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FEATURE ARTICLE
Gap Junctions in Developing Thalamic and Neocortical Neuronal Networks

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The presence of direct, cytoplasmatic, communication between neurons in the brain of vertebrates has been demonstrated a long time ago. These gap junctions have been characterized in many brain areas in terms of subunit composition, biophysical properties, neuronal connectivity patterns, and developmental regulation. Although interesting findings emerged, showing that different subunits are specifically regulated during development, or that excitatory and inhibitory neuronal networks exhibit various electrical connectivity patterns, gap junctions did not receive much further interest. Originally, it was believed that gap junctions represent simple passageways for electrical and biochemical coordination early in development. Today, we know that gap junction connectivity is tightly regulated, following independent developmental patterns for excitatory and inhibitory networks. Electrical connections are important for many specific functions of neurons, and are, for example, required for the development of neuronal stimulus tuning in the visual system. Here, we integrate the available data on neuronal connectivity and gap junction properties, as well as the most recent findings concerning the functional implications of electrical connections in the developing thalamus and neocortex.

Keywords: connexin, electrical synapse, gap junctions, neocortex, thalamus

Introduction

For a long time, electrical synapses in vertebrates and especially mammals have been regarded as reminiscent communication pathways from invertebrates, and much simpler signal transmission devices than the highly complex chemical synapses. Today, we know that gap junctions are abundant and very important during the prenatal phase of neurogenesis (Bittman et al. 1997; Bittman and LoTurco 1999; Kunze et al. 2009), while chemical synapses emerge mainly postnatally along with the establishment of neuronal circuits. However, in adult animals, electrical synapses are still present, connecting to different degrees, neurons in various brain areas (Mercer et al. 2006). Either at young ages or in adult brains, the roles electrical synapses have are diverse, serving as conductors of electrical charges and small molecules (especially IP_3 and Ca^{2+} ions); such signaling is essential for correlated electrical activity, metabolic synchronization, and also for the generation of the left-right patterning of brain structures (Oviedo and Levin 2007).

Gap junction structure, properties, and expression patterns, especially in rodents and humans, have been reviewed by several authors (Kumar and Gilula 1996; Sohl and Willecke 2004; Rackauskas et al. 2010). The structure of gap junction networks (Fukuda 2007), functions of electrical connections in all main brain regions (Galarreta and Hestrin 2001; Hormuzdi et al. 2004; Sohl et al. 2005; Bruzzone and Dermietzel 2006), and the first indications about the involvement of electrical connections in cortical development (Montoro and Yuste 2004; Sutor and Hagerty 2005; Elias and Kriegstein 2008) have been discussed as well. Among different brain areas, the thalamocortical system is one where the properties of gap junctions and electrically constituted networks could prove vital to responsiveness during active sensory exploration or sleep-related sustained inhibition (Long et al. 2004; Cruikshank et al. 2005). In the present review, we describe in more detail the connectivity pattern and the properties of gap junctions between developing neurons of various classes within thalamus and neocortex. We put these details in the context of network activity, brain waves, and the most recent findings concerning gap junction involvement in the functional maturation and stimulus tuning of neocortical pyramidal neurons.

Gap Junction Channel Features and Expression Patterns

The cells of deuterostomes express 2 families of proteins that have the ability to form hexameric channels in the plasma membrane: pannexins and connexins (Cxs). They do not have any homology to each other and, due to extracellular-loop glycosylation differences, it is suggested that only Cxs form junctional channels, whereas pannexins are present only as assemblies equivalent to Cx hemichannels (Rackauskas et al. 2010).

The Cx family consists of 20 members in the mouse. A Cx subunit consists of 4 alpha helical transmembrane domains (M1–M4), 2 extracellular loops (E1–E2), an intracellular loop (I1), and intracellular N- and C-termini. Six such subunits co-assemble to form a hemichannel; hemichannels of adjacent cells interact through noncovalent bonds to form a gap junction channel (Yeager and Gilula 1992; Foote et al. 1998).

Connexin gap junctions are relatively nonselective in their permeability to molecules with diameters of <1.5 nm. Therefore, inorganic ions (Na^+, K^+, Ca^{2+}, etc.), and small molecules such as cAMP and IP_3 can pass through gap junctions (Kumar and Gilula 1996). Some studies have shown, though, that different types of Cxs can co-assemble and form gap junction channels with various permeabilities that can allow the discrimination of second messengers; this diversity could enable the generation of different compartments in which a group of cells are regulated by a certain type of second messenger (Elfgang et al. 1995).

Connexins are expressed in almost every tissue, and a big variety of their isoforms has been reported in neurons and glial cells. Ten such isoforms are expressed in the mammalian

nervous tissue (Nagy and Rash 2000; Hombach et al. 2004), out of which at least 6 being present in neurons: Cx26, Cx32, Cx36, Cx43, Cx45 (Nadarajah et al. 1996, 1997; Condorelli et al. 1998, 2003), and Cx57, specifically expressed in the horizontal cells of the retina (Hombach et al. 2004).

Different types of Cxs are expressed at certain time points during brain development, and, in rodents, their expression profile is most dynamic, during prenatal development and the first 4 weeks after birth (Prime et al. 2000). According to their expression profile in the rodent neocortex, Cxs can be roughly classified into 3 categories: 1) Cxs that show little time-dependant changes or a gradual decline depending on species and/or brain area or cortical layer. Prime et al. (2000) have shown (RT-PCR) that, in rat, Cx45 mRNA levels remain constant beyond the fourth postnatal week. In contrast, Condorelli et al. (2003) suggest a continuous decline from P1 to adult (densitometric mRNA evaluation). In support for both observations, a study in mice (Maxeiner et al. 2003) shows that there is indeed a decrease in the expression of Cx45 throughout the brain from P8 to adult, including the cerebral cortex, but the levels remain quite elevated in the layers II, IV, and VI in the parieto-occipital cortex. 2) Cxs that show a significant increase in expression in the first 2 postnatal weeks, without decline during the following 2 weeks (i.e., Cx32, Cx43; Prime et al. 2000; Condorelli et al. 2003), and 3) Cxs that show an increase in expression during the first 2 postnatal weeks, followed by a decrease during the third and fourth postnatal weeks (i.e., Cx26, Cx36; Nadarajah et al. 1997; Prime et al. 2000).

Principles of Gap Junction Signaling

Dye injection experiments have revealed in the early 1990s (Peinado et al. 1993) that neurons in the P5 rat neocortex (layers II/III and IV) form clusters of cytoplasmatically interconnected cells. But even before neurons are positioned in the correct layer and attempt to form proper connections, they are connected via gap junctions to their clonally related neighboring neurons and radial glial cells (Noctor et al. 2001; Yu et al. 2012).

Gap Junctions and Neuroblast Migration

Although the complete picture of gap junction involvement in neurogenesis has not been elucidated yet, there is strong evidence that their role is very important at this critical stage in cortex formation. Bittman et al. (1997) describe that, in the ventricular zone, gap junction-connected cell clusters appear predominantly during the phase of embryonic neurogenesis and are important for fate determination of neural progenitor cells (e.g., layer specificity). As cells go through G1-S-G2 and M phases of their cell cycle, they couple and uncouple, closely apposed and clonally related cells being metabolically synchronized. Those cells that do not re-enter the S-phase will not rejoin clusters either, suggesting that gap junction coupling of neuroblasts influences the moment when neurons are generated and assume a certain fate. Moreover, it has been shown that as neocortical precursor cells progress through their cell cycle, coupling and uncoupling from their neighbors, the expression levels of Cx26 and Cx43 change as well (Bittman and LoTurco 1999). A decrease in the levels of Cx43 during the G1 phase of the cell cycle increases the probability that cells initiate differentiation and will not recouple to neighboring cells. Additional support for the major role gap junctions play in neurogenesis (this time in adult), comes from work done in hippocampus (Kunze et al. 2009), where mice lacking Cx30 and Cx43 in radial glia-like cells (that would otherwise allow tracer diffusion between cells) display almost complete inhibition of proliferation and a reduced number of granule neurons.

In the anterior extension of the subventricular zone (SVZ), immature cells are migrating towards the olfactory bulb forming the so-called rostral migratory stream. Marins et al. (2009) described that cell migration within this stream is inhibited by the gap junction blocker carbenoxolone. This drug has been often used in order to block the gap junction channel. However, in at least one study (Rouach et al. 2003), it has been shown that carbenoxolone has also unspecific effects, reducing excitability in neurons through a mechanism likely independent of astrocytic or neuronal gap junction blockade. Both Cx43 and Cx45 were present in SVZ explants, with the latter being present mainly at the outer border of the SVZ and in the ependymal lining, regions more populated by radial glia-like cells. One of their most interesting findings concerned the incidence of homocellular and heterocellular couplings and the size of the coupled cell clusters in 2 subregions of the anterior SVZ: SVZb, in the region of the olfactory bulb, and SVZc, where adjacent neocortex is present. In the SVZc, the incidence of heterocellular coupling is higher for neuroblasts and astrocyte-like cells, whereas in the SVZb, homocellular astrocyte-like–astrocyte-like and neuroblast-neuroblast coupling prevails. This may suggest that as they get closer to the olfactory bulb region, the cells that supply this area become more specialized, possibly forming clusters and that gap junction connectivity is maintained mainly between cells with similar functional properties. The reduction in the number of heterocellular electrical connections that takes place as the brain develops, does not mean that this kind of connections are always eliminated nor that the homocellular ones are always maintained. In the retina, for instance, there are highly specialized heterocellular connections occurring between most of the cell types (Bloomfield and Volgyi 2009); and in the adult brain connectiones between pyramidal neurons are most probably absent (Yu et al. 2012). However, the general trend appears to illustrate a loss in electrical connectivity between cells from different classes, as networks mature (e.g., around P14, neocortical pyramidal neurons are connected via gap junctions to interneurons, but in adult animals no such connections have been observed; Meyer et al. 2002). The astrocyte-like–astrocyte-like coupling occurs more often than interneuroblast coupling and the cluster size of the former is also larger, especially in the SVZc.

Apart from the role of gap junctions as intercytoplasmatic communication mediators, there is evidence that they also function as adhesion molecules during development (Elias et al. 2007). With an shRNA approach, it has been shown that knocking down the levels of Cx26 and Cx43 rendered more neurons unable to migrate into the cortical plate, which led to their accumulation in the intermediary zone. The mutation of a conserved tyrosine residue in the third transmembrane domain of Cx26 and Cx43, which renders the whole channel closed, was unable to prevent the proper migration of neurons in the cortical plate; this showed that the aqueous pore between the 2 connecting cells is not important for cell migration. However, the effect of this mutation on the

permeability of the hemichannel was tested in C6 glioma cells using propidium iodide; this mutation could render the pore of the hemichannel impermeable for the staining agent, but might still connect the 2 cells to some extent. The only way the authors were able to interfere with cortical migration was by expressing Cxs with mutations on one of the conserved extracellular cysteines that would make it impossible for hemichannels to adhere to one another. Connexins could be involved in the process of neuronal migration in such a way that they stabilize the dominant branch of a migrating neuron along the radial glial fiber; Cx43 puncta have been shown to be the main Cx present in the dominant leading processes that would be maintained over time, and absent in the transient branches.

It has also been shown that as pyramidal neurons migrate from the proliferative ventricular zone to the cortical plate they change their morphology from bipolar to multipolar and back to bipolar in a process partly dependant on Cx 43 via p27 signaling (Liu et al. 2012). Cell differentiation is another important step in brain maturation; and, although information is lacking in the neocortex about the involvement of Cxs, experiments in striatal neurospheres have shown that neuronal and oligodendrocyte differentiation is partly mediated by gap junctions (Cx36; Hartfield et al. 2011). This demonstrates the involvement of Cxs in all the major steps of cellular maturation.

Electrical Communication Properties

During early postnatal development, many types of already differentiated neurons in the brain are connected via gap junction channels, forming a syncytium, which could be important for the metabolic coordination of clonally related cells (Noctor et al. 2001; Yu et al. 2012). The electrical coupling could be the only reliable pathway of communication, at a time when signaling via chemical synapses is being established (Dupont et al. 2006). In the adult, excitatory neurons from many regions lose their gap junction connectivity (Fig. 1*A*,*C*; Peinado et al. 1993; Connors and Long 2004), although interneurons in the cortex and the thalamic reticular nucleus (TRN) remain still highly connected (Fig. 1*B*,*C*; Fuentealba et al. 2004).

Properties of the electrical connectivity between neurons, such as the type of neurons that are interconnected, the prevalence of electrical coupling (Gibson et al. 1999; Venance et al. 2000; Long et al. 2004), the junctional conductance, the coupling coefficient (Wang et al. 2010), and the asymmetry of coupling have been studied (Haas et al. 2011), during development, in a number of interneurons and pyramidal cells from thalamic nuclei and various neocortical regions (Galarreta and Hestrin 1999; Long et al. 2004; Parker et al. 2009).

Gap Junction Connectivity Rate

Regarding the prevalence of electrical coupling and the type of neurons that are interconnected, studies have shown that electrical connections occur far more often between neurons of the same type and at younger developmental stages. Gap junction-connectivity rate has been evaluated, in most cases, as the fraction of cell pairs that display electrical connectivity. Dye-coupling experiments were also performed, although it may be more difficult to estimate the extent of electrical coupling due to various dye permeability and diffusion properties. It is therefore impossible to compare connectivity rates from studies using different approaches.

In the somatosensory neocortex of P14-P21 rats, as many as 62% of the fast-spiking (FS) interneurons are electrically coupled to each other (Table 1), and low-threshold spiking (LTS) interneurons have an even higher coupling rate (85%). Gap junctions between FS and LTS are much less prevalent (10% or less; Galarreta and Hestrin 1999; Gibson et al. 1999; Fig. 1*B*). Somatostatin neurons of layer II/III of the visual cortex (bipolar interneurons) and of layer IV of the somatosensory cortex (fusiform interneurons) were also found to be interconnected via gap junctions during the third postnatal week (Venance et al. 2000). Bipolar interneurons are interconnected in 67% of the cases. Fusiform interneurons connect to each other and also to excitatory spiny stellate cells; the heterocellular coupling occurs less often (6%), illustrating a similar trend of reduced connectivity between cells from different classes. There are indications that earlier in development, neocortical FS cells may have lower connectivity rates (up to 25%; Pangratz-Fuehrer and Hestrin 2011), although the authors might have characterized only a subpopulation of FS cells. However, high electrical connectivity is maintained between cortical parvalbumin-positive interneurons after the third postnatal week, as animals mature. At P42, cells show a connectivity rate of 89%, with no apparent decrease from 90%, reported at P14 in mouse neocortex (Fig. 1*B*; Meyer et al. 2002).

In the thalamus, during the second postnatal week, TRN neurons have an incidence of coupling in the same range as neocortical interneurons, namely, 71% for cells <5 µm apart, and apparently unchanged from what was observed in the first postnatal week (Long et al. 2004; Parker et al. 2009). However, this value proved quite different from what was previously reported for TRN interneurons, when testing for electrical connectivity cells that were up to 30 µm apart; here, the incidence of coupling was only 31% (Landisman et al. 2002). This observation already indicated that the clusters of interconnected inhibitory neurons in the thalamus are much smaller than what has been observed for neocortical interneurons (Amitai et al. 2002). Similar to other interneuronal populations elsewhere in the brain, inhibitory TRN neurons appear not to lose their electrical connectivity as the system matures. In fact, gap junctions prove to be vital for the generation and synchronization of low-frequency activity in the thalamus (Fig. 1*C*; Fuentealba et al. 2004; Blethyn et al. 2008).

Whereas the electrical coupling between interneurons has been extensively studied in the neocortex, hippocampus, as well as cerebellum, gap junction occurrence and functional implications for pyramidal cells and other excitatory neurons have remained largely unknown (Hestrin and Galarreta 2005; Haas et al. 2011; Postma et al. 2011). Wang et al. (2010) have found that in the prefrontal cortex of young (P14–43) rats and 6- to 9-week-old ferrets, the electrical connectivity rate between pyramidal cells is very low (0.5%–5%); this was in stark contrast to the electrical coupling previously reported for interneurons (at least 50% if both cells belong to the same class of inhibitory neurons; Hestrin and Galarreta 2005). These results are partly supported by far more early observations from dye-coupling experiments, which showed that at P5, pyramidal neurons are extensively connected (Fig. 1*A*), and this level of coupling is maintained until P12; after this time point, a sharp decline was seen until the age of P17 when the injected dye seemed not to spread to neighboring neurons anymore (Peinado et al. 1993). A similar developmental reduction in the occurrence of gap junctions has been reported for the excitatory neurons of the ventrobasal nucleus (VBN) of mouse and rat thalamus. In the interval between P2 and P9, VBN neurons

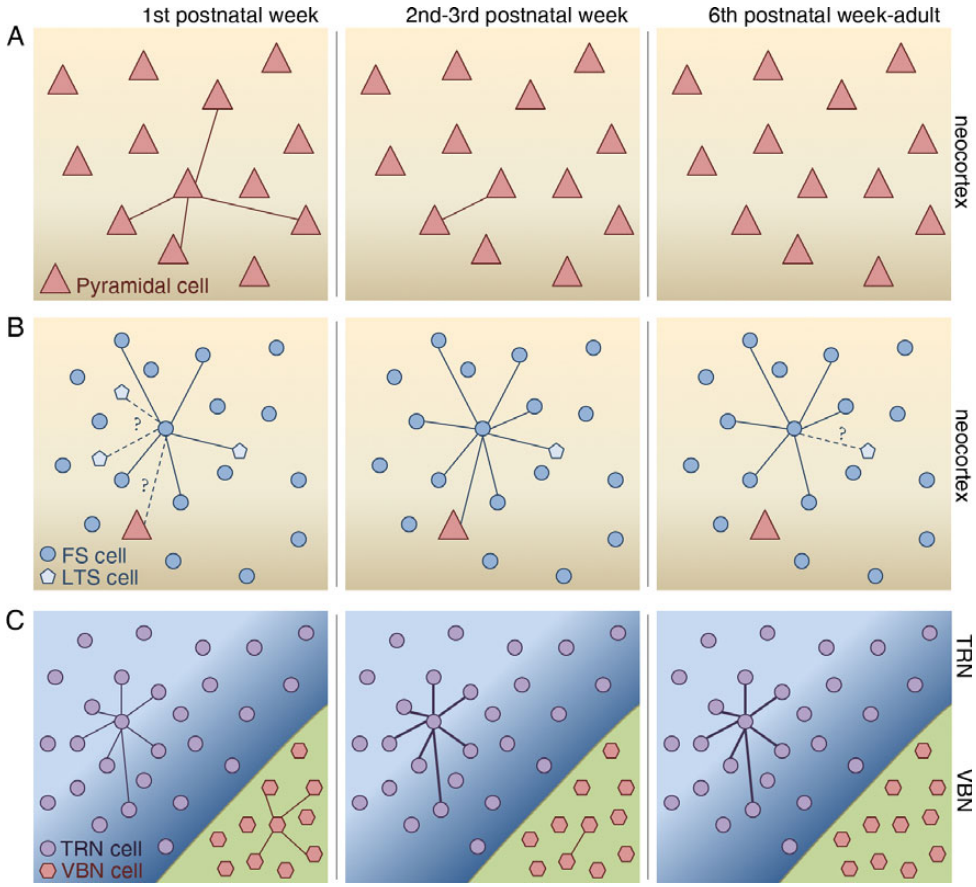

Figure 1. Electrical connectivity changes for cortical and thalamic neurons during development. (*A*) Cortical pyramidal neurons exhibit their highest connectivity rate during the first postnatal week; during the next 2 postnatal weeks, connectivity rates decrease considerably, and become almost absent in adult animals. (*B*) Cortical fast-spiking (FS) cells are possibly less interconnected during the first postnatal week than during the third postnatal week. Neurons of different classes, like low-threshold spiking (LTS) and pyramidal cell (PC), connect to FS cells at much lower rates than other FS cells, during the third week; PCs and LTS neurons possibly connect to FS cells at a similar or higher rate immediately after birth. Upon reaching adulthood, FS cells may lose all connections to neurons of different classes while maintaining high electrical connectivity with each other. (*C*) The inhibitory neurons of the TRN (thalamic reticular nucleus) have a high connectivity rate during the first postnatal week when compared with the excitatory neurons of ventrobasal nucleus (VBN). During the next 2 postnatal weeks, TRN neuron electrical connectivity rate remains the same while the junctional conductance increases; VBN neurons become less electrically connected. In adults, gap junctions appear to be still extensively present within the TRN whereas in the VBN they are absent. Each line connecting 2 cells is equivalent to 5%–10% of connectivity rate; see Table 1 for details. *A*: Peinado et al. 1993; Wang et al. 2010; Yu et al. 2012; *B*: Gibson et al. 1999; Meyer et al. 2002; Pangratz-Fuehrer and Hestrin 2011; *C*: Fuentealba et al. 2004; Parker et al. 2009; Lee et al. 2010.

have an electrical connectivity rate of up to 44%, which decreases to below 8% after P10; no electrical connectivity can be found after P13 (Fig. 1*C*; Lee et al. 2010).

A high degree of specificity of coupling has been demonstrated, where pyramidal cells and interneurons of different classes would preferentially connect to other neurons of the same type (Peinado et al. 1993; Gibson et al. 1999; Venance et al. 2000). Bittman et al. (2002) showed that pyramidal neurons do connect to nonpyramidal cells, as well as to astrocytes, through channels most often constituted of Cx26. Intriguingly, the connections between astrocytes and pyramidal neurons appeared to be unidirectional, with dye transfer only from astrocytes to neurons. This could be an example of asymmetrical gap junctions, the role of which might be to mediate astrocyte-dependent modulation of neuronal network synchronous firing. In layer II/III of the neocortex, at P14, pyramidal neurons were shown to be connected to FS cells in 8%–9% of cases, connectivity that becomes absent after P28 (Fig. 1*B*; Meyer et al. 2002). This could indicate that, earlier in development, different classes of neurons establish heterotypic electrical connections that would later be eliminated. Therefore, since evidence is lacking for the first days after birth, questions remain if the specificity of electrical connections is even lower than at P14 or not.

Whatever the change in connection specificity over time is, the fact that, in rats, during the second postnatal week, thalamic and neocortical inhibitory neurons connect to each other in proportion of 60%–85%, whereas pyramidal neurons remain connected only in 0.5%–5% of cases, make it obvious that the inhibitory and excitatory networks mature following different developmental steps; and that the role of their electrical connectivity is needed at various time points for the proper maturation of the neuronal networks. However, this difference in the electrical connectivity rate between excitatory and inhibitory neurons may be specific only to certain brain areas, such as the neocortex and thalamus; in hippocampus, during the second postnatal week, stratum oriens interneurons present a much lower connectivity rate of 11%–19% (Zhang et al. 2004). Various brain areas could also differ from each other with respect to the changes taking place in the electrically connected neuronal assemblies during development. Whereas, in the neocortex, there is

Table 1
Electrical connectivity rates for cortical and thalamic neurons during development

Brain region	Cell type	Postnatal week		
		1st	2nd–3rd	≥6th
Cortex	PC–PC	30%–40%[a]	0.5%–5%[b]	~ 0%[a]
	FS–FS	26%[c]	62%–90%[d,e]	89%[e]
	FS–LTS	n.d.	10%[d]	n.d.
	FS–PC	n.d.	9%[e]	0%[e]
Thalamus	TRN cell–TRN cell	75%[f]	71%[g]	"high"[h]
	VBN cell–VBN cell	30%–40%[i]	8%[i]	0%[i]

Note: PC, pyramidal cell; FS, fast-spiking interneuron; LTS, low-threshold spiking interneuron; TRN, thalamic reticular nucleus; VBN, ventrobasal nucleus; n.d., not determined.
As determined electrical connectivity rates depend on the distance between the 2 tested cells, we have also specified, where known, the range used by the authors for testing connectivity.
[a]Yu et al. 2012 (10s of μm).
[b]Wang et al. 2010 (0–20 μm).
[c]Pangratz-Fuehrer and Hestrin 2011
[d]Gibson et al. 1999 (<50 μm).
[e]Meyer et al. 2002 (<100 μm).
[f]Parker et al. 2009 (≤2 μm).
[g]Long et al. 2004 (<5 μm).
[h]Blethyn et al. 2008.
[i]Lee et al. 2010 (<5 μm).

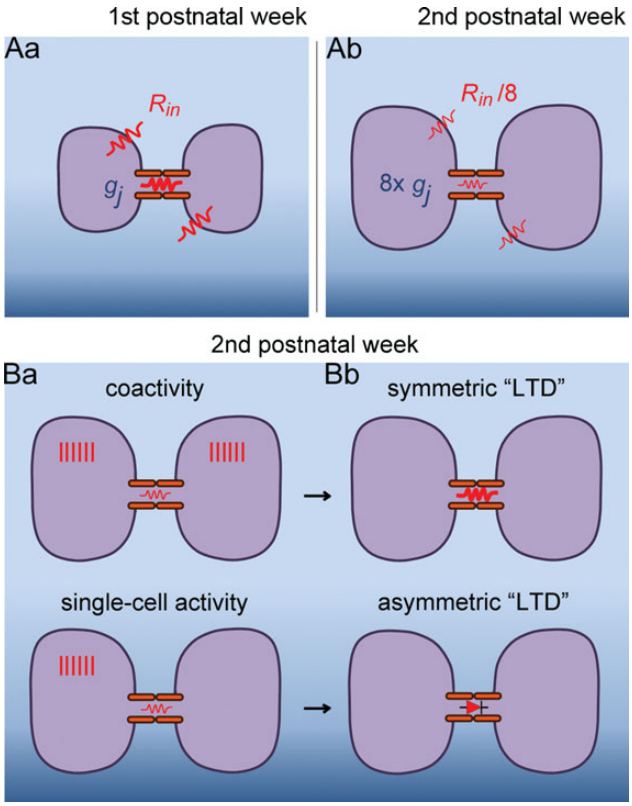

Figure 2. Plasticity of coupling strength as a consequence of development and electrical activity. (*A*) From the first postnatal week (*Aa*) to the second (*Ab*), there is an 8-fold increase in g_j (junctional conductance), possibly as a consequence of a decrease in the R_i (input resistance) of a similar magnitude (Parker et al. 2009). (*B*) After 2 TRN neurons are simultaneously active for a number of times (*Ba*, upper cell pair), the electrical connection between them decreases in strength, symmetrically (*Bb*, upper cell pair); when only one cell in the pair is active (*Ba*, lower cell pair), the electrical connection also decreases in strength, but in an asymmetric way, the formerly active cell being able to receive less input than it can send to the other cell (*Bb*, lower cell pair; Haas et al. 2011).

proof that pyramidal neurons lose possibly all their gap junction connections as networks mature (Yu et al. 2012), in the hypothalamus of adult rats, small clusters of CA3 neurons have been identified by dye injection experiments (MacVicar and Dudek 1980).

Gap Junction Biophysical Properties and Plasticity

The types of neurons connected via gap junctions and the extent of electrical connectivity in different networks reveal important information on gap junction formation and the types of networks that can be modulated through cytoplasm-transferable factors and electrical states; it is, however, important to know the "strength" and the degree of bidirectionality of such connections between neurons. The 2 parameters that are often evaluated are the junctional conductance and the coupling coefficient. The junctional conductance offers a direct measure of the electrical conductivity of the pores formed by different Cxs. The coupling coefficient represents the ratio between the voltage change in the coupled cell and the change in the cell where current injection occurs; therefore, the latter parameter also accounts for the membrane resistances of the 2 cells and reflects the relative influence the 2 neurons can have on one another.

Because of a prolonged presence of gap junctions in TRN interneurons, it was possible to study the age-dependent changes of electrical connectivity, and the possible correlations with modifications in the intrinsic properties of the neuronal membrane. Parker et al. (2009) showed that the junctional conductance increased 6–10 fold in P9–14 neurons when compared with P1–2, whereas the input resistance of these neurons decreased 8 fold as the animals matured, yielding an unvarying coupling coefficient across different ages (Figs 1*C* and 2*Aa*,*Ab*). These results indicate that where gap junctions are still important at older ages, their properties might have been evolutionarily adjusted in order to offset the changes in the biophysical parameters of the membrane. Because of the low-pass filtering, characteristic for gap junctions in inhibitory neurons, and the typical longer membrane time constant of very young neurons, it would be almost impossible to elicit spikes in postjunctional cells upon AP firing in the prejunctional neuron. But, at these young ages, neurons compensate for this apparent impairment by having broader APs, which allows them to transfer enough charge to the postjunctional cell in order to generate the same amount of depolarization as a narrow AP in older neurons with faster membrane time constants (Warren and Jones 1997).

The coupling coefficient between 2 connected cells appears to be different for interneurons and pyramidal cells: pyramidal cells of the neocortex of young rats and ferrets can have a coupling coefficient as high as 10 times the value for interneurons, and a junctional conductance 25 times higher (Wang et al. 2010). The increase in coupling strength would render the 2 connected pyramidal neurons extremely responsive to the electrical changes occurring in their coupled partner cell; occasionally, APs in the prejunctional cell would generate APs in the postjunctional cell, and in addition subthreshold spikelets. In terms of bidirectional coupling, gap junctions between pyramidal neurons of the neocortex present different degrees of asymmetry (Wang et al. 2010), whereas the coupling of FS and LTS interneurons of the somatosensory cortex (Gibson et al. 1999) is similar in both directions between cells of the same type. The coupling strength between neurons in the cerebral cortex and thalamus could also reach levels more specific

to these 2 brain areas, just as it is the case for connectivity rates during development. For instance, Mercer et al. (2006) have described a coupling coefficient for the pyramidal neurons of hippocampus that reaches less than half the value described by Wang et al. for pyramidal cells in the neocortex. Both studies were performed on animals of possibly similar ages.

In 2011, however, Haas et al. (2011) described a mechanism by which 2 electrically coupled neurons can unplug from each other in case of hyperactivity and increase the asymmetry of the electrical synapse. The study was performed on GABAergic neurons of the TRN. The magnitude of the influence the activity in one neuron can have on its coupled neighbor diminishes in both directions regardless of whether both neurons are coactive for a number of minutes or only one in the pair is highly active. However, in the latter case, the expression of long-term depression (LTD) of electrical conductivity at such an electrical synapse is asymmetrical; the amount of influence that the active neuron can receive from its partner (inbound coupling) decreases to a larger extent than the influence the active neuron can exert on the other (Fig. 2*B*). Therefore, while LTD can decrease the connectivity between the 2 active neurons in order to prevent hyperactivity in the network, in the case when only one neuron is active, changes in the gap junction channels could decrease, preferentially, additional electrical drive to the already hyperactive neuron. The authors have also established the importance of Na^+ spikes in the generation of LTD; in the presence of TTX, and when only one neuron was activated, the amount of the influence exerted on its neighbor did not change anymore, while the inbound coupling registered only a minor decrease. By referring to previous experiments (Alev et al. 2008), Haas et al. suggested that phosphorylation changes could account for the changes in Cx channel conductance, and that a switch to non-Cx36 subunits could increase synaptic asymmetry. Recently, it has been shown that due to phosphorylation by calmodulin-dependent protein kinase II (CaMKII) the junctional conductance of Cx36 gap junctions can increase up to 10 fold (Del et al. 2012).

Therefore, although most gap junction connections, either between pyramidal cells or interneurons, seem to have very little asymmetry in terms of junctional conductance (Gibson et al. 1999; Wang et al. 2010), there are examples where connections between different cell types (pyramidal neuron – astrocyte; Bittman et al. 2002) or between neurons of the same type (after periods of certain activity levels; Haas et al. 2011) show a strong asymmetry. This shows that gap junctions are not fixed connections between cells but, just like some classical ion channels (inward rectifiers), are also very selective and plastic in terms of directional selectivity of ionic conductance.

In addition to the electrical synapses that are modulated by the activity states of the connected cells and the intrinsic voltage dependence of the forming Cx subunits (reviewed by Gonzalez et al. 2007), there are neurons which appear to have a more linear, symmetrical and voltage-independent electrical coupling between them (Gibson et al. 2005). By using modeling studies in order to reproduce their experimental findings, Gibson et al. proved such a linear dependence between the voltage changes of 2 coupled FS inhibitory neurons. They also showed that FS cells that are not electrically coupled are able to fire synchronously only at high frequencies, whereas at low frequencies (<100 Hz) their firing is out of phase. However, cells that have strong electrical coupling are able to fire synchronously even at low frequencies, suggesting a possible involvement of gap junctions in the generation of gamma rhythms (30–60 Hz) among layer 4 inhibitory neurons. Considering that FS neurons are required to fire at high frequencies, it is possible that their electrical synapses are constituted of subunits that are not voltage sensitive and cannot mediate neuronal uncoupling like in the case of inhibitory TRN neurons.

Interesting aspects regarding synapse formation and maintenance during development involve the relationship between electrical and chemical synapse formation and the dependence of the latter on the presence and extent of the electrical coupling between neurons at critical steps in ontogeny. Previous studies indicated that electrical synapses, especially between excitatory neurons, represent the main communication pathway in the immature brain, and they decrease in numbers as neuronal networks mature and chemical synapses take over (Peinado 2001; Lee et al. 2010; Yu et al. 2012). Electrical and chemical connections made by excitatory neurons of the thalamic VBN follow such a developmental pattern during the first postnatal weeks. Within the thalamus, VBN neurons make chemical synapses almost exclusively with TRN neurons forming the characteristic disynaptic inhibitory circuit. During the first 6 postnatal days, when electrical synapses are often found between VBN neurons, there is no disynaptic circuit present yet. After P10, as electrical synapses approach the time point of their elimination, disynaptic connections between VBN neurons begin to emerge, illustrating a sequential developmental time course for the 2 types of connections (Lee et al. 2010). However, for the somatostatin-positive inhibitory cells of the neocortex, electrical synapses still represent the major way of communication during the third postnatal week, with the main type of Cx subunit expressed by these neurons being Cx36, and to a smaller extent Cx32 by some bipolar interneurons (Venance et al. 2000). Cortical parvalbumin-positive interneurons also maintain a high degree of electrical connectivity as animals mature (Meyer et al. 2002). Moreover, in one study (Pangratz-Fuehrer and Hestrin 2011), regarding connectivity of FS cells in layer V/VI of mouse visual cortex, electrical synapses between FS cells occur for the first time at P5–6, at a similar time point as the first GABAergic synapses. By the middle of the third postnatal week, the numbers for both types of synapses doubled, illustrating a parallel development of chemical and electrical synapses. The electrical connectivity of FS cells becomes very specific, and no connections can be detected between them and pyramidal cells; in contrast, the chemical synapses of FS cells onto pyramidal cells followed the same developmental pattern as onto other FS cells. However, the study was done in a mouse line (G42, GAD67-GFP) where only 50% of parvalbumin-positive neurons express EGFP; it is possible that their findings characterize only a subset of parvalbumin neurons. More such networks have to be investigated for a complete picture about the developmental relationship between electrical and chemical synapses in the brain; but there are already clear indications, that while excitatory neurons are electrically coupled only during early development, interneurons maintain their gap junctions even as chemical synapses become more prominent.

Although many changes have been shown to take place during development in terms of neuronal electrical coupling, there are only a few examples in which possible mechanisms for electrical coupling and uncoupling have been described (Haas et al. 2011). Apart from fast, activity-related changes in gap junction properties, additional long-term, modulatory effects on gap junction formation or elimination have been

observed (Landisman and Connors 2005; Park et al. 2011). In the somatosensory cortex, activation of type II mGluR receptors, between P1 and P15, induces an increase in the number of gap junctions between neurons and in the expression levels of Cx36, whereas $GABA_A$ receptor activation triggers the opposite effects (Park et al. 2011). Activation of type II mGluRs in the TRN (second postnatal week), however, had different consequences than in the neocortex: application of ACPD ((1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid; mGluRI and II agonist) reduced the electrical coupling responses of TRN cells by 23% (Landisman and Connors 2005). The opposing effects of mGluRII activation in the 2 brain regions indicate that additional research is needed for a full understanding.

Network Events Mediated by Gap Junctions

At a larger scale, gap junctions can be studied in terms of electrical and Ca^{2+} waves, originating at different locations and spreading across several brain areas. During the first postnatal days, the synchronized oscillatory activity of the mouse cerebral cortex is driven by subplate activity and spreads through a gap junction-coupled neuronal network (Dupont et al. 2006). The initiation of the oscillatory activity depends on the activation of muscarinic acetycholine receptors and gap junction-mediated rise in intracellular calcium, and is little affected by blockage of glutamate receptors during the first postnatal days. As the system matures, more glutamate receptors are being incorporated at chemical synapses, which take over the role of coupling neuronal domains through oscillatory activity, by the end of the first postnatal week. However, another study (Garaschuk et al. 2000) points towards a different kind of oscillatory cortical activity that is dependent on glutamate receptor activation even in P1-P2 rats. The reduced frequency of Ca^{2+} transients could indicate an underdeveloped network of chemical synapses at an age when oscillations through the electrical network predominate. A study by Peinado (Peinado 2001) describes 2 distinct types of oscillatory activity, in the neocortex of 1-week-old rats, which differ in their propagation pattern and sensitivity to glutamate receptor and gap junction blockers: “horizontal” waves and “vertical” waves. The former, which can spread over a few millimeters, are not abolished by glutamate receptor antagonists, but their propagation speed is significantly reduced in the presence of the gap junction-blocker carbenoxolone. “Horizontal” waves appeared to be partly mediated by gap junctions between the dendrites of layer IV neurons, as the wave propagation can be markedly disrupted at boundaries between barrels (since the dendrites are more strictly confined to individual barrels; Lubke et al. 2000).

In vivo, 2 independently occurring network events in the mouse primary visual cortex have been described before and at the onset of vision (Siegel et al. 2012). Low-synchronicity events (L-) originate in the retina, whereas high-synchronicity (H-) events are independent of retinal input. The latter have higher amplitudes and are sensitive to gap junction blockers, suggesting their origin might be in the subplate. Since during each H-event, all the neurons in an area are active in a highly correlated manner, the role of such events could be to homeostatically modulate synaptic weights and maintain activity levels within physiological levels.

From the electrical properties of Cx channels and the plasticity they can undergo it would appear as if most of the activity-related changes in gap junction-connected neuronal assemblies were due to spreading of electrical signals through the intercytoplasmic bridges. However, it has been shown at least in one case that the propagating cellular signal that spreads through gap junctions and activates neuronal domains is not actually electrical (Kandler and Katz 1998). In the developing visual cortex, the authors have shown that activation of neuronal domains depends on the release of calcium from internal stores, and extracellular calcium is not required. Although release of calcium from internal stores appears to be essential, it is not calcium that diffuses through gap junctions, but inositol triphosphate (IP_3), the synthesis of which is triggered by the activation of type I metabotropic glutamate receptors (mGluR1 and mGluR5). Since by blocking these receptors only the number, but not the size, of neuronal domains decreased, the authors concluded that mGluR signaling is involved in the initiation but not in the spreading of calcium waves, which is in turn carried by IP_3 diffusion.

Gap Junction-Dependent Stimulus Tuning

Since it became clear that the role of gap junctions in the central nervous system of higher vertebrates consisted of more than being reminiscent ways of communication from invertebrates, their exact functions have been under intense study. Apart from their involvement, early in development, in the migration of neurons to specific layers of the neocortex, the role of gap junctions in the maturation of neuronal networks has remained elusive. As we presented in the sections above, gap junctions are important for network activity, metabolically and electrically coordinating entire groups of cells. But how crucial is this coordination for functional development? Recent studies have addressed the involvement of gap junctions in adequate tuning of neurons in the visual cortex.

A very important role that gap junctions have been shown to play in the development of neuronal networks is to take coupled cells on a similar destiny in their maturation, as Li et al. (2012) have observed in the cortical layer II/III of the visual cortex soon after eye opening (P12–17). Pyramidal neurons that belong to the same clone exhibit similar orientation preferences for visual stimuli, when compared with cells that are not clonally related (Fig. 3*A*). In an accompanying study (Yu et al. 2012), sister neocortical pyramidal neurons have been shown to be preferentially connected to each other rather than with non-sister excitatory neurons. This preferential coupling between sister excitatory neurons is common until P6, after which electrical connectivity becomes scarce. The coupling conductance between sister cells is also substantially higher than between non-sister neurons (about 3 times higher; Fig. 3*Aa*). This facilitates, in sister neurons, AP firing through otherwise subthreshold individual stimuli. Moreover, the authors recorded the native subthreshold activity of neurons in the neocortex and then injected it in pairs of coupled neurons in order to evaluate how the neurons would respond to uncorrelated neuronal activity. The results showed that sister excitatory neurons are able to fire simultaneously in response to uncorrelated neuronal inputs. The development of similar orientation preferences was disrupted in visual cortex neurons expressing a dominant-negative mutant of Cx26, or when a gap junction blocker was administered during the first postnatal week (Fig. 3*B*); these results suggest that the formation of functional minicolumns in the adult requires

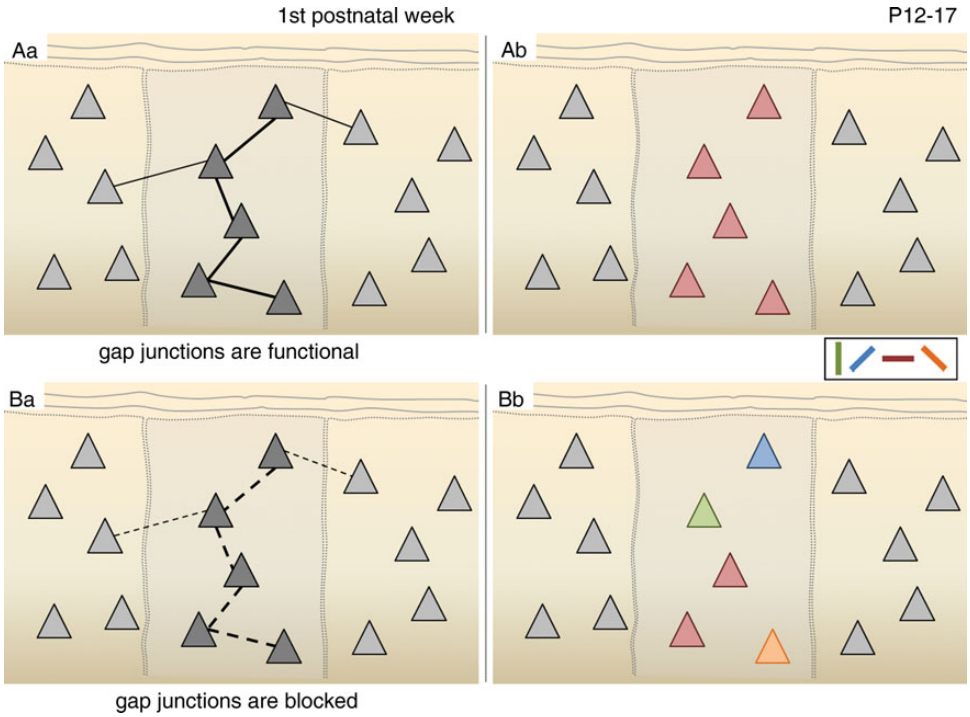

Figure 3. Gap junction presence during the first postnatal week influences orientation selectivity tuning. (*A*) In the visual cortex of the mouse, during the first postnatal week, clonally related neurons are 10 times more likely to connect to each other than to an unrelated neuron; connections between sister cells have a junctional conductance 3-fold higher than other connections (*Aa*) (Yu et al. 2012); if electrical connectivity is maintained, around P12–17, clonally related cells develop identical orientation preferences (*Ab*; Li et al. 2012). If gap junctions are blocked during the first postnatal week (*Ba*), the sister cells develop separately, exhibiting different orientation selectivity preferences (*Bb*).

intercellular communication via gap junctions and possibly simultaneous neuronal firing (Li et al. 2012).

It is well known that inhibition also plays a very important role in shaping neuronal responsiveness and tuning to different stimuli, and such a role of inhibition has been documented, for example, in the case of ocular dominance plasticity occurring in the binocular visual cortex during the "critical period" (Hensch 2004). Postma et al. (2011) showed that synchronous firing of the neurons forming the inhibitory network is essential to the ocular dominance shift. They found that upon afferent stimulation, a fraction of the evoked inhibitory postsynaptic currents is due to coupling via Cx36. A blockage of this coupling, as it happens in Cx36KO mice, would lead to a reduced activation of the inhibitory network and an increased facilitation of inhibition during high-frequency stimulation as a result of desynchronous interneuronal activation. This facilitation would further lead to the inability of generating LTP and induce a failure in strengthening the inputs coming from the nondeprived eye. In addition to failures in synaptic strengthening, the inability of uncoupled neurons to form proper chemical synapses could also lead to alterations in the ocular dominance shift mechanisms. Some studies have shown that electrical and chemical synapses develop in parallel up to a certain age (Pangratz-Fuehrer and Hestrin 2011), but Yu et al. (2012) demonstrated that elimination of electrical coupling between sister pyramidal neurons at early stages of development impairs the subsequent formation of specific chemical excitatory synapses.

The importance of correlated neuronal activity for network tuning and responsiveness could go beyond the developmental time frame of network formation. For instance, the nucleus accumbens (Acb)-mediated pleasure/reward-inducing behaviors were reduced when carbenoxolone was injected in the Acb in young adult rats (Kokarovtseva et al. 2009). Additional experiments could shed light on differences between the influence gap junctions have on neuronal networks that undergo maturation and the ones already mature.

Conclusions

Since the discovery that many neurons form networks of interconnected cytoplasms in the postnatal brains of mammals, a large body of evidence came about showing that gap junctions are not just simple passageways for metabolical and electrical communication; but instead, they are plastic and can respond to different levels of cell activity within relatively short periods of time. From a series of findings, gap junctions can be regarded as implicated in 3 main types of physiological processes, out of which 2 occur primarily during the development of the nervous tissue: 1) neuroblast migration, 2) modulation of cellular and network activity-dependent electrical coupling, and 3) establishment of proper synaptic connectivity and sensory input selectivity of newly formed neurons.

The most recent and interesting functional implications for gap junction connectivity during development have been shown in the visual cortex. By allowing free passage for ions, gap junctions provide the means for electrical summation of subthreshold and uncorrelated inputs that could bring the entire network to AP firing threshold. Correlated activity in the visual cortex, during the first postnatal week, allows neurons to shape their stimulus tuning in a similar way, possibly being one of the steps necessary for the formation of functional columns.

New roles of gap junctions have recently emerged and involve electrical connections in information processing and nervous tissue recovery. Electrical synapses can change signal integration properties of neurons that possess sublinear dendritic integration mechanisms (cerebellar Golgi cells); gap junctions, by spreading the excitatory synaptic charge into the dendrites of other cells, counteract the effects of sublinear dendritic integration, enabling inputs to be more effective (Vervaeke et al. 2012). Apart from the fundamental importance of understanding electrical communication in the mammalian brain, therapeutical implications of gap junctions have also started to surface (Jezierski et al. 2012); amniotic fluid cells express high levels of Cx43 and are able to establish functional gap junctions with cortical neurons following injury, which may help with the reconstruction of damaged nervous tissue. Future studies can bring even more insight into this increasingly interesting field of vertebrate gap junctions.

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