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Regulation of Gap Junction Coupling in the Developing Neocortex

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Abstract

In the developing mammalian, neocortex gap junctions represent a transient, metabolic, and electrical communication system. These gap junctions may play a crucial role during the formation and refinement of neocortical synaptic circuitries. This article focuses on two major points. First, the influence of gap junctions on electrotonic cell properties will be considered. Both the time-course and the amplitude of synaptic potentials depend, *inter alia*, on the integration capabilities of the postsynaptic neurons. These capabilities are, to a considerable extent, determined by the electrotonic characteristics of the postsynaptic cell. As a consequence, the efficacy of chemical synaptic inputs may be crucially affected by the presence of gap junctions.

The second major topic is the regulation of gap junctional communication by neurotransmitters via second messenger pathways. The monoaminergic neuromodulators dopamine, noradrenaline, and serotonin reduce gap junction coupling via activation of two different intracellular signaling cascades—the cAMP/protein kinase A pathway and the IP_3/Ca^{2+}/protein kinase C pathway, respectively. In addition, gap junctional communication seems to be modulated by the nitric oxide (NO)/cGMP system. Since NO production can be stimulated by glutamate-induced calcium influx, the NO/cGMP-dependent modulation of gap junctions might represent a functional link between developing glutamatergic synaptic transmission and the gap junctional network. Thus, it might be of particular importance in view of a role of gap junctions during the process of circuit formation.

Index Entries: Somatosensory cortex; prefrontal cortex; gap junction; development; pyramidal cell; electrotonic cell properties; serotonin; dopamine; noradrenaline; protein kinase A; cAMP; nitric oxide; cGMP; IP_3; protein kinase C.

Developmental Time-Course of Gap Junction Expression in the Neocortex

Gap junctions represent an electrical and metabolic communication system regulating cellular functions in a number of vertebrate central nervous system (CNS) structures. Gap junctions consist of transmembraneous protein complexes (connexons). The connexons of two nearby cells form aqueous pores with an

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extremely low electrical resistance. In addition, gap junctions are easily permeable to small molecules with a mol wt of up to 1500 Daltons *(1)*. The connexons are composed of six protein subunits called connexins. About 15–20 different connexins have been described, and they have been classified according to their molecular weight (e.g., connexin 26 or connexin 43). In the retina *(2–6)*, the teleost Mauthner cell *(7,8)*, the inferior olive *(9–11)*, the mesencephalic nucleus *(12)*, the lateral vestibular nucleus *(13)*, the hypothalamus *(14)*, the nucleus accumbens *(15)*, and the hippocampus *(16,17)*, gap junctional signaling seems to be physiologically important in the adult state. However, in other parts of the CNS, such as the neocortex *(18,19)*, spinal cord *(20–22)*, and olfactory bulb *(23)*, gap junctions seem to have a transient developmental function.

In the ventricular zone, cortical neuroblasts display extensive coupling via gap junctions *(24)*. Following the final mitosis, gap junctional contacts are disrupted and the postmitotic neurons start to migrate into the developing cortical plate *(25)*. Uncoupling of cells is an obvious prerequisite for neuronal migration. Thus, the question arises whether the induction of uncoupling, e.g., by external factors, represents the signal for termination of mitosis or whether entrance of cells into the G_0 phase of mitosis triggers uncoupling. Lo Turco and Kriegstein *(26)* showed that induction of uncoupling via intracellular acidification or halothane stops DNA synthesis in ventricular zone neuroblasts. It seems, therefore, that external factors are capable of triggering both uncoupling and termination of mitosis. However, this is still a correlation, and experimental data providing evidence for a causal relationship are lacking. Nevertheless, one might speculate that external factors that are known to regulate gap junction permeability, e.g., neurotransmitters, interfere with neocortical neurogenesis.

Up to now, there has been no evidence for gap junction coupling between migrating neurons, and for physiological reasons, one should expect no direct connections between these cells. It is not known whether connexin expression is downregulated in migrating neurons, or whether the coupling between cells is suppressed by internal or external factors.

Differentiating postmitotic neurons within the cortical plate are again extensively coupled, both during embryonic *(26)* and early postnatal stages *(18,19,27)*. This new formation of electrotonic contacts between postmitotic neurons indicates that gap junction coupling during the period of synaptogenesis and circuit formation does not simply represent a developmental remnant of coupling between progenitor cells, but probably has an important function during these processes. Connexin expression has been reported to shift from connexin 43 and 26 in undifferentiated neuroepithelial cells to connexin 43 and 32 in more mature brain tissue *(28)*. However, whether connexin expression is different in ventricular neuroblasts and postmitotic cortical neurons remains to be investigated.

During the first postnatal week, deep and superficial pyramidal neurons are coupled via the apical dendrites of deep layer neurons, thus creating the typical columnar appearance of gap junction-coupled cell clusters *(19)*. Dye coupling between deep and superficial pyramidal cells disappears during the second postnatal week. At this stage, concentric coupled cell clusters are found in lamina II/III, whereas deep layer pyramids are already uncoupled *(19,29)*. These observations indicate that the extent of gap junction coupling correlates with the state of differentiation, and thus, it follows the "inside-first outside-last" gradient of cortical development. Whether the initial coupling between deep and superficial layer pyramids reflects a clonal relationship between cells has so far not been investigated. The molecular basis of cell–cell recognition or cell type specificity of gap junction coupling among neocortical neurons is not known. Possible candidates are cell adhesion molecules, which influence both the formation of gap junctions and connexin expression (*see* ref. *30* for review). Furthermore, in the developing neocortex, these molecules seem to be expressed in radially organized cell columns *(30)*.

Dye-coupling between neocortical neurons vanishes by the end of the second postnatal week *(18,19,27)*, although expression of connexin 32 seems to increase until postnatal d 35 in rat neocortex *(31)*. The increased connexin expression might be the result of a large number of closed gap junctions or hemichannels distributed throughout neuronal membranes. The mechanisms leading to the developmental downregulation of gap junction coupling, or at least functional dye transfer, are entirely unknown. Gap junction channels might not completely disappear, but might be kept in a nonfunctional state by some regulatory mechanism affecting either conductance or connexon assembly, and they might be recruited under certain conditions. A recent study by Penttonen et al. *(32)* has shown that dye coupling in the adult rat hippocampus increases following chronic epileptiform discharges. Connexins have high turnover rates *(33)*, and so far it is not clear whether this increase in coupling under pathological conditions is owing to newly synthesized channels, assembly of preexisting hemichannels, or alterations in the modulatory state of "spare" gap junctions. Residual coupling has been occasionally reported in the mature rodent neocortex *(34,35)*. However, experimental evidence for an increase in coupling during epilepsy is so far lacking.

The occurrence of synchronized gap junction-mediated increases in intracellular calcium concentrations in clusters of coupled neurons, so-called neuronal microdomains *(36–38)*, has prompted the hypothesis that gap junctions might preform cortical columns, thus representing a blueprint of the adult neocortical synaptic circuitry. However, the mechanisms of interaction between developing chemical synaptic inputs to neocortical neurons and the gap junction system have hardly been investigated.

In this article, we will first describe the effects of gap junctions on electrotonic cell parameters and the implications for synaptic transmission, and, second, we will discuss a variety of neurotransmitter-activated second messenger pathways that regulate gap junctional communication in rat somatosensory and association cortex.

Influence of Gap Junctions on Electrotonic Cell Properties

During the early postnatal period, gap junctions and developing chemical synaptic transmission coexist in the rodent neocortex. We have addressed the question concerning to what extent these two intercellular communications interact with each other and how far these interactions may guide or regulate the process of synaptic circuit formation in the developing neocortex. The period of circuit formation is characterized by enhanced plasticity of neocortical synapses *(39)*. Gap junctions could interact with these processes in a number of ways: Electrical signals can be directly transmitted via these structures *(18,34)*, biochemical messengers regulating synaptic functions might circulate in a coupled syncytium *(40,41)*, and by influencing the electrotonic structure of differentiating neurons *(27)*, gap junctions might affect the efficacy of chemical synaptic potentials. Both monoaminergic modulatory afferents and glutamatergic synaptic inputs, such as the thalamocortical projection, trigger a multitude of regulatory mechanisms controlling gap junction permeability in the developing mammalian neocortex *(29,42–44)*, and thus strongly interact with the gap junctional system.

To investigate the influence of gap junctions on electrotonic properties of developing neocortical neurons, we have used intracellular acidification as a fast and reversible method to uncouple pyramidal neurons *(27)*. However, intracellular pH may also represent a physiological regulator of gap junctional permeability. During synaptic transmission, local pH transients associated with transmitter release and reuptake occur, which may affect neighboring gap junctions. Intracellular pH changes occur during synchronized neuronal activity, especially during epileptiform activity *(45)*. Under these conditions, the pH regulation of

gap junctional conductance might be strong enough to modulate the propagation of epileptiform potentials effectively. Perez-Velaquez et al. *(17)* have shown that the transmission of epileptiform field burst activity and fast prepotentials, which are thought to represent action potentials propagated through gap junctions, is suppressed by intracellular acidification and enhanced by intracellular alkalinization in rat hippocampal neurons.

Gap junctions certainly affect the propagation of epileptiform activity in the developing neocortex. The immature rat neocortex displays an enhanced vulnerability to convulsant drugs, especially during the second and third postnatal weeks *(46)*. During this developmental period, amplitudes of epileptiform activity and the degree of its synchronization are more pronounced, and epileptiform discharges can be generated by multiple foci *(47)*. These observations might be partially explained by a facilitated spread of depolarizing potentials through gap junctions. However, very extensive electrotonic coupling apparently decreases neuronal input resistance and thus counteracts the ability of the cells to generate large potential changes. This shunting effect might be the reason for the virtual absence of evoked epileptiform discharges observed in slice preparations during the first week of postnatal development of the rat neocortex *(47)*.

The effect of intracellular acidification on gap junction coupling between neocortical neurons has been studied during both embryonic *(24,26)* and postnatal stages *(27,48)*. Prominent increases in input resistance following application of CO_2-saturated extracellular solution were observed in both ventricular zone cells *(24)* and embryonic cortical plate neurons *(26)*. On the other hand, Lucifer yellow coupling between postnatal pyramidal neurons was not significantly affected by application of external solutions containing up to 50% CO_2 *(48)*. In contrast, coupling between astrocytes was completely abolished by this treatment *(48)*. However, a recent study by Peinado et al. *(19)* demonstrated that cortical gap junctions are only weakly permeable for Lucifer yellow, but show high permeability for the smaller neurobiotin molecule. We have reinvestigated the pH sensitivity of gap junctions between neocortical pyramidal cells by filling individual cells with neurobiotin and have applied the anions of weak organic acids to decrease intracellular pH reversibly *(27)*. Under these conditions, dye coupling was reduced by 65%. Thus, neocortical gap junctions are sensitive to intracellular pH changes, but a significant pH sensitivity is only revealed if a tracer of high gap junction permeability is used to determine dye coupling. Gap junction coupling in the neocortex is also suppressed by other well-known gap junction blockers, such as long-chain alcohols, e.g., octanol *(26,27)*, or volatile anesthetics, such as halothane *(19)*.

Gap junctions may affect chemical synaptic signaling in two different ways. First, synaptic potentials might be directly propagated via electrotonic current spread into neighboring neurons; second, the influence of gap junctions on neuronal electrotonic structure may crucially influence the amplitude and time-course of synaptic potentials.

In many cell types, reducing gap junction permeability, e.g., by intracellular acidification or application of octanol, results in an increase in input resistance and a decrease in membrane capacitance large enough to be measured at the whole-cell level *(24,49,50)*. These alterations in input resistance and cell capacitance in turn affect membrane time constants and electrotonic length.

In rat neocortical pyramidal cells, we have observed a 20% reduction in membrane time constant (τ_0) in the majority of cells tested; however, in some neurons, an increase in τ_0 was observed. The first equalizing time constant, τ_1 *(51)*, was dramatically reduced (by 80%), resulting in a reduction of the electrotonic length by approx 20% *(27)*. Input resistance and, hence, neuronal electrotonic structure are determined by the specific membrane resistivity (R_m), the cellular morphology, and the internal resistivity (R_i). By electrotonically coupling adjacent neurons, gap junctions increase the apparent membrane surface area, leading

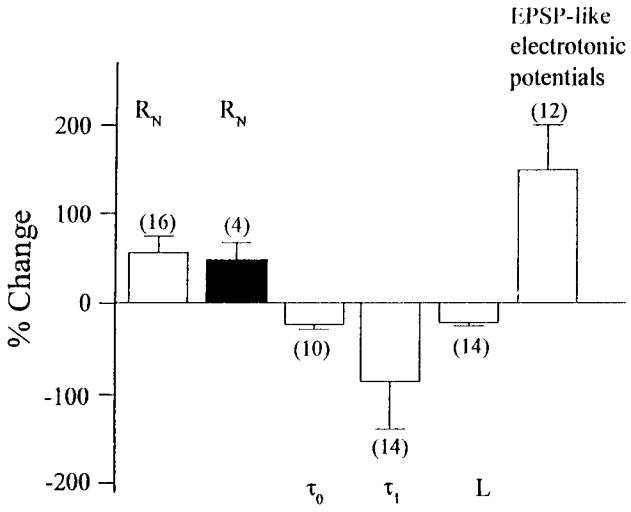

Fig. 1. Summary of the alterations in electrotonic cell parameters observed in developing lamina II/III pyramidal neurons following uncoupling via application of anions of weak organic acids or 1-octanol. Fast electrotonic potentials and the first equalizing time constant τ_1 are predominantly affected by this manipulation.

to a decrease in input resistance and an increase in cell capacitance. Both parameters determine the membrane time constant. Closing of gap junctions entails an increase in input resistance and a decrease in cell capacitance; the effect on membrane time constant thus depends on the relative contribution of both parameters. Figure 1 summarizes the changes in electrotonic parameters observed in developing neocortical pyramidal cells following uncoupling via application of sodium propionate or octanol. These changes in electrotonic cell parameters were exclusively observed in dye-coupled neurons between P1 and P12. In more differentiated neurons, which no longer showed dye coupling, these effects were absent *(27)*, strongly indicating that the effects on electrotonic structure were indeed owing to a reduction in gap junction permeability.

Following gap junction closure, neurons become electrotonically more compact, suggesting an increase in synaptic efficacy. However, one major disadvantage of gap junction blockers currently available is their direct action on chemical synaptic transmission. Octanol, halothane, and weak organic acids reduce excitatory synaptic transmission in the neocortex *(27)*. These effects are still present in more differentiated, uncoupled cells, demonstrating that these substances directly affect chemical signaling. Thus, the lack of specificity of classical gap junction blockers precludes the direct analysis of interactions between the incoming chemical synaptic input and gap junctions.

If synaptic potentials are simulated by injection of fast current transients resembling glutamatergic synaptic currents, these fast electrotonic potentials are reversibly potentiated following gap junction closure (Fig. 2). Again, these effects are absent in more differentiated, uncoupled cells *(27)*. The effect on fast electrotonic potentials is much stronger than on steady-state signals, indicating that the electrotonic effect is frequency dependent. Voltage attenuation in a cable has been shown to be strongly frequency-dependent *(52,53)*. Owing to the low capacitive resistance at high frequencies, current leakage across the cell membrane substantially attenuates fast synaptic signals. Gap junction closure, by increasing input resistance, enlarges the amplitude and prolongs the time-course of fast electrotonic potentials. The slowing of the time-course of synaptic potentials decreases the attenuation of these events. Thus, the physiological consequence of a reduction in gap junction permeability should be an increase in amplitude and a reduction in voltage attenuation of synaptic potentials during propagation toward the cell soma. The prolongation of the time-course of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) facilitates synaptic integration. If, in turn, gap junctional conductance is regulated by synaptic activity, the mechanisms outlined above may contribute to plasticity phenomena. These effects may be very local, and may thus play a role during selective stabilization and potentiation of synaptic inputs. For example, local calcium entry via synaptic NMDA receptors or local activation of protein kinases may close gap junctions in the vicinity of the active synapse. As a consequence, the synaptic input becomes more

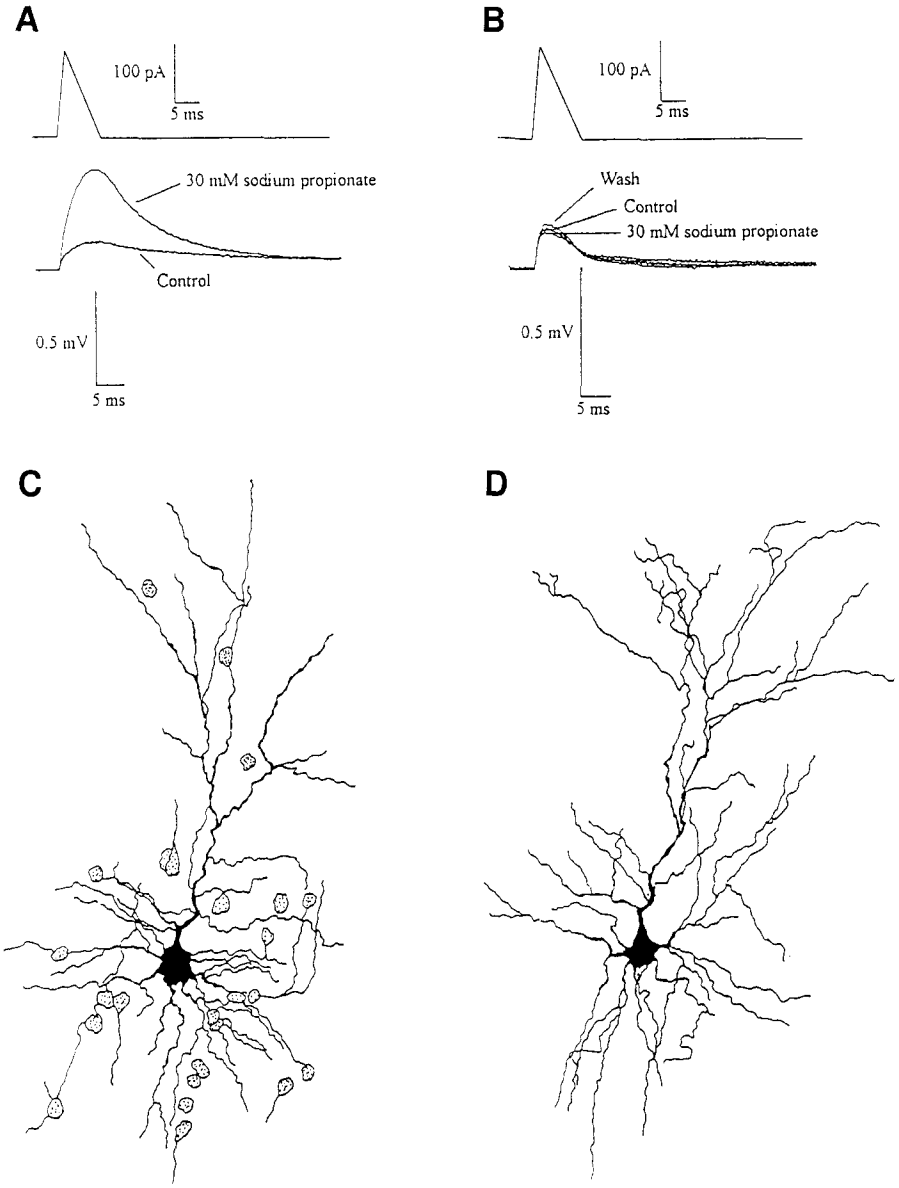

Fig. 2. Comparison of the effects of weak organic acid anions on fast electrotonic potentials in a dye-coupled P11 neuron **(A, C)** and a more mature uncoupled P18 neuron **(B, D)**. Whereas simulated synaptic potentials are strongly potentiated following uncoupling of immature pyramidal cells, these electrotonic effects are no longer observed in more differentiated uncoupled neurons. The recordings shown in (A) and (B) were obtained from the neurons depicted in (C) and (D). Scale bar: 50 μm. Modified after ref. *27*.

efficient, but no longer spreads electrotonically into neighboring neurons. Since gap junction coupling is prominent during the period of circuit formation, the regulation of neuronal electrotonic structure might be an important function of gap junctions during differentiation of synaptic circuitries.

Another important aspect regarding the regulation of junctional permeability is the voltage sensitivity of gap junction channels reported in a number of studies *(54–59)*. Voltage dependence is determined by two independent gates, one contributed by each connexon *(54)*. This property might be extremely important

for the function of neuronal gap junctions, since membrane potential differences between coupled neurons produced by activity-dependent mechanisms, e.g., asymmetric synaptic input or activation of voltage-gated channels, may directly regulate intercellular communication via gap junctions. The situation is even more complicated when gap junctions are heteromers, i.e., both cells contribute hemichannels composed of different connexins. Under these conditions, gap junction channels can exhibit rectification *(57,60)*, which offers even more possibilities for modulation by membrane potential changes generated by synaptic activation. However, the actual mechanisms underlying the complex interplay between developing synaptic inputs and gap junctions remain to be experimentally proven.

Gap junction channels represent relatively large pores, approx 1.5 nm in diameter, passing not only ions, but also metabolites and small molecules up to 1.5 kDa. The permeation of IP_3 and cAMP through gap junctions has already been demonstrated *(61)*. The synchronous gap junction-mediated calcium elevations observed by Yuste et al. *(36,37)* in the immature neocortex seem to be triggered by IP_3 receptor-mediated calcium release from intracellular stores, and propagated by diffusion of IP_3 through gap junctions, which, in turn, results in calcium release and regenerative IP_3 production in coupled cells *(38)*. Thus, coordination of neuronal activity via second messenger transfer seems to represent another important developmental function of neocortical gap junctions. Since low-mol-wt messengers like cAMP are important regulators of gene expression, coupled neurons may not only synchronize their metabolic state, but may also coordinate transcriptional activity. Paysan et al. *(62)* described a clustered expression of $GABA_A$ receptor subunits during development of rat cerebral cortex. These clusters occur during the early postnatal period and disappear during the second postnatal week. Thus, the time-course of coordinated expression of neurotransmitter receptor subunits closely corresponds to the occurrence of microdomains *(36)* and dye-coupled neuronal clusters *(19)*, indicating gap junction coupling. Apart from this, the size and distribution of clusters formed by neurons immunoreactive for the $\alpha 1$ subunit of the $GABA_A$ receptor resembled those of gap junction-coupled clusters. Therefore, these results also point to a synchronization of gene expression in coupled neurons. Gap junction-coupled cells might, thus, express a common molecular phenotype.

Neurotransmitter Regulation of Neocortical Gap Junctions

The conductance state of gap junctions in the neocortex certainly affects electrical and biochemical signaling. An important question, therefore, is which physiological mechanisms might regulate junctional communication. We have already discussed the pH sensitivity of neocortical gap junctions. Further candidates are intracellular calcium elevations and transcellular voltage changes. However, the more important task in view of elucidating the mechanisms guiding circuit formation in the neocortex is to advance the understanding of the interactions between the gap junction system and developing afferent projection systems.

Neurotransmitter receptor activation modulates gap junction coupling in a multitude of cell types. The underlying mechanism in most cases is phosphorylation of the connexin subunits by various protein kinases. The best-characterized pathway is the dopamine D_1 receptor modulation of gap junctional conductance. D_1 receptor activation always results in a reduction in junctional conductance. This action has been shown in retinal horizontal and amacrine cells of various species *(63–67)*. Dopaminergic modulation of gap junctions via D_1 and D_2 receptors has also been described in the rat neostriatum and nucleus accumbens *(15,68,69)*, as well as the teleost Mauthner cell *(70)*.

The effects of the other two monoaminergic neurotransmitters, noradrenaline and serotonin, on gap junction coupling have been less frequently investigated. Noradrenaline has

been shown to increase coupling in rat pinealocytes *(71)*, and in striatal astrocytes, α1-adrenoreceptor activation decreased dye coupling, whereas β1-receptor stimulation enhanced coupling *(72)*. A serotonergic modulation of gap junctions has so far only been described in an invertebrate preparation: 5-HT suppresses the formation of electrical junctions between cultured *Helisoma* neurons *(73)*.

In addition, the inhibitory neurotransmitter GABA seems to regulate gap junction permeability in retinal horizontal cells, since coupling is decreased following application of $GABA_A$ receptor antagonists *(74)*. To date, a cholinergic modulation of gap junctions has been demonstrated only in nonneuronal cells. In pancreatic acinar cells, acetylcholine induces electrical uncoupling *(75)*. In vasopressinergic neurons of the rat supraoptic nucleus, synaptically released glutamate *(76)* and histamine *(77)* increase dye coupling. Even the gonadal steroids estradiol and testosterone have been shown to modulate gap junction coupling among magnocellular neurosecretory cells of the rat hypothalamo-neurohypophysial system *(78)*.

The developing neocortex is invaded by a number of modulatory fiber systems, including cholinergic, noradrenergic, serotonergic, and dopaminergic afferents already at very early developmental stages *(79–83a)*. Innervation commences prenatally and is, particularly in the case of the dopaminergic projection, area-specific. In our recent studies, we have addressed the question concerning whether monoaminergic neurotransmitters modulate dye coupling during the early postnatal period of neocortical development, when synaptic circuitries are formed and gradual uncoupling occurs.

Dopamine

A number of investigations indicate that monoamines, e.g., dopamine, might act as neurotrophic factors during development of the prefrontal cortex. Lesioning of dopaminergic afferents reduces cortical thickness *(84a)* and impairs the development of cortical neurons *(85a)*. Thus, at least the correct structural differentiation of the prefrontal cortex seems to require the presence of monoaminergic afferents.

In the mammalian neocortex, the densest dopaminergic innervation is found in the prefrontal and anterior cingulate areas. In rodents, two classes of dopaminergic afferents to the prefrontal cortex, originating in the A9 and A10 dopaminergic cell groups of the ventral mesencephalon, have been described *(81)*. During the early postnatal period, dopaminergic terminals are present in both superficial and deep cortical layers *(82)*. The strongest expression of both D_1 and D_2 receptors occurs in the deep layers, although these receptors are also expressed in the superficial layers of prefrontal areas *(83b)*. A significant increase in D_1 receptor expression has been observed between the second and third postnatal week when gap junction coupling disappears and synaptogenesis increases *(84b)*.

In acute slices of rat neocortex, dye coupling between superficial pyramidal neurons in the medial precentral area of the prefrontal cortex is significantly reduced following preincubation with dopamine *(29)*. In layers II/III, this effect is observed between postnatal d 7 and 15. In contrast, at this age, the majority of deep layer neurons were already uncoupled, and dopamine had no significant effect on either the incidence or extent of dye coupling. The effects of dopamine on gap junction coupling at younger, e.g., embryonic, stages remain to be investigated. Dopamine-induced uncoupling was mimicked by the adenylyl cyclase activator forskolin as well as the direct protein kinase A (PKA) activator, Sp-cAMPS. The (PKA) inhibitor Rp-cAMPS antagonized the dopamine effect. Thus, uncoupling of immature pyramidal neurons by dopamine seems to involve a PKA-dependent phosphorylation process.

PKA-mediated phosphorylation has been shown to regulate gap junctional conductance in a number of neuronal and nonneuronal preparations. In rat hepatocytes *(85b)* and canine heart cells *(86–89)*, cAMP increases gap junction permeability. In teleost, turtle, and mammalian retinal neurons, PKA-mediated

phosphorylation decreases junctional communication *(63,64,66,90–92)*. In addition, a recent study by Hatton and Yang *(77)* describes a reduction in dye coupling among neurons of the rat supraoptic nucleus following application of 8-Br-cAMP.

In the neocortex, the uncoupling effect of dopamine was mimicked by both the D_1 receptor agonist SKF 38393 and the D_2 receptor agonist quinpirole. Accordingly, the dopamine effect was partially antagonized by the D_1 receptor blocker SCH 23390 and the D_2 receptor antagonist sulpiride *(29)*. Thus, dopamine-induced uncoupling can be mediated by an activation of both D_1 and D_2 receptors. In the neocortex, the D_1 receptor pathway appears to involve G-protein-mediated activation of adenylyl cyclase and PKA. D_2 class receptors are coupled to two major second messenger pathways: a downregulation of cAMP production and activation of phospholipase C-mediated cleavage of phosphatidylinositol 4,5-bis-phosphate into IP_3 and diacylglycerol. In addition, a functional synergism between D_1 and D_2 receptors has been repeatedly described *(93–95)*. Thus far, we have not investigated the intracellular signaling cascade triggering D_2 receptor-induced uncoupling in cortical neurons. This action may involve IP_3 receptor-stimulated calcium release from intracellular stores or liberation of arachidonic acid metabolites from membrane phospholipids.

Second messenger- and neurotransmitter-induced effects on electrotonic cell properties have also been demonstrated *(29,42–44)*. Activation of adenylyl cyclase by application of forskolin induced a reversible increase in input resistance and a potentiation of fast electrotonic potentials comparable to the effects observed following intracellular acidification *(29)*. Following incubation with either dopamine or dopamine receptor agonists, input resistance was also significantly higher compared to control conditions. Thus, uncoupling induced by dopamine, or activation of PKA, induces similar changes in electrotonic properties as uncoupling produced by application of weak organic acid anions.

In conclusion, metabolic and electrical signal transfer via gap junctions in the rodent prefrontal cortex is regulated by dopaminergic afferents. Additionally, electrotonic cell properties may be regulated by dopamine via a reduction in gap junction permeability.

Noradrenaline

A dense dopaminergic innervation is found in only a few cortical areas. If the regulation of gap junctional conductance represents a major developmental function of monoaminergic neuromodulators, an obvious question to ask is whether the more ubiquitous monoamines, noradrenaline and serotonin, also modulate gap junction coupling.

The noradrenergic system has been shown to affect neuronal morphology, synaptogenesis, and circuit formation in rat visual cortex *(96–99)*, and destruction of the monoaminergic innervation disturbs structural plasticity in mouse barrel cortex *(100)*. Noradrenergic afferents have furthermore been implicated in ocular dominance plasticity *(101)* and facilitation of long-term potentiation *(102)*.

In slices of rat somatosensory cortex, dye coupling between lamina II/III pyramidal neurons is significantly reduced following incubation with noradrenaline or the β1-adrenoreceptor agonist isoproterenol *(42)*. This effect is suppressed by the β-receptor antagonist atenolol *(42)*. The β1-adrenoreceptor is positively coupled to adenylyl cyclase and PKA, suggesting that noradrenaline utilizes the same second messenger pathway as dopamine to regulate gap junctional communication. This hypothesis is underscored by the finding that the PKA inhibitor Rp-cAMPS also antagonizes noradrenaline-induced uncoupling *(44)*.

The noradrenergic innervation of the rodent neocortex begins early during ontogenesis, and from postnatal d 5 onward, the entire rat neocortex is innervated by a noradrenergic fiber system that spreads to all cortical layers *(103)*. β-receptor expression increases steeply between P4 and P10 in rat neocortex and

remains high during the following developmental period *(104)*. Thus, during the early postnatal period, β-receptors are highly expressed and noradrenergic fibers have already reached their target layers. Unlike the dopaminergic projection, which is prominent only in the prefrontal and anterior cingulate areas, the noradrenergic system might modulate gap junction coupling in a large number of cortical areas.

The monoamines dopamine and noradrenaline downregulate gap junction coupling via activation of receptor subtypes that increase cAMP production. Since the uncoupling effect of both transmitters is blocked following inhibition of PKA and mimicked following PKA activation *(29)*, uncoupling seems to be mediated by a PKA-dependent phosphorylation process. Thus far, we have not directly demonstrated a PKA-mediated phosphorylation of connexins, i.e., the observed reduction in dye coupling might be indirectly induced via phosphorylation of a different protein. It has been shown that activation of adenylyl cyclase and PKA results in a decrease in the open probability of gap junction channels in retinal neurons *(67,91)*. Thus, the most likely explanation for the reduction in dye coupling induced by PKA in neocortical neurons is phosphorylation of the connexin subunits, which are endowed with several serine phosphorylation sites (*see* ref. *1* for a review).

Since they converge on the same second messenger pathway, dopamine and noradrenaline might have a complementary function as regulators of gap junctional communication in different cortical areas. However, one has to bear in mind that the regulatory efficacy of both transmitters may differ significantly under physiological conditions. Receptor densities may be different, coupling to the intracellular transduction cascade may vary, and developmental changes in modulatory functions might be different for the two projection systems. Thus far, we can only assess the effects on dye coupling following rather long incubation times with the transmitters or receptor-selective agonists. On-line recordings of the junctional current using pair recording paradigms are required to measure the time-course of the modulatory action of neurotransmitters and second messengers.

Serotonin

The neuromodulator serotonin (5-HT) also seems to have a crucial function during neocortical development. At neonatal stages, primary sensory areas of rodent neocortex receive a dense but transient serotonergic innervation arising in the dorsal and median raphe nuclei *(105–107)*. This transient serotonergic hyperinnervation is temporally correlated with an elevation in cortical 5-HT levels *(108)*. During early postnatal development, serotonergic fibers and thalamocortical axon arborizations in the rat primary somatosensory cortex show a parallel anatomical organization *(105,109–111)*. The differentiation of this characteristic organization of serotonergic afferents requires the presence of thalamocortical axons *(112,113)* and disappears by the end of the second postnatal week *(105,112,113)*. The close anatomical alignment of serotonergic and thalamocortical fiber systems suggests a functional role for 5-HT during the formation or refinement of thalamocortical projections in primary sensory areas. Thus, vibrissa-related thalamocortical terminations in the rodent barrel cortex are reduced in size following depletion of 5-HT by 5,7-dihydroxytryptamine (5,7-DHT) injections *(112,113)*. Furthermore, Gu and Singer *(114)* have recently shown that depletion of serotonergic terminals or infusion of serotonin receptor antagonists reduces ocular dominance plasticity in kitten visual cortex. These results suggest an important role of the serotonergic system in primary sensory map formation in the developing neocortex.

The primary mechanism underlying the regulation of developmental plasticity by 5-HT seems to be a modulation of thalamocortical synaptic transmission. Recent electrophysiological studies have demonstrated a presynaptic suppression of thalamocortical transmission in rat somatosensory cortex by 5-HT *(115)*,

involving 5-HT_{1b} receptors located on thalamocortical axons *(116)*.

The transient serotonergic hyperinnervation of the somatosensory cortex appears at a time when widespread gap junction coupling is observed in primary sensory areas of the developing rodent neocortex *(19,106)*. Thus, the modulation of gap junctional communication between immature neurons might be one of the functions of serotonin in regulating developmental plasticity in primary sensory neocortical areas. The regulatory functions of 5-HT during neocortical development are most pronounced during the early postnatal period when synaptic circuitries develop *(117)* and extensive gap junction coupling is still observed *(19)*. During this period, preincubation of cortical slices with 5-HT resulted in a significant reduction in the average number of dye-coupled cells per injection *(118)*. The pharmacological data so far suggest that serotonergic modulation of gap junctions is mediated by 5-HT_2 class receptors. The serotonin effect was mimicked by the 5-HT_2 receptor agonists α-methyl-5-hydroxytryptamine and R (+)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and suppressed by the 5-HT_2 receptor antagonist ritanserin *(118)*. In adult rats, a very high density of 5-HT_2 binding sites has been found in all areas and laminae of the neocortex, with particularly high concentrations in the frontal areas *(119)*. 5-HT_2 receptor mRNA has also been localized in the frontal cortex *(120,121)*. Receptors of the 5-HT_{1A} and 5-HT_{1B} type are also expressed in these cortical areas *(122,123)*. In adult animals, 5-HT_2 and 5-HT_1 class receptors are expressed in both deep and superficial pyramidal cell layers; however, so far the cellular distribution and developmental time-course of 5-HT receptor expression has not been investigated in the rat somatosensory cortex.

The 5-HT_2 receptors are coupled to phospholipase C stimulation and subsequent IP_3 and diacylglycerol (DAG) production *(124,125)*. A rise in cytoplasmic calcium concentration to about 1 μ*M* *(126,127)* closes gap junctions. In addition, many connexins are phosphorylated by protein kinase C (PKC; *1*), which is activated by calcium and DAG. To examine the involvement of this pathway in 5-HT-mediated reduction in gap junction coupling, the whole-cell patch-clamp technique was used to introduce the IP_3 receptor antagonist heparin *(128,129)* into the cell interior. The uncoupling effect of 5-HT was antagonized by addition of heparin to the patch pipet *(118)*. These results indicate that IP_3 receptor stimulation significantly contributes to the uncoupling action of 5-HT. The effect might be mediated by calcium released from intracellular stores acting directly on gap junction channels and/or by activation of PKC. The selective PKC inhibitor NPC 15437 *(130)* also antagonizes the effect of 5-HT on gap junction coupling *(118)*. Thus, activation of PKC probably contributes to the uncoupling effect of 5-HT.

A translocation of PKC from the cytosol to the cell membrane following 5-HT receptor stimulation with either serotonin or the selective 5-HT_2 receptor agonist DOI in cortical slices and synaptosomes has been reported in a previous study by Wang and Friedman *(131)*. Activated PKC may directly phosphorylate gap junction channels, since many connexins have PKC phosphorylation sites *(1)*. PKC-mediated uncoupling has been reported in a variety of cell types and preparations *(132–141)*. However, activation of PKC has different effects on gap junctional conductance in different cell types and seems to depend on the connexin composition. In rat hepatocytes, gap junction coupling was not affected by PKC activation *(142)*, and in cardiac gap junctions, the junctional conductance was even increased *(143)*. Thus far, gap junction channels expressed by developing neocortical neurons have not been isolated, and their connexin composition has not yet been determined. Their biophysical and pharmacological properties *in situ* are unknown. Our dye-coupling data indicate a decrease in junctional permeability following PKC activation, although the effects of PKC-mediated phosphorylation on junctional conductance remain to be investigated.

Calcium ions, which are released from intracellular stores following 5-HT-induced IP_3 pro-

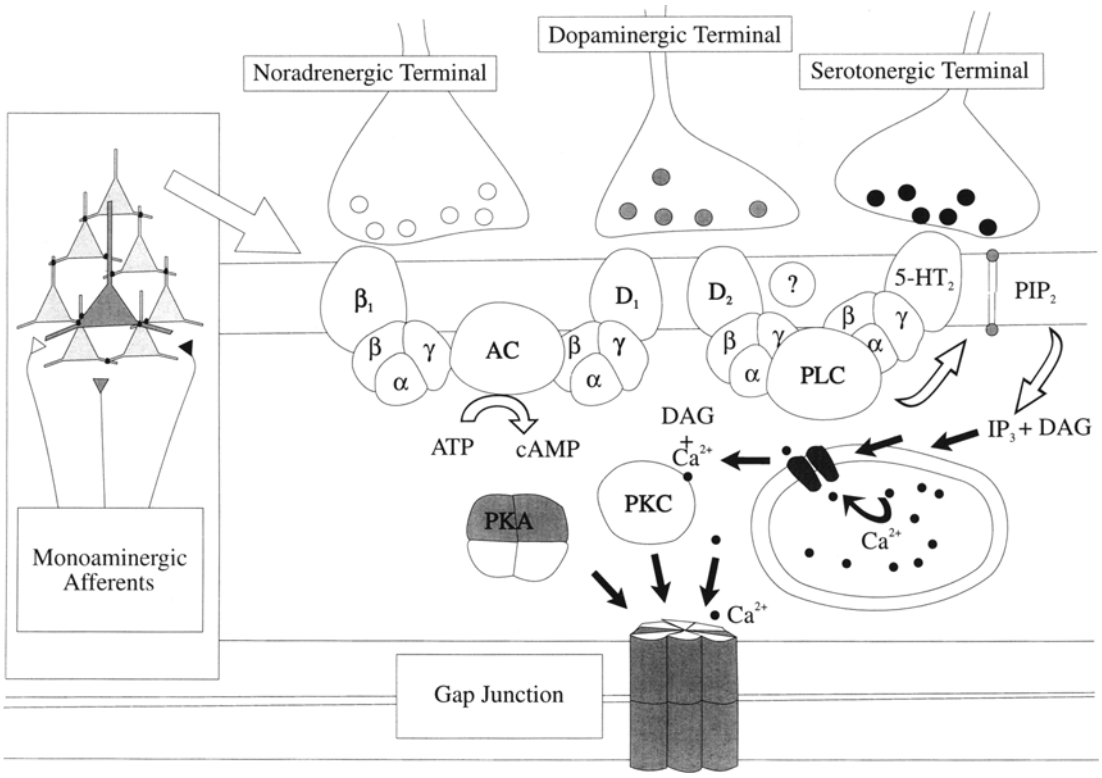

Fig. 3. Summary of those second messenger pathways modulating gap junction coupling that are potentially activated by G-protein-coupled monoamine receptors in neocortical neurons. Dopamine D_1 receptors, as well as β_1-adrenoreceptors, converge on the adenylyl cyclase/PKA signaling system. Serotonin receptors belonging to the 5-HT_2 class, as well as some dopamine D_2 receptors, activate phospholipase C (PLC), resulting in the hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bis-phosphate (PIP_2) and the production of the two second messengers IP_3 and DAG. Gap junctional conductance is regulated by PKA- and PKC-mediated phosphorylation processes, as well as elevated cytosolic calcium concentrations caused, e.g., by IP_3 receptor-mediated calcium release from intracellular stores. Whether uncoupling via D_2 receptor activation is mediated by the phospholipase C pathway has so far not been demonstrated.

duction, might directly affect gap junctional conductance. Uncoupling by calcium has been described in a number of vertebrate nonneuronal tissues *(88,89,126,127,144–146)*, and has furthermore been shown in retinal *(65)* and hippocampal *(147)* preparations. Thus, the uncoupling effect of 5-HT might involve a concerted action of calcium and calcium-activated PKC on neocortical gap junctions.

Conclusions

The modulation of gap junction permeability by monoaminergic transmitters involves two major second messenger pathways—the cAMP/PKA cascade and the IP_3/Ca^{2+}/PKC transduction system (Fig. 3). The PKA pathway is activated by dopamine D_1 receptors and β1-adrenoreceptors. Serotonin does not seem to utilize this pathway in developing neocortical neurons, since inhibition of PKA does not antagonize 5-HT-induced uncoupling *(44)*. The IP_3/Ca^{2+}/PKC signaling system is activated by 5-HT_2 receptors. The dopamine D_2 receptor-induced reduction in dye coupling might also be mediated by this pathway, but this remains to be established experimentally.

Why are two different transduction systems needed to mediate the modulatory effect of monoamines on gap junction coupling? If one

considers the direct action of calcium ions on gap junctions and calcium-dependent PKC activation as simultaneously activated, but separate pathways, we find ourselves dealing with three different modulatory signaling systems. In our dye-coupling assay system, dopamine-induced uncoupling was only partially reversible within 1–2 h *(29)*, whereas 5-HT-induced uncoupling was almost completely reversible within 1 h *(44)*. Evidently, we have to distinguish between short-term and long-term regulatory mechanisms. The direct binding of calcium ions to the connexins, inducing a reduction in gap junction channel permeability, might be the fastest mechanism. However, intracellular diffusion of calcium ions is spatially restricted *(148)*. Thus, the efficacy of calcium-induced uncoupling is likely to depend on the distance between the calcium source and the gap junctions. A local elevation of intracellular calcium concentration, e.g., via NMDA receptor activation, which occurs in the vicinity of the junctional channels, might be more effective than calcium release from remote intracellular stores. The time-course of PKC or PKA activation following receptor stimulation is not known. However, the lower velocity of recoupling following activation of the PKA pathway might indicate that this transduction system exerts long-term effects, probably even irreversible uncoupling or connexin degradation. It may, thus, indeed contribute to the gradual developmental uncoupling observed during the early postnatal period. Nevertheless, both pathways may have a short-term, dynamic regulatory function during the process of circuit formation.

As already discussed in the preceding chapters, a number of investigations have demonstrated the modulation of synaptic transmission and synaptic plasticity by monoaminergic neuromodulators. In addition, disorders in the structural differentiation of the neocortex have been repeatedly shown following elimination of the monoaminergic input systems. These observations already indicate that extrathalamic modulatory projections play an important role during cortical development. Our recent studies have added an additional transient developmental function of monoaminergic transmitters—the modulation of gap junction coupling between immature pyramidal cells. Understanding how far all these multifaceted regulatory mechanisms are coordinated and integrated to allow for the correct differentiation of the complex neocortical cytoarchitecture and synaptic connectivity requires further investigation. In any event, our studies indicate that one function of monoaminergic afferents might be the control of cortical plasticity during early postnatal development via regulation of gap junctional communication between differentiating neocortical neurons.

The early monoaminergic innervation of the developing neocortex is area-specific. Whereas dopaminergic fibers are found predominantly in the prefrontal areas *(85a)*, the transient serotonergic hyperinnervation is restricted to primary sensory areas *(106)*. Recent studies did not point to marked area specificities in gap junction coupling or its developmental time-course *(18,19,27,29,42,43,118)*. However, cortical areas differentiate at different times during development, and area-specific modulatory innervation patterns indicate early functional specializations. It remains to be shown whether gap junction coupling and its modulation by transmitters and second messenger cascades play a similar functional role in the entire neocortex, or whether there are area specific differences associated with differences in extent and time-course of coupling and its regulation.

Regulation of Gap Junctions by the NO/cGMP Pathway

The work discussed shows that gap junctions in the immature neocortex seem to be subject to a multitude of regulatory mechanisms, including intracellular pH *(24,27)* and neurotransmitter receptor-activated phosphorylation by protein kinases *(29,42,44)*. Regarding neurotransmitter modulation of gap junction coupling, we have so far concentrated

on the ascending monoaminergic modulatory systems. However, to gain further insight into the role of gap junctions in the process of circuit formation, an understanding of the interactions of developing thalamocortical and intrinsic synaptic activity with the gap junctional system is of primary importance. In the ferret, gap junctions and spontaneous synaptic currents have been shown to replace each other gradually *(149)*. In the rat, chemical and electrotonic transmissions coexist at least during the first two postnatal weeks.

During the early postnatal period, synaptic potentials in the neocortex are characterized by a prominent NMDA receptor-mediated component *(150)*. Thus, one possible interaction between glutamatergic synaptic transmission and gap junctions might be a transient occlusion of junctional channels by calcium entry via NMDA receptors or calcium-permeable AMPA/kainate receptors. These mechanisms may selectively modify the strength of synaptic inputs by the electrotonic changes induced by gap junction closure. Glutamatergic synaptic activity may also trigger neuronal coactivation via gap junctions. Kandler and Katz *(41)* showed that activation of metabotropic glutamate receptors elicits synchronized calcium elevations (microdomains) in coupled neurons by stimulating IP_3 production and subsequent calcium release from intracellular stores. Another second messenger pathway activated by glutamate receptor-mediated calcium influx, involving the production of nitric oxide (NO) and cGMP, will be discussed in more detail below.

The free radical gas NO has been shown to be involved in plasticity phenomena, as well as in excitotoxicity, acting as a freely diffusing, membrane-permeable messenger molecule *(151)*. NO production is stimulated by Ca^{2+}/calmodulin-dependent activation of nitric oxide synthase (NOS, NADPH-diaphorase). In the neocortex, this enzyme is primarily located in GABAergic and peptidergic interneurons, whereas soluble guanylyl cyclase, the intracellular NO receptor, is found predominantly in pyramidal cells *(151)*. During the period of enhanced synaptic plasticity, evoked excitatory synaptic potentials in rat neocortical neurons show a larger NMDA receptor-mediated component compared to the adult *(152)*, and NMDA currents display slower kinetics *(153)*. Furthermore, fast-spiking neocortical interneurons express calcium-permeable AMPA receptors *(154)*. Calcium entry via glutamate receptors might affect gap junctional communication by calcium ions acting directly on connexons. However, glutamate receptor-mediated calcium influx might also activate the NO/guanylyl cyclase pathway. Thus, these mechanisms might serve as a regulatory link between glutamatergic synaptic activity and gap junctional communication.

Dye-coupled neurons between postnatal d 6 and 10 form concentric clusters around the core cell and are predominantly found in the vicinity of basal and apical dendrites of the injected neuron. Following preincubation with the NO source sodium nitroprusside (SNP), the number of coupled neurons is dramatically reduced *(43)*. Apart from the reduction in cluster size, a striking change in the coupling pattern occurs in many cases. A small number of neurons remain strongly dye-coupled to the injected cell. These often involve 1–3 adjacent layer II/III neurons and occasionally a single layer V neuron. The uncoupling effect of SNP is antagonized by intracellular injection of the guanylyl cyclase inhibitor cystamine, indicating that the effects of NO on dye coupling are mediated by stimulation of guanylyl cyclase in pyramidal neurons. In compliance with these findings, both preincubation of slices with the membrane permeable cGMP analog 8-Br-cGMP and intracellular injection of cGMP resulted in a significant reduction in gap junction coupling. Increasing intracellular cGMP concentrations also mimics the change in coupling pattern observed following incubation with SNP; in many cases 1–3 cells remain strongly dye-coupled *(43)*. In the majority of neurons, intracellular injection of cGMP induces an increase in neuronal input resistance by about 70% *(43)*. This is in line with our previous observations demonstrating an increase in input

resistance following uncoupling via intracellular acidification *(27)*.

Increasing intracellular cGMP has previously been shown to reduce gap junction coupling in retinal horizontal cells *(65)*. In these neurons, guanylyl cyclase is activated by the L-arginine/NO pathway, as well as by arachidonic acid *(50,155)*. 8-Br-cGMP has also been shown to decrease the conductance of rat Cx43 gap junction channels expressed in SKHep1 cells via a phosphorylation-dependent mechanism *(156)*. In contrast, 8-Br-cGMP increased dye coupling in neurons of the rat supraoptic nucleus *(76)*.

Excitatory amino acid receptor stimulation and calcium influx have been shown to increase NOS activity in cortical slices *(157)*, and NOS-positive neurons in rat cerebral cortex express more mRNA encoding the NR1-subunit of the NMDA receptor than non-NOS neurons *(158)*. The number of NOS-expressing cells in the superficial layers of rat neocortex increases during the first postnatal week and thus correlates with laminar differentiation *(159)*. In adult rat primary sensorimotor cortex, the majority of NOS-positive cells are GABAergic local circuit neurons of layers II/III *(160)*; pyramidal cells express NOS only in response to cortical lesions *(161)*. However, soluble guanylyl cyclase, the intracellular NO receptor, is strongly expressed in pyramidal cells *(151)*, suggesting that these neurons represent the major target for NO in the neocortex. Thus, the NO/cGMP-mediated modulation of gap junction coupling represents a directed signaling system, unidirectionally transferring information from interneurons to pyramidal cells (Fig. 4).

The conspicuous change in the structure of gap junction-coupled neuronal assemblies following activation of the NO/cGMP pathway might mimic a developmental refinement process. During the early postnatal period, increasing synaptic activity involving glutamate receptor-mediated calcium entry, NOS, and guanylyl cyclase activation might induce a transition from extensive, nonselective coupling between neighboring, perhaps clonally related cells to a more selective electrotonic coupling pattern involving only a few, perhaps synaptically connected neurons. Although gap junctions probably do not preform the entire columnar circuitry, they might be involved in the shaping of particular sets of synaptic connections, e.g., between neighboring lamina II/III pyramids and the layer III–V loop.

Second messenger modulation of gap junction coupling might provide a functional link between synaptic activity and the state of metabolic and electrotonic coupling. The NO/cGMP system might thus represent an activity-dependent mechanism regulating gap junctional communication during the period of synaptic circuit formation.

Intracellular second messenger signaling systems are functionally interrelated in multiple ways. The regulatory cascades controlling gap junctional conductance in the developing neocortex involve the production or liberation of second messengers, such as cAMP, cGMP, IP_3, and calcium. These molecules regulate the activity of protein kinases, which may phosphorylate connexins, but simultaneously, they activate or inhibit other enzymes, e.g., phosphodiesterases.

The uncoupling effect of a rise in intracellular cGMP concentration, for instance, is suppressed by inhibition of PKA (Sutor and Rörig, unpublished observations). This indicates that the cGMP-induced reduction in dye coupling is indirectly mediated via cAMP and PKA activation. Since a cGMP-inhibited cAMP phosphodiesterase activity is observed already on P6 in rat cortex homogenates (Sutor, unpublished observations), this phenomenon could be explained in the following way: cGMP suppresses the phosphodiesterase-mediated cleavage of cAMP, which, in turn, results in an enhanced activity of PKA. Thus, the common mechanism of dopamine, noradrenaline, and finally, glutamate-triggered gap junction closure might be a PKA-dependent phosphorylation process. However, a contribution of calcium-permeable cyclic nucleotide-gated channels, as well as protein kinase G (PKG) activation, to the cGMP effect cannot be completely ruled out.

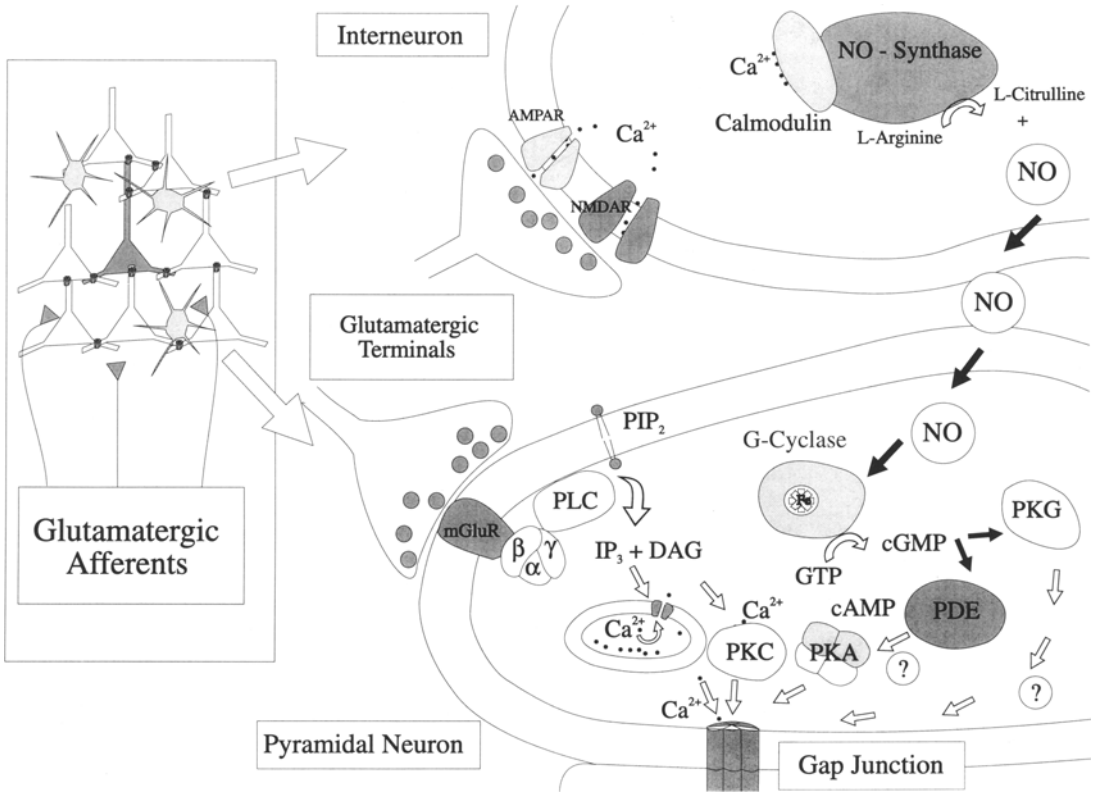

Fig. 4. Potential pathways activated by glutamate receptor stimulation. Synaptic activation of metabotropic glutamate receptors on pyramidal neurons might regulate gap junctional conductance by calcium release from internal stores and activation of PKC. On the other hand, calcium entry via NMDA receptors or calcium permeable AMPA/kainate receptors stimulates NO synthesis in interneurons. NO then activates guanylyl cyclase in pyramidal cells. The mechanism underlying the uncoupling effect of cGMP remains to be clarified; it may involve activation of a cGMP-stimulated protein kinase (PKG) or it may be at least partially mediated by an indirect pathway involving inhibition of a phosphodiesterase (PDE) by cGMP. The rise in intracellular cyclic nucleotide concentrations might then result in uncoupling because of an enhanced activity of PKA.

Functional Significance of the Regulation of Gap Junction Coupling by Synaptic Activity-Dependent Mechanisms

The columnar appearance of both dye-coupled cell clusters *(19)* as well as gap junction-mediated calcium transients *(36,37)* observed in the developing neocortex has provoked the speculation that gap junction coupling of immature neurons precedes the formation of synaptic connections between the same cells. Although the apparent cell-type specificity of gap junction coupling *(19)* argues against a preformation of the entire circuitry constituting a cortical column by the gap junctional network, the conspicuous developmental regulation of coupling points to a function during the period of synaptogenesis. Our understanding of the precise role of gap junctions during the process of circuit formation is still incomplete. However, recent studies have shed some light on the nature of the signals transmitted via gap junctions, as well as the possible interactions between gap junctions and synaptic activity.

Apart from electrical current flow, the transfer of small molecules, e.g., second messengers via gap junction channels, seems to be of primary

importance in the developing neocortex. Recent evidence suggests that IP_3 might be a major candidate *(38)*. The production of this second messenger can be induced by neurotransmitter receptor activation. Stimulation of metabotropic glutamate receptors has already been shown to evoke microdomains via activation of IP_3 production and IP_3 receptor-induced release of calcium ions from intracellular stores *(41)*. A variety of other neurotransmitter receptors coupled to the phospholipase C pathway, such as dopamine D_2 receptors, 5-HT_2 receptors, and so on, also potentially stimulate gap junction-mediated neuronal coactivation.

Thus, one possible functional interaction between gap junctions and chemical synaptic transmission would be a production of gap junction-permeable messengers, triggered by synaptic activation. These messengers might then circulate in the coupled syncytium and synchronize metabolic states or even gene expression. Whether these mechanisms contribute to the differentiation of synaptic connectivity remains to be shown. On the other hand, our studies provided evidence for a 5-HT-induced IP_3 receptor activation, resulting in a reduction of gap junction permeability *(44)*. This observation does not appear to be in line with an IP_3-dependent generation of calcium domains involving an intracellular spread of IP_3 through gap junctions. How can the two effects of activation of IP_3/Ca^{2+}-coupled neurotransmitter receptors on gap junction coupling be functionally reconciled? Since experimental evidence is still lacking, we can only speculate on this point. For discussion of this discrepancy, one has to consider that dye coupling represents an indirect technique, and thus, it does not allow any conclusions about the time course of the 5-HT-induced and IP_3 receptor-mediated effect. Inositol 1, 4, 5- trisphosphate has been shown to act as a long-range messenger, whereas intracellular diffusion of calcium ions is restricted by cytosolic buffer systems and sequestering of calcium into internal stores by calcium pumps *(148)*. Thus, it is possible that IP_3 permeates through gap junctions and initiates calcium release, resulting in the generation of a calcium domain before the calcium concentration in the vicinity of gap junction channels rises to the critical level for gap junction closure. The occurrence of a domain might entail a transient reduction in gap junction permeability following the intracellular calcium elevation, which may limit the duration of the synchronous calcium transient.

Since the cAMP and cGMP molecules are even smaller than IP_3, it is very likely that these substances also permeate through gap junctions. Although the permeation of cyclic nucleotides has so far not been demonstrated, a synchronous spread of second messengers through a gap junction coupled cell assembly and the subsequent closure of gap junctions owing to a rise in intracellular calcium or activation of protein kinases might represent a general principle of interaction between neurotransmitter receptor activation and gap junctions. In this way, the speed of gap junction closure, as well as the duration of recovery, would contribute to the temporal patterning of coactivation in coupled neuronal assemblies. Whether such mechanisms contribute to the generation of synaptically connected networks remains to be shown.

The monoaminergic innervation of the rodent neocortex commences prenatally and is to some extent area specific. We have already mentioned the study by Lo Turco and Kriegstein *(25)*, which demonstrates a termination of mitotic activity in the ventricular zone following uncoupling of neuroblasts. Another function of the early monoaminergic innervation may thus be a regulation of neurogenesis in the ventricular zone via a reduction in gap junction coupling between neocortical progenitor cells. Since cortical areas differ in cell densities and differences in cell cycle kinetics have been proposed to underlie areal specification *(162)*, the control of mitotic activity by different monoamines, e.g., dopamine in the prospective prefrontal areas and serotonin in the prospective primary sensory areas, may contribute to the specification of cortical areas. Neuroblasts also express glutamate and GABA receptors prior to synaptogenesis *(25)*, suggesting that

these transmitters might also regulate gap junction coupling at very early developmental stages by triggering second messenger synthesis or calcium entry. During postnatal development, these mechanisms may initiate the developmental reduction in dye coupling by either keeping gap junctions in a closed state or even by suppressing connexin expression.

In conclusion, the multitude of regulatory pathways we have discussed offers a variety of possible interactions between monoaminergic and glutamatergic afferents and the gap junction system. These mechanisms may comprise both short-term effects regulating metabolite transfer and electrotonic cell parameters, as well as long-term effects probably affecting connexin expression or assembly.

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