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Mechanisms underlying spontaneous patterned activity in developing neural circuits

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Abstract

Patterned, spontaneous activity occurs in many developing neural circuits, including the retina, the cochlea, the spinal cord, the cerebellum and the hippocampus, where it provides signals that are important for the development of neurons and their connections. Despite differences in adult architecture and output across these various circuits, the patterns of spontaneous network activity and the mechanisms that generate it are remarkably similar and can include a depolarizing action of GABA, transient synaptic connections, extrasynaptic transmission, gap junction coupling and the presence of pacemaker-like neurons. Interestingly, spontaneous activity is robust; if one element of a circuit is disrupted another will generate similar activity. This research suggests that developing neural circuits exhibit transient and tunable features that maintain a source of correlated activity during critical stages of development.

Introduction

One way to understand the complexity of neural circuits is to understand how their connectivity emerges during development. The traditional model of brain development includes two phases: an early phase during which a coarse wiring of the nervous system is laid out, followed by a later phase during which the coarse connections are refined. In this model, the developmental events that underlie the coarse wiring are the result of predetermined genetic programs and occur independent of neural activity, whereas the refinement is a result of interactions between the nervous system and the outside world. For example, the traditional view of visual system development is that a genetic program specifies the organization of projections from the retina to the brain and among visual areas within the brain, whereas once vision matures, neural activity driven by visual experience refines the coarse neuronal circuits into their adult pattern of connectivity.

This traditional model is slowly being modified to accommodate an overwhelming number of observations that neural activity and genetic programs interact to specify the composition and organization of neural circuits during all stages of development. Even at extremely early stages, well before synapses form, neurons and neuronal precursors exhibit spontaneous electrical and chemical activity. These early forms of activity, which often occur on a cell-by-cell basis and are not typically correlated across cells, influence developmental events such as neuronal differentiation, establishment of neurotransmitter phenotype, and neuronal migration (for reviews, see[1,2]).

As neurons start to form synaptic connections and functional circuits begin to emerge, spontaneous activity becomes correlated across large groups of neighboring cells. This spontaneous network activity has been observed in many parts of the developing nervous

system, and it serves a variety of purposes. In developing sensory epithelia, in particular the retina[3, 4] and cochlea[5], spontaneous network activity correlates action potential firing among projection neurons during a period of development when these projections are forming sensory maps[6–8]. Spontaneous activity is also observed in the developing spinal cord[9], where it contributes to motor neuron path-finding[10], maturation of synapses[11], and development of pattern-generating circuits within the cord[12, 13]. In forebrain structures such as the hippocampus[14, 15] and the neocortex[16, 17], as well as in the hindbrain[18], the midbrain[19], and the cerebellum[20], it has been postulated that spontaneous activity contributes to the development of local circuits[21, 22]. Each brain area comprises a unique circuit, but there are striking similarities in some aspects of the mechanisms used to generate spontaneous activity.

This Review describes the cellular mechanisms that underlie the generation of correlated firing patterns in immature neural circuits soon after the onset of synapse formation. We do not attempt to review all of the mechanisms that underlie spontaneous activity in multiple brain areas. Rather, our goal is to highlight the remarkable parallels found in the mechanisms used by different circuits. In general, in these developing circuits, transient excitatory networks correlate spontaneous activity in neurons. These networks are formed by different combinations of various mechanisms, such as a depolarizing action of GABA, transient synaptic connections, extrasynaptic transmission, and gap junction coupling. The recurrent excitatory connections in these networks amplify interactions between spontaneously active cells, initiating correlated network activity. In addition, these networks can be resistant to perturbation in the sense that pharmacological or genetic disruptions of critical network components lead to an expression of alternative circuit mechanisms that generate activity similar to the endogenous pattern, suggesting that redundancy is built into neural circuits to ensure that the spontaneous activity is maintained.

Features of spontaneous network activity

Spontaneous network activation has been observed in multiple developing circuits, but has been best characterized in the retina, the spinal cord and the hippocampus. (A wide range of spontaneous activity patterns analogous to those observed in the hippocampus has also been described in the neocortex; for a review, see[23].) The activity patterns in these diverse structures are grossly similar during development. In all three cases, spontaneous network events are comprised of large, slow depolarizations crested by bursts of action potentials (Table 1). Another common feature is that excitatory interneurons have a role in the generation of spontaneous activity. Recently, spontaneous network activity has also been described in developing cochlea[5] and cerebellum[20]. Although the details are not yet fully understood, the strategies used by the cochlea and cerebellum are comparable to those previously described in the retina, spinal cord, and hippocampus. Schematics of the functional circuits that mediate spontaneous network depolarizations in each of these brain structures are provided in Box 1.

Box 1

Circuits mediating spontaneous network activity during development

In the retina (see the figure, part a) three distinct circuits mediate retinal waves at different stages of development (for review, see[6]); the circuit that mediates waves perinatally is not shown. The cholinergic circuit that mediates waves during the first postnatal week consists of cholinergic interneurons (starburst amacrine cells, blue) forming excitatory synaptic connections with other starburst amacrine cells and projection neurons (retinal ganglion cells, grey) (for details, see Figure 2). It is postulated that wave propagation is mediated by excitatory connections among starburst amacrine cells, which in turn release acetylcholine that depolarizes the ganglion cells. Waves are initiated by spontaneous depolarization in starburst amacrine cells that are amplified by recurrent excitatory connections, and the

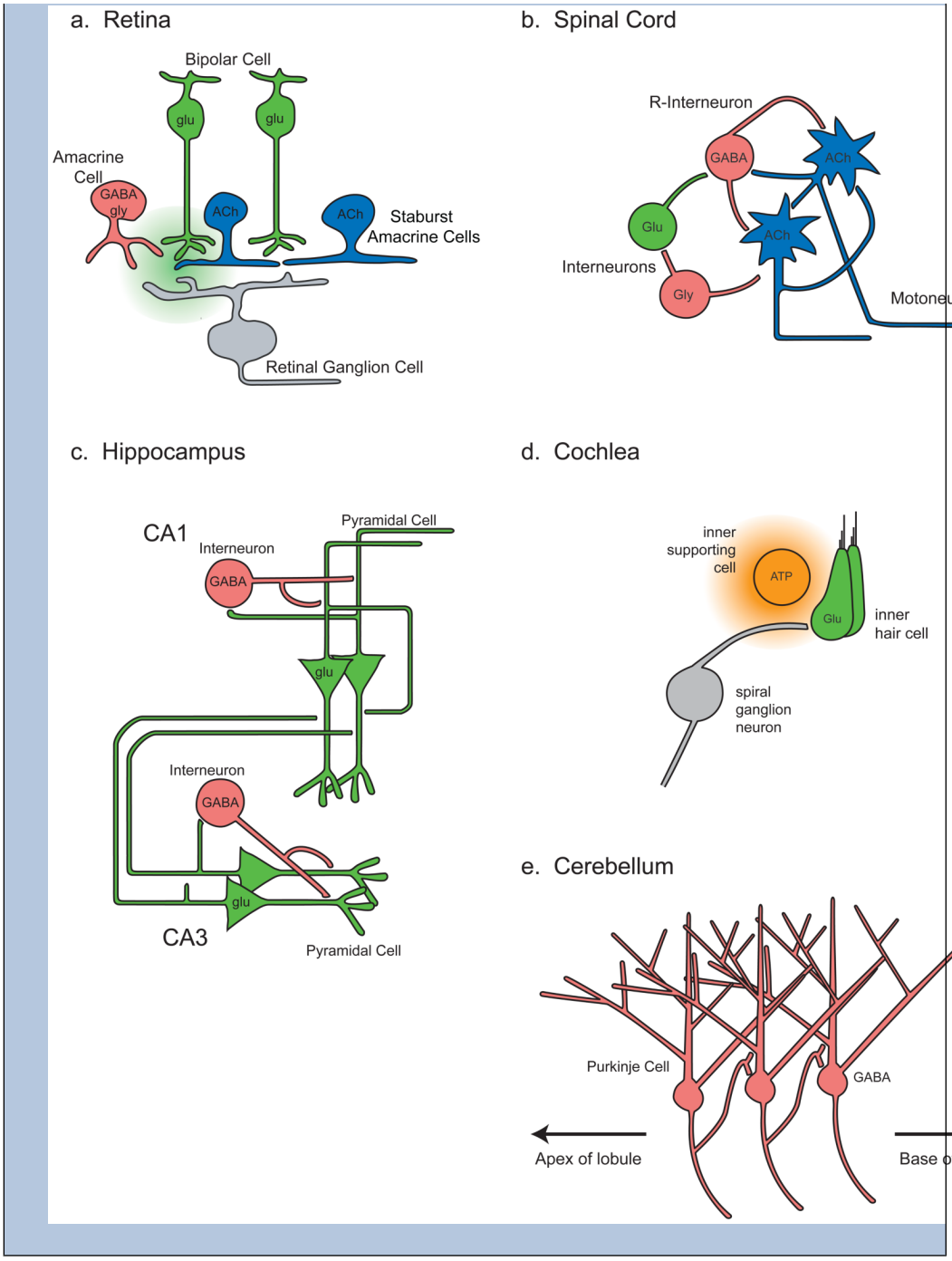
interval between waves is set by a slow after-hyperpolarization in these cells. The circuit that mediates glutamatergic waves, which occur between postnatal day 10 and 14, consists of glutamatergic interneurons (bipolar cells, green), inhibitory interneurons (amacrine cells, pink), and retinal ganglion cells. One hypothesis is that bipolar cells are coupled by high levels of extrasynaptic glutamate (green cloud), which spills out of the synaptic cleft.

In each segment of the developing spinal cord the same circuit mediates spontaneous network activity (see the figure, part b)[66, 80]. The circuit consists of glutamatergic (green) and GABAergic and glycinergic interneurons (represented here as a single cell in red), and cholinergic projection neurons (motor neurons, blue), which transiently make nAChR-mediated connections with local interneurons. It is postulated that spontaneous network events initiate in motor neurons, which depolarize a population of GABAergic interneurons, Renshaw cells (R-interneurons). Later in development the propagation of spontaneous network activity in the spinal cord becomes more dependent on GABAergic and glutamatergic transmission. Event initiation is due to a slow buildup of synaptic activity via recurrent excitatory connections until an event threshold is reached. During an event, strong activation of $GABA_A$ receptors lowers the intracellular chloride concentration, which diminishes the depolarizing force of GABA. The inter-event interval is set by the time it takes to restore chloride concentrations such that the depolarizing action of GABA is restored[71]. Schematic modified from[66]. The circuits that mediate event propagation along the length of the cord are not described here[66].

In the hippocampus, the circuit that mediates giant depolarizing potentials (GDPs) consists of pyramidal cells (green) and local GABAergic interneurons (pink) in both the CA3 region and CA1 regions of the hippocampus (see the figure, part c; for review, see[47, 48, 124]). GDPs are most likely to initiate in the CA3 region, where intrinsic bursting activity in CA3 pyramidal cells is coupled with network interactions mediated by depolarizing GABA and recurrent excitatory connections. The inter-event interval is set by an after-hyperpolarization in CA3 neurons[49, 50].

The cochlear circuit (see the figure, part d) consists of glutamatergic inner hair cells (green), a transient population of inner support cells (orange) located in a developmentally transient structure called Kölliker's organ, and the projection neurons[5] (spiral ganglion cells, gray). Spontaneous network activity in the cochlea is initiated by a diffuse release of ATP (orange cloud) from inner support cells, which drives depolarization in nearby inner hair cells by activating both metabotropic and ionotropic ATP receptors. Inner hair cells in turn release glutamate, which depolarizes spiral ganglion cells via activation of ionotropic glutamate receptors. The mechanisms determining the inter-event interval are not known.

The circuit mediating spontaneous network activity in the cerebellum (see the figure, part e) consists solely of projection neurons[20], which are GABAergic Purkinje cells. Purkinje cells are transiently connected via local axon collaterals, which entrain the spontaneous firing of nearby Purkinje cells via depolarizing GABA signaling. The direction of propagation is dictated by the asymmetric wiring of local collaterals, with Purkinje cells located toward the base of a lobule receiving more connections than those located toward the apex.



The projection neurons of the retina — retinal ganglion cells — exhibit spontaneous bursts of action potentials that are separated by extended periods of silence during development[3]. These bursts of action potentials spread as waves of depolarization across the retina[4, 24], which earned them the name retinal waves (see Supplemental Movie). Retinal waves propagate through the developing visual system, inducing similar burst patterns in the dorsal lateral geniculate nucleus of the thalamus[25, 26] and in visual cortex[27]. Such spontaneous network activation appears very early in development — after retinal ganglion cells have extended axons to their primary targets in the brain — and lasts until the eyes open, which occurs on postnatal day 13–14 in mice. During this time, as the circuits that mediate retinal waves change (Table 1, Box

1), so too do the details of the resulting firing patterns (Table 1). In the last stage, retinal waves briefly co-exist with visual responses, presumably using parallel circuitry.

In spinal cord, motor neurons exhibit episodes of large rhythmic depolarizations that are separated by extended periods of silence, a firing pattern that drives embryonic limb movements[28, 29]. This spontaneous network activity has been observed over an extended period of development, from before motor neurons innervate muscle fibers[30] until central pattern generator circuits are functional, which occurs in late embryonic development[31–33]. As in the retina, the circuits that mediate spontaneous activity in the spinal cord and the resulting pattern of activity change during development[34] (Table 1, Box 1).

In the developing hippocampus, pyramidal cells exhibit two distinct patterns of spontaneous correlated firing[35]. Synchronous plateau assemblies (SPAs), which in mice span the period from a few days before to a few days after birth, are characterized by bursts of plateau potentials and are correlated across 3–7 neurons. Later, hippocampal neurons exhibit giant depolarizing potentials (GDPs), which occur for a week, overlapping briefly with the end of the SPAs (Table 1› Box 1). GDPs are characterized by slow depolarizations that are correlated across many neurons[15, 36, 37].

Prior to the onset of hearing, spontaneous bursts of action potentials have been recorded in the auditory nerve. These bursts follow a pattern similar to those in the retina: short active periods are followed by quiet periods that range from seconds to minutes, depending on the species[38–41]. A recent study revealed that this activity originates in the developing cochlea[5] (Table 1› Box 1). This correlated spontaneous activity dissipates at the onset of hearing[39, 41].

Recently, spontaneous network activation has been characterized in the developing cerebellum[20]. Here, the cerebellar projection neurons, known as Purkinje cells, fire bursts of action potentials that propagate from the apex toward the base of cerebellar lobules. Intervals between bursts are much shorter here compared to the other circuits described above. The spontaneous rhythmic activity in the cerebellum is found in the first postnatal week of development, preceding the formation of the primary inputs to Purkinje cells (Table 1, Box 1).

Pacemaker-like neurons trigger activity

In the absence of external stimuli, what triggers the large correlated depolarizations that characterize spontaneous activity in developing circuits? In the adult nervous system, spontaneous firing in various networks, such as motor circuits[42], is driven by pacemaker neurons. Pacemaker neurons exhibit unstable membrane potentials, caused by a cyclical interplay of depolarizing and hyperpolarizing conductances. Pacemakers in the adult nervous system are typically depolarized by either a hyperpolarization-activated cation conductance[43] or a persistently active sodium conductance (e.g. in respiratory system[44]). Depolarization activates a calcium-activated potassium conductance, generating an afterhyperpolarization (AHP)[45]. The AHP prevents further depolarization, and the duration of the AHP therefore sets the period of depolarizing events. Such a complement of conductances in adult pacemaker neurons typically leads to membrane potential oscillations with a period of tens of milliseconds to seconds. However, spontaneous network depolarizations during development typically have longer intervals between events. To initiate network activity, developing circuits use varying combinations of pacemaker-like intrinsic membrane properties and network interactions.

Perhaps the simplest example of the interaction between pacemaker-like conductances and network properties is found in the developing cerebellum. Purkinje cells spontaneously fire in the absence of synaptic input[46], and therefore serve as the pacemaker-like neurons. During development, network interactions in the form of depolarizing GABAergic synapses (see

below) entrain nearby Purkinje cells to fire such that waves of depolarization propagate down a chain of Purkinje cells[20] (Box 1). Consistent with computational models[20], this leads to an inter-event interval of about 100 msec.

Giant depolarizing potentials (GDPs) in the hippocampus are triggered by an interaction between CA3 pyramidal cells and GABAergic interneurons (Box 1). GABA-induced depolarization causes CA3 pyramidal cells to fire periodic bursts of action potentials (reviewed in[47, 48]). The pacemaker-like bursts of CA3 pyramidal cells, both during development and in the adult, are driven by a persistent sodium current and are terminated by a slow AHP, which lasts 3–4 seconds and is mediated by a calcium-activated potassium conductance[49, 50]. Blockade of the AHP decreases the inter-event interval from 3 seconds to less than 2 seconds, suggesting that the frequency of GDPs is set by the kinetics of these conductances. Similar to cerebellum, network interactions mediated by recurrent excitatory connections between CA3 pyramidal cells and excitatory connections with GABAergic interneurons (see below) entrain depolarizations among neighboring cells, thereby prolonging the AHP and setting the frequency of GDPs. A similar organization is observed in neocortex[51] and midbrain[19], where clusters of pacemaker-like neurons are the sites of repeated event initiation.

The periodicity of spontaneous activity in the developing retina is not fixed by the membrane conductances of the network's pacemaker-like neurons, as it is in cerebellum and hippocampus. Instead, it emerges from an interplay between the connectivity of the network and the properties of the developing retina's pacemaker-like neurons, as has been explored both computationally[52, 53] and experimentally[54]. Early retinal waves are initiated by a class of cholinergic interneurons called starburst amacrine cells[55] (Box 1). In the absence of synaptic input individual starburst amacrine cells spontaneously depolarize approximately every 15 s [54]. It has been postulated that starburst cells, which are densely interconnected through excitatory, cholinergic synapses, depolarize each other, thus generating a retinal wave[56]. During such waves, starburst amacrine cells undergo a large depolarization, which causes a large calcium influx. The calcium triggers a calcium-dependent, slow AHP, which follows the wave-associated depolarization and lasts 15–30 seconds[54], which is roughly the minimum interval between wave initiations. These extremely slow AHPs, which are similar to slow AHPs in the hippocampus, the thalamus, and the peripheral nervous system[57–61], are thought to be regulated by the cAMP/PKA second messenger pathway[62, 63]. Consistent with this hypothesis, elevating cAMP significantly reduces the duration of slow AHPs in starburst amacrine cells[54] and increases the frequency of retinal waves[64]. The AHP makes starburst cells refractory to further depolarization and therefore sets the minimum inter-wave interval. As more starburst cells recover from this refractory period the likelihood of another network depolarization increases [52]. Hence, the minute-long interval between retinal waves is due to pacemaker conductances that are activated by network interactions[65].

In contrast to the retina, hippocampus and cerebellum, no pacemaker-like neuron has been conclusively identified in the developing spinal cord. Some evidence suggests that motor neurons, which are cholinergic, could be responsible for triggering episodes of spontaneous activity: nicotinic acetylcholine receptor antagonists block spontaneous activity early in development [30, 66] and motor neurons are the first population of neurons to be active in each episode [67, 68]. Although motor neurons might trigger episodes of spontaneous activity, recurrent excitatory interactions in the network are thought to set the periodicity of activity. The spinal cord contains cholinergic, glutamatergic, GABAergic, and glycinergic neurons, and all of the connections in the developing spinal cord are excitatory (see below). Immature spinal neurons continuously release neurotransmitters onto one another, but the efficacy of synaptic connections changes as a function of activity[69–73]: immediately after an episode of spontaneous activity the network is the most depressed, so the ongoing synaptic excitation within the network is not powerful enough to trigger another event. As the network recovers

from the previous event the ongoing synaptic excitation increases in efficacy, until eventually the neurons reciprocally excite one another enough to trigger another network-encompassing event. An important component of the network in the spinal cord is a population of GABAergic interneurons that form strong synapses onto motor neurons[67]. During an episode of network activity, which can last as long as 60 seconds, sustained activation of $GABA_A$ receptors on motor neurons leads to a massive efflux of chloride[69]. As an episode progresses the intracellular concentration of chloride is reduced to such an extent that the reversal potential for chloride becomes more negative than before the episode, causing GABA and glycine to be less excitatory. In this scenario, the long interval between events is due to the relatively slow re-accumulation of chloride in motor neuron dendrites via chloride transporters[69, 71, 73]. Evidence for a reduction in the excitatory drive is provided by the reduction of the size of $GABA_A$-mediated postsynaptic currents following a network event. In addition, blockade of the chloride-accumulating transporter NKCC1 (in the presence of ionotropic glutamate receptor antagonists, so that excitatory glutamate transmission was also absent) blocks spontaneous network activity during development[71], indicating that lowering levels of intracellular chloride reduces the excitability of the network.

Recent research has provided a model for the generation of spontaneous bursts of action potentials in the auditory nerve. In the developing rat cochlea, periodic release of ATP from a developmentally transient population of inner supporting cells depolarizes inner hair cells, which then release glutamate onto the afferent dendrites of spiral ganglion neurons and initiate bursts of action potentials[5]. Although ATP-mediated currents occur in hair cells at a rate of about three to four per minute, action potential bursts appear in spiral ganglion neurons only once per minute[5], possibly because only a subset of ATP-mediated currents are large enough to depolarize hair cells sufficiently to trigger glutamate release. At present, little is known about the mechanisms that regulate the timing of ATP release from supporting cells and thus the timing of action potential bursts in the auditory nerve[5].

Transient network features

The patterns of spontaneous network activity observed during development differ in many ways from the activity patterns of the adult nervous system. A dramatic example is found in the retina: here, adult circuits are organized along a “vertical” axis, which limits the lateral spread of excitatory signals in order to preserve a high-acuity representation of visual space. By contrast, during development spontaneous network activity in the form of retinal waves propagates laterally across large areas of tissue that represent several degrees of the visual field. This lateral spread of activity is a result of several connectivity features that are present only during a finite period of development, and which are described below.

Depolarizing GABA

A prominent feature of several developing circuits that is crucial for activity propagation is the excitatory action of GABA and glycine, which in the adult brain act as inhibitory neurotransmitters. This depolarizing action of canonically inhibitory transmitters is primarily due to high intracellular concentrations of chloride at early ages: when a $GABA_A$ receptor is activated, chloride diffuses out of the cell, which causes depolarization. As neurons mature, they change their complement of chloride transporters, which leads to a decrease in intracellular chloride[74]. In the spinal cord, hippocampus, neocortex and cerebellum, the cells that will become inhibitory interneurons in adulthood are a primary source of depolarization during development[47, 75]. In the developing retina, activation of $GABA_A$ receptors on retinal ganglion cells is depolarizing [76, 77], but it is not clear whether GABA signaling is required for cholinergic retinal wave generation. $GABA_A$ receptor antagonists block retinal waves in turtle[78], but not in ferret or mouse (though they do modulate wave properties[64, 79]). There is no evidence for GABA signaling during spontaneous activity in the developing cochlea[5].

Depolarizing GABA is crucial for the generation of GDPs in the developing hippocampus[15, 36, 37]. GDPs are blocked by ionotropic glutamate and $GABA_A$ receptor antagonists and the age at which activation of $GABA_A$ receptors is no longer depolarizing is the age at which GDPs disappear[37]. This is in contrast to the earlier form of spontaneous network activity in the hippocampus — SPAs — which are not dependent on $GABA_A$ signaling, but rather on L-type calcium channel activation and gap junction coupling[35] (Table 1 and below).

Spontaneous activity in the developing spinal cord is also strongly influenced by depolarizing GABA and glycine. In the spinal cord, the frequency of network activation is reduced by $GABA_A$ receptor antagonists[80]. Furthermore, episodes of bursting activity and the underlying waves of depolarization are likely to be triggered at least in part by massive GABA release, and then terminated by a switch in the chloride gradient such that GABA temporarily becomes less excitatory[71, 73]. Also similar to the hippocampus, spontaneous network activity in the spinal cord disappears around the time when activation of $GABA_A$ receptors ceases to be excitatory[31, 33].

Depolarizing GABA is the sole source of coupling involved in generating spontaneous network activity in the developing cerebellum[20]. GABAergic Purkinje cells, which are the primary projection neurons of the cerebellum, make local synaptic connections with neighboring Purkinje cells. These local axon collaterals are not distributed uniformly within the cerebellar network. Instead, the density of Purkinje-Purkinje connections is higher for cells located closer to the base of each cerebellar lobule. Purkinje cells spontaneously spike at all ages, but the existence of depolarizing GABAergic connections between nearby Purkinje cells during the first postnatal week entrains the firing of neighboring Purkinje cells, generating a propagating wave that travels preferentially in the direction of higher-density local connections, i.e. towards the base of the cerebellar lobules. A computational model predicts that when $GABA_A$ signaling becomes inhibitory in the second postnatal week, Purkinje cells would still be entrained, but that the direction of propagation would switch[20], with waves starting from the base of a lobule and propagating toward the apex. Local axon collaterals among Purkinje cells persist until adulthood but form many fewer synaptic connections. Hence, as the cerebellum matures and the functional connections between nearby Purkinje cells are reduced, the substrate for propagation disappears.

Transient connections

A second feature common to many networks that generate spontaneous activity is that they transiently express unique circuit components. These transient components, such as the local axon collaterals of cerebellar Purkinje cells described above and the expression of neurotransmitter receptors, provide a substrate for correlating activity across populations of cells that are not directly connected in adulthood.

The retina provides an example of developmentally transient components that form a substrate for wave propagation. During the first postnatal week in mice, retinal waves propagate via a network of starburst amacrine cells[55]. Immature starburst amacrine cells undergo spontaneous depolarizations and express nicotinic acetylcholine receptors[54, 56] (nAChRs). Starburst cells form a dense, recurrent excitatory network via cholinergic and GABAergic synapses[56] (Box 1). Hence, it has been proposed that cholinergic waves are initiated by spontaneous depolarizations in starburst amacrine cells and propagate via connections with other starburst amacrine cells. However, nAChRs are only expressed at starburst-starburst synapses during development [56]. At the age when starburst amacrine cells stop expressing nAChRs and are therefore no longer connected via excitatory synapses, the cholinergic waves disappear[55] and are replaced by glutamatergic waves, as discussed below.

Similar to the retina, spontaneous network activity in the spinal cord might also depend on connections that exist early in development but that become functionally insignificant in the adult. During development, motor neurons form local excitatory connections with other motor neurons[81] and with local GABAergic interneurons called Renshaw cells[81, 82] (Box 1). Renshaw cells also receive glutamatergic inputs from sensory neurons[81, 83]. Although motor neuron inputs to Renshaw cells persist into adulthood, motor neuron—motor neuron synapses and sensory neuron—Renshaw cell synapses do not remain functional[83].

The developing cochlea uses a similar strategy to sustain spontaneous correlated activity early in development. Prior to the onset of hearing, hair cells are periodically depolarized through activation of purinergic receptors by ATP released from neighboring supporting cells[5]. The supporting cells comprise a transient structure, Kölliker's organ, which is present only during a short period of development[7]. Furthermore, preliminary studies in rats indicate that hair cells express purinergic receptors only for a transient period from a few days after birth to around the time of hearing onset [N. X. Tritsch and D. E. Bergles, "Developmental Regulation of Spontaneous Cochlear Activity", Association for Research in Otolaryngology, Annual Meeting, 2009]. Hence, the transient source of ATP-secreting cells and the transient expression of receptors probably dictate the period of development during which spontaneous activity in the cochlea is present.

Extrasynaptic glutamate

There is growing evidence that extrasynaptic transmission plays a part in propagating the waves of depolarization in developing networks before synaptic structures achieve their mature state[84]. In addition to mediating direct synaptic communication, neurotransmitters released from a presynaptic cell can "spill" out of the synaptic cleft and activate extrasynaptic receptors: on the postsynaptic cell, on the presynaptic terminal and on other neighboring neurons and glia. Extrasynaptic glutamate has been implicated in regulating the early differentiation of neurons in the ventricular zone[85] and might modulate neuronal migration[86]. It is thought that at later developmental stages, retinal waves and hippocampal GDPs are mediated, at least in part, by extrasynaptic glutamate.

In the retina, during the period just prior to eye-opening, spontaneous correlated activity is no longer dependent on acetylcholine release from starburst amacrine cells, but rather on glutamate release from bipolar cells (for review see[6]). In contrast to the starburst amacrine cells, whose processes form a dense lateral network, neighboring bipolar cells are not synaptically connected. Each bipolar cell has a very small axonal process, forming glutamatergic synapses on a small part of the total dendritic tree of its target ganglion cell. Recently we demonstrated that retinal waves are accompanied by large transient increases in extrasynaptic glutamate[87]. This extrasynaptic glutamate provides a possible source of depolarization that is not limited to cells that are directly postsynaptic to bipolar cell release sites.

Does extrasynaptic glutamate mediate wave propagation? Interestingly, elevating extrasynaptic glutamate by pharmacologically blocking glutamate transporters, which tightly regulate glutamate levels outside the synaptic cleft, significantly reduces variability in wave speed, making slow waves faster and fast waves slower[87]. This observation indicates that extrasynaptic glutamate positively and negatively regulates wave propagation. Extrasynaptic glutamate is known to be both excitatory and inhibitory in the adult retina[88–90]. Furthermore, low concentrations of glutamate receptor antagonists have been shown to reduce wave propagation speed in the developing turtle[78] and chick[91] retina, although complete blockade of either AMPA/Kainate or NMDA receptors does not affect wave speed in the developing mouse retina[87]. However, as there is no reliable way to block extrasynaptic glutamate signaling

independently of synaptic glutamate signaling, it is not known whether extrasynaptic glutamate transmission is required for wave propagation.

A role for extrasynaptic glutamate has also been demonstrated in the developing cortex[92, 93], hippocampus[94] and brain stem[95], where increasing extracellular glutamate profoundly alters the patterns of spontaneous network activation. In the hippocampus, episodic elevations of extrasynaptic glutamate levels depolarize interneurons via activation of NMDA receptors, causing an increase in the frequency of events compared to endogenous GDPs[94]. Whether extrasynaptic glutamate has a role in the endogenous activity patterns remains to be determined.

Gap junctions

Several studies have implicated gap junctions as potential substrates for propagating neural activity during development. There are three lines of evidence that support these claims. First, there are several examples of spontaneous network events that persist in the presence of a broad spectrum of neurotransmitter receptor antagonists and are thus non-synaptic. Such non-synaptic waves are detected perinatally in hippocampus[35] and embryonically in retina[96, 97], and they can be induced in cases when the synaptic pathways for mediating waves are disrupted[98] (see next section). Second, spontaneous network activity patterns can be suppressed by pharmacological blockade of gap junctions. Indeed, in the spinal cord, the cochlea and the retina, spontaneous network activity is blocked by gap junction inhibitors[5, 30, 66, 97, 98], at least at some stages of development. Unfortunately, gap junction blockers have several non-specific effects that could underlie the overall reduction of activity, including blockade of voltage-gated calcium channels that mediate synaptic transmission[99, 100], activation of large-conductance calcium-activated potassium channels[100–102], and inhibition of synaptic release[103], which makes these experiments difficult to interpret. Third, transgenic mice lacking specific gap junction proteins (connexins) have altered spontaneous firing patterns. For example, in the spinal cord the expression of a number of connexin proteins in motor neurons changes with development[104], and in mice lacking connexin 40 (Cx40), spontaneous activity is uncorrelated between motor neurons[105]. In mice lacking Cx36, spontaneous network activity in the retina is altered such that retinal ganglion cells fire many more spikes between waves than is observed in wild-type animals[106, 107], suggesting that Cx36-containing retinal gap junctions have a role in mediating the silences between waves.

Homeostatic regulation

One of the striking features of spontaneous network activity during development is its robustness. Throughout their development, circuits use a multitude of strategies to spontaneously generate activity and, although the details of the temporal and spatial correlations change, the overall pattern of activity remains the same – large depolarizations generated by excitatory synaptic inputs are followed by extended periods of silence.

The removal of a crucial component of a circuit showing spontaneous depolarizations often leads to compensation by the remaining components, providing further evidence of the robustness of the network activity[108]. We refer to this compensation as homeostatic regulation under the assumption that the network is adjusting its inputs to achieve a baseline level of activity. This phenomenon was first described in the developing spinal cord, where extended blockade of receptors for a primary excitatory transmitter (acetylcholine during the early stage of development[12, 30] and glutamate or GABA during a later stage[109]) led to an initial block followed by a restoration of spontaneous network activity. Homeostatic compensation has also been observed *in ovo*, where recovery from blockade of glutamate or GABA-A receptors takes significantly longer than that observed in vitro (12 hours vs 30–60 minutes). A recent dissection of mechanisms that underlie a homeostatic phenomenon *in ovo* revealed that changes in

synaptic strength[11, 110] and changes in the expression of ion channels that control cellular excitability in motor neurons[111] compensate for the loss of excitatory transmitter (Figure 1).

Homeostatic compensation has also been observed in the circuits that mediate retinal waves (Figure 2). Transgenic mice lacking choline acetyltransferase (ChAT), an enzyme crucial for acetylcholine production, do not exhibit cholinergic waves. Instead, they exhibit compensatory waves, which are not blocked by any fast neurotransmitter receptor antagonists[112], indicating that the compensatory mechanism here is different from the one observed in the spinal cord and the hippocampus. The compensatory waves are, however, blocked by gap junction antagonists[112], suggesting that they are an extension of an earlier, nonsynaptic wave-generating mechanism that has been observed in embryonic mice[96] and rabbits[97]. One of the interesting features of the compensatory retinal waves is that they require a few days to appear, suggesting that significant circuit rearrangements need to take place. A more complex form of compensation occurs in mice lacking the β2 subunit of nicotinic acetylcholine receptors. Under some experimental conditions, no wave activity is detected in these β2-nAChR-KO mice[96, 113], whereas in other recording conditions[114] -- characterized, for example, by increased temperature[115] -- compensatory waves are observed. As these waves are not blocked by fast-neurotransmitter receptor antagonists[114], they may be the same gap-junction mediated waves as those in transgenic mice lacking ChAT[112]. Although the circuit mediating these compensatory waves is not yet understood, one probable homeostatic mechanism is based on an increased excitability of retinal neurons, because bath application of voltage-gated calcium channel agonists leads to the generation of similar non-synaptic waves in both wild-type and β2-nAChR-KO retinas[98, 116]. Additionally, in β2-nAChR-KO mice retinal waves that are dependent on glutamatergic signaling appear 3–4 days earlier than in wild-type mice [96], indicating that the absence of endogenous signaling induces an early maturation of the next stage of network activity.

The observation that transgenic mice with disrupted cholinergic circuitry exhibit a reappearance of non-synaptic waves suggests that normally, activity in the cholinergic circuit suppresses non-synaptic waves. Similarly, the disappearance of cholinergic waves depends on the maturation of glutamatergic circuits, suggesting that glutamatergic activity suppresses cholinergic circuit activity. Transgenic mice lacking the vesicular glutamate transporter VGLUT1 in bipolar cells continue to exhibit cholinergic waves at the age when cholinergic circuits disappear in wild-type mice[87]. A similar switch from cholinergic to glutamatergic transmission has been observed in the developing hindbrain; however, it is not known whether this transition is influenced by the absence of network activity as in the developing retina and spinal cord[117].

Homeostatic regulation of spontaneous network activity has also been observed in the developing hippocampus. Activity is maintained during acute blockade of GDPs by a strengthening of SPAs[35]. Furthermore, although CA3 pyramidal neurons trigger endogenous GDPs, they are not required for GDP generation—other hippocampal areas, such as CA1, are capable of generating GDPs when they are surgically isolated from CA3, albeit at a lower frequency than endogenous GDPs [47]. This suggests that CA3 pyramidal neurons generate activity at a higher frequency than other hippocampal areas, but that latent circuits present in other areas generate activity in the absence of CA3.

Another instance of homeostatic regulation has recently been observed in the hippocampus. In a knockout mouse lacking the chloride-accumulating transporter NKCC1, activation of $GABA_A$ receptors is never depolarizing, and therefore the major depolarizing drive for GDPs is absent. Nonetheless, GDPs are detectable[118], although fewer hippocampal neurons participate in the events[119]. In NKCC1 knockout mice, the compensatory activity was partially mediated by an increase in the intrinsic excitability of CA3 pyramidal cells rather than by a

change in network properties as seen in the developing spinal cord and retina[118]. To our knowledge, homeostatic regulation of spontaneous network activity has not been observed in the developing cerebellum and cochlea.

The observation that many circuits compensate for the disruption of one form of activity by generating another form leads to the question what aspect of the activity is being homeostatically regulated. In addition, it is not known whether homeostatically generated activity can serve the same function as normal activity. In the case of the retina, the pattern of endogenous and homeostatically generated activity differs. For example, β2-nAChR-KO mice exhibit waves that are larger and faster than waves in wild-type retinas[114, 115], and retinal projections to the brain in β2-nAChR-KO mice are abnormal[6]. This indicates that the feature of activity that is regulated in β2-nAChR-KO mice is not the feature that is required for circuit refinement. Determining what aspects of cellular and/or network function are being homeostatically regulated and what aspects drive circuit maturation will require specific manipulations of endogenous activity patterns.

Conclusions and future directions

A fundamental feature of developing neural circuits is the presence of spontaneous network activity, often taking the form of propagating waves. The circuits that mediate this activity, while differing in the particulars, rely on similar cell-intrinsic and synaptic properties that are observed for only a brief time during development. Robust compensatory mechanisms seem to be in place to ensure that spontaneous network activity is actively maintained throughout this crucial period of development.

Although tremendous insights have been gained into the mechanisms generating spontaneous activity, a remaining question concerns the purpose that this activity serves during development. In developing sensory epithelia, such as the retina and the cochlea, propagating neural activity contains topographic information that may be useful in the establishment of early sensory maps in the brain. Propagating activity in the spinal cord may be used to define circuits within the cord that will serve as central pattern generators in adulthood, or to target motor neurons in neighboring spinal segments to neighboring muscles. Spontaneous activity in the developing cortex and hippocampus may serve to strengthen the networks that mediate oscillatory activity in adulthood. Insights into how spontaneous correlated activity influences the development of neural circuits will require manipulations that alter the pattern of activity rather than block it entirely. Understanding the mechanisms that underlie the generation of correlated patterns will allow us to design such manipulations.

Continued insights into the mechanisms underlying early network activity, as well as an increased awareness of its crucial role in brain development, could have profound implications for clinical treatments of pregnant women. Alcohol, for example, is known to affect network activity patterns, and many regions of the brain are particularly sensitive to fetal alcohol exposure. Alcohol disrupts normal firing patterns in the developing hippocampus[120], and extended fetal exposure prevents the normal development of primary sensory systems[121, 122]. Another potential clinical implication relates to a decrease in spontaneous activity during birth --a transient, neuroprotective effect that is triggered by a large increase in oxytocin, a hormone that modulates the depolarizing action of GABA transmission[123]. A deeper understanding of the mechanisms that mediate spontaneous activity during development will help to prevent neuropathologies associated with fetal exposure to neuroactive pharmacological agents.

Glossary

Central pattern generator	A neural circuit that produces self-sustaining patterns of behaviour independently of sensory input
Gap Junctions	Intercellular channels composed of connexin proteins that are the basis of electrical synapses between neurons
Pacemaker-like neuron	In the adult nervous system, pacemaker neurons possess a set of voltage-gated ion channels that lead to regular patterns of depolarization and hyperpolarization. In developing circuits, pacemaker-like neurons are neurons with unstable membrane potentials, but whose pacemaker properties also depend upon network interactions
Cell autonomous	Describes activity that is generated by a cell independent of any input from other cells, as in pacemaker neurons
Amacrine cell	Retinal interneurons located in the inner nuclear layer of the retina that provide local inhibition in the adult retina
Bipolar cell	An interneuron of the retina that provides excitatory glutamatergic input to retinal ganglion cells. In the adult retina, bipolar cells receive input from photoreceptors
Retinal ganglion cells	The projection neurons of the retina, the axons of which form the optic nerve
Renshaw cells	GABAergic interneurons that receive excitatory input from motor neurons

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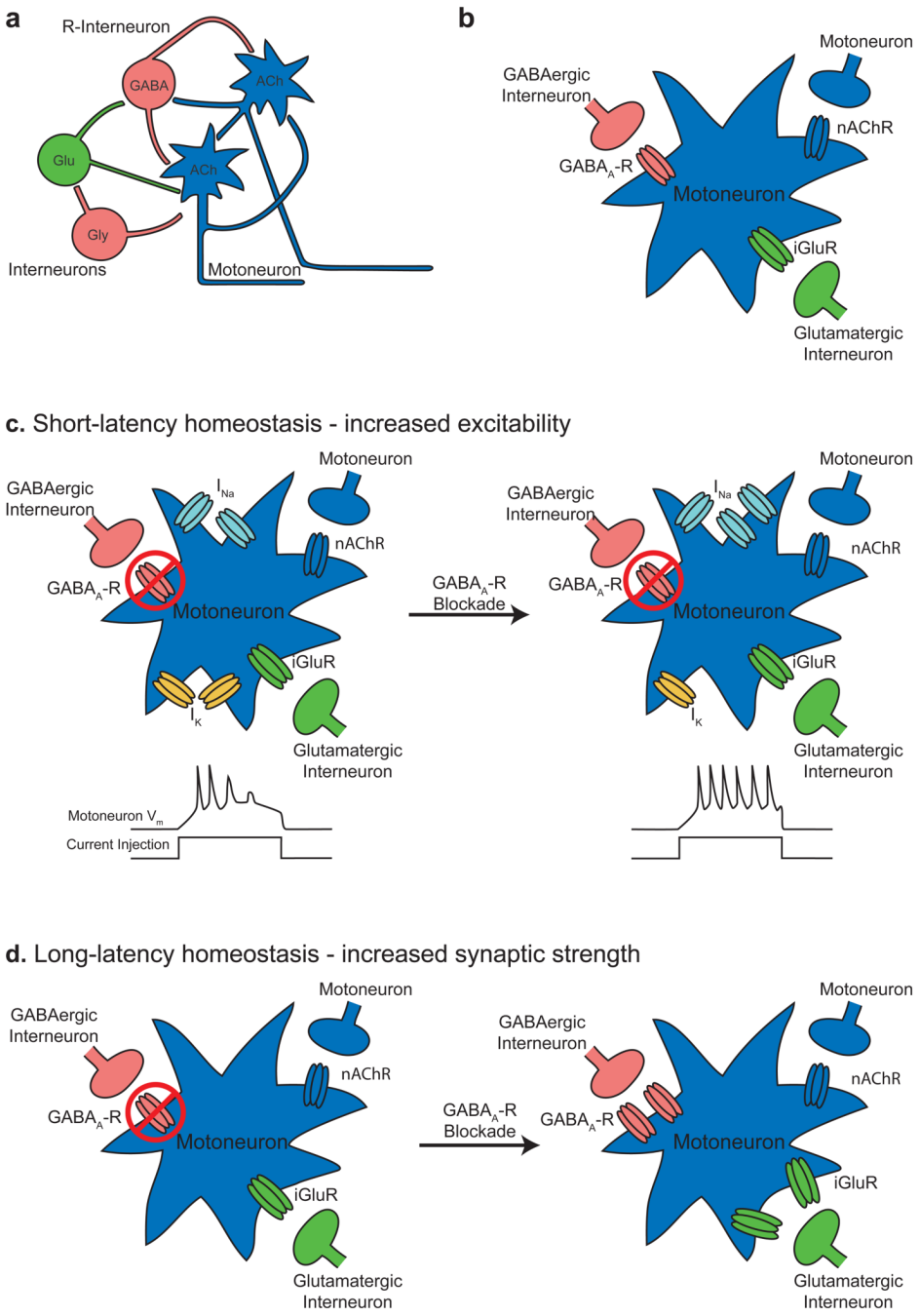

Figure 1. Homeostatic regulation of spontaneous network activity in the chick spinal cord

When a part of the spinal cord network is blocked, activity becomes temporarily less frequent, but recovers to pre-block levels. Here we provide schematics of the changes that take place after activity blockade.

a. Schematic of the circuits that mediate activity in the developing spinal cord. Neurons are color-coded by the transmitter they release. ACh, acetylcholine, blue; Glu, glutamate, green; Gly, glycine, pink; GABA, pink.

b. Motor neurons provide a crucial drive in the generation of activity. They receive input from other motor neurons and from interneurons. nAChR, nicotinic acetylcholine receptor; $GABA_A$-R, $GABA_A$ receptor; iGluR, ionotropic glutamate receptor.

c. When $GABA_A$ receptors are blocked *in ovo* (left), activity becomes temporarily less frequent but recovers [11]. After 12 hours of $GABA_A$-R blockade, motor neurons become more excitable, an effect that is mediated by an increase in the density of sodium current and a decrease in the density of potassium current[111] (right). Bottom: schematics illustrating an increase in motor neuron excitability, with the bottom curve showing current injection into a motor neuron, and the top curve showing membrane potential. A more excitable motor neuron fires more action potentials in response to the same stimulus (right). I_{Na}, sodium current, represented by sodium channels; I_K, potassium current, represented by potassium channels.

d. When $GABA_A$ receptors are blocked for long periods (24–48 hours) glutamatergic and GABAergic postsynaptic currents in motor neurons increase in size[110]. The exact mechanisms underlying this increase in postsynaptic current are not fully understood, but are schematized here as increases in the number of glutamate and $GABA_A$ receptors.

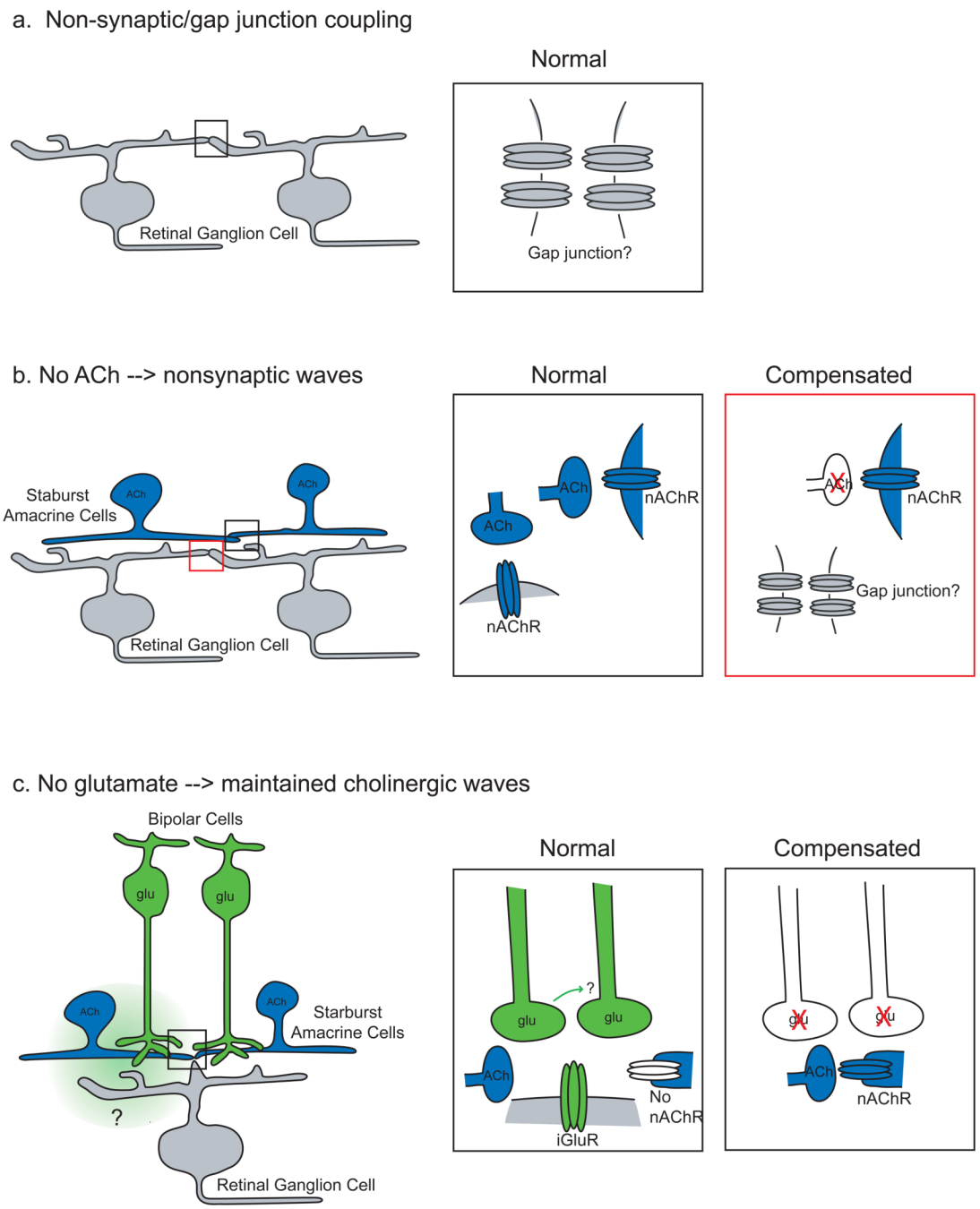

Figure 2. Homeostatic regulation of spontaneous network activity in the mammalian retina

In the absence of a requisite circuit component, the retina regresses to the previous wave-generating mechanism. Here we provide schematics of the circuits that mediate retinal waves at different ages, including the changes that are thought to take place when one form of activity is disrupted.

a. Perinatally in mice, waves are mediated by a non-synaptic circuit, thought to be mediated by gap junction coupling (inset). Here the coupling is shown to be between retinal ganglion cells, although the location of the relevant coupling is not known.

b. During the first postnatal week, starburst amacrine cells (blue) form synaptic connections with other starburst amacrine cells and retinal ganglion cells (gray). Retinas from mice lacking

acetyl choline (Ach; bottom inset) exhibit non-synaptic waves[112], potentially through a reactivation of non-synaptic connections that mediate network activity in the perinatal period (see panel a). Furthermore, blocking nAChRs soon after the onset of cholinergic waves leads to the reappearance of non-synaptic waves[97].

c: In the few days before eye opening in mice, when glutamatergic interneurons begin to form synapses with their postsynaptic targets, waves are mediated by glutamatergic circuits. Inset: Glutamatergic bipolar cells (green), which make glutamatergic synapses onto amacrine and ganglion cells and have no direct connections with each other, release glutamate that is detected both synaptically and extrasynaptically[87]. After the first postnatal week, starburst cells no longer express nAChRs[56]. Retinas from mice in which bipolar cells do not release glutamate (bottom inset) exhibit waves that are mediated by the cholinergic network[87].

Table 1

Summary of important features of spontaneous network activity recorded in rodents.

	Retina			Spinal Cord		Hippocampus		Cochlea	Cerebellum
Stage	**1. E17-P1**	**2. P1–P10**	**3. P10–P14**	**1. E12–E15**	**2. E15–E18**	**1. E18 – P5 (SPAs)**	**2. P3–P10 (GDPs)**	**P7–P10**	**P4–P6**
Description of projection neuron firing patterns	Bursts that propagate over a limited region of the GCL	Bursts that propagate over a large region of the GCL	Clusters of bursts that propagate over a large region of the GCL	Bursts of oscillatory activity that propagate within and between segments	Bursts of oscillatory activity that propagate within and between segments	Ca^{2+} spikes correlated over few pyramidal cells	Bursts correlated across CA3 and CA1 subfields	Bursts of action potentials; correlation pattern unknown	Travelling waves of action potentials that propagate from apex to base of cerebellar lobules
Inter-event interval	30 s	1 – 2 min	1 min	2–3 min	1 min	8 s	3–10 s	5–60 s	100 ms
Mechanisms of Initiation	Unknown	Spontaneous Ca^{2+} spikes in starburst amacrine cells	Unknown	Network interactions	Network interactions	Spontaneous Ca^{2+} spikes in pyramidal cells	Intrinsic bursts in CA3 interneurons	Unknown	Spontaneous firing in Purkinje neurons
Primary source of depolarization	Gap junctions	nACh receptors	iGlu receptors	nACh, $GABA_A$, and glycine receptors	iGlu, nACh, glycine, and $GABA_A$ receptors	L-type Ca^{2+} channels and gap junctions	$GABA_A$ and NMDA receptors	ATP release from supporting cells in Kölliker's organ	$GABA_A$ receptors
State of network at end	Maturation of cholinergic circuit	Maturation of glutamatergic circuits	Onset of vision	Loss of requisite role for nAChR signaling	GABA signaling becomes inhibitory	Maturation of GDP circuits	GABA signaling becomes inhibitory	Kölliker's organ disappears	GABA signaling becomes inhibitory
Recorded in vivo	No	Yes[3]	Yes[26, 27]	Yes (chick[29])	Yes (chick[29])	No	Yes[125]	Yes[41]	No

E, embryonic; P, postnatal; GCL, ganglion cell layer; SPA, synchronous plateau assembly; GDP, giant depolarizing potential; iGlu receptor, ionotropic glutamate receptor; nACh receptor, nicotinic acetyl choline receptor; $GABA_A$ receptor, γ-Aminobutyric acid receptor A

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