Molecular Neurobiology

ISSN0893-7648/04/30(3): 341–357/$25.00

Gap Junctions

Their Importance for the Dynamics of Neural Circuits

Lorena Rela* and Lidia Szczupak

Laboratorio de Fisiología y Biología Molecular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
Instituto de Fisiología, Biología Molecular y Neurociencias, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

Abstract

Electrical coupling through gap junctions constitutes a mode of signal transmission between neurons (electrical synaptic transmission). Originally discovered in invertebrates and in lower vertebrates, electrical synapses have recently been reported in immature and adult mammalian nervous systems. This has renewed the interest in understanding the role of electrical synapses in neural circuit function and signal processing. The present review focuses on the role of gap junctions in shaping the dynamics of neural networks by forming electrical synapses between neurons. Electrical synapses have been shown to be important elements in coincidence detection mechanisms and they can produce complex input–output functions when arranged in combination with chemical synapses. We postulate that these synapses may also be important in redefining neuronal compartments, associating anatomically distinct cellular structures into functional units. The original view of electrical synapses as static connecting elements in neural circuits has been revised and a considerable amount of evidence suggests that electrical synapses substantially affect the dynamics of neural circuits.

Index Entries: Gap junctions; electrical synapse; neural circuit; signal processing; compartmentalization; synchrony; input–output function; rectification; coincidence detection; electrical modulation.

Introduction

Gap junctions are clusters of intercellular channels connecting the cytoplasms of two cells. They allow the flow of metabolites and ions between the connected cells, permitting metabolic and electrical coupling, respectively. The proteins that form vertebrate and invertebrate gap junctions—connexins (*1*) and innexins (*2*), respectively—are very different in terms of sequence (*see also* ref. *3*). But they have similar membrane topology (*4*) and they share the ability to form intercellular channels (*5*,*6*).

Received 3/5/04; Accepted 6/3/04
*Author to whom correspondence and reprint requests should be addressed. E-mail: lrela@fbmc.fcen.uba.ar

Every intercellular channel of the cluster comprises one multimeric hemichannel from each participating cell. Each species contains a family of gap-junction monomers and any given cell usually expresses more than one type of monomer. As a result, cells may generate a diversity of gap junctions (*7*), determined by (a) the expression of different gap-junction monomers (i.e., to form various types of homomeric or heteromeric hemichannels within a given cell); (b) the use of identical or different hemichannels to constitute each intercellular channel between two cells (homotypic or heterotypic channels, respectively); and (c) the homogeneity or heterogeneity of the gap junctions themselves (i.e., clusters containing one type, or more than one type of channel).

During the last 50 yr, considerable knowledge has accumulated, including descriptions of the molecular structure of gap-junction proteins (*8,9*), how structure contributes to the gating properties and permeability of the channels (*10–16*), and how these properties are changed by posttranslational modifications or modulatory ligands (*17–22*). Many genes that code for gap-junction proteins in vertebrates and invertebrates have been identified, and their expression patterns described (*2,7,16,23–27*). Experimental deletion—or interference with the expression—of connexin or innexin genes lead to physiological alterations related to dysfunction of gap junctions (*28,29*). In addition, human pathologies have been described, in which abnormal intercellular communication correlates with specific mutations in connexin genes (*9,29*).

Gap junctions are of particular functional importance for nervous systems, as the electrical coupling mediated by these intercellular channels constitutes a means of communication between neurons, the electrical synapse. This type of synapse transmits signals from one neuron to another by means of the direct flow of current.

Measuring the strength of an electrical synapse requires simultaneous intracellular electrical recordings from the two, coupled cells. Typically, a square current step is injected into one cell (the manipulated cell), and the steady-state change in the membrane potential of both cells is measured. The ratio of the two membrane potential changes (i.e., the proportion of the effective current injected in the manipulated cell that is "seen" by the other cell) is known as the coupling coefficient. It is important to note that the coupling coefficient is influenced both by the conduction properties of the gap junctions themselves, and by the length constant of the cells between the two recording sites.

In contrast to the spike-mediated release of neurotransmitters in chemical synapses, communication mediated by electrical synapses does not require that the presynaptic signal reach firing threshold. Depending on the coupling coefficient, even small subthreshold changes in the membrane potential of one of the neurons may be transmitted to the coupled cell (although it may be hard to detect at the recording site owing to spatial attenuation).

The question regarding the role that electrical synapses play in the nervous systems of higher vertebrates has been discussed since such synapses were initially studied in invertebrates and lower vertebrates (*30–33*).

More recently, dual patch recordings have allowed the discovery of electrical synapses in the nervous systems of developing mammals (*34,35*). The transient character of the massive expression of electrical synapses in some systems suggested that their function was restricted to the immature nervous system, so that a role for such synapses in the development of neuronal connections was postulated (*36,37*).

The demonstration of dye coupling among different cell types (*38*), together with anatomical studies (*39*) have suggested for decades that electrical synapses could be prominent in the adult mammalian retina. However, the existence of electrical coupling between identified neurons in different regions of the adult mammalian brain has been hard to demonstrate because these electrical synapses are found between a restricted population of neurons (*40,41*). Moreover, limitations in the optical techniques has made it difficult to identify

neuron types morphologically (*42*). Recently, the use of infrared differential interference contrast microscopy and, independently, the creation of transgenic mice expressing a green fluorescent protein (GFP) in desired specific types of neurons made it possible to identify neurons more easily, facilitating the performance of dual recordings from specific pairs of neurons (*42–45*). The discovery of electrical synapses between adult neurons in vertebrates suggests that they could have roles in mature circuit function, besides their role during development.

In parallel with these technical improvements that reveal the presence of electrical synapses in the adult mammalian brain, ongoing studies, largely in invertebrate systems have led to a substantial revision of the original view of electrical synapses as static connecting elements in neural circuits. Instead, a considerable amount of evidence suggests that electrical synapses affect the dynamics of brain circuits (*20,46,47*). Accordingly, this review focuses on a very restricted—although highly relevant–aspect of gap-junction physiology: their role in shaping the dynamics of neural networks by forming electrical synapses between neurons. Computational modeling has substantially contributed to the understanding of the role played by electrical synapses in the dynamics of neural networks (*48–55*). However, in this review we focus only on results and interpretations provided by physiological data.

Redefining Synchrony: Compartmentalization

The generally accepted role of electrical synapses in neural circuits is their contribution to the synchronous firing of coupled neurons (*56–64*); because of this, they have been regarded as synchronizing synapses (*65*). It is important to note that other mechanisms for neural synchronization are known, but are not being considered here. Instead, we wish to broaden the view of the function of electrical synapses by considering them as elements that define functional compartments. Two neurons in isolation may be considered as two distinct anatomical compartments, each delimited by its membrane. But, if these neurons are connected by electrical synapses, the voltage differences between them will be reduced, promoting voltage homogeneity, and the pair may constitute a single functional compartment. The degree of voltage homogeneity in that compartment is determined by the coupling strength, which is related to the current flow between the coupled cells through the electrical synapses. Such neuronal compartmentalization was illustrated in networks of synchronized inhibitory interneurons in the mammalian neocortex (*40,66*), where groups of interneurons of the same type are connected by electrical synapses, constituting a syncytium. This syncytial organization has been proposed as a mechanism to amplify inputs and to increase the robustness of outputs (*67–70*), because a single input to one of the cells can spread to the coupled cells recruiting them to form a population that responds coordinately, amplifying the original signal. For example, when electrical connections are established between groups of neurons with axons running in parallel toward a common target, they work as a lateral recruiting system that guarantees robust outputs (*71,72*).

The anatomical location of electrical synapses in the connected neurons relative to their spike initiation sites—or to chemical synaptic inputs—can be an important factor in compartmentalization. Electrical synapses may exert a coupling effect restricted to particular branches of two neurons. As a result, there could be greater voltage homogeneity between parts of two neurons than within each neuron in itself, so that these coupled branches function as a compartment. A paradigmatic example of this situation was encountered in interneurons of the feeding circuit of *Aplysia* (*73*). In this circuit, cerebral-to-buccal interneurons (CBI-5/6) have their somata in the cerebral ganglion and extend arborizations to the buccal ganglion (Fig. 1A). The somata can gen-

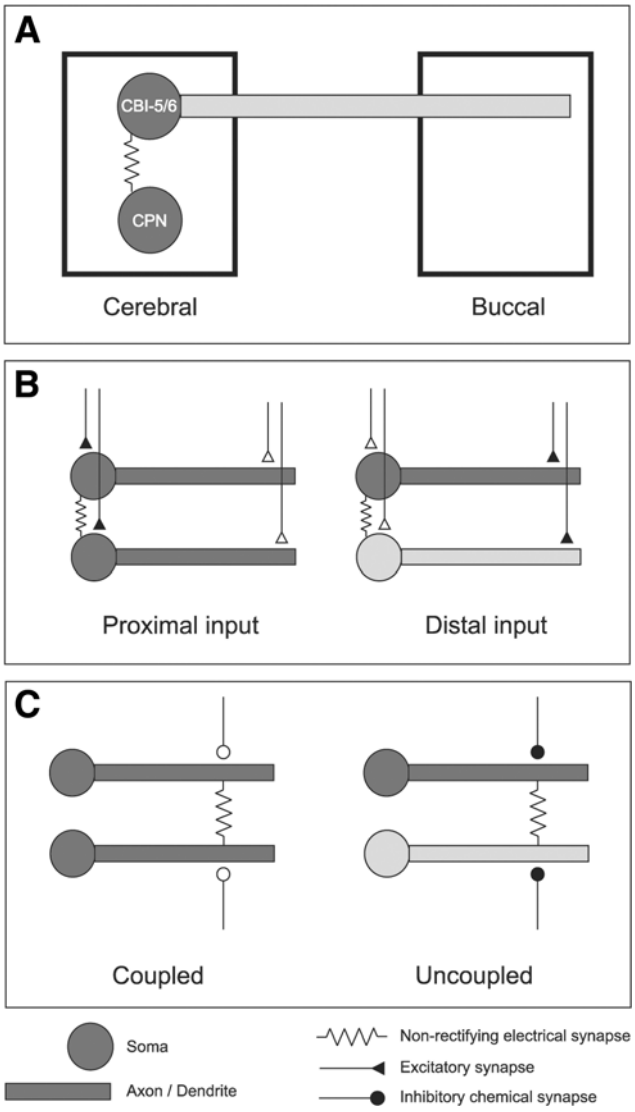

Fig. 1. Compartmentalization and synchrony. (A) Cerebral-to-buccal interneurons (CBI-5/6) extend from the cerebral ganglion (represented by the left rectangle) to the buccal ganglion (represented by the right rectangle) of the mollusk *Aplysia* and are part of its feeding circuit (only one is shown for simplicity). They are electrically coupled to the cerebral-to-pedal neuron (CPN) at the level of the soma, located in the cerebral ganglion. Plateau potentials generated at the soma of CBI-5/6 are transmitted to the coupled neurons recruiting them to form a unique compartment (shown in dark gray). As the spike initiation zone is in the axon, at a site electrically distant from the soma, it is not affected by the plateau potentials and the axon behaves as a different compartment (shown in light gray). **(B)** The medial rectus motoneurons of fish are electrically coupled at the somatic level. They receive excitatory inputs (triangles) at the soma and at a distal dendritic compartment. Suprathreshold inputs (black triangles indicate the active input) at the soma cause both cells to spike synchronously, constituting a single compartment (both cells in dark gray). Suprathreshold inputs at the distal sites cause both cells to spike asynchronously, constituting separate compartments (both cells in different gray hues). **(C)** Expansion motoneurons of *Navanax inermis* are electrically coupled, constituting a single compartment (dark gray). Chemical inhibitory synaptic inputs (circles) act near the coupling site. Activation of these chemical synapses (black circles) 'short circuits' the electrotonic spread through the electrical synapse, splitting the cells in separate functional compartments (different gray hues).

erate plateau potentials that initiate sustained bursts in other neurons in the cerebral ganglion to which CBI-5/6 are electrically coupled. Because these plateau potentials do not reach the distal spike initiation zone in the axons of the CBI-5/6, no output signals are sent to the buccal ganglion. In this example, the somata of CBI-5/6 and their coupled neurons in the cerebral ganglion constitute a compartment that is functionally independent of another compartment formed by the branches of CBI-5/6 in the buccal ganglion.

Another example that shows the importance of the location of electrical contacts relative to active sites and synaptic inputs is provided by goldfish oculomotor neurons, which are electrically coupled at the level of the soma (*31*) (Fig. 1B). It has been proposed that chemical synaptic inputs acting near the soma initiate spikes in this compartment making the coupled neurons fire relatively synchronously (Fig. 1B, left panel). This activity was correlated with saccadic eye movements, which would need a robust output from the oculomotor neurons.

Alternatively, inputs arriving at distal, presumably uncoupled dendrites of these motoneurons would excite distal active zones eliciting asynchronous activity and resulting in slower eye movements (Fig. 1B, right panel).

These two examples illustrate how functional electrical compartments do not necessarily coincide with single neurons. The last one also illustrates that compartments are not fixed structures and electrical and chemical synapses may interact to define them (i.e., when the chemical input reaches the dendrites, the oculomotor neurons behave as two different compartments; when the input reaches the soma, they behave as a unique compartment).

Another case of compartmentalization determined by the interaction between electrical and chemical synapses was described in the mollusk *Navanax inermis* (*74*). Expansion motoneurons mediate the expansion of the pharynx in this organism, as part of its feeding behavior. These motoneurons are electrically coupled, which is important to elicit a robust response. When chemical inhibitory inputs to the expansion motoneurons are activated, the coupling among them is abolished. It was postulated that those synaptic inputs act very close to the coupling site and could "short circuit" the spread of electrotonic current by reducing the membrane resistance in the region where the motoneurons are coupled (Fig. 1C). In this case, chemical synapses regulate the effectiveness of electrical synapses to recruit expansion motoneurons into a single compartment, working as a switch between the coupled (Fig. 1C, left panel) and uncoupled states (Fig. 1C, right panel). A similar network arrangement of chemical and electrical synapses was proposed for the cat inferior olive (*75*).

Producing Complex Input–Output Functions

Any given element of a circuit may, in principle, be characterized by its input–output function (i.e., the relationship between the incoming and outgoing signals). The input–output function of a synapse can be described by the relationship between the presynaptic and postsynaptic changes in membrane potential. For example, at an inhibitory chemical synapse, depolarizing the presynaptic cell typically hyperpolarizes the postsynaptic one, whereas hyperpolarizing the presynaptic cell may cause no change in the postsynaptic one. When neurons are connected by more than one type of synapse, complex input–output functions appear. Several examples of this emergent complexity have been documented at synapses comprising combinations of chemical and electrical synapses. One case is the connection of two neurons (Ai and A neurons) by excitatory chemical and electrical synapses in the prey capture neural network of the mollusk *Clione limacina* (*76*) (Fig. 2A). When a spike is generated in the Ai cell, the A cell shows a complex postsynaptic depolarization owing to the effect of the excitatory neurotransmitter, and also owing to the direct flow of current through the electrical connection. The depolarization elicited in the postsynaptic neuron can in turn be conducted back to the presynaptic one through the electrical synapse, constituting a positive feedback loop that contributes to long-lasting synchronized discharges in the neurons involved. Thus, this arrangement results in a robust input–output function for the connection between the two cells, which is different from the properties of either of the synapses taken alone.

Another example, involving the combination of electrical and inhibitory chemical synapses, was observed for neurons involved in the pyloric rhythm of the lobster (*77*). The pyloric rhythm is a motor pattern used in processing food from stomach to gut. The pyloric late (PL) and lateral pyloric (LP) neurons, which are involved in the generation of this rhythm, are coupled by electrical synapses and also by inhibitory chemical synapses (Fig. 2B). When a hyperpolarizing pulse is applied to a presynaptic PL neuron, the postsynaptic LP neuron hyperpolarizes because of the direct spread of current through the electrical

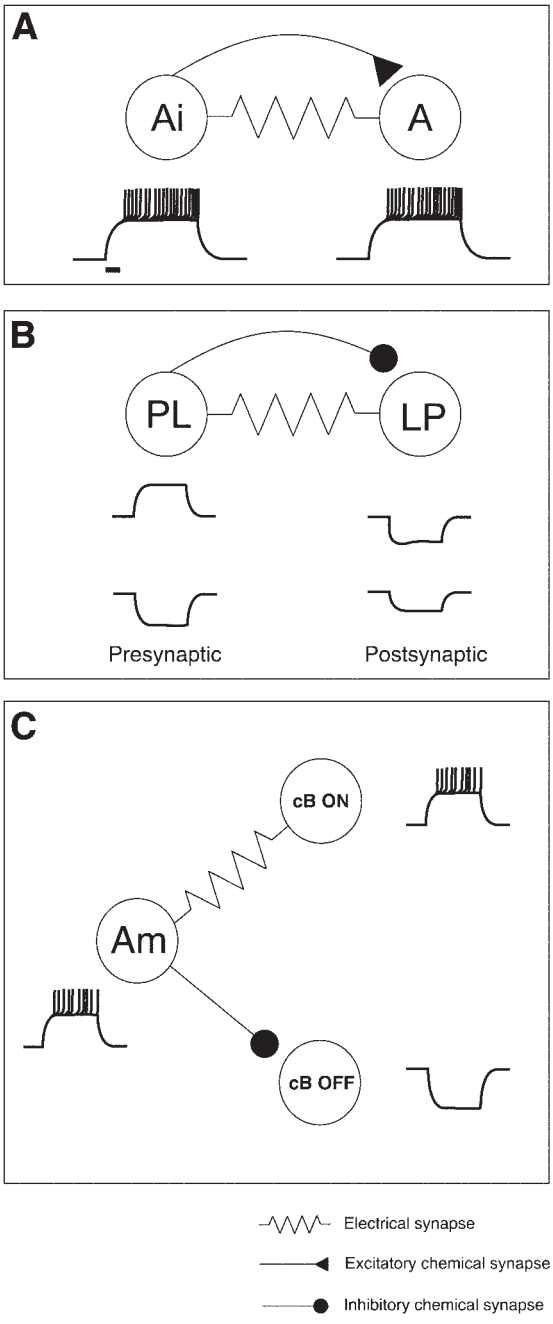

Fig. 2. Complexity of input-output functions. (A) Cerebral A interneurons (Ai) of *Clione limacina* are electrically connected to cerebral A motoneurons (A), defining a single compartment with synchronized activity. Also, Ai excites A via chemical synapses (black triangle). Excitation of A is conducted back to Ai contributing to a positive feedback mechanism that produces long-lasting synchronized activity. The schematic voltage traces on the bottom represent the effect of giving a short-lasting depolarizing current step to Ai (small bar under the left trace) (simplified from *76*). **(B)** Diagram of the synaptic interactions between pyloric late (PL) and lateral pyloric (LP) neurons of the stomatogastric ganglion of the lobster. PL and LP neurons interact via electrical synapses and also by inhibitory chemical synapses (black circle). Underneath the diagram, the schematic traces represent the presynaptic voltage deflection (left traces) and the corresponding postsynaptic responses (right traces). When the PL neuron is hyperpolarized, it inhibits the LP neuron by passing current through the electrical synapse. When the PL neuron is excited, it inhibits the LP neuron via the chemical synapse, which overcomes the depolarizing effect of the electrical synapse (simplified from *77*). **(C)** Amacrine cells of the retina (Am) are connected to ON cone bipolar cells (cB ON) through electrical synapses and to OFF cone bipolar cells (cB OFF) through chemically inhibitory synapses. Thus, excitation of Am cells excites cB ON and inhibits cB OFF simultaneously. The schematic trace on the left represents the presynaptic voltage and the traces on the right represent the parallel postsynaptic responses.

synapse. When a depolarizing step is applied to the PL neuron, the LP neuron also hyperpolarizes, because the chemically mediated inhibition overcomes the electrically mediated excitation. Here again, the combination of the electrical and chemical synapses results in an input–output function different from the functions of either one taken independently.

Another interesting case has been reported for cells in the vertebrate retina (*78,79*). Type AII amacrine cells depolarize when the retina is illuminated, as a result of polysynaptic inputs from the rod photoreceptors. They connect to ON cone bipolar cells (cB ON) via electrical synapses, making them depolarize in response to light by transmitting excitation (Fig. 2C). They also connect to OFF cone bipolar cells (cB OFF) via inhibitory chemical synapses, making them hyperpolarize in response to light by translating excitation into

inhibition. This arrangement of electrical and chemical synapses allows amacrine cells to excite cB ON and inhibit cB OFF simultaneously. In this way, the same excitatory signal is distributed as excitation and inhibition to two functionally different populations of neurons, allowing different and parallel input–output functions. It remains to be studied whether the use of electrical excitatory synapses, instead of chemical ones, is of particular importance for vision.

Symmetrical and Asymmetrical Electrical Synapses

Electrical synapses may be symmetrical, which means that the coupling coefficient between the coupled cells will be of the same magnitude regardless of which of the cells is taken as presynaptic (i.e., which of the cells is the manipulated cell). But this is not always the case, as some electrical synapses are asymmetrical, and the coupling coefficient will attain different values depending on which cell is taken as presynaptic.

This asymmetry may result from differential intrinsic properties of the coupled cells, unrelated to the characteristics of the gap junctions themselves. For example, if the connected cells have different input resistance values (owing to different ion channel densities or different sizes), the electrical connection will be most likely an asymmetrical one. In this case, the coupling coefficient will have a lower value when the cell with the lower input resistance is the postsynaptic one, because the current flowing through the gap junctions is less effective at producing a voltage deflection in that cell (*80,81*).

Asymmetries in the coupling coefficient may also arise when the molecules constituting the gap junctions are voltage-dependent (*13*). In consequence, the conductance of the gap junctions becomes a function of the transjunction potential (i.e., the potential across the gap; but *see also* ref. *12*). In this situation, the channels will limit the flow of current in a specific direction and behave as rectifiers. Lets consider as an example two cells, A and B, connected by rectifying junctions whose conductance increases as the transjunctional potential (V_A–V_B) is more positive. Thus, if the membrane potential of cell A is more positive than that of cell B the gap junction is in its open state and electrical signals can flow in both directions, provided that they do not invert the polarity that grants the open state. However, other types of rectification exist (*82*).

Most of the molecular studies describing the properties of specific gap-junction proteins have been performed by means of electrophysiological recordings from coupled oocyte pairs expressing particular exogenous connexins. It has been postulated that most hemichannels have a voltage sensor (sensing the transjunctional voltage), which is responsible for the transitions between open and closed states. The voltage sensitivity of the whole channel depends on the combined sensitivity of the hemichannels that constitute it. In this way, symmetrical channels (i.e., made by identical hemichannels) will be nonrectifying, and may be voltage-sensitive or not. If there is symmetrical voltage-sensitivity, the coupling coefficient will vary with the transjunctional voltage, but it will be independent of the polarity of this voltage (Fig. 3, left panel). In contrast, rectifying channels are likely to be constituted by voltage-sensitive hemichannels of different gating polarities (*12,20*) (Fig. 3, right panel).

Rectifying electrical synapses, where present, are crucial for the dynamics of neural circuits, because they determine the polarity of current flow among the electrically coupled elements, thus affecting signal processing. Rectifying electrical synapses have been described in a wide variety of organisms. Examples of particular interest are described in the following sections.

Coincidence Detection

The capability to detect coincidental inputs in the nervous system may be important to

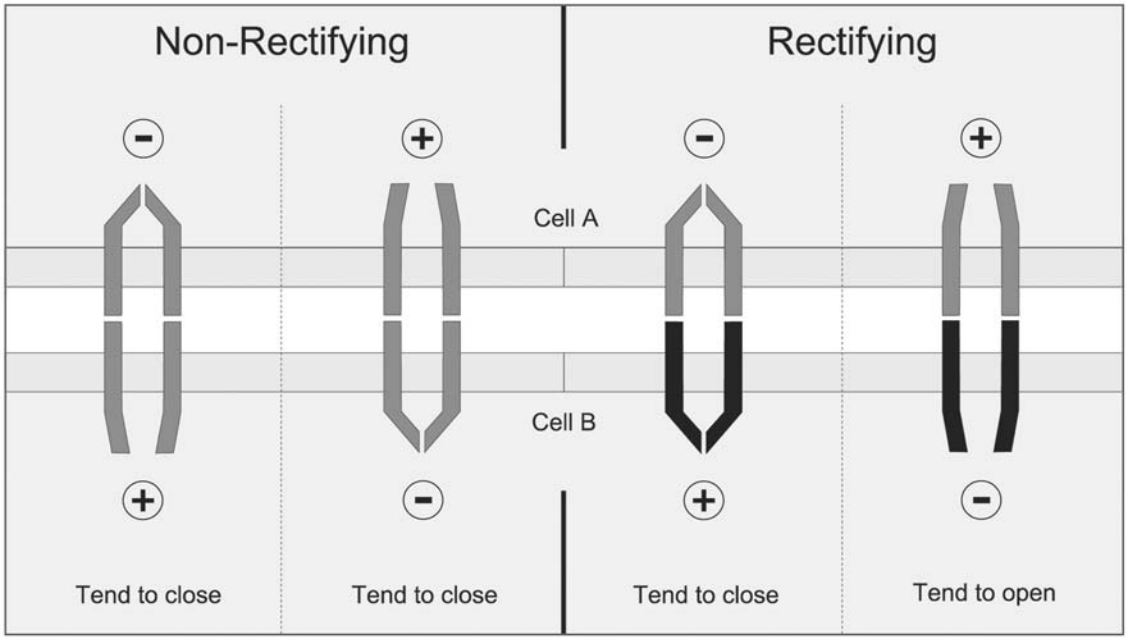

Fig. 3. Voltage-dependence and rectification. Schematic representation of gap junction channels connecting two cells (A and B). The horizontal gray bars represent the membranes of both connected cells. **Left panel:** An example of a voltage-dependent **non-rectifying** channel that closes when the junction potential is increased. Because the channel is symmetrical (composed of identical hemichannels), the behavior is independent of which side is hyperpolarized. Both hemichannels will be on the open state when the voltage difference between the cells is not too high (not shown). **Right panel:** An example of a voltage-dependent **rectifying** channel that closes when cell A is more hyperpolarized than cell B. The channels are composed of hemichannels of different gating polarities. The hemichannels in cell A (gray) will close when this cell is more hyperpolarized and will open when it is more depolarized. The opposite occurs with the hemichannels in cell B (black): they will open when this cell is more hyperpolarized and will close when it is more depolarized (modified from *20*).

achieve adaptive behaviors, like the localization of a sound source (*83*) or memory formation (*84*). A coincidence-detecting element of a neural circuit is one that integrates signals arriving from different sources, producing different responses when these sources are coincident and when they are not. Different coincidence detection mechanisms have been described at the molecular (*85*) and cellular (*83,86*) levels.

Coincidence detection mechanisms are not necessarily complex. A very simple mechanism has been described in the retina that involves nonrectifying synapses between two neurons receiving different excitatory inputs in parallel (*45*) (Fig. 4A). Subthreshold inputs arriving to one of the neurons elicit subthreshold postsynaptic depolarizations. When inputs arrive coincidentally to both coupled neurons, they summate and cause both neurons to fire. By this mechanism, stimuli that are subthreshold can result in suprathreshold responses when arriving coincidentally. The radically different response of the postsynaptic neurons when there are coincident inputs to the system makes them good coincidence detectors.

A more complex coincidence detection mechanism, based on rectifying electrical synapses, was described in the escape circuit of the crayfish (*87*). One type of command

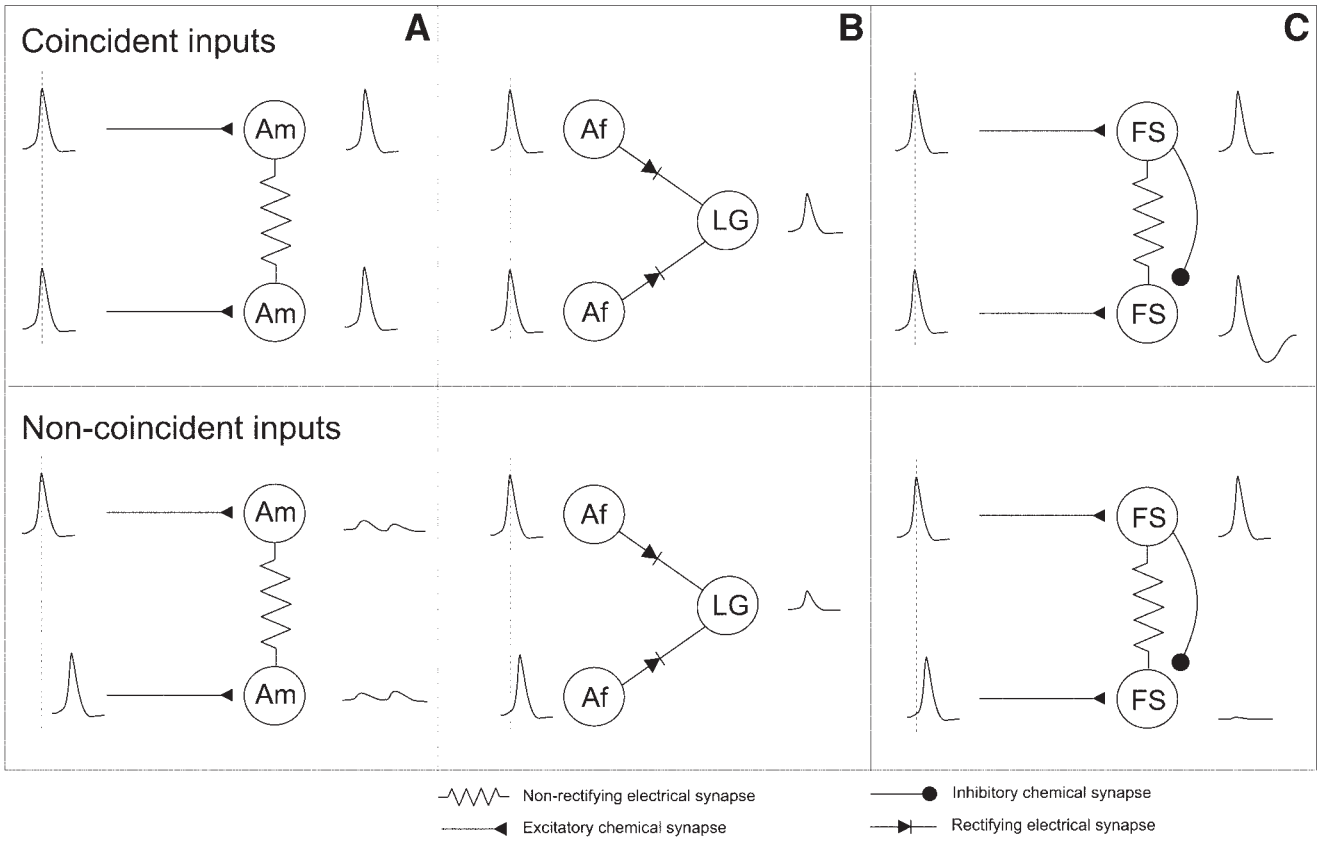

Fig. 4. Coincidence detection. Schematic representations of neuronal circuits that allow detection of coincident inputs. The presynaptic voltage changes are represented to the left of each circuit, whereas the postsynaptic voltage changes are schematized to the right. The vertical dotted lines aid the visualization of coincident or delayed input signals. The top panels show the output of each circuit upon coincident inputs and the bottom panels show the output to non-coincident inputs. **(A)** In the retina, excitatory inputs (black triangles) are transmitted in parallel, to electrically coupled amacrine (Am) cells. The postsynaptic responses elicited by coincident presynaptic activity summate and reach threshold to produce a spike. When the inputs are not coincident the postsynaptic responses do not summate and subthreshold excitatory postsynaptic potentials (EPSPs) are generated. **(B)** In the crayfish, convergent inputs from afferent fibers (Af) are transmitted to one lateral giant (LG) motoneuron. Af are connected to LG through rectifying electrical synapses that conduct depolarizing current from the first to the latter. The postsynaptic responses elicited by coincident presynaptic activity summate producing a large EPSP. When inputs are not coincident, the first one produces a postsynaptic response that reduces the synaptic conductance during the transmission of the second input, thus producing a small EPSP (redrawn from *88*). **(C)** In the neocortex, fast-spiking (FS) neurons may be connected both by non-rectifying electrical synapses and chemical inhibitory synapses (black circle). Excitatory inputs (black triangles) are transmitted to FS cells in parallel. Coincident inputs produce coincident excitation in both postsynaptic cells, which elicits synchronous spikes. The postsynaptic spike in the FS cell on bottom is followed by the inhibition produced by the simultaneous postsynaptic spike of the FS cell on top. When the inputs are not coincident, this inhibition can counteract the delayed excitatory input (redrawn from *89*).

neurons for the escape behavior, the lateral giant (LG) neurons receive inputs from primary afferents through rectifying electrical synapses. These synapses conduct depolarizing signals better from primary afferents to LG neurons than in the opposite direction and the electrical conductance is reduced when the LG neurons are depolarized. This strongly shapes the postsynaptic response in the LG neurons when presynaptic spikes are transmitted from the afferents. For example, consider a single postsynaptic neuron receiving convergent inputs from two afferents through rectifying synapses of this type (Fig. 4B). When coincident spikes arrive from the two afferents, they act as a single large input, producing a large postsynaptic response. In contrast, when spikes arriving from the two afferents are not coincident the second spike arrives when the conductance across the rectifying synapses is reduced (because the first spike depolarized the LG neuron), and produces little additional postsynaptic depolarization. The amplitude of the postsynaptic LG neuron response is dramatically reduced in the case of noncoincident inputs compared to coincident ones. This makes the LG neuron a coincidence detector (*88*).

Another, yet more complex arrangement of neuronal elements, involving the interaction of electrical and chemical synapses, allows fast-spiking (FS) inhibitory interneurons to detect coincidental activity of inputs in the mammalian neocortex (*89*). FS neurons can be connected by nonrectifying electrical synapses and chemical inhibitory synapses. As shown in Fig. 4C, when one FS cell receives signals from another FS cell both through electrical and chemical inhibitory synapses, the postsynaptic response to a presynaptic spike is biphasic. It consists of a fast depolarizing phase, mediated by the electrical synapse, followed by a slow hyperpolarizing phase. This hyperpolarization is caused by the after-hyperpolarization of the presynaptic spike, transmitted through the electrical synapse, combined with the inhibitory postsynaptic potential from the chemical synapse. When coincident afferent inputs arrive to each one of the coupled neurons, the depolarizing phases generated in both cells coincide in time and summate, eliciting synchronous postsynaptic activity. In contrast, when the inputs received by each FS neuron are noncoincident, a possible outcome is that the FS neuron receiving the first input will inhibit the other FS neuron by the time it receives its excitatory input, reducing its excitability and diminishing its ability to produce a spike in response to the delayed input (Fig. 4C). Taking into consideration that the layer of FS cells forms a vast network of interconnected elements, coincident inputs onto many FS cells may cause a strong network output. In contrast, noncoincident inputs probably will cancel out resulting in a very weak output of the network. Thus, this system responds very differently when coincident and non-coincident inputs arrive, behaving as a coincidence detector.

Electrical Neuromodulation

In the previous sections, we have seen examples of how electrical synapses shape the dynamics of neural circuits in vertebrates and invertebrates. In our own work, we have recently characterized a circuit formed by a population of neurons connected by electrical and chemical synapses, that serves a modulatory function in the nervous system of the leech (*90*). An important element of this circuit is a pair of nonspiking (NS) neurons present in each midbody ganglion of the leech nervous system. The NS neurons are coupled to virtually all the excitatory motoneurons via rectifying electrical synapses that conduct only hyperpolarizing current from the NS neurons to the motoneurons, and only depolarizing current in the opposite direction (*91*). Thus, hyperpolarizing the NS neurons may inhibit the whole population of motoneurons in a segment of the animal. This widespread inhibition is probably aided by the fact that excitatory motoneurons are electrically coupled among themselves through nonrectifying electrical synapses (*90,92*).

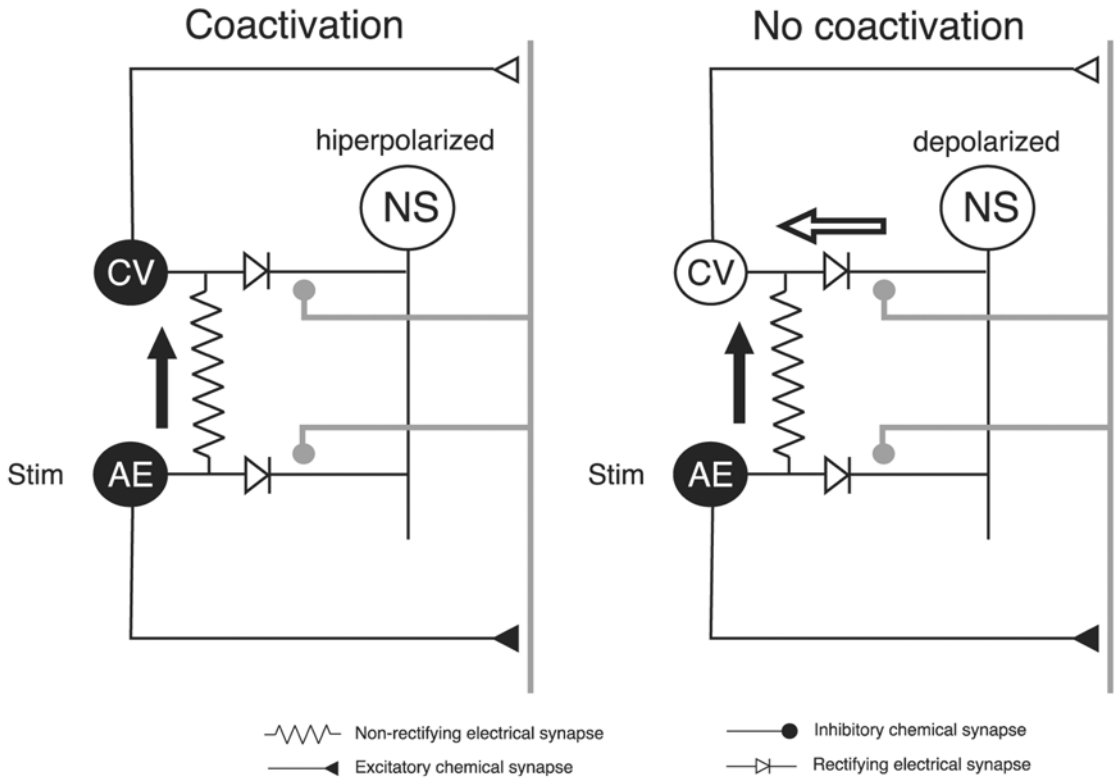

Fig. 5. Electrical neuromodulation. Simplified schematic representation of a circuit in the leech nervous system (only one NS neuron of the pair is shown for simplicity). Both schemes show that motoneurons (AE and CV) are connected through a chemical excitatory synapse (triangles) to an interneuronal layer (gray lines), which is connected to the NS neuron through a chemical inhibitory synapse (small circles). The motoneurons are electrically coupled among themselves through non-rectifying electrical synapses and are connected to the NS neuron through rectifying electrical synapses. The latter conduct hyperpolarizing current from the NS neuron to the motoneurons and depolarizing current in the opposite direction. Excitation of the AE motoneuron (Stim), when the NS neuron is depolarized (right panel), excites the interneuronal layer (black triangle) evoking a hyperpolarization in the NS neuron that is transmitted to the CV motoneurons (open arrow) and counteracts the excitation transmitted through the electrical synapse from AE (black arrow). Instead, when the NS neuron is hyperpolarized close to the reversal potential of the inhibitory signal (left panel) the main input to the CV motoneuron is the excitation transmitted through electrical synapses from the AE motoneuron (black arrow), which results in coactivation of both motoneurons (both somata in black) (redrawn from *90*).

In addition to the electrically mediated interactions, the motoneurons are linked to the NS neurons via inhibitory interneurons that transmit their signals through chemical synapses (Fig. 5). Because of the latter type of interaction, when motoneurons are excited, hyperpolarizing responses are generated in the NS neurons. In turn, this hyperpolarization can be transmitted back to the motoneurons through the rectifying synapses, establishing a negative feedback. As expected from a chemical synapse, the expression of the hyperpolarization elicited by motoneurons in NS neurons depends on the membrane potential of the latter: the amplitude of the response is bigger as the NS neurons are depolarized. In

contrast, when NS neurons are hyperpolarized, approaching the reversal potential of the inhibitory chemical synapse (around –60 mV), the only response elicited by the excitation of motoneurons in NS neurons is a small depolarizing current transmitted through the rectifying synapses. Thus, the membrane potential of NS neurons regulates the extent of the negative feedback.

Given the set of interactions described here, when a motoneuron is excited, it can excite other motoneurons directly, but it can also inhibit them through the activation of the inhibitory interneurons that hyperpolarize the NS neurons. The net result of the two responses depends on the membrane potential of the NS neurons. When the NS neurons are depolarized, active motoneurons transmit signals to other motoneurons via two pathways: direct excitatory signals through electrical connections, and indirect inhibitory signals mediated by the feedback from the chemically hyperpolarized NS neurons. Normally, these two signals counterbalance each other and the excited motoneuron has no net effect on other motoneurons (Fig. 5, right panel). In contrast, if the NS neurons are hyperpolarized, the feedback mechanism is inactivated, and the active motoneuron transmits only the direct excitatory input through electrical synapses to other motoneurons. This has the effect of promoting the co-activation of motoneurons (Fig. 5, left panel).

Thus, this circuit has the capacity to regulate the coactivity of motoneurons, depending on the state of the NS neurons. As a result of the profuse connectivity of NS neurons with all excitatory motoneurons, this circuit could be a suitable way to shape motor outputs, providing to the implementation of behavioral choice (*93*). Previous studies have revealed that leeches execute distinct behaviors by activating neural networks drawn from relatively small sets of segmentally iterated sensory neurons, motoneurons, and interneurons in different combinations (*93*). We are currently investigating the performance of this NS circuit element in association with particular motor behaviors. Because the NS neurons directly influence motor networks, they may function as decision makers at a low level of the decision-making hierarchy (*94*). Returning to the idea that electrical synapses are elements that can redefine neuronal compartments, the NS circuitry represents a good example of how the compartments can be dynamically modified by activity in the nervous system. The motoneurons can constitute a functional unit when the electrical synapses that link them are the preponderant connection. The circuitry around the NS neurons redefines the "motoneuronal compartment" as a function of the activity in the motoneurons and the NS membrane potential.

In evaluating the consequences of using electrical synapses in the distribution of inhibitory inputs to motoneurons, it is important to notice that signals transmitted through electrical synapses do not generate the phasic changes in input resistance that characterize chemical synaptic transmission. Thus, they may constitute an inhibitory mechanism that does not reduce the sensitivity to other concomitant inputs.

We interpret the function of the NS circuit as a neuromodulatory one, because it may affect the dynamics of the motoneuronal network. But in contrast to the known chemical modulatory mechanisms (*95*), it is mediated by electrical synapses and so we refer to this effect as "electrical neuromodulation."

The thorough description of such a network in the leech nervous system was possible because of the relatively simple organization of the nervous system of this annelid, in which neurons can be readily identified from animal to animal. However, such a premotor network mechanism may be encountered in other organisms.

Conclusion

The work described here illustrates that, in contrast to early interpretations, electrical synapses—by themselves or in combination with chemical ones—are not rigid elements of neural circuits limited to ensure synchronicity,

but rather contribute in important ways to change the dynamics of neural circuits (*7,46,65,96*).

A major obstacle to addressing the function of electrical synapses in nervous systems in general is the lack of selective gap junction antagonists, that do not alter (directly or indirectly) the intrinsic properties of neurons, as is the case for most of the drugs available (*97*). Interfering with the expression of specific gap junction genes will be an important experimental approach to elucidate their roles in specific neural circuits. Knockout animals for specific connexins have provided information about these roles, but owing to undesirable developmental effects, like embryo lethality (*29*), this approach is problematic at best. Tissue-specific knockouts, which avoid these problems, gave promising results (*98,99*), but knockouts targeted to different nervous tissues or different developmental stages have not been obtained yet. Finally, the manipulation of connexin genes to produce connexins fused to fluorescent proteins shows promise. This technique enabled the investigators to observe fluorescent plaques that show the location, structure and dynamics of gap junctions in cultured cells expressing these constructs (*100–102*). It remains to be seen whether transgenic animals expressing these fusion proteins are useful for visualizing the sites of contact between coupled neurons *in situ*. That information may be of crucial importance to understand the relation between the anatomical localization of electrical synapses and signal processing in neural circuits

Acknowledgments

The authors thank Dr. María Ana Calviño, Lic. Sergio Daicz, Dr. William Kristan, Dr. Gustavo Murer, Lic. Rafael Pagani, and Lic. Mariano Rodriguez for their valuable comments on the text; and Dr. Lukas Buhler and Dr. David Weisblat for their substantial contribution to the present manuscript. The authors thank the Biblioteca Virtual de Biotecnología para las Américas for providing many of the papers cited in this review. This work was supported with grants from Fundación Antorchas, NIH-FIRCA, Human Frontier for Science Research and Agencia de Promoción Científica y Tecnológica to LS.

References

1 Bennett M. V. L., et al. (1991) Gap junctions: new tools, new answers, new questions. *Neuron* **6,** 305–320.
2. Phelan P. and Starich T. A. (2001) Innexins get into the gap. *BioEssays* **23,** 388–396.
3. Bruzzone R., et al. (2003) Pannexins, a family of gap junction proteins expressed in brain. *Proc. Natl. Acad. Sci. USA* **100,** 13644–13649.
4. Hua V. B., et al. (2003) Sequence and phylogenetic analyses of 4 TMS junctional proteins of animals: connexins, innexins, claudins and occludins. *J. Membr. Biol.* **194,** 59–76.
5. Swenson K. I., et al. (1989) Formation of gap junctions by expression of connexins in xenopus oocyte pairs. *Cell* **57,** 145–155.
6. Landesman Y., et al. (1999) Innexin-3 forms connexin-like intercellular channels. *J. Cell Sci.* **112,** 2391–2396.
7. Dermietzel R. (1998) Gap junction wiring: a "new" principle in cell-to-cell communication in the nervous system? *Brain Research Rev.* **26,** 176–183.
8. Kumar N. M. and Gilula N. B. (1996) The gap junction communication channel. *Cell* **84,** 381–388.
9. Evans W. H. and Martin P. E. M. (2002) Gap junctions: structure and function. *Mol. Membr. Biol.* **19,** 121–136.
10 Moreno A. P., et al. (1994) Gap junction channels: distinct voltage-sensitive and -insensitive conductance states. *Biophys. J.* **67,** 113–119.
11. Veenstra R. D., et al. (1995) Selectivity of connexin-specific gap junctions does not correlate with channel conductance. *Circ. Res.* **77,** 1156–1165.
12. Oh S., et al. (1999) Molecular determinants of electrical rectification of single channel conductance in gap junctions formed by connexins 26 and 32. *J. Gen. Physiol.* **114,** 339–364.
13. Revilla A., et al. (2000) Molecular determinants of membrane potential dependence in vertebrate gap junction channels. *Proc. Natl. Acad. Sci. USA* **97,** 14,760–14,765.

14. Stebbings L. A., et al. (2000) Two drosophila innexins are expressed in overlapping domains and cooperate to form gap-junction channels. *Mol. Biol. Cell* **11,** 2459–2470.
15. Cottrell G. T. and Burt J. M. (2001) Heterotypic gap junction channel formation between heteromeric and homomeric Cx40 and Cx43 connexons. *Am. J. Physiol.-Cell Ph.* **281,** C1559–C1567.
16. White T. W., et al. (2002) Virtual cloning, functional expression, and gating analysis of human connexin31.9. *Am. J. Physiol.-Cell Ph.* **283,** C960–C970.
17. Teranishi T., et al. (1983) Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in the carp retina. *Nature* **301,** 243–246.
18. Neyton J. and Trautmann A. (1986) Physiological modulation of gap junction permeability. *J. Exp. Biol.* **124,** 993–114.
19. Kwak B. R., et al. (1995) Differential regulation of distinct types of gap junctional channels by similar phosphorilation conditions. *Mol. Biol. Cell* **6,** 1707–1719.
20. Bennett M. V. L. (1997) Gap junctions as electrical synapses. *J. Neurocytol.* **26,** 349–366.
21. Bevans C. G. and Harris A. L. (1999) Regulation of connexin channels by PH. *J. Biol. Chem.* **27,** 3711–3719.
22. van Rijen H. V. M., et al. (2000) Human connexin40 gap junction channels are modulated by cAMP. *Cardiovasc. Res.* **45,** 941–951.
23. Bennett M. V., et al. (1994) The connexins and their family tree. *Soc. Gen. Physiol. Ser.* **49,** 223–233.
24. Dermietzel R., et al. (2000) Molecular and functional diversity of neural connexins in the retina. *J. Neurosci.* **20,** 8331–8343.
25. Venance L., et al. (2000) Connexin expression in electrically coupled postnatal rat brain neurons. *Proc. Natl. Acad. Sci. USA* **97,** 10,260—10,265.
26. Rozental R., et al. (2000) Temporal expression of neuronal connexins during hippocampal ontogeny. *Brain Res. Rev.* **32,** 57–71.
27. Stebbings L. A., et al. (2002) Gap junctions in drosophila: developmental expression of the entire innexin gene family. *Mech. Develop.* **113,** 197–205.
28. Trimarchi J. R. and Murphey R. K. (1997) The *shaking-B*^{2} mutation disrupts electrical synapses in a flight circuit in adult *drosophila*. *J. Neurosci.* **17,** 4700–4710.
29. White T. W. and Paul D. L. (1999) Genetic diseases and gene knockouts reveal diverse connexin functions. *Annu. Rev. Physiol.* **61,** 283–310.
30. Furshpan E. J. and Potter D. D. (1959) Transmission at the giant motor synapses of the crayfish. *J. Physiol. (London)* **145,** 289–325.
31. Korn H. and Bennett M. V. L. (1975) Vestibular nystagmus and teleost oculomotor neurons: functions of electrotonic coupling and dendritic impulse initiation. *J. Neurophysiol.* **38,** 430–451.
32. Werblin F. S. (1978) Transmission along and between rods in the tiger salamander retina. *Journal of Physiology* **280,** 449–470.
33. Detwiler P. B. and Hodgkin A. L. (1979) Electrical coupling between cones in the turtle retina. *J. Physiol. (London)* **291,** 75–100.
34. Peinado A., et al. (1993) Extensive dye coupling between rat neocortical neurons during the period of circuit formation. *Neuron* **10,** 103–114.
35. Sutor B. (2002) Gap junctions and their implications for neurogenesis and maturation of synaptic circuitry in the developing neocortex. *Results Probl. Cell Differ.* **39,** 53–73.
36. Personius K. E. and Balice-Gordon R. J. (2001) Loss of correlated motor neuron activity during synaptic competition at developing neuromuscular synapses. *Neuron* **31,** 395–408.
37. Pastor A. M., et al. (2003) Increased electrotonic coupling in spinal motoneurons after transient botulinum neurtotoxin paralysis in the neonatal rat. *J. Neurophysiol.* **89,** 793–805.
38. Vaney D. I. (1991) Many diverse types of retinal neurons show tracer coupling when injected with biocytin or neurobiotin. *Neurosci. Lett.* **125**.
39. Kolb H. and Famiglietti E. V. (1974) Rod and cone pathways in the inner plexiform layer of the cat retina. *Science* **186,** 47–49.
40. Gibson J. R., et al. (1999) Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* **402,** 75–79.
41. Galarreta M. and Hestrin S. (2001) Electrical synapses between GABA-releasing interneurons. *Nat. Rev. Neurosci.* **2,** 425–433.
42. Galarreta M. and Hestrin S. (2002) Electrical and chemical synapses among parvalbumin fast-spiking GABAergic interneurons in adult mouse neocortex. *Proc. Natl. Acad. Sci. USA* **99,** 12,438—12,443.

43. Meyer A. H., et al. (2002) *In vivo* labeling of parvalbumin-positive interneurons and analysis of electrical coupling. *J. Neurosci.* **22,** 7055–7064.
44. Bartos M., et al. (2002) Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. *Proc. Natl. Acad. Sci. USA* **99,** 13,222—13,227.
45. Veruki M. L. and Hartveit E. (2002) AII (rod) amacrine cells form a network of electrically coupled interneurons in the mammalian retina. *Neuron* **33,** 935–946.
46. Marder E. (1984) Roles for electrical coupling in neural circuits as revealed by selective neuronal deletions. *J. Exp. Biol.* **112,** 147–167.
47. Marder E. (1998) Electrical synapses: beyond speed and synchrony to computation. *Curr. Biol.* **8,** R795–R797.
48. Kepler T. B., et al. (1990) The effect of electrical coupling on the frequency of model neuronal oscillators. *Science* **248,** 83–85.
49. Sherman A. and Rinzel J. (1992) Rhythmogenic effects of weak electrotonic coupling in neuronal models. *Proc. Natl. Acad. Sci. USA* **89,** 2471–2474.
50. Vardi N. and Smith R. G. (1996) The aII amacrine network: coupling can increase correlated activity. *Vision Res.* **36,** 3743–3757.
51. Ammermüller J., et al. (1996) Effects of horizontal cell network architecture on signal spread in the turtle outer retina. Experiments and simulations. *Vision Res.* **36,** 4089–4103.
52. Manor Y., et al. (1997) Low amplitude oscillations in the inferior olive: a model based on electrical coupling of neurons with heterogeneous channel densities. *J. Neurophysiol.* **77,** 2736–2752.
53. Traub R. D. and Bibbig A. (2000) A model of high-frequency ripples in the hippocampus based on synaptic coupling plus axon–axon gap junctions between pyramidal neurons. *J. Neurosci.* **20,** 2086–2093.
54. Traub R. D., et al. (2001) Gap junctions between interneuron dendrites can enhance synchrony of gamma oscillations in distributed networks. *J. Neurosci.* **21,** 9478–9486.
55. Lewis T. J. and Rinzel J. (2003) Dynamics of spiking neurons connected by both inhibitory and electrical coupling. *J. Comput. Neurosci.* **14,** 283–309.
56. Mann-Metzer P. and Yarom Y. (1999) Electrotonic coupling interacts with intrinsic properties to generate synchronized activity in cerebellar networks of inhibitory interneurons. *J. Neurosci.* **19,** 3298–3306.
57. Christie M. J., et al. (1999) Electrical coupling synchronizes subthreshold activity in locus coeruleus *in vitro* from neonatal rats. *J. Neurosci.* **9,** 3584–3589.
58. Alvarez V., et al. (2000) Frequency-dependent synchrony in locus coeruleus: role of electronic coupling. *Proc. Natl. Acad. Sci. USA* **99,** 4032.
59. De-Miguel F. F., et al. (2001) Spread of synaptic potentials through electrical synapses in Retzius neurones of the leech. *J. Exp. Biol.* **204,** 3241–3250.
60. Deans M. R., et al. (2001) Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing conexin36. *Neuron* **31,** 477–485.
61. Hormuzdi S. G., et al. (2001) Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron* **31,** 487–495.
62. Long M. A., et al. (2002) Rhythmicity without synchrony in the electrically uncoupled inferior olive. *J. Neurosci.* **22,** 10,898—10,905.
63. Tresch M. C. and Kiehn O. (2002) Synchronization of motor neurons during locomotion in the neonatal rat: predictors and mechanisms. *J. Neurosci.* **22,** 9997—10,008.
64. Friedman D. and Strowbridge B. W. (2003) Both electrical and chemical synapses mediate fast network oscillations in the olfactory bulb. *J. Neurophysiol.* **89,** 2601–2610.
65. Bennett, M. V. L. (1968), in *Physiological and biochemical aspects of nervous integration*, Carlson, F. D. ed., Prentice-Hall, Englewood Cliffs, NJ, p. 73.
66. Galarreta M. and Hestrin S. (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* **402,** 72–75.
67. Koós T. and Tepper J. M. (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nature* **2,** 467–472.
68. Perez Velazquez J. L. and Carlen P. L. (2000) Gap junctions, synchrony and seizures. *Trends Neurosci.* **23,** 68–74.
69. Beierlein M., et al. (2000) A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat. Neurosci.* **3,** 904–910.
70. Amitai Y., et al. (2002) The spatial dimensions of electrically coupled networks of interneurons in the neocortex. *J. Neurosci.* **22,** 4142–4152.

71. Schmitz D., et al. (2001) Axo–axonal coupling: a novel mechanism for ultrafast neuronal communication. *Neuron* **31,** 831–840.
72. Herberholz J., et al. (2002) A lateral excitatory netweork in the escape circuit of crayfish. *J. Neurosci.* **22,** 9078–9085.
73. Perrins R. and Weiss K. R. (1998) Compartmentalization of information processing in an *Aplysia* feeding circuit interneuron through membrane properties and synaptic interactions. *J. Neurosci.* **18,** 3977–3989.
74. Spira M. E., et al. (1980) Synaptic organization of expansion motoneurons of *Navanax inermis*. *Brain Res.* **195,** 241–269.
75. Llinás R., et al. (1974) Electrotonic coupling between neurons in the cat inferior olive. *J. Neurophysiol.* **37,** 560–571.
76. Norekian T. P. (1999) GABAergic excitatory synapses and electrical coupling sustain prolonged discharges in the prey capture neural network of *Clione limacina*. *J. Neurosci.* **19,** 1863–1875.
77. Graubard K. and Hartline D. K. (1987) Full-wave rectification from a mixed electrical-chemical synapse. *Science* **237,** 535–537.
78. Sharpe L. T. and Stockman A. (1999) Rod pathways: the importance of seeing nothing. *Trends Neurosci.* **22,** 497–504.
79. Bloomfield S. A. and Dacheux R. F. (2001) Rod vision: pathways and processing in the mammalian retina. *Prog. Ret. Eye Res.* **20,** 351–384.
80. Heitler W. J., et al. (1991) Different types of rectification at electrical synapses made by a single crayfish neurone investigated experimentally and by a computer simulation. *J. Comp. Physiol. A* **169,** 707–718.
81. Veruki M. L. and Hartveit E. (2002) Electrical synapses mediate signal transmission in the rod pathway of the mammalian retina. *J. Neurosci.* **22,** 10,558—10,566.
82. Acklin S. E. (1988) Electrical properties and anion permeability of doubly rectifying junctions in the leech central nervous system. *J. Exp. Biol.* **137,** 1–11.
83. Joris P. X., et al. (1998) Coincidence detection in the auditory system: 50 years after Jeffress. *Neuron* **21,** 1235–1238.
84. Tsien J. Z. (2000) Linking Hebb's coincidence-detection to memory formation. *Curr. Opin. Neurobiol.* **10,** 266–273.
85. Anholt R. R. (1994) Signal integration in the nervous system: adenylate cyclases as molecular coincidence detectors. *Trends Neurosci.* **17,** 37–41.
86. Agmon-Snir H., et al. (1998) The role of dendrites in auditory coincidence detection. *Nature* **393,** 268–272.
87. Edwards D. H., et al. (1999) Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22,** 153–161.
88. Edwards D. H., et al. (1998) Neuronal coincidence detection by voltage-sensitive electrical synapses. *Proc. Nat. Acad. Sci. USA* **95,** 7145–7150.
89. Galarreta M. and Hestrin S. (2001) Spike transmission and synchrony detection in networks of GABAergic interneurons. *Science* **292,** 2295–2299.
90. Rela L. and Szczupak L. (2003) Coactivation of motoneurons regulated by a network combining electrical and chemical synapses. *J. Neurosci.* **23,** 682–692.
91. Wadepuhl M. (1989) Depression of excitatory motoneurones by a single neurone in the leech central nervous system. *J. Exp. Biol.* **143,** 509–527.
92. Stuart A. E. (1970) Physiological and morphological properties of motoneurones in the central nervous system of the leech. *J. Physiol. (London)* **209,** 627–646.
93. Kristan W. B. and Shaw B. K. (1997) Population coding and behavioral choice. *Curr. Opin. Neurobiol.* **7,** 826–831.
94. Esch T., et al. (2002) Evidence for sequential decision making in the medicinal leech. *J. Neurosci.* **22,** 11,045—11,054.
95. Katz P. S. (1995) Intrinsic and extrinsic neuromodulation of motor circuits. *Curr. Opin. Neurobiol.* **7,** 826–831.
96. Hormuzdi S. G., et al. (2004) Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks. *Biochim. Biophys. Acta* **1662,** 113–137.
97. Rekling J. C., et al. (2000) Electrical coupling and excitatory synaptic transmission between rhythmogenic respiratory neurons in the pre-Bötzinger complex. *J. Neurosci.* **20,** RC113–RC117.
98. Gutstein D. E., et al. (2001) Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ. Res.* **88,** 333–339.
99. Cohen-Salmon M., et al. (2002) Targeted ablation of connexin26 in the inner ear epithelial

gap junction network causes hearing impairment and cell death. *Curr. Biol.* **12,** 1106–1111.
100 Jordan K., et al. (1999) Trafficking, assembly, and function of a connexin43-green fluorescent protein chimera in live mammalian cells. *Mol. Biol. Cell* **10,** 2033–2050.
101 Falk M. M. (2000) Connexin-specific distribution within gap junctions revealed in living cells. *J. Cell Sci.* **113,** 4109–4120.
102. Evans W. H. and Martin P. E. (2002) Lighting up gap junction channels in a flash. *BioEssays* **24,** 876–880.