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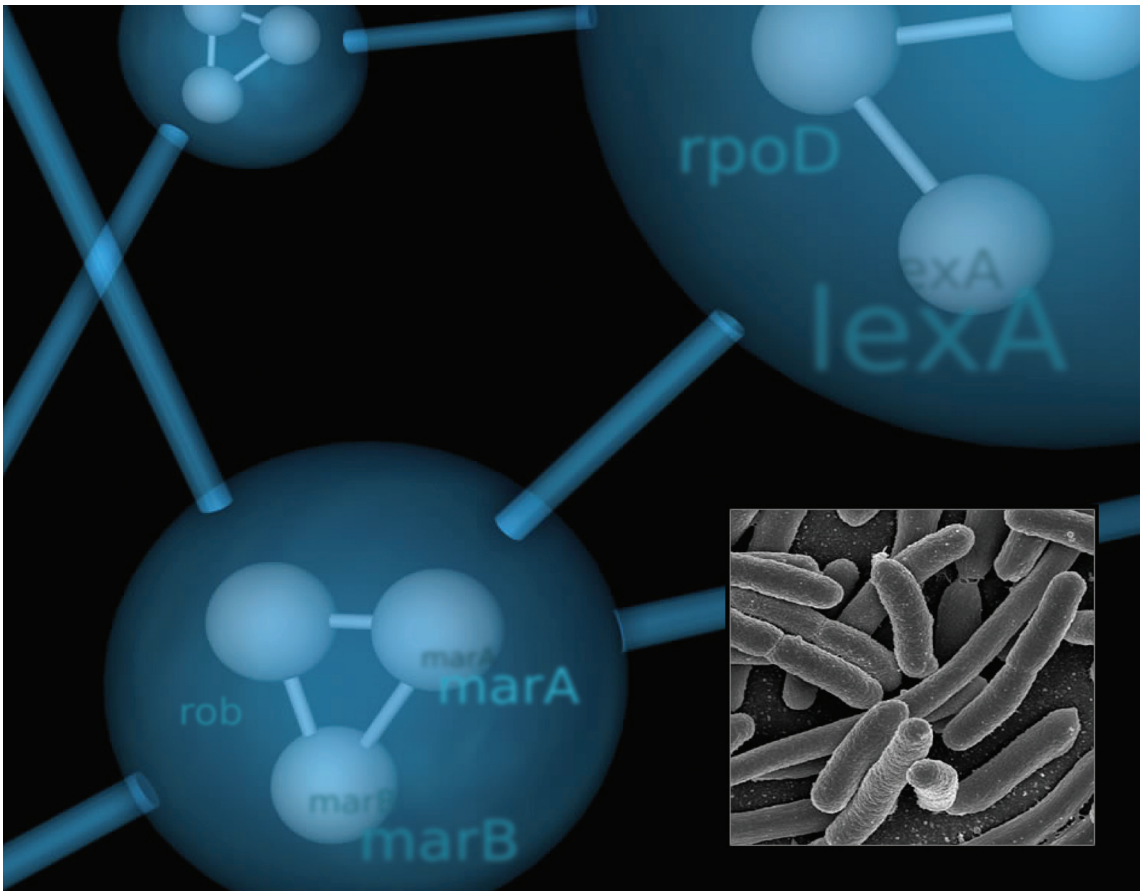

Theoretical models of spontaneous activity generation and propagation in the developing retina†

Keith B. Godfrey and Stephen J. Eglen*

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Spontaneous neural activity is present in many parts of the developing nervous system, including visual, auditory and motor areas. In the developing retina, nearby neurons are spontaneously active and produce propagating patterns of activity, known as retinal waves. Such activity is thought to instruct the refinement of retinal axons. In this article we review several computational models used to help evaluate the mechanisms that might be responsible for the generation of retinal waves. We then discuss the models relative to the molecular mechanisms underlying wave activity, including gap junctions, neurotransmitters and second messenger systems. We examine how well the models represent these mechanisms and propose areas for future modelling research. The retinal wave models are also discussed in relation to models of spontaneous activity in other areas of the developing nervous system.

Introduction

The retina is located at the back of the eye, and its function is to translate patterns of light stimulation into neural activity. Light is first converted into neural activity by photoreceptors, and the neural activity is then modulated by both vertical and horizontal pathways of interconnecting neurons within the retina, before reaching the retinal ganglion cells (RGC). At this point, the visual image has been converted into a pattern of neural activation that is then sent along the optic nerve to the brain. The right panel of Fig. 1 shows a cartoon overview of some of these connections. The retina is a highly complex circuit, and yet is an important model system because of its laminar structure, and compared to many other brain regions, much is known about the different neuronal cell types and their connections. For a recent review of the processing performed in the retina, see ref. 1.

In contrast to its functional role as a device for converting visual signals into neural activity, during development the retina behaves quite differently. Before the photoreceptors are visually responsive and able to pass their information to the RGCs (left panel of Fig. 1), rather than being silent, the RGCs are spontaneously active in such a way that neighbouring RGCs tend to be active at the same time (Fig. 2). These spontaneous activity patterns are termed retinal waves due to the way that they travel across the retinal surface.[2,3] In this context, the term spontaneous refers to the idea that the activity is generated independent of external stimulation (such as light).

Such spontaneous rhythmic activity has also been observed in many other parts of the developing nervous system. In the auditory system, both the cochlea and associated nuclei[4–7] are spontaneously active before the onset of sensory experience. Spontaneous rhythmic activity is also observed in developing motor circuits, such as spinal networks,[8,9] and in non-motor areas such as the hippocampus[10,11] and neocortex.[12,13] This activity plays many instructive and permissive roles in neural development, including regulation of axon growth and pathfinding,[14–17] dendrite growth,[18,19] gene expression,[20–22] and the refinement of synaptic connectivity.[23–27]

While activity has been demonstrated to regulate many subcellular processes, how it contributes to organization on

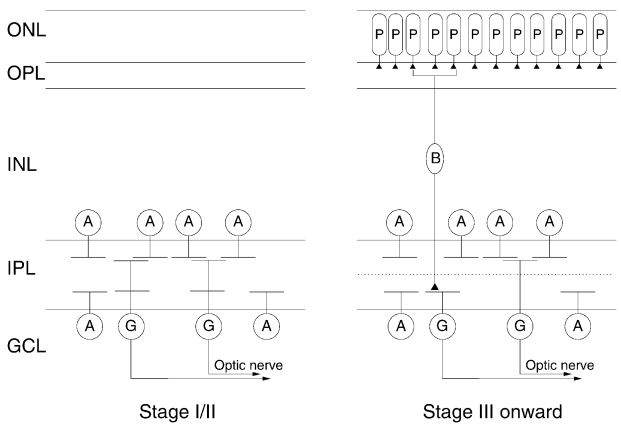

Fig. 1 Cross section of retina at two different stages of development. Left: early network present at the time of stage I/II retinal waves. Right: later network for generation of stage III waves. Figures are illustrative only, and do not include all neuronal types/connections. The retina is divided into three layers (ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer) with two layers of interconnecting processes (OPL: outer plexiform layer; IPL: inner plexiform layer). During stage I/II, spontaneous activity of retinal ganglion cells (G) is driven mostly by the interactions between ganglion cells and the cholinergic amacrine cells (A). At later stages, waves are driven by glutamateric-mediated signals, perhaps coming from bipolar neurons (B), which in turn are connected to the photoreceptors (P).

Cambridge Computational Biology Institute, Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Wilberforce Road, Cambridge, UK CB3 0WA.
E-mail: S.J.Eglen@damtp.cam.ac.uk; Tel: +44 (0) 1223 765761
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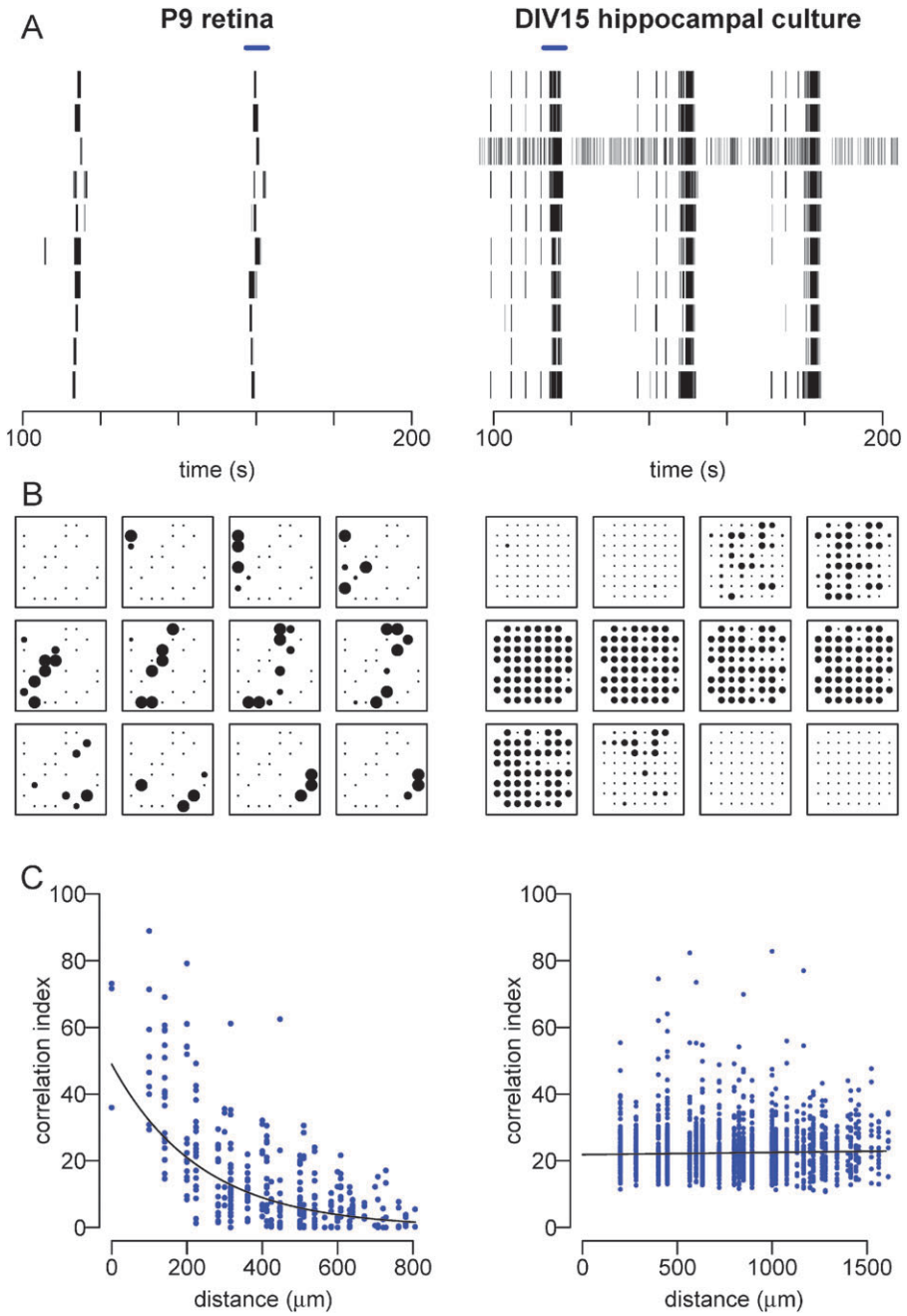

Fig. 2 Spontaneous activity patterns in developing retina (left) and hippocampal cultures (right). (A) Spike trains recorded from a multi-electrode array (MEA). Spikes recorded from ten (of typically sixty) electrodes are shown, one row per electrode. Vertical lines denote times each electrode recorded a spike. One hundred seconds of activity is shown; horizontal blue line indicates the time for which activity is shown in (B). (B) Propagation of activity across the MEA, shown every 0.5 s (row by row). Each electrode is drawn as a dot, with the size of the dot proportional to its firing rate. Inter-electrode spacing is 100 μm on left and 200 μm on right. (C) Correlation index between pairs of neurons as a function of the distance separating each pair of neurons. The solid line shows the best fit to a decaying exponential function. Experimental data provided by Dr Jay Demas and Dr Paul Charlesworth.

a network level remains a mystery. RGC axons produce orderly patterns of connections within the sections of the brain that they project to. Up-regulating retinal activity during development induces changes in these patterns of innervation,[25] as does interfering with its correlation patterns.[26] Pharmacological blocking of retinal activity disrupts normal organization[23,28] as does synchronizing the firing of retinal cells.[29] How these changes in patterns of activity influence the development of retinal projections is unknown. In order to understand the effect of perturbations to normal patterns of activity, normal behaviour must itself be understood.

This review addresses one of the paths of such research, the computational modelling of spontaneous activity in the developing nervous system. Theoretical neuroscience is now an established but still young field.[30] Over the last ten or so years, computational modelling has played an important part

in understanding the mechanisms that are responsible for the generation and propagation of spontaneous activity. Computational models allow us to test what mechanisms are sufficient to generate particular behaviours, or to quantitatively evaluate particular hypotheses that have been proposed. In addition to allowing us to test or predict hypotheses about neural development and functions, these models can also be used to provide simulated datasets to investigate other questions of neural development, such as the development of retinotopic maps.[31,32]

In this article, we review the key computational models of spontaneous retinal activity.[33–36] We then address the molecular mechanisms known to underlie spontaneous activity, including gap junctions, neurotransmitter receptors, and second messenger signalling pathways, and assess how accurately the models represent this data and then propose directions for future models of spontaneous retinal activity. We conclude with a comparison of retinal activity to spontaneous activity as is observed in other developing neural circuits and address how current and future modelling approaches will help to advance our understanding of these processes.

Spontaneous activity in the retina

Retinal waves are characterized by spatially restricted groups of neurons that become electrically active simultaneously, with this activity slowly propagating across the retina.[37] This activity is not the result of pacemaker neurons but rather is an inherent property of the network, and it begins long before the retina is responsive to light (Fig. 1). Waves can initiate at any retinal location, they have no propagation bias[3,38–40] and they have non-repeating boundaries.[34,39]

Fig. 2 shows an example of retinal wave activity, recorded from a postnatal day 9 (P9) mouse retina using a multi-electrode array. On a coarse timescale (Fig. 2A), electrodes seem to be simultaneously active, with elevated firing occurring once a minute. However, when looking in detail at the spatiotemporal properties of this activity, it is clear that there is a propagating wave; Fig. 2B shows in detail a wave moving from left to right, taking about five seconds to propagate across the array. It is thought that these slowly-propagating waves allow for the firing of neurons to provide instructional cues: neurons close to each other are likely to fire at the same time, whereas neurons that are further apart are less likely to fire together. This is quantified using a correlation index (Fig. 2C) which measures the degree to which any two neurons on the array fire synchronously (within a time window of $\pm$50 ms) and is plotted as a function of the distance separating that pair of neurons. The higher the correlation index, the more correlated the firing of a pair of neurons; for two uncorrelated spike trains, the index should be one.[38] For the retinal wave data, the correlation index decays exponentially with the distance separating the neurons. This cue is thought to be instructive for the retinotopic refinement of connections from the retina to their primary target areas in the brain.[27,41]

Retinal waves occur while the retina is maturing and can be categorized into three different developmental stages[41,42] During stages I and II, the retina has few functional cell types (Fig. 1) and retinal waves are produced through the interactions of these cells. As the retina matures, additional cell types are introduced and form connections with this initial network, and these new cells become involved in generating wave activity. Stage I waves are mediated both by the neuromodulator adenosine and by gap junctions and occur while few synapses are identifiable in the retina.[42] At this stage, the circuitry underlying wave generation is restricted to the inner retina (left panel of Fig. 1). Stage II waves begin with the onset of synaptogenesis and are primarily mediated by the neurotransmitter acetylcholine[42] and they have characteristic refractory periods, such that when a wave crosses a section of the retina, several tens of seconds elapse before another wave propagates across the same location.[34,42] The waves also initiate at any location in the retina and propagate across discrete regions, forming "domains".[34] Compensatory mechanisms exist and the retina can sometimes revert back to stage I if the mechanisms necessary for stage II waves become blocked.[42,43] Stage I waves are of higher velocity and have shorter inter-wave intervals than stage II.[42,43]

Stage I and II waves are formed through a coupled network of amacrine and retinal ganglion cells (Fig. 3). The transition to stage III occurs as bipolar cells establish synapses with RGCs and contribute to wave activity (right panel of Fig. 1). At this point in development, the neurotransmitter glutamate becomes involved in mediating retinal waves. Acetylcholine continues to be involved, but the neurotransmitter receptor used in stage II waves (nicotinic) is replaced by a different category of receptor (muscarinic) during stage III.[41,42]

Stage III waves have similar a velocity to stage II waves, but are smaller and less frequent than earlier waves, and only half of the waves propagate a significant distance on the retina.[42] In some species, such as mouse, the retina first becomes sensitive to light near the onset of stage III waves[44] while in other species, such as rabbit, several days elapse before light generates a response.[42] Wave activity generally disappears after eye-opening[38] and spontaneous activity is replaced by visually evoked responses. However, this does not occur in all species. In turtle for example, wave activity does diminish by the time of hatching, but non-propagating patches of spontaneous activity persist for up to 4 weeks.[40]

One salient characteristic of waves is their propagation velocity. Retinal waves travel at 100–300 $\mu m\ s^{-1}$ in many species, including ferret,[34] mouse,[43,45] early chick development,[46] turtle[40] and rabbit.[42] At some ages these velocities are faster, but not significantly so, such as stage I rabbit (450 $\mu m\ s^{-1}$)[42] and stage III chick (500–1500 $\mu m\ s^{-1}$).[47] These velocities are much slower than are explained by electrical gap junctions or chemical synapses with integration times on the order of milliseconds[3] or that are observed after disinhibiting coupled networks of neurons.[48] These velocities are also much faster than can be explained by the extracellular diffusion of excitatory molecules.[48]

Another significant feature of retinal wave activity is its prevalence, being observed in many different species and at different developmental stages. While wave activity has many similarities among the different species and ages where it has been observed, the mechanisms underlying its production vary considerably. Each animal passes through developmental stages where activity is mediated through different cell types

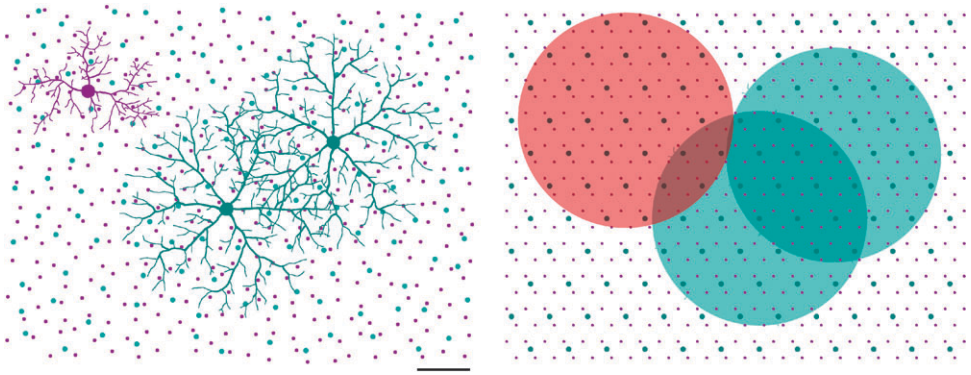

Fig. 3 Spatial arrangement of neurons within the retina. On the left is an example of the distribution, size and spacing of retinal ganglion cells (purple) and starburst amacrine cells (blue). Scale bar is 50 μm. On the right is a representative approximation of the retina as used in computational models. The blue circles indicate the dendritic field of two starburst amacrine cells and the red circle represents the dendritic field of a retinal ganglion cell.

and molecular mechanisms. Differences are also noted between species. For example, using data reported for rabbit[42] as a baseline, the retina becomes light sensitive much sooner in mouse,[44] and waves in chick retina are much faster.[47] Gap junctions are important in rabbits, as in mice[45] and chick,[46] while they seem to not contribute to wave activity in turtle.[49] The mechanisms that underlie differences in wave behaviour between species are not known, and significant functional differences do exist, yet the characteristics of wave behaviour remain similar. Modelling studies have investigated different mechanisms that might underlie wave generation and propagation and analysis of these models helps to provide insight into the functional mechanisms involved.

Models of retinal waves

Some of the first studies reporting spontaneous bursting activity in the retina suggested that the activity might result from ionic imbalances in the extracellular medium[2] or diffusion of excitatory substances, such as potassium ions.[3] Following up on these ideas, Burgi and Grzywacz[33] described a retinal wave model showing that accumulations of extracellular potassium could generate propagating patterns of bursting activity similar to those observed experimentally. In their model, K^+ release from bursting RGCs accumulated in the extracellular space and this accumulation led to the depolarization of neighbouring RGCs, resulting in a propagating wave of activity. The model was supported in part by experiments that blocked the type of K^+ ion channels that become active during RGC bursting.[49] Subsequent studies showing that other cell types are involved in wave activity[50] and that wave activity was mediated by neurotransmitters such as acetylcholine and glutamate[39,51,52] effectively disproved the model. It is still possible that there is a functional role for K^+ accumulation in the generation and propagation of retinal waves, but occurring at the level of amacrine and bipolar cells instead of RGCs. This has not been discounted, but the interaction of K^+ ion channels with mechanisms known to influence wave behaviour[53] also makes it difficult to prove a functional role for extracellular ion accumulation in influencing wave activity.

The first computational model that was able to generate patterns of non-repeating waves that were statistically similar to experimental observations was described by Feller *et al.*[34] and was subsequently analyzed in more detail.[54] This model will be referred to here as the Butts model, after its primary architect. The Butts model generated patterns of activity similar to those observed in newborn ferret, when the retina is still very immature and is not responsive to light. The model required two groups of cells to produce waves: a group of interneurons, amacrine cells, that produced the spontaneous activity; and a second group of neurons, retinal ganglion cells, that acted as a low-pass filter, generating a response only after a sufficient number of local amacrine cells were depolarized (Fig. 3). This model provided a good fit to experimental data, as blocking acetylcholine prevented spontaneous retinal activity[39] and starburst amacrine cells are the only retinal neurons expressing this neurotransmitter.[55] Moreover, patch clamp recordings showed that amacrine cells depolarized with the same regularity as retinal waves[50] and that amacrine cells formed a recurrent excitatory network.[56] The functional mechanism of this model was that amacrine cells spontaneously depolarized, after which time they entered a refractory state where they were non-responsive for a period of time. Wave activity was produced when a sufficient number of nearby amacrine cells became spontaneously active, and wave propagation depended on the local density of non-refractory amacrine cells. The activation threshold of the retinal ganglion cells was sufficiently high that random activity among amacrine cells would not cause an RGC response, but a response was generated when there were sufficient amacrine cells active to generate a propagating wave.

The beauty of this model was its simplicity, having effectively two free parameters that governed wave properties: the amacrine cell activation threshold, and the probability of spontaneous activation. Moreover, it demonstrated how a simple mechanism could generate realistic wave behaviour. Subsequent analysis of the model demonstrated that it had some weaknesses, however. The most notable of these was the sensitivity of the model to changes in parameters, such that small changes to the free parameters could produce large changes in the spatiotemporal wave properties, particularly

when generating waves with physiologically realistic values for size, frequency and velocity.[54] This weakness likely resulted from the simple implementation of the model and was not necessarily indicative of a problem with the underlying mechanism. This is supported by a recent model[36] that uses a similar functional mechanism to describe amacrine cell activity, wave formation and wave propagation. As discussed below, this latter model incorporates many physiological details that were unknown at the time the Butts model was formulated and describes how mechanisms at the molecular level can produce network level wave-like behavior. It was also able to avoid the problem of sensitivity to changes in parameters (M. Hennig, personal communication).

The next model that sought to address retinal wave formation was proposed by Godfrey and Swindale[35] and incorporated experimental data that was unavailable when the Butts model was created. The Godfrey model was based in a large part on the description by Zheng *et al.*[53] of amacrine cell behaviour during waves, which implied an activity-dependent refractory period in amacrine cells. Amacrine cells were observed to have intervals between spontaneous depolarizations that were associated with how much excitation the amacrine cell received. In the model, this was implemented as amacrine cells having a variable refractoriness that was proportional to how much excitation the cell received when it was depolarized. Refractoriness would decay with time and each amacrine cell would depolarize when it decayed to zero. Wave activity was produced when the excitation from depolarizing amacrine cells overcame the refractoriness of other nearby cells, causing them to depolarize in turn. Wave propagation continued so long as the advancing wavefront was able to overcome the refractoriness of the neighbouring cells. The activity-dependent aspect of refractoriness resulted in a differential of refractoriness between cells in the centre and at the boundary of wave domains, resulting in a destabilizing mechanism that inhibited the production of subsequent waves similar to previous ones. Neurons receiving more input during a wave became more refractory, with higher amounts of refractoriness having the effect of inhibiting subsequent responses by the neuron. In this regard, the model bore resemblance to a previous model of spontaneous activity in developing spinal networks[57] which was based on activity-dependent inhibition. See the discussion for further details.

A characteristic of the model was that all amacrine cells within the wave became depolarized and contributed to its passage. Further, amacrine cells did not have a fixed refractory period and could be induced to depolarize multiple times over short intervals, as sometimes happens immediately before a wave.[53] An interesting feature of this model was that it had chaotic properties and was able to produce seemingly random, non-repeating waves in the absence of stochastic mechanisms. Fig. 4 shows samples of simulated wave activity by the model and a comparison of wave properties between experimental and simulated data. The model effectively reproduces the spatio-temporal properties of retinal waves as have been recorded from a range of species.[35]

While of similar algorithmic complexity to the Butts model, the Godfrey model had five free parameters. However, its parameter space was stable, with small changes in parameters producing small changes in wave behaviour over a wide range of parameter settings, and it was able to generate wave activity that had statistical properties similar to waves observed in different species and at different developmental stages. The model also required only a single group of neurons, demonstrating that two types of neurons were not required for the production of wave activity as was previously thought.[34,54] Similar to the Butts model, the Godfrey model only represented the general behaviour of neurons and did not address the underlying mechanisms that could give rise to such behaviours. This generality in both models allows the functional mechanisms to be more easily applied to networks based on other cell groups, but it also restricts the insight that these models can provide. Implementation at the molecular level is necessary to determine how the described mechanisms might be realized physiologically, as well as to assess if the mechanisms are realistic.

Recently Hennig *et al.*[36] described how biophysical mechanisms could generate wave-like behaviour, in particular how calcium channel dynamics in individual amacrine cells can underlie the production of waves. During wave activity, amacrine cells produce bursts of spikes that are generated mainly through calcium currents. Calcium influx in turn activates slow-acting K^+ currents which produce a long lasting hyperpolarizing current in the cell.[53] In the model, this behaviour was realized through representation of fast and slow acting K^+ currents that were activated by intracellular Ca^{2+} concentrations. When a cell was depolarized, it excited its neighbours. Each amacrine cell was also subjected to random excitatory noise, possibly resulting from stochastic ion channel gating. This noise produced periodic and random depolarizations in individual amacrine cells. Excitation from nearby amacrine cells helped other amacrine cells to depolarize, and when enough nearby cells depolarized at the same time, a wave began and propagated through areas of the retina where there were sufficient numbers of local amacrine cells that were non-refractory that they could in turn become depolarized. This mechanism was functionally similar to that used by Butts, with a notable exception being that excitation from depolarized amacrine cells influenced the excitability of nearby cells, effectively amplifying stochastic excitation. In the Hennig model, amacrine cells did not have fixed refractory periods and the excitation of an advancing wave was sufficient to recruit most amacrine cells into a wave.

To assess the phenomenological accuracy of the Hennig model, its behaviour was compared to electrode recordings of mouse and turtle retinas, and it was subjected to simulated pharmacological manipulations, with the results of these manipulations compared to experimental data. Electrode recordings of mouse and turtle retinas were made using multi-electrode arrays that consisted of 60 close-spaced (100–200 μm separation) electrodes, similar to Fig. 2B. The electrode array was sufficiently large to observe spatial patterns of electrical activity on the retina, providing information on many aspects of waves, including wave size and the duration of bursting activity. Recorded waves observed power law distributions of both wave size and duration, a behaviour also reproduced in the model (and other models—see the discussion). Pharmacological manipulations of the simulated

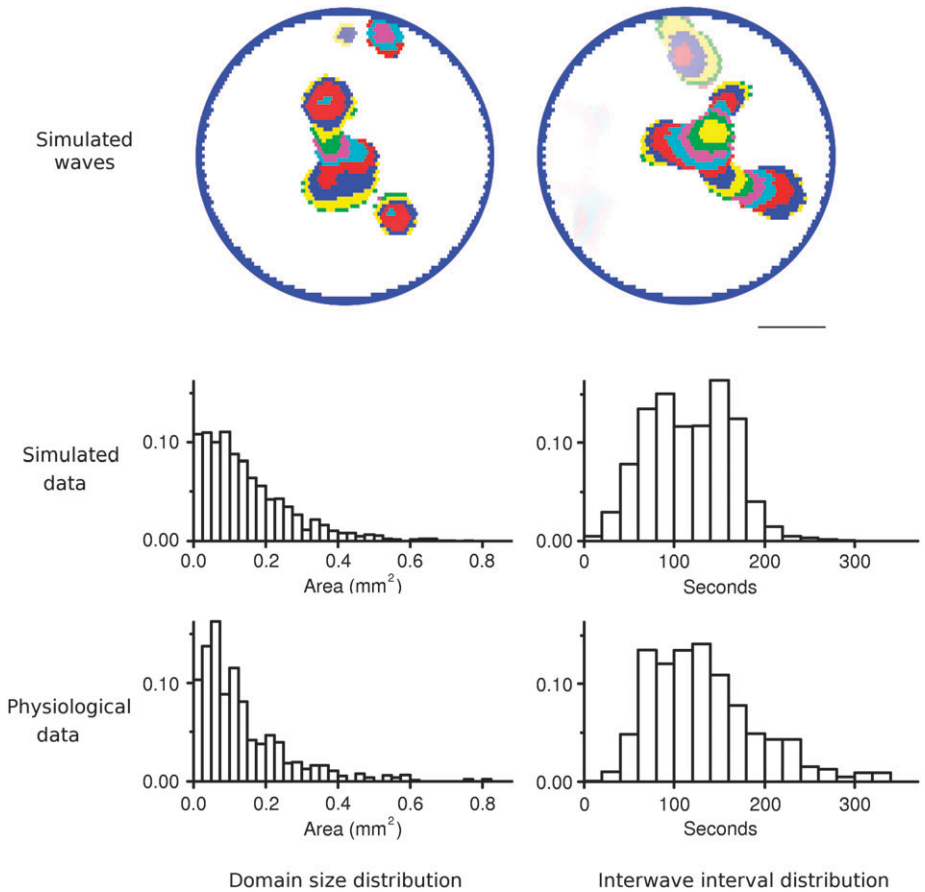

Fig. 4 Wave patterns generated by the Godfrey and Swindale model.[35] Top: example waves. Colors indicate progression of neural activity over 0.5 s intervals. Multiple waves are shown. Scale bar is 0.5 mm. Bottom: comparison of experimental waves and output from simulation. Figure adapted from ref. 35. Experimental data from ref. 34.

retina demonstrated similar behaviour with experiments that blocked acetylcholine as well as those that increased activation of second messenger pathways, resulting in shortened after-hyperpolarizing (AHP) currents. Increasing the sensitivity to acetylcholine did not reproduce experimental findings, however, possibly due to molecular mechanisms that were not represented in the model, such as ion channel desensitization and inactivation.

Analysis of retinal wave models

All of the models described were able to reproduce phenomenological patterns of retinal wave activity to the level that each was analyzed. A more thorough and controlled analysis of these models is possible, which could better compare how each model is able to account for the same experimental observations. However, a more useful task might be to analyse the mechanisms underlying these models relative to the molecular mechanisms known to contribute to wave behaviour. This approach can better identify strengths and weaknesses and also help guide future modelling and experimental efforts. We analyze the models relative to the activities of gap junctions, the neurotransmitter GABA, excitatory neurotransmitters, the neuromodulator adenosine, and intracellular second messenger pathways.

Gap junctions are large molecules that create a pore between two cells, linking their cytoplasm and allowing ions and small molecules to be exchanged between coupled cells. Gap junctions are found throughout the retina during development[45,58] and have been implicated in contributing to wave behaviour. Gap junction blockers prevent or disrupt wave behaviour in stage I, II and III mammalian retinas.[42,45] Mutant mice which lack a particular gap junction gene show greatly altered patterns of retinal activity in stage III waves.[59] Gap junctions blockers suppress waves in chick retinas[46,47] and they also appear to serve as a compensatory mechanism for generating waves, as gap junction mediated waves can appear when neurotransmitter release is blocked during stage II waves.[42,43]

The reason for the importance of gap junctions in generating wave activity is unclear. Heightened cytoplasmic calcium levels appear to cause acetylcholine release by amacrine cells,[56] and gap junctions might facilitate the spread of calcium between cells, acting to synchronize their behaviour. It is also possible that a functional role of gap junctions is to simultaneously depolarize coupled cells, again acting to synchronize their behaviour. The high electrical conductance of gap junctions would seem to support this role, but how much of a factor this might play is unclear as depolarized amacrine cells already release neurotransmitters that have the same depolarizing

effect on neighbouring cells. Whatever their functional role, gap junctions appear to serve an important role in retinal waves as blocking them disrupts wave behaviour in all three wave stages, even though each are mediated by different cellular mechanisms and neurotransmitters.

Modelling studies have not yet explicitly addressed the role of gap junctions in retinal waves. It is possible that their role is implicitly represented in the mechanisms of these models. Without having theories as to the functional role of gap junctions, this is difficult to assess. It seems likely that gap junctions somehow work to synchronize the activity between coupled cells and none of the models provide inter-cell dynamics beyond direct excitation, suggesting that the contribution of gap junctions is not represented. Exploring the functional role of gap junctions is a task that is well suited to computational modelling, and hopefully future models will address this topic.

There are several neurotransmitters released during retinal waves. One transmitter that is common to all wave stages is the traditional "inhibitory" transmitter GABA. GABA typically acts through two distinct mechanisms. In stage I waves, it hyper-polarizes neurons through activation of slow-acting (metabotropic) ion channels that let K^+ ions out of the cell, hyperpolarizing it and inhibiting subsequent activity.[42] In stage III waves, GABA uses fast-acting (ionotropic) ion channels that allow Cl^- ions into the cell, again hyperpolarizing and inhibiting it.[42] During stage II waves, the role of GABA is much less clear and is further complicated by the fact that GABA can have an excitatory role at this age, because the reversal potential for Cl^- is above the resting potential for the cell.[60] In rabbit, amacrine cells co-release GABA with acetylcholine,[56] both ionotropic and metabotropic GABA receptors are present at this stage and GABA contributes a significant component of neurotransmitter input to waves.[42] However, blocking either ionotropic or metabotropic receptors produced minimal effects on wave properties.[42] In ferret, GABA receptors are present[39] however, blocking these receptors either decreases retinal activity[60] or has no effect.[39,61] GABA plays a regulatory role in turtle, underlying the developmental changes in wave propagation patterns.[40] The inconsistent role of GABA suggests the circuit exists in a tenuously-balanced state.[41]

Neurons can use different ratios of ion channels to achieve the same qualitative cellular behaviour, and that the homeostatic mechanisms balance these ratios to maintain a desired operating behaviour.[62] It is possible that GABA receptors play a similar balancing role during stage II waves. Modelling studies do not explicitly address GABA, but because of its co-release with acetylcholine and its apparent excitatory, neutral or homeostatic role, the models can be considered to implicitly represent it. Modelling studies exploring the role of GABA in waves should focus on stage I or III waves, or retinal waves in turtles, where GABA plays an important regulatory role.[40]

Stage II waves in many mammalian species are mediated by the neurotransmitter acetylcholine. With the exception of the Burgi model, all retinal wave models are based upon acetylcholine release. Acetylcholine acts on the nicotinic receptor,[39,42] an ionotropic, non-selective cation channel. The receptor responds quickly to binding by agonists, with a response time of a few milliseconds. Inducing calcium release in an amacrine cell causes a large acetylcholine-mediated postsynaptic current in nearby amacrine cells within 10s of milliseconds.[56] This fast-excitatory behaviour is inconsistent with inter-cell excitation represented in the models, which assume excitatory mechanisms with a rise time measured in the hundreds of milliseconds. The slow time course of excitation in the models produces long, slow depolarizations as are observed experimentally[56] and the low propagation velocity in these models (100–300 μm s^{-1}) appears to depend on this slow rate of excitation. Models using fast excitation have much faster propagation velocities, such as 100–200 mm s^{-1} in the hippocampus.[63] As suggested by the Godfrey model, higher wave velocities result from shortening the time course for excitation.

The slow rise time of excitation and long depolarization in amacrine cells, required by models and as is observed experimentally in the retina,[56] suggests the involvement of metabotropic receptors. Metabotropic receptors influence cellular behaviours indirectly through activation of second messenger molecular pathways and take longer to become active and, generally, have longer lasting affects. A similar slow time course for excitation is observed in the developing auditory system: the cochlea uses both ionotropic and metabotropic receptors to generate spontaneous activity[64] with metabotropic receptor activation inducing a significant rise in intracellular calcium through activation of second messenger pathways.[65] Assuming the presence of a similar mechanism in the retina, a likely candidate for this metabotropic mechanism is through the adenosine pathway.

Adenosine is a neuromodulator that is released during retinal waves and that influences wave properties.[61] The characteristic long depolarizations in amacrine cells are observed in the presence of GABA, glutamate, acetylcholine and gap junction antagonists,[53] suggesting that they are not the result of fast neurotransmitters such as acetylcholine. Adenosine receptors are G protein-coupled receptors that influence cellular behavior through activation of second messenger pathways. In the retina, one of the actions of adenosine is the activation of the second messenger cyclic-AMP (cAMP),[61] which in turn reduces AHP currents in amacrine cells.[53] Consistent with this role, adenosine agonists increase wave frequency, while adenosine receptor antagonists either reduce waves to barely detectable levels[61] or completely and reversably block them.[42] A similar behaviour was noted with manipulations of cAMP, where it was also observed that wave size and velocity are also affected,[42,61] demonstrating a clear role for adenosine in wave behaviour. In muscle tissue, adenosine and cAMP have also been shown to desensitize acetylcholine receptors.[66] It is unclear that this desensitization mechanism has a significant role in the retina as amacrine cells are sensitvie to acetylcholine depolarization immediately after a wave,[53] although it could still play a role in terminating amacrine cell depolarization. It is quite possible that activation of adenosine receptors and second messenger pathways modulate other cellular mechanisms, such as the activity of Ca^{2+} ion channels.[67]

Modelling studies do not directly address adenosine or its effects, or explicitly represent any second messenger pathways. It is possible that adenosine's primary effect is on the slow time course of excitation. If so then it is implicitly represented in the existing models. However, its role in reducing the AHP current[53] suggests that it has a broader role in the regulation of wave activity. Models which represent AHP currents or their effects, *e.g.* ref. 35 and 36, should take into account the role of adenosine, as it has a clear role on the mechanisms represented in these models. The Hennig model does suggest a link between the slow AHP currents and second messenger pathways, as would be activated by adenosine. In support of this, decreasing the duration of the slow AHP in the model does produce similar results to pharmacological activation of the adenosine pathway experimentally.[36] Unfortunately, the model does not provide dynamic control over these currents as would be experienced by the activity dependent release of adenosine. A reasonable scenario is that adenosine release is both activity-dependent and is non-specific, acting to synchronize the AHP state of neighbouring cells and thus directly influence wave initiation or propagation.

Future models that investigate the mechanisms underlying retinal waves should address the role of a synchronizing mechanism between nearby amacrine cells that is based on a mechanism other than acetylcholine-mediated excitation, such as through adenosine or gap junctions. These mechanisms are clearly important for generating wave behaviour and theoretical studies which are able to account for how these mechanisms influence wave properties can help to guide future experiments. Models based on mechanisms that are not biologically accurate can themselves be useful for generating phenomenological patterns of activity that can be used as input to models of the retinocollicular or retinogeniculate pathway[31,32] and can shed light into possible functional mechanisms underlying wave behaviour. Models that seek to explain the physiology underlying the production of waves, however, should represent the known biological behaviours, especially those that influence the mechanisms represented in the model. For example, representation of a refractory period without considering the role of adenosine reduces the likely accuracy of how refractoriness is implemented. It is possible that a model represents refractoriness in such a way that it generates waves in a manner that does not require the functional role played by adenosine. In this case, the model would likely use a different functional mechanism than is used by biology and thus the model would provide little, or even misleading, insight into how refractoriness operates in the cell. The same argument holds true for gap junctions. If gap junctions are required for wave propagation, but models do not require an equivalent role as is played by gap junctions, it suggests that the models produce waves through different functional mechanisms than biology. It does not necessarily follow that models that fail to represent gap junctions or adenosine or any other requisite mechanism are flawed, as the role of the missing mechanisms might be implicitly represented in such models. However, these implicit assumptions, and how omitted mechanisms might fit into a model, should be addressed in the presentation and analysis of the models.

Discussion

Spontaneous activity is present not only in the developing retina, but also in many other parts of the developing nervous system. In this section, we briefly describe activity in two other areas, hippocampal cultures and spinal cord, particularly as there has been considerable effort to model these activity patterns. In-depth reviews of spontaneous activity can be found elsewhere.[68,69]

Spontaneous activity is prevalent in developing cultured networks, for example cultures generated from dissociated hippocampal neurons.[70] In these preparations, hippocampal neurons are extracted just before birth in a mouse, and cultured onto a multi-electrode array (MEA). This preparations allows for the regrowing network to be monitored over long periods of time, typically up to a month. The dissociated neurons reform connections once on the array, and then exhibit spontaneous activity after around seven days *in vitro* (DIV). An example of this spontaneous activity is shown in Fig. 2A, where after fifteen days *in vitro* (DIV15) the MEA records spontaneous activity every 30 s or so. (Note, however, that the third electrode reported almost constant activity, indicating some neurons may be continuously firing.) Hence, on a broad timescale, the retina and hippocampal cultures exhibit similar patterns. However, when examining the spatiotemporal properties of the activity on a finer timescale, the two systems are quite different. Whereas in retinal waves, activity propagates relatively slowly, in the cultured neurons, almost all electrodes are simultaneously active (Fig. 2B and C). In particular, comparing the correlation index values for retina with hippocampal cultures, the flat correlation index profile suggests that relative firing of a pair of neurons no longer varies with distance separating the neurons. These patterns of correlated activity observed in hippocampal cultures have also been observed in cortical cultures, and have been described as "neuronal avalanches".[71,72] Models for the development of such neuronal avalanches have recently been described,[73,74] and warrant further study to compare their properties with the retinal wave models described here. In particular, the Hennig model of retinal waves also shows power law distributions for event size and duration,[36] and yet the models are based on quite different mechanisms; it is possible then that many different mechanisms could underlie power law distributions.

Motorneurons in the developing spinal cord are also known to generate spontaneous activity.[69] Dynamical systems approaches have been used to model the generation of this activity, assuming groups of purely excitatory units are connected to each other, combined with slow, activity-dependent depression to terminate bursting events.[57,75] In these models, the major goal was to investigate the generation of episodic bursting rather than the propagation of activity across groups of units. The advantage of using differential equation models is that it allows for a thorough characterization of the behaviour of the network's behaviours as various key parameters of the system vary. One key prediction of the model is that the activity-dependent depression is more likely to be driven by a slow synaptic mechanism rather than a loss of intrinsic cellular excitability.[57] An interesting area of future research would be to apply these dynamical systems approaches to spontaneous activity from other areas (such as retina or hippocampal cultures).

Computational modelling has been instructive in helping us understand the mechanisms that underlie the generation and propagation of spontaneous neural activity in developing nervous systems. In this article, although we have focused on models of retinal waves, we believe the issues faced in accounting for these spontaneous activity are rather general and occur in other parts of the developing nervous system, such as spinal cord and even cultured networks. Finally, we have outlined several areas of future research where the models can be extended to account for recent experimental findings.

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References

1 H. Wässle, *Nat. Rev. Neurosci.*, 2004, **5**, 747–757.
2 L. Maffei and L. Galli-Resta, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 2861–2864.
3 M. Meister, R. O. L. Wong, D. A. Baylor and C. J. Shatz, *Science*, 1991, **252**, 939–943.
4 A. W. Gummer and R. F. Mark, *Neuroreport*, 1994, **5**, 685–688.
5 W. R. Lippe, *J. Neurosci.*, 1994, **14**, 1486–1495.
6 T. A. Jones, S. M. Jones and K. C. Paggett, *J. Neurosci.*, 2001, **21**, 8129–8135.
7 T. A. Jones, P. A. Leake, R. L. Snyder, O. Stakhovskaya and B. Bonham, *J. Neurophysiol.*, 2007, **98**, 1898–1908.
8 M. J. O'Donovan and N. Chub, *Semin. Cell Dev. Biol.*, 1997, **8**, 21–28.
9 N. C. Spitzer and X. Gu, *Semin. Cell Dev. Biol.*, 1997, **8**, 13–19.
10 O. Garaschuk, E. Hanse and A. Konnerth, *J. Physiol.*, 1998, **507**, 219–236.
11 X. Leinekugel, R. Khazipov, R. Cannon, H. Hirase, Y. Ben-Ari and G. Buzsaki, *Science*, 2002, **296**, 2049–2052.
12 R. Yuste, A. Peinado and L. C. Katz, *Science*, 1992, **257**, 665–669.
13 A. Peinado, *J. Neurosci.*, 2000, **29 RC54**, 1–6.
14 S. M. Catalano and C. J. Shatz, *Science*, 1998, **281**, 559–562.
15 E. S. Ruthazer, C. J. Akerman and H. T. Cline, *Science*, 2003, **301**, 66–70.
16 S. Cohen-Cory and B. Lom, *Int. J. Dev. Biol.*, 2004, **48**, 947–956.
17 M. G. Hanson and L. T. Landmesser, *J. Neurosci.*, 2006, **26**, 12769–12780.
18 H. T. Cline, *Curr. Opin. Neurobiol.*, 2001, **11**, 118–126.
19 K. Haas, J. Li and H. T. Cline, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 12127–12131.
20 A. Buonanno and R. D. Fields, *Curr. Opin. Neurobiol.*, 1999, **9**, 110–120.
21 H. T. Cline, *Trends Neurosci.*, 2003, **26**, 655–661.
22 P. Vanhoutte and H. Bading, *Curr. Opin. Neurobiol.*, 2003, **13**, 366–371.
23 A. A. Penn, P. A. Riquelme, M. B. Feller and C. J. Shatz, *Science*, 1998, **279**, 2108–2112.
24 L. I. Zhang and M. Poo, *Nat. Neurosci.*, 2001, **4**, 1207–1214.
25 D. Stellwagen and C. J. Shatz, *Neuron*, 2002, **33**, 357–367.
26 T. McLaughlin, C. L. Torborg, M. B. Feller and D. D. M. O'Leary, *Neuron*, 2003, **40**, 1147–1160.
27 E. S. Ruthazer and H. T. Cline, *J. Neurobiol.*, 2004, **59**, 134–146.
28 A. R. Chandrasekaran, D. T. Plas, E. Gonzalez and M. C. Crair, *J. Neurosci.*, 2005, **25**, 6929–6938.
29 J. T. Schmidt and M. Buzzard, *J. Neurobiol.*, 1993, **24**, 384–399.
30 L. F. Abbott, *Neuron*, 2008, **60**, 489–495.
31 S. J. Eglen, *Philos. Trans. R. Soc. London, Ser. B*, 1999, **354**, 497–506.
32 T. Elliott and N. R. Shadbolt, *J. Neurosci.*, 1999, **19**, 7951–7970.
33 P. Y. Burgi and N. M. Grzywacz, *J. Neurosci.*, 1994, **14**, 7426–7439.
34 M. B. Feller, D. A. Butts, H. L. Aaron, D. S. Rokhsar and C. J. Shatz, *Neuron*, 1997, **19**, 293–306.
35 K. B. Godfrey and N. V. Swindale, *PLoS Comput. Biol.*, 2007, **3**, e245.
36 M. H. Hennig, C. Adams, D. Willshaw and E. Sernagor, *J. Neurosci.*, 2009, **29**, 1077–1086.
37 R. O. L. Wong, *Annu. Rev. Neurosci.*, 1999, **22**, 29–47.
38 R. O. L. Wong, M. Meister and C. J. Shatz, *Neuron*, 1993, **11**, 923–938.
39 M. B. Feller, D. P. Wellis, D. Stellwagen, F. S. Werblin and C. J. Shatz, *Science*, 1996, **272**, 1182–1187.
40 E. Sernagor, C. Young and S. J. Eglen, *J. Neurosci.*, 2003, **23**, 7621–7629.
41 C. L. Torborg and M. B. Feller, *Prog. Neurobiol.*, 2005, **76**, 213–235.
42 M. M. Syed, S. Lee, J. Zheng and Z. J. Zhou, *J. Physiol.*, 2004, **560**, 533–549.
43 R. C. Stacy, J. Demas, R. W. Burgess, J. R. Sanes and R. O. L. Wong, *J. Neurosci.*, 2005, **25**, 9347–9357.
44 D. Kerschensteiner and R. O. L. Wong, *Neuron*, 2008, **58**, 851–858.
45 J. H. Singer, R. R. Mirotznik and M. B. Feller, *J. Neurosci.*, 2001, **21**, 8514–8522.
46 M. Catsicas, V. Bonness, D. Becker and P. Mobbs, *Curr. Biol.*, 1998, **8**, 283–286.
47 W. T. Wong, J. R. Sanes and R. O. L. Wong, *J. Neurosci.*, 1998, **18**, 8839–8852.
48 S. I. Firth, C. T. Wang and M. B. Feller, *Cell Calcium*, 2005, **37**, 425–432.
49 E. Sernagor and N. M. Grzywacz, *J. Neurosci.*, 1999, **19**, 3874–3887.
50 Z. J. Zhou, *J. Neurosci.*, 1998, **18**, 4155–4165.
51 W. T. Wong, K. L. Myhr, E. D. Miller and R. O. L. Wong, *J. Neurosci.*, 2000, **20**, 351–360.
52 Z. J. Zhou and D. Zhao, *J. Neurosci.*, 2000, **20**, 6570–6577.
53 J. Zheng, S. Lee and Z. J. Zhou, *Nat. Neurosci.*, 2006, **9**, 363–371.
54 D. A. Butts, M. B. Feller, C. J. Shatz and D. S. Rokhsar, *J. Neurosci.*, 1999, **19**, 3580–3593.
55 R. H. Masland and M. Tauchi, *Trends Neurosci.*, 1986, **9**, 218–223.
56 J. Zheng, S. Lee and Z. J. Zhou, *Neuron*, 2004, **44**, 851–864.
57 J. Tabak, W. Senn, M. J. O'Donovan and J. Rinzel, *J. Neurosci.*, 2000, **20**, 3041–3056.
58 A. A. Penn, R. O. L. Wong and C. J. Shatz, *J. Neurosci.*, 1994, **14**, 3805–3815.
59 C. L. Torborg, K. A. Hansen and M. B. Feller, *Nature Neurosci.*, 2005, **8**, 72–78.
60 K. F. Fischer, P. D. Lukasiewicz and R. O. L. Wong, *J. Neurosci.*, 1998, **18**, 3767–3778.
61 D. Stellwagen, C. J. Shatz and M. B. Feller, *Neuron*, 1999, **24**, 673–685.
62 E. Marder and J. M. Goaillard, *Nat. Rev. Neurosci.*, 2006, **7**, 563–574.
63 R. D. Traub, R. Miles and R. K. S. Wong, *Science*, 1989, **243**, 1319–1325.
64 N. X. Tritsch, E. Yi, J. E. Gale, E. Glowatzki and D. E. Bergles, *Nature*, 2007, **450**, 50–56.
65 F. Mammano, G. I. Frolenkov, L. Lagostena, I. A. Belyantseva, M. Kurc, V. Dodane, A. Colavita and B. Kachar, *J. Neurosci.*, 1999, **19**, 6918–6929.
66 S. Pitchford, J. W. Day, A. Gordon and D. Mochly-Rosen, *J. Neurosci.*, 1992, **12**, 4540–4544.
67 T. Takahashi, *Cell Calcium*, 2005, **37**, 507–511.
68 M. B. Feller, *Neuron*, 1999, **22**, 653–656.
69 M. J. O'Donovan, *Curr. Opin. Neurobiol.*, 1999, **9**, 94–104.
70 L. Valor, P. Charlesworth, L. Humphreys, C. Anderson and S. Grant, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 4658.
71 J. Beggs and D. Plenz, *J. Neurosci.*, 2003, **23**, 11167–11177.
72 V. Pasquale, P. Massobrio, L. Bologna, M. Chiappalone and S. Martinoia, *Neuroscience*, 2008, **153**, 1354–1369.
73 L. Abbott and R. Rohrkemper, *Prog. Brain Res.*, 2007, **165**, 13–19.
74 J. Thivierge and P. Cisek, *J. Neurosci.*, 2008, **28**, 7968.
75 J. Tabak, J. Rinzel and M. J. O'Donovan, *J. Neurosci.*, 2001, **21**, 8966–8978.