expression of proteins in cortical neurons responsible for their aggregation into barrel walls and orienting their dendrites toward barrel hollows.