Ion Channel Development, Spontaneous Activity, and Activity-Dependent Development in Nerve and Muscle Cells

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neuronal development. The configuration of voltage- and ligand-gated ion channels that are expressed early in development regulate the timing and waveform of this activity. They also regulate Ca\(^{2+}\) influx during spontaneous activity, which is the first step in triggering activity-dependent developmental programs. For these reasons, the properties of voltage- and ligand-gated ion channels expressed by developing neurons and muscle cells often differ markedly from those of adult cells. When viewed from this perspective, the reasons for complex patterns of ion channel emergence and regression during development become much clearer.

I. INTRODUCTION

The complex patterns of ion channel development that occur in many excitable cells belie the notion that the development of physiological properties in these cells can be understood as a simple linear continuum to a single, final set of properties. This perplexing complexity of ion channel development can now be viewed in light of our understanding of the critical roles of spontaneous activity in early development. Most nerve and muscle cells generate spontaneous electrical activity during at least one discrete stage of their development, and this activity is of fundamental importance to their later development. Spontaneous activity regulates a large variety of developmental processes, and in doing so it occurs with patterns and waveforms that cannot support the mature forms of activity and information processing of the cell.

For this reason, the configuration of ion channels and receptors expressed at early stages of development are optimized to mediate spontaneous activity and unique to the early stages when this kind of activity occurs. This optimization creates the appropriate electrical waveform of spontaneous activity, synchronizes it among cells, and mediates the Ca\(^{2+}\) influx that transduces activity into developmental programs. In addition to optimization of immature ion channels and their incompatibility with many mature functions, two other general principles will arise repeatedly in this review. The first is coordination of the development of multiple channel types, both ligand- and voltage-gated, which must cooperate to create periods of spontaneous activity. The second is the self-limiting nature of spontaneous activity. The transition between this immature period of spontaneous activity and the mature, information processing functions of the cells is critical. It is managed in part by making the expression of mature ion channels and receptors, whose expression tends of terminate spontaneous activity, dependent on the spontaneous activity created by their immature predecessors.

The existence of distinct electrical properties early in development that are geared toward creating spontaneous activity has important clinical implications, especially in the field of pediatric seizure disorders.

In this review, we analyze how the patterns of ion channel development give rise to spontaneous activity and how that activity carries out its developmental functions. In section ii we use several cell types to illustrate the wide variety of developmental processes regulated by spontaneous activity, and how the waveform of activity in different cells is regulated by the expression patterns of ion channels early in development. In section iii, we discuss the mechanisms by which spontaneous activity controls developmental processes. In section iv, we propose some general principles that govern how the ion channels expressed at early stages differ from mature channels and how they regulate spontaneous activity. In section v, we discuss the critical role played by spontaneous activity in regulating the maturation of ion channels so that cells successfully make the transition between embryonic and mature signaling properties. Finally, in section vi, we discuss clinical ramifications of the idea that immature neurons have electrical properties that favor spontaneous activity.

II. COREGULATION OF ION CHANNEL DEVELOPMENT, SPONTANEOUS ACTIVITY, AND ACTIVITY-DEPENDENT DEVELOPMENT

In this section, we discuss 16 cell types that illustrate different aspects of how ion channel development regulates spontaneous electrical activity, which in turn regulates some important aspect of subsequent development.

A. Oocyte Maturation and the Block to Polyspermy

We often think of complex patterns of ion channel development as characteristic of the terminal differentiation of neurons and muscle cells. But they actually begin even before fertilization. The ways in which ion channel properties are modulated during development of oocytes can be understood in the same context that governs similar later events: by knowing the developmental function of electrical signals at these stages and asking how particular ion channels ensure that the properties of those signals are consistent with that function.

I. Nature and developmental function of spontaneous activity

In many organisms (including echinoderms, amphibians, nemertean worms, but not mammals), fertilization is accomplished by a large, long-lasting depolarization known as the fertilization potential (see Refs. 264, 265 for review). Fertilization potentials last from several minutes
to more than 1 h, depending on the species. The fertilization potential is mediated by sodium, nonspecific cation, or chloride channels that are gated by the rise in [Ca\(^{2+}\)], that occurs at fertilization, with contributions at early times by voltage-gated Ca\(^{2+}\) or Na\(^{+}\) channels and possibly by channels donated to the oocyte membrane by the sperm. The long duration of the fertilization potential is caused by the long duration of the [Ca\(^{2+}\)], transients at fertilization and in many egg cells by the virtual absence of delayed K\(^+\) currents to repolarize the membrane. In addition, the resting resistance of most oocytes is very high, creating a long time constant. It is an interesting, but as yet unexplained, observation that oocytes (such as those of mammals) that do not depolarize at fertilization still have voltage-gated channels and are excitable to direct stimulation (477). This suggests that electrical activity has functions in oocytes that we do not yet understand.

While the fertilization potential is not strictly spontaneous because it is triggered by sperm binding, it emphasizes that electrical activity plays a role in development from the earliest stages. A variety of experiments have shown that fertilization potential mediates the fast block to polyspermy in many organisms, acting to prevent supernumerary sperm from fusing with the oocyte at short times before physical mechanisms of polyspermy block have been established (264, 292, 266, 267; see Ref. 265 for reviews). Direct current injection experiments have shown that the depolarization alone is sufficient to block sperm entry (264).

2. Relationship to channel development

The populations of ion channels in the egg cell membrane at the time of fertilization result from a complex earlier process of development that has been studied in detail in starfish. At the end of oogenesis in starfish, the fully grown, immature oocyte awaits a hormonal signal that will trigger its maturation and ovulation in preparation for fertilization. In starfish this hormone is 1-methyladenine (1-MA; Ref. 275). Maturation involves breakdown of the nuclear membrane, the initiation of meioisis, and other events that prepare the egg to be fertilized, events that are similar to those triggered in mammalian oocytes by progesterone.

Voltage-clamp of oocytes of the starfish Leptaserias shows that the fully grown, immature oocyte has only two depolarization-activated currents: an inward Ca\(^{2+}\) current and a transient outward (A-type) K\(^+\) current. Action potentials can be elicited by depolarization, but because of the large ratio of A-current to Ca\(^{2+}\) current, they do not overshoot 0 mV. During maturation, which can be triggered in vitro and takes only 30–45 min, the A-current decreases by \(-50\%\), while the Ca\(^{2+}\) current remains unchanged. This increases the amplitude of the action potential so that it now overshoots 0 mV (424). The decrease in A-current is caused by loss of plasma membrane during maturation: cell capacitance decreases by precisely the same amount and with the same time course as the A-current, and electron micrographs show an almost complete elimination of the microvilli that characteristic the surface of the oocyte before maturation (422; see Ref. 530). Quantitative measurements of membrane surface area from the micrographs show a close correspondence with the change in surface area measured electrically by capacitance. The selective loss of the A-current during maturation is necessary for the mature egg to be fertilized successfully. Without the resulting increase in action potential amplitude, the fast electrical block to polyspermy is much less efficient, fertilization is polyspermic, and abnormal development ensues (414, 415). Thus a prefertilization process of ion channel development that relates to the first activity-dependent developmental event in the life of the organism is required for embryonic life to begin.

The lack of decrease in Ca\(^{2+}\) current during this massive membrane loss implies that Ca\(^{2+}\) channels are protected from endocytosis, possibly by cytoskeletal anchoring. During oogenesis in this species, the A-current increases gradually, accurately tracking membrane area during a 2-yr growth interval just as it tracks the 30-min period of membrane loss during maturation. The Ca\(^{2+}\) current, on the other hand, appears abruptly at the end of the growth phase, dissociated from membrane addition just as it is from membrane loss during maturation (421). The appearance of the Ca\(^{2+}\) current coincides with the migration of the nucleus to the animal pole at the end of oogenesis. This raises the possibility that Ca\(^{2+}\) channels are inserted into the membrane only at the animal pole and are then protected from endocytosis by mechanisms that anchor the nucleus in that position (529). Protection from endocytosis by cytoskeletal anchoring or by accessory subunits influences ion channel development during terminal neuronal differentiation as well (6).

Selective loss of some currents and preservation or increases in the amplitudes of others have also been observed during maturation of amphibian oocytes (25). This kind of selective modulation also extends to exogenous channels expressed in oocytes (80, 536).

The patterns of ion channel development in this relatively simple system encapsulate many of the principles that are seen in more complex central nervous system structures. Individual currents may increase and decrease during development, changing at distinct stages and with specific relationships to other cellular events, such as changes in membrane surface area or cell cycle progression and arrest. The timing and specificity of such changes dictate changes in action potential threshold and waveform in ways that are critical for the developmental roles of electrical signaling at specific stages.
B. Early Postfertilization Changes and Selective Channel Elimination

Complex changes in ion channel expression also occur between the time of fertilization and the beginning of nervous system formation. Most of the information about ion channel development at these early cleavage stages comes from work in ascidian embryos. Ascidians are chordates whose embryos have long been a classic preparation in which to study development, partly because many cell lineages are committed very early in development without substantial cell interactions (520; a notable exception is the nervous lineage, which does require induction by the notochord). Some species have the additional advantage of an intensely pigmented muscle lineage, so muscle lineage cells can be recognized even at early cleavage stages and in dissociated preparations.

Before fertilization, eggs of the ascidian Bottlenia villosa express $\text{Na}^+$, $\text{Ca}^{2+}$, and inwardly rectifying $\text{K}^+$ currents. After fertilization these three currents are eliminated, each at a specific stage. The $\text{Na}^+$ current is lost within 2 h, at the time of first cleavage, but the $\text{Ca}^{2+}$ current is retained (51, 240, 120). The $\text{Ca}^{2+}$ current is eliminated later, at gastrulation, and the inward rectifier even later, at neurulation. The inward rectifier is particularly interesting. In the 12 h before it is eliminated, the inward rectifier is maintained in all cells of the embryo at a constant density. Because the total surface area of all cells in the embryo at gastrulation is ~10 times that of the egg, there must have been a 10-fold upregulation of the inward rectifier during this period, either by addition of new channels or unmasking of preexisting ones. Then, after this intense period of upregulation, the inward rectifier is eliminated in all cells in only a few hours (51, 208). This kind of active maintenance of channel density followed by abrupt disappearance suggests roles both for the presence of the channel early and its absence later. In this case, it is likely that maintenance of the inward rectifier, which is the only resting conductance of these cells, combined with the loss of the $\text{Na}^+$ current, prevents generation of action potentials during the cleavage phase of early development. As discussed below, the disappearance of the inward rectifier is the trigger for spontaneous activity in these cells. Superimposed on all of these changes is the cyclical appearance and disappearance of a hyperpolarization-activated $\text{Cl}^-$ channel with each cell division (52, 598).

A very interesting analogous result to the above selective elimination of $\text{Na}^+$ current ($I_{\text{Na}}$) after fertilization, but retention of $\text{Ca}^{2+}$ current ($I_{\text{Ca}}$), is seen in another ascidian, Ciona intestinalis. The egg cell of this species does not have a low-threshold $I_{\text{Na}}$, but rather a second, low-threshold $I_{\text{Ca}}$ similar to the T-type $\text{Ca}^{2+}$ current. It is this low-threshold $I_{\text{Ca}}$ that is lost after fertilization in Ciona, while the high-threshold $I_{\text{Ca}}$ is retained (11). This suggests that the functional significance in both species is the elimination of the low-threshold inward current that could lead to aberrant spiking during cleavage, independent of the identity of the channel that carries it. Such aberrant spiking might be triggered by the activation of mechanosensitive ion channels, which are a prominent feature of ascidian oocytes (423).

Some of the most extensive work on ion channel development in early embryos has been done by Takahashi and colleagues, working on cleavage-arrested embryos of the ascidian Halocynthia roretzi. The egg cell of this species expresses $\text{Na}^+$, $\text{Ca}^{2+}$, delayed $\text{K}^+$, and inwardly rectifying $\text{K}^+$ currents (460). If embryos at various early stages of development are cleavage-arrested with cytochalasin, the cells differentiate into a variety of mature cell types without dividing. This differentiation is accompanied by specific changes in the patterns of ion channel expression (245, 456). So, for example, if cleavage is arrested at or before the four-cell stage, cells develop into an epidermal type, characterized by expression of $\text{Ca}^{2+}$, inwardly rectifying $\text{K}^+$, and $\text{Ca}^{2+}$-activated $\text{K}^+$ currents. Later cleavage arrest yields cells that differentiate into neural or muscle types, which express different patterns of ion currents. The cleavage-arrested one-cell embryo is large enough that the time course of ionic current expression can be followed in real time as it differentiates from egg to epidermal cell (244). The egg $\text{Ca}^{2+}$ current disappears before gastrulation, and then a mature epidermal form reappears later. The mature $\text{Ca}^{2+}$ current shows $\text{Ca}^{2+}$-dependent inactivation, whereas the egg form shows voltage-dependent inactivation (423). Oddly enough, in the closely related species H. awrantium, the egg-type $\text{Ca}^{2+}$ current reappears and then it subsequently changes into the mature form (243). The $\text{Na}^+$ current disappears entirely from the cleavage-arrested egg as it develops, since the epidermal cell type into which it develops does not express a functional $\text{Na}^+$ current. The disappearance of the $\text{Na}^+$ current is very gradual and follows a complex time course, with a transient peak of density as the larval tail elongates (243). In a further elegant series of experiments, this group developed an in vitro two-cell neural induction system, in which individual cleavage-arrested neural- and notochord-lineage cells are placed into physical contact and subsequently separated to study the exact developmental timing of induction, as well as the timing of competence in each cell (454, 455, 457). With the use of this system, it was shown that neural induction triggered the expression of a neural-type $\text{Na}^+$ channel whose biophysical properties were distinct from the $\text{Na}^+$ channel expressed in the egg (461, 462).

Early postfertilization channel development has also been studied in mammalian oocytes. These cells express functional $\text{Ca}^{2+}$ currents and generate action potentials (459, 477, 630), acquiring this property during the growth
phase of oogenesis (162). After fertilization, this Ca$^{2+}$ current shows a transient increase in density near the second meiotic division (627) and then decreases in amplitude and disappears by the eight-cell stage (413).

Some of these changes in ion channel expression in cleavage-stage embryos may be related to the fact that electrical activity and Ca$^{2+}$ influx appear to play a role in neural induction. Induction by the lectin ConA is accompanied by long-lasting increase in [Ca$^{2+}$], and is inhibited by L-type Ca$^{2+}$ channel blockers. L-type Ca$^{2+}$ channel agonists can trigger neural induction of ectoderm (426). L-type Ca$^{2+}$ channels are expressed in the embryo in dorsal ectoderm at the appropriate stages (154), and induction by mesoderm in vivo activates these channels and causes [Ca$^{2+}$]$_i$ transients in ectoderm (321, 322). In vivo imaging of Xenopus embryos reveals spontaneous [Ca$^{2+}$]$_i$ transients in the presumptive forebrain region of the dorsal ectoderm, although not in the presumptive spinal cord. L-type Ca$^{2+}$ channel blockers produce defects in anterior nervous system structures (322).

Later, specific patterns of ion channel expression appear in presumptive neural tissue. Outward K$^+$ currents and T-type Ca$^{2+}$ currents are expressed in rat floor plate epithelium (188). As discussed below, proliferative cells of the cortical ventricular zone also express delayed outward K$^+$ currents (480).

The above studies show that complex patterns of ion channel development are triggered by fertilization. These proceed throughout the early cleavage stages of embryogenesis, and usually involve specifically timed elimination of voltage-gated currents, as well as cyclical activation of channels by the cell cycle. Thus the electrophysiological properties seen at the start of terminal differentiation in many excitable cells reflect a complex history of ion channel development that begins even before fertilization. Aside from polyspermy block, the roles of electrical signaling during oogenesis, maturation, and early cleavage stages are as yet poorly understood.

C. Retina and Refinement of Visual Connections

1. Retina: nature of spontaneous activity

It has been known for quite some time that the mammalian retina generates spontaneous activity early in development, before it is capable of responding to light, and that this activity involves retinal ganglion cells (RGCs) and cholinergic synaptic transmission (381). With the understanding that the spontaneous activity was intimately involved in the patterning of retinogeniculate connections (535), more attention began to be paid to its mechanisms. Using extracellular unit recording, Galli and Maffei (192) and Maffei and Galli-Resta (362; both in rat retina) demonstrated that neighboring RGCs showed temporally correlated action potentials during this activity, and proposed that such correlations might act in a Hebbian manner to strengthen their connections to downstream targets. We now know that this activity takes the form of spontaneous waves of action potentials and [Ca$^{2+}$]$_i$ transients (169, 616). Such spontaneous activity before the onset of patterned vision occurs in a wide variety of vertebrates, including mouse (17), rat (192), rabbit (637), ferret (389), cat (389), chick (620), turtle (533), and salamander (76) (see Refs. 167, 615, for reviews).

The basic properties of retinal waves have been established using a combination of multielectrode array recording, whole cell recordings from single cells, and [Ca$^{2+}$]$_i$ imaging. Activity in the form of bursts of action potentials lasting 2–4 s, occurring at intervals of 1–2 min, sweeps across large regions of the retina at speeds of 80–140 μm/s (389). RGCs participate in this activity, generating brief bursts of action potentials riding on a large depolarizing wave (618). These waves spread across “domains” in the retina, initiated apparently at random in different regions (169, 170). Some kind of refractory period prevents the rapid reoccurrence of waves in single regions. The idea of some form of postevent refractoriness as a determinant of the interval between waves is likely to hold in other spontaneously active developing structures, like the spinal cord (576). In retina it is supported by the finding of postburst depression of RGC-RGC synapses, whose time course of recovery is similar to the interval between waves (234), and by computational models (85, 86). In chick retina the propagation of these waves seems somewhat more widespread, with activity often moving outward to the edges of the retina (90, 535, 620). Although early experiments indicated that this activity was sensitive to block by tetrodotoxin (TTX), thus implying that the [Ca$^{2+}$]$_i$ transients required Na$^+$-dependent action potential activity (389), it is now understood that this is not strictly true. TTX reduces the amplitude of the [Ca$^{2+}$]$_i$ transients during activity (563), indicating that although RGC action potentials are not essential for wave propagation, they are necessary for the full amplitude of Ca$^{2+}$ entry during waves and probably also to permit detection of the waves with extracellular recording methods.

Although spontaneous activity persists for a long time during development before the establishment of patterned vision, the mechanisms generating that activity and propagating it across the retina change dramatically. At early stages, experiments in ferret and rabbit retina indicate that ACh is the primary transmitter involved in wave propagation, consistent with the fact that the retinal circuitry at those stages is mainly dependent on the cholinergic starburst amacrine cells (168, 619, 637). At later times in this early period, GABA becomes involved as an excitatory transmitter in the waves (563, 619), reflecting the presence of GABAergic amacrine cells. At these
stages (and perhaps later), the spread of activity appears to involve endogenous adenosine acting via A2 receptors to increase intracellular levels of cAMP (563).

At later stages, as glutamatergic synapses between bipolar cells and RGCs develop, glutamate becomes essential for wave generation and ACh becomes less important (619, 637). GABA also becomes inhibitory (178), reflecting its transition from excitatory to inhibitory action due to changing intracellular chloride concentration (see sect. ivC). The appearance of glutamatergic bipolar cell participation in the activity also correlates with the appearance of differences in the participation of ON and OFF RGCs in waves (323), reflecting a developing role for the waves in segregation of ON and OFF terminals within the lateral geniculate nucleus (see below). This developmental change in the transmitters and circuitry underlying spontaneous activity is also seen in spinal cord and reflects a remarkable stability in spontaneous activity even as the cells and circuits mediating it undergo developmental changes. The timing of this change in retina is plastic. When the early cholinergic waves are eliminated in mouse knockouts of the β2-subunit of the nicotinic ACh receptor, glutamate-dependent waves appear several days earlier than normal (17). This may reflect a form of compensation that ensures stability of spontaneous activity during certain critical stages of development, even in the face of disruptions to the mechanisms that create it.

Gap junctional communication also seems to be involved in retinal waves. In chick, blockers of gap junctions suppress spontaneous waves (620). In salamander retina, they disrupt the short-time correlations between firing of neighboring cells (76), and in mouse they increase the interval between waves and decrease the number of cells participating in a wave (544).

As is true in developing cortex (see sect. ivE), the retina can be induced to generate similar waves under conditions that increase neuronal excitability (L-type Ca^{2+} channel agonists). Also as in cortex, these waves are not generated by the same mechanisms as normal spontaneous activity. In retina, the induced waves persist in the presence of antagonists of L-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA), N-methyl-D-aspartate (NMDA), glycine, and GABA receptors, which block normal waves at various stages of development. Like normal waves, however, induced waves are suppressed by gap junction blockers and by agents that disrupt the action of adenosine (544). The overlap in properties of these two forms of activity implies that retinal circuitry has several mechanisms of propagating waves of activity, involving classical chemical synaptic transmission pathways, electrical communication via gap junctions, and spread of activity via adenosine action on the cAMP second messenger system. The degree to which each participates may depend on developmental stage as well as the physiological states of the participating cells.

These results, like those in cortex, emphasize the difference between the kinds of activity that neuronal circuits are capable of generating and the activity that actually occurs. Inducing activity by artificial means may yield valuable information about the underlying functional circuitry and potential mechanisms of activity that a given structure may draw upon in creating spontaneous activity. Studying the actual spontaneous activity itself reveals how the structure makes use of that circuitry and those mechanisms.

An interesting recent finding points to the possibility that retinal waves may also propagate into the retinal ventricular zone (VZ), providing some kind of feedback to the zone from which retinal cells arise (574). Waves in the VZ showed close spatial and temporal relation to the retinal waves, and pharmacological studies indicated that the VZ waves involve muscarinic ACh receptors, and likely require the retinal waves, but not vice versa. Mature retinal glia can also generate [Ca^{2+}i] waves, which can modulate retinal ganglion cell light responses (439, 440).

2. Retina: refinement of retinal ganglion cell connections by activity

The parameters of retinal waves, such as propagation speed and emerging differences in the participation of various types of RGCs, are critical for how activity encodes RGC identity to target structures. The establishment of correct patterns of connections between RGCs and their primary targets in the brain is one of the best known examples of how intrinsic molecular tags and electrical activity cooperate during brain development. To understand the roles of electrical activity in RGC projections, it is important to understand the anatomical and functional differences among the various species of animals in which the work has been done. Three points are particularly important: 1) the location to which RGC axons project. In cold-blooded vertebrates (amphibians, fish), RGCs project to the optic tectum, which is the main visual processing center in these species. In rodents and birds, RGCs project both to the tectum (superior colliculus) and to the lateral geniculate nucleus in the thalamus (LGN). The relative projections to those two structures differ among species (200, 337). In the higher mammals (cats, ferrets, primates), the visual cortex has evolved as the main processing center, and RGCs project primarily to the LGN as the synaptic relay center that sends visual information to the cortex. The superior colliculus in the higher mammals and birds serves important functions in the control of eye movement, but not as a processing center for visual perception.

2) The second important point is the developmental timing and initial accuracy of RGC projections to their primary targets in relation to eye opening and the appearance of patterned visual input (see Refs. 543, 615 for...
In cold-blooded vertebrates, the retinotopic map of initial RGC projections is fairly accurate, although there is substantial remodeling and refinement of that map, partly to compensate for retinal and tectal growth (494). This period of refinement occurs after the establishment of patterned vision, and thus the activity dependence of refinement in these animals could reflect activity driven by visual input, although the relative roles of visually driven and spontaneous activity are not entirely clear (see Ref. 296). In rodents, RGC projections to the superior colliculus are less accurate initially, and subsequent refinement takes place before the retina has become functional. Birds are similar in this regard. Thus the activity dependence of refinement reflects spontaneous retinal activity. In higher mammals, RGC projections to the LGN are also initially fairly inaccurate, and refinement occurs before the establishment of functional vision.

3) The degree of binocular input to the RGC projection site is important. Although adult cold-blooded vertebrates have binocular pathways in the tectum, there is not the eye-specific layering that is seen in the LGN of higher mammals. However, eye-specific tectal fields can be created by surgical manipulations that create direct binocular innervation of the tectum (tectal ablation, third eye implants) (see, e.g., Refs. 495, 499, 512, 513 for reviews). Thus both retinotopic projections and eye-specific termination fields can, and have been, studied in these animals. In rodents, although there is binocular projection to the LGN, it is small compared with that in higher mammals, and so true eye-specific layers are not present, although eye-specific fields do exist and are studied (200; see, e.g., Ref. 255). In birds, retinotectal projections are almost exclusively contralateral, a pattern that arises by elimination, in an activity-dependent manner, of ipsilateral projections that are present earlier (159, 615, 624). In higher mammals with substantial binocular vision, true binocular projections to the LGN exist in the form of eye-specific layers, created in part by activity-dependent pruning of initial connections (see below; Ref. 615).

Axons of RGCs projecting to the tectum in lower mammals, chicks, and cold-blooded vertebrates undergo a period of refinement into restricted terminal zones in a retinotopic pattern. In fish in which this pattern is being reestablished during regeneration of cut optic nerves, blockade of retinal activity by TTX injection prevents this topographic map refinement, although axon outgrowth and initial projections to the tectum are normal (397). The same activity dependence has also been shown during initial development of this map in zebrafish, by abolishing RGC activity using the macho mutant, which reduces RGC Na\(^+\) currents and blocks their activity, or TTX (199). Similar experiments have been done in developing chick, in experiments using TTX or the Na\(^+\) channel opener grayanotoxin to disrupt retinofugal activity (295). To test the hypothesis that patterned visual input provides activity that is correlated between neighboring RGC axons as a means of map refinement, fish were raised in stroboscopic light to synchronize activity across wide regions of RGCs. Stroboscopic light, but not diurnal light or darkness, phenocopied the TTX effects on map refinement (118, 525). Thus artificially synchronizing all RGC inputs does disrupt map formation, but on the other hand, the retinotopic map forms normally in darkness. These results imply a more complex scheme in which spontaneous activity even during stages when visual input is functional, is necessary for map refinement. Recent experiments in regenerating fish optic nerve add another layer of complexity: the retinas do not generate spontaneous waves of activity during regeneration, and in fact, overall firing rates of RGCs were depressed as their tectal projections refined during regeneration. Furthermore, blocking retinal activity during this period did not affect activity in tectal neurons, making a Hebbian scheme of refinement more difficult to envisage (296).

In amphibians there is direct evidence that coactivity of adjacent RGCs during spontaneous retinal waves serves to mediate long-term changes in synaptic efficacy where the two RGCs converge onto a single tectal neuron (634). These experiments were done in frog at early stages when RGC axon terminals are still widespread in the tectum, and synapses have a combination of NMDA and AMPA receptors. Repetitive stimulation of a single input to a tectal neuron causes homosynaptic potentiation, which requires action potentials in the postsynaptic neuron, but which does not affect other inputs. Pairing of two inputs potentiates both as long as the postsynaptic cell spiked. This included potentiation of a previously subthreshold input as long as it fired within 20 ms before the suprathreshold input. Simultaneous stimulation of two subthreshold inputs could potentiate both as long as they summed to trigger postsynaptic activity.

In mice, the retinotopic map in the superior colliculus (tectum) is refined to a much greater degree during early development, before development of visual input (see above). Nonetheless, activity is still required from RGCs for map refinement, as shown by using mice lacking the \(\beta_2\)-subunit of the ACh receptor, which is essential for spontaneous waves of activity at these early stages (388). This activity-dependent refinement requires functional NMDA receptors (543).

When innervation of one tectum by both eyes is induced, eye-specific layers are formed (see above), implying that differential activity between the two eyes can drive segregation of their projecting axons, since presumably the molecular targeting cues are the same for axons from the two eyes. Blocking RGC activity with TTX prevents eye-specific segregation (67) and can even reverse segregation that has already occurred (495). These results imply that activity is not simply permissive for formation of retinotectal maps predetermined by other factors, but...
that activity can instruct the formation of a map that does not occur under normal circumstances. Block of tectal NMDA receptors can also desegregate the RGC terminals in the presence of continued afferent activity, and exogenous NMDA can sharpen the borders of eye-specific regions (113). Activation of NMDA receptors appears to act by a combination of elimination of inappropriate axonal branches and stabilization of appropriate ones (488, 512). Interestingly, nitric oxide does not seem to be involved downstream of these NMDA effects (499).

In higher mammals, such as cats, ferrets, and primates, there is extensive binocular innervation of the LGN by RGCs, and eye-specific layers form in each LGN before visual input is possible (see Refs. 513, 615 for reviews). These layers form by pruning of an initial innervation pattern in which axon terminals originating from different eyes are intermixed (560). Blockade of spontaneous activity originating in the retina at the stages during which this layering occurs (see above) prevents eye-specific layers from forming and leaves the earlier, wider branching patterns of axons in the LGN in place (535, 561). Although the original experiments were done with intracranial infusions of TTX, later intracocular TTX injections confirmed that the activity in question does indeed arise from the retina (476). The long-term effects of TTX treatment on layering are not completely clear. Layers appear to form almost normally at long times during chronic TTX treatment (119), but activity block after layering is complete can cause desegregation (97). The mechanisms by which activity acts involves competition between RGC terminals from different eyes: increasing activity in one eye expands its territory in the LGN, but increasing activity in both eyes leaves layering mostly intact (476, 562). Even so, NMDA receptor activity does not appear to be involved in this competition-based formation of layers (548), although it does seem to be involved in formation of LGN sublaminae containing different RGC cells types (see below). After the period of eye-specific layer formation, retinal spontaneous activity continues, although its mechanism changes from cholinergic to glutamatergic (see above). Selective elimination of the early, cholinergic activity in mice deficient in the β2-subunit of the ACh receptor disrupts layering, demonstrating that the early retinal activity is required (432, 508). Interestingly, in these experiments, eye-specific patches of RGC terminals were still formed in the LGN (432).

The patterns of spontaneous activity recorded from the retina at these stages seem well-suited to encode both spatial location of RGCs within one eye and ipsilateral versus contralateral identity. The movement of waves of activity across one retina results in contiguous RGCs showing correlated activity (362), and the short duration of the waves compared with their frequency of occurrence would result in activity from the two eyes occurring at different times. Confirming this is the finding that disrupting correlations in activity between neighboring RGCs while leaving the overall frequency of activity intact did not disrupt eye-specific layering (253). Reinforcing the idea of the role of correlated activity strengthening retinogeniculate synapses in the Hebbian-like mechanism is the finding that retinal activity is passed onto geniculate neurons, and that stimulated RGC firing at frequencies near those occurring during spontaneous waves can induce long-term potentiation (LTP)-like synaptic strengthening (425). However, findings that blockade of RGC activity by TTX does not completely eliminate eye-specific layer formation at early stages, and that even in the chronic presence of TTX, delayed layer refinement does occur (although not to normal levels of sharpness)(119), indicate that neuronal activity may not be the sole player in regulating retinogeniculate mapping. It is also possible that redundant mechanisms exist that can at least partially compensate for loss of activity (see Ref. 513). Recent evidence suggests the involvement of immune system molecules in activity-dependent LGN layering (255).

Activity also is involved in the finer-grain segregation of mammalian retinogeniculate connections. ON- and OFF-center RGCs innervate distinct sublaminae in the LGN (567), and this segregation requires activity in the retina before visual input is functional (124, 221). Unlike eye-specific layer formation (548), formation of ON and OFF sublaminae does require activity of NMDA receptors (221), and subsequent activation of the neuronal nitric oxide (NO) synthase-NO-cGMP pathway (123, 125, 320). Although these findings reinforce the idea of LTP-like synaptic strengthening by correlated pre- and postsynaptic activity, ON/OFF segregation does not involve the activation of “silent” synapses by induction of AMPA receptors in LGN neurons (247). Segregation of ON and OFF RGC connections in the geniculate appears to rely on their different patterns of activity, which in turn appears to be due to a divergence of their intrinsic ion channel properties early in retinal development (436).

Activity-dependent refinement and pruning of axons in the visual system is not restricted to RGC connections. Spontaneous activity regulates branching and organization of LGN axon projections in the visual cortex. Block of activity by TTX prevents correct branching and causes some axons to project to the subplate of areas outside of their normal visual cortex target area (89, 238).

In many of these experiments, as well as those described below, one must be aware that blocking spontaneous activity with TTX, for example, does not necessarily eliminate all periodic activity in the system. Ca2+-dependent action potentials, spontaneous transmitter release, and possibly other forms of activity may still occur and be responsible for some developmental phenomena. In systems such as the retinotectal and retinogeniculate pathways, the assumption is that activity in the
form of Na$^+$-dependent action potentials must propagate
along axons to carry out its developmental functions. In
other preparations, however, local effects of activity may
be more resistant to TTX.

3. Retina: outgrowth of retinal ganglion cell axons

It has been known for some time that direct electrical
stimulation can reversibly arrest axon outgrowth and
cause growth cone filopodial retraction in both inverte-
brate and vertebrate neurons (115, 176). As in other cases
of activity-dependent events, stimulation in a burst, or
phasic, pattern is more effective than tonic stimulation
(176). But early in development, activity may stimulate
initial axon outgrowth. Recent experiments indicate that
activity interacts with various trophic factors in intricate
ways to regulate axon outgrowth and pathfinding. In reti-
nal ganglion cells, peptide trophic factors stimulate axon
outgrowth, but only at slow rates in the absence of activ-
ity. Electrical stimulation at physiological frequencies
greatly speeds outgrowth stimulated by these factors
(201). Interestingly, the pattern of stimulation that proved
most effective was brief bursts of action potentials deliv-
ered at 1-min intervals, closely approximating the pattern
of spontaneous synchronous activity seen normally in
developing retina and many other areas of the mammalian
central nervous system (see sect. IV A2; Ref. 201).

4. Retina: dendritic patterning of RGCs

The spontaneous activity that sweeps across the de-
veloping retina appears to have a function in the elabora-
tion of dendritic trees in the retina, as well as on the
patterns of RGC axon elaboration in the LGN. Retinal spontaneous activity helps to segregate the dendrites of
ON and OFF RGCs within the retina, much as activity
segregates their axon terminals into different LGN sub-
laminae, although the degree to which activity instructs
this is a matter of debate. Bodnarenko and Chalupa (54)
and Bisti et al. (43) report a strong requirement for
metabotropic glutamatergic transmission in this process,
whereas Bansal et al. (17) find more subtle effects. Block-
ing activity in RGCs with TTX also eliminates their ability
to extend dendrites into RGC-free areas created by injury
(142).

5. Retina: relationship to channel development

Ion channel development in various retinal cell types
is likely timed to regulate spontaneous activity, although
many details remain unclear. In cat and mouse retinal
ganglion cells, a negative shift in the voltage dependence
of activation and a positive shift in the inactivation curve
of $I_{\text{Na}}$ combine with increased $I_{\text{Na}}$ density to help bring
about the early appearance of repetitive firing ability (510,
546). Later expression of Ca$^{2+}$-activated K$^+$ currents and
speeding of recovery from inactivation of $I_{\text{Na}}$ may con-
tribute to changing the firing patterns of retinal ganglion
cells from bursting during spontaneous retinal waves to
more sustained firing needed for encoding visual informa-
tion (509, 604, 605).

Divergence of ion channel properties in different reti-
cal cell types is also likely to regulate how spontaneous
activity occurs, although not always in obvious ways.
Late-emerging differences in the intrinsic properties of
ON and OFF RGCs allow them to participate differentially
in spontaneous retinal waves of activity, a difference
which probably instructs their differential projections in
the LGN (436). More perplexing are changes in excitabil-
ity of starburst amacrine cells. In the rabbit, these cells
express large Na$^+$ currents and action potentials just
before eye opening, and then lose $I_{\text{Na}}$ and excitability over
the next several weeks (636). Because spiking ability
coincides with the period of spontaneous retinal activity,
it was presumed that the transient expression of $I_{\text{Na}}$ al-
ows starburst amacrine cells to participate in this activ-
ity. Direct recordings showed, however, that despite their
ability to spike, these cells do not generate action poten-
tials during retinal waves (635; see Ref. 563). This may
imply a different function for Na$^+$ currents, perhaps in the
development of intrinsic properties of these cells.

Optimization of channel properties is also likely to
occur downstream in the visual system to tune the re-
sponses of LGN neurons to spontaneous activity in RGCs.
During these stages, LGN neurons express NMDA recep-
tors containing the NR2B subunit, which gives glutamate-
induced synaptic currents a much longer time course than
in the adult. This would clearly favor temporal summa-
tion, and in fact, such summation is observed in neonatal
rat LGN in response to retinal spontaneous activity (349;
see sect. IV B).

D. Hippocampus and Excitatory GABA Responses

1. Nature of spontaneous activity

During the first postnatal week, rat hippocampal neu-
rons generate spontaneous and highly synchronous
bursts of activity known as early network oscillations
(ENOEs) or giant depolarizing potentials (GDPs) (35, 194).
These take the form of large synaptically driven depolar-
izations and bursts of action potentials, with associated
bursts of [Ca$^{2+}$], transients, occurring at an overall fre-
cquency of 0.4 – 2/min (194). GDPs occur in the entire pop-
ulation of CA1 and CA3 pyramidal cells, in interneurons,
and in hilar, septal, and dentate gyrus neurons (194, 284,
324, 326, 391, 566). GDPs are primarily GABAergic events,
but also have substantial NMDA components and, at least
at later stages, AMPA components as well (35, 55, 194,
326). Similar events have been recorded in vivo in both
anesthetized and freely moving neonatal rats (325). The
disappearance of spontaneous GDPs at the end of the second postnatal week correlates closely with the change in intracellular Cl\textsuperscript{−} concentration that converts GABA action from excitatory to inhibitory (283). [Note that there is some disagreement as to the timing of GDP disappearance and GABA switchover. Khazipov et al. (283) postulate that earlier estimates are biased by the use of intracellular recording methods, which might disrupt intracellular Cl\textsuperscript{−} concentration and/or introduce leak resistance effects into the measurements.]

It is not completely clear whether the synchronous GDPs that occur throughout the hippocampus are driven by a specific pacemaker region. Strata et al. (566) presented evidence that hilar neurons serve this function, at least for GDPs in the CA3 region. In hippocampal slices, surgical isolation of the hilus from CA3 abolished CA3 GDPs but left those in hilar neurons intact. Paired recordings showed that hilar GDPs preceded CA3 GDPs with a consistent 5- to 10-ms latency. Dye injections showed gap junctional coupling among hilar neurons and block of gap junction channels with octanol suppressed GDPs. Finally, voltage clamp of hilar neurons showed the presence of a putative pacemaker current (I\textsubscript{h}), and Cs\textsuperscript{+} block of I\textsubscript{h} slowed or blocked GDPs. Other experiments point to different pacemaker regions. Leinekugel et al. (324) used an intact two hippocampi plus septum preparation to ask whether septal neurons might act as pacemakers for spontaneous GDPs. They found that spontaneous GDPs propagate temporally to the hippocampi from the septum and that when partially isolated, the septum maintains a higher GDP frequency than the hippocampi. When the hippocampi were completely isolated from the septum, however, the hippocampi retained the ability to generate GDPs while the septum did not. They propose a pacemaker role from the septum, but an additional requirement of activity generated in the hippocampus to perhaps raise the level of excitability in the septum so that it can serve its pacemaking function. These two results are not necessarily incompatible. Strata et al. (566) worked in transverse slices, with CA3-hilus connections intact but with no septal-hippocampal connections. It remains possible that the hilar neurons generate a pacemaker signal that propagates to both the CA3 region and to the septum, which in turn propagates a pacemaker-like signal back into the hippocampus. Work by Menendez de la Prida and Sanchez-Andres (392, 393) and Menendez de la Prida et al. (391) makes an equally convincing case that GDPs are an emergent network property that can be generated by almost any subset of the hippocampal circuit. This hypothesis is based on data showing that isolated “islands” of CA1, CA3, and dentate gyrus can each generate spontaneous GDPs. Further data from paired recordings show that GDPs are triggered when the overall network activity rises to a level that can generate a threshold frequency of excitatory postsynaptic potentials (EPSPs) within participating neurons. Thus the origin of spontaneous GDPs in the neonatal hippocampus remains somewhat of a mystery, with evidence pointing to more than one potential pacemaker region and to a network property that can operate without a single discrete pacemaker.

2. Developmental roles of spontaneous activity

Much of this work focuses on the apparent paradox of “silent synapses” early in development (see Ref. 226). In the hippocampus, the large majority of synapses at P0 are pure NMDA, or silent, synapses, so named because in the absence of other receptor types, glutamate cannot activate them due to the voltage-dependent Mg\textsuperscript{2+} block of the NMDA receptor. It is known that repeated pairing of presynaptic activity with postsynaptic depolarization can “AMPA-ify,” or induce, functional synapses by inducing functional AMPA receptors. The problem is that, early in development, there may not be a sufficiently high density of AMPA receptors on the postsynaptic cell for repeated presynaptic activity to depolarize the postsynaptic cell enough to trigger induction. It has been proposed (226) that spontaneous, GABAergic GDPs might provide the coincidence pre- and postsynaptic depolarization to activate Hebbian mechanisms of synapse strengthening. Indeed, it has been recently shown that pairing of mossy fiber stimulation with spontaneous GDPs can induce a form of LTP, including induction of previously silent synapses (279).

At least one of the activity-dependent developmental programs in hippocampal neurons may serve to make activity self-limiting. Spontaneous activity in hippocampus depends on excitatory GABA\textsubscript{A} actions (see sect. ivC). Application of GABA\textsubscript{A} blockers blocks activity and delays the appearance of KCC2 chloride pump mRNA, whose expression lowers intracellular Cl\textsuperscript{−} concentration and converts GABA\textsubscript{A} action to inhibition. Enhancing activity by KCl depolarization accelerates the switchover and the KCC2 mRNA expression (193).

3. Relationship to channel development

Spontaneous activity in hippocampus relies on the excitatory action of GABA, which is unique to developing neurons (reviewed in sect. ivC). It has been known for some time that GABA\textsubscript{A} actions are excitatory early in development, due to the high intracellular Cl\textsuperscript{−} concentration at these stages (reviewed in Refs. 34, 469, 470). Some of the early reports of this phenomenon were of experiments done in hippocampus (430, 431). That spontaneous activity in hippocampus depends on excitatory GABA transmission is apparent from both the effects of GABA\textsubscript{A} blockers on activity (see above) and from the fact that the developmental disappearance of spontaneous GDPs parallels closely the switchover to inhibitory GABA\textsubscript{A} action (283).
The developmental profile of the hyperpolarization-activated cation current $I_h$ may also influence hippocampal spontaneous activity. $I_h$ is well known as a pacemaker current in a variety of cells (473, 504), and in particular, hilar neurons in the hippocampus appear to rely on $I_h$ to drive spontaneous GDPs in other hippocampal regions (566). $I_h$ density peaks in the early postnatal hippocampus (596) and thus may play a role in spontaneous activity. Another potential pacemaker current, the T-type Ca$^{2+}$ current, is also present at higher density in neonatal hippocampal neurons than later (94).

E. Cerebral Cortex and Coordinated Na$^+$ and Resting Channel Development

1. Cortex: nature of spontaneous activity

There are a large number of reports of spontaneous activity in rat and mouse cortical neurons, particularly in the first postnatal week. Most of these involve activity that is synchronous in small contiguous clusters of neurons, or involves more subtle correlations of activity among slightly more scattered cells. In many of these instances, activity is not actually spontaneous, but is elicited by altered ionic conditions, ion channel blockers, or transmitter agonists. These experiments show that the perinatal neocortex has the ability to generate activity with a variety of complex forms of spatial and temporal synchronicity.

A) NEURONAL DOMAINS. In rat cortex during the first postnatal week, clusters of 5–50 cells in a columnar orientation generate synchronous [Ca$^{2+}$]$_i$ transients (632). Different clusters can be seen to generate this activity apparently randomly, with an interval of ~4 min between events in different clusters. Although columnar in orientation, the clusters do not correspond to obvious functional units such as barrels. This activity does not appear to be caused by synchronous electrical activity, but rather by an inositol 1,4,5-trisphosphate (IP$_3$)-mediated [Ca$^{2+}$]$_i$ release that spreads from a trigger cell through the cluster, which seems to be defined by gap junctional coupling (277, 631). A similar form of activity is seen in mouse cortex at these stages, and knockout experiments suggest the involvement of the $\varepsilon_2$ subunit of the NMDA receptor in regulating the spread of the [Ca$^{2+}$]$_i$ transient (453), although how is unclear. It is possible that these coupled units and the [Ca$^{2+}$]$_i$ signaling within them might represent a precursor to a modular architecture in the mature cortex.

B) CORRELATED ACTIVITY PATTERNS AMONG LAYER I NEURONS. A very interesting observation in the context of early cortical development is the presence of correlated patterns of [Ca$^{2+}$]$_i$ transients among neurons of layer I, including both Cajal-Retzius and non-Cajal-Retzius cells (4, 531). This activity is not synchronous across large numbers of cells, but correlations among groups of cells can be detected by comparing correlation coefficients in pairs of cells to those expected from random activity at the same mean frequency (531). This pattern of activity in layer I was only rarely observed spontaneously, but could be evoked by high levels of extracellular K$^+$ (50 mM). The [Ca$^{2+}$]$_i$ transients were quite long in duration (>100 s), and were not blocked by TTX, although TTX did block the correlations among the transients (531). Correlations among cells involved chemical synaptic transmission, including glutamate, GABA, and ACh receptors. Correlations were not blocked by blockers of gap junction channels, indicating that direct electrical communication is not involved, thus distinguishing this activity from the neuronal domains discussed above. It is not clear whether these [Ca$^{2+}$]$_i$ transients are caused by Ca$^{2+}$ entry during electrical activity, or represent release from internal stores. Their long duration suggests the latter, but their sensitivity to block by Ni$^{2+}$ suggests that Ca$^{2+}$ entry may at least be the initial trigger for internal Ca$^{2+}$ release. This hypothesis is also more compatible with the action of TTX in blocking correlations among cells within the network. It seems possible that Ca$^{2+}$-dependent activity in cell bodies may trigger the long [Ca$^{2+}$]$_i$ transients, but axonal Na$^+$-dependent action potentials may be required to propagate the activity to other cells in the correlated network. The function of this type of activity in layer I is not clear, although communication between layer I cells and the apical dendrites of developing pyramidal neurons is likely to be involved (531). Activity that is synchronous between layer I neurons (both Cajal-Retzius and non-Cajal-Retzius) can be evoked under low-Mg$^{2+}$ conditions, which activates silent NMDA receptors (549). The question remains, though, as to whether this activity is intense enough under normal (not high [K$^+$]) conditions to carry out the proposed functions. The possibility of a deep cortical trigger for layer I activity is raised by the work of Dammerman et al. (131), who reported that electrical stimulation of GABAergic axons passing through layer I could excite cortical pyramidal neurons in neonatal rat cortex. These fibers arise in the zona incerta (ZI) of the thalamus and could represent a subcortical pathway capable of driving activity across large regions of the developing cortex. This hypothesis is strengthened by the direct recording of spontaneous activity in ZI neurons (131).

C) INDUCED LARGE-SCALE WAVES OF ACTIVITY. Cortical slices can generate waves of activity (measured either as electrical activity or [Ca$^{2+}$]$_i$ transients) when treated with cholinergic agonists (287) or TEA (475). Although induced under artificial conditions, these waves reveal the capabilities of the neonatal cortex to initiate and propagate large-scale waves, synchronous among many neurons, over large physical distances. These waves require the activity of voltage-gated Na$^+$ channels and seem to be propagated via glutamatergic synapses more than
GABAergic ones. In the case of TEA, gap junctions seem also to be involved in wave propagation (475). The triggering of these waves by cholinergic agonists suggests that these agonists may substitute for cholinergic inputs from subcortical structures that are disrupted in brain slices (287). Such inputs may serve to raise the overall level of excitability in the neonatal cortex to a point where glutamatergic synaptic interactions can synchronize large neuronal populations. Cholinergic agonists can trigger such waves only during the first postnatal week, indicating that the intrinsic neuronal properties and synaptic circuitry of the neonatal cortex are specifically optimized for such functions.

D) SPONTANEOUS WIDESPREAD SYNCHRONOUS ACTIVITY. Two reports indicate that both rat and mouse neonatal cortical neurons can generate spontaneous [Ca\(^{2+}\)] transients that show widespread synchrony among a very large percentage of neurons in the cortex (121, 195). These [Ca\(^{2+}\)] transients result from electrical activity, as judged both by TTX sensitivity and by extracellular field potential recordings. Although GABA is excitatory to cortical neurons at these stages (E18-P5), activity is not blocked by GABA\(_A\) antagonists, at least in rat. Activity is blocked by antagonists to NMDA and non-NMDA glutamate receptors. Unlike in the retina, the pharmacological profile of activity in cortex does not change as development progresses (195). The [Ca\(^{2+}\)] transients occur at low frequencies of \(\approx 1/\text{min}\) to \(\approx 1/12 \text{ min}\) and propagate across the cortex at \(\approx 1.5-2.5 \text{ mm/s}\). The activity emerges in the cortex just before birth, peaks at P0, and ceases by about P5 (121, 195). In each case, transients were studied in somewhat elevated (4.5–5 mM) \([K^+]\). The posterior-anterior propagation of this activity, starting in the entorhinal cortex (195), might suggest that hippocampal activity acts as a pacemaker for cortical activity. Cortical activity, however, does not consistently propagate in this direction and occurs at lower frequencies than that in hippocampus (195). Analysis of slices in which the participation of neurons in synchronous activity was \(<50\%\) showed that participating neurons were spatially clustered, implying a network in which mechanisms of synchronicity are widely distributed and are weaker between distant neurons than contiguous ones (121).

A recent report documents synchronous [Ca\(^{2+}\)] transients induced by blockers of glutamate transport in P0-P5 rat cortex (139). This raises the question of how to interpret these experiments where activity is induced by slightly elevated external \([K^+]\) or glutamate transport blockers. It is likely that both stimuli compensate for loss of activity during preparation and maintenance of slices, due to loss of extracellular glutamate by diffusion or loss of subcortical excitatory inputs. But it is also possible that cortex in vivo is not spontaneously active, or at least less so than in vitro. In that case, activity induced by these stimuli indicate that in vivo cortex is only marginally subthreshold for generating this kind of widespread spontaneous activity. The former possibility is strengthened by the finding that spontaneous synchronized activity recorded in hippocampal slices also occurs in vivo (325).

E) SPONTANEOUS ACTIVITY IN CULTURED CORTICAL NEURONS. A strikingly similar form of widespread spontaneous activity appears in cortical neurons from E15-E16 rat brain that have been in culture for 1–3 wk (467, 602), somewhat later chronologically than widespread activity in acute slices of neonatal cortex. The spontaneous [Ca\(^{2+}\)] transients occur at similar frequencies and had similar durations to those found in intact neonatal cortex. It is unclear whether this activity arises from the same mechanisms as that in cortical slices. In culture, this activity seems to be driven by a pacemaker population of neurons in the subplate and is as a result blocked by GABA\(_A\) antagonists. GABA\(_A\) blockers do not block similar activity in rat neocortical slices, indicating that a GABAergic subplate pacemaker is not necessary in that preparation.

2. Cortex: developmental effects of spontaneous activity: migration

In the embryonic mammalian neocortex, excitatory pyramidal neurons are produced in the VZ and migrate radially through the intermediate zone (IZ) to form the layers of the cortical plate (CP) (10). A second major path of migration involves inhibitory interneurons that arise in the ganglionic eminences (GEs) and migrate tangentially into the neocortex (374, 375). The wealth of information about the timing of cell cycle events and migratory pathways (see, e.g., Refs. 578, 579) has greatly facilitated studies of ion channel development in this preparation.

Experiments by Komuro and Rakic (297, 299, 300) have shown that migration of cerebellar granule cells depends on Ca\(^{2+}\) influx through N-type Ca\(^{2+}\) channels and NMDA receptors (see sect. II F). It is likely that neuronal migration in cortex is similarly activity dependent. NMDA receptor activity stimulates chemotactic movements of mouse neocortical VZ cells (but not cortical plate cells from the same stages) (32). Application of NMDA receptor antagonists to intact slices blocks migration of VZ cells into the cortical plate, showing that endogenous glutamate levels are acting as a migratory stimulus. The existence of high levels of glutamate in the cortical plate at these stages is consistent with its role as a migratory attractant. [Interestingly, the situation is different in rat, where GABA seems to serve the role as a chemoattractant (29–31).]

Experiments on identified tangentially migrating neurons (arising in the GEs) show that they possess functional NMDA, AMPA, and GABA\(_A\) receptors, all of which can cause [Ca\(^{2+}\)] transients when stimulated (396, 552). They also express functional voltage-gated Na\(^+\) channels that are activated during GABA\(_A\) receptor stimulation and...
participate in creating GABA-induced $[\text{Ca}^{2+}]_i$ transients (552). Neurons of this tangential migratory stream make close contact with neurites of corticofugal axons arising from the CP. This suggests that glutamatergic CP neurons, arising from the cortical VZ by radial migration, can release glutamate onto the processes of tangentially migrating GABAergic neurons (396). That this process might affect migration is indicated by the finding that activation of AMPA receptors causes neurite retraction in these tangentially migrating neurons (483). It is also possible that bidirectional communication exists between these cell types, because AMPA receptor activation also leads to GABA release from the tangentially migrating neurons, which could influence radially migrating neurons from the cortical VZ as well as have autocrine effects on the tangential stream (484).

3. Cortex: developmental effects of spontaneous activity: later differentiation of neurons and circuits

After cortical neurons enter the cortical plate, spontaneous activity takes the form of the widespread, synchronous burst of action potentials and $[\text{Ca}^{2+}]_i$ transients occurring at P0, as described above. Although few clear functions for this activity have yet been reported, there are several likely possibilities.

The effect of synchronous activity in promoting survival is seen in cultured cortical neurons, in which TTX reduces, and KCl depolarization enhances, survival. Furthermore, survival is selective for neurons that are active synchronously with other neurons (601). Activity-dependent survival of cortical neurons requires Ca$^{2+}$ influx via voltage-gated Ca$^{2+}$ channels and/or NMDA receptors (196, 465, 577). Brain-derived neurotrophic factor (BDNF), parathyroid hormone-related peptide, and pituitary adenylate cyclase activating polypeptide (PACAP) have each been implicated as mediators of activity-dependent survival (196, 309, 464, 577), which also seems to involve p38-mitogen-activated protein kinase, which phosphorylates and activates the transcription factor MEF2 (365).

Other activity-dependent processes that occur near birth in large numbers of cortical neurons include dendritic arborization (which involves Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels, activation of calmodulin kinase IV and CREB phosphorylation; Ref. 493), and myelination (140).

Finally, as discussed above for the hippocampus, activity that is synchronous in pre- and postsynaptic partners may serve in a Hebbian fashion to strengthen synapses that are initially too weak to establish paired activity on their own (see Ref. 226). Indeed, in mouse barrel cortex, the critical period for plasticity and refinement of thalamic inputs overlaps closely with the period during which LTP can be experimentally elicited (122; but see Ref. 503 for the contrary result in visual cortex).

4. Cortex: activity and neural migration: relationship to channel development

Proliferative cells of the neocortical VZ express delayed K$^+$ currents (5, 402, 480), BK Ca$^{2+}$-activated K$^+$ currents (399, 402), and excitatory GABA receptor (468). BK channels in VZ cells often show a high-frequency flicker mode of gating that is rarely seen in mature cells (399). It is not clear to what extent proliferative VZ cells express voltage-gated Ca$^{2+}$ currents. Flow cytometry experiments coupled with KCl depolarization and $[\text{Ca}^{2+}]_i$ imaging did not detect VZ Ca$^{2+}$ currents (371). On the other hand, patch-clamp recordings using monovalent permeation through Ca$^{2+}$ channels in divalent free solutions to greatly increase current amplitudes did detect multiple Ca$^{2+}$ current types in VZ cells (480). This may be a species difference, since the latter experiments were done in mouse and the former in rat, or simply a detection difference. In either case, the fraction of VZ cells expressing Ca$^{2+}$ currents in the patch-clamp experiments was low enough that the possibility that only VZ cells that have exited the cell cycle express Ca$^{2+}$ currents could not be ruled out (480).

Some caution is needed in interpreting results obtained in VZ recordings, for several reasons. First, after E12 (in mouse), a significant fraction of VZ cells has exited the cell cycle and begun to migrate (579). Conclusions about delayed K$^+$ currents in proliferative cells are drawn from experiments showing that these currents are expressed at E9, before any VZ cells exit the cell cycle (Albrieux and Moody, unpublished data), and from the fact that 100% of VZ cells express delayed K$^+$ currents at E12-E14, when the majority of VZ cells are still proliferative (480). Similar logic applies to the finding of Ca$^{2+}$-activated K$^+$ currents in VZ cells (81, 399). A second problem arises from extensive electrical coupling among VZ cells (353). If this is accompanied by electrical coupling, then currents that occur in only a few cells might be shunted by the low input resistance of coupled clusters and thus not detected. Or, currents might be assigned to the wrong cell type due to spread from a different, but coupled, type. This problem is not significant in mouse VZ, where electrical coupling is much weaker than in rat, although dye coupling is similar (480). Taking advantage of this property, we were able to show that a subset of VZ cells express functional Na$^+$ currents starting at E14 (480) and that the fraction of total VZ cells represented by this subset approximated those that have exited the cell cycle at that stage. This implies that Na$^+$ current expression is a very early event following cell cycle exit in...
cortical neurogenesis. A similar conclusion was reached in rat by physically uncoupling cells (404).

As neuronal precursors migrate out of the VZ into the IZ, Na\(^+\) currents and ionotropic amino acid receptors are strongly upregulated, while outward I\(_K\) density is unchanged and dye coupling is greatly reduced (372, 373, 480, 481). Each of these changes would increase responses to transmitters and other depolarizing influences. It is not known whether Ca\(^{2+}\) currents are upregulated as cortical neurons begin to migrate.

5. Cortex: perinatal spontaneous synchronized activity: relationship to channel development

After migrating neurons enter the cortical plate, we begin to see a new pattern of ion channel development, which appears to be related to the occurrence of spontaneous, synchronous activity, centered around P0 (121, 195). Between E16 and P2 in pyramidal neurons, I\(_{\text{Na}}\) density shows its largest increase while I\(_K\) density does not change. A few days after this increase in I\(_{\text{Na}}\) density, the resting resistance of the neurons falls by almost five-fold (481). The occurrence of spontaneous, synchronous [Ca\(^{2+}\)]\(_i\) transients closely tracks these changes. [Ca\(^{2+}\)]\(_i\) transients first appear at E16, as I\(_{\text{Na}}\) starts to increase, and their occurrence declines markedly after P1, when input resistance falls (121, Fig. 9). These data suggest that a threshold I\(_{\text{Na}}\) density is at least permissive for activity to occur and that the decrease in resting resistance postnatally reduces the ability of the neurons to respond to synaptic inputs and to their own sodium currents to the point where spontaneous synchronous activity ceases. The role of I\(_{\text{Na}}\) may not be simply to increase neuronal excitability so that [Ca\(^{2+}\)]\(_i\) transients can occur. Analysis of data at early embryonic stages indicates that spontaneous transients occur in many cells, but asynchronously, and then later become synchronized during the increase in I\(_{\text{Na}}\) (121). This suggests that the increase in I\(_{\text{Na}}\) density may serve more to increase transmitter release and synchronize cells than to render them sufficiently excitable to generate [Ca\(^{2+}\)]\(_i\) transients in the first place. Ca\(^{2+}\) currents with both transient and sustained components also appear in cortical neurons just as spontaneous activity starts (Moody, unpublished data).

After the period of spontaneous, synchronous activity is over in the first few postnatal days, the electrical properties of cortical neurons continue to develop over the next several weeks. The hyperpolarization-activated cation current I\(_K\) appears at about P0 and develops rapidly near the end of the first postnatal week (481) (it is present earlier in Cajal-Retzius cells; Ref. 360). The persistent Na\(^+\) current increases threefold in density over the first three postnatal weeks (9). Overall Ca\(^{2+}\) current density also increases, although the subtype composition of the currents remains relatively constant (352). Between P5 and P7, there is an abrupt increase in the density of BK-type Ca\(^{2+}\)-activated K\(^+\) channels (278). It is during this period that the mature diversity of cortical neuron firing types emerges, including the “fast spiking” and “regular spiking” phenotypes. This differentiation is driven in part by cell-specific expression of a slowly inactivating, 4-aminopyridine (4-AP)-sensitive K\(^+\) current, identified from single-cell RT-PCR analysis as Kv3.1 (382).

It is not only the voltage-gated channels whose patterns of expression appear optimized for spontaneous activity and Ca\(^{2+}\) entry at early stages in the cortex. Ligand-gated channels, too, have unique properties in embryonic and perinatal cortical neurons. AMPA receptors in a variety of neurons, including those of brain stem, cerebellum, hippocampus, retina, and cortex, lack the GluR2 subunit early in development (91, 161, 312, 318, 343, 346). Unlike GluR2-containing receptors, these AMPA receptors are permeable to Ca\(^{2+}\). Because AMPA receptors do not show a voltage-dependent Mg\(^{2+}\) block, this form can admit Ca\(^{2+}\) to cells even at negative potentials (see Ref. 146). NMDA receptors in embryonic and early postnatal cortical neurons also have a unique subunit composition and properties that are different from those in the mature cells. NMDA receptors in immature neurons contain NR2B (or NR2D) subunits, which give the channel a much slower deactivation time (127, 239, 626). As neurons mature, deactivation kinetics speed as a result of substitution of the NR2A subunit (182, 246). The exact developmental function of NMDA receptors containing the NR2B subunit and the long time course of the currents they mediate is not entirely clear. The immature subunit composition would clearly favor temporal summation, and in fact, such summation is observed in neonatal rat LGN in response to spontaneous activity from retinal ganglion cells (349). It would also, as in the case of the ACh receptor, be better matched to the high input resistance and long time constant of immature cells (see sect. ivB). In addition, the longer duration EPSCs in immature cells would be expected to admit more Ca\(^{2+}\) to cells during activity, possibly allowing more effective triggering of gene expression and synapse stabilization (122, 289, 487). But it is not entirely clear that developmental plasticity in synapse function is tightly related temporally to the period of immature NMDA receptor expression (see Ref. 503), or that in cases where a good temporal relation exists under normal conditions, that the long-duration immature NMDA responses are required for the plasticity (347).

Finally, as in hippocampal neurons, the high intracellular [Cl\(^-\)] in immature cortical neurons makes GABA\(_A\) action excitatory (307, 353, 355, 359, 468, 469, 470), although as discussed above it is not clear the degree to which excitatory GABAergic transmission participates in cortical spontaneous activity.
In summary, the spontaneous synchronized activity in P0 neocortex occurs at a time when the properties of a large number of voltage- and ligand-gated channels all favor such activity: Na\(^+\) currents that are newly increased in density, newly developed Ca\(^{2+}\) currents, high input resistance, long-lasting NMDA responses, Ca\(^{2+}\)-permeable AMPA receptors, and excitatory GABA\(_A\) transmission.

**F. Cerebellar Neurons, Ca\(^{2+}\) Currents, and Neuronal Migration**

A variety of studies, mostly done in granule cells of the developing cerebellum, have shown that Ca\(^{2+}\) influx through both voltage- and ligand-gated channels is an essential regulator of neuronal migration (300). Cerebellar granule cells express N-type Ca\(^{2+}\) channel protein just after they exit the cell cycle and upregulate the number of those channels as they migrate (297). Selective blockers of N-type Ca\(^{2+}\) channels decrease the rate of granule cell migration up to 75%. Blockers of L- or T-type Ca\(^{2+}\) channels have no effect (297). Ca\(^{2+}\) imaging studies show that migrating granule cells exhibit spontaneous [Ca\(^{2+}\)] transient lasting ~1 min at an average frequency of ~13/h. At the peak of each [Ca\(^{2+}\)] transient, the forward speed of the cell is high, and during each trough between [Ca\(^{2+}\)] transients, the cell is stationary or moves slightly backwards (299). Suppressing transients with conotoxins or creating transients with high KCl slowed or speeded migration, respectively (299). Ca\(^{2+}\) influx into granule cells during activation of the NMDA receptor has similar effects on migratory rate (298). Spontaneous opening of NMDA channels has been recorded in granule cells in cerebellar slices, and the frequency of these openings increases dramatically as the cells enter the migratory phase (507). N-type Ca\(^{2+}\) channel activity has also been implicated in neuronal migration in Caenorhabditis elegans, where loss-of-function alleles of the N-type Ca\(^{2+}\) channel gene unc-2 disrupt migration of specific neurons without altering axonal outgrowth (580).

The mechanisms by which activity and [Ca\(^{2+}\)] transients regulate migration are not yet clear. Direct effects of [Ca\(^{2+}\)] on cytoskeletal elements and cell motility are likely candidates, but other, more complex events are also probably involved. For example, expression of cell adhesion molecules and extracellular glycoproteins that are known to affect neuronal migration are activity dependent at the appropriate developmental stages and in response to physiologically relevant levels of activity (260, 515). Neuronal migration in other areas of the central nervous system (CNS), such as the olfactory placode, hypothalamus pathway, is similarly inhibited by blocking electrical activity (191).

**G. Hindbrain and Synchronized Activation of Motor Neurons and Cranial Nerves**

1. **Nature of spontaneous activity**

The hindbrain is unique in that neurons are born within transient compartments (rhombomeres) that determine the neuronal fate of progeny within them. In addition, cells are unable to mix between the rhombo-meric compartments until the transient borders disappear. Neurons differentiate first in the even-numbered rhombomeres, later in the odd-numbered rhombomeres. In chicks, the hindbrain is segmented between HH stages 12 and 24, in mice between E8.5 and E11.5, and in zebrafish, between hpf 18 and 36 h postfertilization (hpf). Motor neurons in the hindbrain include somitic motor neurons, which innervate somitically derived muscle, much as in the spinal cord; in addition, there is a population of branchiomeric motor neurons that innervate muscles derived from the branchial arches (reviewed in Ref. 189). The branchiomeric trigeminal motor neurons derive from r2, facial motor neurons from r4. Early segmental identity in the hindbrain is determined by the Hox genes, a family of closely related transcription factors that are differentially expressed in the rostral-caudal axis of many animals, including vertebrates. These genes dictate later expression of segment-specific genes, which play roles in segment-specific neuronal fate and determination.

**B) CHICK HINDBRAIN.** Extracellular motor root recordings [in elevated external K\(^+\) (8 mM)] in chick hindbrain branchiomeric nerves did not show any activity until stage 24, when periodic episodes of activity were observed with an interval rate of ~1 min (averaged over stages 24–26) (98, 99, 183). These changed over developmental time (up to stage 36), with more bursts of activity in each episode, with the interval between episodes lengthening to 2.4 min. These episodes were synchronous between the different pairs of recorded motor roots (V versus VII, IX, X or XII), and synchronous between the two sides of the hindbrain (183, 186). Different sections of the hindbrain, when transected along the midline or divided in the rostrocaudal dimension, were each able to generate independent rhythms, and were no longer synchronized with the other cranial roots. It was not clear which region of the hindbrain had the strongest ability to lead the other portions. However, when the VIIth nerve region was isolated, it had the ability to maintain the same rhythm seen previously in the intact tissue, and rostral isolated fragments had a faster rhythm than isolated caudal fragments. The synchronous behavior between the nerve roots was present through several important developmental events in the hindbrain: neuronal migration and differentiation, and the formation of brain stem nuclei (186).
With the use of $[\text{Ca}^{2+}]_i$ imaging in mouse hindbrain with motor neurons identified by retrograde dextran labeling, the development of activity in identified branchiomeric motor neurons was monitored at earlier stages (218). To quantify synchronicity, $[\text{Ca}^{2+}]_i$ records were idealized so that each $[\text{Ca}^{2+}]_i$ transient in an individual cell that crossed a criterion threshold was given an amplitude of 1.0, and all $[\text{Ca}^{2+}]_i$ points below threshold were given an amplitude of 0.0. By averaging such idealized records from all cells in a given experiment, a summary record was created in which the amplitude of each $[\text{Ca}^{2+}]_i$ transient had a value between 0.0 and 1.0, indicating the fraction of neurons that participated synchronously in that event. These experiments showed that E9.5 neurons had very slow and infrequent $[\text{Ca}^{2+}]_i$ transients, while E10.5 events were much shorter in duration. At E11.0, events were much more frequent, and abruptly at E11.5, all motor neuron transients became highly synchronized expressing $[\text{Ca}^{2+}]_i$ transients every 1–2 min. This is the stage at which rhombomere borders disappear. The events at E9.5 and 10.5 were dependent on extracellular $\text{Ca}^{2+}$ but were not blocked by TTX, while the activity at E11.5 was completely blocked by low doses of TTX. These results indicate that between E10.5 and E11.5, TTX-sensitive Na$^+$ channels become functionally expressed in the developing neurons, while during the interval of E11.0–11.5, mechanisms of synchronization become abruptly expressed.

Analysis of mice in which specific Hox genes have been inactivated have shown the roles of certain classes of segment-specific neurons in controlling respiratory rhythm in neonatal animals. Krox-20 is a transcription factor normally expressed in rhombomeres 3 and 5 (before and during segmentation) and regulates Hox-related genes. Deletion of Krox-20 leads to deletion of r3 and r5, and a severe phenotype in which newborn animals have reduced respiratory rate and increased periods of apnea, often dying within hours of birth (263). In addition, the jaw-opening motor patterns in the newborn Krox-20 (−/−) animals were reduced. Naloxone, an inhibitor of the opioid system regulating respiration, had the effect of increasing the respiration rate and increasing viability in these animals (184). Anatomical and physiological studies indicated that nonnordaradnergic reticular neurons, which were postulated to be specific respiratory rhythm-promoting nuclei in the brain stem, were deleted or reduced, and the respiratory rhythm was significantly slower than in control littermates. In Krox-20 mutants, although the facial motor nucleus appears fairly normal (due most likely to the population of r4-derived neurons), the trigeminal motor nucleus is dramatically reduced. This is probably caused by a trophic or inductive factor normally present in r3 that is needed for normal trigeminal neuron development in r2. Since in chick the expression of a GABAergic inhibition leading to the more “mature” respiratory

Central pattern generator driving all of the nerves synchronously was not the underlying mechanism for synchronous nerve root activity. Instead, they propose that each region is able to generate rhythmic activity, perhaps in a manner similar to that seen in early spinal cord (see below) and that the activity is coordinated between segments by a different subset of neurons. In the hindbrain, there is possible input from reiterated sets of reticular neurons that are present in the units of tissue derived from each rhombomere. It is postulated that the early rhythm in hindbrain motor neurons is modified in the older animal to play a role in the oral components of the respiratory rhythm. This work and the relationship to late fetal and adult respiratory rhythms are reviewed in Chamagnat and Fortin (95).

Experiments using a combination of ventral root and single cell recording from developing hindbrain (185; reviewed in Ref. 62) revealed that the initiation of the more mature episodes containing high-frequency bursting depended on the development of a GABAergic input that matures value. The burst interval is regulated by $I_h$. During development in the segmented hindbrain, the expression of the ability of the motor neurons to generate the response to GABA and the high-frequency response is determined by the odd-numbered rhombomeres. In rhombomeres that are isolated and then allowed to develop in vitro, only the odd-numbered rhombomeres were able to express the high-frequency rhythm. In a more intact hindbrain, high-frequency rhythm expression depends on interactions between adjacent even- and odd-numbered rhombomeres; in isolated segmental configurations where a rostral odd-numbered rhombomere was paired with the neighboring caudal even-numbered rhombomere the high-frequency rhythm occurred, while the opposite pairing expressed only the “immature” low-frequency rhythm.

C) MOUSE HINDBRAIN. In a similar mouse hindbrain preparation, nerve root recording at embryonic (E) days 10.5, 12.5, and 14.5 showed spontaneous activity; at embryonic day 10.5 (E10.5), different nerve roots were not synchronized, and rostral root frequency was faster than that recorded in more caudal roots, demonstrating a possible rostral-to-caudal developmental sequence. At E12.5 low-frequency activity synchronized between different nerve roots was recorded, with a slightly longer interepisode interval than the more caudal lower frequency events at E10.5 (interval of 75 s; Refs. 1, 98, 99). The activity at E12.5 is similar to that seen in chick stage 24, and in both animals this is recorded soon after the disappearance of the rhombomere boundaries. At E14.5, the high-frequency activity is recorded, which is synchronized between nerve roots; this is also similar to the timing of the appearance of high-frequency activity in chick.
rate requires the presence of a specific odd-even rhombomere patterning (185), it is possible that the mature respiratory rate requires r3 and r5 and is deleted in Krox-20 (−/−) mice.

A similar analysis of early gene expression and the regulation of rhythmic activity is seen in mice in which the Hoxl1 gene, normally expressed from the posterior of the mouse up to the r3-r4, is deleted. The resultant homozygous mice die near birth from anoxic episodes and have malformations in the inner ear, bones of the skull, and deletion or misplaced cranial nerves, stemming from the reduction of r4 and deletion of r5 (88, 106, 358, 376). Neonatal mutant mice express an unusual pattern of respiratory rhythm, due most likely to an increased input from an ectopic group of reticular neurons that mediate the respiratory rhythm (62, 150). These ectopic neurons are most likely derived from r3-r4 neurons that acquire an inappropriate, more rostral, fate.

Additional gene deletion experiments examining respiratory patterns have examined the deletion of the NMDA-R1, Phox2a (expressed in cranial sensory and motor nuclei), BDNF (required neurotrophic factor for the survival of chemosensory neurons in the respiratory system), and kreisler (r5-specific transcription factor) genes; in the cases of NMDA-RA, Phox2a, and BDNF, respiratory drive is strongly decreased (63, 158, 184, 597), while in kreisler-deleted neonates the respiratory and jaw opening rate is unusually high (98, 99). Given that NMDA receptors are likely involved in the generation of early embryonic rhythms and that certain subclasses of sensory neurons may also drive rhythmic activity, it would be interesting to examine the expression of the early embryonic rhythmic pattern in these mutated animals to determine the changes that may mediate the alterations in respiratory rhythm.

2. Developmental roles of spontaneous activity

Spontaneous activity in developing motor neurons coincides with the onset of neuromuscular junction formation and spontaneous movement in the head and limbs. At the onset of synchronized activity in the mouse hindbrain, trigeminal motor growth cones are initiating synaptic connections with their peripheral motor targets, and spontaneous neural activity may be important in neuromuscular junction formation. In addition, trigeminal and facial motor neurons undergo dramatic somal migration within the hindbrain, and activity-dependent processes, with concomitant fluctuations in [Ca²⁺], may play a role in the correct navigation of the cells within the growing hindbrain. The importance of these neuronal populations undergoing appropriate developmental sequences is shown by deletions in hindbrain-specific genes (see above). Functions needed at birth, such as respiratory and feeding patterns, may require the endogenous rhythms that are expressed early in hindbrain development.

3. Relationship to channel development

Relatively little is known about the ion channels that play roles in the expression of spontaneous activity in hindbrain neurons. Synchronization of [Ca²⁺] transients in mouse hindbrain occurs abruptly at E11.5 and requires the expression of TTX-sensitive Na⁺ channels, which are not important in the nonsynchronized activity seen at earlier stages (218). In addition, GABAergic inputs and modulation by I₉ are required in chick hindbrain to alter the rhythm from an immature low-frequency type of activity to a mature pattern (185). However, other ion channels and receptors have not been carefully characterized yet.

H. Spinal Cord and Emerging Motor Patterns

1. Nature of spontaneous activity

The developing spinal cord expresses both spontaneous neuronal activity as well as activity that is driven or modulated by a variety of excitatory agents, including NMDA, serotonin, high K⁺, or electrical or sensory stimulation. Spontaneous activity in spinal and hindbrain motor neurons is accompanied, at the appropriate developmental stage, by movement of the muscles innervated by the active neurons. The expression of spontaneous motility in most vertebrates develops in a rostral to caudal fashion, initially as relatively small random movement and proceeding to highly patterned motility with alternating flexor and extensor motor output as well as activity that alternates on the two sides of the body. This may be dictated by the general rostral-caudal developmental pattern of development, including the ability to generate spontaneous electrical activity. In chick, motility begins at day 3.5 of incubation in one area of the body, while later involving the whole body (222, 223). In mouse, spontaneous motility begins at embryonic day (E)12.5, is usually initiated near the head (70% of the movements), and is propagated in a rostrocaudal direction. Those movements that initiate at other positions often propagate in the same direction (571). The similarities in generation of spontaneous movement in chick and mouse (similar Carnegie stages, initiation ~1 day after muscle innervation by the motor neurons, rostrocaudal pattern of motility) suggest that there is conservation of this developmental feature, and thus, that it is important to development in general. Because spontaneous activity continues through the period of innervation, developmental changes in the pattern of that activity (described below) are likely to influence muscle, as well as neuronal, differentiation.

A) ZEBRAFISH SPINAL CORD. An elegant series of experiments using video microscopy has elucidated the se-
quence of motor behaviors in the developing zebrafish (516). The first spontaneous activity consists of alternating trunk contraction was seen at 17 hpf; this activity increased in frequency and then decreased to <0.1 Hz after 10 h (27 hpf). During this time, at 21 hpf, touch responses, consisting of coiling of the body, were elicited. These responses increased in speed of contraction until 48 hpf. In addition, after 26 hpf, the embryos were able to respond to touch by swimming. Each of these behaviors appeared abruptly, as if the circuit were wired correctly with no experience-dependent modification, and then became functional. The behaviors were arrested by application of nicotinic synaptic blockers. Lesioning the brain above the level of the hindbrain did not modify any of the behaviors, and additionally removing the hindbrain did not affect the spontaneous contractions (but did block the other two behaviors). Removal or lesion of the rostral hindbrain did not affect touch responses or swimming, while lesioning at the level of the caudal hindbrain interfered with both behaviors (516).

The earliest spontaneous activity in zebrafish is correlated with spontaneous inward currents recorded from motor neurons (517); these spontaneous inward currents occur on the same developmental time scale as the early alternating trunk contractions and are distinct from synaptic events, which are glycine mediated. The spontaneous inward currents remain during block of synaptic release (low Ca$^{2+}$/high Mg$^{2+}$), but were abolished by TTX. The frequency of the events was affected by holding potential, suggesting that the activity is an intrinsic property or arises as an emergent property of a local circuit, rather than being driven by a remote, upstream pacemaker. Heptanol and cytoplasmic acidification blocked the events, suggesting the involvement of gap junctional coupling. Given the small number of motor neurons at these stages, a local circuit composed of electrically coupled premotor neurons and motor neurons seems a strong candidate for the mechanism mediating spontaneous activity.

In addition to spontaneous depolarizations in the motor neurons, several classes of interneurons in the early spinal cord also express periodic depolarizing events, especially those neurons that are ventrally located (518). These events are independent of synaptic inputs (in fact, were still present in the presence of botulinum toxin), but were completely abolished by gap junction blockers. The active ventral interneurons were shown to be electrically coupled to other active interneurons and motor neurons on their ipsilateral side by the use of paired patch recording. The authors propose that spontaneous activity is triggered by Ca$^{2+}$ channel-mediated events in combination with Na$^+$ currents, which are then spread synchronously throughout the network as well as being strengthened by electrical coupling.

B) CHICK SPINAL CORD. Spontaneous bursts of activity can be recorded from ventral roots in chick spinal cord at stage 24 [embryonic day (E)4], although due to their small size, this is done only with some difficulty. From stages 25 to 28.5 (E6), the interval between episodes of activity lengthens, changing from 1- to 2-min intervals at stage 25 to intervals of ~7 min at stage 28.5, with more bursts recorded during each episode. The mechanism underlying the bursts included synaptic interactions and gap junctional coupling (408). Although NMDA or kainate receptor stimulation modulates the activity, application of receptor blockers either alone or in combination did not abolish the spontaneous activity, indicating that though glutamate receptors are present at stages 25 and 28, they are likely not the primary mechanism that generates the spontaneous activity. GABAergic circuits were involved in the spontaneous activity between stages 21 and 25, since both stimulators and blockers of GABA receptors altered the activity, with the effect of blockers being stronger at the later stages; glycinergic inputs regulate spontaneous activity after stage 28 (228). However, the strongest candidate for mediating the spontaneous activity was nicotinic AChR input, specifically those receptors that do not contain the α7-subunit, since α-bungarotoxin (BTX) did not block the activity. This is in contrast to the later (E10–12) chick spinal cord activity where α-BTX does block spontaneous activity (316).

Spontaneous activity is also recorded in older (E7-E11) chick embryos, and by E10 episodes comprised of several cycles of bursting activity occur every 10–20 min (575). These episodes are generated by networks of neurons, residing primarily in the ventrolateral regions of the spinal cord that include interneurons, since the activity can be recorded in ventral roots or the ventrolateral funiculus, an interneuron tract (449). The network properties that drive spontaneous activity include synaptic interactions between neurons that are primarily excitatory, and different transmitters may be able to substitute as drivers of activity if one subset of transmitters is removed. For example, if glutamate and nicotinic receptors are blocked, after a period of time, GABAergic/glycinergic inputs are able to replace the element of excitability and initiate spontaneous activity (107, 448, 575). Each episode is preceded by a heightened state of excitability as shown by recordings using intracellular techniques, and after each episode, levels of spontaneous activity are much reduced (449). Experiments using combined imaging and recording techniques showed that motor neurons, perhaps due to their intrinsic higher excitability, were able to stimulate network episodes by activating R-interneurons which then spread excitation through the network of neurons via functionally excitatory GABAergic inputs (610). Intervals between episodes are mediated by a form of cellular depression that includes alterations in the drive.
ing force for Cl⁻, reducing the effective input for GABA (108).

C) MOUSE SPINAL CORD. Developmental aspects of mouse spinal cord locomotor physiology have been reviewed recently (58, 612). Spontaneous activity is recorded in lumbar motor nerves in the E11.4–14.5 spinal cord and consisted of both major episodes that propagate throughout the lumbar cord, and local episodes, recorded on a single motor nerve (227). Intervals between major episodes increased over the developmental time (from 2 min at E11.5 to ~8 min at E14.5), as did the episode duration. The spontaneous episodes were synchronized between the two sides of the spinal cord, an indication that reciprocal inhibitory synapses between the two sides had not yet been expressed. At a higher concentration of extracellular K⁺ (4.5 mM) in spinal cord preparations with attached medulla, similar spontaneous activity was recorded at E12.5 and E13.5 (633). However, at E14.5, an additional class of episodes was observed, with relatively long episode durations compared with the events at all other stages, and longer postepisode silent period (interval of ~11 min, compared with the interval for the shorter duration events of ~1 min). These events were not recorded at any other stage. At E15.5–16.5, the short-duration episodes occurred at long intervals of 10–15 min, and the spinal cord engaged in activity <1% of the time; at E17.5, short-duration events again became quite frequent but relatively irregular, with intervals of 1 min, and the spinal cord experienced a much higher rate of activity. Spontaneous activity was expressed equally in the rostral-caudal spinal cord at the E12.5–13.5, shifted to rostral predominance at E15.5–16.5, and then to caudal predominance at E17.5. Activity at E14.5 showed no clear rostral or caudal directionality, and this stage appeared to mark a transitional time, where two distinct rhythms are expressed as development of a more mature phenotype appears (633); the immature regular short-duration events recorded at E12.5–13.5 gave way after E14.5 to more mature irregular short-duration events.

Applying agonists or antagonists of glutamate receptors did not modulate the spontaneous activity, suggesting that glutamate receptors are not yet expressed in the circuit generating spontaneous activity (227). [At later developmental stages, glutamate inputs are required for rhythmic activity (71).] In addition, GABA antagonists lengthened the interval between episodes but did not block activity, indicating that inputs from this transmitter system can modulate spontaneous activity but are not required for the expression of that activity.

Glycinergic transmission was required for the propagation of activity throughout spinal cord segments, but not for the generation of local episodes. This implies a model in which local episodes are dependent on a network composed of motor neurons and GABAergic interneurons, while propagated activity is driven by the two inhibitory transmitters, glycine acting between segments and GABA acting within each segment. The group of glycineric interneurons expressed a dihydro-β-erythroidine hydrobromide (DHβE)-sensitive ACh receptor that allowed inputs from motor neurons to elicit propagated episodes (227).

At all stages examined, application of serotonin was able to elicit activity; this activity was synchronized on the two sides of the cord in early animals (E13), but alternated between the two sides of the cord in later animals (72). E15 appears to be a transitional stage, where serotonin-stimulated activity was not completely synchronized between the two sides; strict synchronicity was reestablished in the presence of strychnine, suggesting that the mature activity that alternates between the two sides of the spinal cord is mediated by glycineric contralateral interneurons, and develops soon after E15. Thus the time point of E14.5–15 is again suggested to be a crucial time point in the maturation of rhythmic patterns. That serotonin can initiate activity demonstrates that those receptors are present and able to modulate rhythmic activity well before the innervation of the spinal cord by the descending serotonergic inputs from the raphe (16).

In the neonatal spinal cord, activity more representative of the adult pattern is seen; this latter pattern consists of evoked locomotor-like activity with extensors and flexors acting in an alternate pattern and with alternating responses from the two sides of the cord. Although periods of spontaneous activity are observed at postnatal day (P)0–4, recordings in the lumbar spinal roots show that they are considerably more variable than the patterns recorded in the earlier embryonic animals, with additional variability in the appearance of alternating patterns, either left-right or flexor-extensor alternation [613 (4 K⁺)]. Spontaneous activity is clearly alternating in lumbar roots at P0–2, while sacral roots are less mature in their patterns. Raising the probability of NMDA receptor channel opening by perfusing Mg²⁺-free solution increases the rhythmogenicity [56 (4 K⁺)], and the probability of alternating rhythm in the caudal spinal cord (72).

Stimulation of sensory inputs also elicits left-right alternations in lumbar and sacral spinal cord, as shown with a combination of [Ca²⁺] imaging and ventral root recording (59). In response to stimulation, alternating activity is recorded that propagates from rostral to caudal in the spinal cord. Spontaneous activity recorded under these conditions also moved from rostral to caudal. Propagation of the signal under conditions of sensory stimulation occurred at the rate of 15 μm/ms, although there was an apparent increase in velocity of propagation in the lower lumbar region (58). Several experiments also demonstrate that the sequence of rostral to caudal developmental events may also dictate the ability of different
regions of the spinal cord to generate rhythmic activity at specific developmental stages (56, 443).

Rhythmic activity is consistently evoked by bath application of drugs, although activity generated by such pharmacological manipulations was much slower than that seen spontaneously (613; reviewed in Ref. 58). Combinations of serotonin, NMDA, and dopamine were able to elicit patterned alternating activity, which was slower in frequency than that generated spontaneously. Different combinations of pharmacological agents modified the periods of overlap during alternating activity. Midline sectioning of the cord generated slower cycles of activity on the hemi-cords. Application of MK801 or AP5 slowed but did not block activity, while CNQX completely blocked the activity (613). This evidence indicates that NMDA receptors likely modulate activity but are not required for its generation, whereas non-NMDA (AMPA/kainate) receptors are required for the activity (56, 72).

D) RAT SPINAL CORD. Several recent reviews have described the maturation of locomotor networks in the developing rat (110, 111), the spatial distribution of the pattern generation mechanism, especially in the hindlimb region (285), and the ability of sensory inputs to modify motor output (474). We will concentrate on the spontaneous activity present in the early embryo.

In the rat, spontaneous motor neuron output is present at E13 (209), before responsive muscle contraction. In isolated E13.5–15.5 spinal cords, spontaneous activity is recorded (437; –6 K⁺) that is present on cervical and lumbar roots, in which the cervical segments lead the lumbar segments. At E16.5, the lumbar roots begin to lead the cervical roots and have additional spikes of activity that are not propagated to the cervical segments. The role of the leading segments was elucidated by transection experiments; dividing the cord at E14.5 showed that although both rhythms were slowed, the cervical rhythm was more rapid than the lumbar. Separation at E15.5 demonstrated equal abilities of the two cord portions to generate spontaneous activity, and the cervical rate was slowed to a greater extent than the lumbar rate. Division at E16.5 showed that the lumbar cord was able to independently generate activity, and at a much higher rate than the cervical cord. Thus the ability of different regions of the spinal cord to drive spontaneous activity changes over development. These results have been extended by experiments exploiting recording from a more complete rostral-caudal range (498). These showed that at E14.5, thoracic segments lead both cervical and lumbar regions, and that until approximately E17, the spinally generated patterns were also recorded in the brain stem, on cranial nerve XII. Imaging with voltage-sensitive dyes combined with field recording showed that E15 embryos had synchronized spontaneous voltage fluctuations on the two sides of the cord, while spontaneous fluctuations in postnatal animals alternated on the two sides, with additional alteration between the medial and lateral portions of each side (141). These spontaneous fluctuations were dependent on extracellular Ca²⁺ and could also originate in quadrants dissected from the whole cord, implying that the networks responsible for mediating spontaneous activity are distributed throughout different regions of the cord.

Pharmacological experimentation examined the role of different transmitter systems in causing the spontaneous activity. Kynurenate or CNQX had little effect on the activity at E14.5 or E15.5. At E16.5, activity in cervical regions was blocked by these antagonists, although it sometimes reappeared in the presence of blocker; in the lumbar cord, kynurenate or CNQX did not block activity (437, 498). At later stages, glutamate receptor blockade abolishes activity; thus there is a rostral to caudal progression in the development of glutamate-mediated spontaneous activity in the spinal cord. Activity up to stage E17.5 was blocked by strychnine (437, 498) and bicuculline (498), indicating that the classic inhibitory transmitters lead to spontaneous activity at early stages. In addition, because of the change in the Cl⁻ reversal potential during the same range of developmental stages, GABAergic blockers initially caused an early abolition of activity, while the same blockers used at later stages caused an augmentation of activity (311, 498). Ren and Greer (498) also showed that nicotinic receptor blockade led to cessation of spontaneous activity at E13.5–E17.5.

Spontaneous activity in the early embryonic rat spinal cord is synchronized between different segments of the spinal cord and between the left and right sides of the animal. More mature patterns of activity (post E17.5), most of which are induced by application of excitatory neurotransmitters (most often glutamate or serotonin), are recognized by the alternation of activity between the two sides of the animal and between antagonistic motor neuron groups. The application of strychnine, a blocker of glycinergic receptors, caused alternating activity to become synchronized, implying that glycinergic inputs, inhibitory at this stage (E20.5), were required to produce the alternating patterns between the two sides of the cord (311). Because both glutamatergic and glycinergic stimulation induced activity, these authors proposed that these two transmitter systems were involved in stimulating activity; that activity is initially synchronized, while later in development it becomes alternating because of the more negative reversal potential for Cl⁻. The switch between synchronized and alternating patterns was also shown by [Ca²⁺], imaging of the commissural interneurons in the spinal cord, which have increases in [Ca²⁺] in synchrony with ventral root activity, and mediate the relationship between the two sides of the cord. At earlier stages (E15.5), these commissural neurons synchronize the two sides via excitatory synaptic GABAergic inputs, shown by removal of extracellular Ca²⁺ or application of antago-
nists in a split-bath recording arrangement. At E18.5–20.5, the effect of GABA is superseded by glycine, mediating alternation of activity in a very similar way to the GABA effect. At E16.5, slices of spinal cord were used to demonstrate that the mechanisms mediating the synchronous activity of the two sides of the cord were contained within one-half (anterior-posterior slices) of a single segment (200 μm slice), but not within one-quarter of a segment (100 μm slice).

Electrical coupling between neurons is one mechanism that allows synchronization of activity. In motor neurons, synchronized or correlated activity may be crucial in the ability of presynaptic neurons to initiate the between-cell communication required for the establishment of end plates. Correlated activity causes the postsynaptic cell to express correct junctional proteins and signals, and a polyinnervated synapse is formed. In the developing rat spinal cord, electrical coupling in the form of halothane-sensitive electrical coupling is mediated by specific classes of connexins, which are down-regulated during the initial steps of synapse elimination in rats (96). During the initial period of synapse elimination (postnatal week 1), spontaneous activity is relatively low but highly correlated temporally (478). Experiments in awake moving mouse pups during the period of synapse elimination in the lumbar spinal cord have shown that the junctional coupling decreases as synapse elimination occurs in mice (478) and that pharmacological blockade of gap junctions in mice abolished the correlated activity in the motor neurons, perhaps disrupting normal synaptic connectivity (479). Thus a mechanism that seems to be crucial during early development to direct synapse formation may lessen in importance as end plates become singly innervated. However, there is evidence that electrical coupling is used in adult motor neuron behavior, in either normal or abnormal situations (286).

2. Developmental roles of spontaneous activity

In addition to the known role of pruning of inappropriate or supernumerary inputs to the NMJ, spontaneous motor neuron activity may function at earlier stages to enable correct matching of pre- and postsynaptic partners. The pathfinding of motor axons into their motor units in E4 chick lumbar spinal cord requires the correct expression of spontaneous activity, which at this early stage is mediated by glycinergic receptors. The mechanism of this pathfinding includes the activity-dependent processing of cell adhesion molecules and guidance molecules used to sort axons at the initial exit point of the neural tube (228). Appropriate matching axons with target motor units ensure that the spontaneous muscular movements occur; these may influence bone and connective tissue growth in the animal.

3. Relationship to channel development

The repertoire of excitatory inputs that mediate spontaneous activity in spinal cord neurons give rise to a secondary feature, which is that they are functionally redundant: blockage of two inputs (glutamatergic and nicotinic) slows spontaneous activity only temporarily, with reactivation of the same rate of activity by increased glycinergic or GABAergic input (chick, Ref. 448). Functional circuitry also changes with development, with AChR subunit switching occurring between E4 and E10 (chick; Ref. 316) and excitatory inputs switching from GABA to glycine (chick; Ref. 228). Chick motor neurons express large overshooting action potentials at E4, contributed by Na⁺ channels; expression of the Na⁺ currents increases over several days, with later differential increases in K⁺ channel expression shortening the action potential (387). Early expression of Ca²⁺ channels in chick motor neurons are dominated by high-voltage-activated channels, while more mature motor neurons largely express N- and L-type Ca²⁺ channels (387).

I. Cochlear Hair Cells and the Loss of Excitability After Activity

1. Nature of spontaneous activity

During development, inner hair cells (IHCs) of the cochlea show spontaneous action potentials from E18 to P6 with a peak frequency of ~5 Hz (368, 369). A single spontaneous action potential is capable of triggering exocytosis of transmitter from the IHC (40, 369). This implies that spontaneous activity is communicated to brain stem auditory nuclei, and indeed, such activity has been recorded in the brain stem at these stages (216, 272, 304, 341). This spontaneous activity itself is modulated by cholinergic innervation from the superior olive that is itself a transient developmental phenomenon (198, 542). Thus efferent spontaneous activity arising in the brain stem is likely to interact with afferent spontaneous activity in IHCs.

2. Developmental roles of activity

Spontaneous activity in IHCs is involved in transneuronal survival and death, a phenomenon extensively studied in the auditory system (see Ref. 511 for review). Neurons of the VIIIth nerve, which are activated during spontaneous activity of hair cells, make synapses in the brain stem in the cochlear nucleus. Functional deafferentation, either by cochlear ablation or block of VIIIth nerve activity, causes pronounced and rapid neuronal death in the cochlear nucleus (see Ref. 428), with first events in the postsynaptic cells visible within 12 h. These effects are specific to developing animals and can be rescued by providing synaptic activity onto cochlear neurons but not
by stimulating them directly. The survival promoting action of VIIIth nerve activity relies on stimulation of metabotropic glutamate receptors, which act to keep [Ca\(^{2+}\)]\(_i\) in cochlear neurons at low levels (639). Neuronal cell death appears to involve activation of caspase-3 (429). These studies demonstrate an intimate relationship between developmental patterns of spontaneous activity, the resulting activity in the VIIIth nerve, and neuronal cell survival in the auditory brain stem.

3. Relationship to channel development

Mature IHCs are the primary auditory sensory receptors in the cochlea. They have an impressive ability to transduce high-frequency mechanical inputs into membrane potential oscillations and transmitter release. To do this, they rely on an interplay between inward Ca\(^{2+}\) currents and outward Ca\(^{2+}\)-activated K\(^+\) currents to create rapid oscillations in membrane potential, rather than full action potentials (see Ref. 173 for review). Individual IHCs have specific frequency tuning curves, which are created in part by variability in kinetics of the Ca\(^{2+}\)-activated K\(^+\) current. This results from alternative splicing of the channel transcript and modification of the channel properties by accessory subunits (489–491). Although IHCs do not generate action potentials in the adult, they do early in development (190, 368, 369). In mouse, the period during which IHCs are capable of generating full action potentials extends from about E18 to P12. Thus the onset of spontaneous activity appears to coincide with the appearance of spike-generating ability, but the cessation of spontaneous activity occurs before spike-generating ability is lost (368, 369). The events that terminate the ability to generate action potentials in IHCs have some features in common with those that modify firing properties in Xenopus spinal neurons and ascidian muscle (see sect. II, K and L). During the period of action potential generation, the dominant outward K\(^+\) current is voltage-gated and slowly activating (190, 308, 368, 550). Later, a rapidly activating Ca\(^{2+}\)-activated K\(^+\) current appears (at about P10-P12 in mouse and 2 days prehatching in chick), which effectively terminates spike-generating ability. The expression of I\(_{K(Ca)}\) correlates approximately with the onset of hearing. Its expression requires the preceding period of spontaneous activity created in part by its absence. In mice lacking Cav1.3 Ca\(^{2+}\) channels and hence these early action potentials and spontaneous activity, I\(_{K(Ca)}\) fails to develop (73). In addition, at least in mouse, resting potential (V\(_\text{rest}\)) becomes more negative with development and the inactivation versus V\(_\text{curve}\) for the voltage-gated outward K\(^+\) current shifts more positive, thus increasing the magnitude of K\(^+\) current available during depolarization (368). Superimposed on these changes is the transient expression of an inwardly rectifying K\(^+\) current, which appears at E15.5 (mouse) and disappears after the onset of hearing at P12 (367). Loss of inward currents also contributes to the loss of excitability. Both Na\(^+\) and Ca\(^{2+}\) currents appear in these cells just before birth and reach peak densities at P5, correlating closely with the period of spontaneous activity. Both I\(_{Na}\) and I\(_{Ca}\) are then dramatically downregulated between P5 and P12 (the onset of hearing), I\(_{Ca}\) by ~70%, and I\(_{Na}\) completely (369). This coordinated developmental appearance of rapidly activating outward K\(^+\) currents and downregulation of both inward currents serves to terminate spike-generating ability and allows their maturation as functional transducers of high-frequency inputs.

In cells like IHCs, which are excitable only transiently during development, the presumption is strong that electrical activity is serving a developmental function. It will be very interesting to explore other nonneurally derived cells that are inexcitable in the mature state, to see whether early expression of ion channels creates similar early periods of excitability and signaling. This point is discussed further in section vD2.

J. Dorsal Root Ganglion Cells, Myelination, and Cell Adhesion Molecules

1. Nature of activity

Embryonic dorsal root ganglion neurons begin to generate spontaneous action potentials at about E16, when axon terminals begin to reach the periphery. Initially, activity occurs at relatively low frequencies (<0.5 Hz) but speeds to frequencies of up to 10 Hz near birth (see Ref. 174 for review).

2. Developmental roles

In cultured DRG neurons, direct stimulation of the neurons at 0.1 Hz reduces myelination, but 1-Hz stimulus has no effect. These frequencies are similar to those that trigger expression of cell adhesion molecules and to normal frequencies of spontaneous activity in DRG neurons at these stages (565). Further experiments on DRG neurons showed that direct electrical stimulation of the neurons induces [Ca\(^{2+}\)]\(_i\) transients in both the neurons and cocultured Schwann cells, secondary to the release of ATP from the neurons (564). The Schwann cell [Ca\(^{2+}\)]\(_i\) transients result in CREB phosphorylation and arrest of the Schwann cell in a nondividing “predifferentiated” state. Shrager and Novakovic (541) reported that myelin development in embryonic spinal cord slices was unaffected by TTX treatment, but as the authors point out, growth factors present in the culture medium might substitute for activity in inducing myelination.

The expression of different cell adhesion molecules in DRG neurons is activity dependent and shows differential frequency sensitivity (260). N-cadherin and L1 are
both downregulated by 0.1 Hz activity, although only L1 is downregulated at 1.0 Hz, and NCAM expression is not regulated by activity. It is possible that activity-dependent changes in cell adhesion are involved in defasciculation of DRG axons in the periphery and in Na⁺ channel clustering at nodes of Ranvier (361). Activity has also been implicated in differentiation of the dopaminergic phenotype in DRG neurons (79).

3. Relationship to channel development

In DRG neurons, the Na⁺ channel Nav1.3 is expressed early, peaking at E17 and then disappearing by about P15. The timing of Nav1.3 expression corresponds with a period of spontaneous activity. Because Nav1.3 has particularly rapid recovery from inactivation, it may help to induce spontaneous, repetitive firing in developing DRG neurons (128, 171). The expression (measured as both mRNA and protein levels) of two other Na⁺ channel types, Nav1.8 and Nav1.9, is downregulated by activity in DRG neurons (291), although it is not yet known whether spontaneous activity actually downregulates these channels during normal development. Nav1.8 and Nav1.9 show fairly negative voltage dependence and slow inactivation kinetics, so changes in their expression would have marked effects on excitability in the neurons. Similarly, different Ca²⁺ current subtypes show distinct developmental regulation in DRG neurons (241), and Ca²⁺ current expression shows similar dependence on the specific patterns of stimulation as do other activity-regulated molecules (331).

K. Amphibian Spinal Neurons, Transmitter Phenotype, and Low-Frequency Spontaneous Activity

I. Nature of activity

Developing Xenopus embryonic spinal neurons generate a variety of waveforms of spontaneous [Ca²⁺]ₐ transients (212, 214). [Ca²⁺]ₐ “spikes” are clearly triggered by spontaneous action potentials that propagate throughout the cells. These occur at very low, but reproducible, frequencies of 1–3/h during the first 10 h in culture after neurons are removed from the neural plate. They also occur in the embryonic spinal cord in vivo (558). Neurons in single cell cultures show normal differentiation of activity-dependent properties, indicating that spontaneous activity is cell-autonomous (237). When imaged in vivo, [Ca²⁺]ₐ transients are seen to be synchronous among small clusters of neurons in the spinal cord, with cluster size and position consistent with the hypothesis that neurons that differentiate together as a small group generate spontaneous [Ca²⁺]ₐ transients in synchrony (558). This finding is important in that it shows that complex forms of synchrony of spontaneous activity in vivo can emerge out of subpopulations of interconnected, but independent, oscillators. This emphasizes that intrinsic properties that are instructive for spontaneous activity interact with the emerging synaptic circuitry of the nervous system to create appropriate patterns of activity.

In contrast to Ca²⁺ “spikes,” [Ca²⁺]ₐ “waves” occur locally in the soma and growth cone and appear to result from Ca²⁺ entry through a channel open at the resting potential (214). Waves have a longer duration than spikes (33 vs. 9 s). Waves in the growth cone occur at higher frequencies than soma spikes (8–10 vs. 1–3/h). Growth cone filopodia also show local [Ca²⁺]ₐ transients driven by substrate interaction (203). These findings indicate that within the complex spatial structure of developing neurons, several types of spontaneous activity can occur at the same time.

2. Developmental roles

A series of elegant experiments in which naturally occurring spikes and waves were blocked and then artificially stimulated [Ca²⁺]ₐ transients imposed at various frequencies showed that these events are both necessary and sufficient for several aspects of neuronal differentiation. When spikes and waves are eliminated in 0 Ca²⁺ medium, the normal speeding of delayed K⁺ current activation does not occur, fewer neurons develop the GABA phenotype (quantified by both GABA immunoreactivity and GAD transcripts), and neurites are abnormally long (145, 248, 249, 557, 607).

To demonstrate sufficiency of [Ca²⁺]ₐ transients, transients were artificially imposed on neurons in Ca²⁺-free medium by brief applications of high K⁺ + Ca²⁺ medium (214). When transients were imposed at normal spike frequencies, K⁺ current speeding and GABA phenotype were both rescued. Remarkably, imposed transients at 1/h did not rescue K⁺ kinetics at all, whereas transients at 2/h rescued kinetics completely (see Fig. 4b in Ref. 214). This indicates a very sensitive and finely tuned mechanism for decoding even very low frequencies of Ca²⁺ transients. Similar results were obtained for rescue of the GABA phenotype, with 1 transient/h not rescuing and 3/h restoring GABA phenotype completely (see Fig. 4c in Ref. 214). Neurite length, however, was not rescued by spike-frequency transients, but when wave frequencies of 8–9/h were imposed, neurite length reverted to control. Neurite outgrowth appears to be regulated by growth cone [Ca²⁺]ₐ waves via calcineurin-mediated dephosphorylation of GAP-43 (319). This shows that the two types of transients encode different aspects of neuronal differentiation, partly by virtue of their different frequencies. More recently, Borodinsky et al. (65) showed that activity-dependent regulation of transmitter phenotype in this system was “homeostatic,” with high levels of activity
increasing expression of inhibitory transmitters at the expense of excitatory transmitters, and blockade of activity doing the opposite.

Similar roles of activity and/or \([\text{Ca}^{2+}]_i\) transients in reducing neurite outgrowth have been reported in retina (91), cerebellar Purkinje neurons (242, 523; but see Refs. 496, 493), and neuronal cell lines (101). In dentate granule cells, dendritic spine maturation depends on neuronal activity (151).

Other neurons also show activity-dependent transmitter phenotype. For example, mouse spinal neurons show spontaneous activity in culture, and blocking that activity with TTX downregulates enkephalin expression (2). This effect can be rescued by \([\text{Ca}^{2+}]_i\) channel agonists.

Activity also affects the response of Xenopus neuron growth cones to guidance molecules. In culture, axons are repulsed by netrin-1 early in development but attracted only 8–10 h later (409). At the early stages, simulation inverts the normal repulsion by netrin to attraction. At late stages, electrical stimulation increases the sensitivity of axons to attraction by netrin, and blocking \([\text{Ca}^{2+}]_i\) channels inverts the attractive response to repulsion (252). These responses involve a complex interaction between the type of netrin receptor, \([\text{Ca}^{2+}]_i\), and intracellular levels of cAMP and cGMP (444). Overexpression of the UNC5 Netrin receptor converts the response of the axon to netrin from attraction to repulsion and inverts the effect of netrin on the growth cone L-type \([\text{Ca}^{2+}]_i\) current from augmentation to reduction (444). Similarly, increasing the ratio of intracellular cAMP/cGMP shifts the axonal response from attraction to repulsion.

3. Relationship to channel development

Xenopus spinal neurons, including the transient population of sensory Rohon-Beard neurons, become excitable shortly after their last round of DNA synthesis, at the neural tube stage. (It may well be that their precursors show a complex pattern of channel development, as discussed in section II, A and B). During the early stages of excitability, Xenopus spinal neurons generate long-duration action potentials that are primarily \([\text{Ca}^{2+}]_i\) dependent. During the next 24–48 h, the action potential shortens by \(\sim 100\)-fold and becomes more \(\text{Na}^+\) dependent and less \(\text{Ca}^{2+}\) dependent (556). These changes are all cell-autonomous, proceeding normally in single-cell cultures (237).

Under voltage clamp, the major change during this period is a large increase in density and activation rate of the delayed \(\text{K}^+\) current and an increase in \(\text{Na}^+\) current density. The high-threshold \(\text{Ca}^{2+}\) current does not change in amplitude (19, 451). Thus the shortening of the action potential reflects the changes in delayed \(\text{K}^+\) current amplitude and kinetics. The loss of apparent \(\text{Ca}^{2+}\) dependence of the action potential seen in current clamp reflects not a loss of the \(\text{Ca}^{2+}\) current but the truncation of action potential duration by the changes in the delayed \(\text{K}^+\) current to a duration at which the \(\text{Ca}^{2+}\) current contributes little to the spike waveform (18, 19). Modeling studies indicate that the increase in amplitude of the delayed \(\text{K}^+\) current plays a bigger role in spike shortening than the speeding of \(\text{K}^+\) current kinetics (350). Although all spinal neurons show spike shortening in this preparation, different molecular subtypes of \(\text{K}^+\) channels are involved in different cells, including xKv1.1, xKv2.1, and xKv3.1 (47, 500, 600). This diversity of outward \(\text{K}^+\) currents may help create the different patterns of spontaneous \([\text{Ca}^{2+}]_i\) transients seen in different classes of spinal neurons (65).

The long duration of the early action potentials is critical for the developmental effects of spontaneous activity. Modeling studies show that the long-duration action potentials do indeed admit more \(\text{Ca}^{2+}\) (350). Misexpression of a mature delayed \(\text{K}^+\) channel shortens the immature action potential and eliminates at least some aspects of the activity-dependent developmental events (271), but only in culture, not in vivo (270). It would be interesting to know whether expressing the mature \(\text{K}^+\) current simply shortens the action potential, or might also act to reduce spontaneous activity itself by reducing the time during which net inward current flows during depolarization (see, e.g., Ref. 208).

These studies suggest several important principles. One is that action potentials may exist in an “embryonic” form that is more efficient in carrying out its developmental role, that is to occur spontaneously and admit \(\text{Ca}^{2+}\) to the cell in amounts and patterns to trigger activity-dependent developmental programs. Another is that spontaneous activity can be instructive, not just permissive, in its developmental role, as shown by the ability of artificially imposed activity to rescue development in cells where activity is blocked. A very interesting unresolved question is what regulates the very low but stable frequencies of spontaneous action potentials in these cells.

I. Ascidian Muscle, Inward Rectifier, and Activity-Dependent Ion Channel Development

1. Nature of spontaneous activity

Muscle cells of developing ascidian embryos generate spontaneous bursts of action potentials for a period of 6–8 h just following exit from the cell cycle at the start of neurulation. This activity takes the form of bursts of action potentials at 2–4 Hz lasting an average of 20 s separated by silent periods of slightly more than 1 min (129). Activity occurs well before the appearance of innervation and the development of contractility. Because these action potentials are \(\text{Ca}^{2+}\) dependent (ascidian muscle cells do not express \(\text{Na}^+\) currents at any stage of development), this activity presumably results in sponta-
neous \([\text{Ca}^{2+}]_i\) transients, although these have not been measured directly. Spontaneous activity in these cells occurs in isolated cells developing in culture, and thus is cell autonomous (129). Whether activity is synchronous among muscle cells in vivo is not known.

2. Developmental roles of spontaneous activity

One of the major functions of spontaneous activity in ascidian muscle is to trigger the later expression of the \(\text{Ca}^{2+}\)-activated K\(^+\) current, which shortens action potential duration and helps to terminate the period of spontaneous activity. This is discussed in the next section. Activity-blocked cells also fail to organize their actin filaments properly and are only minimally contractile compared with normal muscle.

3. Relationship to channel development

We discussed above the early postfertilization changes in ion channel properties in embryos of the ascidian Boltenia villosa. In this embryo, the period between fertilization and gastrulation is characterized by the sequential elimination of the Na\(^+\) current, then the Ca\(^{2+}\) current, and then at the start of neurulation, the inwardly rectifying K\(^+\) current (51, 208). These experiments were part of a larger study of ion channel development in the muscle cells of this embryo.

Taking advantage of the endogenous pigment that marks muscle-lineage cells in this embryo, we used perforated patch methods to voltage clamp muscle-lineage cells at all stages of development. In the gastrula, these cells are inexcitable and express only an inwardly rectifying K\(^+\) current, which is their sole resting conductance. This inexcitability, reflecting as it does a complex history starting at fertilization during which these cells have lost their previously acquired depolarization-activated currents, emphasizes that electrophysiological studies that commence at the start of their terminal differentiation can miss a complex preceding history of ion channel development.

A few hours after gastrulation, three simultaneous events occur that dramatically change the electrical properties of these cells (129, 208). 1) The inward rectifier disappears, leaving the cells with almost no detectable resting conductance. 2) A high-threshold, inactivating calcium current appears and rapidly reaches much higher density than found in the oocyte. 3) A slowly activating, voltage-gated delayed K\(^+\) current appears for the first time in development. If transcription or translation is blocked, the Ca\(^{2+}\) and outward K\(^+\) currents fail to appear, whereas the inward rectifier disappears normally. This combination of events causes the cells to become excitable and, because of the loss of their resting conductance, spontaneously active.

This period of spontaneous activity ends because of two near-simultaneous events that occur 6–8 h after the inward rectifier disappears. The inward rectifier reappears (an event that requires transcription), and a rapidly activating, \(\text{Ca}^{2+}\)-activated K\(^+\) current appears for the first time in the development of the cells. These two events terminate spontaneous activity and shorten the duration of the action potential by \(\sim 10\)-fold. In addition, a new Ca\(^{2+}\) current appears at this time and increases rapidly in density to contribute \(>80\%\) of the total Ca\(^{2+}\) current. It can be distinguished from the immature Ca\(^{2+}\) current present at earlier stages by voltage dependence (20 mV more positive), lack of inactivation, and differential conotoxin sensitivity (129).

If spontaneous activity is blocked, the cells fail to develop the rapidly activating \(\text{Ca}^{2+}\)-activated K\(^+\) current, and as a result, the action potential fails to acquire the short duration required for the mature contractile function of the muscles in larval swimming (129). These results contrast somewhat with Xenopus spinal neurons, in which action potential shortening occurs normally when activity is blocked (45). This is probably because in Xenopus neurons, K\(^+\) current speeding and increased density are two separable phenomena. In ascidian muscle, however, the increase in density is due to the expression of a new, more rapidly activating K\(^+\) current, so the two are linked.

We next asked to what extent the channels present in immature and mature muscle are optimized for their particular functions. Immature muscle generates bursts of spontaneous activity but has no need to respond to neural input, since the nervous system has no neuromuscular connections at these stages and the muscle cells are not yet contractile. Activity is serving a purely developmental function. Mature muscle has very different requirements. It must respond to neural input and be able to contract at 10 Hz or more to mediate larval swimming, which is essential for dispersion and maintenance of genetic diversity.

To ask how channels at each stage are optimized for these functions, we used action potential waveform clamp (386). In this method, action potentials or bursts of spontaneous activity are recorded from cells under normal conditions, and then are digitized and replayed into cells as voltage-clamp commands. We replayed these waveforms into cells of the same and different stages than those that generated them. The utility of the method derives from the fact that the waveforms are replayed into cells under conditions that isolate the voltage-gated Ca\(^{2+}\) currents (e.g., intracellular Cs\(^+\)). The current flowing during an action potential or activity waveform command under these conditions (after leak and capacitative current subtraction) represents the Ca\(^{2+}\) current flowing during activity, and thus the flux of Ca\(^{2+}\) into the cell.
Using this strategy, we reached the following conclusions (130).

1) Long-duration immature action potentials do not admit more Ca\(^{2+}\) to the cell than short-duration mature action potentials, because during development the amplitude of Ca\(^{2+}\) current increases in exact proportion to the decrease in action potential duration. Ca\(^{2+}\) influx during long-duration immature action potentials, however, is spread over a much longer period of time. Part of this is due to the obvious fact that the action potential is simply longer. Part is due to the fact that the immature, inactivating Ca\(^{2+}\) channels reopen in transiting from the inactivated to the closed states (547), thus creating a postspike burst of Ca\(^{2+}\) entry at voltages where driving force on Ca\(^{2+}\) is high.

2) During bursts of spontaneous action potentials in immature cells, Ca\(^{2+}\) enters the cells almost continuously, including during the period between individual action potentials. This is due to the more negative voltage dependence of the immature Ca\(^{2+}\) current compared with the mature one. The potentials at which the former is activated but the latter would not be falls precisely in the range of potentials of the interspike voltage trajectory during spontaneous activity. Shifts in channel voltage dependence are common in development (see sect. IV).

3) The burst length of spontaneous activity is set by accumulating inactivation of the immature Ca\(^{2+}\) current. If brief voltage-clamp commands mimicking spontaneous action potentials are delivered at burst frequencies, the Ca\(^{2+}\) current declines by 50% by the end of a burst equal in action potential number to the average burst of spontaneous activity. Blocking inactivation by using Ba\(^{2+}\) as the permeant ion eliminated this effect. Bursts of action potentials in mature cells did not cause accumulating inactivation of the mature Ca\(^{2+}\) current. The lack of inactivation of the mature Ca\(^{2+}\) current, in contrast, allows rapid repetitive firing, essential for rapid contraction relaxation cycles in larval swimming.

4) The increased amplitude and activation rate of the composite outward K\(^{+}\) current in mature muscle restricts Ca\(^{2+}\) entry to the brief duration of the spike itself, guaranteeing rapid relaxation of muscle after activation.

The work on ascidian muscle emphasizes the concept that many properties of the ion channels present at each stage of development are finely tuned to mediate specific patterns of spontaneous activity that occur at that point in development. We also see in these cells the complex transition between immature and mature electrical properties, critical to the cell because the properties of ion channels present in the immature state are not compatible with mature function. An important, and probably generally applicable, concept arising from this work is the feedback between embryonic ion channels, spontaneous activity, and mature ion channels. Embryonic channels regulate spontaneous activity, which in turn triggers expression of mature ion channels that terminate the activity. This principle is expanded on in section V.

M. Insect Neurons and the Refinement of Dendritic Trees During Metamorphosis

Some motor neurons in insects are preserved during metamorphosis, and their structure and connections are remodeled to serve new functions (587). In Manduca motor neuron, MN5 innervates slow crawling muscle in the larva and fast flight muscle in the adult. In making this transition, MN5 acquires a higher firing threshold and much decreased excitability to make the transition from a tonically firing slow motor neuron to one that fires only once or twice per wingbeat during flight. This is accomplished by an 80% decrease in resting resistance combined with a large increase in the magnitude of voltage-gated K\(^{+}\) currents (155). Like most insect neurons, the soma of MN5 does not generate full-size action potentials at either the larval or adult stages. However, for a brief period in pupal life, the soma can generate action potentials, and does so spontaneously. The development of Ca\(^{2+}\) currents is coordinated with these changes. A small, sustained Ca\(^{2+}\) current is expressed in larval MN5, but disappears by early pupal stages. Then, at the time of soma excitability and spontaneous firing, a large Ca\(^{2+}\) current with both transient and sustained components appears, which is maintained into adulthood. Soma excitability is eliminated after the pupal stages by a large increase in outward K\(^{+}\) currents. Some excitability could be restored by blocking these K\(^{+}\) currents. Although the developmental role of spontaneous activity is unclear in this cell, the period during which it occurs correlates with the end of a period of extensive dendritic remodeling and branch growth. Ca\(^{2+}\) imaging experiments indicate that during the period of dendrite extension, local dendritic [Ca\(^{2+}\)]\(_i\) transients occur, suggesting a localization of Ca\(^{2+}\) channels in dendritic compartments. At the end of the period of dendritic growth, soma activity appears and Ca\(^{2+}\) transients now are generated throughout the cell (156).

This work emphasizes that large, transient changes in firing properties may occur in particular compartments of individual cells during development. It would be interesting to know whether transient periods of excitability are a general property of developing arthropod neuronal somata, which are generally inexcitable in the adult. It is known that normally passive, nonspiking crayfish neuronal somata become capable of generating action potentials after axotomy (315).

N. Mammalian Muscle and Activity-Dependent Fusion of Myoblasts

During development of vertebrate skeletal muscle, mononucleated myoblasts fuse into multinucleated myo-
tubes. The fusion process is driven by a specific change in membrane potential determined by the coordinated activity of three types of ion channels (37, 41, 179, 342). As part of the program to achieve competence to fuse, myoblasts hyperpolarize to a membrane potential of around \(-65\) mV. This hyperpolarization is achieved in two stages, due to the effects of newly expressed EAG and Kir 2.1 channels. Just before fusion, T-type (\(\alpha_{1H}\)) Ca\(^{2+}\) channels are expressed by the myotubes. The K\(^+\) channel-mediated hyperpolarization brings the membrane potential into the range for the “window current,” the steady Ca\(^{2+}\) current generated through the T-type Ca\(^{2+}\) channel at potentials at which its activation and inactivation versus voltage curves overlap. At these potentials, T-type channels open but do not inactivate completely. The window Ca\(^{2+}\) current thus generated provides a steady influx of Ca\(^{2+}\) into the cell. This increases \([Ca^{2+}]_i\), which is the primary trigger for fusion. It is not entirely clear whether the membrane potential is held in this range for window currents during fusion, but since TTX does not block fusion, Na\(^+\)-dependent action potentials are not required. Thus Ca\(^{2+}\) entry occurs in the absence of action potentials, using the outward K\(^+\) current to balance Ca\(^{2+}\) influx, allowing the steady entry of Ca\(^{2+}\) at high driving force. Similar mechanisms allow hair cells to generate rapid membrane potential oscillations in response to high-frequency sound, and allow crustacean slow muscle fibers to generate large, graded Ca\(^{2+}\) entry for graded contractility (12, 173, 419, 420).

T-type currents are especially prominent at early stages of development in a variety of cells. Their potential roles in shaping spontaneous activity and Ca\(^{2+}\) influx are discussed in section ivD1.

O. Amphibian Muscle and Multiple Windows of Activity-Dependent Development

In embryonic Xenopus muscle, the delayed K\(^+\) current develops in two phases, with a multi-hour lag between two periods of increasing density (339). The inward Na\(^+\) current continues to increase in density during this lag, suggesting a period of increased excitability due to the temporary increase in inward-to-outward current ratio. Blocking the Na\(^+\) current during this time suppresses the second phase of K\(^+\) current development, implying the existence of spontaneous activity at those stages, although it was never directly measured (339). Activity block also suppresses development of the inwardly rectifying K\(^+\) channel.

Results like these would normally be interpreted to indicate that the second phase of delayed K\(^+\) current development depends on activity, whereas the first phase does not, since it was not affected by blocking activity. There is a problem with this interpretation, however. Because the first phase of K\(^+\) current development occurs before any inward currents are present, there is no activity to block. Thus it is possible that both phases could depend on activity, but only the second phase does so normally because that is when activity is possible. To test this idea, we expressed a mammalian brain Na\(^+\) channel in developing muscle to create spontaneous activity at abnormally early stages and indeed found that the first phase of development of the endogenous delayed K\(^+\) current was advanced to earlier stages (as was development of the inward rectifier) (338). This emphasizes the point that developmental events may be responsive to activity even if activity does not normally occur at those stages. Blocking endogenous activity will not reveal this. Although this “latent” activity dependence may not operate under normal circumstances, it may under pathological conditions, such as the early occurrence of seizure activity in the developing brain, or the exposure to excitatory pharmacological agents.

P. Cajal-Retzius Cells, Rohon-Beard Neurons, and Activity-Dependent Cell Death

A large body of literature has demonstrated that electrical activity and depolarization enhance the survival of embryonic neurons, leading to the hypothesis of selective survival of neurons that successfully make synaptic connections and thus participate in activity (466). Activity does not always promote survival of developing neurons, however. In two populations of temporary neurons that undergo programmed cell death as populations, zebrafish Rohon-Beard neurons, and mammalian Cajal-Retzius neurons, there is evidence for excitotoxic effects of spontaneous activity. In Cajal-Retzius cells, in vivo application of NMDA blockers protects against normal apoptosis (405). In culture, cell death is prevented by TTX or AMPA receptor blockers, but not by NMDA receptor blockers (137). The discrepancy between these two results may be a species difference (rat vs. mouse) or the choice of NMDA blockers (Mienville and Pesold used a noncompetitive antagonist and Del Rio et al. a competitive blocker). In zebrafish Rohon-Beard neurons, blocking activity by eliminating Na\(^+\) currents either pharmacologically or genetically reduces cell death (573).

In both types of neurons, early activity-dependent apoptosis may be related to their unique configuration of ion channels expressed at early stages compared with neighboring neurons. Cajal-Retzius neurons upregulate NMDA receptors markedly between E18 and P11, and during these stages they have significantly more positive resting potentials than non-Cajal-Retzius neurons (405). It is not clear what channels mediate the more positive resting potential. Their resting potentials are in the range that can remove the voltage-dependent Mg\(^{2+}\) block of the...
NM1 receptor, thus promoting tonic Ca\textsuperscript{2+} influx. Ca\textsuperscript{2+} influx may be further promoted by the early expression of repetitive firing ability in these cells. Voltage-clamp studies combined with single-cell RT-PCR show that Cajal- Retzius neurons express Na\textsuperscript{+} currents of the Nav1.3 isoform very early in development, by E12 (5). Voltage-clamp measurements on neonatal Cajal-Retzius cells show that they express the pacemaker current $I_h$ and repetitive firing ability earlier than other cortical neurons (287, 360). Finally, excitatory GABA responses persist in Cajal-Retzius cells much later than in other cells, presumably because they show delayed expression of the KCC2 chloride pump (400).

Rohon-Beard neurons show the same delayed switch-over to inhibitory GABA action (505), and additionally have shown to have a different incidence and pattern of spontaneous [Ca\textsuperscript{2+}], transients at early stages (65).

Q. Summary

Several common themes emerge from the above studies of spontaneous activity.

Activity tends to occur as bursts of action potentials with long interburst intervals. For most cells studied, interburst intervals are between ~1 and 20 min. The exceptions appear to be in two sensory systems, DRG cells and cochlear hair cells, where action potentials occur in steady patterns with frequencies of 0.5–10 Hz. There may, however, be longer-interval patterns to these high frequencies, which approximate the interburst intervals in other cell types. It is very interesting that imposed stimulus intervals for either [Ca\textsuperscript{2+}], transients or action potential bursts of ~1/min are particularly effective at triggering various downstream events such as specific gene expression (see sect. IIIA2).

Establishing such long intervals is likely to involve some kind of long-term depression or inactivation that affects synaptic transmission, voltage-gated ion channels, ion concentration gradients, or all three. Studies in spinal cord neurons, retinal ganglion cells, and ascidian muscle support this idea.

Spontaneous activity is often synchronous among populations of cells. Synchronicity among small clusters of cells, sometimes created by spread of waves of activity across a tissue, is likely to encode spatial information to downstream targets or to coordinate other developmental events among contiguous cells.

Spontaneous activity is quite robust under conditions in which circuitry is changing. This is seen in many different ways. In ascidian muscle and Xenopus neurons, activity persists even in individual, isolated cells. In cortex and retina, activity can be reestablished in dissociated cultures (234, 467, 602), although it is possible that the activity may be different in some respects from that in situ. In retina and spinal cord, activity persists during periods of development when significant changes in the synaptic circuitry and transmitters occur. Finally, in spinal cord at least, activity is reestablished during chronic application of receptor blockers that initially suppress activity. [This latter property is reminiscent of mature motor pattern generators, such as the crustacean stomatogastric ganglion, in which activity reemerges after initially being suppressed by eliminating neuromodulatory input (202).]

Spontaneous activity regulates a wide variety of developmental processes at all stages. For example, it is now clear that as central axons grow, navigate, and prune, an intricate series of regulatory events occurs involving trophic factors, neuroattractant and repulsive molecules, electrical activity, and intracellular second messenger levels. Pruning of inappropriate connections and strengthening of appropriate synaptic connections is only one of the later activity-dependent events in this process.

As discussed in section III, many but not all of the effects of activity are mediated by Ca\textsuperscript{2+} entry through voltage- and ligand-gated channels. And many, but not all, involve specific gene expression triggered by Ca\textsuperscript{2+}-dependent second messenger systems.

Finally, as discussed at length in sections IV and V, spontaneous activity relies on combinations of voltage- and ligand-gated ion channels and electrical and chemical synaptic circuitry that are unique to those stages of development. These serve dual roles. The first is to generate the appropriate patterns of electrical activity needed to trigger activity-dependent developmental programs. The second is to ensure the correct magnitude, frequency, and spatial distribution of Ca\textsuperscript{2+} entry during that activity. Ca\textsuperscript{2+} entry is regulated both by the Ca\textsuperscript{2+}-permeable channels themselves and by their responses to the voltage profile of activity as shaped by other, non-Ca\textsuperscript{2+}-permeable channels.

III. HOW SPONTANEOUS ACTIVITY CARRIES OUT ITS DEVELOPMENTAL FUNCTIONS

The wide variety of developmental events that spontaneous activity initiates are nearly all secondary to the Ca\textsuperscript{2+} influx during the activity. In many cases the resulting transient increases in [Ca\textsuperscript{2+}] are linked to the expression of specific genes. In other cases, Ca\textsuperscript{2+} activates cytoskeletal elements or exocytosis to carry out its developmental roles. The variety of activity-dependent developmental events and the feedback loops that connect them are discussed in sections III, IV, and V and are summarized in Figure 1.
A. Role of \([\text{Ca}^{2+}]_i\) Transients

Nearly all activity-dependent developmental events that involve gene expression are triggered initially by \([\text{Ca}^{2+}]_i\) influx and the resulting transient increases in \([\text{Ca}^{2+}]_i\). The responses of cells to \([\text{Ca}^{2+}]_i\) transients are determined by their amplitude, frequency, pathway of entry, and spatial location.

1. Amplitude

Obviously, enough \(\text{Ca}^{2+}\) must enter the cell to trigger the appropriate downstream events. For example, many instances of activity-dependent development rely on \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release from internal stores (CICR) (249), which requires a threshold amount of \(\text{Ca}^{2+}\) entry to occur. Usachev and Thayer (590, 591) have clearly shown such a threshold behavior of CICR in DRG neurons, where a difference between seven and nine action potentials in a single burst crossed the threshold \(\text{Ca}^{2+}\) entry required for CICR and resulted in a fivefold increase in the amplitude of the \([\text{Ca}^{2+}]_i\) transient. Thus the structure of spontaneous bursts may be tightly controlled so that this threshold is reliably crossed. Triggering sufficient CICR may be important to initiate regenerative \([\text{Ca}^{2+}]_i\) waves (60), perinuclear \(\text{Ca}^{2+}\) “puffs” (340), or \([\text{Ca}^{2+}]_i\) waves that propagate over the cytoplasm to engulf the nucleus (588), to create nuclear \(\text{Ca}^{2+}\) transients that can activate specific transcriptional events (see Refs. 100, 232). The dual tasks of the CICR system in this context are to avoid spurious \(\text{Ca}^{2+}\) release, or “noise,” and to maintain a safety factor that ensures that \(\text{Ca}^{2+}\) entry, even during low-frequency spontaneous activity, is securely able to trigger CICR. The IP\(_3\)-releasable store uses cooperative IP\(_3\) plus \(\text{Ca}^{2+}\) binding and IP\(_3\) receptor inactivation to guard against inappropriate release of \(\text{Ca}^{2+}\) into the cytoplasm (366). The CICR system in developing Xenopus neurons has a very high safety factor, such that reductions of \(\text{Ca}^{2+}\) influx during activity of up to 100-fold only reduce the \([\text{Ca}^{2+}]_i\) transient in the nucleus by less than 2-fold and the \([\text{Ca}^{2+}]_i\) transient in the cytoplasm by less than 5-fold (see Fig. 4 in Ref. 249).
Other processes downstream of Ca\(^{2+}\) entry are graded with the amplitude of the [Ca\(^{2+}\)]\(_i\) transient. So, for example, the amount of calmodulin translocation to the nucleus, which is critical in activity-dependent gene expression (395), is controlled by [Ca\(^{2+}\)]\(_i\), in a graded manner in the physiological range of 0–600 nM [Ca\(^{2+}\)]\(_i\) (334). Amplitude of the initial [Ca\(^{2+}\)]\(_i\) transients may also determine the identity of the downstream second messenger pathways that are activated. Wu et al. (622) showed that large increases in [Ca\(^{2+}\)]\(_i\) in hippocampal neurons (triggered by a brief KCl depolarization) result in CREB phosphorylation lasting more than 1 h. This long-lasting response was triggered by sequential activation of a fast calmodulin kinase (CaMK) pathway followed by a slower, but longer lasting, mitogen-activated protein kinase (MAPK) activation. Smaller amplitude [Ca\(^{2+}\)]\(_i\) transients, however, triggered only the CaMK pathway and resulted in more transient CREB phosphorylation.

2. Frequency

Encoding the frequency of activity into the amplitude of a downstream response requires some sort of molecular integration mechanism, many of which exist in developing nerve and muscle. The simplest integrator is the membrane time constant, which is long in many immature cells due to their high input resistance (see sect. ivD3), combined with the slow kinetics of many immature ligand-gated channels (see sect. iv, A and B). The next level of integration comes in the form of summation of [Ca\(^{2+}\)]\(_i\) transients during repetitive action potentials, because the rise and fall of [Ca\(^{2+}\)]\(_i\) is slow compared with the voltage transient of the action potential. Longer time integration occurs in this system because the [Ca\(^{2+}\)]\(_i\) transient caused by CICR has a much longer duration than the transient caused by Ca\(^{2+}\) entry during the burst (see Fig. 2 in Ref. 591). Secondary to either type of [Ca\(^{2+}\)]\(_i\) transient are cascades of second messenger systems whose responses long outlast the transients themselves, and thus provide temporal amplification of the initial response. These can create a 10-fold or more amplification of the time course of the initial [Ca\(^{2+}\)]\(_i\) transient, even at the level of the first stage of protein kinase activity (407, 623).

In addition to this kind of simple integration based on the progressively slower kinetics of processes downstream of Ca\(^{2+}\) entry, other specific properties of various second messenger systems create even more long-lasting “memories” of activity in very few molecular steps. Three examples illustrate these kinds of processes. First, cooperative autophosphorylation of CaMK occurs at high levels of calmodulin (CaM) occupancy, rendering CaMK constitutively active even after CaM dissociation (160, 229). This can create long-lasting increases of CaMK activity during low-frequency [Ca\(^{2+}\)]\(_i\) transients. Second, pro tease, kinase, or phosphatase activity can similarly create constitutively active second messengers. This is seen, for example, in the Ca\(^{2+}\)-dependent cleavage by calpain of protein kinase C (PKC) (572). Third are physical translocation processes, such as the movement of CaM into the nucleus under the influence of [Ca\(^{2+}\)]\(_i\) (126, 334), which can have a “priming” effect so that normally slow responses to [Ca\(^{2+}\)]\(_i\) transients become more rapid when the [Ca\(^{2+}\)]\(_i\) transients are repeated (395). Another example of translocation is the NF-ATc transcription factor, which in hippocampal neurons moves to the nucleus on Ca\(^{2+}\) entry under the influence of the phosphatase calcineurin, which unmaskes nuclear localization sequences on the protein. This results in a residence of NF-ATc in the nucleus for more than 2 h after a 3-min depolarization of the cell (206).

By integrating and encoding even very low frequency spontaneous activity using these, and other as yet undiscovered mechanisms, developing neurons and muscle cells have established a set of developmental responses that are finely tuned to both the frequency and temporal patterns of spontaneous activity (see Ref. 82 for review). These responses often show not only the ability to create long-lasting changes in gene expression triggered when the frequency of activity rises above a certain value, but the further ability to frequency tune different responses to different frequencies, or different patterns, of activity. This kind of frequency tuning has been known for some time in the control of fast- and slow-twitch skeletal muscle, where cross-innervation can modify contractile properties to be appropriate for the identity of the innervating motor neuron. Stimuli delivered in patterns appropriate to the innervating motor neuron can mimic the effects of cross innervation on muscle gene expression (157). Expression of different cell adhesion molecules depends on the frequency of activity in DRG neurons, as discussed in section uJ. In T lymphocytes, activation of the transcription factors NFAT, Oct/OAP, and NF\(_\kappa\)B by [Ca\(^{2+}\)]\(_i\) transients is sharply tuned to transient frequencies near 1/min (149, 332). Optimal frequencies of near 1/min have been reported in several other systems. In DRG neurons, for example, MAPK activation, CREB phosphorylation, and c-fos expression are all triggered more efficiently by bursts of action potentials delivered at 1-min intervals than they are by the same number of action potentials at steady frequency (175, 537). BDNF release from hippocampal neurons (see below) is best triggered by brief high-frequency bursts at 20-s intervals (14). This optimization of responses at near 1/min burst frequencies is particularly interesting because spontaneous, synchronous bursts of action potentials and/or [Ca\(^{2+}\)]\(_i\) transients occur at those frequencies in developing retina, hindbrain, hippocampus, and cortex (see sect. ii).
3. Physical pathway of Ca\(^{2+}\) entry

Many experiments have shown that the route through which Ca\(^{2+}\) enter the cell determines the patterns of gene expression triggered by activity. The L-type Ca\(^{2+}\) channel and the NMDA receptor are particularly effective pathways for activity-mediated transcriptional events involving CREB phosphorylation (for reviews, see Refs. 136, 611). Thus synaptic stimuli are much more effective in hippocampal neurons in triggering nuclear CREB phosphorylation than action potentials evoked antidromically, because synaptic events preferentially activate both L-type Ca\(^{2+}\) channels and NMDA receptors (134, 394). L-type Ca\(^{2+}\) channel blockers preferentially block CaM translocation to the nucleus in hippocampal neurons following depolarization (135, 395) and the tonic phase of CREB phosphorylation following depolarization of cortical neurons (148). The preferential linkage of the L-type channel to CREB phosphorylation and CRE-dependent transcription occurs even when several other Ca\(^{2+}\) channel types contribute to the [Ca\(^{2+}\)]\(_i\) transient caused by activity (148). L-type Ca\(^{2+}\) channels are also linked to activation of the transcriptional domains of upstream regulatory factors that cooperate with CREB in activation of BDNF transcription (103) and to the translocation of NF-AT transcription factors to the nucleus (206). Similar preferential block of activity-mediated transcriptional events is seen for the NMDA receptor (135, 233). This specificity is not related to differences in the global cytoplasmic [Ca\(^{2+}\)]\(_i\) transients created by Ca\(^{2+}\) entry through different channels. In hippocampal neurons CREB phosphorylation is sustained following Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, but transient after influx through NMDA receptors, a difference which remains when the different stimuli are adjusted so that both events produce the same [Ca\(^{2+}\)]\(_i\) transient (231). Furthermore, blocking the global cytoplasmic [Ca\(^{2+}\)]\(_i\), transient with a slow Ca\(^{2+}\) buffer that leaves the submembrane microdomain [Ca\(^{2+}\)]\(_m\), transient in place does not prevent activity-dependent transcriptional events following stimuli that activate L-type Ca\(^{2+}\) channels and NMDA receptors (134, 230). The preferential linkage between L-type Ca\(^{2+}\) channels and CREB phosphorylation relies on a combination of the interaction of the “IQ motif” in the \(\alpha_{1C}\) subunit with calmodulin (148) and a PDZ interaction sequence at the carboxy terminus of the same subunit (608). A similar situation exists for the NR1 subunit of the NMDA receptor (see Ref. 116 for review).

The linkage between these specific channels and transcriptional events is by no means simple or absolute. Evidence in AtT20 cells and hippocampal neurons indicates that rapid nuclear [Ca\(^{2+}\)]\(_i\) transients not linked to Ca\(^{2+}\) entry through specific channel types may be required to activate CREB binding protein which participates along with CREB phosphorylation in activating gene expression (100, 231, 232). CREB phosphorylation itself may be mediated by both nuclear Ca\(^{2+}\) and by second messenger pathways activated by submembrane Ca\(^{2+}\) (230, 395). In some activity-dependent genes, such as BDNF, multiple upstream regulatory elements may respond to different aspects of activity (103, 104, 583). In addition, the specific linkage between L-type Ca\(^{2+}\) channels may be malleable with activity. Following initial CaM translocation to the nucleus under the influence of Ca\(^{2+}\) entry through L-type channels in hippocampal neurons, additional stimuli that closely follow the first one induce CREB phosphorylation that is quicker than the first and less dependent on L-type Ca\(^{2+}\) channels (395).

Complicating the issue of specific communication between channel types and intracellular messenger pathways leading to gene transcription are two additional factors: differences among cell types and different stimulus paradigms. For example, CaM translocation to the nucleus following L-type Ca\(^{2+}\) channel activation is more pronounced in hippocampal neurons than in dentate gyrus neurons, probably related to the tonic levels of CaM in the nucleus in the absence of stimulation (395). The stimulus used to depolarize the cell is also very critical in judging the conclusions drawn about channel specificity. In petrosal ganglion neurons, patterned electrical stimuli and tonic depolarization by KCl both induced activity-dependent gene expression similarly, and as expected the effect of patterned electrical stimuli was blocked by TTX, whereas that of KCl depolarization was not. Less expected, however, was the finding that the effects of KCl depolarization required L-type Ca\(^{2+}\) channels but those of patterned electrical stimuli required N-type Ca\(^{2+}\) channels. This emphasizes that KCl depolarization is not a physiological stimulus and its tonic nature may inactivate channels such as the N-type Ca\(^{2+}\) channel and thus prevent assessment of its participation in activity-dependent events (78). Further studies showed that the two types of stimuli activated gene expression using different patterns of second messenger pathways (78, 79). Although this kind of problem clearly does not explain all results indicating channel specificity (see, e.g., Refs. 134, 394), it does emphasize that an unphysiological stimulus paradigm may preferentially activate channel types that are not the ones used under physiological conditions. In other cell types, P/Q-type Ca\(^{2+}\) channels preferentially activate gene expression over N-type channels, relying on carboxy-terminal sequences (569), and possibly on their selective coupling to different intracellular Ca\(^{2+}\) stores (521). The confounding effects of stimulus specificity has also been raised in hippocampal neurons by experiments of Hardingham et al. (233). These experiments showed that NMDA channel activation was equally effective as L-type Ca\(^{2+}\) channel activation in stimulating CREB activity and BDNF expression, contrary to some previous results (e.g., Refs. 196, 231). This discrepancy is due to the use of
bath-applied glutamate, as opposed to synaptic stimulation, to activate NMDA receptors. Bath glutamate also activates nonsynaptic NMDA receptors, which trigger a CREB shutoff pathway. Activation of NMDA receptors can also serve to refill intracellular Ca\(^{2+}\) stores in preparation for subsequent release by other triggers (588).

4. Spatial distribution of Ca\(^{2+}\) entry

Activity-dependent developmental events not only depend on how much, how often, and how Ca\(^{2+}\) enters the cell, but on where it enters as well. In addition to the “microdomains” of Ca\(^{2+}\) entry, where complexes of channel proteins and intracellular signaling molecules exist (see above; Ref. 46), the gross spatial distribution of Ca\(^{2+}\) entry is tightly regulated during development and allows distinct developmental functions to be carried out by different types of activity simultaneously within the same cell. Good examples of spatially localized [Ca\(^{2+}\)]\(_i\) transients are seen in Xenopus spinal neurons and insect motor neurons, as discussed in section II, K and M.

Although these local [Ca\(^{2+}\)]\(_i\) transients often act via local signal transduction pathways to affect processes such as growth cone motility, local action does not necessarily preclude the involvement of new gene expression. Local [Ca\(^{2+}\)]\(_i\) transients can affect nuclear events at a distance (see above). In long-term synaptic plasticity, local transmitter action even at distant synaptic terminals can trigger gene expression, and the products of that expression can act specifically at the terminals that initiated the events because of a process of “tagging” that occurs when the terminals are initially activated (377). If homologous processes exist in developing neurons, then local [Ca\(^{2+}\)]\(_i\) transients could trigger local developmental events that depend on global gene expression.

B. Developmental Regulation of Intracellular Ca\(^{2+}\) Stores and Buffering

Superimposed on all of the above patterns of [Ca\(^{2+}\)]\(_i\) transients are developmental changes in intracellular Ca\(^{2+}\) release mechanisms. Ca\(^{2+}\) release mechanisms show rapid and regionalized developmental changes (166), and developmental changes in both Ca\(^{2+}\) release mechanisms and Ca\(^{2+}\) buffering molecules are influenced by activity (69, 398).

C. Release of Developmentally Active Neurotransmitters

Not all effects of activity are mediated by the actions of [Ca\(^{2+}\)]\(_i\) transients on gene expression. In addition to rapid effects of [Ca\(^{2+}\)]\(_i\) on cell motility (see, e.g., Ref. 319), increases in [Ca\(^{2+}\)]\(_i\) can trigger secretion of developmentally active molecules. In many cases these molecules are transmitters, which in early development have profound neurotrophic actions on cell proliferation, migration, and other processes (31, 32, 355; see Refs. 34, 469, 470 for reviews). In addition to demonstrating the developmental effects of exogenously applied neurotransmitters such as GABA and glutamate, it has been shown in many cases that receptors for those transmitters are tonically activated in developing neurons by endogenously released agonists (48, 49, 180, 181, 300, 354, 355, 592; see Refs. 469, 470 for brief reviews). Evidence for endogenous activation is seen, for example, in migrating granule neurons of the cerebellum, where the frequency of opening of NMDA channels increases as cells enter the migratory phase (507). In some cases, these transmitters may be released in a nonsynaptic, activity-independent manner (see, e.g., Ref. 138), but in others their presence may be regulated by vesicular release and possibly by electrical activity in growing axons (see, e.g., Ref. 396).

D. Neurotrophins as Major Activity-Dependent Pathway

Neurotrophins are one of the most important class of molecules whose secretion and action are controlled by spontaneous activity. They are involved in complex feedback loops in the developing nervous system and are major players in mediating activity-dependent developmental phenomena (see Ref. 585 for review). As discussed above, the transcription of BDNF is triggered by patterned electrical activity and [Ca\(^{2+}\)]\(_i\) transients, as is the transcription of other neurotrophins (356). In addition, BDNF secretion is stimulated by activity, and preferentially by certain patterns of activity (patterned bursts as opposed to tonic depolarization or steady firing) that mimic the patterns of spontaneous activity recorded in many areas of the CNS (50-Hz bursts lasting 2 s repeated every 20 s) (14, 15; see also Refs. 8, 50). Furthermore, electrical activity can induce expression of the trk neurotrophin receptors (8, 42), thus providing a mechanism by which neurotrophins selectively affect electrically active neurons (383).

The multiplicity of interactions between spontaneous activity and neurotrophins creates the possibility of complex autocrine and paracrine feedback loops in the developing nervous system, and indeed such feedback loops appear to be widespread. In hippocampal pyramidal neurons, activity-dependent Ca\(^{2+}\) entry through L- and Q-type Ca\(^{2+}\) channels increases the number of calbindin-positive neurons that develop, an effect that is mediated by secretion of NT-3 and its activation of trkC receptors (68). A similar autocrine feedback loop involving BDNF probably regulates neurite extension during development of embryonic cortical neurons (196) and DRG neurons (625).
One of the more intriguing feedback systems involves BDNF effects on the development of GABAergic transmission, which appears to involve both positive- and negative-feedback loops that change rapidly with development. During stages when GABA is excitatory, due to high intracellular chloride concentration (see sect. IVC), GABA can stimulate BDNF expression in a variety of neurons, an effect that disappears as GABA converts to an inhibitory transmitter (38, 447). During these stages, BDNF can acutely increase GABA miniature end plate current frequency, thus creating a positive-feedback loop (447). But BDNF also stimulates expression of the KCC2 Cl transporter, which exports chloride ions and converts GABA to an inhibitory transmitter, thus terminating its ability to stimulate BDNF expression (3).

Because ion channels and [Ca\(^{2+}\)]\(_i\) transients show complex spatial distribution within developing neurons, it is not surprising that interactions between electrical activity and neurotrophin effects can be heterogeneous within single cells. In cortical pyramidal neurons, for example, spontaneous action potentials support BDNF effects on apical, but not basal, dendritic growth (383).

Completing one of the many feedback loops between neurotrophins and electrical activity and its resultant Ca\(^{2+}\) entry are effects of neurotrophins on various ion channels. In cortical neurons, NT-3 application (but not other neurotrophins) causes a prolonged stimulation of the Ca\(^{2+}\)-activated K\(^+\) current (250). BDNF, on the other hand, rapidly excites cortical, hippocampal, and cerebellar neurons at very low concentrations (273), an effect mediated by the Nav1.9 sodium channel (53). In BDNF knockout mice, the development of repetitive firing ability in retinal ganglion neurons is substantially delayed due to late upregulation of the Na\(^+\) current (510), which would likely have a profound impact on activity-dependent wiring in the retina-LGN circuit. An intriguing compensatory action of neurotrophins acting in a paracrine manner is seen in Xenopus neuromuscular synapses (441). In this system, blockade of neuromuscular transmission results in spike broadening in the presynaptic neurons, an effect mediated by a decrease in the delayed K\(^+\) current secondary to a loss of a trophic action of NT-3.

E. Relationship Between Synaptic Plasticity in the Adult and Developing Nervous Systems

The close relationship between developmental and adult neuronal plasticity has been appreciated for some time (276), but recent studies have brought this relationship into clearer focus (see Refs. 435, 555). Much of this work focuses on the apparent paradox of “silent synapses” early in development, as discussed above in section II, D and E. This work raises the possibility that one role of widespread synchronous activity in the CNS is to provide pairing of pre- and postsynaptic activity to strengthen appropriate synaptic connections during development. As discussed in section IV, evidence exists in the retinotectal system of amphibians that pairing of inputs to tectal neurons, possibly as a result of spontaneous activity, can trigger long-term increases in synaptic efficacy. Constantine-Paton and Cline (117) provide a critical review of the differences between adult and developmental forms of plasticity.

IV. SOME PRINCIPLES OF HOW ION CHANNELS DEVELOP TO REGULATE SPONTANEOUS ACTIVITY

The studies of ion channel development in the cells discussed above suggest some important general principles that govern how ion channel development is related to spontaneous activity. We summarize these principles in general form here, and then in the rest of this section review examples of how they are applied in a variety of cells.

Developing neurons (and muscle cells, and probably other cell types as well) pass through at least one transient state during which their firing properties are different from the mature state. During this time, spontaneous activity is favored. This temporary state is created by the transient occurrence of a different configuration of ion channel expression than that found at maturity, and in some cells by a transient pattern of synaptic interactions that are different from those found at maturity.

Differences in the function of immature ion channels may be created by differences in the intrinsic properties of channels, in the concentration gradients of permeant ions, in the timing of expression of some channel types relative to others, in the spatial distribution of channels, or in the coupling of channels to intracellular events.

The pattern of channel expression at stages during which spontaneous activity occurs is optimized to produce the correct patterns of activity in the context of the synaptic circuitry that exists at those stages (if any does exist), and to ensure the appropriate magnitude, kinetics, physical pathway, and spatial distribution of Ca\(^{2+}\) entry during that activity to trigger downstream developmental programs.

The properties created at early stages to carry out these functions are unlikely to be compatible with the mature functions of the cell, so the transition between the immature and mature patterns of ion channel expression is critical.

This transition is mediated in part by making the expression of certain mature ion channels dependent on the spontaneous activity created by the immature ion channels.
A. Immature Voltage-Gated Channels With Properties Different From Their Mature Counterparts

The kinetics of ion channels expressed in many immature cells are slower than in mature cells. This was illustrated above in the ascidian and Xenopus examples. In both cases, outward K⁺ currents are small and slowly activating early in development and speed later. Speeding of outward currents during development is very common (19, 208, 368, 451). Small, slowly activating outward currents have several effects in immature cells, not all of them obvious. First, they may allow more Ca²⁺ to be admitted during activity compared with a short-duration spike. For example, if the long-duration spike in developing Xenopus neurons is truncated by experimentally expressing a rapidly activating K⁺ current, activity-dependent developmental events are disrupted (271). It is not always true, however, that long-duration immature action potentials admit more Ca²⁺ than their brief counterparts, because increases in inward Ca²⁺ currents during development may compensate for spike shortening (130). In these cases, it may be the slower kinetics, rather than the magnitude, of Ca²⁺ entry that is critical (130). Second, slow outward currents increase the time during which net inward current flows during a depolarizing stimulus (see Fig. 7 in Ref. 208). This makes the cell more responsive to inward current flows during a depolarizing stimulus (see Fig. 7 in Ref. 208). This makes the cell more responsive to slow depolarizations, which may be common as pacemakers for spontaneous activity early in development. Third, inactivation of Ca²⁺ currents during longer spikes may allow Ca²⁺ channel reopening during the falling phase of the action potential (547), admitting disproportionately large amounts of Ca²⁺ because of the large driving force. Finally, by broadening the action potential, slow outward currents can cause cumulative inactivation of inward currents during bursting, which may be an important mechanism controlling the patterns of spontaneous activity in developing cells. This is seen in ascidian muscle, where the slow outward current is expressed at the same time as an inactivating Ca²⁺ current, and Ca²⁺ current inactivation appears to set burst duration during spontaneous activity (130). Kinetic changes in other currents can have profound effects on the development of firing patterns as well. In rat retinal ganglion neurons, speeding of recovery from inactivation of I₅Na appears to play a major role in the postnatal appearance of sustained, repetitive firing ability (605).

In other cases, the voltage dependence of channels rather than their kinetics is different in the immature cell. Shifts in voltage dependence of channels are very common during development, including differential shifts in inactivation and activation curves. Again, one example is seen in ascidian muscle, where the immature Ca²⁺ current activates at more negative potentials than the mature Ca²⁺ current, allowing it to admit Ca²⁺ between spikes in a burst (130). A high percentage of neonatal cerebellar Purkinje neurons are spontaneously active, even when completely isolated from synaptic inputs. The Na⁺ current in these cells has a more negative voltage dependence of activation, but the same inactivation versus voltage (h_inf) relation, as that in the inactive cells (438). This results in a “window current” where the two relations overlap in immature cells. The window current, which drives spontaneous activity, is absent in inactive cells. Hippocampal astrocytes show a pronounced negative shift in the h_inf curve for the Na⁺ current during development, probably due to a developmental switch in Na⁺ channel types (551). Chick cardiac ventricular muscle cells also show a negative shift in the h_inf curve of the Na⁺ current with development, which reduces the overlap, or window current, where the activation and inactivation curves overlap (514). The large window current early in development triggers spontaneous activity, which is absent in the mature cells. A similar shift occurs during development of hair cells. In addition to the outward K⁺ current speeding during development, part of the program that eliminates excitability in these cells after the period of spontaneous activity is over is a positive shift in the h_inf curve for the outward current coordinated with a negative shift in the resting potential (369). These two changes make more outward current available during depolarization, thus helping to eliminate spiking. In cat retinal ganglion cells, a negative shift in the voltage dependence of activation and a positive shift in the inactivation curve of I₅Na combine with increased I₅Na density to help bring about the early appearance of repetitive firing ability (546). A similar negative shift in the I₅Na activation curve combined with increased I₅Na density is seen in mouse retinal ganglion cells (510). Furthermore, developmental changes in the intrinsic properties of ON and OFF RGCs allow them to participate differentially in spontaneous retinal waves of activity, a difference which probably instructs their differential projections in the LGN (436). It is likely that many cases of variations in properties of immature voltage-gated channels from their mature counterparts are caused by the differential expression of accessory, or beta subunits (225, 489–491, 534).

B. Immature Ligand-Gated Channels With Properties Different From Their Mature Counterparts

There are also substantial differences in the kinetics of ligand-gated channels between immature and mature cells. Fetal muscle expresses ACh receptors with a subunit composition α₂βγδ, with the γ-subunit giving them a longer open time (177, 410). Later the receptors switch to the adult α₂βδε form, which has a shorter open time. This appears to be an example of impedance matching to the
low conductance of the immature muscle resting membrane so that synaptic currents are long enough in duration to allow ACh-triggered activity necessary for muscle maturation (268). Interestingly, in slow-twitch muscle fibers, this switch is delayed, and in true slow extraocular muscles, the γ-form persists into adulthood (412). This may reflect differences in activity-dependent suppression of γ-subunit synthesis due to differences in muscle activity among these types (412). It also may have a functional role in matching ACh currents to the higher resting resistance of the slow muscle membrane. In fast-twitch muscle, nerve-induced activity suppresses the γ-subunit synthesis and increases resting Cl current (236, 581). This coordinates the development of the high mature resting conductance (see sect. nD3) with the suppression of the long-open time embryonic ACh channel, which is no longer required to compensate for the long immature membrane time constant.

Similar developmental changes occur in the NMDA receptor. During the development of many neuronal types, the deactivation kinetics of NMDA currents become much faster (e.g., Ref. 239). [Deactivation kinetics are a property of the receptor, not of the time course of glutamate or NMDA persistence (see Ref. 127).] NMDA receptors are heteromeric ion channels, consisting of an essential NR1 subunit and one or more NR2 subunits. NR1 subunits are expressed in multiple splice variants, while NR2 subunits form a multigene family consisting of at least four members (NR2A, B, C, D). The subunit composition of the receptor has large effects on channel kinetics, especially deactivation times, with NR2B- and NR2D-containing receptors showing much slower deactivation kinetics than NR2A-containing receptors (626; see Ref. 127 for review). The speeding of deactivation kinetics during early development in most cells results from a subunit swap, with the NR2B subunit that is present in immature cells being replaced by the NR2A subunit (182, 246, 593). The exact developmental function of NMDA receptors containing the NR2B subunit and the long time course of the currents they mediate is not entirely clear. The immature subunit composition would clearly favor temporal summation, and in fact, such summation is observed in neonatal rat LGN in response to spontaneous activity from retinal ganglion cells (349). It would also, as in the case of the ACh receptor, be better matched to the high input resistance and long time constant of immature cells. In addition, the longer duration excitatory postsynaptic currents in immature cells would be expected to admit more Ca²⁺ to cells during activity, possibly allowing more effective triggering of gene expression and synapse stabilization (122, 289, 487). But it is not entirely clear that developmental plasticity in synapse function is tightly related temporally to the period of immature NMDA receptor expression (see Ref. 503), or that in cases where a good temporal relation exists under normal conditions, that the long-duration immature NMDA responses are required for the plasticity (347).

AMPA receptors also show pronounced developmental changes in function. AMPA receptors lacking the GluR2 subunit are permeable to Ca²⁺, unlike GluR2-containing AMPA receptors. Because AMPA receptors do not show a voltage-dependent Mg²⁺ block, this form can admit Ca²⁺ to cells even at negative potentials (see Ref. 146 for review of AMPA receptors). A variety of neurons, including those of brain stem, cerebellum, hippocampus, retina, and cortex, express AMPA receptors lacking the GluR2 subunit early in development (91, 161, 312, 318, 343, 346) and are thus permeable to Ca²⁺. In the migratory IZ of the developing cortex, tangentially migrating neurons originating in the basal telencephalon express Ca²⁺-permeable AMPA receptors and are in close proximity to axons originating in the cortical plate, which could release glutamate to activate these receptors and possibly influence migration (396; see also Ref. 552).

C. Different Immature Channel Function Due to Different Ion Gradients Early in Development

Profound changes in the function of ligand-gated channels need not rely on differences in channel structure. They can be created by the timing of expression of pumps that create the ionic gradients that determine current flow through the channels. The best known and perhaps most important example of this is the excitatory action of the transmitters GABA and glycine early in development (reviewed in Refs. 34, 469, 470). It has been known for some time that GABA action was depolarizing and functionally excitatory in many developing neurons (35, 44, 307, 353, 355, 359, 430, 431, 446). Experiments using the gramicidin perforated patch method of whole cell recording, which does not disrupt intracellular chloride levels, subsequently showed that the excitatory action of GABA was not caused by a unique GABA receptor, but by elevated intracellular Cl⁻ concentrations early in development (468, 505). The high [Cl⁻]i in immature neurons is maintained by a Na⁺-dependent Cl⁻ uptake pump (NKCC1 in rat) (505, 539). The switchover to inhibitory GABA action takes place in rodent cortex and hippocampus during the first two postnatal weeks and results from expression of the K⁺-dependent Cl⁻ extrusion pump KCC2 (254, 274, 502, 621). In cortex, the timing of this switch is controlled by the state of differentiation of the neurons, not by chronological age of the animal (539). In spinal cord neurons, the timing of the switch is influenced by astrocytes (332).

Interestingly, the switch from excitatory to inhibitory GABA action occurs at different times in different regions of the brain, even when strong synaptic pathways connect the two. So, for example, neurons of the rat visual cortex
show strong excitation by GABA at P0, whereas lateral geniculate neurons are inhibited by GABA at the same stage (256). Other neurons show long delays in the switch-over correlated with substantial late developmental changes in their function, such as hypothalamic gonadotropin-releasing hormone (GnRH) neurons, which switch at puberty (224). Of particular note is the late persistence of excitatory GABA action in temporary neuronal populations, such as the Rohon-Beard neurons of the amphibian spinal cord (505) and Cajal-Retzius cells of the cortex (400)(see sect. 111). The switch-over in GABA action is reversible under some circumstances, as indicated by the reappearance of excitatory GABA responses in neurons after various forms of traumatic injury (594). Other examples of this “dedifferentiation of channel function” are seen in certain experimental models of epilepsy (see sect. vi).

GABA itself seems to be involved in the switch-over of the Cl decay gradient. In hippocampal neurons, application of GABA_A blockers delays the change and delays the appearance of KCC2 mRNA, whereas depolarizing the cells with KCl accelerates the switch-over and the KCC2 mRNA expression (193).

During this period when GABA exerts a depolarizing action, it has many trophic effects on neurons and neuronal precursors (see Refs. 34, 469, 470 for reviews). These include inhibition of DNA synthesis and stimulation of migration in cortical ventricular zone precursor cells (29, 30, 235, 355). The fact that DNA synthesis in cortical precursor cells in cortical explants can be stimulated by bicuculline (355) indicates endogenous activation of GABA receptors, possibly by GABA released from axons of GABAergic cells in the early cortex or subcortical structures (114, 131, 595). These effects rely on the depolarizing action of GABA and its ability to increase [Ca^{2+}]_i via voltage-gated Ca^{2+} channels (see, e.g., Refs. 355, 468), and hence are restricted to the period when the intracellular Cl^- concentration is sufficiently high (see Refs. 34, 469, 470 for reviews). The high affinity and lower levels of desensitization of the immature GABA_A receptor (471) are likely to contribute to the ability of immature neurons and precursor cells to respond to low, nonsynaptically released levels of GABA. Some trophic effects of GABA may be indirect, given that GABA can stimulate BDNF expression specifically in immature cells via depolarization and activation of L-type Ca^{2+} channels and MAPK-dependent CREB phosphorylation (38, 447). Because BDNF can markedly stimulate GABA release, an interesting positive feedback exists in immature neurons relating GABA excitatory transmission and the trophic effects of BDNF (447).

As discussed in Ben-Ari (34), Owens and Kriegstein (469, 470), and elsewhere, the term excitatory is ambiguous when applied to the actions of GABA in immature cells. If the Cl^- potential is positive to threshold, GABA may depolarize a cell and directly excite it by, for example, moving the membrane potential into a range that opens voltage-gated Na^+ and Ca^{2+} channels and causes a rise in [Ca^{2+}]_i. But at the same time, the GABA reversal potential may be negative to the glutamate reversal potential, and hence, GABA may reduce glutamatergic excitation. Even in this situation, if the depolarizing action of GABA is sufficient to remove the Mg^{2+} block of the NMDA channel, GABA may potentiate NMDA responses while shunting both NMDA and non-NMDA glutamate responses. As chloride is pumped out of immature cells and GABA makes the transition to an inhibitory transmitter, functional inhibition will develop before GABA becomes hyperpolarizing, when the GABA reversal potential becomes negative to threshold for a particular cell.

The excitatory action of GABA in immature cells encapsulates four important principles that we discussed in section i, and which have appeared in many contexts throughout this review.

1. Optimization

Depolarizing GABA action is necessary for its developmental effects.

2. Coordination

For GABA to act in this way, expression of GABA_A receptors must be coordinated with that of Cl^- pumps that create, and then reverse, the immature Cl^- gradient, and with voltage-gated Ca^{2+} channels that GABA excitation acts on to admit Ca^{2+} to cells.

3. Self-limiting nature of activity

GABA itself appears to participate in triggering the reversal of the Cl^- gradient and its conversion to an inhibitory transmitter (193).

4. Incompatibility of immature and mature properties

Clearly, excitatory GABA action would be incompatible with mature nervous system function, as evidenced by the fact that KCC2 knockout mice die at birth from respiratory failure (254).

D. Nonlinear Developmental Profiles of Channels That Create Early Periods With Unique Firing Properties

In addition to making use of channels that are structurally or functionally different from their mature counterparts, immature cells often express mature-type channels in such different patterns that their firing properties are unique to their stage of development. This is most often seen either as the transient expression of a partic-
ular channel type or as large temporal disparities in the times of functional expression of different channels.

1. Transient channel expression: disappearing channels

When a channel functionally disappears during development, the inference is strong that it serves a developmental function during the period when it is present. We discussed several examples of disappearing channels above: downregulation of A-currents during starfish oocyte maturation (sect. iiA), the disappearance of Na$^+$ and Ca$^{2+}$ currents during early postfertilization development of ascidian embryos (sect. iiB), the downregulation of Na$^+$ and Ca$^{2+}$ currents in cochlear hair cells (sect. iiI), and the transient disappearance of inwardly rectifying K$^+$ currents in immature ascidian muscle (sect. iiJ).

Disappearance of Na$^+$ currents and consequent loss of excitability occurs in many cells. Some glial cells, for example, express functional $I_{Na}$ at early stages, but not later (22, 306; but see Ref. 64). In hippocampal astrocytes, $I_{Na}$ shows a biphasic expression pattern: $I_{Na}$ density is high at P0 and P7, but absent at P4-P5, suggesting a switch in $I_{Na}$ subtype (551). This is supported by the finding that the inactivation versus voltage curve is 20 mV more negative at P7 than at P0. It is not known whether the gap in $I_{Na}$ expression has a functional significance, but it is reminiscent of the gap in inward rectifier expression in developing ascidian muscle, created by the time lag between disappearance of the maternally coded channel and expression of the zygotic form (208). Another example of loss of $I_{Na}$ and excitability is seen in the starburst amacrine cells of the retina, as discussed in section iiC. Hair cells express $I_{Na}$ at P0-P9, but lose it by P18. The absence of $I_{Na}$ at maturity in these cells is understandable, since full action potentials are not compatible with the ability of membrane potential oscillations to follow high-frequency sounds. The early function of the Na$^+$ current probably relates to activity-dependent cell survival in the auditory brain stem, among other things (e.g., Ref. 639). A similar pattern is seen in utricular hair cells, where it has been shown that action potential activity at early stages releases BDNF (92). In the pacemaker sinoatrial node cells of the heart, a sodium current is present at early developmental stages and contributes to pacemaking activity, but is absent at later stages (24). Rat pituitary melanotropes are spontaneously active early in postnatal development, before dopaminergic innervation arrives from the hypothalamus. Secreted dopamine from hypothalamic afferents downregulates the Na$^+$ current and shuts off spontaneous activity (351).

Transient expression of Na$^+$ currents during development can also be subtype specific. In DRG neurons, for example, Nav1.3 is expressed early, peaking at E17, and then has disappeared by about P15. The timing of Nav1.3 expression corresponds with a period of spontaneous activity in DRG neurons. Because Nav1.3 has particularly rapid recovery from inactivation, it may help to induce spontaneous, repetitive firing in developing DRG neurons (128, 171). Interestingly, Nav1.3 shows this pattern of transient expression in a variety of CNS structures (28). Nav1.3 is also expressed at very early stages in Cajal-Retzius neurons of the preplate (5), where it might contribute to postnatal activity-dependent cell death in this temporary neuronal population (405).

The inwardly rectifying cation current $I_{h}$ is downregulated during the development of cardiac ventricular myocytes (629) and hippocampal neurons (596). $I_{h}$ serves pacemaking functions in many cells (473, 504). As an inward (depolarizing) current activated by hyperpolarization, $I_{h}$ can create a situation where there is net inward current at all potentials near rest, thus ensuring spontaneous, repetitive firing. Thus a developmental downregulation suggests an early role for $I_{h}$ in pacemaking spontaneous activity. Ventricular myocytes do in fact show spontaneous pacemaking at early stages, when $I_{h}$ is present, and lose it as $I_{h}$ disappears (629). In the hippocampus, $I_{h}$ in hilar neurons has been implicated as a pacemaker for spontaneous, synchronous activity (566). The experiments of Vasilyev and Barish (596) also illustrate the importance of measuring cell surface area in developmental studies of ionic currents. In the hippocampus, $I_{h}$ density shows an early peak, but $I_{h}$ amplitude does not. The impact of a current on firing properties is more closely related to its density, and as cells grow during development, it is important to know whether changes in current amplitude keep pace with, fall short of, or exceed the amount of membrane added. A similar finding was made for the T-type Ca$^{2+}$ current in Muller glial cells: current amplitude held constant during early postnatal development, but because there was extensive cell growth, current density fell substantially (75). Although current amplitude and capacitance (a measure of surface area) are not always measured over the same compartments because they are measured at different frequencies, at least an approximate indication of true current density should be monitored along with current magnitude in developmental studies where cell size and/or shape are changing. This can sometimes give valuable clues as to the mechanism underlying changes in relative amplitudes of different current types, as discussed above for the case of starfish oocyte maturation (422).

The current that most often declines or disappears with development is the T-type, or low voltage activated, Ca$^{2+}$ current, implying that it plays important developmental roles. Reduction in T-type currents occurs during the early development of spinal neurons (213, 380, 387), embryonic skeletal muscle (26, 39, 204), cardiac myocytes (172, 217, 327), Muller glial cells (75), cortical and hippocampal neurons (94, 584), vestibular neurons (93), neu-
The inference that the T-type current is serving some developmental function has been confirmed in several cell types. In amphibian spinal neurons, the T-type current is expressed during the period of spontaneous activity, and blocking it blocks activity (213). This implies a role for the current in pacemaking activity, as might be expected from a low-threshold inward current. But it is also likely to serve a role in mediating Ca\(^{2+}\) entry during activity, because the channel opens when Ca\(^{2+}\) driving force is high and because the overlap of activation and inactivation curves creates a window current near the resting potential (20, 21, 39). The idea of Ca\(^{2+}\) entry through steady T-type currents at near-resting potential has also received strong support from studies showing that the resting potential of myoblasts is in the range of voltages where the T-type Ca\(^{2+}\) channel window current exists, and that Ca\(^{2+}\) influx by this pathway is essential for myoblast fusion (41; see sect. III). In developing cardiac myocytes, Ca\(^{2+}\) influx through the T-type channel mediates atrial natriuretic factor release (327).

In general, the developmental role of the T-type Ca\(^{2+}\) channel is likely to be a combination of three of its common functions: a low-threshold inward current that amplifies depolarizing inputs, increases excitability, and by virtue of its inactivation, creates bursting behavior out of tonic inputs; a Ca\(^{2+}\) entry pathway that operates at high driving force, and hence can mediate large amounts of Ca\(^{2+}\) influx during activity (386); and a channel whose inactivation and activation relations overlap near the resting potential, creating window currents and the potential for steady Ca\(^{2+}\) entry at near-resting potentials. These roles are not trivial to separate with blocker experiments, and probably use of methods such as action potential waveform voltage clamp combined with [Ca\(^{2+}\)]\(_i\) imaging will yield clearer results.

Ligand-gated channels are also often expressed transiently during development. This is seen in the auditory brain stem, where synapses between the MNTB and the lateral superior olive change from GABAergic to glycinergic during development (303). It presumably also occurs in other situations where the transmitter phenotype of a presynaptic input changes (see, e.g., Ref. 614; see also chick spinal cord discussion in sect. II). GnRH neurons migrating from the olfactory placode are spontaneously active and express functional GABA\(_A\) receptors only during the period of migration; this appears to be significant developmentally since GABA inhibits migration (191, 313).

2. Relative timing differences of the development of different channels

Transient periods of unique levels of excitability or firing patterns need not be created by transient channel expression. Another common method of creating early periods of heightened excitability is to delay major increases in outward currents relative to the expression of inward currents. Action potential shortening, as is seen in Xenopus spinal neurons and ascidian muscle, are examples (see sect. II, K and L). In ascidian muscle, the late expression of large and rapidly activating Ca\(^{2+}\)-activated K\(^+\) currents both shortens spike duration and decreases cell responsiveness to slowly depolarizing inputs (208). Late expression of Ca\(^{2+}\)-activated K\(^+\) currents may also contribute to changing the firing patterns of retinal ganglion cells from bursting during spontaneous retinal waves to more sustained firing needed for encoding visual information (510, 604). And in embryonic Xenopus muscle, time lags between two phases of delayed K\(^+\) current development relative to Na\(^+\) current development creates a window of spontaneous activity (see sect. II).

In more extreme cases, late expression of outward currents helps to eliminate the ability to generate action potentials entirely, as seen for example in cochlear hair cells and insect motor neuron somata (see sect. II, I and M).

There are other cell types in which excitability in the sense of the ability to generate full action potentials must be eliminated by increasing expression of outward currents without eliminating or reducing inward currents. In hair cells, some Ca\(^{2+}\) current is retained because the interaction between Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels and the subsequent activation of I\(_{K(Ca)}\) underlies the oscillatory behavior on depolarization (173). Crustacean slow muscle fibers are another example. These cells contract on depolarization due to Ca\(^{2+}\) entry through Ca\(^{2+}\) channels, but must do so in a graded fashion. They therefore must have Ca\(^{2+}\) entry graded with depolarization, which is difficult to achieve with action potentials. They also have very slow contractile apparatus, which brief action potentials cannot activate. They therefore eliminate the ability to generate action potentials by expressing large outward K\(^+\) currents. When these are blocked pharmacologically or by anoxia-induced intracellular acidification, the ability to generate action potentials appears (420). Indeed, in the immature state, the crayfish superficial flexor muscle does generate full action potentials (Moody, unpublished data), and presumably the late expression of the outward currents eliminates action potentials at later stages (see Ref. 12). Other cell types in which blockade of outward K\(^+\) currents reveals action potential generating ability may pass through similar immature stages in which action poten-
tials, either spontaneous or evoked, can be generated (see Ref. 163).

Although differential timing of functional ion channel development is likely often to be created by direct transcriptional control of channel α-subunits, other mechanisms also play important roles. Accessory, or β, subunits show complex patterns of developmental regulation and regulate the functional properties of channels, their insertion into the membrane, and their susceptibility to activity-dependent regulation (see, e.g., Refs. 6, 84, 489, 490, 491, 522, 534; see Refs. 225, 258, 586 for reviews). Other less common forms of regulation may exist. In ascidian embryos, a truncated form of L-type Ca$^{2+}$ channel mRNA is expressed early in development (458) and can suppress expression of the full-length form. The downregulation of this truncated form appears to function as a late, positive regulator of a rapid rise the developmental appearance of the fully functional L-type Ca$^{2+}$ channel.

3. Late appearance of mature, low resting resistance

The resting conductances of a cell have a profound influence on excitability because they affect the size and duration of synaptic inputs, the response of the cell to its own inward and outward voltage-gated currents, the passive spread of voltage within the cell, and the effective electrical coupling across gap junctions. There is a strong tendency toward high input resistance in immature neurons and muscle cells. This is seen in the development of mammalian central neurons (33, 379, 481, 589, 638), insect neuronal somata (155), and vertebrate skeletal muscle (236). Developmental decreases in resting resistance can be quite large, fivefold or more in some cases. The coincidence of high resting resistance and recently increased Na$^+$ currents may underlie widespread, spontaneous, synchronous activity in cortical neurons, and the decline in resistance may help terminate that activity (481; see sect. uE). In insect neuronal somata, a fivefold decrease in resting resistance is one factor that eliminates soma excitability and spontaneous activity during development (155). In skeletal muscle, the late development of large resting Cl$^-$ conductance in fast-twitch muscle fibers is essential for their ability to generate single twitches in response to single excitatory postsynaptic potentials (486).

The apparently simple phenomenon of a decrease in input resistance can exert complex effects on cellular properties. In cortical pyramidal cells, the 10-fold decrease in resistance shortens the length constant and electrically isolates the apical dendrites from the soma, changing the roles of regenerative responses in the dendrites in the processing of incoming synaptic events (638).

E. Changes in the Spatial Distribution of Channels During Development

By changing the spatial distribution of channels during development, cells may effectively eliminate ionic currents and truncate their functions in certain regions of the cell without globally up- or downregulating them. Although changes in physical location of channels during development are best known for ligand-gated channels in muscle, it also occurs for voltage-gated channels in neurons. For example, in hippocampal neurons, the N-type Ca$^{2+}$ channel is diffusely distributed throughout the cell early in development, but becomes punctate at presumed future regions of vesicle release later, upon neuron-neuron contact (13). During these same stages, L-type channels remain restricted to the soma (485).

F. Changes in the Coupling of Channels to Intracellular Events During Development

As the expression and spatial distribution of channels changes during development, so does the expression of other molecules involved in second messenger cascades and vesicle release. As a result, the functional coupling between ion channels that admit Ca$^{2+}$ to the cell and downstream cellular events may change over time. The result is a change in function of a given channel type even during periods of relatively constant expression. This is seen most commonly as changes in the type of Ca$^{2+}$ channel that mediates transmitter release (207, 261, 527, 545) or in the efficiency with which metabotropic transmitter receptors couple with activation of second messenger systems (442, 526). It is not just coupling of ion channels to subsequent events in the cell, but also coupling of cellular events to channel modulation that can change with development. For example, voltage-gated Na$^+$ channels in immature cortical neurons are subject to activity-dependent endocytosis at early stages, but are protected later when their β-subunit is expressed (6).

G. Differences in Intracellular Trafficking of Channel Subtypes

Although it is clear that the biophysical properties of channels expressed at early stages may be optimized to regulate spontaneous activity, it has more recently become appreciated that different channel types, even within the same family, may also differ substantially in intracellular trafficking. Thus a cell’s choice of channel types to express may govern how efficiently that channel is expressed at the level of the plasma membrane and how that expression is regulated by events such as glycosylation or the presence of β-subunits. Within the Kv1 family of delayed K$^+$ channels, Kv1.4 is much more efficiently
trafficked to the plasma membrane in a variety of heterologous systems than are Kv1.1 or Kv1.2, which tend to be retained more in the endoplasmic reticulum (363). Between Kv1.1 and Kv1.2, the expression of Kv1.2 at the plasma membrane is more subject to modulation by the formation of heterotetramers and by the presence of β-subunits than for Kv1.1 (363). Mutating a single amino acid (A352) within the pore region (S5-S6 loop) can greatly increase the trafficking of Kv1.1 to the plasma membrane (364). This traffic control region interacts with a glycosylation site in the S1–S2 extracellular linker of the membrane (364). This traffic control region interacts with the protein in controlling functional expression. It is intriguing that the same region of the protein that controls trafficking also determines dendrotoxin (DTX) sensitivity, suggesting when the mature channel density set point has been reached is to switch the subunits that are expressed, changing to a channel type for which translational or trafficking control operates around a different channel density.

A recent paper by Blaine et al. (47) implicates carboxy-tail sequences in the Kv2 family as regulators of channel density control during development of Xenopus spinal neurons. They found that excess transcripts of some Kv1 and Kv2 family members could increase functional channel density at any stage of development, but Kv2.2 specifically could only increase functional channel density in immature neurons. This suggests that one solution to the problem developing neurons have of determining when the mature channel density set point has been reached is to switch the subunits that are expressed, changing to a channel type for which translational or trafficking control operates around a different channel density.

V. ACTIVITY-DEPENDENT ION CHANNEL DEVELOPMENT AS PART OF THE ESSENTIAL TRANSITION BETWEEN IMMATURE AND MATURE PHYSIOLOGICAL PROPERTIES

Like their mature counterparts (370), developing neurons show both stability and plasticity in their firing patterns: stability in that they can maintain consistent patterns of spontaneous activity for many days during critical periods of development, and plasticity in that they successfully make the transition out of this early state of spontaneous activity into their mature firing patterns. Because early patterns of spontaneous activity are likely to be incompatible with the mature functions of the cell (see below), the transition between these two states of activity is critical. The complex sequence with which ion channels develop in a given neuron is best viewed in the context of early periods of spontaneous activity followed by the required transition to the mature physiological state. Because activity-dependent ion channel development is part of this transition process, it is also best viewed in the same context. In many cases, the activity-dependent channel expression is compensatory, the effect of the change being to reduce or eliminate the activity that triggered it.

A. Voltage- and Ca\(^{2+}\)-Gated Channels

The apparent compensatory nature of activity-dependent channel development is readily seen in the case of outward \(K^+\) currents and inward \(Na^+\) currents. The ascidian muscle and hair cell examples discussed in section II, I and L, illustrate this for the Ca\(^{2+}\)-activated \(K^+\) current \([I_{K(Ca)}]\). In each case spontaneous activity is eliminated by the activity-dependent expression of a rapidly activating \(I_{K(Ca)}\). In other cell types, spontaneous firing may change in pattern during development, rather than be eliminated entirely, but the same principles may hold. Cerebellar Purkinje neurons, for example, are spontaneously active in both the immature and mature states, but the pattern of activity changes from steady firing to more bursting behavior. It is likely that activity-dependent developmental expression of a Ca\(^{2+}\)-activated \(K^+\) channel participates in this transition as well (433). \(I_{K(Ca)}\) development also depends on early periods of spontaneous activity in mammalian spinal motor neurons (378), although its role in terminating or modifying the activity is not yet understood.

Similar changes in firing properties during development can be created by activity-dependent expression of voltage-gated \(K^+\) currents. Inferior colliculus neurons express high levels of the voltage-gated \(K^+\) current Kv3.1, whose rapid kinetics contribute to high-frequency firing abilities of these cells during auditory processing. During development, midbrain auditory neurons fire spontaneously at low frequencies (304), and this activity may contribute to Kv3.1 maturation, given evidence that Kv3.1 expression is enhanced by depolarization and Ca\(^{2+}\) entry (344). Activity-dependent \(K^+\) current development occurs at other levels of the central auditory system as well, such as in the chick nucleus magnocellularis (357). Similarly, activity dependence of the second phase of expression of a delayed \(K^+\) current in embryonic Xenopus muscle appears to terminate a period of spontaneous activity (see sect. II).

Activity can be very specific in its effects on \(K^+\) channel development. In hippocampal neurons, six members of the Kv1 family all appear during postnatal development, in a specific pattern, and contribute to a seven- to eightfold increase in macroscopic outward current. But only Kv1.1, Kv1.2, and Kv1.4 show activity dependence, and for those, block of activity prevents their developmental appearance at early stages, but does not down-regulate them in more mature cells (211). Similarly, in

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cerebellar neurons, the Kv3.1b splice variant increases after P8, but not Kv3.1a. Although both are regulated by basic fibroblast growth factor (bFGF), depolarization prevents the FGF effect on Kv3.1a, but not on Kv3.1b (345), so it is possible that a suppressive effect of activity contributes to the specific developmental expression of splice variants of the same channel. Activity can also show opposite effects on K⁺ channel expression in different cell types. Depolarization, acting via cAMP, upregulates Kv1.5 transcription in cardiac muscle but downregulates it in GH3 cells (328, 427). [Interestingly, this effect in GH3 cells seems to be an unusual case in which the effects of activity are not secondary to Ca²⁺ entry (328).] The effects of activity on K⁺ channel expression are likely to be due to several mechanisms, including transcriptional (328, 427), mRNA stability (7), or with multiple posttranscriptional effects probably acting simultaneously in the same cell (66).

Voltage-gated Na⁺ channels also show activity-dependent expression, especially early in development. If the effects of activity are compensatory, as they appear to be by upregulating outward K⁺ channel expression, one would expect activity to downregulate Na⁺ channel expression. That is what is commonly seen. In developing central neurons (hippocampus and cortex), activity triggered by Na⁺ channel agonists causes a rapid internalization of Na⁺ channel proteins and a downregulation of α-subunit mRNAs (132, 133, 197, 317, 472). This phenomenon occurs only at early stages of development (132) and is specific for Na⁺ channels (472). The loss of Na⁺ channel sensitivity to activity-induced internalization with development parallels the appearance during development of the β₃-subunit, implying a protective or stabilizing role for the β₃-subunit (6). As is the case for Kv1.5 downregulation in GH3 cells (328), Na⁺ channel internalization triggered by activity does not seem to be secondary to Ca²⁺ influx (472). However, a similar endocytic internalization of Na⁺ channels in chromaffin cells is mediated by increased [Ca²⁺]ᵢ (294). Depolarization of neurons with KCl could mimic the effects of Na⁺ channel agonists on Na⁺ channel downregulation, but, oddly enough, KCl depolarization relies partly on cAMP for its effects, whereas Na⁺ channel agonists do not (197). Differences in transduction pathways triggered by different types of depolarizing stimuli are also seen in other cells (see Ref. 78, sect. VII.A.3). Na⁺ currents in developing cortical neurons also show a corresponding upregulation when electrical activity is blocked chronically with TTX (143). Similar activity-dependent downregulation of Na⁺ channels occurs in skeletal and cardiac muscle cells (77, 105, 452, 538), where there is direct evidence that the effects are secondary to Ca²⁺ entry. Activity has been reported to upregulate Na⁺ channel density in a Ca²⁺-dependent manner in GH3 cells (417). As with outward K⁺ currents, activity-dependent Na⁺ channel downregulation can be highly subtype specific, as seen for example in embryonic dorsal root ganglion neurons (see sect. VII).

Calcium currents seem to show more variable activity-dependent developmental effects than Na⁺ or K⁺ currents, with both up- and downregulation by activity evident, sometimes in the same cells (see, e.g., Ref. 164). This may reflect the somewhat more complex and varied roles of Ca²⁺ currents in neuronal function in both immature and mature states. In some cases, there is clear evidence that activity mediated by immature patterns of Ca²⁺ channel expression triggers later development of mature Ca²⁺ current expression. This occurs in the NG108–15 neuronal cell line, which expresses only functional T-type Ca²⁺ currents in the undifferentiated state, but during differentiation adds a variety of high-voltage-activated Ca²⁺ current types (101). Blocking the T-type Ca²⁺ current at early stages greatly suppresses the later appearance of functional HVA Ca²⁺ currents. As with other channels, the effects of activity on Ca²⁺ channel development may be subtype specific (144, 164, 187), even to the point of selectively stabilizing one form of T-type Ca²⁺ channel mRNA but not another (528). In DRG neurons, Ca²⁺ currents show similar dependence on the specific patterns of stimulation as do other activity-regulated molecules (331).

In many cells, inwardly rectifying K⁺ channels provide all or part of the resting conductance. These channels close with small depolarizations, allowing cells to maintain a high resting K⁺ conductance and yet still present a high input resistance to depolarizing stimuli. In embryonic amphibian skeletal muscle, Iₜhr development is suppressed by blocking spontaneous activity and accelerated by providing activity at abnormally early stages (338, 339). In mammalian skeletal muscle, innervation upregulates and denervation downregulates Iₜhr expression (205), effects that appear to be secondary to activity-induced increases in [Ca²⁺]ᵢ that stabilize IRK1 mRNA (540).

Chloride channels also contribute to resting conductance, particularly in skeletal muscle, and their development appears to be dependent on innervation-induced electrical activity (236, 293, 482).

There is some evidence that development of the hyperpolarization-activated cation channel, Iₜhr, which acts as a pacemaker current in cardiac and other cells (473, 504), can be influenced by activity (74, 102; see sect. VI).

Regulation of voltage-gated ion channels by electrical activity need not be limited to the cells that generate the activity. T-type Ca²⁺ currents and inwardly rectifying K⁺ currents in glial cells show long-term upregulation as a result of activity in associated neurons (27, 301, 302).

Although activity-dependent control of channel spatial distribution is most commonly associated with ligand-gated channels (see sect. VII.B), it also operates for voltage-gated channels. In hippocampal neurons, Kv2.1 channels are clustered at the soma and proximal dendrite, whereas
Kv2.2 channels are more uniformly distributed (336). 

\[
\text{Ca}^{2+} \text{ entry triggered by glutamate or by kainate-induced seizure activity declusters Kv2.1 (as well as inducing a large hyperpolarizing shift in its current-voltage relation), apparently by triggered calcineurin-based channel dephosphorylation of sequences in the carboxy tail region (411).}
\]

B. Ligand-Gated Channels

During development of the mammalian neuromuscular junction, activity-dependent regulation of ACh receptor expression plays a major role. Before innervation, receptors are of the fetal form, with a subunit composition \(\alpha_2\beta\gamma\delta\). Innervation initiates a complex set of changes resulting in the suppression of extrajunctional fetal receptors, stimulates synthesis of receptors in nuclei underlying the synapse, and then triggers a change in subunit composition to that junctional receptors change to the \(\alpha_2\beta\delta\varepsilon\) form, suppressing synthesis of the \(\gamma\)-subunit and stimulating synthesis of the \(\varepsilon\)-subunit (410). Suppression of synthesis of ACh receptors from extrajunctional nuclei involves multiple regulatory elements within the first 81 upstream base pairs, at least for the \(\delta\)-subunit (603). Suppression results from nerve-stimulated activity, acting via CaM kinase II, which phosphorylates myogenin and reduces its DNA binding ability (412, 581). Both \(\gamma\) and \(\varepsilon\)-subunits cluster at the end plate, but synthesis of the \(\gamma\) is suppressed in the perinatal period, whereas synthesis of the \(\varepsilon\)-subunit is stimulated. ARIA induces both genes at the end plate, but electrical activity specifically suppresses ARIA induction of the \(\gamma\), but not the \(\varepsilon\), subunit (412). The \(\delta\)-subunit is regulated oppositely in extrajunctional nuclei (suppression by activity) versus junctional nuclei (enhancement by nerve related factors), and these two aspects of regulation involve different upstream elements (582). Thus nerve-induced activity is involved in a very complex process of maturing the composition and spatial distribution of ACh receptors in skeletal muscle, and in coordinating the passive properties of the muscle membrane with the properties of ACh receptors. Activity stimulates development of the mature low resting resistance, in the form of Cl\(^-\) channels, and participates in the spatial restriction and subunit swapping of the ACh receptor so that its open time matches the impedance of the muscle membrane (268).

Similarly, the speeding of kinetics of the NMDA receptor discussed above in section IV/B depends on electrical activity (87, 487). The developmental upregulation of the NR2A subunit is prevented by blocking activity with either L-type Ca\(^{2+}\) channel or NMDA receptor blockers (246), and reexpression of NR2B-like properties can be induced in neurons after the swap to NR2A by blocking ongoing activity with TTX (290). More details of the mechanism of this change in subunit composition have been discovered using green fluorescent protein-tagged subunits in hippocampal slices (23). They found that the insertion of NR2B-containing receptors into the postsynaptic membrane does not require activity and that NR2B-containing receptors can only replace other NR2B-containing receptors, but not those containing NR2A. In contrast, NR2A-containing receptor insertion does require synaptic activity, and these receptors can replace NR2B-containing receptors.

In the AMPA-type glutamate receptor, the developmental switch to GluR2-containing AMPA receptors, which eliminates this Ca\(^{2+}\) permeability, depends on electrical activity (343). In addition to mediating the subunit swap, activity can also recruit AMPA receptors rapidly (within minutes) to the postsynaptic membrane of immature neurons (335).

C. Summary

Developmental changes in the expression of a wide variety of voltage-, Ca\(^{2+}\), and ligand-gated channels are dependent on electrical activity. These are summarized in Table 1. The pattern that emerges from studies of activity-dependent channel expression in developing nerve and muscle suggests two conclusions. First, activity-dependent channel development helps to mediate the essential transition between immature and mature patterns of channel expression. As a result, many of the changes are compensatory, serving to eliminate the very activity that triggered them. A second conclusion is more speculative. Activity-dependent expression of channels that eliminate spontaneous activity may be a monitoring system, by which the cell detects when spontaneous activity has successfully triggered the required developmental programs. By making expression of a channel that terminates activity one of these programs, the system can compensate for variations in the intensity and timing of activity by extending or truncating the period over which the activity occurs.

VI. CLINICAL IMPLICATIONS OF ACTIVITY-DEPENDENT NERVOUS SYSTEM DEVELOPMENT

Understanding how the time course and mechanisms by which the intrinsic properties of central neurons develop is essential to understanding both the etiology and sequelae of pediatric seizure disorders. Immature animals, including humans, are more susceptible to seizures than adults (251, 269, 416, 553). In humans, seizure susceptibility peaks in the first few months after birth, and then declines from 5 years through adolescence (553). Seizure incidence has been reported to be between ~2

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and 5 per 1,000 live births, and about one-third of neonates with seizures progress to status epilepticus (416).

The attribution of heightened early seizure sensitivity to particular immature neuronal properties has been problematic because of differences in methods used to trigger seizures, differences in the time course of development of neuronal properties in various regions of the brain, and difficulties in measuring certain neuronal properties accurately over time. A recent paper by Khazipov et al. (283) has attempted to resolve some of these issues by using relatively noninvasive extracellular recording methods in hippocampus to measure the developmental time courses of spontaneous activity (GDPs; see above), GABA inhibition, and seizure susceptibility. Using single- and multi-unit recording to estimate the functional sign of GABA action, they concluded that GABA was uniformly excitatory through P10 and underwent a gradual transition to inhibition by P15. This is a somewhat later transition than previous estimated. Comparing this time course to that of GDP occurrence and susceptibility to seizures induced by elevated KCl, they found a close correspondence. GDPs were present in all slices from P0 to P9 and

Data shown are as discussed in section V. See text for definitions.

TABLE 1. Activity-dependent development of voltage- and ligand-gated channels

<table>
<thead>
<tr>
<th>Effect of Activity</th>
<th>Cell</th>
<th>Effect on Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Ascidian muscle</td>
<td>Speeds overall K(^+) current activation. Helps terminated activity by reducing response to slow depolarization. Shortens action potential duration and reduces Ca(^{2+}) entry.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Cochlear hair cells</td>
<td>Helps eliminate both spontaneous activity and excitability.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Spinal motor neurons</td>
<td>Not yet clear.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Cerebellar Purkinje cells</td>
<td>May help change spontaneous activity to a bursting pattern.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Xenopus muscle</td>
<td>May help terminate spontaneous activity. TXT reduces and Na(^+) channel overexpression increases ( I_{\text{K(Ca)}} ) expression.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{K(Ca)}} )</td>
<td>Xenopus spinal neurons</td>
<td>Effect on spontaneous activity unclear. Subunit specific: activity affects only Kv1.1, 1.2, and 1.4.</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Na}} )</td>
<td>Cortex, hippocampal neurons</td>
<td>Effect on spontaneous activity unclear. Rapid endocytosis followed by slower downregulation of transcription, both caused by increased activity. Specific to early stages when ( \beta )-subunit is absent. Activity block causes ( \uparrow I_{\text{Na}} )</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Ka}} )</td>
<td>DRG neurons</td>
<td>May help terminate spontaneous activity due to negative voltage dependence of Nav1.8 and 1.9. Subtype specific for Nav1.8 and 1.9, but not for Nav1.3.</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Na}} )</td>
<td>Skeletal muscle</td>
<td>Unclear.</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Na}} )</td>
<td>Cardiac muscle</td>
<td>Unclear.</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Na}} )</td>
<td>Hypothalamic neurons</td>
<td>Activity increases development of LVA current.</td>
</tr>
<tr>
<td>( \downarrow I_{\text{Na}} )</td>
<td>Xenopus muscle</td>
<td>Probably helps reduce aberrant activity created by Na(^+) channel misexpression.</td>
</tr>
<tr>
<td>( \uparrow I_{\text{CI}} )</td>
<td>Skeletal muscle</td>
<td>Early lack of resting Cl conductance is impedance matched by long open-time ACh channels. ( \uparrow I_{\text{CI}} ) by innervation, via activity, limits muscle action potentials and contraction following stimulus.</td>
</tr>
<tr>
<td>( \downarrow \text{ACh channel open time} )</td>
<td>Skeletal muscle</td>
<td>Activity-dependent swap from ( \delta )- to ( \epsilon )-subunit. Long embryonic open time for impedance matching to high embryonic ( R_{\text{m}} ). Note activity also increases resting Cl conductance, so mature short open-time ACh receptor is impedance matched to low, mature ( R_{\text{m}} ).</td>
</tr>
<tr>
<td>( \uparrow \text{NMDA receptor deactivation rate} )</td>
<td>Cortical neurons</td>
<td>Activity dependent swap from NR2B to NR2A subunit. Slowly deactivating embryonic receptor may aid in summation of EPSPs and increase Ca(^{2+}) entry, but relation to developmental plasticity is unclear at present.</td>
</tr>
<tr>
<td>Eliminate of embryonic AMPA receptor</td>
<td>Cerebellar neurons</td>
<td>Activity-dependent upregulation of GluR2-containing receptors. Likely that Ca(^{2+}) entry through embryonic receptor occurs under physiological conditions in immature neurons.</td>
</tr>
<tr>
<td>Switch of GABA from excitatory to inhibitory action</td>
<td>Hippocampal neurons</td>
<td>Activity-dependent expression of KCC2 Cl pump, which lowers [Cl], and converts GABA action to hyperpolarizing.</td>
</tr>
</tbody>
</table>

The pattern of seizures in the immature brain is different than in the mature brain, with the majority of early-onset seizures being neocortical, rather than hippocampal, in origin (416). In addition, antiepileptic drugs that work in adults are not necessarily effective in early-onset seizures (269). The neonatal brain is less prone to certain types of seizure-induced damage, such as cell death (251), although more prone to other kinds of damage (210, 282, 524).

It is almost certain that the increased susceptibility of the immature brain to seizures is caused in part by the unique intrinsic properties of developing neurons, in particular their propensity to generate spontaneous synchronized activity. It is equally likely that the different long-term response of the immature brain to early seizure activity results at least in part from the fact that seizure-like activity disrupts normal activity-dependent developmental processes.
gradually disappeared from P10 to P16, closely following the GABA switchover. Seizure susceptibility was bell-shaped between P6 and P16, with a peak at P11. This implies that the switch of GABA to inhibition may govern the decline of seizure susceptibility, whereas the onset may rely on other factors, such as the emergence of synaptic circuitry or early developmental changes of intrinsic neuronal properties. They also found, as have others, a mixed functional excitatory and inhibitory action of GABA even at stages when it generates depolarizing responses (see sect. III). This work also emphasizes the point that early seizure activity occurs at the same stages as normal spontaneous activity and indeed has been shown to disrupt spontaneous GDPs in hippocampus (281).

Several experiments indicate the early seizure activity has long-term deleterious effects on processes that are known or likely to be dependent on spontaneous activity. Hippocampal slices cultured under conditions that trigger seizure-like activity (picrotoxin) show inhibited growth of mossy fibers to their normal targets, but increased targeting to other areas (257), reminiscent of improper targeting of thalamic axons to visual cortex in activity-blocked seizures, even though they do not block the seizure activity itself (406). Repetitive seizures in hippocampus also reduce neurogenesis (385). Other long-term effects are more subtle. Repetitive hippocampal seizure activity does not cause substantial acute cell loss, indicating the lower sensitivity to seizure-induced cell death in the immature brain, but it does increase in the sensitivity of the hippocampus to later seizure-induced cell death (524).

Some of the most interesting and relevant studies revolve around the question of how repeated seizures at early stages can induce independent seizure foci, and how that phenomenon might relate to activity-dependent development of ion channels. Reactive febrile seizures are the most common seizure type in children, and it is a matter of some controversy whether they increase the later incidence of limbic epilepsy. Two recent studies indicate that experimental hyperthermia-induced seizures in rats can lead to long-term effects on neuronal excitability. Tested 1 wk after the seizures, hippocampal neurons showed a small positive shift in the inactivation versus voltage curve for I h (102), resulting in increased postinhibitory rebound. This finding clarified how hyperthermia-induced seizures can increase circuit excitability while also increasing inhibition. It also emphasizes the fact that even subtle changes in the properties of currents measured under voltage clamp can have marked effects on the firing properties of neurons, especially in complex circuits. These effects are caused by a specific downregulation of HCN1 channel subtype and increased HCN2 subtype in both CA1 and CA3 neurons (74). Other studies have also implicated seizure-induced changes in intrinsic neuronal properties as possible mechanisms of seizure-induced hyperexcitability. Long-term postseizure increases in burst generation (147), decreases in I[K(Ca)] expression (262, 599), increased input resistance (262), upregulation of T-type Ca2+ currents, and downregulation of N-type currents (568) have all been reported. Many of these changes effectively reverse developmental events that have been shown to help terminate normal spontaneous activity, such as I[K(Ca)] upregulation, early developmental prominence of T-type Ca2+ currents, and high input resistance of immature neurons. They thus suggest a close relationship between the disruption of activity and activity-dependent processes and the aftereffects of seizure activity. Another excellent example of how seizure activity in one set of neurons can induce independent seizure foci in another by interfering with normal developmental processes is seen in a recent paper by Khalilov et al. (282). Using an intact two-hippocampi preparation in which the two structures can be independently exposed to drugs, they showed that repeated kainate-induced seizures in one hippocampus can induce independent seizures in the other. This effect was due purely to the propagation of TTX-sensitive action potentials to the “naive” hippocampus, but could be prevented by blocking NMDA receptors. The specific requirement for NMDA receptor activation was indicated by the fact that NMDA blockers did not prevent acute propagation of the seizures to the naive hippocampus, but did prevent the establishment of an independent seizure focus there. The establishment of the mirror focus was accompanied by a positive shift in Cl– potential, indicating the possibility that the incoming activity had delayed the normal developmental reduction in intracellular Cl– concentration that shifts GABA action from excitatory to inhibitory. This may be another example of the “dedifferentiation” of electrical properties created by injury. Seizure activity can also trigger changes in the spatial distribution of channels (411).

Other work indicates that this kind of activity-dependent effect of seizures on ion channels may underlie some instances of the development of resistance of seizures to antiepileptic drugs. In human hippocampi from patients that had developed carbamazepine-resistant seizures, the normal use-dependent block of Na+ channels by this drug had disappeared, suggesting that seizure activity had triggered the modification of Na+ channels (497).
VII. SUMMARY

The patterns of ion channel development in excitable cells appear quite complex when viewed from the perspective of a straightforward progression to the mature state. The many instances of transient up- and downregulation of channels and of channels in immature cells that have very different properties from their mature counterparts are hard to reconcile with such a straightforward progression. When one views cells as existing in multiple electrophysiological states during development, however, the complexity of ion channel development makes much more sense. Early in development, the functional channel populations present in a cell are optimized to create periods of spontaneous electrical activity and Ca\(^{2+}\) influx. This spontaneous activity is critical for many aspects of development beginning from the earliest stages and extending to late in terminal differentiation. The patterns of ion channel development are tightly coordinated with these periods of spontaneous activity and interact with emerging synaptic circuitry to create the appropriate patterns of electrical activity and calcium influx. The critical transition between immature and mature electrical states is managed in part by activity-dependent expression of mature ion channels. The fact that neurons and circuits may exist in distinct immature states during which activity occurs and plays important developmental roles is likely to be critical to the understanding of the physiology of infant and childhood neurological pathologies.

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