The Role of Neural Activity in Cortical Axon Branching

NAOFUMI UESAKA, EDWARD S. RUTHAZER, and NOBUHIKO YAMAMOTO

Axonal branching is an important process for establishing the final pattern of connections between a neuron and its target cells. Cortical connections between upper-layer cells in the neocortex have provided insights into the cellular mechanisms by which electrical activity regulates neural connectivity, including branch formation. Recent evidence further indicates that spontaneous firing and synaptic transmission contribute to axonal branching of cortical neurons through postsynaptic activation. NEUROSCIENTIST 12(2):102–106, 2006. DOI: 10.1177/1073858405281673

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During development, axons grow along specific pathways, reach their target zones, and form branches to make synaptic contacts with a variable number of cells. The territory and density of axonal branching are the first determinants of the pattern and strength of neuronal connectivity.

A characteristic feature of branch formation is stereotyped branching, which is found in topographic and layer-specific projections. These stereotyped branching patterns are thought to be regulated by genetically specified molecules expressed in particular locations and developmental stages (reviewed in Sanes and Yamagata 1999; Yamamoto 2002). However, such molecular expression is not the only determinant of axon branching. Since the pioneering work of Hubel and Wiesel on the effect of visual deprivation on the developing mammalian visual cortex (Hubel and others 1977), it has been demonstrated that electrical activity contributes to neural connectivity including axonal branching (reviewed in Katz and Shatz 1996; Erzurumlu and Kind 2001; Ruthazer and Cline 2004). These studies have been performed primarily in thalamocortical projections and retinotectal projections in vertebrate systems. Intracortical connections, in particular, the connection between layer 2/3 cells, are also a suitable system with which to explore this issue, inasmuch as its connection pattern, function, and development have been well examined. In addition, this connection has been shown to be highly plastic (reviewed in Foeller and Feldman 2004). In this article, we review the mechanisms by which neural activity regulates axonal branching, focusing on horizontal connections in upper-layer neurons.

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Horizontal Axon Branching during Development

Pyramidal neurons in layers 2/3 project long axon collaterals horizontally (horizontal axons), which form synaptic connections with cells in the same cortical layer. In the visual cortex, these horizontal connections are thought to interconnect columns with similar orientation preference (Gilbert and Wiesel 1989).

During development, horizontal axons extend initially in all directions in layer 2/3 and begin to form branches. As development proceeds, branch morphology is altered: Some axons have complicated branching, whereas other branches appear to be eliminated (Callaway and Katz 1990). Finally, a few horizontal axons establish elaborate terminal arbors. These aspects suggest that horizontal axon branching is an ongoing, dynamic process that takes place continuously throughout postnatal development. We recently demonstrated this dynamic property in an organotypic slice culture of the cortex by labeling a small number of layer 2/3 cells with a fluorescent protein (Fig. 1). Most branches grew or retracted for several days after initial branch formation. Even in later stages, more than 80% of branches still exhibited growth or retraction, although the remaining ones became relatively stable. Similar dynamic axon branching has been also shown in the retinotectal projection (O'Rourke and Fraser 1990). Thus, the formation of precise connection patterns seems to be sculpted by dynamic branch behavior.

Involvement of Activity in Horizontal Axon Branching

During postnatal stages while horizontal axons exhibit dynamic aspects of branching, various sensory stimuli are

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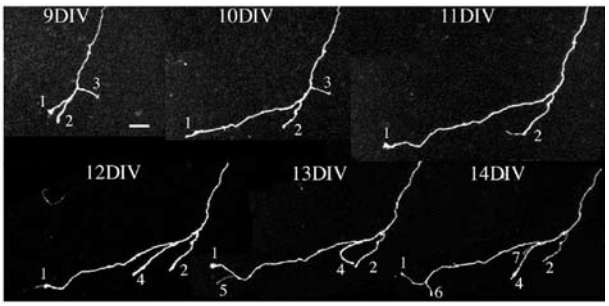

Fig. 1. Dynamics of horizontal axon branching. Axon terminal of upper layer neurons in an organotypic culture was imaged daily over six days (9 days in vitro to 14 days in vitro). This axon was labeled with yellow fluorescent protein (YFP) by introducing a YFP-containing vector (from Uesaka and others 2005). Note that branches show dynamic behavior both extending and retracting. Scale bar represents 50 μm. In each panel, the *bottom right* is pial side and the *top left* is ventricular side. From Uesaka and others (2005) with permission from the *Journal of Neuroscience*.

received from the outside world. An attractive hypothesis is that neural activity induced by sensory stimuli influences neuronal connections including branch and synapse formation. Several studies have demonstrated that sensory-evoked firing activity affects the development of horizontal axons (Callaway and Katz 1991; Lowel and Singer 1992; Ruthazer and Stryker 1996; Keller and Carlson 1999). For example, binocular lid suture after the time of normal eye opening leads to horizontal connections that are less well clustered than in normal animals (Callaway and Katz 1991). Prior to the stages when sensory-evoked activity is necessary for horizontal connection refinement, spontaneous firing activity has been shown to affect horizontal connections. The early emergence of clustered horizontal connections, which occurs in the absence of retinal inputs, nonetheless requires neuronal activity in the cortex because the clustering of horizontal connections was not observed after intracortical infusion of tetrodotoxin (TTX), a sodium channel blocker (Ruthazer and Stryker 1996). The requirement of spontaneous activity for the refinement process has been documented in other CNS areas (Penn and others 1998; Hata and others 1999; for review, see Feller 1999) and may represent a common feature in precise neuronal circuit formation.

We have demonstrated a role of neural activity in branch formation by observing single-labeled horizontal axons in cultured cortical slices for more than a week (Uesaka and others 2005). Simultaneously, development of spontaneous firing activity was examined using multielectrode culture dishes with which electrical activity of cortical neurons can be recorded chronically. Spontaneous activity appeared after around one week in vitro when horizontal axon branching first emerged. In later stages, when axonal branches were more elaborate but still dynamic, the firing rate of spontaneous activity notably increased and was maintained (Fig. 2). Thus, there is a strong correlation between the occurrence of firing activity and branch formation. Further evidence clearly showed that firing activity was required for horizontal axon branching. Complete blockade of spontaneous activity by the addition of TTX to the culture medium resulted in a dramatic decrease in axonal branching (Fig. 3). In other words, neural activity promotes branch formation of horizontal axons.

However, it is not commonly reported that electrical activity increases axonal branching. It has been shown that activity blockade causes an increase of branch dynamics and expansion of axonal arbors in retinotectal and thalamocortical projections (Antonini and Stryker 1993; Cohen-Cory 1999). This paradoxical effect on axonal branching in vitro might be attributed to different patterns of neural activity. In the culture conditions, synchronous activity was found rather frequently in the cortical explant. As exemplified in Figure 4, firing activity was quite synchronized between the cortical cells that were located in different places. On the other hand, it is likely that firing activity is less synchronous when external specific inputs such as sensory inputs are present (Chiu and Weliky 2001). Such synchronous and asynchronous activity may influence the degree of correlated firing between presynaptic axons and postsynaptic cells (see below).

The Role of Firing and Synaptic Activity in Axonal Branching

A plausible mechanism for activity-dependent axonal branching is that the degree of correlated and uncorrelated activity between pre- and postsynaptic cells may regulate the strength of connectivity. Correlated activity could reinforce connections by increasing branching and subsequent synaptic contacts. Long-lasting changes in synaptic efficacy or long-term potentiation (LTP) of synapses can be induced in this manner in many regions of the brain, including the cortex (reviewed in Foeller and Feldman 2004). Although it has not been demonstrated that synaptic changes really lead to formation and stabilization of axonal branches, a few studies suggest that horizontal connections in layer 2/3 in the visual cortex are regulated by correlated and uncorrelated patterns of neural activity, which may induce LTP and long-term depression, respectively (Lowel and Singer 1992).

Many studies show that the N-methyl-D-aspartate (NMDA) type glutamate receptor is responsible for detecting correlated activity between pre- and postsynaptic cells and is required for the induction of LTP (reviewed in Malenka 1994). In the somatosensory cortex, NMDA receptors play a role in the formation of whisker-specific neural patterns (reviewed in Erzurumlu and Kind 2001). Moreover, there is direct evidence that axonal branching depends on NMDA receptor activation (reviewed in Ruthazer and Cline 2004).

In addition to involvement of NMDA receptors, we suggested that a mechanism mediated by non–NMDA receptor activation contributes to branch formation of horizontal axons (Uesaka and others 2005). In the study of horizontal axon branching in culture, blockade of non-NMDA or α-amino-3-hydroxy-5-methyl-4-

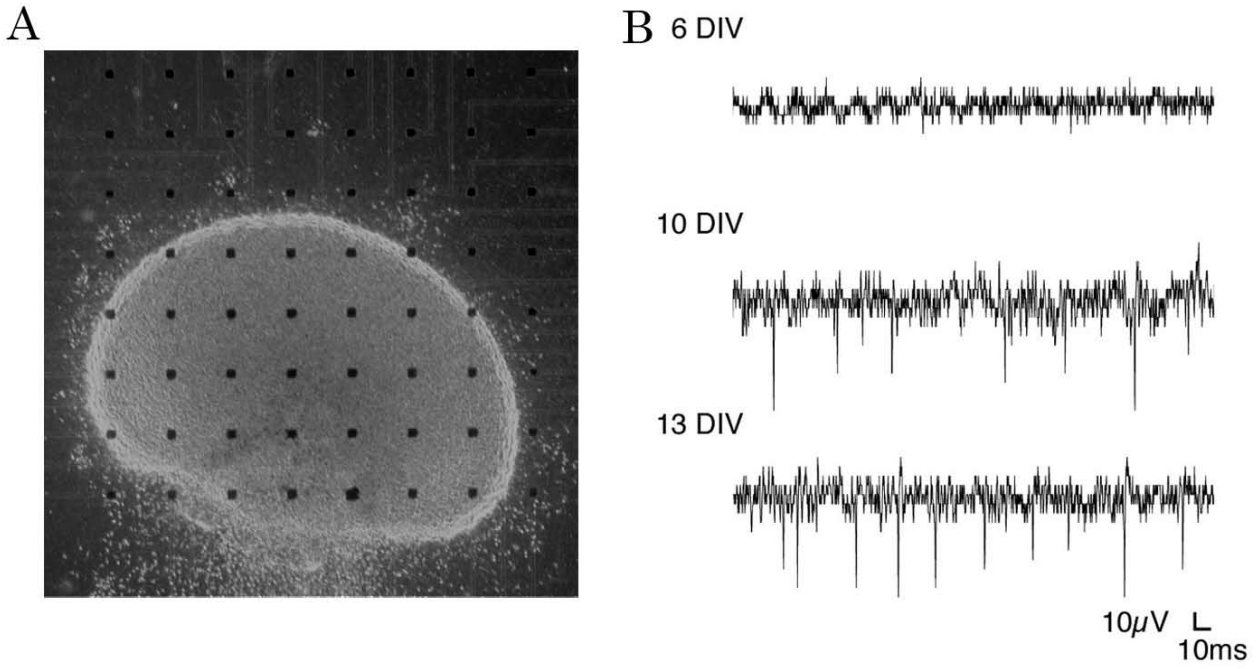

Fig. 2. Development of spontaneous activity of upper layer neurons. *A*, A cortical slice cultured on multielectrode dish is shown. *B*, Spontaneous firing activity was recorded for more than a week by using the multielectrodes, which are embedded in a culture dish. Right panel from Uesaka and others (2005) with permission from the *Journal of Neuroscience*.

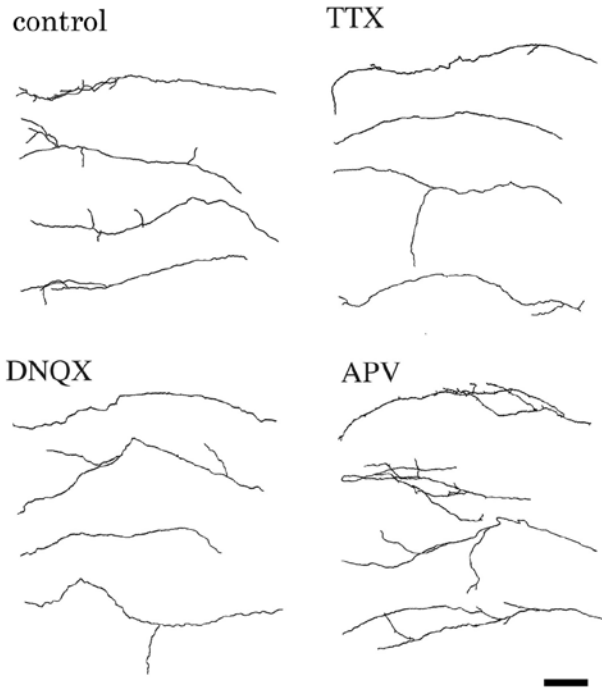

Fig. 3. Axonal branching under pharmacological treatments. Axonal branches were examined after two weeks in vitro. For pharmacological experiments, tetrodotoxin (TTX); 6, 7-dinitroquinoxaline-2, 3-dion (DNQX); and 2-amino-5-phosphonovaleric acid (APV) were added to the culture medium. Note that branching is reduced in the presence of TTX or DNQX. APV and DNQX are an NMDA receptor antagonist and non–NMDA receptor antagonist, respectively. Scale bar represents 200 μm. From Uesaka and others (2005) with permission from the *Journal of Neuroscience*.

isoxazolepropionic acid (AMPA) receptors reduced axonal branch formation by an amount comparable to that of activity blockade by TTX treatment (Fig. 3). By contrast, treatment with antagonists of NMDA receptors did not cause a significant change in axonal branching. Because the spontaneous activity observed during the non–NMDA receptor blockade was not different from that in an untreated group, the failure of axon arbors to branch cannot be simply attributed to the lack of spontaneous activity in these cultures. Thus, synaptic transmission through AMPA receptors is involved in regulating branch formation of horizontal axons.

Possible Mechanisms for Translating Neural Activity into Axonal Branching

Although the mechanism for translating neural activity into axonal branching remains unknown, activity-induced Ca^{2+} elevation seems to play a key role in activating intracellular signaling pathways. One mechanism by which Ca^{2+} elevation arises is calcium influx through NMDA receptors. Ca^{2+} influx through NMDA receptors activates calcium/calmodulin-dependent protein kinase type II (CaMKII), which is known to retrogradely affect branch formation of frog retinotectal axons (Zou and Cline 1996). Thus, Ca^{2+} influx through NMDA receptors can induce a signaling cascade that regulates the intracellular machinery required for axonal branching.

In addition, AMPA receptors can also induce Ca^{2+} elevation directly or indirectly. AMPA receptor activation could cause a depolarization, which in turn could open voltage-dependent calcium channels (VDCCs). It

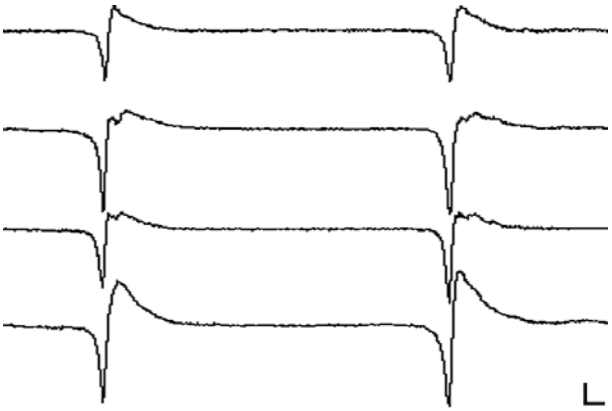

Fig. 4. Synchronous neural activity in cultured cortical slices. Spontaneous firing patterns were recorded at multiple electrode positions. Synchronized neural activity was evident, although the onset of activity differs among electrodes. Scale bars represent 200 μV and 20 ms.

has been shown that AMPA receptors lacking GluR2 are highly permeable to calcium and could induce NMDA receptor-independent LTP (Jia and others 1996). Calcium entry through VDCCs or calcium-permeable AMPA receptors therefore could participate in activity-dependent remodeling of horizontal axons.

How does postsynaptic activation lead to presynaptic events such as axonal branching? One possible explanation is that postsynaptic activation may regulate secretion of retrograde signals from the postsynaptic cells to presynaptic cells. A number of studies have shown that retrograde signaling is essential for neural development and plasticity including presynaptic development (reviewed in Schinder and Poo 2000; Schmidt 2004). Applying this model to horizontal axons, glutamate receptor–mediated depolarization and calcium influx would release a retrograde signal. One candidate molecule of these factors is brain-derived neurotrophic factor (BDNF) because the expression and secretion have been shown to be activity-dependent (reviewed in Schinder and Poo 2000). We demonstrate that BDNF expression in the neocortex increases dramatically during the second postnatal week, when axonal branching of horizontal axons is formed (Hanamura and others 2004). Moreover, horizontal axon branching was altered when BDNF was applied exogenously to cultured cortical slices (unpublished observations). Thus, BDNF might regulate axonal branching by activity-dependent secretion from postsynaptic cells, although the mechanism by which BDNF acts on a subset of presynaptic terminals remains unknown.

Interplay of Activity-Dependent and Independent Processes

There is little doubt that neural activity, including firing and synaptic activity, influences axonal branching. However, it is unlikely that axonal branching is regulated by activity exclusively. Horizontal axon branching in the cultures is observed even under TTX treatment, although the number of branches is reduced considerably. This supports the view that axonal branching is also regulated by molecules, the expression of which is governed by a developmental program. Indeed, some molecules have been shown to induce axonal branching in the CNS (Dent and others 2004; reviewed in Yamamoto 2002).

To date, we have demonstrated the existence of a molecule that promotes thalamocortical axon branching that is expressed in the target layer of the cortex (Yamamoto and others 1997; Yamamoto and others 2000). Such lamina-specific expression of molecules is thought to be regulated developmentally as an intrinsic property of the neocortex (reviewed in Yamamoto 2002). It is likely that horizontal axon branching in the upper layers could also be regulated by layer-specific molecular cues. In support of this idea, lamina-specific elongation and branching of horizontal axons have been reproduced in organotypic slice cultures.

There is also evidence that blockade of electrical activity degrades layer specificity of axonal branches in the neocortex (Herrmann and Shatz 1995; Dantzker and Callaway 1998). One possible mechanism is that neural activity may play a role in regulating the expression pattern of molecular cues that are expressed in a layer-specific manner. In the absence of electrical activity, the layer-specific expression pattern of molecular cues may be distorted. Alternatively, neural activity may be involved in normal molecular expression in growing axons, including receptor molecules, by which growth cones can recognize their target cells. In accordance with this view, it has been demonstrated that electrical activity can alter growth cone responses to guidance cues (Ming and others 2001). Thus, it is likely that an activity-dependent mechanism supersedes developmentally regulated molecules such as lamina-specific cues.

In conclusion, neural activity including spontaneous firing and synaptic transmission contributes to axonal branching of cortical neurons during postnatal development. Postsynaptic activation through glutamate receptors and the subsequent retrograde messages would be required for the conversion of electrical signals into the molecular signals that regulate axonal branching. An activity-independent mechanism also cooperates with the activity-dependent process for generating appropriate branching patterns.

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