COUPLING BETWEEN NEURONS OF THE DEVELOPING RAT NEOCORTEX¹

B. W. CONNORS,² L. S. BENARDO,³ AND D. A. PRINCE

Department of Neurology, Stanford University School of Medicine, Stanford, California 94305

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Abstract

We have estimated the prevalence of coupling between neurons of the rat neocortex during postnatal development. Single intracellular injections of the fluorescent dye Lucifer Yellow CH resulted in dye coupling among 70% of neurons from 1 to 4 days of age. Dye coupling dropped to 30 to 40% by 10 to 18 days and occurred in 20% of injected adult neurons. The number of neurons per dye-coupled aggregate also decreased. Whereas three to seven coupled neurons were common in cortex of 1 to 4 days, aggregates of more than two neurons were exceptionally rare in adults. The frequency of dye coupling did not vary systematically with cortical depth at any age. When chemical synaptic activity was blocked, most 4-day neurons exhibited short latency antidromically evoked depolarizations which were relatively insensitive to repetitive activation and membrane polarization. These depolarizations may represent electrotonically conducted spikes from coupled neurons. No such potentials were found in adult neurons. The results suggest that neuronal coupling is extensive in immature rat neocortex, but that coupling declines at a time just before the numbers of chemical synapses increase most rapidly.

The morphology of cellular development in the neocortex is well described. Autoradiographic investigations have revealed that neuroblasts migrate radially from their origins in the deep ventricular germinal zone to form the cortical plate, with each cell assuming a final position superficial to that of its predecessors (Angevine and Sidman, 1961; Berry and Rogers, 1965; Hicks and D'Amato, 1968). Studies using the Golgi technique have meticulously charted the ensuing progression of complexity and diversity of neuronal shapes (e.g., Ramon y Cajal, 1911; Eayrs and Goodhead, 1959; Miller, 1981). However, very little is known about the types of neuronal interactions which must certainly occur during cortical development nor the specific roles these may play in the genesis and function of immature neocortical circuits. With Golgi-electron microscopic methods, it has become possible to document the ontogeny of structurally defined synaptic connections between cortical neurons (Parnavelas et al., 1978; Miller and Peters, 1981), but the data are still quite sparse.

One form of cell-to-cell communication which is particularly prominent in early development is that subserved by the low resistance intermembranous channels which often aggregate to form gap junctions (Loewenstein, 1981). In fact, as pointed out by Bennett et al. (1981), the presence of this form of intercellular coupling has been observed in every early embryo which has been studied, from annelids to mammals. The developmental distribution of coupling in both space and time may be quite specific, although the electrical and/or chemical communicative roles which it serves are very poorly understood. Some of the most intriguing examples of developmental changes in coupling have been observed in nonmammalian nervous systems (e.g., Dixon and Cronly-Dillon, 1972; Lo Presti et al., 1974; Goodman and Spitzer, 1979). Examples in developing mammalian nervous systems are more poorly documented. Motoneurons of the newborn rat spinal cord appear to be extensively coupled to one another (Fulton et al., 1980), but it is not clear whether the coupling changes with maturity.

Morphological evidence in adult primates (Sloper, 1972; Sloper and Powell, 1978; Smith and Moskovitz, 1979) and adult rats (Peters, 1980), as well as physiological data from adult guinea pigs (Gutnick and Prince, 1981), suggests that electrical coupling may have a significant role in the function of mature neocortex. To assess the prevalence of coupling in developing neocortex we have used intracellular injections of the junction-

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² To whom correspondence should be addressed.

³ Present address: College of Physicians and Surgeons of Columbia University, New York, NY 10032.

permeable fluorescent dye Lucifer Yellow CH (Stewart, 1978) and indirect electrophysiological tests. These results have appeared in abstract form (Benardo et al., 1982).

Materials and Methods

All experiments were performed *in vitro* on slices of neocortex obtained from albino rats (Sprague-Dawley). Animals ranged in age from 1 day (0 to 24 hr following time of birth) to adulthood (>60 days), and ages were not controlled for gestation time.

Techniques for maintaining neocortical slices *in vitro* have been previously described (Connors et al., 1982). Animals were decapitated and their brains rapidly removed and placed in ice-cold Ringer solution. Using a scalpel, a block of tissue was dissected from the sensorimotor area, or its presumed precursor. Slices of 500- μ m nominal thickness were cut in the coronal plane on a McIlwain tissue chopper and immediately placed in a recording chamber maintained at $36 \pm 1^\circ\text{C}$. Slices were continuously superfused with solution saturated with 95% O_2 5% CO_2 .

Extracellular stimulation (200- μ sec duration pulses) was applied via a sharpened monopolar tungsten electrode placed in cortical layers deep to the recording site. Intracellular recordings were obtained with microelectrodes pulled from 1-mm thin walled capillary tubing (Frederick Haer, Inc.). Electrodes were filled with either 4 M potassium acetate (40 to 50 megohms) or a 5% solution of Lucifer Yellow CH (Aldrich Chemical Co., Inc.) in distilled water (150 to 250 megohms). All recordings and dye injections reported here were obtained from neurons, identified physiologically by the presence of action potentials, evoked synaptic potentials, and relatively high input resistances. Glia were encountered in older cortices but were easily distinguished by standard criteria previously confirmed in the neocortical slice preparation (Gutnick et al., 1981). Dye was injected into neurons via a bridge circuit by applying 750-msec hyperpolarizing current pulses of 2 to 4 nA at a rate of 1 Hz. The injection period was terminated at 5 min or if the membrane potential dropped below about 20 mV. The current intensity and duration of each injection were recorded. Dye injections were limited to four to five per slice and were spaced far enough apart that specific identification of each site was unambiguous after processing the tissue. Following dye injection, slices remained in the recording chamber for a minimum of 30 min. Slices were fixed overnight in 4% buffered formalin (3°C), dehydrated, cleared, and whole mounted for viewing under epifluorescence (Stewart, 1978).

Results

Dye coupling. Injection of Lucifer Yellow CH into adult neocortical neurons most often stained single pyramidal-shaped cells (Fig. 1A) and occasional single non-pyramidal cells. In 20% of the successfully recovered injections ($n = 46$), however, more than one cell was stained following a single intracellular recording. The majority of these dye-coupled aggregates consisted of pairs of cells; in only one case were three cells stained and never more than three. Somata of stained cells were

located from 150 to 1600 μ m below the pia, and the laminar distribution of dye-coupled neurons was coextensive with that of uncoupled neurons. The presence of neuronal dye coupling in adult rat neocortex confirms similar observations in the homologous part of adult guinea pig neocortex (Gutnick and Prince, 1981; L. S. Benardo, B. W. Connors and D. A. Prince, unpublished experiments). There are quantitative differences, however. In guinea pigs, coupling resulted from 44% of the injections, and coupled neurons were usually confined to superficial cortical layers (<400 μ m deep) (Gutnick and Prince, 1981). Most strikingly, coupled aggregates in the guinea pig commonly consisted of three to six neurons, in contrast to the overwhelming incidence of coupled pairs in the rat.

When Lucifer Yellow CH was injected into cortical neurons 10 to 18 days of age (Fig. 1, B and C), the incidence of dye coupling was slightly greater than in the adult (28 to 40%). Coupled aggregates still consisted primarily of pairs, but a few groups of three to six neurons were encountered. In contrast, neuronal dye coupling in cortices from 1- to 4-day animals was quite common. Figures 2 and 3 illustrate several examples of the staining patterns which were recovered. Many cells appeared to be immature forms of pyramidal neurons (Fig. 2, A and B; Fig. 3, A and C), whereas others were more stellate shaped (Fig. 3B). In almost every case, the intensity of staining was great enough to show an overlap of dendritic fields and, in some pyramidal cells, the extension of adjacent apical dendrites into apparent bundles (cf. Ronney et al., 1979). Because dendrites usually coursed near the somata of coupled cells also, it is impossible to state whether the sites of coupling were dendro-dendritic or dendro-somatic. The average size of each neuronal aggregate was also greater in very immature neocortex. Coupled groups of 3 to 10 neurons were often recovered, although pairs of cells were still the most prevalent group size. As with the more mature neocortex, coupled neurons were coextensive in subpial depth (150 to 750 μ m) with uncoupled neurons. However, only coupled cells were encountered at the most superficial depths (50 to 150 μ m).

Figure 4 graphically illustrates the developmental course of the incidence of dye coupling. Because the occurrence of coupling dropped dramatically between 4 and 10 days, data were grouped to illustrate the distribution of cells per coupled aggregate (Fig. 5). Thus, within the 10 days following birth, both the percentage of cells coupled and the number of cells per coupled group decreased.

As in the adult guinea pig cortex (Gutnick and Prince, 1981), coupled rat neurons of all ages were often arranged in vertical arrays (Fig. 2, A and B; Fig. 3A). Along the radial axis intersomatic distances ranged from less than one cell radius (i.e., overlapping) up to 200 to 300 μ m, whereas the lateral spread of cell bodies in a group rarely exceeded 50 μ m and was usually less. There was no consistent change in either the radial or lateral extent of cell aggregates during development.

Because estimates of the amount of injected dye (calculated from the current-time product) were variable between injections, we were concerned that the degree

Figure 1. Lucifer Yellow-stained neocortical neurons. *A*, Single pyramidal neuron of adult cortex. *B*, Pair of dye-coupled neurons from deep layers of 10-day-old cortex. *C*, Single spindle-shaped nonpyramidal cell from 10-day cortex. Calibration bars = 50 μ m.

of dye coupling might be a function of the quantity of dye injected. However, there was no correlation between the estimated amount of dye injected and the number of coupled cells recovered when data from a given age (and thus from cells of similar volumes) were pooled. When control ejections of dye were made extracellularly at all ages, there was usually no staining recovered after processing the tissue. Occasionally a single faintly stained neuron could be discerned, but this was not age dependent.

Electrophysiological evidence for coupling. The most direct test for the presence of electrical coupling between cells is the demonstration that passage of current into one cell appropriately alters the membrane potential of the coupled cell by a pathway other than the extracellular space or a chemically mediated synapse (Bennett, 1977). The technical difficulties inherent in simultaneously recording from pairs of immature neocortical cells, together with the low probability of blindly impaling two coupled cells (cf. MacVicar and Dudek, 1981; Knowles and Schwartzkroin, 1981), prompted us to concentrate our efforts on a less direct test. This entailed antidromically

activating a group of neurons while recording intracellularly from a single neuron. With chemical synaptic activity suppressed, action potentials should, given sufficiently high electrical coupling coefficients, be propagated electrotonically from coupled neighbors to the recorded cell (Bennett, 1977).

Although intracellular recordings were obtained from 1-day cortical neurons, most of the data reported here were made from 4-day neurons because of their greater stability. Using potassium acetate electrodes, well impaled neurons in normal medium had membrane potentials comparable to those of adult neocortical neurons *in vitro* (Connors et al., 1982) and input resistances ranging above 100 megohms. Current injections revealed a prominent delayed rectification, and high threshold action potentials could be evoked (Fig. 6*A*). Stimulation in the deeper layers usually elicited long lasting (100- to 200-msec) EPSPs (Fig. 6*B*), which proved to be very sensitive to repetitive activation, i.e., marked attenuation occurred at stimulation rates greater than 0.5 Hz. In many cells an apparent antidromic spike could be evoked, although it usually failed to invade the soma completely (Fig. 6*B*).

Figure 2. A, Dye coupling between three neurons of 1-day cortex. The apical dendrites of each cell intertwined as they headed toward the pia (top left). Photograph is a composite of two planes of focus. B, Neurons of 4-day cortex. In this example seven neurons were stained from a single dye injection. Most cells are out of the plane of focus. Calibration bars = 50 μ m.

When polarized with current passed through the recording electrode, the antidromic spike could be blocked and the EPSP amplitude grew with hyperpolarization and diminished with depolarization, although reversals could not be demonstrated under these conditions.

When slices were bathed in a medium containing 1 mM Ca^{2+} plus 3 mM Mn^{2+} for 30 min or more, long duration chemically mediated EPSPs were abolished, as noted previously in adult guinea pig cortex (Gutnick and Prince, 1981). Neurons were still readily impaled, and in 12 of 15 neurons of 4-day-old cortex it was possible to elicit subthreshold depolarizing potentials antidromically (Fig. 7A). In most cells these consisted of one to three separate all-or-none components, each with a discrete threshold. The neuron illustrated in Figure 7A is unusual in having five to six components, with varying latencies. The duration of the antidromic events was shorter than the EPSPs in normal medium and typically lasted 20 to 60 msec. Unlike chemically mediated synaptic potentials, these events would withstand short periods of stimulation at rates up to 20 Hz without attenuation. The discrete and long (1.5- to 4.5-msec) latencies of antidromic potentials indicate that they do not arise from

direct excitation of dendrites or somata. When electrodes were withdrawn from the neurons, extracellular antidromic field potentials consisted of small (≤ 0.5 mV) positive-negative events which lasted 2 to 4 msec. Thus, the intracellularly recorded potentials are not significantly changed when the field potentials are subtracted from them.

When polarized, antidromic potentials were either unaffected or slightly decreased in amplitude (Fig. 7B). When changes did occur, they could not be explained by measurable changes in membrane rectification. It was sometimes possible to abolish one or more components of the responses by hyperpolarization, but in only one cell was the antidromic potential completely blocked. Such blockable components may have been antidromically conducted spikes from the axon of the recorded neuron or electrotonic potentials from neurons so tightly coupled that current injected through the recording electrode was sufficient to block spike invasion of the coupled cell. The relative voltage insensitivity of the remaining portions of the antidromic potentials suggests that they are not chemically mediated synaptic potentials, and it is consistent with the concept that they are electrotoni-

Figure 3. Dye coupling in 4-day cortical neurons. *A*, Large immature pyramidal cell with smaller, less densely stained, pyramidal cell located adjacent to its apical dendrite. *B*, Three dye-coupled nonpyramidal cells located 500 μm from pial surface. *C*, Dye-coupled cells located 225 μm from pia. Both had apical dendrites which extended into layer I; cell on *left* had axon which could be traced to deepest layers. Calibration bars = 50 μm in *A* and *B*; 25 μm in *C*.

cally conducted from neighboring coupled neurons. The long duration of the antidromic potentials relative to full action potentials is consistent with the low pass filter characteristics of electrotonic junctions.

Another indirect test of electrical coupling is the demonstration that the suspected coupling potential cannot be collided with a preceding orthodromic impulse elicited in the impaled neuron (Baker and Llinas, 1971; Gutnick and Prince, 1981; Taylor and Dudek, 1981). Application of this approach in 4-day cortex was hampered by the high thresholds of the immature neurons, which appeared to be even higher when slices were bathed in Mn^{2+} -containing solution. Nevertheless, in two Mn^{2+} -treated neurons it was possible to show that antidromic

potentials persisted even when the interval between the preceding orthodromic spike and the antidromic potential was sufficiently short to have produced collision (i.e., $<2L + R$, where L is the antidromic potential latency and R is the estimated refractory period of the axon, which may be several milliseconds in immature central axons; cf., Foster et al., 1982). In another neuron (Fig. 7C) the antidromic potential had two components with different thresholds and latencies. When preceded by an orthodromic spike the slower, presumably axonal, component could be shown to collide, leaving the faster,

Figure 4. Incidence of dye coupling as a function of age. The ordinate is the percentage of successful Lucifer Yellow injections which yielded more than one stained neuron. Numbers in parentheses stand for the number of successful dye injections for each point.

Figure 5. Frequency histograms of the number of stained neurons per successful dye injection. Data have been grouped from cortices 1 to 4 days of age (left) and those 10 days or older (right).

Figure 6. Intracellular recordings from 4-day neuron. A, Superimposed voltage (upper) and current (lower) traces during intracellular current injection. Input resistance in the hyperpolarizing direction was about 100 megohms. Largest current pulse in the depolarizing direction evoked an action potential. B, Focal stimulation to deep cortical layers evoked long EPSPs (different cell). Superimposed traces obtained with increasing stimulus intensity. Highest intensity evoked a short latency all-or-none antidromic spike.

Figure 7. Mn^{2+} -resistant antidromically evoked potentials in 4-day cortical neurons (three different neurons). *A*, Stimulus applied to deep layers, increasing intensity. Five to six discrete depolarizing events were evoked at relatively high thresholds. *B*, When antidromic potentials were superimposed on hyperpolarizing current pulses, they showed diminished amplitude despite constancy of membrane conductance. *C*, Partial collision of antidromic potentials. Initial orthodromic potential was evoked with a short intracellular current pulse, and antidromic potentials were evoked at progressively shorter interstimulus intervals (double vertical lines are stimulus artifacts). At longer intervals there were two discrete components to the potential; at the short interval the second component was blocked (arrow), leaving the short latency component intact.

presumably coupling, component intact (Fig. 7*C*, arrow). In two other neurons most or all of the antidromic potentials could be collided and thus may have represented partial invasion of axonal spikes from the same cell. Alternative interpretations of both the positive and negative results are possible (Taylor and Dudek, 1982). For example, if electrical coupling ratios are high, the orthodromic spike in a collision test might occlude the coupling potentials because it would trigger spikes in the coupled neurons. Conversely, low coupling ratios would result in antidromic coupling potentials too small to detect. A low safety factor for antidromic propagation would also preclude the electrotonic propagation of these potentials into neighboring neurons.

Of 15 recordings from adult neurons bathed in Mn^{2+} -containing solution, six showed no response to intense

stimulation of deeper layers, whereas in eight it was possible to elicit an antidromic action potential which fully or partially invaded the site of recording. Antidromic spikes had single discrete thresholds of stimulus intensity and could be blocked by hyperpolarization or collision with an orthodromic spike. In one neuron antidromic stimulation generated two subthreshold components, both of which were eliminated in an all-or-none fashion when the membrane was hyperpolarized 10 to 20 mV from resting potential. These may have been antidromically conducted spikes from two axon collaterals of the recorded neuron.

Discussion

We have presented two types of evidence for a relatively high incidence of electrical coupling among immature rat neocortical neurons: the large percentage of Lucifer Yellow-injected cells which are dye coupled, and the frequent observations of Mn^{2+} -resistant, antidromically evoked subthreshold depolarizations. The results of similar experiments in more mature rat neocortex stand in stark contrast. The incidence of dye coupling was very much lower, and there were virtually no clear examples of possible coupling potentials when chemical synapses were suppressed. The latter finding is somewhat surprising, considering the significant degree of dye coupling in adults, but may indicate that whatever neuronal coupling does remain in the mature rat cortex is either electrically weak or so strong that the coupled cells effectively function as one. Such a high electrical coupling coefficient would seem unlikely with a dendro- or soma-dendritically located coupling site, but further studies are necessary to clarify this issue. Nevertheless, the developmental trend in both the dye coupling and electrophysiology is quite similar: both indicate a large decrease in neuronal coupling between birth and 10 days.

An important morphological correlate of these data would be the identification of neuronal gap junctions with electron microscopy. Although these have not yet been described for immature rat neocortex, such specializations have been seen in frontal cortex of adult rats (Peters, 1980), as well as auditory (Smith and Moskowitz, 1979) and sensorimotor cortex of adult primates (Sloper, 1972; Sloper and Powell, 1978). On the earlier end of the developmental scale, interdendritic gap junctions have been identified in neocortex of fetal sheep (Mollgard and Moller, 1975) and humans (Mollgard, 1975) and on the ventricularly directed processes of cortical neuroblasts in embryonic mice (Shoukimas and Hinds, 1978). The difficulties involved in anatomically visualizing gap junctions among a complex neuropil make it very possible that their apparent absence in the developing rat is simply a matter of experimental neglect. Alternatively, functional coupling can sometimes persist in cellular systems devoid of organized gap junctions (e.g., Williams and De Haan, 1981), presumably because the intermembranous channels exist at a low spatial density.

The detection of coupled cells by the spread of a dye molecule depends critically upon several factors (cf. Bennett et al., 1978). The amount of dye injected, the junctional and nonjunctional dye permeabilities, the rate of dye destruction, the volumes of the coupled cells, and the incubation time preceding fixation will help to determine

whether the fluorescence level in all cells of a coupled aggregate appear above the detection level. If these factors were held constant, one could expect to have a reliable indicator of the relative degree of coupling between different cell populations. However, in a developing system such constancy is unlikely. Nevertheless, several considerations lead us to believe that our observed degrees of dye coupling are valid.

Stewart (1978) has demonstrated that the cell membrane permeability of Lucifer Yellow is extremely low, allowing dye to leak from cells only over periods of many hours to days. Cytoplasmic diffusion, however, seems to be relatively unimpeded, and dye passage between cells known to be electrically coupled by gap junctions occurs rapidly (i.e., over minutes) (Spray et al., 1979; Schuetze and Goodenough, 1982). These properties are not likely to change significantly during cortical development. Of more concern, however, is the large change in neuronal volume which occurs postnatally (Miller, 1981). If the amount of dye per injection was kept constant, its cytoplasmic dilution would be increased in large, mature cells, allowing the possibility that coupled cells would be less detectable. In the ideal steady state experiment, the amount of dye per unit volume of coupled cytoplasm would be kept relatively constant, but uncertainties regarding cell volumes (soma + dendrites) and the actual amount of dye delivered at each intracellular injection make such a controlled experiment unfeasible. However, the staining intensity of coupled neurons was often quite uniform, suggesting that chemical coupling coefficients (as distinct from electrical coupling coefficients) were quite high under the conditions of long equilibration times. This was especially true of older neurons (i.e., >10 days), where more dye could be injected and the number of cells per aggregate were generally smaller. Data also indicated that within a given age, and thus among neurons of similar volume, there was no correlation between the amount of injected dye and the number of coupled cells.

The developmental sequence of electrical coupling we have described may faithfully represent the situation which obtains for the neocortex *in situ*; alternatively, the procedure of slicing and incubating the tissue may induce the formation of cytoplasmic bridging or even *de novo* synthesis of gap junctions. Although our data do not address this issue, dye injections into hippocampal pyramidal cells yielded similar incidences of coupling *in vivo* and *in vitro* (MacVicar and Dudek, 1980; MacVicar et al., 1982). The possibility remains that immature tissue is more labile in its response to the trauma of the isolation process.

A related, and less soluble, question is whether the injection electrode itself induced the coupling by damaging and fusing closely apposed dendrites and/or somata (cf., Kaneko et al., 1981). This seems improbable because the density of dendritic and axonal arborizations is substantially lower in immature cortex than in that of adults (Eayrs and Goodhead, 1959), whereas the incidence and number of neurons per dye-coupled aggregate decreased dramatically with age. Moreover, most of the coupled cells had well separated somata, making it unlikely that

the electrode was able to slip unobserved from one neuron to another during the injection of dye or during electrode withdrawal (cf., Andrew et al., 1982). Some of the neurons within a single dye-filled aggregate also displayed distinct variations in fluorescence intensity. This would be consistent with the limited rate of dye diffusion one might expect between weakly coupled cells, rather than cells with syncytially fused membranes. Nevertheless, the possibility that neocortical dye coupling is an experimental artifact still remains. In hippocampal CA1 pyramidal cells, discrepancies between dye coupling and electrophysiological data have led Knowles et al. (1982) to conclude that at least some of the multiple-cell stains in that area are spurious. In the neocortex, however, alternative explanations would have to account for the wide variations in dye coupling which occur during stages of maturity, between different architectonic areas (M. J. Gutnick, personal communication), and between species (cf., this study and that of Gutnick and Prince, 1981).

The roles played by intercellular coupling in development are still largely speculative. The aqueous channels assumed to form the connection can easily pass small inorganic ions; this property is exploited by a variety of neuronal systems to produce functional electrical synapses (Bennett, 1977). Yet channel permeability extends to much larger molecules (Finbow and Pitts, 1981; Schwarzmann et al., 1981) and the intriguing correlative evidence in developing systems suggests that gap junctions serve as a pathway for organic or inorganic informational substances (for references see Bennett et al., 1981). Our evidence for the importance of cellular coupling in the developing neocortex is similarly correlative. At birth, rat neocortex contains only a very few ultrastructurally defined chemical synapses, but the absolute number increases many-fold between postnatal days 10 and 30 (Eayrs and Goodhead, 1959; Aghajanian and Bloom, 1967; Armstrong-James and Johnson, 1970; Wolff, 1978). In rat visual cortex, lateral geniculate afferents begin to form synapses between postnatal days 6 and 8 (Lund and Mustari, 1977). Our data show that dye coupling is most prevalent at 1 to 4 days and falls to nearly adult levels by about 10 days. Thus, loss of neuronal coupling may coincide with, or slightly precede, the explosive synaptogenesis of this formative period. The electrical recordings obtained when chemical synapses were suppressed suggest that the immature coupling coefficients may be high enough to effect significant electrical communication. This might partially substitute for the weakness of intrinsic chemical synaptic connectivity in the rat neonate and may have a role in the synchronization of neonatal cortical discharges (cf., Crain, 1952; Armstrong-James and Williams, 1963; Crain and Bornstein, 1974). Inasmuch as the large decrease in coupling also occurs before most extrinsic cortical connections have formed (Lund and Mustari, 1977; Wise and Jones, 1978), it is unlikely that coupling in very immature cortex is behaviorally significant. Rather, the coupling may have a role in the formation of neuronal connectivity by facilitating some developmentally crucial signal, either electrical or chemical.

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