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Review

Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks

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Abstract

Gap junctions consist of intercellular channels dedicated to providing a direct pathway for ionic and biochemical communication between contacting cells. After an initial burst of publications describing electrical coupling in the brain, gap junctions progressively became less fashionable among neurobiologists, as the consensus was that this form of synaptic transmission would play a minimal role in shaping neuronal activity in higher vertebrates. Several new findings over the last decade (e.g. the implication of connexins in genetic diseases of the nervous system, in processing sensory information and in synchronizing the activity of neuronal networks) have brought gap junctions back into the spotlight. The appearance of gap junctional coupling in the nervous system is developmentally regulated, restricted to distinct cell types and persists after the establishment of chemical synapses, thus suggesting that this form of cell–cell signaling may be functionally interrelated with, rather than alternative to chemical transmission. This review focuses on gap junctions between neurons and summarizes the available data, derived from molecular, biological, electrophysiological, and genetic approaches, that are contributing to a new appreciation of their role in brain function.

Keywords: Connexin; Coupling; Gap junction; Neuron; Oscillation; Retina

1. Introduction

The synapse has been defined as a specialized structure that mediates a functional interaction between two neurons or between a neuron and another cell type. This zone of contact presents two distinctive elements, the pre-synaptic terminal and the post-synaptic target site, separated by a synaptic cleft [1]. The nature of synaptic transmission was vigorously debated by some of the finest neuroscientists of the last century, who argued either in favor of an electrical mode implying that the action potential in the pre-synaptic neuron induces a passive current flow into the post-synaptic cell, or in favor of a chemical substance, liberated from the pre-synaptic cell upon arrival of an action potential, which interacts with the post-synaptic cell and propagates the stimulus. For some time the strong case made by the unequivocal evidence for chemical transmission in the vertebrate brain and at the neuromuscular junction led to the generalization that all synaptic transmission would be chemical. Then, a direct demonstration of electrical synaptic transmission was first obtained at the giant motor synapse in the crayfish, where it was shown that the post-synaptic response arose in a fraction of a millisecond after pre-synaptic stimulation [2], and these findings were shortly confirmed in vertebrates [3–5]. It is now accepted that either view overestimated just one type of synaptic transmission, as both mechanisms, chemical as well as electrical, co-exist (see Ref. [5] for a thorough and entertaining discussion on the nomenclature of synaptic transmission).

Electrical and chemical synapses differ not only in the molecular mechanisms of information transfer, but also in their morphological organization (Fig. 1). At chemical synapses, there is no continuity between the cytoplasm of the two cells at the synapse, and the distance separating the pre- and post-synaptic membranes, namely the synaptic

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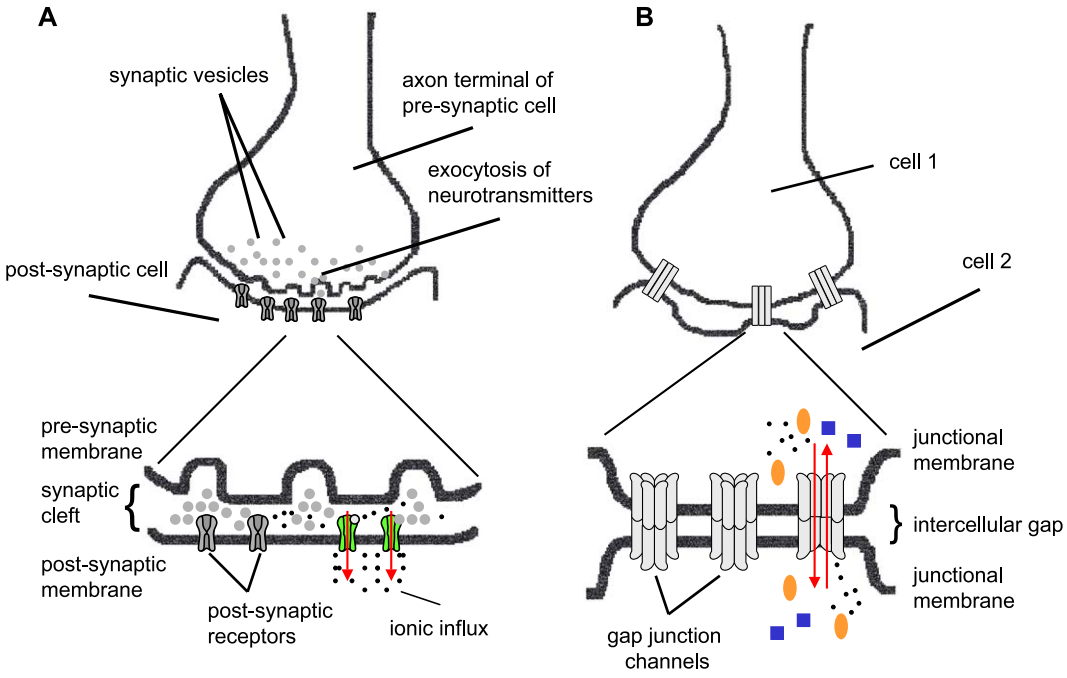

Fig. 1. Synaptic transmission can be chemical and electrical. Schematic drawing depicting the principal features of the two types of synapses. (A) At chemical synapses, an action potential arriving at the pre-synaptic terminal triggers the exocytosis of vesicles filled with neurotransmitters (gray), which are then released in the synaptic cleft. Transmitters diffuse and bind to specific receptors on the post-synaptic cell, where they gate (viz., open or close) ion channels either directly or indirectly, thereby affecting its membrane conductance. In this example, the opening of a ligand-gated channel (green) triggers ionic influx (black) in the post-synaptic cell. (B) At electrical synapses, gap junction channels allow a direct communication between the cytoplasm of the two coupled cells. In addition to ions (black circle), and metabolites (blue), small second messenger molecules (orange) can also diffuse through gap junction channels. Whereas chemical transmission is unidirectional, electrical synapses usually pass signals equally well in both directions.

cleft, is in the order of 20–40 nm. In contrast, electrical synapses are characterized by an area of very close apposition, in the order of 2–4 nm between the pre- and post-synaptic membranes. Within this area of apposition the two cells communicate through gap junctions, cell-to-cell pores that serve as conduits between the cytoplasm of the two cells. The structural proteins comprising these channels, called connexins (Cx), form a multigene family whose members are distinguished according to their predicted molecular mass in kDa (e.g. Cx32, Cx43) [6–9]. The family of connexin genes comprises 21 members in the human and 20 in the mouse genome, 19 of which can be considered as orthologue pairs on the basis of their sequence [10]. Intercellular channels span two plasma membranes and result from the association of two half channels, called connexons, contributed separately by each of the two participating cells. Each connexon, in turn, is a hexameric assembly of connexin subunits. Intercellular channels are defined as homotypic, when the two connexons have the same molecular composition, or heterotypic, when the connexons differ. Connexins have evolved a code of compatibility that permits only selective interactions between connexons, so that the establishment of electrical coupling is also dependent on the pattern of connexin expression between neighboring cells [7,9]. Gap junction channels have relatively large pores (16–20 Å of diameter) that allow ions as well as small molecules (in general ≤1 kDa) to pass from one cell to the other, although important differences exist between connexins [11–15]. Hence, these intercellular channels are also involved in the transmission of metabolic signals between cells, by permitting the passage of second messengers such as inositol trisphosphate (IP3) and cyclic adenosine monophosphate (cAMP) [16–20].

Electrical synapses function as low pass filters, that is they preferentially transmit low-frequency stimuli (but not exclusively, as we will discuss their role in mediating high-frequency oscillations in the hippocampus in Section 3.1) that allow the rapid transfer of a pre-synaptic impulse into an electrical excitatory post-synaptic potential in the post-junctional cell [5,21]. If the current transmitted to the post-synaptic cell is sufficient to depolarize the membrane above a certain threshold, activation of voltage-gated ion channels will lead to the generation of action potentials. Since ionic current flow can occur freely between the two cells, electrical transmission via the intercellular channels can be bi-directional (Fig. 2). In fact, it is the distinctive reciprocity of the stimulus supported by electrical but not chemical neurotransmission that, together with the transfer of sub-threshold potentials favoring synchronous activity, may well be one of the advantages of electrical synapses. It should be emphasized, however, that electrical communication cannot be equated to mutual excitation. If a more depolarized cell

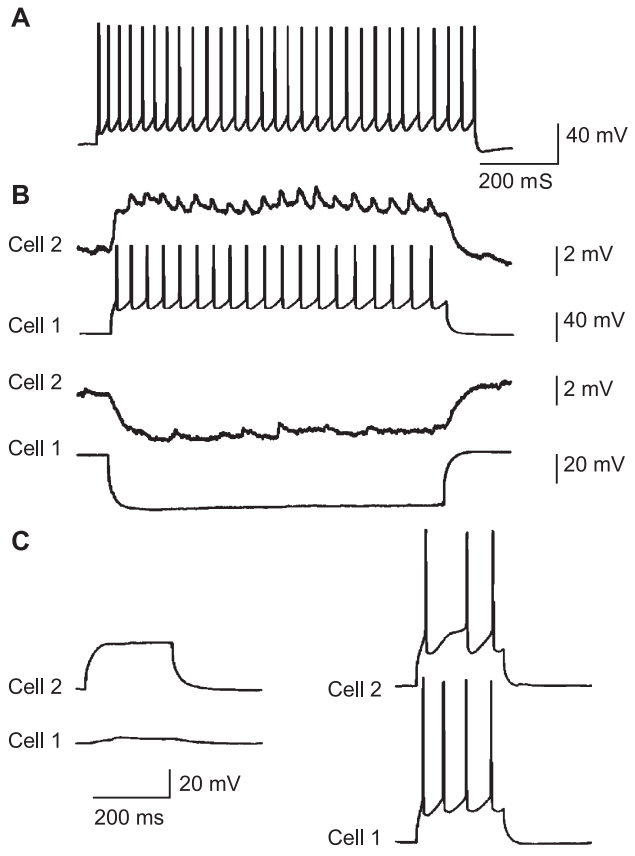

Fig. 2. Electrical coupling between mouse hippocampal interneurons. Dual whole-cell patch-clamp recordings performed in brain slices on pairs of fast-spiking interneurons from the dentate gyrus region of the hippocampus have demonstrated that the vast majority of cell pairs are electrically coupled [30,41]. (A) Fast-spiking interneurons in the dentate gyrus are identified on the basis of their morphology, location and action potential firing patterns (illustrated here by a representative trace). (B) Electrical coupling between fast-spiking interneurons is reciprocal. Voltage responses in cell 1 following depolarizing (upper traces) or hyperpolarizing (lower traces) current injections are reflected in cell 2, albeit with a significant reduction in amplitude. (C) Electrical coupling is likely to promote the generation of action potentials. When cell 2 of a pair is injected with sub-threshold current pulses, no action potentials are recorded in either cell (left traces). In pairs of electrically coupled interneurons a sub-threshold depolarizing current, however, facilitates the generation of action potentials when concomitant firing is evoked in the second interneuron (reprinted from Ref. [39], with permission from Elsevier).

can excite a less depolarized cell to which it is coupled, the opposite also occurs, as the less depolarized cell tends to inhibit the more depolarized partner. Moreover, in some cases electrical synapses are not bi-directional but actually rectifying, that is the efficacy of transmission in one direction is greater than in the other, as is the case at the giant motor synapse of the crayfish [2].

Electrical communication has been regarded for a long time as a property of the invertebrate brain where faster transmission is needed to accomplish simple, reactive tasks. This scenario may not always hold true, as it has been pointed out that the delay of stimulus propagation at electrical synapses can also be longer than that of chemical transmission, particularly at mammalian body temperature [21]. Nevertheless, electrical synaptic transmission—it was argued—would not be well-suited for the more complex integrative processes of higher organisms, which would benefit from the higher diversity and fine-tuning provided by chemical synapses. More importantly, with the exception of the mixed excitatory synapse between auditory efferents and the Mauthner cell of the goldfish, where short-term potentiation of both the electrical and chemical components has been demonstrated [22,23], electrical coupling does not usually exhibit the activity-dependent plasticity of chemical synapses, hence implying that there is no learning through electrical transmission. Several lines of evidence have progressively contributed to the modification of this minimalist view of the role of gap junctions in shaping neuronal activity. The first major breakthrough has been the identification of a novel connexin highly expressed in the vertebrate central nervous system (CNS) and unambiguously present in neurons [24–26]. The second finding has been the demonstration that morphologically identifiable gap junctions between neurons are more abundant than previously d [27,28]. Another key progress, owing to the technological advantage brought by infrared differential interference contrast microscopy and by transgenic technology, has been the direct demonstration of electrical synapses between identified gap junction-coupled neuronal pairs of the young rodent brain [29–35]. Finally, both computer simulations and electrophysiological recordings have recently emphasized a key role for electrical synapses in synchronizing large neuronal ensembles at different frequency bands [35–44], which have been proposed to underlie a variety of cognitive processes, such as perception, memory, and learning. Electrical transmission should be viewed, therefore, as a complementary form of communication, not alternative to chemical signaling, with which it interacts.

Gap junctions and direct intercellular communication in the CNS are not limited to neurons. In fact, both macroglial cell types (astrocytes and oligodendrocytes) are coupled via connexins [45–56] and these connections establish compartments of communicating cells that persist throughout adulthood [57–61]. It suffices to say that morphological, biochemical and functional studies have indicated that there are qualitative and quantitative differences between classes of glia, with each cell type expressing a repertoire of specific connexins and a distinct level of junctional coupling [62–67]. Since several articles have described in detail the proposed roles of connexins in the regulation of neuronal homeostasis, in neuroprotection and in several pathological conditions of the nervous system [68–86], we will present only a general overview of the morphological and functional incidence of coupling between CNS neurons, followed by a more focused discussion of the evidence implicating a role for electrical synapses in synchronous oscillatory activity in cortical brain regions and in the dynamic control of retinal circuits.

2. Gap junctions and connexins between neurons

A number of reports have appeared over the years, describing the presence of gap junctions and the expression of distinct connexins in different regions of the adult mammalian brain, such as the hippocampus, inferior olive, locus coeruleus, hypothalamus, spinal cord, and olfactory bulb [27,87–109]. These studies have employed a wide array of techniques, e.g. in situ hybridization, immunocytochemistry, electron microscopy, freeze-fracture immunolabeling, electrophysiology, dye coupling, which are ultimately providing a morphological, functional and molecular description of neuronal coupling both in vitro and in vivo. Since gap junctions form a morphologically distinct structure, the most convincing method to visualize them between neurons has been thin-section and freeze-fracture electron microscopy. A systematic analysis of freeze-fracture replicas of the rat spinal cord has demonstrated that mixed synapses are relatively abundant between several classes of neurons [27,28,110]. Consistent with these observations, electrical and dye coupling between neurons is often restricted to cells of the same class [29,30,32,33,35,111–113], but several examples of intercellular communication between different types of neurons have been convincingly documented both during development and in the adult [33,34] (the retina is a special case that will be treated separately in Section 4). Although the occurrence of another form of heterocellular coupling, between neurons and astrocytes has been reported [114–119], this issue remains unresolved and no convincing morphological evidence of gap junctions between these two cell types has yet been found [59,120,121] (see Ref. [84] for a comprehensive discussion of this topic). It is possible that the discrepancy between the functional and the morphological observations reflects the limits of the techniques or, alternatively, that coupling between neurons and glia occurs during a narrow temporal window and/or in restricted brain areas.

Although freeze-fracture allows the detection of junctions with a small number of channels, it has been pointed out that efficient intercellular coupling can be achieved with only few gap junction channels that may be very difficult to visualize and even the most accurate analysis of large plasma membrane areas may underestimate the incidence of gap junctional communication. Hence, the use of complementary approaches, including paired recordings in the whole-cell patch-clamp mode, injection of gap junction-permeable fluorescent tracers, imaging techniques, in situ hybridization and immunocytochemistry, is warranted to assess the incidence of neuronal coupling *in* the CNS.

2.1. Gap junctions in the developing CNS

Gap junction-dependent neuronal communication is widespread in the developing CNS when chemical synapses are immature and their number still very low. It has been noted that the prevalence of gap junctional coupling is well correlated with specific developmental events (including neurulation, cellular and regional differentiation, migration, axon guidance, and the formation of neuronal circuits) and that the basic properties of these channels are well suited to mediate the transfer of developmental signals [57,122–127]. Studies both in vitro and in vivo have shown that progenitor cells, neuroblasts and proliferating cells located in several areas of neurogenesis are strongly coupled. In contrast, coupling is down-regulated as differentiation proceeds in different model systems, strongly suggesting a role for intercellular communication during proliferation and differentiation of multipotent neural progenitors [128–139]. Furthermore, there is an inverse correlation between connexin expression and cell proliferation, suggesting that coupling and cell cycle of neural progenitors may be interdependent [73,130,140]. It is tempting to postulate that gap junctions establish communication compartments that isolate groups of coupled cells engaged in a coordinated activity from other populations, which participate in distinct processes. Coupling in the developing neocortex is regulated by cholinergic and monoaminergic transmitters during the period of formation of synaptic circuits in an area-specific manner [141–143]. Incubation with specific agonists reduces gap junction communication presumably via activation of downstream protein kinases, a finding indicative that connexin phosphorylation may result in the short-term modulation of electrical coupling between neurons and contribute to the control of cortical plasticity during the first weeks of postnatal development [144–146].

The impact of gap junctions in building the functional architecture of the nervous system was first inferred by demonstrating that electrical coupling actually precedes the establishment of chemical transmission between nerve and muscle cells in culture, hence providing a route for the exchange of signals involved in synapse formation [147]. Thus, at early stages of development it has been proposed that gap junction channels are not only important for electrical synchronization, but are chiefly used as a biochemical means that allows neuronal ensembles to exchange small second messenger molecules that shape their activity [125,148]. Support for this hypothesis comes from work that, by taking advantage of more sensitive tracers and imaging techniques, has led to the crucial observation that gap junctions produce large functional clusters of coupled neurons, most often arranged in vertical columns that span several cortical layers [149,150]. Of note, these discrete regions of the developing neocortex can exhibit large and synchronous increases in cytosolic free Ca^{2+} levels that are suppressed by gap junction blockers, but not by abolishing chemical synaptic transmission. Cortical domains consist of short-range Ca^{2+} waves that depend on the intercellular passage of IP3, the ensuing IP3-induced Ca^{2+} release from intracellular stores and the regenerative formation of IP3 in the coupled cells [125,150,151]. Interestingly the ability of these neurons to transfer electrical signals is relatively weak, so that the voltage response in the post-synaptic cell is only

a small percentage of the voltage change elicited upon current injection in the pre-synaptic cell. By contrast, Ca^{2+} waves are propagated very efficiently, thus indicating that gap junction channels at these stages couple cells both biochemically and metabolically, providing an intercellular pathway for morphogens and other instructive cues that influence a wide variety of cell functions.

Another aspect of the involvement of electrical synapses during development has emerged from studies of electrical and dye coupling among motor neurons [152–154] and of their possible role in the formation and editing of neuromuscular synapses. A key feature of this process is the activity-dependent elimination of functional synapses from circuits. In the case of the neuromuscular junction, each muscle fiber is initially innervated by two to eight motor neurons and subsequently, with synaptic editing, the single innervation pattern of adult organisms is established [155,156]. Chang et al. [157] have found that neonatal motor neurons are transiently coupled, and that this coupling disappears by the end of the first postnatal week. Thus, during the formation of spinal neural circuits, the activity of motor neurons innervating the same muscle is temporally correlated via these electrical synapses. Synchronous activation of the post-synaptic cell would not allow the muscle fiber to distinguish between the competing neurons, whereas the progressive disappearance of gap junction coupling and, hence, of temporally correlated activity after birth would trigger synaptic competition. The post-synaptic muscle fiber would then discriminate the strength of the different inputs and eliminate the weakest synapses [158]. A similar scenario may be envisaged to explain the fact that, after nerve damage and motor neuron degeneration, motor axons can regenerate and re-innervate fibers showing a period of multiple innervation (more than one neuron per muscle fiber) that coincides, as during development, with the transient re-establishment of coupling between motor neurons [159]. Since most of the results are based on pharmacological manipulation of gap junction coupling with chemical blockers (e.g. alkanols, liquorice derivatives, arylaminobenzoates) whose specificity is unclear, the causality of these two events has not been fully established [160,161].

Further progress in unraveling the role of connexins in the developing nervous system may come from the systematic and detailed analysis of mutant animals with targeted deletion of the connexins that have been detected in discrete neuronal populations. All the available evidence indicates that connexin expression is a dynamic process that results in the spatial and temporal regulation of gap junction coupling in different brain areas. Thus, one can speculate that this form of intercellular communication provides a selective signaling pathway whose properties are determined by the molecular identity of the connexins available to the cells in direct contact. A corollary to this hypothesis is that connexins are differentially deployed to fulfill specific tasks. Thus, if the panoply of connexins expressed at any given time by a group of neurons is of importance, one can postulate that altering such composition in vivo would result in the development of functional abnormalities that demonstrate the stringency of connexin channel requirements in different brain regions. This hypothesis could be tested by replacing one connexin gene with another by genetic knock-in, a powerful approach that has already been successfully applied and has revealed unexpected phenotypes in other organs [162–165].

2.2. The molecular identity of neuronal connexins

A proper understanding of the contribution of gap junction channels to the functioning of the normal and pathological CNS requires that the cellular and developmental distribution of connexins be unambiguously defined. The identity of connexins expressed in neurons, however, has remained controversial and discrepancies persist concerning the distribution of several candidate neuronal connexins. Thus, transcripts for Cx26, Cx32, Cx33, Cx36, Cx40, Cx43, Cx45 and Cx47 have been detected in the CNS either by single cell reverse transcription-polymerase chain reaction (RT-PCR) or by in situ hybridization and have been proposed to be expressed in some neuronal populations [33,115,138,166–169]. Although in situ hybridization using radioactive labeled oligoprobes has proved a reliable method to visualize cells expressing connexin mRNA (Fig. 3), low mRNA levels may be a serious limitation and the presence of mRNA does not mean that the protein is made. By contrast, in situ hybridization experiments using non-radioactive riboprobes for detection of connexin genes have sometimes proven prone to cross-hybridization artifacts that cannot be fully overcome by extensive RNAse digestion, increase in hybridization temperature or probe concentrations. The use of antibodies to visualize connexin expressing cells may also be problematic in the brain [119,121,170,171], where the anatomical complexity of cell–cell interactions (connexins may be located on termini far away from the cell soma) and the scarcity of protein levels have proved a serious obstacle that has slowed down progress in this area. In fact, few connexins have passed more stringent investigations that have combined standard biochemistry, molecular biology and immunocytochemistry with genetic approaches based on the expression of a reporter gene (such as the lacZ gene, which encodes *E. coli* β-galactosidase) to trace the expression pattern of genes of interest (Table 1).

Several connexins have been identified in progenitor cells, but it is unclear which ones are important and which ones are dispensable. The two family members which have been more frequently linked to expression in progenitor cells are Cx43 and Cx26, but the specificity of the antibodies used, or the use of in vitro models has not allowed to draw a clear conclusion as to their expression in vivo [130,134,138,140]. The case of Cx26 is particularly perplexing, as the relative abundance of Cx26 protein expression observed by immunocytochemistry in neuronal populations during early brain development

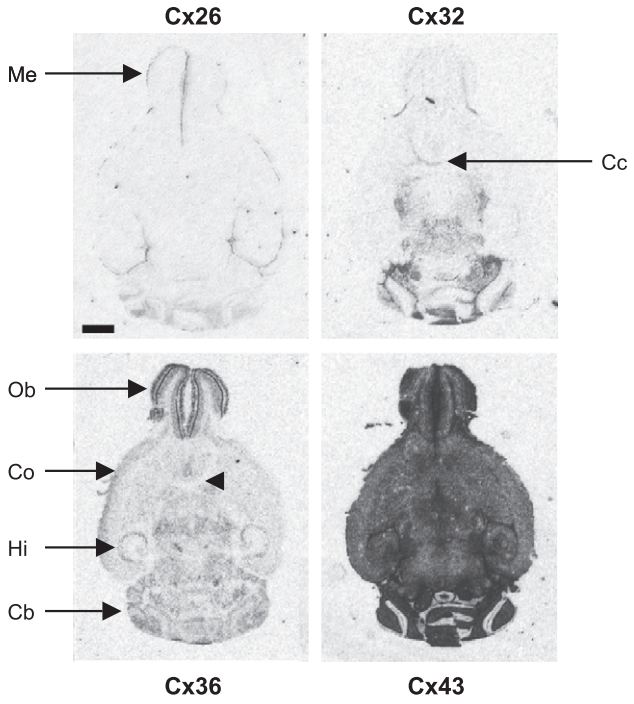

Fig. 3. Connexins are differentially distributed in the mouse brain. The profile of mRNA expression was determined by radioactive in situ hybridization in horizontal brain sections obtained from rats at postnatal day 90. X-ray autoradiograms illustrate the differences between the localization of Cx26, whose transcript is detected only in the meningeal layer (Me) [173], and Cx43, which is highly expressed in astrocytes [105]. Cx36 mRNA is high in the olfactory bulb (Ob) and also present in other regions, including cortex (Co), hippocampus (Hi) and cerebellum (Cb). Note that the signal for the neuronal Cx36 [176] is absent from white matter structures (arrowhead), such as corpus callosum (Cc), where labeling is evident for Cx32, which is expressed by oligodendrocytes [52]. Scale bar is 2 mm.

[119,171] does not correlate with either the very low levels of mRNA detected by in situ hybridization or the cellular distribution determined by the expression of a reporter gene at comparable ages [172,173]. Thus, by using a genetic approach, it has been recently reported that the expression of Cx26 is neither neuronal nor glial but is restricted to the meninges in both embryonic and adult brain [173]. This finding is consistent with the first immunohistochemical analysis of connexin distribution in the brain [105], which excluded a glial or neuronal expression of Cx26 in the adult CNS (Fig. 3).

The expression of Cx36 in the CNS was demonstrated by several groups and has been verified using different techniques (Table 1). In a series of studies, Condorelli et al. [25,174] have presented a detailed analysis of Cx36 distribution in the CNS and have shown that this connexin is expressed in the spinal cord, brainstem nuclei, scattered cells in the cerebellar granule layer, hypothalamus, mesencephalic and diencephalic structures, basal ganglia, neocortex, retina and olfactory bulb. By combining in situ hybridization for Cx36 mRNA with immunohistochemistry for a general neuronal marker, they found that Cx36 is expressed only in neurons [175,176]. Analysis of developing brain further revealed that Cx36 reaches a peak of expression in the first 2 weeks of postnatal life, and decreases sharply during the third week. Similar results have been obtained with two antibodies directed against the cytoplasmic loop of the protein, either by freeze-fracture immunolabeling or by comparing the pattern of staining in wild-type and Cx36 null-mutant mice [110,120,177,178]. These findings have been further verified in transgenic animals in which the coding region of Cx36 had been replaced by a reporter gene [112,179,180].

By combining in situ hybridization, immunocytochemistry and analysis of a reporter gene, Cx43 has also been detected in both mature and immature olfactory receptor neurons, as well as basal cells in the olfactory epithelium of adult mice (Table 1). The levels of Cx43 mRNA in the nasal cavity show regional differences that are consistent with the distribution of a lacZ reporter gene driven by the proximal 6.5 kb of the Cx43 promoter in transgenic animals. Furthermore, lacZ is expressed in cells that are positive for the olfactory marker protein, thus indicating that Cx43 is expressed in mature olfactory receptor neurons [181]. Some caution should be exerted in the interpretation of these findings, since this construct does not contain the entire regulatory elements of the Cx43 gene, thus raising the possibility that a certain degree of ectopic expression caused by chromosomal sequences surrounding the insertion site of the transgene may occur. This problem may be minimized by applying the bacterial artificial chromosome technology, which has a higher chance to result in transgene expression patterns faithfully recapitulating, for the most part, the cellular distribution of the wild-type gene [182]. Using a different strategy that involved the conditional replacement of the Cx43 coding region by a lacZ reporter gene, mimicking the transcriptional activity of the endogenous Cx43 gene, it has been shown that Cx43 is expressed in some neurons of the olfactory bulb, substantia nigra, ventral posterolateral thalamic nuclei, and globus pallidus, whereas it is notably absent from principal cells of the mouse cortex and hippocampus [183].

More recently, a third connexin, Cx45, has been identified in neurons by both in situ hybridization and a genetic approach with lacZ as the reporter gene (Table 1). In young animals, at postnatal day 8 (P8), a strong signal is present in most brain areas, including the thalamus, hippocampus, striatum, cerebral cortex and cerebellum [172,184]. In contrast, in adult animals (P28 and older), Cx45 distribution becomes restricted to the stratum pyramidale in the CA3–CA4 region of the hippocampus, the thalamus as well as in the granular and molecular layers of the cerebellum [184]. In one study, with the exception of few oligodendrocyte precursor cells, β-galactosidase activity was associated with the expression of a neuronal marker and never co-localized with antigenic determinants of astrocytes and adult oligodendrocytes [184]. In another study, however, hybridization signals were also detected in non-neuronal cell types in

Table 1
Distribution of connexins in neurons of the central nervous system

Connexin	mRNA	Protein	Reporter gene
Cx36	Inferior olive, olfactory bulb, cerebral cortex, CA3 region of the hippocampus, hilus of the dentate gyrus, parvalbumin containing GABAergic neurons in the strata radiatum and oriens of the hippocampus, cerebellum, striatum, pineal gland, principal accessory nuclei, inner nuclear layer of the retina, cerebellar cortex, spinal cord gray matter [25,26,33,42,172,174–176,342]; lumbar spinal motor neurons [157]; forebrain, midbrain, sympathetic and spinal ganglia, spinal cord (E9.5–E12.5) [343]; suprachiasmatic nucleus [344]; olfactory epithelium, ventral and lateral regions of turbinates [345].	Inferior olive [110,175,177]; retinal inner and outer plexiform layers, AII amacrine cells [110,112,175,177,178,266,270,271]; cerebral cortex [178]; hippocampus, cerebellum [177]; anterior pituitary, pineal gland [175]; spinal cord [110]; olfactory nerve bundles underlying the olfactory epithelium, olfactory nerve layer and glomerular layer of the olfactory bulb, glomerular layer of the accessory olfactory bulb, vomeronasal nerve [175,345].	Retinal photoreceptors, cone bipolar cells, AII amacrine cells [272]; reticular thalamus [179]; inferior olive [180]; cortex, co-localization with somatostatin and parvalbumin neurons [112]; olfactory epithelium and olfactory bulb [345].
Cx43	Olfactory epithelium (sustentacular cells, mature and immature olfactory receptor neurons, basal cells) [181].	Mature olfactory receptor neurons [181].	Olfactory epithelium (sustentacular cells, mature and immature olfactory receptor neurons, basal cells) [181]; olfactory bulb [183].
Cx45	Motor neurons [157]; retina [267,269]; dopaminergic neurons of the midbrain floor [346]; cerebral cortex, granular and molecular layers of the cerebellum [172]; olfactory epithelium and mature olfactory neurons (co-localization with olfactory marker protein) [185].	Inner and outer plexiform layers of the retina [267]; motor neurons [157]; dopaminergic neurons of the midbrain floor [346]; neurons of the olfactory epithelium, proximal processes of mitral cells in the olfactory bulb [185].	Ganglion cells and the inner nuclear layers of the retina [267,278]; widespread expression during embryogenesis and up to P15, CA3–CA4 region of hippocampus, thalamus and cerebellum (basket and stellate cells) in the adult [184].

The identity of gap junction proteins expressed in neurons remains controversial and discrepancies persist concerning the distribution of several candidate neuronal connexins. A selected compilation of the expression profiles of three connexins, for which standard molecular biology and immunocytochemistry techniques have been combined with genetic approaches based on the expression of a reporter gene to trace their cellular distribution, is presented here. E=embryonic day; P=postnatal day.

several brain regions, such as cerebral cortex, thalamus, amygdala, hippocampus, hypothalamus and striatum [172]. Cx45 is also present in the mouse olfactory epithelium where its distribution largely overlaps with that of cells expressing olfactory marker protein mRNA, indicating that a substantial number of mature olfactory neurons express Cx45, and in the olfactory bulb, where it is presumably expressed by mitral cells [185].

It is clear that a more refined map of the distribution of connexins in neurons will require a combinatorial approach including anatomical, molecular and functional characterization of connexins in identified neuronal populations [186,187].

3. Electrical signaling and synchronous oscillatory activity

Since their discovery, the functional implication of electrical synapses has often been discussed in the context of the speed of signal transmission they provide, and of the precise temporal synchronization of the firing of coupled cells. Therefore, it is perhaps not surprising that connexins are gaining recognition for their ability to shape synchronous rhythmic activity in the CNS. Oscillations occurring at different frequencies have been recorded in vivo in various brain regions such as the olfactory bulb, hippocampus, thalamus, cortex, and cerebellum [188–192]. They reflect the temporal coordination of the activity of neuronal populations and, because they may display task or stimulus dependence, have been implicated as a mechanism that selects subsets of neurons for further joint processing and eventual stimulus representation [193,194]. Temporal correlations of neuronal activity have also been suggested to be a mechanism that conveys the strength rather than the nature of the signal [195]. The importance of oscillations is also implied from the association of abnormal network activity patterns with pathologies of the CNS [196,197], perhaps reflecting the cognitive and motor deficits associated with them.

The identity and characteristics of the neuronal subtypes involved in the generation of specific oscillatory patterns, and the contribution of these to various aspects of learning, memory and behavior are active areas of research. Oscillatory activity at specific frequency bands is correlated with different behavioral states [36]. For example, in the hippocampal formation of the mouse, theta (9–12 Hz) and gamma (40–90 Hz) oscillations were shown to occur during exploration and REM sleep, whereas ripples (200 Hz; also referred to as ultrafast oscillations elsewhere in the text) were recorded in the immobile awake and sleeping animal [198]. The segregation of these network patterns is hypothesized to have a functional significance. As suggested by Buzsaki and Chrobak [36], the acquisition of information represented by alterations in synaptic strength may take place during the theta and gamma phases, whereas the

consolidation of these patterns and their transfer to other brain structures may occur in the immobile animal. It is likely that distinct cellular mechanisms and molecules are involved in generating oscillations. However, inhibitory synaptic inputs in the hippocampus and neocortex have been assigned a prominent role in entraining networks of principal cells [36,43,199,200]. In combined interneuronal–principal cell networks, it has been suggested that an oscillatory synaptic input is imposed onto principal cells by GABAergic neuronal "supernetworks" resulting in a periodic fluctuation of the membrane potential of principal cells [201]. This notion has received considerable computational and experimental support. For instance, in vivo studies have demonstrated that the discharges/membrane potentials of hippocampal excitatory and inhibitory cells may be locked to different phases of gamma or theta oscillations [191,202], and have suggested that gamma synchrony of the CA1 region is brought about by CA3 interneurons [191].

The ability of electrical synapses to promote ionic coupling and bi-directional current flow make them particularly suited for synchronizing the discharges of interconnected cells. A variety of experimental and simulation studies support the notion that gap junctions may bring about synchrony in larger networks [40,41,44,203]. These include the findings that the incidence of morphologically and functionally identified gap junctions between neurons throughout the brain, mainly comprising Cx36 and Cx45, is more common than hitherto suspected [29–35,42,98,110,120,175,176,184,204–212], and that synchrony and oscillations of specific neuronal populations are altered in Cx36 knockout animals [35,42,112,179,180,210,213]. In networks containing large numbers of neurons, the transmission of electrical signals directly through gap junctions decreases the temporal heterogeneity of discharges, thereby enhancing synchrony [206]. Recent studies on Cx36 suggest that the formation of gap junction coupled clusters comprising cell types with similar properties is responsible for the decrease in the heterogeneity of drives in oscillating networks. These findings are discussed below.

3.1. Cx36 participates in specific oscillatory networks

Experiments in mice lacking Cx36 have demonstrated that the disruption of oscillogenesis is observed only in models of gamma frequency, whereas electrical communication within the excitatory neuronal network, as measured using ultrafast population activity, is normal [42,213] (but see Ref. [214]). A comparison of the cellular components of the two forms of oscillations described in these studies is particularly informative. Pharmacologically induced gamma oscillations depend upon both chemical synaptic inhibition and gap junctional coupling [41]. They may be brought about by the rapid curtailment of gap junctional potentials by inhibitory post-synaptic potentials (IPSPs) in networks containing GABAergic synapses and gap junctions in spatial proximity [32]. Ultrafast oscillations, on the other hand, are suggested to be an emergent property of a coupled pyramidal cell network. In vitro, they have been shown to occur in the absence of chemical neurotransmission [39,42,214] and require gap junctions between the axons of principal cells [40,203,215]. Thus, the finding that Cx36 in the hippocampus of the adult mouse is expressed only by interneurons and is necessary for the coordination of inhibitory networks, provides an explanation for the specific requirement of Cx36-containing gap junctions in gamma oscillations (Fig. 4). It also raises the possibility, however, that another intercellular channel protein is needed for mediating ultrafast oscillations in the hippocampus. While the identity of this protein is as yet unknown, the segregation of inhibitory and excitatory cell populations in the hippocampus into two separate electrically coupled networks suggests that this may be a fundamental mechanism to allow appropriate entrainment of pyramidal cell discharges by interneurons.

3.2. Cellular assemblies communicating through gap junctions mature during development

The distribution of Cx36 as revealed by in situ hybridization, immunohistochemistry, and Northern blot analysis indicates that the gene is regulated during development [26,42,175]. Expression in the postnatal brain is highest 2 weeks after birth and then decreases significantly in older animals. Importantly, this decrease in expression is not associated with a uniform decline in transcript levels but appears to reflect a continual refinement in the cell-specific distribution of Cx36 gene expression. Thus, the widespread distribution of Cx36 RNA in layers 2–6 of the P7 rat cortex becomes confined to scattered cells at P16, and in the hippocampus, Cx36 expression in the stratum pyramidale shows a continuous restriction until it is expressed only by interneurons in the adult. A developmental decrease in the expression of another neuronal connexin, Cx45, has also been recently described [184]. Consistent with these observations, the prevalence of functional coupling in the rodent brain also appears to show developmental and cell- and brain region-specific regulation. As determined by the strength of coupling and by the ease of finding connected pairs of neurons, coupling between basket cells in the dentate gyrus declines during postnatal development, whereas similar studies examining a different type of interneuron in the cortex indicates that coupling between these cells is not developmentally regulated [34]. Importantly, the incidence of coupling between excitatory and inhibitory cells declines with age [33,34] so that in the adult most electrical coupling exists between homogeneous cell types. These results suggest that the formation of gap junction clusters is refined over time and that connexins are expressed in a much larger subset of neurons in the developing brain at a time when neurons undergo morphological changes and the brain circuitry is being edited. It is

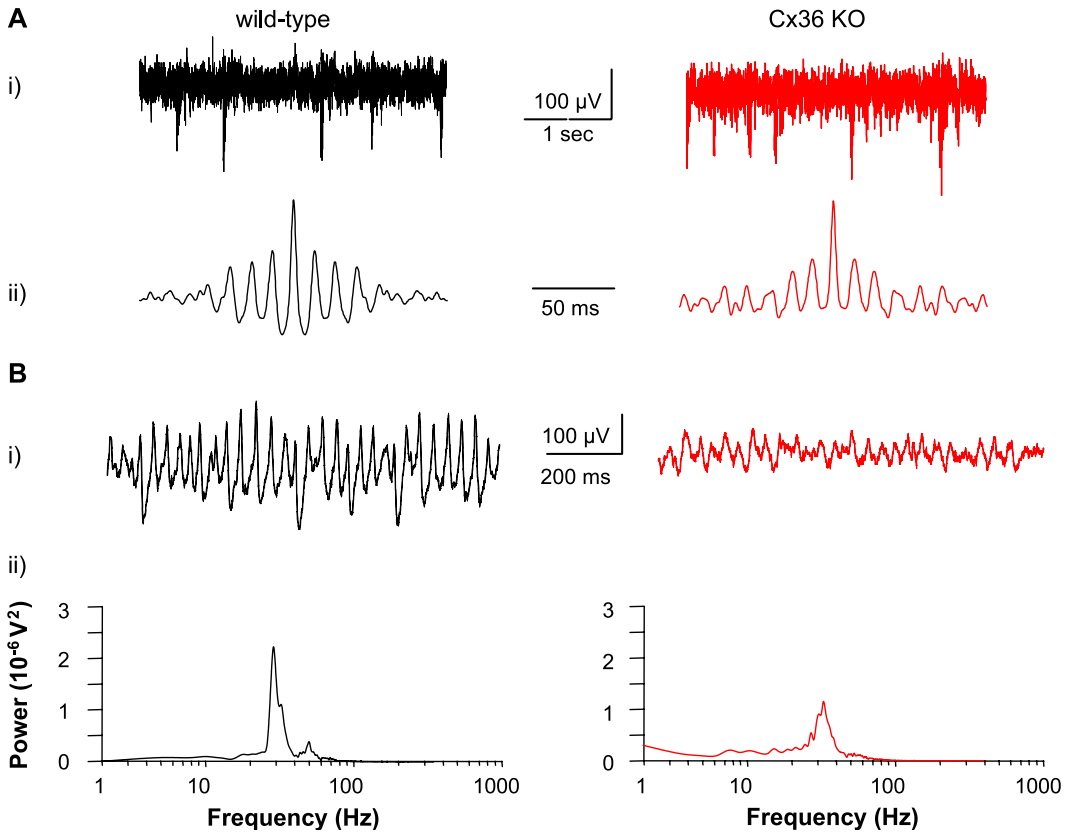

Fig. 4. Cx36 is involved in gamma frequency (30–80 Hz), but not ultrafast (150–200 Hz) oscillations. Extracellular field recordings were obtained in brain slices prepared from wild-type (black traces) and Cx36 knockout (KO) animals (red traces). (A) Effect of Cx36 deletion on hippocampal ultrafast population activity. Representative traces (i), taken from the CA3 region of the hippocampus, and the corresponding autocorrelations shown underneath (ii) illustrate that the characteristic bursts of high-frequency ripples are present in both wild-type and Cx36 KO mice and provide evidence for maintained ultrafast activity in hippocampal networks of mutant animals, despite the ablation of the major neuronal connexin. (B) Effect of Cx36 deletion on hippocampal population gamma activity induced by carbachol. Representative traces (i) taken from the stratum radiatum of the CA3 area illustrate that, in slices of wild-type animals, superfusion with 20 μM carbachol evokes the typical pattern of gamma frequency population activity. Although intra-area synchrony remains unaffected, the amplitude of the oscillatory activity, shown in the corresponding power spectra underneath (ii), is greatly reduced in Cx36 KO animals. These data provide genetic evidence for the role of a specific connexin in mediating synchronous oscillatory activity in large-scale neuronal networks in the hippocampus (reprinted from Ref. [39], with permission from Elsevier).

tempting to speculate that connexins are involved in some of these processes and indeed the presence of gap junction-dependent dye coupling and synchronous Ca^{2+} transients within cell assemblies of the immature cortex has been suggested to be important for the formation of functional assemblies in the adult [125,150]. The lack of gross morphological or physiological deficits in the absence of Cx36, however, precludes a role for this protein.

3.3. Restriction of gap junctional communication to inhibitory cell populations

Recent studies have identified and characterized the chemical and electrical synaptic connectivity of two cortical microcircuits, each containing an excitatory cell and two interneuron subtypes [30,31,35,112]. In both circuits, Cx36-containing gap junctions were observed to be critical for eliciting agonist induced supra- or sub-threshold oscillations in a particular interneuron subtype (low-threshold spiking (LTS) cells in layer 4 and multipolar bursting (MB) cells in layer 2/3), which drove synchronized responses in the other two cells (Fig. 5). Thus, in layer 4 of the neocortex, the addition of ACPD, a metabotropic glutamate receptor agonist, selectively induced synchronized depolarizing responses in LTS cells, which communicate among themselves solely by electrical synapses. Synchronized responses did not depend on action potentials or chemical neurotransmission but were strongly attenuated by octanol, which blocks electrical coupling, suggesting that ACPD-induced synchrony is an intrinsic property of the LTS cell network dependent upon gap junction communication between cells in the network. ACPD-induced responses in LTS cells were highly correlated with population IPSPs in fast-spiking (inhibitory) and regular-spiking (excitatory) cells, the other two cell types of the layer 4 circuit, which are abundantly innervated by LTS cells [31]. Similar

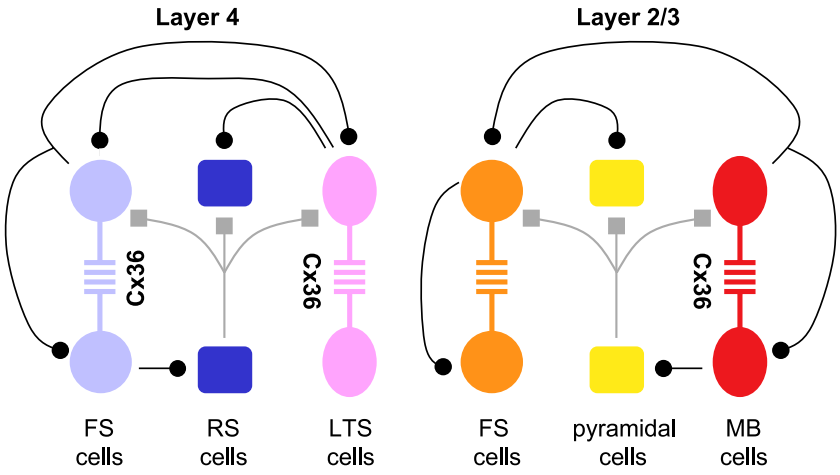

Fig. 5. Electrical synapses control the oscillatory activity of cortical microcircuits. The excitatory (gray), inhibitory (black), and electrical (parallel lines between connected cells) synaptic connections between the indicated cell types in the two cortical layers are illustrated [31,35]. Cx36-containing gap junctions have been functionally demonstrated to occur between three of the four inhibitory cell types. In layer 4 of the neocortex, FS and LTS give rise to independent networks of electrically coupled interneurons. FS and LTS cells are reciprocally connected by inhibitory synapses, but only FS cells show homologous chemical connectivity. Metabotropic agonists drive the LTS population to generate synchronized low frequency inhibitory outputs in a circuit of excitatory neurons, as well as in the inhibitory FS cells [31]. In layer 2/3 of the frontal and somatosensory cortex FS cells and MB cells form reciprocal synapses with neighboring pyramidal cells. Each interneuron population is connected by both chemical and electrical synapses. Interestingly, synaptic inputs between MB and FS cells are unidirectional, with MB innervating FS cells but not vice versa. Carbachol, but not metabotropic agonists, induces rhythmic and synchronous activity within the theta frequency band [35]. As discussed in the text, gap junctional communication is exhibited by all GABAergic interneurons in these circuits, but is restricted such that it occurs only between cells of the same inhibitory subtype. FS, fast spiking cell; RS, regular spiking cell; LTS, low-threshold spiking cell; MB, multipolar bursting cell.

observations were made for the cells in layer 2/3, where MB cells form a novel interneuron network that, upon cholinergic drive, can generate rhythmic and synchronous theta frequency activity providing temporal coordination of the local pyramidal cell output [35]. In addition to electrical coupling, GABAergic neurotransmission appears to be an important requirement in networks of MB cells for the generation of rhythmic activity, as is the case for fast spiking cells, albeit in a different frequency range [32]. Importantly, examination of electrical coupling between the cells indicates that each interneuron subtype forms a homologous cell population networked by gap junctions, and that Cx36 connected at least three of the four interneuron subtypes investigated. Surprisingly, although low-threshold spiking and fast spiking cells in cortical layer 4 express Cx36 and form Cx36-containing gap junctions, the two cell types are not coupled to each other suggesting that functional intercellular channels do not form between the two. Since the two cell types are spatially interspersed and form GABAergic synapses onto one another, a mechanism must exist to ensure that connexons, assembled in the two cells, segregate into spatially distinct gap junction domains. A spatial segregation of Cx29 and Cx32 immunoreactivity in Schwann cells has recently been described [55], and it is possible that a similar mechanism may underlie the separation of non-isotypic gap junctions in neurons. Since the vast majority of electrical coupling has been described for homogeneous cell types, coupling between heterogeneous cells being much less prevalent (except in the retina, as discussed in Section 4), it is likely that some specific mechanism may be used to limit gap junctional communication between networks.

Thus, these findings indicate that electrically coupled cellular assemblies are dynamically altered during development and form networks of homogeneous cell types that may participate in different oscillations. For Cx36, the evidence suggests that it forms intercellular channels only between interneurons in the cortex and hippocampus and that unknown mechanisms organize Cx36-coupled interneurons into many heterocellular arrays each containing synchronous populations of homogeneous cells. One consequence of this functional re-organization of interneuronal networks is that any heterogeneity in the oscillatory drive resulting from the enormous diversity of interneurons is nullified allowing specific interneuron populations to coordinate their discharges at determined phases of the oscillatory rhythms as was described in a recent study [202].

4. Electrical signaling in the retinal circuitry

The retina was one of the first parts of the vertebrate brain where electrical synapses were demonstrated and has remained one of the best model systems to analyze the function of electrical synapses in the nervous system, chiefly because gap junctions are present from early developmental stages and are conspicuously found in adult animals between nearly all neuronal cell types [216]. Although there are more than 50 types of retinal neurons, they can be classified into five major classes [217–219]: photo-

receptors (cones and rods), bipolar cells (8–10 types), horizontal cells (at least 2 types), amacrine cells (at least 28 types), and ganglion cells (12 types). These retinal neurons are arranged into five layers: three nuclear layers, where the cell bodies of the neurons are found and two synaptic layers, where the projections and contacts of the different neurons are seen (Fig. 6). The visual information flows in a vertical way, which is the main pathway, from the light-stimulated photoreceptors to bipolar cells, to ganglion cells. This vertical pathway undergoes spatial and temporal modulation by lateral-spreading information across the networks formed by horizontal and amacrine cells [220].

4.1. Electrical wiring of the retina

A combination of functional analysis (electrophysiological measurements) and morphological examination (electron microscopy and freeze-fracture analysis) has led to the discovery and demonstration of electrotonic junctions between retinal neurons [93,221–225]. Subsequently, by injecting biocytin (MW=373 Da) and neurobiotin (MW=286 Da) in the retinas of cats and rabbits, it was demonstrated that this type of coupling in the retina occurs not only for photoreceptor cells but also for many other types of neurons. Interestingly, the use of a larger tracer,

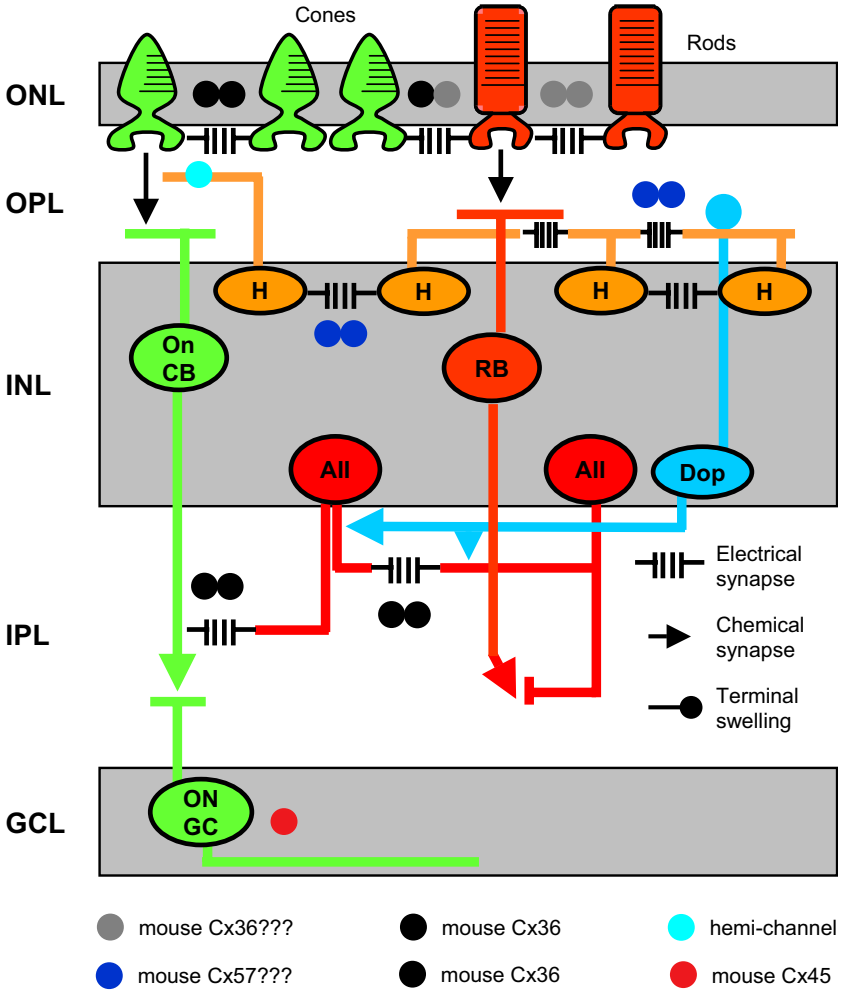

Fig. 6. Retinal neurons express multiple connexins in a cell-specific manner. In this simplified scheme depicting a very small part of the synaptic circuitry of the murine retina, the photopic, cone-driven pathway is shown in green, whereas the scotopic, rod-driven pathway is shown in red. Rod bipolar cells do not synapse directly on ganglion cells, but via AII amacrine cells that feed rod signals to cone bipolar cells. The suggested localization of connexins (color-coded) as hemi-channels in horizontal cells (gray circle) and at gap junctions between retinal neurons is presented [267,270–272,328,347]. Cx45 is expressed in two groups of bistratified ganglion cells that show homotypic coupling [278], whereas the cellular localization of mouse Cx57 remains uncertain [10]. The distribution of fish connexins has been omitted for clarity's sake. Dopaminergic cell dendrites form chemical synapses with AII amacrine [340], but no synaptic output has been identified for dopaminergic processes in the outer plexiform layer, where they end with small swellings [341]. The number of cells shown for each neuronal type does not imply any quantitative view of their relative abundance. Chemical synapses are shown as arrows, electrical synapses as parallel lines. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; CB, cone bipolar cell; RB, rod bipolar cell; H, horizontal cell; AII, amacrine cell of that subtype; Dop, dopaminergic amacrine cell; GC, ganglion cell.

lucifer yellow (MW=457 Da), showed a very restricted diffusion to neighboring cells (virtually no coupling), thus indicating that these junctions in neurons do not share the same permeability range of those present in most other organs [226–228]. This was the first functional indication that the molecular sieve of gap junction channels is dependent on the molecular identity of the constitutive connexins [13–15,229].

These early morphological and functional studies have established that virtually every single type of neuron is coupled in a homologous (between cells of the same type) or heterologous (between different types of cells) fashion by gap junctions [230–233]. In the outer plexiform layer of the retina, the synaptic endings of cones (pedicles) are electrically coupled to each other as well as to the synaptic terminals of rods (spherules) [221,223,234–239]. Horizontal cells of the same subtype are connected between their cell bodies, dendrites and axon terminals through large gap junctional plaques containing thousands of connexons [222,240–246]. Bipolar cells are also coupled by gap junctions between their axons or dendrites in homologous [247–250] and heterologous fashion [251]. Amacrine cells, which are a large and heterogeneous group of interneurons that control the lateral signaling pathway of the inner retina are reciprocally connected via dendro-dendritic gap junctions [226,252–256] and, furthermore, establish electrical synapses with ON-center cone bipolars [257,258] (discussed in Section 4.4). In spite of the absence of ultrastructural reports of morphological gap junctions between ganglion cells, electrophysiological and tracer coupling studies have revealed the presence of both homologous (α to α and γ to γ) and heterologous (both α and γ ganglion cells to amacrine) coupling [226,227,256,259–261].

4.2. The expression of connexins in the retina

The fish retina has been the source from which the first neuronal connexin, skate Cx35, was cloned [24]. Both the gene structure and amino acid sequence of Cx35 (the fish ortholog of mouse Cx36) revealed that it is evolutionarily divergent from all previously identified connexins, hence accounting for the failure of previous strategies to identify it. Subsequent studies [262–265] have demonstrated that more connexins are predominantly expressed in the fish retina, thus providing further evidence that retinal neurons are endowed with a repertoire of distinct connexins that may account for a functional diversification of gap junction-mediated intercellular communication in neuronal networks. By contrast, only Cx36 has been found to be prominently expressed in neurons of the mammalian retina [25,112,178,266–272], as more stringent experiments have progressively eliminated several other candidate connexins.

One of the obvious questions arising from the cloning and functional expression work is whether retinal neurons show a cell-specific pattern of connexin expression. The fish retina has once more been generous in yielding useful information. For example, in situ hybridization has shown that two connexins are expressed in the zebrafish inner nuclear layer. Thus, zebrafish (zf) Cx55.5 labels a band of regularly spaced cells that, according to their localization and spatial distribution in the inner nuclear layer, as well as some morphological features, most likely represent horizontal cells [264]. Moreover, the unitary conductance of zfCx55.5 is in agreement with the electrophysiological data reported for gap junction channels in horizontal cells [264,273–275]. The spatial distribution of labeled cells, however, alternates with signal-free areas, suggesting that only a subtype of horizontal cells may express zfCx55.5. More recently, transcripts for a second connexin, zfCx52.6, have been detected using two different techniques at the border of the inner nuclear and outer plexiform layers, exactly at the site where horizontal cells are localized [265]. In contrast to the clustered expression of zfCx55.5, zfCx52.6-positive signals are detected as linearly arranged cell lines covering most of the retinal circumference. This topological distribution strongly indicates that the expression of zfCx52.6 is restricted to horizontal cells [265]. Despite intense efforts, the identity of the connexin(s) that compose electrical synapses between horizontal cells in mammals remains unknown. If one were to extrapolate the results obtained in zebrafish, one possible candidate would be Cx57, which on the basis of sequence homology is the closest mouse relative of zfCx55.5 and zfCx52.6 [264,265,276]. Interestingly, a strong signal for Cx57 mRNA has been detected by RT-PCR [269], but the distribution of this connexin in the retina has not been examined thus far.

In the mammalian retina, Cx36 is expressed by several classes of neurons (Fig. 6), particularly amacrine cells of the AII (rod)-type, and participates in both homologous and heterologous gap junctions with other AII amacrine and cone bipolar cells, respectively [270–272] (see Table 1). By generating mice in which the Cx36 coding sequence was replaced with histological reporters, it has now been shown that Cx36 is expressed in at least five different cell types. Thus, analysis of the reporter distribution has demonstrated that, besides AII amacrine cells, Cx36 is expressed in photoreceptors—whether rods, cones, or both is still a matter of debate—two kinds of cone bipolar cells and a number of cells within the ganglion cell layer [272]. Using a similar genetic approach, the presence of Cx45 in the retina has been studied by examining the expression of the reporter gene β-galactosidase. Cx45 promoter activity appears to be confined to a small number of cells in the inner nuclear and ganglion cell layers [267]. Since it has been calculated that over half of the neurons in the ganglion cell layer are displaced amacrine cells [277], it is possible that the positive β-galactosidase activity may originate from these cells. More recent work has revealed that Cx45 is expressed in two groups of bistratified ganglion cells that show homotypic coupling [278]. Should these cells represent direction-selective ganglion cells, as proposed by the authors, this

observation would lend support to the notion that coupling might be involved in the generation of direction-selective responses [278].

Despite the paucity of molecular information on the constituents of gap junctions in the mammalian retina, tracer coupling experiments suggest that distinct classes of channels are present. In one elegant study, a series of structurally related tracers of increasing size was used to compare the relative permeability of gap junctions between different types of neurons [217]. The basic assumption was that the rate of diffusion would decline with tracers of larger size and, by normalizing the data to the smallest tracer, one could then derive for each gap junction type a profile relating tracer size to permeability. If gap junction channels in the different networks analyzed (four homologous and one heterologous) consisted of the same connexin type, they should in theory produce similar, if not identical slopes of decline in the coupling rate. Instead, the experimental results show that the permeability of these channels can be separated into three groups: A-type horizontal cells exhibit the smallest decline with increasing tracer size, B-type horizontal and AII amacrine cells have an intermediate slope, whereas the diffusion of tracers from AII amacrine to ON-bipolar cells falls sharply with the larger molecules. The simplest interpretation of these results is that at least three connexins are differentially deployed in the mammalian retina, although the authors do point out that alternative explanations, such as cell-specific post-translational modifications of a single connexin, or different heteromeric/heterotypic combinations of just two connexins, cannot be ruled out [217].

4.3. Gating retinal connexins

Gap junction communication between retinal neurons is regulated via multiple independent mechanisms and distinct pharmacological properties have been described in different gap junctional pathways of the vertebrate retina [243,273,279–288]. Scores of publications have documented that homologous coupling between horizontal and AII amacrine cells is modulated by cAMP, cyclic guanosine monophosphate (cGMP) and intracellular acidification [255,273,289–295]. As an example, let us consider the release of the neurotransmitter dopamine upon stimulation of the vertebrate retina by light from interplexiform cells, an amacrine subtype [296–299]. This catecholamine modulator, which is responsible for many of the events that lead to neural adaptation to light, closes gap junction channels between both horizontal and AII (rod-driven) amacrine cells, thus restricting lateral signaling at the outer and inner plexiform layers. The action of dopamine is mediated via the D1 receptor subtype that triggers the activation of adenylate cyclase and the ensuing increase in cytosolic concentrations of cAMP, which, in turn, stimulates protein kinase A (PKA), a cAMP-dependent protein kinase [255,273,300–303]. It is believed that this signaling cascade promotes phosphorylation of the connexins present in these cell types and, consequently decreases both the duration and frequency of channel openings [274,301]. Although direct phosphorylation of one of the retinal connexins by PKA has not yet been demonstrated, it has been recently reported that perch Cx35, the fish ortholog of Cx36, is gated by cAMP and that a specific PKA consensus sequence present in the middle cytoplasmic portion is required for channel gating [304]. Dopamine also has an opposite effect on either rod–cone or horizontal cell electrical synapses, which is mediated via a D2 receptor mechanism, whereby D2 agonists increase and D2 antagonist decrease coupling [287,305]. Two other messenger molecules have received special attention. Thus, the nitric oxide transmitter system reduces the macroscopic junctional conductance between horizontal cells and at the cone bipolar to AII amacrine electrical synapse, an effect that appears to be mediated by activation of the cGMP/cGMP-dependent protein kinase G (PKG) pathway [273,295,306,307]. More recently another signaling molecule, retinoic acid, has been added to the list of gap junction modulators in the retina [288,308]. Retinoic acid, which is a potential endogenous neuroactive substance in the vertebrate retina, mimics the effects of background light on horizontal cell responses and acts independently of other known second messenger systems (e.g. Ca^{2+}, PKA, PKG, PKC and calmodulin kinase), suggesting that it closes electrical synapses between fish horizontal cells via a direct gating mechanism [309].

As is the case with more orthodox ion channels (e.g. sodium, potassium, calcium), the conductance of intercellular channels is affected by a difference of potential, or transjunctional voltage, between the coupled cells. The kinetics and steady-state properties of voltage dependence have revealed that a wide range of voltage gating behaviors exists [15]. Since one distinct feature of the neuronal Cx36 and of its fish orthologs is their weak voltage sensitivity [177,262,266,310], it had been speculated that electrical coupling between retinal neurons may not be easily disrupted by shifts in membrane potential that occur during periods of visual activity. This possibility has received some support from electrical recording between pairs of retinal neurons, where it has been shown that, by-and-large, junctional currents are nearly ohmic over a certain range ($\pm$60 mV) of transjunctional potentials [239,273,292,311,312] (but see also Ref. [303]). By contrast, the electrical properties of retinal connexins recently isolated from fish clearly indicate that neurons are also endowed with voltage-sensitive electrical synapses (Fig. 7). Of note, zfCx55.5 exhibits a unique feature that has not been reported for any other gap junction channel thus far [264]. Instead of closing in response to transjunctional voltage, zfCx55.5 is characterized by a voltage-induced opening in homotypic configuration and forms rectifying channels [2,313,314] in heterotypic settings. Since zfCx55.5 revealed high levels of expression in different layers of the retina, where it

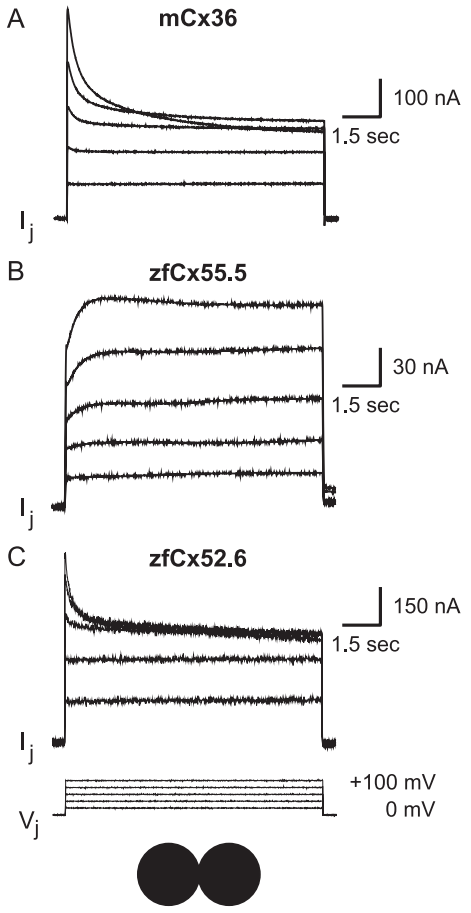

Fig. 7. The voltage-gating behavior of retinal connexins is diverse. *Xenopus* oocytes were injected with RNA encoding either mouse Cx36 (mCx36), zebrafish (zf) Cx55.5 or zfCx52.6 [265], and then paired in homotypic configuration for measurements of junctional currents by dual voltage clamp. The two paired oocytes (symbolized by the black circles) were initially clamped at −40 mV to ensure zero transjunctional voltage (V_j). While one cell was held at a constant potential, depolarizing V_j steps of 10 s duration were sequentially applied in 20 mV increments (bottom traces) to the other cell and the resulting junctional currents (I_j) were recorded. In the case of mCx36 (A) and zfCx52.6 (C), currents reflect a voltage-induced closure for V_j steps greater than +40 mV [265,266]. The kinetics of channel closure, however, differ significantly. Thus, fitting the current decay of these representative traces to a second order exponential function, the calculated rate of channel closure of zfCx52.6 at a V_j of +100 mV yields time constants of 0.25 and 6.84 s for the fast (τ_1) and slow (τ_2) components, respectively, whereas the time constants of mCx36 with the same imposed V_j are 0.32 and 3.04 s for τ_1 and τ_2, respectively. By contrast, zfCx55.5 (B) exhibits an opposite voltage gating behavior (viz., a V_j-induced channel opening), a unique feature that has not been reported for any other gap junction channel thus far [264].

can interact with other connexins in heterotypic configurations, such rectifying synapses may well exist in vivo.

Recent studies have pointed out to a previously neglected property of connexins, namely that they appear to be active in a variety of cells also in the non-junctional plasma membrane as unpaired connexons, or hemi-channels [315,316]. While the first evidence was obtained from the functional expression of Cx46 in *Xenopus* oocytes [317], voltage-clamp recordings from solitary retinal neurons have demonstrated the presence of large voltage-dependent membrane currents that exhibit some properties pointing to connexins as the prime suspects [318,319]. Hence, these conductances are activated by reducing extracellular Ca^{2+} concentration and, in parallel, cells become permeable to lucifer yellow, a hallmark of channels composed of connexins. Remarkably, hemi-channel currents are suppressed by the application of dopamine and prove sensitive to the same experimental manipulations that affect gap junction channels, such as changes in cAMP, cGMP, intracellular pH [318,319], with the exception of quinine modulation [320,321]. Consistent with these observations, functional expression of several retinal connexins has resulted in the development of voltage activated non-junctional membrane currents in *Xenopus* oocytes [265,266,304,322,323], although the ability to assemble functional hemi-channels is a property shared by many members of the connexin family (reviewed in Refs. [315,316,324]). As we will discuss in the next section, this property may be essential for an aspect of neuronal signaling in the outer retinal layer and not simply reflect an artifact of the isolation procedure of horizontal cells or the peculiarity of the heterologous expression system.

4.4. Function of gap junctions in the retina

It is widely accepted that coupling through connexin channels in the retina is an important element of transmission of the visual stimulus as well as signal processing from the outer to the inner retinal layers [216–220]. There are multiple pathways that incorporate electrical synapses in the transmission of visual signals from photoreceptors to ganglion cells, but three aspects of this circuitry and their functional implications have been investigated in great detail: coupling between photoreceptors and the improvement of signal-to-noise ratio under different light conditions, coupling between horizontal cells and the mechanism of lateral inhibition and, finally, electrical synapses of AII amacrine cells and the transmission of rod-mediated signals in the mammalian retina.

Current flow through coupled rod photoreceptors reduces the amplitude of the normal hyperpolarization resulting from absorption of even a single photon, while spreading its response to neighboring cells. Thus, by coordinating the voltage responses of a network of coupled rods, gap junctions would improve the detection of diffuse light, given that all the rods would respond equally to the stimulus, but would be detrimental in the case of a small localized spot of light, when coupling would reduce the amplitude of the photoreceptor response and worsen the signal-to-noise ratio [220,325]. Electrical synapses are also utilized by cones in several vertebrate species. Since the visual acuity depends on the quality of the signals generated by cone photoreceptors (e.g. the ability to resolve two points as discrete entities depends on the difference in signal intensity between neighboring cells), it has long been assumed that

electrical coupling between cones would impair spatial resolution by reducing the differences between signals in neighboring cells. By contrast, recent data suggest that the opposite occurs [239]. Because of random photon absorptions and fluctuations of signaling molecules and ion channels, the electrical noise of each individual cone is asynchronous and independent of the others, unless it is coupled to neighboring cones. It has been proposed that this coupling leads to a drastic reduction of the noise level that far exceeds the reduction of signal differences that are also caused, thereby improving visual resolution [239]. Finally, heterologous coupling between rods and cones is likely to be important under mesopic light conditions. In this situation, it has been postulated that rod signals utilize electrical connections between rods and cones to reach ganglion cells directly via cone bipolar cells, bypassing the conventional rod-dependent route [237].

Horizontal cells are a key element for the generation of center-surround antagonism in the outer retina. This feedback or lateral inhibition is a property shared by many retinal neurons whereby a light spot in the periphery, or surrounding annulus, evokes a response of opposite polarity to that elicited by illumination of the central zone. Horizontal cells receive excitatory chemical inputs from photoreceptors in the center and then feed back inhibitory signal to cones in the surround zone, blunting their response to light stimuli. This antagonism is responsible for the organization of bipolar and ganglion cell receptive fields and is thought to represent a key initial step of encoding spatial information and contrast in visual signals [216, 217]. Two mechanisms are involved in this pathway and they may both depend on connexin channels. First, homologous coupling between horizontal cells allows enlargement of the receptive field far beyond the limits of their dendritic tree, thus allowing the lateral flow of post-synaptic signals over distances up to several millimeters. In fact, the size of the horizontal cell receptive field is regulated in parallel to the extent of electrical and dye coupling by dark- and light-induced changes [281,296,326,327]. Second, hyperpolarization of horizontal cells modulates the voltage-dependent Ca^{2+} channels of the cones through an ephaptic interaction [328]. Ephapses represent a non-synaptic mode of neural transmission in which electrical impulses or changes in ionic concentration in the vicinity of one cell affect the electrical activity of an adjacent one. This model postulates that, in the carp retina, opening of connexin hemi-channels (i.e. connexons) in horizontal cell dendrites creates a current sink that would result in a more negative potential in the extracellular space near the synaptic ribbon [328]. This would, in turn, depolarize the cone pedicle and increase glutamate release, which is precisely what would be expected if the light stimulus to the cone were reduced. This feedback response, therefore, reduces the size of the light-induced signals that are transmitted to bipolar cells, and subsequently to ganglion cells. The experimental evidence in support of this idea is both ultrastructural and pharmacological. An ortholog of Cx26 has been localized, in carp and turtle, to the membrane of the lateral processes of horizontal cells in the close vicinity of voltage-gated Ca^{2+} channels of the cone terminals and all feedback-mediated responses are abolished by the gap junction blocker carbenoxolone, which also closes hemi-channels [328,329]. Since Cx26 is not present in mouse horizontal cells, it will be interesting to elucidate whether a distinct connexin takes up this role in the mammalian retina (perhaps Cx57) or whether a different mechanism of inhibition is in place.

Another type of retinal interneuron, the AII (rod) amacrine cell, is also extensively connected to neighboring cells of the same class through gap junctions that are permeable to neurobiotin, but not to lucifer yellow [226,255,330]. A direct electrophysiological demonstration that these gap junctions are the morphological correlate of electrical synapses between AII amacrine cells has been obtained only recently, by recording from cell pairs of rat retinal slices [312]. Coupling between identified AII cells is always strong when cells have overlapping dendritic trees (visualized by tracer injection at the end of the experiment), possesses the expected characteristics of a low-pass filter, and is not gated voltage over the range of transjunctional voltages tested. From this analysis, it has been proposed that electrical synapses between AII amacrine cells mediate synchronized activity with respect to spiking and subthreshold membrane fluctuations [312]. Although gap junction channels perform a similar task in interneuron networks in other areas of the brain (see Section 3), an important difference is that the latter networks are, in most instances, connected by both electrical and chemical synapses (see Section 3.3), whereas there is no evidence of inhibitory chemical synapses between AII amacrine cells. As is the case for horizontal cells, the release of dopamine by light triggers a D1-receptor signaling cascade (increased cAMP levels and PKA activity) that closes gap junction channels between AII (rod-driven) amacrine cells, thus restricting lateral signaling at the inner plexiform layer and providing at the same time a flexible switch between sensitivity and spatial resolution in the rod pathway. It is noteworthy that Cx36 bears the same PKA consensus sequence in the middle cytoplasmic loop that confers cAMP sensitivity to perch Cx35 [304].

A well-known mechanism that the retina uses in order to optimize function in different light conditions is the shift from the use of rods at low light (scotopic) levels, to the use of cones at high (photopic) light levels. In this mechanism the dynamic modulation of gap junctions between the bipolar and amacrine cells is pivotal, since heterologous electrical coupling between AII amacrine and ON-center bipolar cells constitutes the route to ganglion cells for the rod-driven signaling pathway. In fact, while retinal cone bipolar cells synapse directly on ganglion cells, rod bipolar cells do not; rather, they synapse on AII amacrine cells, which in turn are connected by gap junctions both to each other and to the ON-center cone bipolar cells [253,256,

258,331]. Since both rod and cone inputs ultimately converge on cone bipolar cells, a mechanism must exist to distinguish between the rod and cone inputs which are active under different light conditions. AII/AII junctional communication is reduced by dopamine or forskolin, indicating a cAMP-mediated regulation of these gap junction channels. Forskolin, however, has little effect on AII/cone bipolar communication. By contrast, cGMP agonists inhibit intercellular communication from AII cells to the cone bipolar cells, but not between AII cells [294]. In addition, the gap junctions joining these cells to each other and to cone bipolar cells exhibit a remarkably distinct size selectivity. Neurobiotin (MW=286, charge +1) passes easily between both AII/AII and AII/cone bipolar junctions, whereas biotin-X cadaverine (MW=442, charge +1) passes readily only through AII/AII gap junctions [294]. Although these data had been originally interpreted as evidence that the repertoire of connexins composing channels between AII cells would differ from that utilized at heterologous AII/cone bipolar electrical synapses, more recent work has demonstrated that Cx36 is present in at least two classes of cone bipolar cells, including ON-center cone bipolar coupled to AII amacrine cells [272]. In accordance with these findings, simultaneous whole-cell recordings from pairs of AII amacrine cells and ON-center cone bipolar cells in rat retinal slices have provided direct evidence for strong electrical coupling with symmetrical junction conductance that displayed characteristics similar to those previously reported for homologous AII/AII cells [311]. It is difficult, therefore, to account for the different gating properties of the two types of electrical synapses made by AII amacrine cells. One possibility is that they depend on the cellular segregation of either scaffolding or effector proteins, which would result in the formation of signalosomes with distinct capabilities for each synaptic connection. Alternatively, cone bipolar cells may also express another connexin and the resulting heterotypic/heteromeric channels may display a different sensitivity to PKA activation. It is clear that, from a physiological standpoint, the molecular composition of electrical synapses in the rod pathway is a very important issue in the retina circuitry. Given the heterogeneity of the cone bipolar cells (both ON- and OFF-center), it is likely that the definitive connexin map has not yet been established.

A direct assessment of the contribution of Cx36 to the transmission of visual signals along the rod pathway has become possible by analyzing a number of parameters in Cx36 knockout mice [272,332]. The take-home message is that both lines of mutant animals show functional deficits indicating that Cx36 is necessary for the propagation of rod signals to ON-center ganglion cells. Two separate lines of evidence that deletion of Cx36 disrupts junctional coupling between neighboring AII cells and between AII-cone bipolar cells were provided. First, dye coupling experiments in which neurobiotin was injected into single AII cells show that, while the tracer diffuses to many neighboring AII and cone bipolar cells in wild-type animals, it remains virtually confined to the injected cell in the Cx36 knockouts [272]. Second, the lack of Cx36 interferes with the transfer of the neurotransmitter glycine from AII amacrine to cone bipolar cells. The observation that many bipolar cells contain and accumulate glycine in the absence of specific transporters, which are obviously expressed by the glycinergic AII amacrine cells, had first led to postulate [333], and then to functionally demonstrate [334] the presence of a biochemical coupling pathway between these two cell types. Consistent with this hypothesis, glycine immunoreactivity is limited to AII cells and cannot be detected in cone bipolar cells of Cx36 null mice, indicating that AII-cone bipolar gap junctions have been functionally eliminated [272,332]. Electrical recordings reveal a complete elimination of rod-mediated, but not cone-dependent, responses at the ganglion cell level. Since only the response of low-sensitivity ganglion cells, which are driven by cones, is maintained in the Cx36 knockout retina, these data have been interpreted as an indication that both the primary rod pathway (via rod bipolar−AII−cone bipolar cells), which conveys the most sensitive response, and the alternative pathway (via direct communication to cones), which funnels responses of intermediate sensitivity, require Cx36-based electrical synapses [272]. Furthermore, recordings of electroretinograms showed a reduction in the activity of ON-center bipolar cells that is exclusively dependent on cone activation [332]. Together, these observations suggest that Cx36 may actually be present in cone photoreceptors and, therefore, play a processing role in the cone pathway. The completion of a map of connexin distribution in the mammalian retina and the generation of mutant animals with cell-specific deletion of connexin genes will allow to further dissect the relative contribution of selected gap junctions to the integration and processing of visual stimuli.

5. The unanswered questions

The presence of electrical synapses in the adult vertebrate brain is no longer in doubt and the emerging consensus has placed them as key players in different neuronal circuits. It should be kept in mind that gap junctional channels have some peculiar properties that set them apart from other ionic channels, as they allow not only electrical but also biochemical coupling. The next task on the agenda is to assign specific cellular functions to the presence of connexins in different neuronal populations and, further, to evaluate their role at the system and behavioral levels. This is not going to be a free ride and will most certainly take some time, as finding an answer to these questions entails a multidisciplinary approach that can be successful either with the synchronous activity of a large laboratory, or with the coordinated effort of a research network that is bi-directionally interconnected with electrical synapses and not inhibitory inputs. Rather than presenting a list of all the many

question marks that await an answer, we feel that two issues deserve special attention.

First: is the quest for neuronal connexins over? Considering the diversity of cell subtypes in the CNS, it remains puzzling that mammalian neurons utilize a small repertoire of connexins. It is possible that the systematic investigation of some of the new members that have been identified through database searches will lead to a more refined atlas of connexin expression. Another possibility is that other proteins form a different class of electrical synapses with distinct properties. Gap junction-based intercellular channels have been conserved throughout evolution as the cellular basis of direct cell–cell communication. Although these channels underlie similar functions in all multicellular organisms, vertebrates and invertebrates use two unrelated gene families to accomplish the same task [335]. Thus, database searches of the sequenced genomes of *Drosophila melanogaster* and *Caenorhabditis elegans* have confirmed that there are no connexins in invertebrates. Yet, innexins are expressed in the CNS of invertebrates and their role in wiring neuronal circuits has been firmly established. An intriguing group of proteins, which share some structural features with innexins, has been recently found through a database search to be present in both invertebrates and vertebrates [336]. Although multiple alignment show significant homology only in a very small region, the predicted four-transmembrane topology and the conservation of two cysteine residues in the extracellular loops raises the possibility that these proteins, which have been named pannexins [336], may be in fact vertebrate innexin equivalents and belong to the same superfamily. Our initial studies suggest that pannexins constitute an additional group of gap junction channel-forming proteins [337]. Thus, electrical recordings from single and paired *Xenopus* oocytes have shown that pannexin1 forms both hemi-channels and intercellular channels alone and in combination with pannexin2. Based on the high degree of co-expression of these two genes in several brain areas, we speculate that they may represent the molecular correlate of a novel class of electrical synapses [337]. It will be interesting to determine in which networks pannexins are the molecular correlate of inter-neuronal communication.

Second: how are connexins transported to their final destination, placed at synaptic contacts and retrieved, which proteins do they interact with during their cellular journey? So far, there is scarce information but the identification of their interacting partners (α and β tubulins, the c-Src tyrosine kinase, the ZO-1 scaffolding protein) has recently begun [338], and there is also initial evidence for a spatial interaction between electrical and chemical synapses, as connexins and NMDA glutamate receptors are closely associated at mixed synapses in the fish brain [339]. If precise connectivity has to be provided, a reliable delivery system needs to be in place for connexins and/or pannexins. In this respect, one could speculate that connexins are confined to the dendritic compartment, whereas pannexins are axonal, and that they are endowed with separate sorting mechanisms. Besides subcellular segregation into spatially distinct gap junction domains, it will be important to investigate which mechanisms are operative to preclude the inclusion of wrong cell types within gap junction communicative networks which, in the adult brain occur mainly within homogeneous cell types.

Finally, if one considers the experimental evidence indicating that connexins are important for neurophysiology (and we certainly do), it is also likely that they may be involved in pathology and several instances implicating connexins in disorders of the CNS have recently been reviewed [84]. Based on the notion that connexins represent a more evolved form of communication (unicellular organisms have the basic machinery for chemical, but not electrical transmission) [5], it is not unreasonable to propose that proper functioning of electrical synapses in the brain will have an impact of cognitive functions and performance. Thus, the development of specific pharmacological tools becomes indispensable. In this respect, the ability of connexins to form also hemi-channels may come handy for devising new screening strategies aimed at identifying molecules with connexin-selective actions.

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