REVIEWS

Electrical activity in early neuronal development

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The construction of the brain during embryonic development was thought to be largely independent of its electrical activity. In this view, proliferation, migration and differentiation of neurons are driven entirely by genetic programs and activity is important only at later stages in refinement of connections. However, recent findings demonstrate that activity plays essential roles in early development of the nervous system. Activity has similar roles in the incorporation of newly born neurons in the adult nervous system, suggesting that there are general rules underlying activity-dependent development. The extensive involvement of activity makes it likely that it is required at all developmental stages as a necessary partner with genetic programs.

Early neuronal development may be divided into three major phases occurring sequentially but partially overlapping. Proliferation from stem cells generates large numbers of neurons. Migration of neurons to achieve their final locations follows on the heels of proliferation. Differentiation includes maturation of electrical excitability, transmitter specification, and outgrowth of axons and dendrites. Here I consider newly discovered functions of electrical activity in these events that follow neural induction and precede synapse formation.

The activity dependence of synapse formation and neuronal survival at later stages of development has been recognized for some time and has been recently reviewed[1–4]. Proliferation, migration and differentiation all depend to various extents on forms of electrical activity, both embryonically and in the adult. Given the involvement of ion channel signalling in neural induction[5], it appears that electrical activity plays a role at all stages of development. Research now focuses on the mechanisms by which this activity exerts its effects.

Conventional forms of excitability

In the past, electrical activity was viewed exclusively as the activity of action potentials in which the inward current is carried by Na ions. Because tetrodotoxin blocks voltage-gated Na channels and the action potentials they generate, tetrodotoxin was the principal assay used to determine whether or not a developmental process is activity-dependent. Recognition of the role of Ca as a second messenger coupled with the development of techniques for imaging intracellular Ca ion concentrations led to the discovery of spontaneous fluctuations in intracellular Ca at early stages of neuronal development (Box 1). Examination of the origins of these fluctuations revealed roles for neurotransmitters that depolarize neurons and activate voltage-gated Ca channels that promote entry of Ca, or that bind to receptors that flux Ca. The transmitters GABA (γ-aminobutyric acid) and glycine activate receptors that generate depolarizing chloride currents at early stages, while glutamate receptors generate Na and Ca currents that depolarize neurons throughout development. As a result, tests of the roles of these forms of excitability in neuronal development require blockade not only of voltage-gated Na channels, but voltage-gated Ca channels and neurotransmitter receptors, for both of which there are large families. Proliferation, migration, ion channel expression, neurotransmitter specification, axon pathfinding and dendrite outgrowth are all regulated by the expanded repertoire of conventional forms of excitability.

Novel forms of excitability

The discovery of previously unknown forms of excitability has also contributed to the appreciation of the role of electrical activity in neuronal development. Here excitability is defined as the influx of ions, often Ca ions, via mechanisms that do not depend on classical voltage-gated or neurotransmitter-gated channels. For example, TRP (transient receptor potential) channels are involved in the generation of growth cone Ca transients. Metabotropic transmitter receptors, mechanoreceptors, and transmitter transporters acting in reverse are less-well-studied components generating electrical signals that can shape neuronal development. Tests for the roles of Ca transients in

Box 1 | Spontaneous activity before synapse formation

Observations of spontaneous electrical activity at early stages of development before synapse formation suggested that it could have roles in the assembly of the nervous system. Electrical recordings and optical imaging of intracellular Ca revealed the presence of extensive neurotransmitter-evoked activity in a range of CNS structures in both vertebrates and invertebrates before the appearance of conventional synaptic structures. Endogenous release of a neurotransmitter activates NMDA receptors in embryonic turtle cortex[72] and generates Ca transients via activation of metabotropic glutamate receptors in embryonic mouse cortex[73]. Endogenous transmitters also engage $GABA_A$ receptors that lead to depolarization of neuronal progenitors in the postnatal mouse subventricular zone and rostral migratory stream[74].

Spontaneous Ca transients are also triggered by substrate interactions and by mechanisms that are still unknown. Ca transients are observed in embryonic mouse neural crest[75], *Xenopus* and zebrafish spinal neurons[20,32,42,76–78], chick dorsal root ganglion neurons[31] and hamster cortical neurons[33]. Several different patterns of Ca transients are observed in precursor cells of the embryonic rat neocortical ventricular zone[79]. In tunicates and invertebrate nervous systems, spontaneous Ca elevations are generated in cells as varied as embryonic ascidian muscle and pupal moth antennal-lobe and fly mushroom-body neurons[19,80,81].

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developmental processes now include imaging of intracellular Ca during the relevant period of development to learn whether such forms of excitability are present. It seems likely that further classes of Ca transients await identification.

Neuronal proliferation

Neurons are generated in vast numbers in the embryonic brain, as asymmetric divisions of neural progenitors give rise to neurons at a prodigious rate. Strikingly, this process is regulated by electrical signalling. GABA and glutamate released at this early stage before synapse formation have different effects on neurogenesis in different regions. GABA and glutamate depolarize embryonic rat cortical progenitor cells in the ventricular zone, stimulate elevations of intracellular Ca, and inhibit DNA synthesis. Blockade of $GABA_A$ receptors and the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainate class of glutamate receptors increases DNA synthesis, indicating a role for endogenously released transmitters in regulating proliferation[6]. Similarly, release of GABA from differentiating neuroblasts depolarizes their progenitor cells in the postnatal mouse subventricular zone and limits proliferation[7] (Fig. 1). In contrast, GABA induces proliferation of postnatal rat immature cerebellar granule cells by depolarization and activation of Ca channels[8]. Disruption of Ca wave propagation through embryonic rat radial glia that are neuronal progenitors decreases neuronal proliferation in the ventricular zone[9]. Roles for other neurotransmitters and the mechanisms by which activity stimulates or inhibits proliferation are now subjects of interest. The sign of the effects of electrical activity can be regulated by the ratio of 3′, 5′-cyclic AMP to cyclic GMP[10,11].

Neuronal migration

Neurons are generated in a restricted set of locations and generally migrate to specific sites from which they extend processes to establish synaptic connections. Some neurons migrate radially and others migrate tangentially. Both the rate and extent of migration are regulated by neurotransmitters released at this early point in development. Radial migration of postnatal mouse cerebellar granule cells along Bergmann glia depends on activation of voltage-gated *N*-type Ca channels and the NMDA (*N*-methyl-D-aspartate) class of glutamate receptors, which generate fluctuations in intracellular Ca ions that are positively correlated with their rate of migration[12]. Release of GABA and glutamate in a manner that does not require conventional SNARE-dependent exocytosis of vesicle contents promotes migration of embryonic mouse hippocampal pyramidal neurons[13,14]. Somatostatin increases the frequency of Ca transients and the initial rate of postnatal murine granule cell migration as well as the loss of Ca transients that triggers the completion of migration[15] (Fig. 2). In contrast, GABA slows the rate of migration in other regions of the brain. Astrocyte-like cells regulate the rate of tangential migration by uptake of GABA in the postnatal mouse subventricular zone and rostral migratory stream; migration is reduced by inhibiting uptake and increased by blocking $GABA_A$ receptors[16]. The signal transduction pathways by which neurotransmitters stimulate or inhibit neuronal migration are now being investigated[17,18]; the ratio of cAMP to cGMP appears to regulate the sign of this process as well.

Neuronal differentiation

Voltage-gated ion channel expression. Neuronal differentiation has many components, one of which is the specification of ion channels that govern excitability. Spontaneous electrical activity can regulate the level of excitability of cells by controlling the expression of ion channels generating it. The best-studied example is the development of embryonic ascidian muscle, in which Ca currents are expressed before the appearance of rapidly activated outward and inwardly rectifying potassium currents, engendering a period of spontaneous activity. When Ca-dependent action potentials are blocked, developmental expression of the rapidly activated potassium current is specifically suppressed[19]. In embryonic *Xenopus* spinal neurons the delayed rectifier current plays the central role in developmental conversion of Ca-dependent action potentials to Na-dependent spikes. Ca influx through voltage-gated channels at early stages of development drives an increase in the rate of activation of this current[20,21]. These results demonstrate feedback loops by which early forms of electrical activity regulate intrinsic excitability and can limit the embryonic period during which activity triggers developmental programs.

Neurotransmitter specification. A second feature of neuronal differentiation is the specification of neurotransmitter phenotype. This process depends on early electrical activity in a number of systems, in conjunction with cell-specific transcription factor expression or action of trophic factors. Alterations in activity can change the numbers of neurons expressing excitatory and inhibitory transmitters. Different classes of embryonic *Xenopus* spinal neurons generate

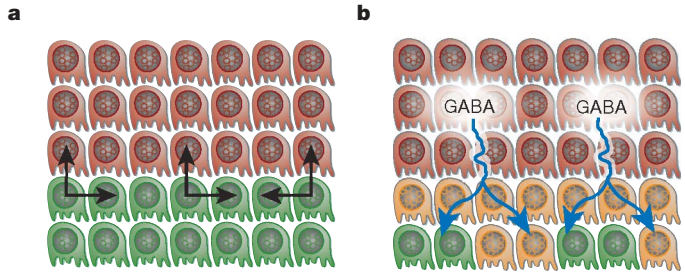

Figure 1 | Spontaneous release of neurotransmitters from neuroblasts generates elevations of calcium in embryonic progenitor cells that suppress their proliferation. **a**, Cerebral cortical progenitor cells (green) in the ventricular zone divide asymmetrically (black arrows) to give rise to neuroblasts (red) in rodent brains. **b**, Neuroblasts release GABA (blue arrows), depolarizing progenitors and stimulating elevations of Ca (yellow) that inhibit DNA synthesis and proliferation. Adapted from ref. 7, with permission.

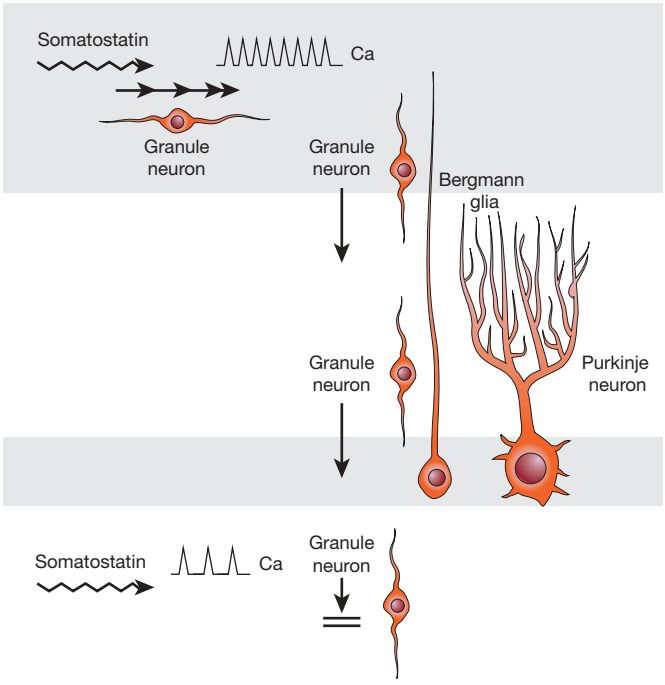

Figure 2 | Neurotransmitter modulation of embryonic neuronal migration by stage-specific regulation of calcium transients. Spontaneous release of somatostatin increases the frequency of Ca transients (Ca) and enhances tangential migration of granule neurons on the superficial layer of the mouse cerebellum. Somatostatin suppresses Ca transients and slows radial migration of granule neurons in the deep layer at a later stage of development. Adapted from ref. 15, with permission.

spontaneous Ca spikes at different frequencies, before synapse formation. In a homeostatic manner, spike suppression increases the incidence of excitatory transmitter expression and decreases the incidence of inhibitory transmitters. Conversely, increasing spike production decreases the incidence of neurons expressing excitatory transmitters and increases the incidence of neurons expressing inhibitory transmitters[22] (Fig. 3). Embryonic chick and rat neurons generate developmentally transient Ca-dependent action potentials that may produce Ca spikes like those described for *Xenopus* spinal neurons and regulate transmitter expression similarly[23–25]. Results from studies of other systems are consistent with activity-dependent homeostatic regulation of transmitter expression. Stimulation of embryonic rat sensory petrosal ganglion neurons *in vitro* induces expression of tyrosine hydroxylase[26], a synthetic enzyme for dopamine that acts as an inhibitory transmitter for these neurons. Physiological stimulation of these neurons *in vivo* induces tyrosine hydroxylase expression exclusively in cells expressing *Phox2a/2b* (paired-like homeobox 2a and 2b) transcription factors[27], illustrating the partnership between transcription factor expression and activity.

Transmitter receptor populations change to match activity-dependent changes in transmitter expression at the vertebrate neuromuscular junction. Matching of transmitters with their cognate receptors appears to be achieved by a process of selection. Embryonic *Xenopus* muscle cells initially express receptors for glutamate, GABA and glycine as well as acetylcholine; acetylcholine receptor expression prevails as maturation progresses. However the receptor populations remaining in muscle cells parallel changes in transmitter phenotype when neuronal activity is perturbed. Glutamatergic, GABAergic and glycinergic synaptic currents are recorded from muscle cells when early neuronal Ca-dependent activity is manipulated to increase the number of neurons expressing these transmitters[96]. These results are consistent with release of glutamate from neonatal mammalian motorneurons[28,29] and glutamatergic transmission at adult vertebrate neuromuscular junctions[30].

Axon pathfinding. A third aspect of neuronal differentiation is the targeting of axons that neurons extend to synapse with other neurons, muscles or glands. Both novel and conventional forms of activity are involved in pathfinding of axons via the migration of growth cones at their tips. Intracellular Ca has diverse effects on axon extension, turning and branching that may depend on concentration and route of entry. Growth cone motility and neurite extension are inhibited by application of transmitters that elicit depolarizations and Ca increases, and axon extension is inhibited by spontaneous growth cone Ca transients as well[20,31–33]. TRPC5-receptor-operated ion channels appear to constitute the Ca entry pathway generating spontaneous growth cone transients in cultured hippocampal neurons[34].

Axons turn in response to diffusible guidance molecules. Growth cones of cultured *Xenopus* spinal neurons exhibit Ca-dependent attraction to gradients of acetylcholine and of glutamate[35]. Ca also mediates growth cone turning of these neurons stimulated by an extracellular gradient of netrin-1 (ref. 36), a diffusible guidance factor *in vivo*. The ratio of cAMP to cGMP modulates the activity of voltage-gated Ca channels that regulate Ca increases and growth cone turning[37]. The turning responses to netrin-1, brain-derived neurotrophic factor and MAG (myelin-associated glycoprotein) are mediated by TRPC1 channels[38,39] in *Xenopus* spinal neurons and TRPC3 channels in rat cerebellar granule neurons[40] (Fig. 4a). Stimulation of action potentials enhances netrin-1 attraction and converts MAG repulsion to attraction, by elevating both Ca and cAMP (ref. 41), demonstrating an intersection of conventional and novel forms of excitability. Axons also turn in response to different substrates. Filopodia that extend from neuronal growth cones sample the environment for extracellular guidance cues, and generate localized transient elevations of intracellular Ca at their tips that propagate back to the growth cone in embryonic *Xenopus* spinal neurons. The frequency of filopodial transients is substrate-dependent and reduces filopodial motility, promoting turning when stimulated differentially within filopodia on one side of the growth cone[42].

Axons branch both at their tips and interstitially, and both classes of branching are activity-dependent. Axons of retinal ganglion cells arriving in the optic tectum of *Xenopus* tadpoles add branches at their tips equally but eliminate them selectively through a NMDA-receptor-dependent mechanism in regions of the tectum constructed to be dominated by the opposite eye[43]. Competition between neighbouring axons growing and branching in the zebrafish optic tectum involves activity-dependent neurosecretion that implicates the role of neurotransmitters[44]. Interstitial or collateral branching at points along axons involves chemotropic signals[45]. Netrin-1 increases interstitial branching of cultured hamster somatosensory cortical neurons, stimulating repetitive Ca transients that are spatially and temporally correlated with the sites of axon protrusion; suppression of these transients blocks branching[46].

Axon guidance by both substrate and diffusible molecules requires expression of appropriate receptors, and receptor expression is activity-dependent in a number of cases[47,48]. This is an important but relatively unexplored subject.

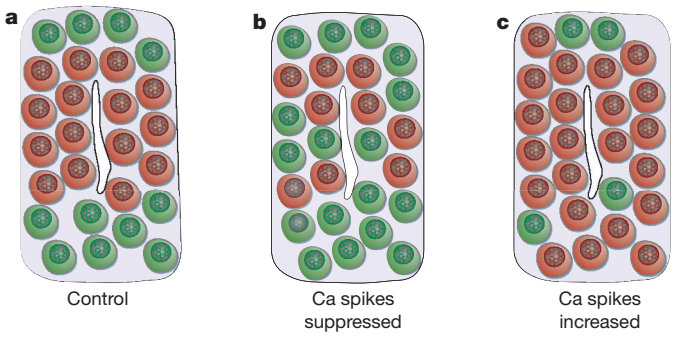

Figure 3 | Calcium-spike-dependent homeostatic specification of embryonic neurotransmitter expression. **a**, Normal expression of excitatory transmitters (green) and inhibitory transmitters (red) in the frog spinal cord. **b**, Suppressing production of spontaneous Ca spikes increases the number of neurons expressing excitatory transmitters and decreases the number of neurons expressing inhibitory transmitters. **c,** Increasing the frequency of Ca spikes decreases the number of neurons expressing excitatory transmitters and increases the number of neurons expressing inhibitory transmitters. Adapted from ref. 22.

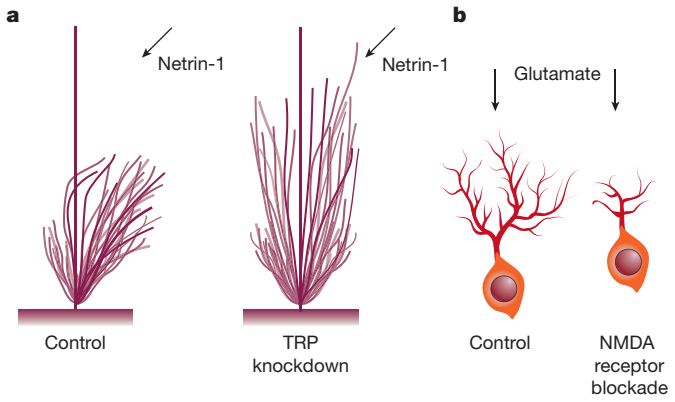

Figure 4 | Turning of embryonic axonal growth cones and elaboration of dendritic arbours depend on elevation of intracellular calcium. **a**, Growth cone trajectories of frog spinal neurons during exposure to a gradient of the guidance molecule netrin-1 (arrows) show turning in controls that is absent when synthesis of Ca-permeable TRP channels is knocked down. Adapted from ref. 38, with permission. **b**, Blocking Ca-permeable glutamate receptors (NMDAR) reduces the growth of dendrites in the frog brain. Adapted from ref. 49, with permission; copyright 1999 by the Society for Neuroscience.

Dendrite outgrowth. A fourth facet of neuronal differentiation is the formation of dendrites, which are generally shorter than axons, more extensively branched, and specialized for the receipt instead of transmission of synaptic signals. Their growth and stability depend on activation of neurotransmitter receptors and Ca elevation. Blockade of NMDA receptors on neurons in the *Xenopus* tadpole optic tectum, during the period of synaptogenesis in which axons are innervating the tectum and growth cones are releasing transmitter, suppresses development of their dendritic arbours by curbing addition of new branches and extension of pre-existing branches[49] (Fig. 4b). Cholinergic neurotransmission evokes local Ca-induced Ca release that stabilizes developing dendrites of retinal ganglion cells during early stages of synapse formation in the embryonic chick retina[50]; blockade of glutamatergic transmission suppresses dendrite motility at later stages of development[51]. Dendrites of postnatal rat hippocampal neurons in slices generate Ca transients in their filopodia during synapse formation that are correlated with reduced filopodial motility[52], similar to the effect of Ca transients on axonal filopodia[42].

Activation of transmitter receptors also stimulates global Ca increases throughout dendritic arbours that signal to the nucleus and regulate dendrite outgrowth. Ca-induced dendritic growth in cultured embryonic rat cortical neurons is regulated by activation of a transcriptional program that involves CaM (Ca/calmodulin-dependent protein) kinase IV and CREB (cAMP responsive element binding protein)-mediated signalling to the nucleus[53]. Targeted disruption of CREST, a Ca-responsive transactivator, compromises dendritic growth and branching in mouse hippocampal and cortical neurons[54]. Thus activity-dependent transcriptional regulation via the activity of conventional transmitter-activated channels is a key aspect of dendrite formation. Activity of novel channels may also be involved, given the expression of TRP channels on dendrites in the mature nervous system.

Adult stem cells

The discovery of adult neurogenesis[55] led to investigation of the steps regulating proliferation, migration, and differentiation of newly born neurons as they develop in the adult brain. Remarkably, early stages of development of these neurons in the dentate gyrus of the hippocampus and in the olfactory bulb are also activity-dependent.

Neuronal precursors and differentiating neurons express voltage-gated Na and Ca and transmitter-activated channels similar to those in embryonic cells[56,57], which may give rise to spontaneous activity and have functions similar to those in the embryonic nervous system. Altering excitability affects proliferation. Activating NMDA receptors decreases proliferation and blocking NMDA receptors or lesioning excitatory afferents increases proliferation of granule cells in adult rat dentate gyrus[58]. In contrast, serotonin stimulates hippocampal rodent neurogenesis[59,60]. Seizure activity and voluntary wheel running increase the number of new neurons and odour deprivation reduces it[61,62], suggesting that physiological activity regulates adult neurogenesis. Perturbing excitability also perturbs migration. Seizures disrupt migration from the subgranular proliferative zone to the inner granule cell layer in the dentate gyrus[61]. Odour deprivation reduces expression of tenascin-R and disrupts radial migration of neuroblasts in the mouse olfactory bulb[63].

Depolarization by neurotransmitters is important for neuronal differentiation from adult stem cells. Excitation of cultured mouse hippocampal proliferating precursors via NMDA receptors and voltage-gated Ca channels represses glial fate genes and stimulates expression of the neural fate gene *NeuroD*[64]. GABAergic synaptic activation of precursor cells *in vivo* is excitatory owing to their increased intracellular chloride concentration and stimulates Ca influx and expression of *NeuroD* that promotes neuronal differentiation[65]. Progeny of adult rat hippocampal neural stem cells co-cultured with neurons and astrocytes from neonatal hippocampus become electrically active and form synaptic connections that appear to contribute to their functional integration into pre-existing circuits[66]. Indeed, integration of newly generated neurons into existing networks is activity-dependent. GABAergic depolarization of newborn mouse granule cells in the adult dentate gyrus is necessary for synapse formation and dendritic development *in vivo*[67]. Thus many features of activity-dependent early development involving conventional ion channels are recapitulated in adult neurogenesis; roles for novel forms of activity remain to be investigated.

Conclusions and perspectives

Now that we recognize the involvement of electrical activity in many aspects of early neuronal development, we can ask whether electrical activity has a specific function or functions. Has evolution simply opportunistically seized on what was useful at the time—selecting signalling by transcription factors or signalling by ion channel activity for various purposes? Or are there particular benefits to the organism of signalling by ion channels that generate elevations of intracellular Ca?

On the one hand, signalling by electrical activity may refine signalling via gene expression, providing feedback loops to validate or fine-tune steps of development driven by genetic programs. Analogous to processes of development at later stages of differentiation, genes establish an initial landscape that subsequent activity reshapes. In this way electrical activity can act to ensure the efficiency and fidelity of brain assembly.

On the other hand, electrical activity seems likely to operate at a more fundamental level, integrated with gene expression. Genes encoding ion channels, receptors and ligands that activate them generate electrical activity leading to a wide range of elevations of intracellular Ca (ref. 68; Box 2). Different patterns of Ca elevation can regulate gene expression in a cell-autonomous manner. However, transient elevations of intracellular Ca also regulate cellular secretion and organization of the cytoskeleton that controls motility. Secretion of diffusible molecules and cellular motility that generates cell–cell contacts enable inductive interactions among cells that regulate neuronal development at all levels, from cell proliferation to post-translational protein modification. In this way brief electrical activity can act non-cell-autonomously to stimulate ligand–receptor binding with consequences that play out over longer, developmentally relevant timescales (Fig. 5).

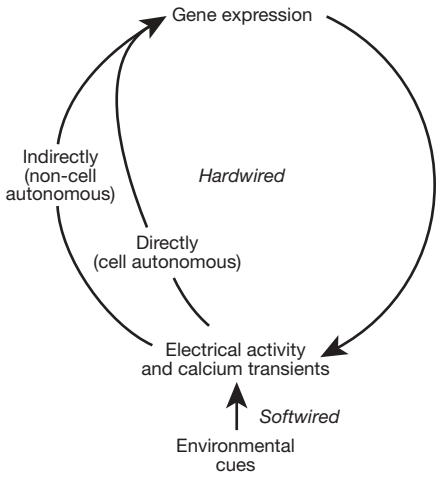

Figure 5 | The partnership of gene expression with electrical activity. Gene expression leads to synthesis of ion channels, receptors and ligands; secretion of ligands and cell–cell interactions drive electrical activity that generates transient elevations of Ca. Ca transients in turn regulate gene expression both directly in a cell-autonomous manner and indirectly, non-cell-autonomously, via inductive interactions. This hardwiring of early development is likely to be complemented by softwiring driven by stimulation of electrical activity by environmental cues.

Box 2 | Spontaneous activity following synapse formation

Spontaneous activity is also observed after synapse formation. Ca transients generated at these later stages of development may influence aspects of differentiation (for example, neurotransmitter specification) that are regulated by early spontaneous activity and affect development of neighbouring neurons that are at earlier stages of development. Rhythmic spontaneous electrical activity and Ca transients are observed early in embryonic mouse and chick spinal cord development[82,83]. Voltage-sensitive dyes allow optical identification of transiently expressed Ca-dependent action potentials and spontaneous large-scale correlated wave activity in the embryonic chick and rat brainstem[84,85]. Synchronized Ca transients stimulated by serotonin are observed in mouse cranial motor neurons at embryonic stages of development[86]. Synchronized Ca oscillations activated by AMPA and NMDA receptors are propagated from posterior to anterior cortex in the newborn rat[87]. Similar transients are generated by voltage-gated Na and Ca channels in mouse cortex and the degree of synchrony peaks at the time of birth[88]. Postnatally, spontaneous giant depolarizing potentials are observed in synchronized populations of rat hippocampal neurons[89] that generate Ca transients[90]. Developing retinal ganglion cells propagate spontaneous bursts of action potentials and ensuing Ca elevations in waves across the retina[91,92]. Spontaneous synchronous and sequential Ca transients driven by intrinsic currents and circuit mechanisms are observed in mouse visual cortex in networks of pyramidal cells[93], with precise repetitions of sequences of activation in mouse and cat cortex[94]. Recurrent Ca transients involving thousands of cells occur in cortical neurons of non-anaesthetized newborn mice during resting periods[95]. It is of great interest to understand further the functions of these forms of spontaneous activity.

This process is probably important, because the complexity of the brain renders the number of genes in the genome insufficient to program the organization of the nervous system directly on a single-gene-to-single-component basis. Local communication is required to specify developmental choices in a temporally and spatially appropriate manner. Cellular and subcellular Ca signalling controls DNA synthesis[6–9,58–62,64,65], regulates messenger RNA and protein levels[47,48,54,69], and stimulates phosphorylation and dephosphorylation[53,70,71]. Thus electrical activity and gene expression appear to be full partners in neuronal development. We can look ahead to identification of the inducing molecules released in an activity-dependent manner and the cell–cell contacts driven by activity that drive inductive interactions in early neuronal development.

Activity-dependence of early neural development does not necessarily challenge the idea that development is hardwired. Development operates on a fixed agenda if activity simply implements the outcome of genetic programs or even if it is an equal partner, turning on and being turned on by genetic programs. Hardwiring will be broken, however, when environmental cues alter activity that affects development (Fig. 5). Because synapses have not yet formed at early stages, these cues must reach developing neurons by other means, such as paracrine action of transmitters, circulation of maternal hormones, or (in lower animals) fluctuations in temperature. These stimuli can be predicted to alter early activity, but the extent to which this regulates and softwires early development remains to be determined.

1. Zito, K. & Svoboda, K. Activity-dependent synaptogenesis in the adult mammalian cortex. *Neuron* **35,** 1015–1017 (2002).
2. Goda, Y. & Davis, G. W. Mechanisms of synapse assembly and disassembly. *Neuron* **40,** 243–264 (2003).
3. Mennerick, S. & Zorumski, C. F. Neural activity and survival in the developing nervous system. *Mol. Neurobiol.* **22,** 41–54 (2000).
4. Vanhoutte, P. & Bading, H. Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. *Curr. Opin. Neurobiol.* **13,** 366–371 (2003).
5. Webb, S. E., Moreau, M., Leclerc, C. & Miller, A. L. Calcium transients and neural induction in vertebrates. *Cell Calcium* **37,** 375–385 (2005).
6. LoTurco, J. J., Owens, D. F., Heath, M. J., Davis, M. B. & Kriegstein, A. R. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* **15,** 1287–1298 (1995).
7. Liu, X., Wang, Q., Haydar, T. F. & Bordey, A. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nature Neurosci.* **8,** 1179–1187 (2005).
8. Fiszman, M. L., Borodinsky, L. N. & Neale, J. H. GABA induces proliferation of immature cerebellar granule cells grown *in vitro*. *Dev. Brain Res.* **115,** 1–8 (1999).
9. Weissman, T. A., Riquelme, P. A., Ivic, L., Flint, A. C. & Kriegstein, A. R. Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. *Neuron* **43,** 647–661 (2004).
10. Erhardt, N. M. & Sherwood, N. M. PACAP maintains cell cycling and inhibits apoptosis in chick neuroblasts. *Mol. Cell. Endocrinol.* **221,** 121–134 (2004).
11. Gibbs, S. M. Regulation of neuronal proliferation and differentiation by nitric oxide. *Mol. Neurobiol.* **27,** 107–120 (2003).
12. Komuro, H. & Rakic, P. Intracellular Ca^{2+} fluctuations modulate the rate of neuronal migration. *Neuron* **17,** 275–285 (1996).
13. Demarque, M. *et al.* Paracrine intercellular communication by a Ca^{2+}- and SNARE-independent release of GABA and glutamate prior to synapse formation. *Neuron* **36,** 1051–1061 (2002).
14. Manent, J. B. *et al.* A noncanonical release of GABA and glutamate modulates neuronal migration. *J. Neurosci.* **25,** 4755–4765 (2005).
15. Yacubova, E. & Komuro, H. Stage-specific control of neuronal migration by somatostatin. *Nature* **415,** 77–81 (2002).
16. Bolteus, A. J. & Bordey, A. GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. *J. Neurosci.* **24,** 7623–7631 (2004).
17. Chen, Y. *et al.* Reelin modulates NMDA receptor activity in cortical neurons. *J. Neurosci.* **25,** 8209–8216 (2005).
18. Haase, A. & Bicker, G. Nitric oxide and cyclic nucleotides are regulators of neuronal migration in an insect embryo. *Development* **130,** 3977–3987 (2003).
19. Dallman, J. E., Davis, A. K. & Moody, W. J. Spontaneous activity regulates Ca^{2+}-dependent K^+ current expression in developing ascidian muscle. *J. Physiol. (Lond.)* **511,** 683–693 (1998).
20. Gu, X. & Spitzer, N. C. Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients. *Nature* **375,** 784–787 (1995).
21. Desarmenien, M. G. & Spitzer, N. C. Role of Ca and protein kinase C in development of the delayed rectifier potassium current in *Xenopus* spinal neurons. *Neuron* **7,** 797–805 (1991).
22. Borodinsky, L. N. *et al.* Activity-dependent homeostatic specification of transmitter expression in embryonic neurons. *Nature* **429,** 523–530 (2004).
23. Ahmed, Z., Walker, P. S. & Fellows, R. E. Properties of neurons from dissociated fetal rat brain in serum-free culture. *J. Neurosci.* **3,** 2448–2462 (1983).
24. Nerbonne, J. M. & Gurney, A. M. Development of excitable membrane properties in mammalian sympathetic neurons. *J. Neurosci.* **9,** 3272–3286 (1989).
25. McCobb, D. P., Best, P. M. & Beam, K. G. The differentiation of excitability in embryonic chick limb motoneurons. *J. Neurosci.* **10,** 2974–2984 (1990).
26. Brosenitsch, T. A. & Katz, D. M. Physiological patterns of electrical stimulation can induce neuronal gene expression by activating N-type calcium channels. *J. Neurosci.* **21,** 2571–2579 (2001).
27. Brosenitsch, T. A. & Katz, D. M. Expression of Phox2 transcription factors and induction of the dopaminergic phenotype in primary sensory neurons. *Mol. Cell. Neurosci.* **20,** 447–457 (2002).
28. Nishimaru, H., Restrepo, C. E., Ryge, J., Yanagawa, Y. & Kiehn, O. Mammalian motor neurons corelease glutamate and acetylcholine at central synapses. *Proc. Natl Acad. Sci. USA* **102,** 5245–5249 (2005).
29. Mentis, G. Z. *et al.* Noncholinergic excitatory actions of motoneurons in the neonatal mammalian spinal cord. *Proc. Natl Acad. Sci. USA* **102,** 7344–7349 (2005).
30. Brunelli, G. *et al.* Glutamatergic reinnervation through peripheral nerve graft dictates assembly of glutamatergic synapses at rat skeletal muscle. *Proc. Natl Acad. Sci. USA* **102,** 8752–8757 (2005).
31. Gomez, T. M., Snow, D. M. & Letourneau, P. C. Characterization of spontaneous Ca transients in nerve growth cones and their effect on growth cone migration. *Neuron* **14,** 1233–1246 (1995).
32. Gomez, T. M. & Spitzer, N. C. *In vivo* regulation of axon extension and pathfinding by growth-cone Ca transients. *Nature* **397,** 350–355 (1999).
33. Tang, F., Dent, E. W. & Kalil, K. Spontaneous calcium transients in developing cortical neurons regulate axon outgrowth. *J. Neurosci.* **23,** 927–936 (2003).
34. Greka, A., Navarro, B., Oancea, E., Duggan, A. & Clapham, D. E. TRPC5 is a regulator of hippocampal neurite length and growth cone morphology. *Nature Neurosci.* **6,** 837–845 (2003).
35. Zheng, J. Q., Wan, J. J. & Poo, M. M. Essential role of filopodia in chemotropic turning of nerve growth cone induced by a glutamate gradient. *J. Neurosci.* **16,** 1140–1149 (1996).
36. Hong, K., Nishiyama, M., Henley, J., Tessier-Lavigne, M. & Poo, M. Calcium signalling in the guidance of nerve growth by netrin-1. *Nature* **403,** 93–98 (2000).
37. Nishiyama, M. *et al.* Cyclic AMP/GMP-dependent modulation of Ca^{2+} channels sets the polarity of nerve growth-cone turning. *Nature* **423,** 990–995 (2003).
38. Wang, G. X. & Poo, M. Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. *Nature* **434,** 898–904 (2005).
39. Shim, S. *et al.* XTRPC1-dependent chemotropic guidance of neuronal growth cones. *Nature Neurosci.* **8,** 730–735 (2005).

40. Li, Y. *et al.* Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. *Nature* **434,** 894–898 (2005).
41. Ming, G., Henley, J., Tessier-Lavigne, M., Song, H. & Poo, M. Electrical activity modulates growth cone guidance by diffusible factors. *Neuron* **29,** 441–452 (2001).
42. Gomez, T. M., Robles, E., Poo, M. & Spitzer, N. C. Filopodial calcium transients promote substrate-dependent growth cone turning. *Science* **291,** 1983–1987 (2001).
43. Ruthazer, E. S., Akerman, C. J. & Cline, H. T. Control of axon branch dynamics by correlated activity *in vivo*. *Science* **301,** 66–70 (2003).
44. Hua, J. Y., Smear, M. C., Baier, H. & Smith, S. J. Regulation of axon growth *in vivo* by activity-based competition. *Nature* **434,** 1022–1026 (2005).
45. O'Leary, D. D. *et al.* A target-derived chemoattractant controls the development of the corticopontine projection by a novel mechanism of axon targeting. *Dev.*, Suppl. 2, 123–130 (1991).
46. Tang, F. & Kalil, K. Netrin-1 induces axon branching in developing cortical neurons by frequency-dependent calcium signaling pathways. *J. Neurosci.* **25,** 6702–6715 (2005).
47. Itoh, K., Stevens, B., Schachner, M. & Fields, R. D. Regulated expression of the neural cell adhesion molecule L1 by specific patterns of neural impulses. *Science* **270,** 1369–1372 (1995).
48. Hanson, M. G. & Landmesser, L. T. Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron* **43,** 687–701 (2004).
49. Rajan, I. & Cline, H. T. Glutamate receptor activity is required for normal development of tectal cell dendrites *in vivo*. *J. Neurosci.* **18,** 7836–7846 (1998).
50. Lohmann, C., Myhr, K. L. & Wong, R. O. Transmitter-evoked local calcium release stabilizes developing dendrites. *Nature* **418,** 177–181 (2002).
51. Wong, W. T., Faulkner-Jones, B. E., Sanes, J. R. & Wong, R. O. Rapid dendritic remodeling in the developing retina: dependence on neurotransmission and reciprocal regulation by Rac and Rho. *J. Neurosci.* **20,** 5024–5036 (2000).
52. Lohmann, C., Finski, A. & Bonhoeffer, T. Local Ca transients regulate the spontaneous motility of dendritic filopodia. *Nature Neurosci.* **8,** 305–312 (2005).
53. Redmond, L., Kashani, A. H. & Ghosh, A. Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. *Neuron* **34,** 999–1010 (2002).
54. Aizawa, H. *et al.* Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. *Science* **303,** 197–202 (2004).
55. Altman, J. & Das, G. D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124,** 319–335 (1965).
56. Schmidt-Hieber, C., Jonas, P. & Bischofberger, J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* **429,** 184–187 (2004).
57. Esposito, M. S. *et al.* Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.* **25,** 10074–10086 (2005).
58. Cameron, H. A., McEwen, B. S. & Gould, E. Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. *J. Neurosci.* **15,** 4687–4692 (1995).
59. Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.* **20,** 9104–9110 (2000).
60. Santarelli, L. *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301,** 805–809 (2003).
61. Parent, J. M. *et al.* Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* **17,** 3727–3738 (1997).
62. van Praag, H., Christie, B. R., Sejnowski, T. J. & Gage, F. H. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl Acad. Sci. USA* **96,** 13427–13431 (1999).
63. Saghatelyan, A., de Chevigny, A., Schachner, M. & Lledo, P. M. Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain. *Nature Neurosci.* **7,** 347–356 (2004).
64. Deisseroth, K. *et al.* Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* **42,** 535–552 (2004).
65. Tozuka, Y., Fukuda, S., Namba, T., Seki, T. & Hisatsune, T. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* **47,** 803–815 (2005).
66. Song, H. J., Stevens, C. F. & Gage, F. H. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nature Neurosci.* **5,** 438–445 (2002).
67. Ge, S. *et al.* GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* **439,** 589–593 (2006).
68. Berridge, M. J., Bootman, M. D. & Roderick, H. L. Calcium signalling: dynamics, homeostasis and remodeling. *Nature Rev. Mol. Cell Biol.* **4,** 517–529 (2003).
69. Watt, S. D., Gu, X., Smith, R. D. & Spitzer, N. C. Specific frequencies of spontaneous Ca^{2+} transients upregulate GAD 67 transcripts in embryonic spinal neurons. *Mol. Cell. Neurosci.* **16,** 376–387 (2000).
70. Wu, G. Y. & Cline, H. T. Stabilization of dendritic arbor structure *in vivo* by CaMKII. *Science* **279,** 222–226 (1998).
71. Lautermilch, N. J. & Spitzer, N. C. Regulation of calcineurin by growth cone calcium waves controls neurite extension. *J. Neurosci.* **20,** 315–325 (2000).
72. Blanton, M. G., Lo Turco, J. J. & Kriegstein, A. R. Endogenous neurotransmitter activates *N*-methyl-D-aspartate receptors on differentiating neurons in embryonic cortex. *Proc. Natl Acad. Sci. USA* **87,** 8027–8030 (1990).
73. Flint, A. C., Dammerman, R. S. & Kriegstein, A. R. Endogenous activation of metabotropic glutamate receptors in neocortical development causes neuronal Ca oscillations. *Proc. Natl Acad. Sci. USA* **96,** 12144–12149 (1999).
74. Wang, D. D., Krueger, D. D. & Bordey, A. GABA depolarizes neuronal progenitors of the postnatal subventricular zone via $GABA_A$ receptor activation. *J. Physiol. (Lond.)* **550,** 785–800 (2003).
75. Carey, M. B. & Matsumoto, S. G. Spontaneous calcium transients are required for neuronal differentiation of murine neural crest. *Dev. Biol.* **215,** 298–313 (1999).
76. Gu, X., Olson, E. C. & Spitzer, N. C. Spontaneous neuronal calcium spikes and waves during early differentiation. *J. Neurosci.* **14,** 6325–6335 (1994).
77. Conklin, M. W., Lin, M. S. & Spitzer, N. C. Local calcium transients contribute to disappearance of pFAK, focal complex removal and deadhesion of neuronal growth cones and fibroblasts. *Dev. Biol.* **287,** 201–212 (2005).
78. Ashworth, R. & Bolsover, S. R. Spontaneous activity-independent intracellular calcium signals in the developing spinal cord of the zebrafish embryo. *Dev. Brain Res.* **139,** 131–137 (2002).
79. Owens, D. F. & Kriegstein, A. R. Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone. *J. Neurosci.* **18,** 5374–5388 (1998).
80. Mercer, A. R. & Hildebrand, J. G. Developmental changes in the electrophysiological properties and response characteristics of *Manduca* antennal-lobe neurons. *J. Neurophysiol.* **87,** 2650–2663 (2002).
81. Jiang, S. A., Campusano, J. M., Su, H. & O'Dowd, D. K. *Drosophila* mushroom body Kenyon cells generate spontaneous calcium transients mediated by PLTX-sensitive calcium channels. *J. Neurophysiol.* **94,** 491–500 (2005).
82. Hanson, M. G. & Landmesser, L. T. Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. *J. Neurosci.* **23,** 587–600 (2003).
83. O'Donovan, M. J., Bonnot, A., Wenner, P. & Mentis, G. Z. Calcium imaging of network function in the developing spinal cord. *Cell Calcium* **37,** 443–450 (2005).
84. Momose-Sato, Y., Sato, K. & Kamino, K. Optical identification of Ca-dependent action potentials transiently expressed in the embryonic rat brainstem. *Neuroscience* **90,** 1293–1310 (1999).
85. Momose-Sato, Y., Mochida, H., Sasaki, S. & Sato, K. Depolarization waves in the embryonic CNS triggered by multiple sensory inputs and spontaneous activity: optical imaging with a voltage-sensitive dye. *Neuroscience* **116,** 407–423 (2003).
86. Hunt, P. N., McCabe, A. K. & Bosma, M. M. Midline serotonergic neurones contribute to widespread synchronized activity in embryonic mouse hindbrain. *J. Physiol. (Lond.)* **566,** 807–819 (2005).
87. Garaschuk, O., Linn, J., Eilers, J. & Konnerth, A. Large-scale oscillatory calcium waves in the immature cortex. *Nature Neurosci.* **3,** 452–459 (2000).
88. Corlew, R., Bosma, M. M. & Moody, W. J. Spontaneous, synchronous electrical activity in neonatal mouse cortical neurones. *J. Physiol. (Lond.)* **560,** 377–390 (2004).
89. Ben-Ari, Y., Cherubini, E., Corradetti, R. & Gaiarsa, J. L. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol. (Lond.)* **416,** 303–325 (1989).
90. Kasyanov, A. M., Safiulina, V. F., Voronin, L. L. & Cherubini, E. GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. *Proc. Natl Acad. Sci. USA* **101,** 3967–3972 (2004).
91. Stellwagen, D. & Shatz, C. J. An instructive role for retinal waves in the development of retinogeniculate connectivity. *Neuron* **33,** 357–367 (2002).
92. Torborg, C. L., Hansen, K. A. & Feller, M. B. High frequency, synchronized bursting drives eye-specific segregation of retinogeniculate projections. *Nature Neurosci.* **8,** 72–78 (2005).
93. Cossart, R., Aronov, D. & Yuste, R. Attractor dynamics of network UP states in the neocortex. *Nature* **423,** 283–288 (2003).
94. Ikegaya, Y. *et al.* Synfire chains and cortical songs: temporal modules of cortical activity. *Science* **304,** 559–564 (2004).
95. Adelsberger, H., Garaschuk, O. & Konnerth, A. Cortical calcium waves in resting newborn mice. *Nature Neurosci.* **8,** 988–990 (2005).
96. Borodinsky, L. N. & Spitzer, N. C. Activity-dependent neurotransmitter–receptor matching at the neuromuscular junction. *Proc. Natl Acad. Sci. USA*, in the press.

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