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Shaping brain connections through spontaneous neural activity

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Abstract

An overwhelming number of observations demonstrate that neural activity and genetic programs interact to specify the composition and organization of neural circuits during all stages of development. Spontaneous neuronal activities have been documented in several developing neural regions in both invertebrates and vertebrates, and their roles are mostly conserved among species. Among these roles, Ca^{2+} spikes and levels of electrical activity have been shown to regulate neurite growth, axon extension and axon branching. Here, we review selected findings concerning the role of spontaneous activity on circuit development.

General introduction

How neuronal activity influences developmental events in the nervous system is one of the most intriguing issues in neurobiology. Roger Sperry hypothesized that fundamental neuronal circuits are established by a specific affinity between growing axons and their target cells, based on the finding that regenerating retinal ganglion cell (RGC) axons correctly reach their original target zone even after the eyeball is rotated in the frog (Sperry, 1963). This 'chemoaffinity hypothesis' became the basis for studies that pursued the molecular mechanisms of neuronal wiring. Within the past two decades, numerous molecules that are involved in axon navigation and specific connectivity have been discovered in the developing brain.

On the other hand, neuronal activity such as firing and synaptic responses is also known to contribute to neuronal wiring. Hubel and Wiesel showed that visual cortical circuitries are modified by visual inputs (Hubel & Wiesel, 1970; Hubel *et al.*, 1977). In higher mammals, axons of lateral geniculate nucleus (LGN) neurons serving the left and right eyes are segregated and form eye-specific projection patterns in the primary visual cortex. After monocular deprivation during infancy, LGN axons serving the open eye expand their territory while those serving the closed eye shrink. Similarly, visual experience during development also affects orientation selectivity of cortical neurons (Blakemore & Cooper, 1970). These early findings in the visual cortex clearly indicates the importance of sensory-evoked activity in shaping brain connectivity. Moreover, evidence has been accumulating that electrical activity including spontaneous firing plays a crucial role not only in late developmental stages but also in earlier stages. In this review, we focus on the various forms of early neural activity displayed by the developing brain and on the potential roles in neuronal wiring.

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Spontaneous activity modulating early developmental processes

Spontaneous activity, which is present early in the embryonic brain, and experience-driven activity during the postnatal period are both critical for circuit development. Here we begin with a discussion of the most extensively studied forms of spontaneous activity during early development.

Until recently it was believed that electrical activity contributes only to the refinement of connections once an axon has reached its target. For example, the retina produces spontaneous waves of electrical activity whose blockade inhibits layer-specific segregation of retinal axons in target structures (Wong *et al.*, 1993; Feller *et al.*, 1997; Penn *et al.*, 1998; Bansal *et al.*, 2000; Rossi *et al.*, 2001; see in the later section). These and many other studies show clearly that patterned electrical activity is important in the fine-tuning of connections within a target tissue. However, it remained unclear whether this activity, if present earlier, could modulate pathfinding decisions. Pioneering discoveries from Landmesser's group revealed that it could. Rhythmic bursts of activity, similar to retinal waves, are present in developing chick spinal cord neurons when motor axons have just exited the spinal cord. Altering this activity both before and during this exit period resulted in axon guidance errors (Hanson & Landmesser, 2004), demonstrating that, in contrast to the generally accepted view, spontaneous activity is required for the proper unfolding of earlier development programs.

The importance of spontaneous activity in the establishment of major axonal tracts in the developing mouse brain was appreciated

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only 5 years ago, when it was demonstrated that blocking this activity in neonatal visual or somatosensory cortex affects the growth of callosal axons and their arbors in the contralateral hemisphere (Mizuno *et al.*, 2007; Wang *et al.*, 2007). Another key axonal connection that deserves attention regarding the role of spontaneous activity is the development of thalamocortical connectivity. Thalamocortical axons (TCAs) reach the cortex around embryonic day (E) 15 in the mouse, and establish functional transient connections with subplate neurons before they invade their ultimate target within the cortical plate (Friauf *et al.*, 1990; López-Bendito & Molnár, 2003). The hypothesis was that synaptic contacts within the subplate could support activity-dependent interactions during the process of thalamocortical target selection. Indeed, studies in cat fetuses in which the sodium channel blocker tetrodotoxin (TTX) was infused into the brain, to block all activity at the time when the first LGN axons have just reached the subplate, suggested that this might be the case (Catalano & Shatz, 1998). Surprisingly, however, the number of LGN axons reaching the visual cortex was significantly decreased in TTX-infused animals; instead, several axons projected abnormally to other cortical areas, creating aberrant topography (Catalano & Shatz, 1998). Thus, this is further clear evidence that neural activity is required for early developmental events, such as initial targeting of axons, during the formation of neural circuits. But what is the exact nature of the requisite neural activity? Evoked neurotransmitter release seems to be unnecessary for these early interactions and topographic arrangements of the thalamocortical fiber pathways, as shown by an analysis of *Snap-25*-knockout (KO) mice (Molnár *et al.*, 2002). As Snap-25-deficient neurons are able to generate spontaneous activity, this alone may be sufficient to ensure correct thalamocortical pathfinding.

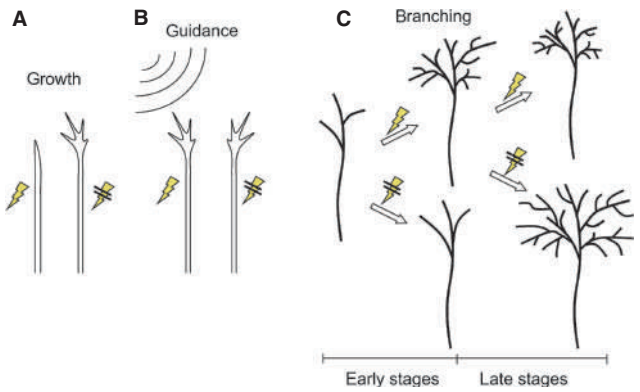


FIG. 1. Representation of the effects of electrical stimulation on axon behaviour during distinct phases of connectivity development. (A) Trains of action potentials can negatively influence the growth capacity of isolated snail neurons (Cohan & Kater, 1986). (B) Electrical activity has also been shown to modulate axon responses to guidance cues. For example, electrical stimulation of *Xenopus* spinal cord neurons can change their response to the axon guidance molecule Netrin 1. When axons are stimulated with a series of action potentials, the response to Netrin 1 changes from repulsion to attraction (Ming *et al.*, 2001). (C) The influence of neural activity on axon branch formation is developmentally regulated. At early stages, activity normally acts as a positive regulator of thalamocortical and horizontal axon branching (Herrmann & Shatz, 1995; Uesaka *et al.*, 2005, 2007), whereas at later stages activity blockage promotes axon branching.

Role of calcium in axon growth and guidance

Calcium activity

Accumulating evidence indicates that calcium activity in growth cones plays a crucial role in axon growth and guidance during development. Axonal growth cones are responsible for the proper growth of axons during nervous system development, and it was during this process when the importance of calcium signaling was first discovered. Seminal work by Kater *et al.* in the late 1980s in isolated cell culture showed that intracellular calcium concentration is a key modulator of axonal growth cone elongation and neurite sprouting (Mattson *et al.*, 1988a,b). They first showed that stimulating individual snail neurons, isolated in culture, with a train of action potentials could suppress neurite elongation and growth cone motility (Fig. 1; also Cohan & Kater, 1986), and thereby demonstrated for the first time that electrical activity can influence structure and connectivity within the nervous system. Subsequent advances in imaging methods facilitated the investigation of growth cone regulation by calcium. Gomez & Spitzer (1999) were the first to show, in *Xenopus* spinal cord neurons, that growing axons display spontaneous calcium transients in their growth cones *in vivo*. These growth cone calcium transients are modulated by components of the extracellular matrix, such as laminin or L1, that are expressed on axons, and they are stimulated by molecules such as Netrin 1 (Hong *et al.*, 2000; Gomez *et al.*, 2001). Furthermore, the frequency of Ca^{2+} activity is a key determinant of this process. Whereas growth cones with a high frequency of Ca^{2+} transients grow more slowly or retract, those generating a lower frequency of transients advance more rapidly (Gomez & Spitzer, 1999). Remarkably, this change in frequency of Ca^{2+} transients has been implicated not only in axonal growth but also in neurotransmitter specification of neural subtypes. A change in frequency of Ca^{2+} transients occurs in raphe and spinal cord neurons of *Xenopus in vivo*, and disrupting this change affects neurotransmitter specification (Borodinsky *et al.*, 2004; Demarque & Spitzer, 2010). Interestingly, our own studies demonstrate that changes occur in the frequency of Ca^{2+} spikes in the soma of developing mouse thalamic neurons, and that these events contribute to modulation of the growth rate of TCAs *in vivo* (E. Mire, C. Mezzera & G. López-Bendito, unpublished data). Thus, it seems plausible that a conserved mechanism to control axonal extension during circuit formation might depend on the modulation of the frequency of Ca^{2+} spikes in the soma and/or growth cones of developing neurons.

Several lines of evidence have demonstrated the existence of intracellular Ca^{2+} spikes well before synapse formation, suggesting a role for Ca^{2+} in the assembly of neural circuits. The development of novel techniques for imaging intracellular Ca^{2+} ion concentration has helped to identify Ca^{2+} as a second messenger inside the cell that mediates a wide spectrum of cellular functions. Ca^{2+} transients are documented in both invertebrate and vertebrate neurons, such as *Xenopus* and zebrafish spinal neurons (Gu *et al.*, 1994; Gu & Spitzer, 1995; Gomez & Spitzer, 1999; Hua *et al.*, 2005; Muto *et al.*, 2011), chick dorsal root ganglion cells (Gomez *et al.*, 1995) and precursor cells of rat embryonic neocortex (Owens & Kriegstein, 1998).

Ca^{2+} spikes are produced at distinct frequencies by developing neurons. In the developing hindbrain and spinal cord in *Xenopus*, their frequency is approximately 2–5/h (Borodinsky *et al.*, 2004; Demarque & Spitzer, 2010), considerably lower than that reported for mouse cortical progenitors or migrating cortical interneurons (60–150/h; Weissman *et al.*, 2004; Bortone & Polleux, 2010; Martini & Valdeolmillos, 2010). What is the origin of this activity? At these early embryonic stages, it is unlikely that the generation of spontaneous Ca^{2+} spikes will depend on the contribution of synaptic transmission. The stimulus that induces Ca^{2+} signals more probably originates from Ca^{2+} influx at the plasma membrane, triggered by membrane depolarization, through Ca^{2+} channels. The best-known Ca^{2+} channels in the plasma membrane that are involved in controlling developmental processes such as neuronal motility are voltage-dependent Ca^{2+} channels (VDCCs). Other neuronal events such as

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endogenous release of neurotransmitters may be also regulated by VDCCs (Ben-Ari, 2001).

In cells possessing gap junctions, the high degree of coupling between cells allows intracellular spread of Ca^{2+} activity leading in most cases to the production of Ca^{2+} waves. Correlative spontaneous activity in the developing retina, spinal cord and hippocampus is generated by excitatory synaptic connections (Meister *et al.*, 1991; Yuste *et al.*, 1992; O'Donovan *et al.*, 1998), whereas gap junctions mediate coactivation of the neurons within discrete domains in several brain areas such as the locus coeruleus, the developing neocortex and, to a limited extent, the retina (Blankenship *et al.*, 2011). However, gap junctions play a critical role in the coordination of electrical activity in the zebrafish embryo spinal cord (Drapeau *et al.*, 2002; Saint-Amant, 2006).

Other modes of calcium activity

It has been ascertained in the last few years that cells can exhibit excitability (i.e., the influx of ions, often Ca^{2+} ions) via mechanisms that do not depend on classical voltage-gated or neurotransmitter-gated channels, suggesting that novel forms of spontaneous activity should be considered. Spontaneous electrical activity can regulate the level of excitability of cells by controlling the expression of pertinent ion channels For example, transient receptor potential channels (TRPCs) are involved in the generation of growth cone Ca^{2+} transients. In particular, Ca^{2+} influx via TRPCs appears to be a critical component of the signaling cascade that mediates the guidance of growth cones and the survival of neurons in response to chemical cues such as neurotrophins or Netrin 1 (Wang & Poo, 2005). Depolarizations applied to the growth cones of *Xenopus* spinal neurons result in the activation of L-type VDCC currents after exposure to Netrin 1 (Nishiyama *et al.*, 2003); thus, TRPCs may further increase Ca^{2+} influx through other VDCCs, such as L-type VDCCs. Moreover, Ca^{2+} release from intracellular stores and the resulting elevation of calcium concentration can also contribute to modulating developmental processes such as growth cone movement (Takei *et al.*, 1998). Drug-induced depletion of internal Ca^{2+} stores and inhibition of D-myo-inositol-1,4,5-trisphosphate (IP3) signaling suppress neurite extension (see more in next sections). Transmitter transporters, metabotropic transmitter receptors and mechanoreceptors are less well studied molecules generating electrical signals that can shape neuronal development.

Calcium ion concentration in axon guidance

Deeper insight has been gained in the last few decades into the mechanisms by which Ca^{2+} influences axon guidance and growth cone steering. Second-messenger networks involving Ca^{2+} influx, and secondary Ca^{2+} release depending on cyclic AMP (cAMP) and cyclic GMP, are the mechanisms through which growth cones read signals encountered along the pathway. Extracellular gradients of nerve growth factor (Gundersen & Barrett, 1980; Song & Poo, 1999), brain-derived neurotrophic factor (BDNF; Song *et al.*, 1997; Song & Poo, 1999), myelin-associated glycoprotein (MAG) (Ming *et al.*, 2001) and Netrin 1 (Song & Poo, 1999; Hong *et al.*, 2000; Nicol *et al.*, 2011), among others, all induce growth-cone turning responses depending on Ca^{2+} signaling (Fig. 1). Furthermore, irrespective of whether these molecules are attractive or repulsive, the Ca^{2+} signals are triggered and increased on the side of the growth cone facing the molecular gradient, and thus the amplitude of the Ca^{2+} signal seems to be the key determinant of whether a molecule exerts an attraction or a repulsion on growth cone steering (Hong *et al.*, 2000). However, how do Ca^{2+} elevations on a single side of the growth cone trigger bidirectional turning? One of the key factors is whether Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores takes place. High amplitude of Ca^{2+} induces CICR and triggers the turning of growth cones toward the side of Ca^{2+} signals, mediating attraction (Hong *et al.*, 2000; Akiyama *et al.*, 2009). Repulsion is not accompanied by CICR and is mostly generated by lower-amplitude Ca^{2+} events. However, several other parameters, such as the source of Ca^{2+} signals or exocytic and endocytic trafficking, are also relevant in the control of signaling events in the growth cone (Tojima *et al.*, 2011). Finally, it is also possible that, as well as controlling changes in growth cone steering, Ca^{2+} changes in the soma of developing neurons could trigger changes in the expression of genes, such as those encoding guidance receptors, that may influence pathfinding decisions (see below).

Role of neuronal activity in circuit formation and axon branching

The necessity of patterned spontaneous activity

After growing axons reach their target areas, spontaneous as well as sensory-evoked neuronal activity further refines the connectivity with target cells by regulating the size and complexity of axon arbors.

In the retinotectal system, RGC axons form appropriate branches and make synaptic contacts with tectal cells in a topographic manner. Since the enunciation of the chemoaffinity hypothesis, this topographic connection has been exploited to study target recognition mechanisms. After extensive molecular screening, interactions between Eph receptors (receptor tyrosine kinases) and their ligands (ephrins) were shown to be crucial for map formation (Nakamoto *et al.*, 1996; Feldheim *et al.*, 2000, 2004). However, this is not the only determinant: spontaneous activity also contributes to map formation.

Spontaneous firing activity has been found to be generated in the rat retina even in embryonic stages (Galli & Maffei, 1988). The Shatz, Wong and Feller groups further demonstrated that RGCs display a characteristic aspect of spontaneous activity, using whole-mount retinal tissue (Meister *et al.*, 1991; Wong *et al.*, 1993; Feller *et al.*, 1997). Interestingly, the spontaneous firing was synchronous in adjacent regions and formed waves. It should be emphasized that this firing activity occurs without visual input. The importance of such synchronous activity for retinotopic mapping has been shown using KO mice lacking the $\beta 2$ subunit of the nicotinic receptor, in which firing activity is not synchronous in adjacent cells in the retina (Grubb *et al.*, 2003; McLaughlin *et al.*, 2003; Chandrasekaran *et al.*, 2009; Stafford *et al.*, 2009). In wild-type mice, RGC axons initially extend so as to cover the entire tectum. Interstitial branches then appear along the anteroposterior axis (Yates *et al.*, 2001), after which overshooting axons and collateral branches outside the target region are gradually eliminated while branching within the target region becomes more complex. Finally, elaborate arbors arise that are confined to the corresponding tectal zone. In $\beta 2$-KO mice, however, branch addition and elimination do not occur precisely, as a result of which the localization of arbor formation is broadened (McLaughlin *et al.*, 2003; Dhande *et al.*, 2011). Thus, waves of correlated firing activity contribute to the fine-tuning of RGC axon arbor formation. A similar activity-dependent confinement has also been observed in visual map formation in the retinogeniculate projection, although the activity dependency is less prominent than that in the retinocollicular system (Pfeiffenberger *et al.*, 2005).

Crair *et al.* have shown the combined effect of targeting molecular cues and neuronal activity (Chandrasekaran *et al.*, 2005). In transgenic mice in which guidance molecule distribution is disrupted in the retina

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by uniform expression of bone morphogenic protein (BMP), RGC axons form arbors in several ectopic target zones. By crossing BMP-transgenic with β2-KO animals, the ectopic projections were accentuated. A cumulative influence has also been demonstrated in the pharmacological blockade of β2 subunit in Eph receptor-KO mice (Pfeiffenberger *et al.*, 2005). It has also been demonstrated that ephrin-A-induced repulsion for retinotectal map formation is regulated by spontaneous retinal activity via cAMP oscillations (Nicol *et al.*, 2007). Thus, activity-dependent and molecular cues cooperate in map formation.

In higher mammals such as cats and ferrets, the LGN has an obvious laminar structure. In these animals, lamina A receives inputs from the contralateral retina whereas lamina A1 input is from the ipsilateral retina. RGC axons form branches not only in a topographic fashion but also in a layer-specific manner. This segregation is established during development (Sretavan & Shatz, 1986). Initially, RGCs extend axons in both layers, and collateral branches emerge from the entire axonal fragment in the LGN. As development proceeds, exuberant contralateral branches are eliminated in inappropriate layers, while branches are more highly elaborated in the target layer. The role of electrical activity in this segregation was tested by infusion of TTX during embryonic stages (Shatz & Stryker, 1988; Sretavan *et al.*, 1988). Collateral branching in the inappropriate layer was not eliminated in TTX-treated animals (see Fig. 1), and the ipsi- and contralateral RGC axons were not segregated in a lamina-specific manner.

Further evidence indicates that patterned activity is required for eye-specific segregation in the LGN, as is the case with retinotopic map formation. Indeed, disruption of patterned activity with pharmacological treatments disorganized the segregation pattern in ferret LGN (Penn *et al.*, 1998; Huberman *et al.*, 2002; Stellwagen & Shatz, 2002). Similar experiments were carried out in the mouse retinogeniculate system (Pfeiffenberger *et al.*, 2005). Left and right eye RGC axons are also segregated in mouse LGN, although there is no laminar structure in this species. The segregation is obscure in pharmacologically treated mice, in which patterned activity is diminished. Genetic removal of the β2 subunit of nicotinic receptor has also shown the necessity of patterned activity for the segregation (Rossi *et al.*, 2001; Pfeiffenberger *et al.*, 2006).

Whether electrical activity is permissive or instructive for neural circuit formation is the subject of debate. In the former case, the topographic map and branch segregation could be affected by the total amount of electrical activity, but not by the difference of patterned activity. In the latter case, they could be influenced by the patterned activity. As described above, firing itself is not diminished in β2-KO mice while synchronicity in adjacent regions is disrupted, suggesting that electrical activity acts as an instructive rather than a permissive signal. Recent investigations with genetic and optogenetic manipulations further support this view (Xu *et al.*, 2011; Zhang *et al.*, 2011).

Another interesting point is that the influence of neural activity on retinotectal map formation differs between species. In the goldfish, RGC axons form terminal arbors in their target regions from the onset, and are barely affected by the abolition of action potentials (Stuermer *et al.*, 1990). Aberrant branches and their subsequent elimination are also observed in amphibian RGC axons (Fujisawa, 1987), but overshooting is less prominent. In chick embryos, RGC axons overshoot the target zone and form side branches, after which the overshooting axons and branches are eliminated (Nakamura & O'Leary, 1989). This process is closer to that found in rodent RGC axon remodeling. However, the extent of overshooting appears to be smaller in the avian than in the rodent system. Thus, the necessity of electrical activity for fine-tuning may have increased during animal evolution.

Positive and negative regulation by electrical activity

Remodeling of TCA arborization is well known to take place in the developing visual cortex. After monocular deprivation, the extent and complexity of individual LGN axon branching also expand or contract according to the input (Antonini & Stryker, 1993; Antonini *et al.*, 1999). Such morphological change is directly attributable to the visual input. Consistent with the notion of the influence of electrical activity, binocular blockade disrupts segregation completely (Stryker & Harris, 1986). It should be noted that this remodeling occurs during a restricted developmental stage called the critical period (Hensch, 2004); that is, TCAs exhibit dynamic changes after fundamental branches are established. This raises the question of how the eye-specific projection is formed initially. Katz *et al.* inferred the existence of a molecular cue, which would distinguish between the ipsi- and contralateral sides, based on their finding that eye-specific projections are formed even in eye-enucleated animals (Crowley & Katz, 2000). Their interpretation assumes that geniculate activity is almost silenced by the removal of retinal inputs; however, it is unlikely that thalamic or cortical cell activity is abolished by eye removal. Indeed, activity blockade at early stages failed to generate eye-specific projections (Huberman *et al.*, 2006), which indicates that neural activity is necessary for both maintenance and segregation during remodeling of TCA arborization.

Neural activity is also influential for fundamental branch formation of TCAs in earlier developmental stages. It has been shown that geniculocortical axons form many fewer branches when TTX is infused into the kitten visual cortex during the period in which these axons form branches (Fig. 1; Herrmann & Shatz, 1995). Such activity-dependency of TCA branching has been shown in organotypic co-culture preparations of the thalamus and cortex (Uesaka *et al.*, 2007). In this co-culture system, lamina-specific connections recapitulate those found *in vivo* (Yamamoto *et al.*, 1989, 1992; Molnár & Blakemore, 1991; Bolz *et al.*, 1992). After a few weeks in culture, synaptic and action potentials can be evoked by applying electrical stimulation to thalamic explants, indicating that functional thalamocortical and intracortical connections are also established *in vitro* (Yamamoto *et al.*, 1989, 1992). Uesaka *et al.* (2007) used this culture system to examine how neuronal activity influences branch formation. First, spontaneous activity was measured using multi-electrode dishes in which small electrodes are implanted in the culture dish. Firing activity hardly occurred during the first week in culture but gradually increased during the second week. Interestingly, a time-lapse experiment showed that TCAs also begin to form branches during the second week in culture. The intriguing question of how electrical activity is involved in branch formation of TCAs was answered by treating the cultures with TTX or glutamate receptor blockers during the second week in culture. Branching decreased substantially in either treatment (Fig. 1), indicating that firing and synaptic activity in the early developmental stages are required to promote TCA branching.

The necessity of neuronal activity for branch formation has also been demonstrated in intracortical circuits. Cortical neurons in the upper layers extend primary axons towards the ventricular surface to reach other cortical areas. Upper-layer cells also extend long-range axon collaterals (horizontal axons) that run horizontally in layer 2/3 and form branches in the same layer (Gilbert & Wiesel, 1979; Rockland & Lund, 1982; Burkhalter & Charles, 1990; McGuire *et al.*, 1991; Lohmann & Rorig, 1994). It has been reported that these axon arbors develop during postnatal stages by growth and retraction (Callaway & Katz, 1990; Durack and Katz, 1996). Moreover, it is likely that neuronal activity is involved in this process, because the organization of these connections is altered by deprivation of visual

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experience (Callaway & Katz, 1990; Lowel & Singer, 1992) and by blockade of action potentials (Ruthazer & Stryker, 1996). An *in vitro* study using organotypic slice cultures has also demonstrated that horizontal axon branching is markedly inhibited by activity blockade (Fig. 1; Uesaka *et al.*, 2005).

Thus, neuronal activity acts as a positive regulator in TCA and horizontal axon branching. In contrast, activity blockade often promotes RGC axon branching in the tectum and LGN (Reh & Constantine-Paton, 1985; Cohen-Cory, 1999). This apparent discrepancy may be explained by the difference in the timing of activity blockade. The pharmacological treatment was applied before the onset of axon branching for TCAs and horizontal axons, whereas RGC axons were treated after primary branches had formed. In support of this explanation, our preliminary result indicates that TCA branching in culture is promoted by activity blockade after a few weeks in culture, when fundamental branches are already formed (C. Terada, Y. Maeda & N. Yamamoto, unpublished data). Synaptic maturation, which is accompanied by branch formation, may thus account for the difference. In addition, characteristics of spontaneous activity differ between developmental stages (Huberman *et al.*, 2008).

Correlation of pre- and postsynaptic activity

The role of pre- and postsynaptic activity in the eye-specific patch formation of LGN axons has been studied from the physiological point of view. Hata and Stryker have addressed this issue by manipulating neuronal activities in the kitten visual cortex (Hata *et al.*, 1999; Haruta & Hata, 2007). Interestingly, silencing cortical cell activity by muscimol infusion inhibited branching of LGN axons serving the open eye, but did not result in shrinkage of the axon arbor serving the closed eye. Thus, TCA branching is preserved or expanded when pre- and postsynaptic activities are correlated.

As described above, TCA branching is inhibited when both pre- and postsynaptic cell activity is suppressed by pharmacological blockade in culture (Uesaka *et al.*, 2007). Yamada *et al.* (2010) asked whether thalamic (presynaptic) or cortical (postsynaptic) cell activity is required for branch formation by overexpressing Kir2.1, an inward-rectifying potassium channel, in either thalamic or cortical cells in organotypic co-cultures. Kir2.1 transfection suppresses firing activity by hyperpolarizing membrane potential. In the case of transfection into thalamic cells, the transfected thalamic cell axons underwent less branching. Similarly, TCA branching decreased substantially in Kir2.1-transfected cortex. Therefore, both pre- and postsynaptic activity is necessary for TCA branching. This is not consistent with the correlation hypothesis, because TCA branching is inhibited when both activities are suppressed. However, the preliminary result that TCA branching is promoted by blocking both pre- and postsynaptic activity after a few weeks in culture (see above) suggests that correlated activity may predominate in branch remodeling at later developmental stages (Fig. 1). In accordance with this view, a correlation-based mechanism is likely to be predominant for RGC axon arbor formation in *Xenopus* (Ruthazer *et al.*, 2003).

On the other hand, studies in the zebrafish retinotectal projection system support the view that presynaptic axons compete with one another for target cells (Hua *et al.*, 2005; Ben Fredj *et al.*, 2010). Indeed, branch formation is suppressed in individually silenced RGC axons, but this inhibition is relieved when adjacent RGC axons are also suppressed. This result favors the mechanism of competition between presynaptic axons rather than the correlation mechanism.

How is activity converted to molecular signals?

Activity-dependent transcriptional regulation

The signaling mechanisms that link Ca^{2+} influx to transcription have been studied extensively. However, while the pathways mediating the activity-dependent gene regulation involved in plasticity are well known (Flavell & Greenberg, 2008; Lin *et al.*, 2008), it is still unclear how activity modulates transcription during development. A recent study demonstrated that neurotransmitter specification relies on activity-dependent regulation of the *tlx3* gene through a variant of the cAMP response element (CRE) motif in its promoter (Marek *et al.*, 2010). This CRE variant binds cJun, which in turn represses *tlx3* transcription.

Interestingly, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor has recently emerged as a major regulator of axon elongation (Gutierrez *et al.*, 2005; Gavalda *et al.*, 2009; Gutierrez & Davies, 2011). NF-κB is as an activator of gene transcription and has been demonstrated to either promote or inhibit axon growth depending on the phosphorylation status of specific residues in NF-κB subunits. Interestingly, these contrasting effects on axon growth have been recorded in the same population of neurons but at distinct developmental stages (Gutierrez *et al.*, 2008), suggesting that a switch in the NF-κB signaling network might contribute to changes in axonal behavior. Among other factors, fluctuations in Ca^{2+} levels have recently been shown to modulate NF-κB activity (Riquelme *et al.*, 2011). In fact, the activation of NF-κB occurs at a low frequency of these Ca^{2+} oscillations (Dolmetsch *et al.*, 1997), whereas the stimulation of CaM kinase II and of the nuclear factor of activated T cells (NFAT) and organic cation transporter transcription factors requires high frequencies of these Ca^{2+} transients (Dolmetsch *et al.*, 1998; Lin *et al.*, 2008; De Konnick and Schulman, 1998).

Chromatin remodeling is an essential component of appropriate gene expression programs and subsequent cellular responses. Histone-modifying enzymes are known to act as transcriptional regulators to remodel chromatin structure in cooperation with the basal transcription machinery (Strahl & Allis, 2000). Two families of antagonistic enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs), catalyze the acetylation and deacetylation of histones and thereby act as transcriptional activators and repressors, respectively (Turner, 2000). In particular, class IIa HDACs bind several transcription factors to regulate cell type-specific gene expression by shuttling between nucleus and cytoplasm (West *et al.*, 2002; Yang & Gregoire, 2005). In the nervous system, firing activity has been shown to trigger nucleocytoplasmic translocation of class IIa HDACs, which is involved in cell survival (Chawla *et al.*, 2003; Linseman *et al.*, 2003; Bolger & Yao, 2005; Morrison *et al.*, 2006).

HDAC9, a class IIa HDAC, is localized in the nucleus of cortical neurons at the early postnatal stages in mice, but gradually translocates to the cytoplasm (Sugo *et al.*, 2010). Moreover, this translocation is accelerated by depolarization and is reversed by activity blockade. As HDAC9 has been shown to repress the action of myocyte-specific enhancer factor 2, a transcription factor, downstream gene expression could be up-regulated by its nucleocytoplasmic translocation (Zhou *et al.*, 2001). In developing cortical neurons, HDAC9 translocation up-regulates the expression of immediate–early genes such as *c-fos* and *arc* (Sugo *et al*., 2010). Acting together with a particular set of transcription factors, HDACs can stimulate subsequent gene expression, which leads to morphological changes.

As class II HDACs translocate from the nucleus to the cytoplasm, HAT activity could become dominant. CRE-binding protein (CREB) is known to induce activity-dependent gene expression and subsequent cellular events by binding to CRE (Mayr & Montminy, 2001; Lonze & Ginty, 2002). In this reaction, the nuclear protein calcium-responsive

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transactivator (CREST)-mediated CREB-binding protein (CBP), which has HAT activity, functions as a cofactor for CREB. Moreover, CREST promotes dendritic growth with CBP, in response to neural activity and the subsequent calcium influx (Aizawa *et al.*, 2004). Conversely, NFAT is activated by calcinulin-mediated dephosphorylation and is involved in dendritic development as a negative regulator (Schwartz *et al.*, 2009). Such regulation of gene expression by histone-modifying enzymes and transcription factors could also affect axonal development.

Activity-dependent expression of adhesion molecules, neurotransmitters and neurotrophins

As described above, Ca^{2+} signaling regulates key features of neuronal development including cell proliferation and migration, axon guidance, branching formation, cell survival and neurotransmitter specification (Spitzer, 2006). Neural activity has been shown to regulate these processes by modulating gene expression, and in recent years we are starting to elucidate how earlier forms of spontaneous activity also modulate these processes. Using transcriptional assays, several groups have found that certain transcription factors and cell adhesion molecules are preferentially activated by rhythmic stimuli and distinct patterns of neural impulses (Itoh *et al.*, 1995, 1997; Dolmetsch *et al.*, 1998; Chang & Berg, 2001). Furthermore, the delivery to the axon surface of neurotrophic factors that promote dendritic and axonal growth, such as candidate plasticity gene 15, is also regulated by activity-dependent mechanisms (Cantallops & Cline, 2008). However, not only the activity *per se* but also its pattern specifies distinct programs of gene regulation. In developing *Xenopus* spinal cord, expression of the GAD67 protein is sensitive to the frequency of Ca^{2+} transients (Watt *et al.*, 2000), and altering the pattern of rhythmic activity in immature *Xenopus* spinal cord neurons also alters neurotransmitter expression, probably through transcriptional regulation (Borodinsky *et al.*, 2004; Marek *et al.*, 2010) (a).

BDNF is a strong candidate molecule to account for the activity-dependent mechanisms involved in circuit remodeling. Indeed, there is a novel Ca^{2+}-responsive sequence 1 and a CRE in the promoter region of *bdnf* (Shieh *et al.*, 1998; Tao *et al.*, 1998, 2002). In the visual cortex, the expression of BDNF, but not nerve growth factor, is altered by visual experience (Castren *et al.*, 1992; Schoups *et al.*, 1995). In particular, monocular deprivation elicits a striking decrease in BDNF mRNA and protein in the cortex (Bozzi *et al.*, 1995; Sala *et al.*, 1998). In the somatosensory cortex, whisker stimulation enhances *bdnf* expression in the corresponding barrel (Rocamora *et al.*, 1996). Such up-regulated BDNF could promote the growth of TCAs. BDNF is also involved in activity-dependent branching of frog RGC axons (Cohen-Cory, 1999), but probably not in initial growth and branching in the mammalian cortex. *bdnf* is barely expressed in the rodent cortex during the early postnatal stages (Hanamura *et al.*, 2004), indicating that increasing neuronal activity during the late developmental stages accelerates *bdnf* expression. In contrast, neurotrophin-3 is expressed only in the early developmental stages and is thought to act as an activity-independent factor (Hanamura *et al.*, 2004). Collectively, these are important discoveries as they reveal that spontaneous activity is required for the normal pattern of expression of adhesion molecules, neurotransmitters and neurotrophins.

Activity-dependent modification in growing axons

In addition to regulating the expression of activity-dependent molecules in target postsynaptic cells, firing activity can modify the expression of receptor molecules, and thus the behavior of their downstream molecular pathways, in presynaptic axons. Hanson & Landmesser (2004) found that the frequency of rhythmic bursting in chick spinal motoneurons was critical for expression of the axon-guidance receptor EphA4 (Fig. 2). Altering this activity at the time of motor axon guidance resulted in abnormal pathfinding errors and a significant change in the expression of both EphA4 and NCAM that might contribute to the pathfinding process as receptor molecules. We have recently found that altering spontaneous Ca^{2+} activity in the developing thalamus leads to a considerable increase in the expression of the guidance receptor Robo1, and ultimately to changes in the growth rate of TCAs (E. Mire, C. Mezzera & G. López-Bendito, unpublished data). Such a regulatory mechanism for receptor molecules could affect axon growth and branching via local cytoplasmic signaling and gene expression, which lead to short- and long-term morphological changes, respectively.

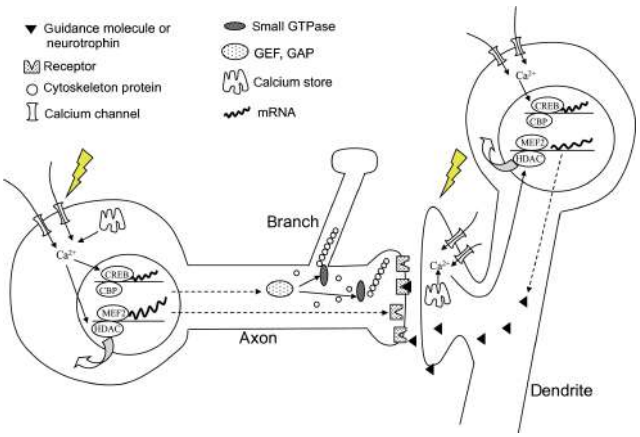


FIG. 2. Possible mechanisms of activity-dependent gene expression and axonal remodeling. Neuronal activity induces nucleocytoplasmic translocation of class II HDACs, whereupon particular transcription factors such as myocyte-specific enhancer factor 2 can bind to their target sequence and up-regulate downstream gene expression. Simultaneously, HAT activity is increased and this promotes the activity of transcription factors such as CREB. In postsynaptic target cells, an activity-dependent regulation may alter the expression of guidance molecules or neurotrophins. In presynaptic growing axons, such a regulatory mechanism may affect the expression of their receptors. Cytoskeleton protein polymerization or depolymerization may also be affected by altered expression of regulatory proteins such as GEFs or GAPs.

Another regulatory mechanism for morphological changes of presynaptic growing axons is that firing activity may influence cytoplasmic signaling, which regulates states of cytoskeleton proteins (Dent *et al.*, 1999; Dent & Kalil, 2001). Small GTPases regulate the polymerization and depolymerization of cytoskeleton molecules, leading to morphological changes (Hall & Lalli, 2010), and it would be interesting to determine their role in activity-dependent processes. In particular, Rho family small GTPases are well characterized in the CNS. Ohnami *et al.* (2008) studied the role of RhoA in horizontal axon branching in cortical slice cultures (see above). Branch formation of horizontal axons is considerably increased by the expression of constitutively active RhoA, and slightly inhibited by the dominant-negative form. Activators and inhibitors of endogenous RhoA signaling also promote and inhibit branching, respectively. Moreover, the level of endogenous active RhoA is dependent on neural activity. Thus, RhoA signaling acts as a positive regulator for activity-dependent axon branching in cortical neurons (Fig. 2). However, it has been shown that RhoA acts as a negative regulator of axonal growth (Bito *et al.*, 2000) and dendritic branching (Li *et al.*, 2000; Nakayama

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et al., 2000; Sin *et al.*, 2002). Rho GTPases may regulate cytoskeleton organization in contrasting ways, depending on cell type and developmental stages.

Although it is not known whether other Rho family members also exhibit activity-dependent behavior, a key issue is how the active form is produced in response to neuronal activity. The active and inactive forms of Rho GTPases are modulated by activators (guanine nucleotide exchange factors; GEFs) and inactivators (GTPase-activating proteins; GAPs; Garcia-Mata & Burridge, 2007). Interestingly, GEFs and GAPs are members of the GEF and GAP families, each comprised of numerous members, and can transmit specific signals from receptor molecules (Fig. 2). For example, ephexin is a well-characterized GEF under EphA4, and specifically transmits ephrin signals (Shamah *et al.*, 2001). Which GEF(s) function endogenously in activity-dependent mechanisms remains unknown, and the role of these proteins in activity-dependent processes poses a challenge for the future.

Concluding remarks

There is now no doubt that spontaneous activity in all its forms plays a key role in shaping brain connectivity. Outstanding challenges are to decipher the mechanisms through which this activity affects developing circuits, and how genetic programs and spontaneous activity interact to this end. How is spontaneous activity initiated and developmentally controlled in distinct populations of neurons? Is the presence of activity more important than its pattern? We will need to take a multidisciplinary approach to be able to answer these and other important questions in the future.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CRE, cAMP response element; E, embryonic day; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; KO, knockout; LGN, lateral geniculate nucleus; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RGC, retinal ganglion cell; TCA, thalamocortical axon; TTX, tetrodotoxin; VDCC, voltage-dependent Ca^{2+} channel.

References

Aizawa, H., Hu, S.C., Bobb, K., Balakrishnan, K., Ince, G., Gurevich, I., Cowan, M. & Ghosh, A. (2004) Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. *Science*, **303**, 197–202.

Akiyama, H., Matsu-ura, T., Mikoshiba, K. & Kamiguchi, H. (2009) Control of neuronal growth cone navigation by asymmetric inositol 1,4,5-trisphosphate signals. *Sci. Signal.*, **2**, ra34.

Antonini, A. & Stryker, M.P. (1993) Rapid remodeling of axonal arbors in the visual cortex. *Science*, **260**, 1819–1821.

Antonini, A., Fagiolini, M. & Stryker, M.P. (1999) Anatomical correlates of functional plasticity in mouse visual cortex. *J. Neurosci.*, **19**, 4388–4406.

Bansal, A., Singer, J.H., Hwang, B.J., Xu, W., Beaudet, A. & Feller, M.B. (2000) Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. *J. Neurosci.*, **20**, 7672–7681.

Ben Fredj, N., Hammond, S., Otsuna, H., Chien, C.B., Burrone, J. & Meyer, M.P. (2010) Synaptic activity and activity-dependent competition regulates axon arbor maturation, growth arrest, and territory in the retinotectal projection. *J. Neurosci.*, **30**, 10939–10951.

Ben-Ari, Y. (2001) Developing networks play a similar melody. *Trends Neurosci.*, **24**, 353–360.

Bito, H., Furuyashiki, T., Ishihara, H., Shibasaki, Y., Ohashi, K., Mizuno, K., Maekawa, M., Ishizaki, T. & Narumiya, S. (2000) A critical role for a Rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. *Neuron*, **26**, 431–441.

Blakemore, C. & Cooper, G.F. (1970) Development of the Brain depends on the visual environment. *Nature*, **228**, 477–478.

Blankenship, A.G., Hamby, A.M., Firl, A., Vyas, S., Maxeiner, S., Willecke, K. & Feller, M.B. (2011) The role of neuronal connexins 36 and 45 in shaping spontaneous firing patterns in the developing retina. *J. Neurosci.*, **31**, 9998–10008.

Bolger, T.A. & Yao, T.P. (2005) Intracellular trafficking of histone deacetylase 4 regulates neuronal cell death. *J. Neurosci.*, **25**, 9544–9553.

Bolz, J., Novak, N. & Staiger, V. (1992) Formation of specific afferent connections in organotypic slice cultures from rat visual cortex cocultured with lateral geniculate nucleus. *J. Neurosci.*, **12**, 3054–3070.

Borodinsky, L., Root, C., Cronin, J., Sann, S., Gu, X. & Spitzer, N. (2004) Activity-dependent homeostatic specification of transmitter expression in embryonic neurons. *Nature*, **429**, 523–530.

Bortone, D. & Polleux, F. (2010) KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. *Neuron*, **62**, 53–71.

Bozzi, Y., Pizzorusso, T., Cremisi, F., Rossi, F.M., Barsacchi, G. & Maffei, L. (1995) Monocular deprivation decreases the expression of messenger RNA for brain-derived neurotrophic factor in the rat visual cortex. *Neuroscience*, **69**, 1133–1144.

Burkhalter, A. & Charles, V. (1990) Organization of local axon collaterals of efferent projection neurons in rat visual cortex. *J. Comp. Neurol.*, **302**, 920–934.

Callaway, E.M. & Katz, L.C. (1990) Emergence and refinement of clustered horizontal connections in cat striate cortex. *J. Neurosci.*, **10**, 1134–1153.

Cantallops, I. & Cline, H.T. (2008) Rapid activity-dependent delivery of the neurotrophic protein CPG15 to the axon surface of neurons in intact *Xenopus* tadpoles. *Dev. Neurobiol.*, **68**, 744–759.

Castren, E., Zafra, F., Thoenen, H. & Lindholm, D. (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. *Proc. Natl. Acad. Sci. USA*, **89**, 9444–9448.

Catalano, S.M. & Shatz, C.J. (1998) Activity-dependent cortical target selection by thalamic axons. *Science*, **281**, 559–562.

Chandrasekaran, A.R., Plas, D.T., Gonzalez, E. & Crair, M.C. (2005) Evidence for an instructive role of retinal activity in retinotopic map refinement in the superior colliculus of the mouse. *J. Neurosci.*, **25**, 6929–6938.

Chandrasekaran, A.R., Furuta, Y. & Crair, M.C. (2009) Consequences of axon guidance defects on the development of retinotopic receptive fields in the mouse colliculus. *J. Physiol.*, **587**, 953–963.

Chang, K.T. & Berg, D.K. (2001) Voltage-gated channels block nicotinic regulation of CREB phosphorylation and gene expression in neurons. *Neuron*, **32**, 855–865.

Chawla, S., Vanhoutte, P., Arnold, F.J., Huang, C.L. & Bading, H. (2003) Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J. Neurochem.*, **85**, 151–159.

Cohan, C. & Kater, S. (1986) Suppression of neurite elongation and growth cone motility by electrical activity. *Science*, **232**, 1638–1640.

Cohen-Cory, S. (1999) BDNF modulates, but does not mediate, activity-dependent branching and remodeling of optic axon arbors in vivo. *J. Neurosci.*, **19**, 9996–10003.

Crowley, J.C. & Katz, L.C. (2000) Early development of ocular dominance columns. *Science*, **290**, 1321–1324.

De Konnick, P. & Schulman, H. (1998) Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science*, **279**, 227–230.

Demarque, M. & Spitzer, N.C. (2010) Activity-dependent expression of Lmx1b regulates specification of serotonergic neurons modulating swimming behavior. *Neuron*, **67**, 321–334.

Dent, E.W. & Kalil, K. (2001) Axon branching requires interactions between dynamic microtubules and actin filaments. *J. Neurosci.*, **21**, 9757–9769.

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Dent, E.W., Callaway, J.L., Szebenyi, G., Baas, P.W. & Kalil, K. (1999) Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. *J. Neurosci.*, **19**, 8894–8908.

Dhande, O.S., Hua, E.W., Guh, E., Yeh, J., Bhatt, S., Zhang, Y., Ruthazer, E.S., Feller, M.B. & Crair, M.C. (2011) Development of single retinofugal axon arbors in normal and beta2 knock-out mice. *J. Neurosci.*, **31**, 3384–3399.

Dolmetsch, R.E., Lewis, R.S., Goodnow, C.C. & Healy, J.I. (1997) Differential activation of transcription factors induced by Ca2+ response amplitude and duration. *Nature*, **386**, 855.

Dolmetsch, R.E., Xu, K. & Lewis, R.S. (1998) Calcium oscillations increase the efficiency and specificity of gene expression. *Nature*, **392**, 933–936.

Drapeau, P., Saint-Amant, L., Buss, R.R., Chong, M., McDearmid, J.R. & Brustein, E. (2002) Development of the locomotor network in zebrafish. *Prog. Neurobiol.*, **68**, 85–111.

Durack, J.C. & Katz, L.C. (1996) Development of horizontal projections in layer 2/3 of ferret visual cortex. *Cereb. Cortex*, **6**, 178–183.

Feldheim, D.A., Kim, Y.I., Bergemann, A.D., Frisen, J., Barbacid, M. & Flanagan, J.G. (2000) Genetic analysis of ephrin-A2 and ephrin-A5 shows their requirement in multiple aspects of retinocollicular mapping. *Neuron*, **25**, 563–574.

Feldheim, D.A., Nakamoto, M., Osterfield, M., Gale, N.W., DeChiara, T.M., Rohatgi, R., Yancopoulos, G.D. & Flanagan, J.G. (2004) Loss-of-function analysis of EphA receptors in retinotectal mapping. *J. Neurosci.*, **24**, 2542–2550.

Feller, M.B., Butts, D.A., Aaron, H.L., Rokhsar, D.S. & Shatz, C.J. (1997) Dynamic processes shape spatiotemporal properties of retinal waves. *Neuron*, **19**, 293–306.

Flavell, S.W. & Greenberg, M.E. (2008) Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annu. Rev. Neurosci.*, **31**, 563–590.

Friauf, E., McConnell, S.K. & Shatz, C.J. (1990) Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. *J. Neurosci.*, **10**, 2601–2613.

Fujisawa, H. (1987) Mode of growth of retinal axons within the tectum of *Xenopus* tadpoles, and implications in the ordered neuronal connection between the retina and the tectum. *J. Comp. Neurol.*, **260**, 127–139.

Galli, L. & Maffei, L. (1988) Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. *Science*, **242**, 90–91.

Garcia-Mata, R. & Burridge, K. (2007) Catching a GEF by its tail. *Trends Cell Biol.*, **17**, 36–43.

Gavalda, N., Gutierrez, H. & Davies, A.M. (2009) Developmental switch in NF-kappaB signalling required for neurite growth. *Development*, **136**, 3405–3412.

Gilbert, C.D. & Wiesel, T.N. (1979) Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature*, **280**, 120–125.

Gomez, T.M. & Spitzer, N.C. (1999) In vivo regulation of axon extension and pathfinding by growth-cone calcium transients. *Nature*, **397**, 350.

Gomez, T.M., Snow, D.M. & Letourneau, P.C. (1995) Characterization of spontaneous calcium transients in nerve growth cones and their effect on growth cone migration. *Neuron*, **14**, 1233–1246.

Gomez, T.M., Robles, E., Poo, M. & Spitzer, N.C. (2001) Filopodial calcium transients promote substrate-dependent growth cone turning. *Science*, **291**, 1983–1987.

Grubb, M.S., Rossi, F.M., Changeux, J.P. & Thompson, I.D. (2003) Abnormal functional organization in the dorsal lateral geniculate nucleus of mice lacking the beta 2 subunit of the nicotinic acetylcholine receptor. *Neuron*, **40**, 1161–1172.

Gu, X. & Spitzer, N.C. (1995) Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca2+ transients. *Nature*, **375**, 784–787.

Gu, X., Olson, E.C. & Spitzer, N.C. (1994) Spontaneous neuronal calcium spikes and waves during early differentiation. *J. Neurosci.*, **14**, 6325–6335.

Gundersen, R.W. & Barrett, J.N. (1980) Characterization of the turning response of dorsal root neurites toward nerve growth factor. *J. Cell Biol.*, **87**, 546–554.

Gutierrez, H. & Davies, A.M. (2011) Regulation of neural process growth, elaboration and structural plasticity by NF-kappaB. *Trends Neurosci.*, **34**, 316–325.

Gutierrez, H., Hale, V.A., Dolcet, X. & Davies, A. (2005) NF-kappaB signalling regulates the growth of neural processes in the developing PNS and CNS. *Development*, **132**, 1713–1726.

Gutierrez, H., O'Keeffe, G.W., Gavalda, N., Gallagher, D. & Davies, A.M. (2008) Nuclear factor kappa B signaling either stimulates or inhibits neurite growth depending on the phosphorylation status of p65/RelA. *J. Neurosci.*, **28**, 8246–8256.

Hall, A. & Lalli, G. (2010) Rho and Ras GTPases in axon growth, guidance, and branching. *Cold Spring Harb. Perspect. Biol.*, **2**, a001818.

Hanamura, K., Harada, A., Katoh-Semba, R., Murakami, F. & Yamamoto, N. (2004) BDNF and NT-3 promote thalamocortical axon growth with distinct substrate and temporal dependency. *Eur. J. Neurosci.*, **19**, 1485–1493.

Hanson, M.G. & Landmesser, L.T. (2004) Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron*, **43**, 687–701.

Haruta, M. & Hata, Y. (2007) Experience-driven axon retraction without binocular imbalance in developing visual cortex. *Curr. Biol.*, **17**, 37–42.

Hata, Y., Tsumoto, T. & Stryker, M.P. (1999) Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited. *Neuron*, **22**, 375–381.

Hensch, T.K. (2004) Critical period regulation. *Annu. Rev. Neurosci.*, **27**, 549–579.

Herrmann, K. & Shatz, C.J. (1995) Blockade of action potential activity alters initial arborization of thalamic axons within cortical layer 4. *Proc. Natl. Acad. Sci. USA*, **92**, 11244–11248.

Hong, K., Nishiyama, M., Henley, J., Tessier-Lavigne, M. & Poo, M. (2000) Calcium signalling in the guidance of nerve growth by netrin-1. *Nature*, **403**, 93–98.

Hua, J.Y., Smear, M.C., Baier, H. & Smith, S.J. (2005) Regulation of axon growth in vivo by activity-based competition. *Nature*, **434**, 1022–1026.

Hubel, D.H. & Wiesel, T.N. (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol.*, **206**, 419–436.

Hubel, D.H., Wiesel, T.N. & LeVay, S. (1977) Plasticity of ocular dominance columns in monkey striate cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **278**, 377–409.

Huberman, A.D., Stellwagen, D. & Chapman, B. (2002) Decoupling eye-specific segregation from lamination in the lateral geniculate nucleus. *J. Neurosci.*, **22**, 9419–9429.

Huberman, A.D., Speer, C.M. & Chapman, B. (2006) Spontaneous retinal activity mediates development of ocular dominance columns and binocular receptive fields in v1. *Neuron*, **52**, 247–254.

Huberman, A.D., Feller, M.B. & Chapman, B. (2008) Mechanisms underlying development of visual maps and receptive fields. *Annu. Rev. Neurosci.*, **31**, 479–509.

Itoh, K., Stevens, B., Schachner, M. & Fields, R. (1995) Regulated expression of the neural cell adhesion molecule L1 by specific patterns of neural impulses. *Science*, **270**, 1369–1372.

Itoh, K., Ozaki, M., Stevens, B. & Fields, R. (1997) Activity-dependent regulation of N-cadherin in DRG neurons: differential regulation of N-cadherin, NCAM, and L1 by distinct patterns of action potentials. *J. Neurobiol.*, **33**, 735–748.

Li, Z., Van Aelst, L. & Cline, H.T. (2000) Rho GTPases regulate distinct aspects of dendritic arbor growth in *Xenopus* central neurons in vivo. *Nat. Neurosci.*, **3**, 217–225.

Lin, Y., Bloodgood, B.L., Hauser, J.L., Lapan, A.D., Koon, A.C., Kim, T.K., Hu, L.S., Malik, A.N. & Greenberg, M.E. (2008) Activity-dependent regulation of inhibitory synapse development by Npas4. *Nature*, **455**, 1198–1204.

Linseman, D.A., Bartley, C.M., Le, S.S., Laessig, T.A., Bouchard, R.J., Meintzer, M.K., Li, M. & Heidenreich, K.A. (2003) Inactivation of the myocyte enhancer factor-2 repressor histone deacetylase-5 by endogenous Ca(2+)//calmodulin-dependent kinase II promotes depolarization-mediated cerebellar granule neuron survival. *J. Biol. Chem.*, **278**, 41472–41481.

Lohmann, H. & Rorig, B. (1994) Long-range horizontal connections between supragranular pyramidal cells in the extrastriate visual cortex of the rat. *J. Comp. Neurol.*, **344**, 543–558.

Lonze, B.E. & Ginty, D.D. (2002) Function and regulation of CREB family transcription factors in the nervous system. *Neuron*, **35**, 605–623.

López-Bendito, G. & Molnár, Z. (2003) Thalamocortical development: how are we going to get there? *Nat. Rev. Neurosci.*, **4**, 276–289.

Lowel, S. & Singer, W. (1992) Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. *Science*, **255**, 209–212.

Marek, K., Kurtz, L. & Spitzer, N.C. (2010) cJun integrates calcium activity and tlx3 expression to regulate neurotransmitter specification. *Nat. Neurosci.*, **13**, 944–950.

Martini, F.J. & Valdeolmillos, M. (2010) Actomyosin contraction at the cell rear drives nuclear translocation in migrating cortical interneurons. *J. Neurosci.*, **30**, 8660–8670.

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Mattson, M.P., Dou, P. & Kater, S.B. (1988a) Outgrowth-regulating actions of glutamate in isolated hippocampal pyramidal neurons. *J. Neurosci.*, **8**, 2087–2100.

Mattson, M.P., Guthrie, P.B. & Kater, S.B. (1988b) Components of neurite outgrowth that determine neuronal cytoarchitecture: influence of calcium and the growth substrate. *J. Neurosci. Res.*, **20**, 331–345.

Mayr, B. & Montminy, M. (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.*, **2**, 599–609.

McGuire, B.A., Gilbert, C.D., Rivlin, P.K. & Wiesel, T.N. (1991) Targets of horizontal connections in macaque primary visual cortex. *J. Comp. Neurol.*, **305**, 370–392.

McLaughlin, T., Torborg, C.L., Feller, M.B. & O'Leary, D.D. (2003) Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron*, **40**, 1147–1160.

Meister, M., Wong, R.O., Baylor, D.A. & Shatz, C.J. (1991) Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science*, **252**, 939–943.

Ming, G., Henley, J., Tessier-Lavigne, M., Song, H. & Poo, M. (2001) Electrical activity modulates growth cone guidance by diffusible factors. *Neuron*, **29**, 441–452.

Mizuno, H., Hirano, T. & Tagawa, Y. (2007) Evidence for activity-dependent cortical wiring: formation of interhemispheric connections in neonatal mouse visual cortex requires projection neuron activity. *J. Neurosci.*, **27**, 6760–6770.

Molnár, Z. & Blakemore, C. (1991) Lack of regional specificity for connections formed between thalamus and cortex in coculture. *Nature*, **351**, 475–477.

Molnár, Z., Lopez-Bendito, G., Small, J., Partridge, L., Blakemore, C. & Wilson, M. (2002) Normal development of embryonic thalamocortical connectivity in the absence of evoked synaptic activity. *J. Neurosci.*, **22**, 10313–10323.

Morrison, B.E., Majdzadeh, N., Zhang, X., Lyles, A., Bassel-Duby, R., Olson, E.N. & D'Mello, S.R. (2006) Neuroprotection by histone deacetylase-related protein. *Mol. Cell. Biol.*, **26**, 3550–3564.

Muto, A., Ohkura, M., Kotani, T., Higashijima, S., Nakai, J. & Kawakami, K. (2011) Genetic visualization with an improved GCaMP calcium indicator reveals spatiotemporal activation of the spinal motor neurons in zebrafish. *Proc. Natl. Acad. Sci. USA*, **108**, 5425–5430.

Nakamoto, M., Cheng, H.J., Friedman, G.C., McLaughlin, T., Hansen, M.J., Yoon, C.H., O'Leary, D.D. & Flanagan, J.G. (1996) Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo. *Cell*, **86**, 755–766.

Nakamura, H. & O'Leary, D.D. (1989) Inaccuracies in initial growth and arborization of chick retinotectal axons followed by course corrections and axon remodeling to develop topographic order. *J. Neurosci.*, **9**, 3776–3795.

Nakayama, A.Y., Harms, M.B. & Luo, L. (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.*, **20**, 5329–5338.

Nicol, X., Voyatzis, S., Muzerelle, A., Narboux-Neme, N., Sudhof, T.C., Miles, R. & Gaspar, P. (2007) cAMP oscillations and retinal activity are permissive for ephrin signaling during the establishment of the retinotopic map. *Nat. Neurosci.*, **10**, 340–347.

Nicol, X., Hong, K.P. & Spitzer, N.C. (2011) Spatial and temporal second messenger codes for growth cone turning. *Proc. Natl. Acad. Sci. USA*, **108**, 13776–13781.

Nishiyama, M., Hoshino, A., Tsai, L., Henley, J., Goshima, Y., Tessier-Lavigne, M., Poo, M. & Hong, K. (2003) Cyclic AMP/GMP-dependent modulation of Ca2+ channels sets the polarity of nerve growth-cone turning. *Nature*, **423**, 990–995.

O'Donovan, M.J., Wenner, P., Chub, N., Tabak, J. & Rinzel, J. (1998) Mechanisms of spontaneous activity in the developing spinal cord and their relevance to locomotion. *Ann. NY Acad. Sci.*, **860**, 130–141.

Ohnami, S., Endo, M., Hirai, S., Uesaka, N., Hatanaka, Y., Yamashita, T. & Yamamoto, N. (2008) Role of RhoA in activity-dependent cortical axon branching. *J. Neurosci.*, **28**, 9117–9121.

Owens, D.F. & Kriegstein, A.R. (1998) Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone. *J. Neurosci.*, **18**, 5374–5388.

Penn, A.A., Riquelme, P.A., Feller, M.B. & Shatz, C.J. (1998) Competition in retinogeniculate patterning driven by spontaneous activity. *Science*, **279**, 2108–2112.

Pfeiffenberger, C., Cutforth, T., Woods, G., Yamada, J., Renteria, R.C., Copenhagen, D.R., Flanagan, J.G. & Feldheim, D.A. (2005) Ephrin-As and neural activity are required for eye-specific patterning during retinogeniculate mapping. *Nat. Neurosci.*, **8**, 1022–1027.

Pfeiffenberger, C., Yamada, J. & Feldheim, D.A. (2006) Ephrin-As and patterned retinal activity act together in the development of topographic maps in the primary visual system. *J. Neurosci.*, **26**, 12873–12884.

Reh, T.A. & Constantine-Paton, M. (1985) Eye-specific segregation requires neural activity in three-eyed Rana pipiens. *J. Neurosci.*, **5**, 1132–1143.

Riquelme, D., Alvarez, A., Leal, N., Adasme, T., Espinoza, I., Valdes, J.A., Troncoso, N., Hartel, S., Hidalgo, J., Hidalgo, C. & Carrasco, M.A. (2011) High-frequency field stimulation of primary neurons enhances ryanodine receptor-mediated Ca2+ release and generates hydrogen peroxide, which jointly stimulate NF-kappaB activity. *Antioxid. Redox Signal.*, **14**, 1245–1259.

Rocamora, N., Welker, E., Pascual, M. & Soriano, E. (1996) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. *J. Neurosci.*, **16**, 4411–4419.

Rockland, K.S. & Lund, J.S. (1982) Widespread periodic intrinsic connections in the tree shrew visual cortex. *Science*, **215**, 1532–1534.

Rossi, F.M., Pizzorusso, T., Porciatti, V., Marubio, L.M., Maffei, L. & Changeux, J.P. (2001) Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc. Natl. Acad. Sci. USA*, **98**, 6453–6458.

Ruthazer, E.S. & Stryker, M.P. (1996) The role of activity in the development of long-range horizontal connections in area 17 of the ferret. *J. Neurosci.*, **16**, 7253–7269.

Ruthazer, E.S., Akerman, C.J. & Cline, H.T. (2003) Control of axon branch dynamics by correlated activity in vivo. *Science*, **301**, 66–70.

Saint-Amant, L. (2006) Development of motor networks in zebrafish embryos. *Zebrafish*, **3**, 173–190.

Sala, R., Viegi, A., Rossi, F.M., Pizzorusso, T., Bonanno, G., Raiteri, M. & Maffei, L. (1998) Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex. *Eur. J. Neurosci.*, **10**, 2185–2191.

Schoups, A.A., Elliott, R.C., Friedman, W.J. & Black, I.B. (1995) NGF and BDNF are differentially modulated by visual experience in the developing geniculocortical pathway. *Brain Res. Dev. Brain Res.*, **86**, 326–334.

Schwartz, N., Schohl, A. & Ruthazer, E.S. (2009) Neural activity regulates synaptic properties and dendritic structure in vivo through calcineurin/NFAT signaling. *Neuron*, **62**, 655–669.

Shamah, S.M., Lin, M.Z., Goldberg, J.L., Estrach, S., Sahin, M., Hu, L., Bazalakova, M., Neve, R.L., Corfas, G., Debant, A. & Greenberg, M.E. (2001) EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. *Cell*, **105**, 233–244.

Shatz, C.J. & Stryker, M.P. (1988) Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. *Science*, **242**, 87–89.

Shieh, P.B., Hu, S.C., Bobb, K., Timmusk, T. & Ghosh, A. (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. *Neuron*, **20**, 727–740.

Sin, W.C., Haas, K., Ruthazer, E.S. & Cline, H.T. (2002) Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature*, **419**, 475–480.

Song, H.J. & Poo, M.M. (1999) Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.*, **9**, 355–363.

Song, H.J., Ming, G.L. & Poo, M.M. (1997) cAMP-induced switching in turning direction of nerve growth cones. *Nature*, **388**, 275–279.

Sperry, R.W. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. USA*, **50**, 703–710.

Spitzer, N. (2006) Electrical activity in early neuronal development. *Nature*, **444**, 707–712.

Sretavan, D.W. & Shatz, C.J. (1986) Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers within the cat's lateral geniculate nucleus. *J. Neurosci.*, **6**, 234–251.

Sretavan, D.W., Shatz, C.J. & Stryker, M.P. (1988) Modification of retinal ganglion cell axon morphology by prenatal infusion of tetrodotoxin. *Nature*, **336**, 468–471.

Stafford, B.K., Sher, A., Litke, A.M. & Feldheim, D.A. (2009) Spatial-temporal patterns of retinal waves underlying activity-dependent refinement of retinofugal projections. *Neuron*, **64**, 200–212.

Stellwagen, D. & Shatz, C.J. (2002) An instructive role for retinal waves in the development of retinogeniculate connectivity. *Neuron*, **33**, 357–367.

Strahl, B.D. & Allis, C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41–45.

Stryker, M.P. & Harris, W.A. (1986) Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. *J. Neurosci.*, **6**, 2117–2133.

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Stuermer, C.A., Rohrer, B. & Munz, H. (1990) Development of the retinotectal projection in zebrafish embryos under TTX-induced neural-impulse blockade. *J. Neurosci.*, **10**, 3615–3626.

Sugo, N., Oshiro, H., Takemura, M., Kobayashi, T., Kohno, Y., Uesaka, N., Song, W.J. & Yamamoto, N. (2010) Nucleocytoplasmic translocation of HDAC9 regulates gene expression and dendritic growth in developing cortical neurons. *Eur. J. Neurosci.*, **31**, 1521–1532.

Takei, K., Shin, R.M., Inoue, T., Kato, K. & Mikoshiba, K. (1998) Regulation of nerve growth mediated by inositol 1,4,5-trisphosphate receptors in growth cones. *Science*, **282**, 1705–1708.

Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J. & Greenberg, M.E. (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron*, **20**, 709–726.

Tao, X., West, A.E., Chen, W.G., Corfas, G. & Greenberg, M.E. (2002) A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron*, **33**, 383–395.

Tojima, T., Hunes, J.H., Henley, J.R. & Kamiguchi, H. (2011) Second messengers and membrane trafficking direct and organize growth cone steering. *Nat. Rev. Neurosci.*, **12**, 191–203.

Turner, B.M. (2000) Histone acetylation and an epigenetic code. *BioEssays*, **22**, 836–845.

Uesaka, N., Hirai, S., Maruyama, T., Ruthazer, E.S. & Yamamoto, N. (2005) Activity dependence of cortical axon branch formation: a morphological and electrophysiological study using organotypic slice cultures. *J. Neurosci.*, **25**, 1–9.

Uesaka, N., Hayano, Y., Yamada, A. & Yamamoto, N. (2007) Interplay between laminar specificity and activity-dependent mechanisms of thalamocortical axon branching. *J. Neurosci.*, **27**, 5215–5223.

Wang, G.X. & Poo, M.M. (2005) Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. *Nature*, **434**, 898–904.

Wang, C.-L., Zhang, L., Zhou, Y., Zhou, J., Yang, X.-J., Duan, S.-m., Xiong, Z.-Q. & Ding, Y.-Q. (2007) Activity-dependent development of callosal projections in the somatosensory cortex. *J. Neurosci.*, **27**, 11334–11342.

Watt, S.D., Gu, X., Smith, R.D. & Spitzer, N.C. (2000) Specific frequencies of spontaneous Ca^{2+} transients upregulate GAD 67 transcripts in embryonic spinal neurons. *Mol. Cell. Neurosci.*, **16**, 376–387.

Weissman, T.A., Riquelme, P.A., Ivic, L., Flint, A.C. & Kriegstein, A.R. (2004) Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. *Neuron*, **43**, 647–661.

West, A.E., Griffith, E.C. & Greenberg, M.E. (2002) Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.*, **3**, 921–931.

Wong, R.O., Meister, M. & Shatz, C.J. (1993) Transient period of correlated bursting activity during development of the mammalian retina. *Neuron*, **11**, 923–938.

Xu, H.P., Furman, M., Mineur, Y.S., Chen, H., King, S.L., Zenisek, D., Zhou, Z.J., Butts, D.A., Tian, N., Picciotto, M.R. & Crair, M.C. (2011) An instructive role for patterned spontaneous retinal activity in mouse visual map development. *Neuron*, **70**, 1115–1127.

Yamada, A., Uesaka, N., Hayano, Y., Tabata, T., Kano, M. & Yamamoto, N. (2010) Role of pre- and postsynaptic activity in thalamocortical axon branching. *Proc. Natl. Acad. Sci. USA*, **107**, 7562–7567.

Yamamoto, N., Kurotani, T. & Toyama, K. (1989) Neural connections between the lateral geniculate nucleus and visual cortex in vitro. *Science*, **245**, 192–194.

Yamamoto, N., Yamada, K., Kurotani, T. & Toyama, K. (1992) Laminar specificity of extrinsic cortical connections studied in coculture preparations. *Neuron*, **9**, 217–228.

Yang, X.J. & Gregoire, S. (2005) Class II histone deacetylases: from sequence to function, regulation, and clinical implication. *Mol. Cell. Biol.*, **25**, 2873–2884.

Yates, P.A., Roskies, A.L., McLaughlin, T. & O'Leary, D.D. (2001) Topographic-specific axon branching controlled by ephrin-As is the critical event in retinotectal map development. *J. Neurosci.*, **21**, 8548–8563.

Yuste, R., Peinado, A. & Katz, L.C. (1992) Neuronal domains in developing neocortex. *Science*, **257**, 665–669.

Zhang, J., Ackman, J.B., Xu, H.P. & Crair, M.C. (2011) Visual map development depends on the temporal pattern of binocular activity in mice. *Nat. Neurosci.*, **15**, 298–307.

Zhou, X., Marks, P.A., Rifkind, R.A. & Richon, V.M. (2001) Cloning and characterization of a histone deacetylase, HDAC9. *Proc. Natl. Acad. Sci. USA*, **98**, 10572–10577.

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