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Review

Involvement of gap junctions in the development of the neocortex

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Abstract

Gap junctions play an important role during the development of the mammalian brain. In the neocortex, gap junctions are already expressed at very early stages of development and they seem to be involved in many processes like neurogenesis, migration and synapse formation. Gap junctions are found in all cell types including progenitor cells, glial cells and neurons. These direct cell-to-cell connections form clusters consisting of a distinct number of cells of a certain type. These clusters can be considered as communication compartments in which the information transfer is mediated electrically by ionic currents and/or chemically by, e.g., small second messenger molecules. Within the neocortex, four such communication compartments can be identified: (1) gap junction-coupled neuroblasts of the ventricular zone and gap junctions in migrating cells and radial glia, (2) gap junction-coupled glial cells (astrocytes and oligodendrocytes), (3) gap junction-coupled pyramidal cells (only during the first two postnatal weeks) and (4) gap junction-coupled inhibitory interneurons. These compartments can consist of sub-compartments and they may overlap to some degree. The compartments 1 and 3 disappear with ongoing develop, whereas compartments 2 and 4 persist in the mature neocortex. Gap junction-mediated coupling of glial cells seems to be important for stabilization of the extracellular ion homeostasis, uptake of neurotransmitters, migration of neurons and myelination of axons. Electrical synapses between inhibitory interneurons facilitate the synchronization of pyramidal cells. In this way, they contribute to the generation of oscillatory network activity correlated with higher cortical functions. The role of gap junctions present in neuroblasts of the ventricular zone as well as the role of gap junctions found in pyramidal cells during the early postnatal stages is less clear. It is assumed that they might help to form precursors of the functional columns observed in the mature neocortex. Although recent developments of new techniques led to the solution of many problems concerning gap junction-coupling between neurons and glial cells in the neocortex, there are many open questions which need to be answered before we can achieve a comprehensive understanding of the role of gap junctions in the development of the neocortex.

Keywords: Gap junction; Neocortex; Development; Interneuron; Pyramidal cell; Glia

Contents

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1. Introduction

The embryonic development of the brain and the subsequent maturation of the specific brain areas is an evolving process which involves a diversity of molecular and cellular mechanisms. These mechanisms also include gap junction-mediated coupling between developing neurons as well as developing glial cells. This cell-to-cell communication is well recognized [1–5] and represents an additional information transfer system complementary to that created by chemical synapses. During the developmental process of almost all regions of the brain, gap junction-mediated cell coupling has been shown to be correlated with neurogenesis, migration, cellular differentiation, arealization and circuit formation [3,4,6,7].

The developmental processes leading to the formation of different brain areas with different functions share many similar molecular and cellular mechanisms, particularly during the period of embryonic development. However, in order to create morphologically and functionally unique neuronal and glial networks which endow a given brain region with the ability to serve a set of specific functions within the concerted action of the brain, these processes necessarily diverge depending on the brain area. The intention of this review is to evaluate the significance of gap junction-mediated cell coupling during the maturation of the neocortex. Therefore, the role of gap junctions will be discussed in context with the developmental processes leading to the maturation of the cerebral cortex.

Within the mature neocortex, neurons communicate predominantly via chemical synapses and glial cells interact mainly via gap junctions. Particularly during the first stages of neocortical development, chemical synapses between neurons are scarce and, in recent years, experimental data have accumulated which provide evidence for the existence of a non-synaptic neuronal communication system within the immature neocortex (for review see Ref. [3] and Refs. [8–10]). During some developmental periods, neurons are aggregated into clusters with cells coupled to each other via gap junctions. Gap junctions are specialized cell-to-cell contacts that enable direct intercellular information transfer by means of ionic currents and/or second messengers [1,11]. Gap junction-coupled functional syncytia of neurons or neuroblasts are found not only during early embryonic stages of development, e.g., during the period of neurogenesis [12], but also during postnatal stages in which formation of synaptic circuits occurs [13,14]. In addition, it has been shown that gap junctions connect inhibitory interneurons in the young yet mature neocortex [15,16,16a] and that the various subtypes of glial cells are coupled by gap junctions [17]. Thus, it has become obvious that gap junctions form distinct communication compartments [2,18] between distinct cell types and during distinct time periods of neocortical development. These compartments may overlap in space and time and they may interact to some extent. At least during the later stages of neocortical development and in the mature neocortex, these communication compartments co-exist with the communication system formed by chemical synapses.

The size of a communication compartment formed by gap junctions, i.e., the number of cells within a cluster, is obviously correlated with its function. For example, it has been estimated that each neocortical inhibitory interneuron is coupled to between 20 and 40 neighboring interneurons with intersomatic distances to the 'mother cell' of less than 200 μm [19]. In Cx36-deficient mice, gap junction-coupling among neocortical inhibitory interneurons has been shown to be nearly absent [20]. Simultaneously, facilitation of synchronous neocortical network activity was found to be reduced, although occasional weak coupling between neocortical interneurons has been observed in these mice [21]. Obviously, a certain number of coupled cells is required within a communication compartment in order to efficiently fulfill the corresponding function. The factors determining the size of functional gap junction-coupled clusters are still unknown. However, it has been shown that neurotransmitters and neuromodulators can regulate the size of such clusters and, hence, their efficiency (for review, see Refs. [8,9]).

The mature mammalian neocortex represents a large intricate, but well-organized network of interacting neurons and glial cells. Some areas of the neocortex are directly and primarily involved in basic sensory or motor functions (e.g., Gyrus postcentralis and Gyrus precentralis, respectively), other areas, in particular the associative cortices, are predominantly involved in higher brain functions (e.g., cognition and speech). These functional differences of neocortical areas are reflected to some extent by their cytoarchitectonic structure [22]. Based on the specific distribution of certain neuronal cell types (e.g., pyramidal cells and interneurons) and both afferent as well as efferent fibers, the so-called isocortex can be subdivided into six horizontal layers (Laminae I–VI). Laminae II, III, V and VI predominantly contain projection neurons (i.e., pyramidal cells) with axons arising from the neocortex and projecting to different brain areas. Projection neurons are excitatory and use L-glutamate as a neurotransmitter [23]. In addition, local circuit neurons or interneurons of variable morphology are present in all neocortical layers. They are mainly inhibitory and use γ-aminobutyric acid (GABA) as a neurotransmitter [23]. In some areas of the neocortex, in particular the primary sensory areas, the interneurons are concentrated in Lamina IV. Regions of the neocortex, with such a prominent Lamina IV, are designated as granular cortices. In other areas, e.g., the primary motor area, Lamina IV is poorly developed or absent and the interneurons are evenly distributed within all layers (agranular cortex). Where present, Lamina IV serves as the main input relay station of the neocortex. Lamina I is characterized by a low cell density and by large bundles of tangentially oriented neurites originating from apical dendrites of Laminae II/III and Lamina V pyramidal cells as well as from axons of cortical and subcortical neurons projecting to the neocortex. In addition, Lamina I contains GABAergic interneurons connected by chemical as well as electrical synapses [23a].

In addition to this horizontal organization, vertically oriented modules have been described in the neocortex and designated as cortical columns [24–26]. These columns span the neocortex from the white matter to the pial surface and are interconnected by horizontal fibers [27]. Most probably, these columns

represent the functional units of the neocortex. Interestingly, neocortical columns seem to contain a constant number of cells [28]. These are assembled into neuronal networks [29] that provide the basis for the higher integrative and cognitive functions of the mammalian brain. Neocortical columns and networks result from complex developmental processes comprising, inter alia, genesis of neurons and glial cells, cell migration, cell differentiation, activity-dependent formation and elimination of synapses and apoptosis [30].

1.1. Connexins, connexons, gap junctions and their expression in the central nervous system

Two cells (neurons or glial cells) in close apposition (about 2 nm) can be connected via gap junctions. These channel-like structures enable a direct cytoplasmic connection between the cells and, hence, a direct intercellular communication pathway [11]. Gap junction channels consist of two hemichannels (connexons), each of which is composed of six subunit proteins, called connexins. Twenty connexin genes have been described in the mouse genome and 21 in the human genome [11]. In addition to the connexins, a second group of protein subunits, the pannexins, have been described (for review, see Ref. [11]). Of the pannexins identified, pannexins 1 and 2 are capable of forming gap junction-like structures and they have been shown to be expressed in the mammalian central nervous system [31].

Gap junction channels allow the permeation of molecules up to 1000 Da in size, including ions and second messengers. A homomeric connexon consists of six identical connexins, heteromeric connexons are composed of different connexins. Homomeric or heteromeric connexons can create homotypic (two identical connexons) or heterotypic (two different homomeric or heteromeric connexons) gap junction channels [32]. The electrical properties as well as the permeability of gap junction channels depend on its subunit composition [33].

Up to now, at least six connexins (Cx26, Cx29, Cx30, Cx32, Cx36, Cx43) have been ultrastructurally identified as unequivocally defined gap junctions in neurons and glial cells. Another five connexins have been shown to be expressed in neural tissue (Cx31, Cx37, Cx45, Cx47, Cx57) [4]. Gap junctions between neurons are formed almost exclusively by Cx36. However, depending on the type of neuron, also Cx45 or Cx57 might build neuronal gap junctions [11]. Astrocytic gap junctions are created predominantly by Cx43, Cx30 and perhaps Cx26 [4,17]. Oligodendrocytes are coupled to each other by gap junctions consisting of Cx32, Cx29 and Cx47 subunits [4,17]. This short summary shows that the expression of a certain connexin subunit seems to be restricted to a certain type of cell. However, it cannot be excluded that the expression pattern differs during the development of the central nervous system [4].

1.2. Gap junctions and communication compartments in the neocortex

As outlined above, gap junctions may create communication compartments within a homogeneous (or heterogeneous) cell population. A probable function of such a compartment might be to ensure the synchrony of cell activity – in the broadest sense – in a specific cell population at a given time. Within the neocortex, gap junctions have been shown to exist between neuroblasts of the ventricular zone, between astrocytes and between oligodendrocytes, between pyramidal cells during early stages of postnatal development and between inhibitory interneurons of the mature cortex (for review, see Refs. [3,10]). In addition, gap junctions play an important role during cell migration [7] and are important components for radial glia function [34]. Thus, one might define at least four different gap junction-dependent communication compartments in the neocortex: (1) ventricular zone, migrating cells and radial glia, (2) glial cells (with at least two sub-compartments among astrocytes and oligodendrocytes, respectively), (3) pyramidal cells during the first 2 postnatal weeks and (4) inhibitory interneurons in the mature neocortex (with sub-compartments among different types of interneurons). Each of these compartments already exists during the development of the neocortex. While the compartments 1 and 3 disappear during the course of development, compartment 2 persists and can be found in the adult neocortex. The ‘interneuron compartment’ (compartment 4) is established in parallel or following the maturation of inhibitory interneurons.

The development of neocortical networks can be divided roughly into two principal periods (for review, see Refs. [30,35,36]). The first period includes generation of neurons and glial cells and migration of the cells to their final position within the neocortex. At about the same time, a coarse pattern of afferent and efferent fiber connections is established. These processes are predominantly determined by activity-independent mechanisms [37]. In rodents, this developmental stage involves the prenatal period and the first postnatal week [38]. The second principal period of neocortical development is characterized by the maturation of the final synaptic connections. These processes require patterned neuronal activity [39] and they depend on interactive mechanisms comprising growth and withdrawal of axon branches as well as synapse formation and elimination [37]. In rodents, synapse formation mainly occurs during the course of the second and third postnatal weeks [38]. However, there exists evidence that synaptic connections are formed during very early stages of neocortical development [40] and morphological studies have shown that genesis of neocortical synapses begins prenatally and the first synapses are found in the marginal zone and in the subplate (for review, see Ref. [38]).

1.2.1. Compartment 1: Ventricular zone, migrating cells and radial glia

The projecting neurons (i.e., pyramidal cells) [36,41], glial cells [42] and probably some of the interneurons [41] originate from the neuroepithelium of the ventricular zone, which lines the wall of the rostrodorsal telencephalic forebrain vesicles [36]. Almost all of the neocortical interneurons are generated in the medial ganglionic eminence, from where they migrate tangentially to the neocortex (for review, see Ref. [43]). Early generated postmitotic neurons leave the ventricular zone and migrate

towards the pial surface of the cerebral wall, where they form a primordial superficial layer, the so-called preplate [44]. This plexiform layer also contains monoaminergic fibers projecting from the midbrain and pons to the telencephalon. With ongoing development, an additional transient layer, the intermediate zone, appears between the preplate and the ventricular zone, which consists mainly of tangentially oriented fibers.

As the next major step in rodent corticogenesis, later-generated neurons enter the preplate to form the cortical plate, splitting the preplate into a superficial marginal zone, which becomes neocortical Lamina I, and a deeper layer called subplate. Almost simultaneously, the subventricular zone, where most of the cortical glial cells are generated, becomes evident.

In rats, neocortical neurons are generated between embryonic day 12 (E12) and E21 (gestation time: 21 days). The first neurons to develop are cells that differentiate as Cajal–Retzius cells [36] and into other types of neurons. These cells appear at E12 and arrive at the marginal zone at E13. Neurons of the cortical plate are developed between E15 and E21. The cortical plate, which becomes Laminae II–VI of the mature neocortex, develops in an "inside-first-outside-last" sequence. Neurons generated between E15 and E18 migrate to the cortical plate in about 2 days and form Laminae V and VI of the adult neocortex. Those cells that are generated at E19–E21 migrate through the existing neuron population of the cortical plate and reach the superficial layers within three to 10 days. These neurons form Laminae II and III of the mature rat neocortex. After E19, no neurons are generated within the ventricular zone. Within the subventricular zone, production of neurons and glial cells continues until E21. These neurons migrate to the superficial layers of the cortical plate where they arrive between postnatal day 3 (P3) and P5. After E21, only glial cells are generated in the subventricular zone.

Electrophysiological recordings from neuroblasts of the ventricular zone revealed that these cells display membrane input resistances much lower than expected given the small diameter of these cells [12,45]. Injections of gap junction-permeable tracers into one cell led to the occurrence of tracer signals in neighboring cells. This so-called dye-coupling is taken as an indication of direct cell-to-cell connectivity, theorized to be mediated through gap junctions. Application of drugs known to block gap junction permeability resulted in a marked increase in membrane input resistance of ventricular zone cells [12,45], suggesting that the low input resistances of these cells are the functional consequences of intense coupling of neuroblasts via gap junctions.

Neuroblasts are coupled to build clusters of up to 90 cells. The clusters form vertically oriented columns within the ventricular zone [12] and it has been shown that cell clustering is restricted to neural progenitor cells and radial glia [46]. At the beginning of neurogenesis in the rat neocortex (E12–E13) almost every neuroblast belongs to a cell cluster. Then, the number of cells within a cluster decreases with ongoing development. In addition, between E14 and E19, the number of clusters within the ventricular zone gradually declines and, at developmental stages close to birth (E19), no gap junction-coupled clusters are found in the ventricular zone [12]. After E19, the ventricular zone stops generating neurons [30]. Thus, in the ventricular zone, the cluster size as well as the number of cells within a cluster temporarily correlates with the degree of neurogenesis.

Cell clusters within the ventricular zone are created by gap junctions composed of Cx43 [47]. Postmitotically, the Cx43 expression in these cells declines and other connexins (e.g. Cx26, Cx36, Cx45) begin to appear [48].

Since cell clusters in the ventricular zone appear predominantly during the period of neurogenesis, it seems obvious that these clusters are involved in this process. Accordingly, it has been shown that gap junction-mediated communication between ventricular zone cells regulates cell division [46]. Therefore, gap junction coupling of neuroblasts within the neuroepithelium seems to be important for fate determination of neuronal progenitor cells (e.g., layer and column specificity). Coupling of ventricular zone neuroblasts depends on the cell cycle. During the S-phase, cells are coupled into clusters and they remain coupled through the G_2-phase. The M-phase is characterized by uncoupling and the cells recouple through the G_1-phase. In early neurogenesis, recoupling is complete by the next S-phase, whereas, at later stages of neurogenesis, the rate of recoupling declines and, during the G_2-phase, the number of uncoupled cells increases. If ventricular zone cells do not reenter the S-phase, then they do not rejoin clusters [46]. Thus, the function of gap junction-mediated coupling of neuroblasts into clusters seems to be the synchronization of the cell cycle of closely apposed and clonally related cells. At least to a certain extent, gap junction coupling of neuroblasts influences the generation dates of neurons and, in this way, their fate.

Signals which might control the coupling and uncoupling of ventricular zone cells are GABA and glutamate. Both, $GABA_A$ receptors and glutamate receptors of the AMPA subtype are present on ventricular zone neuroblasts [12,49]. In vitro studies showed that GABA, as well as glutamate, induces large inward currents associated with high amplitude depolarizations. The latter results in an increase in the intracellular calcium concentration predominantly by activation of voltage-gated calcium channels. The large amplitudes of the currents result from the activation of a large number of cells coupled to each other via gap junctions. Thus, the amplitude of these currents and, hence, their physiological effect seem to be a function of the cluster size. The GABA- and glutamate-induced depolarizations of ventricular zone cells are accompanied by a decrease in the number of cells that synthesize DNA [49]. It has been concluded that, by depolarization of the neuroblasts' membrane potentials, these two neurotransmitters decrease the number of cells in the S-phase and, thereby they reduce the cluster size. Those cells that do not rejoin the clusters start their migration into the preplate or cortical plate of the developing neocortex.

Thus, a possible feedback regulation of DNA synthesis might affect, directly or indirectly, the degree of gap junction-mediated cluster formation in the ventricular zone. Both the GABAergic and the glutamatergic transmitter systems differentiate early in development [50] and migrating neurons send axons back to the superficial part of the ventricular zone [51]. The growth cones of these axons might release neurotransmitters which influence cell division within a cluster of neuroblasts coupled to each other by

gap junctions. GABA and glutamate are possible candidates for such feedback signals.

During the last few years, our understanding of neuronal migration in the developing neocortex has been changed profoundly due to recent studies indicating that different neuronal cell types adopt different migratory modes (for review, see Refs. [43,52,53]). In addition, it has been shown that radial glial cells, which build a scaffold for neuronal migration, act as progenitors for the generation of neurons and astrocytes [41,54–56].

At very early developmental stages, when only the ventricular zone and preplate are present, neurons seem to migrate by so-called somal translocation [52]. In stages where the cortical plate has already been created, pyramidal neurons undergo several phases of migration involving radial movement from the site of origin to the subventricular zone, followed by a pause in migration within the intermediate zone and subventricular zone. Finally, the pyramidal cells migrate along radial glia to the cortical plate. Some of the neurons move backwards from the intermediate/subventricular zone to the ventricular zone before they migrate to their final position within the cortical plate [43]. Very recently, it has been shown that calcium waves propagate through radial glial cells and that these calcium waves require the presence of connexin hemichannels [34].

Recently, it has been shown that neuronal migration is altered in the neocortex of Cx43 null mutant mice [7]. Dividing cells were labeled with bromodeoxyuridine (BrdU) during neocortical neurogenesis and the distribution of BrdU-labeled cells was studied at early postnatal stages. In Cx43 null mutant mice, BrdU-positive cells accumulated within the intermediate zone and the inner part of the cortical plate suggesting a significant delay in neocortical neuronal migration.

1.2.2. Compartment 2: Glial cells

Most of the astrocytes arise from ventricular zone cells. At the beginning of neurogenesis, radial glial cells appear, which have many characteristics in common with astrocytes (for review, see Ref. [57]). As already mentioned, radial glial cells serve as progenitors for neurons [41] and, in addition, after cessation of neurogenesis some of these cells are transformed into astrocytes. Another population of astrocytes is generated along with oligodendrocytes in the subventricular zone and migrate into the gray and white matter of the neocortex [58].

Astrocytes cannot be considered as a homogeneous cell population. With respect to gap junction coupling, experimental data suggest that astrocytes which express glutamate transporters are able to form gap junctions with each other and that those astrocytes, which express glutamate receptors, remain uncoupled (for review, see Ref. [17]). Thus, the gap junctional communication compartment ‘Glial cells’ may not include all astrocytes present in a brain area. In vivo, coupling-competent astrocytes typically create clusters with up to 100 cells. The connexins Cx43, Cx30 and Cx26 have been shown to be expressed in astrocytes [17,59].

Non-myelinating oligodendrocytes form only a few gap junctions with other oligodendrocytes, whereas myelinating oligodendrocytes are usually coupled to each other by gap junctions [59]. However, the number of coupled cells is low (2–4). Oligodendrocytes express Cx32, Cx29 and Cx47 [4,17]. Gap junctions between astrocytes and oligodendrocytes are common and it has been proposed that oligodendrocytes communicate with each other via astrocytes [4].

Neuroglial cells fulfill a large variety of functions including stabilization of the extracellular ion homeostasis, uptake of neurotransmitters, axon guidance, scaffold for migrating neurons and myelination. Many new experimental data derived by combination of advanced molecular and cellular biological techniques applied to neural tissues of genetically altered mice have changed our understanding of the role of gap junctions in glia function. These new developments have been summarized in a recent review [17] and will not be discussed here. In this context, however, some observations made in neocortical neurons of Cx32 null mutant mice are of interest.

As expected, the neocortex of adult Cx32-deficient mice displayed minor but significant changes in myelination [60]. The volume fraction of myelin was found to be reduced by 19% and there were slight alterations in the structure of the myelin sheaths. However, this dysmyelination is certainly not sufficient to significantly influence fiber conduction velocity [60]. Recently, it has been shown that mice lacking both Cx32 and Cx47 die about 6 weeks after birth, obviously because of severe disturbances in myelination of central nervous system axons [61]. Thus, the minor changes in myelination observed in Cx32 null-mutant mice can be explained by a compensation of Cx32 function by Cx47 in oligodendrocytes [17].

Interestingly, in addition to the effects on myelination, alterations of neuronal properties have been observed in neocortical pyramidal cells of Cx32-deficient mice [60]. The neurons were found to be electrotonically more compact, i.e., more excitable. Furthermore, neuronal inhibition seemed to be less synchronized resulting in a strong potentiation of glutamatergic synaptic transmission. In about 50% of the neurons investigated, it was impossible to evoke compound inhibitory postsynaptic potentials (IPSPs) by electrical stimulation [60]. Instead, one stimulus induced a barrage of IPSPs lasting for 1–2 s. These IPSPs displayed short durations (<10 ms) and small amplitudes (<10 mV). Since Cx32 is a connexin expressed only in oligodendrocytes, at least in adult animals [4,17], these changes in neuronal properties are hard to explain. One might speculate that the compensation of the Cx32 function by Cx47 starts with a delay during postnatal development of the neocortex resulting in a retarded myelination of fibers and, hence, in an abnormal activity pattern within a certain time window during the period of circuit formation. This might influence synaptogenesis, in particular the genesis of inhibitory synapses. Another possibility is that Cx32 is transiently expressed in developing neocortical neurons.

Independent of the underlying mechanisms, these experiments demonstrated that the deletion of Cx32, which is thought to be expressed only in oligodendrocytes, persistently influences the electrophysiological properties of pyramidal cells of the mature neocortex. It would be of interest to investigate if Cx47 knockout mice display similar changes in neuronal properties.

1.2.3. Compartment 3: Pyramidal cells during early postnatal stages

The first postnatal week of rodent neocortical development is characterized by a high incidence of gap junction-mediated coupling between pyramidal cells in deep and superficial layers [62–64]. Up to postnatal day 5 (P5), gap junction-coupled cell clusters appear as columns spanning the neocortex from the developing white matter to the marginal zone. With ongoing development, these cell clusters gradually disaggregate. In deep layers, uncoupling seems to commence during the second half of the first postnatal week and by the beginning of the second week, most pyramidal cells in Lamina V are not connected to another neuron. At the same time, concentric clusters of coupled cells are still found in Laminae II and III and, on average, these clusters consist of 30–40 neurons [64]. Gap junction-mediated coupling between neocortical Laminae II/III neurons disappears by the end of the second postnatal week.

Although there exists no direct evidence, it can be assumed that Cx36 is mainly responsible for gap junction-mediated coupling between pyramidal cells during the early postnatal development. However, it cannot be excluded that other connexins (e.g., Cx45 or Cx57) or even pannexins are involved. Since Cx26 expression is high during the postnatal period of neocortical development, it has been suggested that Cx26 contributes to the gap junction-mediated coupling of pyramidal cells [48].

The high incidence of gap junction-mediated coupling between pyramidal cells occurs simultaneously to the period of circuit formation within the developing neocortex. The development of synaptic circuits in the neocortex starts during the period of neurogenesis and neuronal migration. During embryonic stages, cortically evoked potentials associated with postsynaptic discharges of cortical neurons have been described [22] and neuronal responses to afferent fiber activation can be observed as early as P1 [40]. Even neuroblasts of the ventricular zone are endowed with important prerequisites for chemical synaptic transmission (i.e., the presence of neurotransmitters and their receptors). They express glutamate receptors of the AMPA and kainate subtype, but obviously no glutamate receptors of the NMDA subtype [65]. In addition, $GABA_A$ receptors have been found in neuroblasts of the ventricular zone [12,49,65].

The first excitatory synapses appear in the cortical plate between E19 and P0 [38]. At the same time, cortical plate cells start to express NMDA receptors [65]. Tetrodotoxin-insensitive miniature excitatory postsynaptic currents (mEPSCs) can be detected as early as P3 and they consist of both a fast AMPA receptor-mediated component and a slow NMDA receptor-mediated component [66,67]. In addition to cortical plate neurons, subplate neurons respond to activation of afferent and efferent fibers with the generation of excitatory postsynaptic potentials (EPSPs) and with antidromic action potentials [68,69], indicating the presence of functional excitatory synapses.

The first postnatal week of rodent neocortex development is characterized by the predominance of excitatory synaptic transmission [67,70–74]. Activation of afferents predominantly evokes EPSPs in immature neocortical neurons and, in addition, during the early postnatal period, immature neocortical neurons seem to be under the influence of tonically released glutamate [73].

Inhibitory postsynaptic potentials (IPSPs) mediated by $GABA_A$ receptors gradually appear beginning from P4 to P8 [75]. At P0, GABAergic neurons are found in the deep layers of the rodent neocortex and the release of endogenous GABA from cortical slices is evident [38]. In immature rat neocortical neurons (P1–P7), we observed a significant reduction in membrane noise following application of the $GABA_A$ receptor antagonist bicuculline [Sutor, unpublished observations] suggesting that immature neocortical neurons seem to be affected by tonically released GABA. In immature neocortical neurons, spontaneous $GABA_A$ receptor-mediated synaptic potentials have been described to occur at P4 [71]. This indicates the presence of functional GABAergic synapses at this early developmental stage. However, at this time, the frequency of mIPSCs is very low [Sutor, unpublished observations] suggesting a low density of GABAergic synapses. This low density of GABAergic synapses is reflected in the fact that IPSPs can hardly be evoked up to P7–P9 [70–73]. Starting from the end of the first postnatal week, the number of GABAergic interneurons gradually increases and attains the mature pattern around P12–P15 [38]. Simultaneously, both the number of $GABA_A$ binding sites and the number of inhibitory Gray type II synapses increases in the developing neocortex [38].

In summary, synaptic circuits of the neocortex start to develop during the time of neurogenesis and migration of pyramidal cells to their final positions. The majority of local circuit neurons (i.e. GABAergic interneurons) start to mature by the end of the first postnatal week. A consequence of the "inside-first-outside-last" pattern of cortical development is that deep layer pyramidal neurons are at their positions and form synaptic connections with other cells, while those of Laminae II and III are still migrating. A similar, but inverse observation has been made concerning the gap junction-mediated coupling of pyramidal cells in the developing neocortex. In deep layer neurons, dye-coupling disappears about a week earlier than in pyramidal cells of upper cortical layers [64].

The most important afferent input to the neocortex originates from the thalamus. Thalamocortical afferents terminate mainly on neurons of Lamina IV and lower Lamina III as well as on neurons of Lamina VI. Thalamocortical synapses are excitatory and are found predominantly on dendritic spines [22]. Thalamic neurons are generated during the same developmental period as cortical neurons, but the generation of thalamic neurons terminates earlier than that of neocortical neurons [30]. Thalamocortical fibers enter the immature neocortex before the migration phase of their neocortical target neurons is finished. After a "waiting period", during which they transiently form synaptic contacts with subplate neurons, thalamocortical afferents grow into the cortical plate and establish synapses with newly arrived cortical neurons [37,39]. In rodents, the establishment of thalamocortical connections occurs mainly during the first postnatal week.

Interestingly, at the same time, the incidence of gap junction-mediated coupling between neocortical pyramidal cells is high [64].

Monoaminergic projections to the cerebral cortex occur very early in development [30]. This innervation appears to be an important determinant for the development of the neocortex [9,30]. Noradrenergic fibers from the locus coeruleus, dopaminergic fibers from the rostral mesencephalon and serotonergic fibers from the mesencephalic raphe nuclei have been detected in the developing rat neocortex as early as E17 [30]. The presumed trophic function of these monoaminergic afferent systems might be mediated, at least in part, by modulation of gap junctions. It has been demonstrated that noradrenaline [76] dopamine [77] and serotonin [78] are able to modulate the permeability of neuronal gap junctions between pyramidal cells in the immature rat neocortex.

Why are pyramidal cells coupled by gap junctions during the first weeks of postnatal development? Until now, there is no conclusive answer to this question. Resting membrane potential, input resistance and membrane time constants of rat neocortical Laminae II/III pyramidal cells develop during the first 3 postnatal weeks [64,79]. Within this period, the neurons' membrane potentials become more negative, the input resistances decrease and the membrane time constants become shorter. Simultaneously, the typical regular pattern of action potential discharge, which is used to identify these cells electrophysiologically, appears. By the end of the third postnatal week, rat neocortical Laminae II/III pyramidal cells represent, at least with respect to their intrinsic neuronal properties, a fairly homogeneous neuronal population. Similar developmental changes have been described for the electrophysiological properties of rat Lamina V pyramidal cells [80,81]. However, in these cells, the maturation of intrinsic neuronal properties seems to be almost complete by the beginning of the second postnatal week. This temporally correlates with the disappearance of the gap junction-mediated coupling of these neurons [81]. A similar correlation was observed in pyramidal cells of superficial cortical layers [64]. The intrinsic properties of these cells varied markedly during the first two postnatal weeks. Beginning in days P12–P14, this variability starts to decline and, by P21, it attains values similar to those observed in the adult rat neocortex. Simultaneously, the size of neuronal clusters within the superficial layers of the neocortex decreases and disappears around P16. Thus, gap junction-mediated coupling of pyramidal cells into clusters might be important for the maturation of intrinsic membrane properties. Interestingly, determinations of intrinsic membrane properties of neocortical neurons of Cx32-deficient mice [60] revealed significant differences between those of neurons recorded from wild-type animals. The neurons were electrotonically more compact and, hence, more excitable. These alterations appeared to be independent of other disturbances detected in these knockout mice. This observation seems to be particularly noteworthy, since Cx32 is not expressed in neurons, at least not in mature neurons [11].

It has been suggested that gap junctions between neocortical pyramidal cells play a role in the maturation of the columnar organization of the neocortex and there is good evidence which substantiate this hypothesis (for reviews, see Refs. [9,14]). The postnatal development of cortical columns seems to be controlled by coordinated patterns of spontaneous neuronal activity. Using calcium-imaging techniques, columnar and circular domains of spontaneously coactive neurons have been described in the developing neocortex [13]. These domains occur before and persist through the main period of circuit formation [14]. In tangential slices of the immature neocortex, the diameters of the circular shaped domains are in the range of 50–100 μm, and in coronal slices of very young animals, the domains may span the entire cortex. In older rats, they cover several neocortical layers. The generation of these domains is independent of action potentials and is suppressed by compounds known to reduce gap junction permeability [81]. The neurons within such a domain are obviously coupled to each other via gap junctions. Whether the domain size corresponds to the size of the clusters formed by gap junction coupling of immature pyramidal cells is not known. It is assumed that the occurrence of these functional domains is triggered by one or a relative few neurons [14]. In addition, it has been shown that the spread of activity is mediated by a second messenger (inositol triphosphate) permeating through gap junctions rather than by electrical current [82]. Similar to the calcium waves observed in astrocytes [17], it should be considered that the calcium changes within neuronal domains might be mediated by extracellular pathways involving gap junction hemichannels.

1.2.4. Compartment 4: GABAergic Inhibitory Interneurons

As already mentioned, most of the neocortical interneurons derive from the medial ganglionic eminence in the ventral telencephalon. They migrate tangentially, enter the subventricular zone of the developing neocortex, move to the ventricular zone (at least part of them), and reach their position within the neocortex by radial migration [53,53a]. GABAergic interneurons appear in the lower cortical plate around postnatal days 3–4 (P3–4) and gradually increase in number until P25 [38]. In the mature neocortex, they can be found in all neocortical layers.

Based on morphology, electrophysiological behavior and expression of calcium-binding proteins and neuropeptides, GABAergic local circuit neurons have been classified into different subgroups [83,84]. The GABAergic inhibitory system controls network excitability in the neocortex and a decrease in the efficiency of neuronal inhibition leads to the induction and spread of epileptiform activity. Local circuit neurons represent only 10–15% of the total neuron population in the neocortex [38]. Therefore, the generation of efficient inhibition requires the synchronization of their activity.

Electrophysiological experiments provided evidence that neocortical GABAergic interneurons are coupled to each other via gap junctions. [15,16,16a]. One group of GABAergic interneurons, the so-called fast-spiking cells (FS cells), display a high incidence of coupling among each other. Similarly, another class of interneurons, the so-called low-threshold-spiking cells (LTS cells), form a network via gap junctions. The coupling is cell type-specific (i.e., coupling exists between FS

cells or between LTS cells, but not between FS cells and LTS cells) and, in addition, mixed connections (i.e., chemical and electrical synapses) have been detected between the same neurons. Investigations using Cx36 null-mutant mice revealed that gap junctions between GABAergic interneurons are composed of Cx36.

Thus, in the rodent neocortex, gap junctions create at least two distinct electrically coupled networks of inhibitory interneurons. The electrophysiological properties of electrical synapses between neocortical interneurons and the putative functions of these networks have been discussed recently in comprehensive reviews by Connors and Long [21], Bennett and Zukin [33] and Hestrin and Galarreta [85].

2. Conclusions

During every developmental period, gap junctions are expressed in almost every cell type of the neocortex. They are present in astrocytes and oligodendrocytes, in pyramidal cells and inhibitory interneurons and in progenitor cells. In some cell types, e.g., glial cells and inhibitory interneurons, they persist after cessation of the maturation period. These cell-to-cell contacts directly connect a distinct number of cells of a given type, thereby creating communication compartments which can be subdivided into several sub-compartments.

The glial compartments fulfill a variety of functions which require the direct exchange of ions or small molecules like second messengers. Gap junction-mediated coupling of inhibitory interneurons facilitates synchronous activity of neocortical networks which is thought to be correlated with higher brain functions.

The role of gap junctions in neuroblasts of the ventricular zone and in pyramidal cells of the postnatal neocortex is less clear. Coupling of ventricular zone cells influences the fate determination of neuronal progenitor cells and might regulate to some extent the layer and column specificity of neurons. The gap junction-coupled clusters of neuroblasts form vertically oriented columns within the ventricular zone and one might speculate that these columns represent precursors of the functional columns observed in the mature neocortex [12]. Similarly, at early postnatal stages, gap junction-coupled pyramidal cell clusters sometimes appear as columns spanning the neocortex from the developing white matter to the marginal zone [63,64]. Therefore, it has been suggested that these neuronal clusters may represent developmental blueprints for the adult functional architecture of the neocortex [13,14]. It will be of interest to investigate the expression and the formation of neocortical columns in Cx36-deficient mice or in mice lacking more than one of those connexins usually expressed in neurons.

During the past few years, the development of powerful new techniques (e.g. patch clamp recordings in conjunction with infrared-DIC microscopy and genetically altered mice) helped to elucidate the role of gap junctions in the development of the neocortex. However, there are still many open questions. Although the cell type-specific expression of connexins seems to be by far clearer than a few years ago, the possibility still exists that a connexin is expressed in a certain cell type only during certain stages of brain development (e.g., Cx32). In addition, we do not know whether certain types of gap junctions prefer signal transfer using electrical current or biochemical messengers and it is unknown, whether such a possible preference changes with maturation of the neurons or glial cells. Furthermore, there exist only a few studies on the regulation of gap junction coupling in neocortical neurons. With respect to the probable correlation between the size of a gap junction-coupled cell cluster and its function, this seems to be the most important issue to investigate.

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