REVIEW ARTICLE

CANADIAN ASSOCIATION OF NEUROSCIENCES REVIEW: Postnatal Development of the Mammalian Neocortex: Role of Activity Revisited

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ABSTRACT: The mammalian neocortex is the largest structure in the brain, and plays a key role in brain function. A critical period for the development of the neocortex is the early postnatal life, when the majority of synapses are formed and when much of synaptic remodeling takes place. Early studies suggest that initial synaptic connections lack precision, and this rudimentary wiring pattern is refined by experience-related activity through selective elimination and consolidation. This view has been challenged by recent studies revealing the presence of a relatively precise pattern of connections before the onset of sensory experience. The recent data support a model in which specificity of neuronal connections is largely determined by genetic factors. Spontaneous activity is required for the formation of neural circuits, but whether it plays an instructive role is still controversial. Neurotransmitters including acetylcholine, serotonin, and γ-Aminobutyric acid (GABA) may have key roles in the regulation of spontaneous activity, and in the maturation of synapses in the developing brain.

RÉSUMÉ: Développement postnatal du néocortex chez les mammifères: révision du rôle de l'activité. Le néocortex des mammifères est la plus grosse structure du cerveau et il joue un rôle stratégique dans la fonction cérébrale. La période postnatale est critique pour son développement. La majorité des synapses se forment à ce moment-là ainsi qu'une grande partie du remodelage synaptique. Plusieurs études ont suggéré que les connections synaptiques initiales manquent de précision et que ce « câblage » rudimentaire du cerveau est raffiné par l'activité reliée à l'expérience, par élimination et consolidation sélective. Cette notion a été remise en question parce que des études récentes ont démontré la présence de motifs relativement précis de connections avant le début de l'expérience sensorielle. Les données récentes sont en faveur d'un modèle où la spécificité des connections neuronales est déterminée en grande partie par des facteurs génétiques. L'activité spontanée est requise pour la formation de circuits neuraux, mais son rôle informatif demeure controversé. Des neurotransmetteurs comme l'acétylcholine, la sérotonine et l'acide g-aminobutyrique (GABA) pourraient jouer un rôle important dans la régulation de l'activité spontanée et dans la maturation des synapses du cerveau en développement.

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The neocortex is the largest structure in the mammalian brain, occuping about two-thirds of the total brain volume. A pinnacle of vertebrate evolution, the neocortex plays central roles in many functions including perception, decision making, learning and memory. The extraordinary computational power of the neocortex is only matched by its structural complexity. The human neocortex, for example, has roughly 20 billion neurons that are interconnected in highly specific ways, through tens of trillions of synapses. A major challenge in neurobiology is to understand how such a neuronal network is formed during development. Two broad mechanisms—activity-independent and activity-dependent—are thought to govern the process of brain development.[1] The early stages of neural development, including pattern formation, neurogenesis, and migration, are largely controlled by genetic programs and require little activity. Neuronal activity on the other hand, plays a key role in the formation, refinement, and consolidation of neuronal connections. In mammals, the neocortex is very immature at birth, and the majority of synapses are formed during early postnatal life. These newly formed synapses are highly unstable,

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and undergo extensive remodeling during development. A large number of studies, pioneered by Hubel and Wiesel[2-4] in the visual cortex, have shown that experience during a critical period in early life plays a key role in the refinement of neuronal circuits. While experience-dependent mechanisms remain the key subject of the field, recent evidence increasingly suggests an important role for spontaneous activities in brain development. In this article, I begin with a brief overview of the postnatal development of the neocortex, then I discuss recent findings on the role of neuronal activity in the maturation of neuronal circuits in the mammalian brain, in particular the neocortex. Much of the discussion is based on studies in non-primates; the development of primate and human brains has been the subject of excellent recent reviews.[5,6]

Structural and functional maturation of the neocortex

The neocortex is a laminated structure that can be generally divided into six layers.[7,8] In adult, layer I contains few cells and is made up mostly of apical dendrites of pyramidal neurons of layer II/III and layer V. Layer II/III is populated by small pyramidal neurons that provide both short- and long-range projections to other parts of the neocortex. With the exception of the motor cortex, layer IV is densely populated by spiny stellate cells that are the targets of thalamocortical projections. The primary output of the neocortex is provided by large pyramidal neurons in layer V which project to many subcortical structures including the striatum, the brain stem, and the spinal cord. Layer VI can be further divided into layer VIa (upper part), and layer VIb (lower part). Layer VIa contains medium size pyramidal neurons that provide reciprocal projections to the thalamus, whereas layer VIb contains heterogeneous populations of pyramidal neurons. Besides the excitatory neurons described above, inhibitory GABAergic interneurons, which represent between 10 to 20% of the total neuronal population, are distributed throughout the cortex.

The formation of cortical layers follows an inside-outside pattern.[9,10] Layer I and layer VI are formed first, followed by layer V and layer IV, and finally layer II/III. The neocortex at birth is still poorly differentiated. In rats, only layer I and layer VI can be clearly identified, and many neurons are still in the process of migration. The migration of neurons is accomplished rapidly in the rat brain, so that by the end of the first week, all cortical layers are formed.[11,12] In primates, including humans, the formation of cortical layers occurs before birth. Based on this and several other criteria, it has been suggested that rodent brains are not as advanced as those of primates at birth.[13] In other words, the early postnatal life in rodents would correspond to the perinatal period in primates with regards to brain development.

The end of neuronal migration marks the beginning of a period of exuberant growth in the neocortex. Once reaching their destinations, neurons expand rapidly at soma and dendrites. A well-known example is layer V pyramidal neurons in the rat. Between the middle of the first week to the end of the third week, the surface of soma doubles, while the length of the apical dendrite increases by 5 fold.[14-16] The basal dendrites show an even larger increase: the total length increases by about 10 fold during the same period, and much of the increase can be attributed to the addition of new dendritic branches. This period of rapid development ends around P21 (21 days after birth) in rats; neurons grow at a much slower pace afterward. Some areas of the cortex, such as the prefrontal cortex, even show significant regression.[11,17]

In parallel with morphological development, the functional properties of neurons change dramatically during early life. Between P3 and P21 in rats, the input resistance of layer V pyramidal neurons decreases almost 20 fold—a result of both neuronal growth and an increase in ion channel expression in cell membrane.[14,15,18] During the same period, action potentials (APs) change in several ways. The peak amplitude of AP increases by 30 mV, while the duration decreases by 3 fold. An important implication of this change, is that compared with mature neurons, each AP results in a much larger entry of Ca^{2+} in immature neurons. Together, available evidence suggests that compared with adult, neurons in neonatal brains are more likely to fire spontaneously at low frequency, and this spontaneous firing can trigger large rises in intracellular [Ca^{2+}].

The rapid growth of dendrites during early life is also accompanied by a surge of the number of synapses. In the rat somatosensory cortex, the density of synapse stays low during the first week after birth, but increases by 4 fold between P10 and P18 to reach the adult level.[19] This increase in synaptic density is correlated with a rise in synaptic responses. In the rat prefrontal cortex for example, evoked excitatory synaptic responses increase by about 10 fold between P7 and P18.[15] Clearly, this increase in evoked response is the combined result of both an increase in the number of synapses and an increase in the strength of individual synapses. The initial surge of synapse number is followed by a period of refinement during which specific sets of synapses are reinforced, whereas others are weakened and eliminated.

Role of activity in the formation of neuronal circuits: instructive or permissive

The idea that activity plays an instructive role in the formation of neuronal circuits comes from the pioneering studies of Hubel, Wiesel, and their colleagues[2-4] in the mammalian visual cortex, where they found that closing one eye during a critical period after birth causes the total loss of vision of the deprived eye. Using radioactive proline as a transneuronal tracer, they showed that in layer IV of the visual cortex, axonal terminal fields corresponding to the deprived eye shrink dramatically, whereas those corresponding to the normal eye expand accordingly.[20-22] In addition, they found that in normal kittens and monkeys, the terminal fields of the two eyes overlap extensively in early life, and segregate gradually into eye-specific columns (ocular dominance columns) during development. These findings provided the foundation for the dominant theory that early life experience during a critical period refines synaptic connections in the immature brain by selectively strengthening appropriate synapses while eliminating others.

Several lines of evidence challenged the view about an instructive role of experience in the formation of eye-specific columns. In the visual cortex of monkeys, the segregation of the afferents from the dorsal lateral geniculate nucleus (dLGN) was found to be already present in the visual cortex in uterus, long before any visual experience.[23,24] (Figure 1) Indeed, both the anatomy of ocular dominance columns and their physiological function were well established at birth—much earlier than the

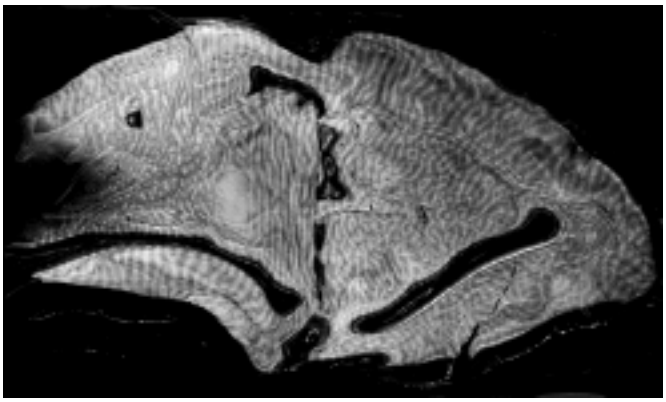

Figure 1: *Segregation of ocular dominance columns occurs before birth in the macaque monkey. Autoradiography of a flattened section through the primary visual cortex of a dark-reared newborn monkey injected with radioactive proline in one eye. The adult-like ocular dominance columns can be clearly identified by the presence of alternating dark and light strips. Reproduced with permission from Reference 24, copyright 1996 Society for Neurosciences.*

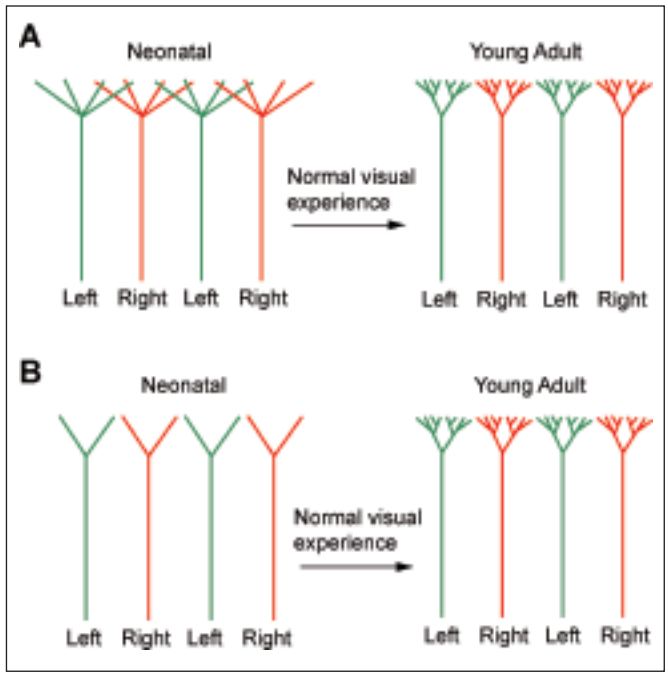

Figure 2: *Two distinct models for the development of ocular dominance columns in the visual cortex. In the classic model (A), the initial pattern of innervation lacks precision, which results in extensive overlap between the left and the right eye's inputs. Eye-specific segregations are achieved through a process of synaptic reorganization, where visual experience has an instructive role. B illustrates a different model where eye-specific segregations are already present before visual experience, but these synapses are highly plastic. The primary role of normal visual experience is to consolidate the existing connections, whereas abnormal visual experience during the critical period can alter the pattern of connections through activity-dependent mechanisms.*

beginning of the critical period determined by the experiments using monocular visual deprivation.[25]

This distinction between the formation of ocular dominance columns and the critical period has been confirmed in ferrets and cats. Using injections of anterograde traces in the dLGN of ferrets, Crowley & Katz[26] have shown that ocular dominance segregation occurs shortly after the arrival of dLGN axons in layer IV of the visual cortex, and precedes the onset of the critical period by almost two weeks. Using improved transneuronal tracing methods and optical imaging of neuronal activity, Crair et al[27,28] have shown that ocular dominance segregation occurs in cats at P14, prior to the onset of the critical period at P21. The discrepancy between these and earlier results can be explained, in part, by the fact that the transneuronal tracers (radioactive amino acids) used in early studies tend to spill over into adjacent, inappropriate dLGN layers in very young animals.

In contrast to the original view that visual experience drives the segregation of dLGN axons, it is now clear that ocular dominance columns are established before the onset of visual experience and the critical period.[29] The recent data proposes a new model where ocular dominance columns are formed through rapid and precise elaboration of axon arbors, in the absence of visual activity.[30] (Figure 2) These newly formed cortical circuits are highly plastic, and can be modified extensively by experience during the critical period. Thus, although not involved in the initial formation of ocular dominance columns, visual experience plays a key role in determining neural connectivity in young adults through synaptic remodeling.

Prior to experience-driven activity, neurons in the developing brain are spontaneously active; many studies have shown that this spontaneous activity is required for the development of neuronal circuits in the brain. Whether spontaneous activity plays an instructive role is still a matter of intense debate. The best-studied example is the projection from retinal ganglion cells to the dLGN. Early in development, binocular innervations to the dLGN are intermingled extensively, before segregating progressively into eye-specific layers.[23,31] This eye-specific segregation is complete before eye opening, thus excluding a role for visual experience. On the other hand, experiments pioneered by Rakic[32] in monkeys have shown that fetal monocular enucleation prevents the segregation of retinogeniculate axons, which underscores the importance of binocular interactions in this process. What is the nature of this binocular interaction? One of the possibilities was that spontaneous activities in the two eyes mediate competitive, binocular interactions in the fetal brain. In support of this idea, Shatz and Stryker[33] found that infusion of tetrodotoxin—a toxin that blocks action potential—into the brain of fetal cats blocked the segregation of retinogeniculate axons. The presence of spontaneous activity in retinal ganglion cells was first demonstrated in the embryonic rat, and was later confirmed in many species including cat, ferret, mouse and rabbit.[34] More interestingly, recordings using multi-electrode array and Ca^{2+} imaging revealed that spontaneous activities in neighboring cells are correlated (Figure 3), and this activity can propagate across the retina in waves.[35,36]

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The finding of retinal waves during early development prompted the idea that correlated spontaneous activities in the eyes are essential for eye-specific segregation of retinogeniculate axons in the dLGN.[37] This hypothesis is supported by four lines of evidence. First, the occurrence of retinal waves coincides with eye-specific segregation in the dLGN.[38] Second, binocular blockade of retinal waves with nicotinic cholinergic antagonists prevents eye-specific segregation.[39] Third, mice lacking functional nicotinic cholinergic receptors in the retina show little eye-specific segregation.[40] Fourth, inducing an imbalance in spontaneous retinal activity between the two eyes results in an expansion of the axon terminal field of the more active eye.[41]

None of these studies, however, have examined specifically the role of patterned activity in eye-specific segregation of retinogeniculate projections, since the manipulations used in these studies also change significantly the overall level of activity in the eye. A recent study has attempted to selectively disrupt correlated activity in the developing retina through immunotoxin depletion of cholinergic amacrine cells in the retina.[42] This treatment abolishes correlated firings of neighboring ganglion cells with little effect on the overall level of activity in the retina. Surprisingly, despite the absence of correlated activity, axons from left and right eyes segregate normally in the dLGN. This finding suggests that correlated activity is not required for eye-specific segregation. It is therefore possible that the formation of the retinotopic map is guided by molecular cues, and spontaneous activities play a permissive, but essential role in the process. It is interesting to note that in the spinal cord of Xenopus, patterns of spontaneous activity regulate the neurotransmitter phenotype,[43,44] and that normal patterns of spontaneous activity is required for proper axon pathfinding and the expression of guidance molecules such as EphA4 (ephrin receptor A4) and polysialic acid on NCAM (neural cell adhesion molecule) in the spinal cord of the chick embryo.[45]

Much less is known about the role of spontaneous activity in the developing neocortex. Ca^{2+}-imaging studies in brain slices have revealed the presence of distinct domains of spontaneous active neurons in the cortex of neonatal rats.[46,47] These slowly propagating calcium waves persist in the presence of tetrodotoxin, but are blocked by gap junction blockers. An obvious implication is that gap junctions may play a key role in intercellular communications in the neonatal cortex, but a role for Ca^{2+}-dependent action potentials cannot be excluded. So far, it is not clear whether such domains also exist in vivo, let along their role in the development of the neocortex. Based on studies in other structures, it is tempting to suggest that spontaneous Ca^{2+} waves have an essential role in the differentiation of neurons and the formation of synapses in the neocortex.

Regulation of spontaneous activity in the developing brain

Regardless its role in circuitry formation, spontaneous activity appears to be a general feature of the developing brain. An important question is how such spontaneous activity is regulated. As discussed above, immature neurons have much higher input resistance, which makes them more responsive to fluctuations in membrane conductance. These fluctuations may have different origins, including fast synaptic transmission and slow neuromodulatory effects. With the notable exception of the retina, however, little is known about the mechanisms involved. A key question regarding waves of spontaneous activities is how does a wave start. Neurons in the brain fire spontaneously at low frequency, but activities in a single cell are unlikely to be sufficient for wave generation—correlated firings among a small group of neighboring cells are required. This could happen by chance, but the probability is very low. A more efficient way is to have a mechanism that synchronizes the activities among a group of neurons. This is indeed the case in the retina where each cholinergic amacrine cell provides excitatory inputs to many retinal ganglion cells. Blocking nicotinic acetylcholine receptors

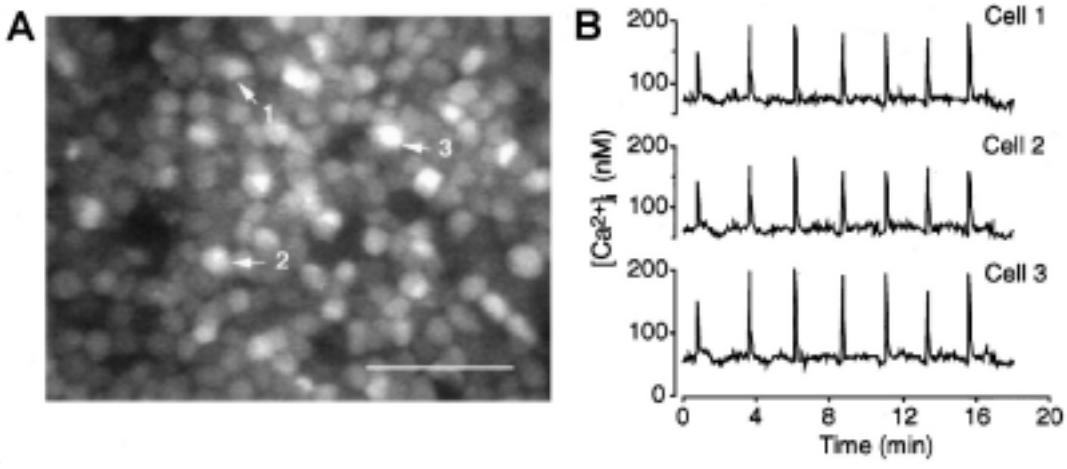

Figure 3: *Correlated spontaneous activity in the embryonic retina. A: a field of cells in the ganglion cell layer of an E16 chick retina that has been loaded with Ca^{2+} indicator Fura-2. B: Spontaneous $[Ca^{2+}]i$ rises from three cells indicated with arrows in A. Activities in these cells are periodic and highly synchronized. Reproduced with permission from Reference 36, copyright 1998 Society for Neurosciences.*

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abolishes correlated firing in the developing retina, as does immunotoxin depletion of cholinergic amacrine cells.[42,48] In addition to acetylcholine, retinal waves are also enhanced by endogenous adenosine through an up-regulation of cAMP, but it is not clear how this action of adenosine is regulated in vivo.[49]

The neocortex receives widespread cholinergic innervation from the nucleus basalis of Meynert located in the basal forebrain.[50,51] This cholinergic innervation is already present at the end of first week after birth in rats, with characteristics very similar to those observed in adults.[52] Both nicotinic and muscarinic acetylcholine receptors are expressed in large quantities in the neocortex during early life.[53,54] Although either type of receptors can mediate excitatory synaptic transmission, only muscarinic receptors appear to be involved in acetylcholine-induced excitatory responses in pyramidal neurons of the developing cortex. In brain slices obtained from neonatal rats, applications of muscarinic agonists result in recurrent calcium waves that propagate slowly to a large number of neurons in the cortex.[55] Whether this mechanism is implicated in vivo is still unknown.

The role of serotonin

A neurotransmitter that has received increasing attention is serotonin (5-hydroxytryptamine, 5-HT). The mammalian brain is widely innervated by 5-HT axons from the raphe nuclei in the brain stem. 5-hydroxytryptamine axons interact with other neurons in complex ways through at least 14 different types of receptors.[56] Not surprisingly, 5-HT is implicated in a wide range of physiological functions such as appetite, sleep, reproduction, mood, learning and memory. It has been shown that brain 5-HT systems appear very early in development.[57,58] Clusters of 5-HT neurons are present in the hindbrain of rats as early as E13, and in humans, at five weeks gestation. 5-hydroxytryptamine projections begin soon after, reaching the cortical plate in rats by E18. This 5-HT innervation intensifies after birth, and reaches the highest level toward the end of the second week in rats.[59] Direct measurements of 5-HT content reveal transient increases of brain 5-HT level during early life. In mice, the 5-HT concentration in the neocortex rises to more than twice the adult level during the first week after birth.[60] In humans, the level of 5-HT in the brain increases during the first two years and then declines to the adult level after the age of five.[61,62] These transient increases of 5-HT innervation and synthesis coincide with up-regulation of receptor expression and function. For example, the expression of 5-HT_{2A}, a major type of 5-HT receptors, increases to twice the adult level in the rat neocortex during the first two weeks after birth.[63,64] When measured by agonist-induced inositol phosphate production, activities of 5-HT_2 receptors in the neonatal cortex are ten-fold higher than in adults.[65] Thus, 5-HT signaling is at its highest during critical stages of postnatal development, and this transient up-regulation appears to be evolutionally conserved.

The early development of a 5-HT system and the transient up-regulation of 5-HT signaling suggests a role for 5-HT in brain development.[66,67] Consistent with this idea, pharmacological manipulations of 5-HT systems in vivo and in vitro produced a variety of effects on the development of neurons and synapses.[68] The strongest support has been provided by recent studies using genetically modified mice. Mice pups with a deletion in the gene

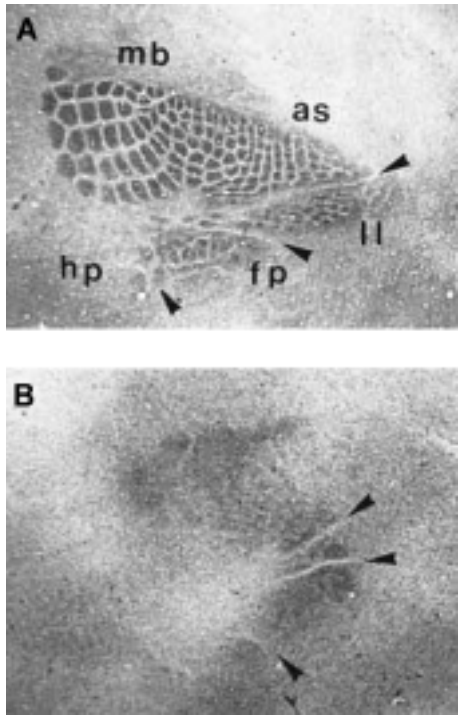

Figure 4: *The lack of cortical somatosensory map in mice deficient in monoamine oxidase A (MAOA). A: normal pattern of the somatosensory map in the cortex of a wildtype mouse stained for cytochrome oxidase activity. The different regions of the somatosensory representation are: the large mystacial vibrissae (mb), the anterior snout (as), the lower lip (ll), the forepaw (fp), and the hindpaw (hp). B: altered patterning in the somatosensory cortex of a MAOA-deficient mouse. The normal pattern can be restored in these mice by neonatal administration of inhibitors of 5-HT synthesis. Reproduced with permission from Reference 70, copyright 1996 Elsevier.*

encoding monoamine oxidase A (MAOA), an enzyme that degrades serotonin and norepinephrine, show a nine-fold increase of brain 5-HT.[69,70] The mutant mice have severe defect in the somatosensory cortex, including a total lack of somatotopic projections of thalamocortical axons (Figure 4); and the effect can be reversed by lowering brain 5-HT levels in the mutant pups with inhibitors of 5-HT synthesis.[70] For reasons not completely clear, the increase of 5-HT in MAOA mutant mice is confined to early development, and by seven months, there is no longer any difference in brain 5-HT level between wild-type and mutant mice. However, adult mutant mice show distinct behaviors, including much enhanced aggression in males, thus suggesting long-lasting consequences of 5-HT-related developmental defects.[69] Interestingly, deficiency in MAOA has been associated with aggressive behavior in men of a Dutch family.[71]

Several other lines of transgenic mice have provided further support. Mice deficient in 5-HT_{1A} receptor have high levels of anxiety-like behavior in adults, but it was unclear whether the behavioral change is caused by the lack of 5-HT_{1A}-mediated signaling in development.[72-74] Using an inducible and

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conditional knock-out mouse line, Gross et al[75] have shown that the lack of 5-HT_{1A} receptors in the forebrain during development is responsible for the behavioral change observed in adults. Deficiency in 5-HT transporter—a key regulator of 5-HT transmission and the target of many antidepressants such as Prozac—has been associated with high levels of anxiety and depressive syndrome in humans. Interestingly, mice lacking 5-HT transporter show abnormal emotional behavior, and this effect is mimicked by transient inhibition of 5-HT transporter with Prozac during early development.[76] These findings underscore the role of 5-HT in the development of emotional and cognitive behaviors.

How 5-HT influences brain development remains largely unknown. The effects of 5-HT in the brain are mediated predominantly by G-protein coupled receptors that are known to regulate the activity of protein kinases including, PKA (protein kinase A), PKC (protein kinase C), and MAPK (mitogen-activated protein kinase). Thus, 5-HT may directly regulate neuronal growth and differentiation through intracellular signaling pathways. Indeed, this has been shown in many studies using neural cell and explant cultures.[68] However, the action of 5-HT in vivo is clearly more complex, with multiple interactions between activity-dependent and –independent mechanisms.

Several studies suggest a role for 5-HT in synaptic plasticity. 5-Hydroxytryptamine, presumably via 5-HT_{2C} receptors, has been shown to promote long-term potentiation (LTP) or long-term depression (LTD) in the visual cortex of kittens; and blocking 5-HT_2 receptors reduces ocular dominance plasticity.[77,78] 5-Hydroxytryptamine is also involved in the plasticity of the rat visual cortex, but with a different role. Endogenous 5-HT, via 5-HT_{1A} and 5-HT_{2C} receptors, is responsible for the developmental decline of LTP in the rat visual cortex.[79] The reason for the discrepancy is unknown. In the somatosensory cortex, the defect in thalamocortical projection in MAOA mutant mice involves 5-HT_{1B} receptors, which are highly expressed at terminals of these axons during early development.[80,81] This latter observation suggests a role for 5-HT-mediated presynaptic regulation of synaptic transmission during development, but whether LTP or LTD is involved remains unclear.

5-Hydroxytryptamine may be a key regulator of spontaneous activity in the developing neocortex. As mentioned above, the neocortex has high levels of 5-HT and its receptors during early development. Recently, 5-HT has been shown to strongly promote neuronal activity in the prefrontal cortex during a critical period of development.[82,83] The prefrontal cortex shows largest expansion in mammalian evolution; in humans, it counts for 30% of the neocortex, and occupies a large area in the frontal lobe. This area of the brain is known to have a key role in cognitive functions;[84,85] and developmental defects in the prefrontal cortex are thought to be involved in schizophrenia and other mental disorders.[86-89] In the rat, the critical period of development for the prefrontal cortex is the first two weeks after birth, where the largest changes in neurons and synapses take place.[15] Interestingly, 5-HT produces strong excitatory responses during this period.[82,83] The excitatory effect by 5-HT is at its highest between P10 and P14, and declines rapidly afterward.[82] Pharmacological analysis suggests that the excitatory effects of 5-HT are largely mediated by 5-HT_{2A} receptors, a conclusion that is consistent with results from previous studies on receptor expression and signaling during development.[63-65] This finding raises the possibility that 5-HT, through up-regulation of neuronal activity in the developing cortex, promotes the differentiation of neurons and the formation of synapses. So far, evidence supporting this idea has only been provided by in vitro studies; it is not clear whether and how 5-HT regulates neuronal activity in vivo during development. The recent development of imaging techniques makes it possible to monitor simultaneously in vivo, activities from a large number of neurons in the neocortex. A key remaining obstacle here is that anesthetics commonly used in in vivo studies strongly suppress 5-HT transmission; and the remaining endogenous 5-HT transmission may not be sufficient to produce any significant effect. Semi-chronic or chronic recordings may be required to address this problem.

The role of GABA

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. Upon synaptic release, GABA binds to $GABA_A$ receptors, and opens ion channels permeable to chloride (Cl^-). In adult brain, the [Cl^-] gradient across cell membranes is very large, with 140 mM external and about 7 mM internal. Under such conditions, opening of $GABA_A$ receptor channels results in a net influx of Cl^-, thus causing a hyperpolarization of neurons. Recent studies have revealed, however, an excitatory effect of GABA during early development.[90,91] In the hippocampus of neonatal rats for example, GABAergic transmission induces depolarization and triggers action potentials in young neurons; and this effect is mediated by $GABA_A$ receptors.[92] How does the same synapse produce totally opposite effects at different stages of development? The answer turns out to be a developmental shift in intracellular concentration of Cl^-, $[Cl^-]_i$.[93,94] Compared with adults, $[Cl^-]_i$ in neonatal neurons is 20 to 40 mM higher; under such conditions, opening of $GABA_A$ channels in neurons leads to an outward flow of Cl^-, thus producing a depolarization that is often sufficient to trigger action potentials. In young neurons, $[Cl^-]_i$ is kept elevated by importing Cl^- through the Na^+-K^+-2Cl^- co-transporter (NKCC1); as the animal matures, neurons start to express another transporter, the K^+-Cl^- co-transporter (KCC2), which lowers $[Cl^-]_i$ by exporting Cl^-.[95] In other words, expression of KCC2 in neurons is responsible for the shift from excitation to inhibition of GABA action during development.

A development shift in $[Cl^-]_i$ has been observed in many species including zebrafish, Xenopus, chick, and primates, suggesting it as a general phenomenon, well conserved throughout evolution.[91] The fact that GABA produces depolarization and intracellular Ca^{2+} oscillations in immature neurons raises the possibility that GABA serves as a developmental signal in the brain. But, so far, it is still not clear what its role might be. One hypothesis suggests that the excitatory effect of GABA is required for the formation of neural networks. The rationale here is that GABA-induced depolarization and Ca^{2+} oscillation promote the development of glutamatergic synapses in immature brain; and as excitatory glutamatergic transmission becomes stronger, GABA will gradually shift to an inhibitory mode in order to maintain the homeostasis of neural networks.[91] This elegant hypothesis,

however, has received little direct support, other than the fact that in the hippocampus, GABAergic synapses seem to appear before glutamatergic ones during development.[96]

Using hippocampal cultures, Ganguly et al[97] have shown that the shift from excitation to inhibition requires endogenous GABAergic transmission and activation of $GABA_A$ receptors. Chronic treatment with bicuculline and picrotoxin, two selective antagonists of $GABA_A$ receptors, blocks the shift in GABA action, the expression of KCC2, and the changes in $[Cl^-]_i$. Intriguingly, the developmental change in GABA action seems to be independent of endogenous glutamatergic transmission, since chronic blockade of glutamate receptors has no effect. More surprisingly, chronic blockade of action potentials with tetrodotoxin also does not affect the developmental shift of GABA signaling. Thus, neither the maturation of excitatory glutamatergic transmission, nor the developmental change in network activity, affects the shift in GABA signaling. Instead, the excitatory action of GABA is, by itself, both necessary and sufficient to induce the shift in $[Cl^-]_i$. Therefore, the primary role of GABA in development seems to be self-regulatory—responsible for the maturation of GABA signaling in the brain.[97]

In addition to $GABA_A$-mediated effects discussed above, metabotrophic $GABA_B$ receptors are also involved in GABA signaling during development. $GABA_B$ receptors are present early during development in many parts of the brain.[98] In the rat hippocampus, for example, $GABA_B$ receptors are detected as early as E14.[99] As in the adult brain, the effects of $GABA_B$ signaling are inhibitory during development, with both pre- and postsynaptic actions.[100] Therefore, $GABA_B$ receptors may have an important role in the regulation of network activity during development. Indeed, endogenous $GABA_B$ signaling has been found to inhibit spontaneous retinal waves.[101,102]

Maturation of glutamatergic synapses

The large majority of synapses in the brain are glutamatergic. In the neocortex, for example, glutamatergic synapses account for about 85% of the total synapses. The period immediately after birth is critical for the formation and maturation of glutamatergic synapses. In rat neocortex, much of the increase in synaptic density occurs during the second week after birth;[19,103] in humans, the rapid increase in synaptic density occurs between 30 gestational weeks and two years after birth.[6]

The events that lead to the formation of glutamatergic synapses remain largely unknown. At vertebrate neuromuscular junctions, the contact between the incoming axon and the postsynaptic muscle fiber initiates, through the extracellular matrix protein agrin, a cascade of signaling events that leads to synaptic formation.[104] At central synapses, however, the role of the extracellular matrix is still poorly understood. Instead, attention has turned to membrane proteins that are capable of trans-synaptic signaling. The idea here is that axon terminals express a set of membrane proteins that, upon contact, would interact with partners expressed in the dendrites, thereby initiating bi-directional signaling required for synaptogenesis.[105] Several candidates have been proposed, including cadherin, β-neurexin/neuroligin, SynCAM (synaptic cell-adhesion molecule), and EphrinB/EphB2. So far, much of the evidence comes from in vitro studies using cell culture, and definitive answers from in vivo studies are still missing.

The initial assembly is followed by a period of maturation where synapses grow in size and gain strength, with changes taking place at both pre- and postsynaptic sites. In the rat neocortex, the mean number of synaptic vesicles per synapse increases by over 3-fold during the first month after birth.[103,106] On the other hand, the functional maturation of glutamatergic synapses, as measured with electrophysiology, is largely due to postsynaptic changes. At a mature synapse, glutamate released by the axon terminal binds to α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors at the postsynaptic site. AMPA receptors are ligand-gated ion channels that are permeable to Na^+ and K^+, whereas NMDA receptors are highly permeable to Ca^{2+}, but are blocked by Mg^{2+} at resting membrane potentials. Intriguingly, immature synapses often have only NMDA receptors, without AMPA receptors, which make these synapses non-functional—'silent'—at resting membrane potentials (Figure 5). Discovered first in rat hippocampus, the silent synapse is a well-conserved phenomenon that has been found in many species.[107-109] The fraction of silent synapses decreases progressively with development. In rat somatosensory cortex, 50% of thalamocortical synapses are silent at P3-5, but only about 10% at P9-10.[110] This change is correlated with an increase in the density of postsynaptic AMPA receptors, as well as the morphological maturation of dendritic spines where the majority of glutamatergic synapses are located. Thus, the recruitment of AMPA receptors by silent synapses seems to be a key step in synaptic maturation.

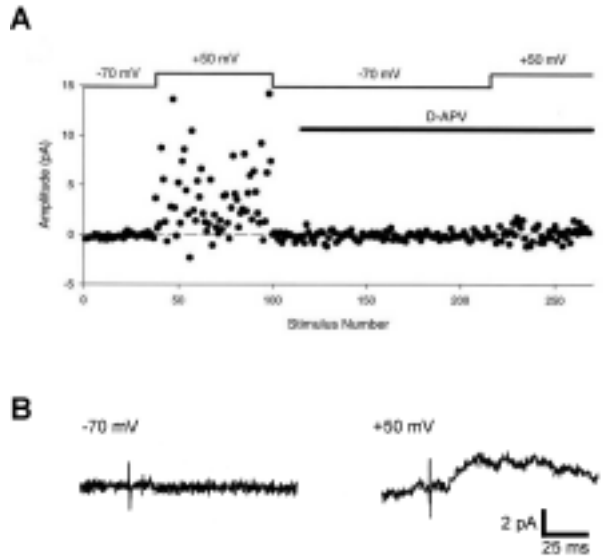

Figure 5: *The presence of silent synapses in the somatosensory cortex of neonatal rats. A: synaptic currents recorded from a layer 4 cell at two different holding potentials, -70 mV and +50 mV. Synaptic responses were detected at +50 mV, but not at -70 mV; the selective NMDA receptor antagonist, D-APV, blocked the synaptic responses at +50 mV. B: traces of synaptic responses at -70 mV (left) and +50 mV (right). Reproduced with permission from Reference 111, copyright 1997 Elsevier.*

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Mechanisms by which AMPA receptors are recruited at the synapse are subjects of intense investigation, with a focus on activity-dependent processes.[111] Repeated pairing of synaptic release with strong postsynaptic depolarization—a condition known to activate NMDA receptors at the synapse—has been shown to convert silent synapses into non-silent ones.[107,108, 110] This effect requires NMDA receptor activation, and seems to involve recruitment of AMPA receptors. In a series of seminal studies, Malinow and colleagues examined the underlying molecular mechanisms using a combination of molecular biology, electrophysiology, and optical imaging.[112-115] Using the AMPA receptor subunit GluR1 tagged with green fluorescence protein (GFP), they showed that GFP-tagged GluR1 subunits are rapidly recruited into dendritic spines upon high frequency stimulation of presynaptic axons, and the effect requires NMDA receptor activation. In addition to this activity-dependent recruitment, the delivery of AMPA receptors can be achieved through activity-independent and subunit-specific mechanisms. Thus, AMPA receptors composed of GluR1 and GluR2 are delivered through activity-dependent processes, whereas those composed of GluR3 and GluR2 do not require activity for their synaptic delivery.[112] It is important to mention that these results have been obtained in tissue cultures with recombinant AMPA receptors that are significantly different from native receptors in structure. Despite these caveats, it is clear that synaptic recruitment of AMPA receptors is a highly regulated process.

Synaptic NMDA receptors also undergo developmental modifications. In the neocortex at birth, NMDA receptors consist predominantly of NMDA receptor subunit 1 (NR1) and subunit 2B (NR2B); with age, NR2A is expressed progressively at the synapse. One consequence of this developmental change in NMDA receptor composition is that the time course of NMDA receptor-mediated synaptic current shortens significantly with age,[116,117] thus resulting in less Ca^{2+} entry during each synaptic event. Because of the essential role of NMDA receptors in brain development and long-term plasticity, it was proposed that the developmental change in NMDA receptor composition controls the process of circuit formation and plasticity.[118] In particular, it was thought that the duration of the critical period is determined by the expression of NR2A. This hypothesis was supported by the observation that either dark rearing, a condition known to extend the critical period, or intraocular tetrodotoxin injection, which blocks activity in the eye, delays the developmental change in NMDA receptors.[116,119-121] A number of studies, however, have challenged this idea. In the ferret visual cortex, the onset rather than the end of binocular plasticity, is correlated with the expression of NR2A.[122] Moreover, NR2A knockout mice show no change in the duration of the critical period in the somatosensory cortex, despite the fact that the maturation of synaptic NMDA response is delayed.[123] These results suggest that the developmental change in NMDA receptor composition is not required for the termination of the critical period; instead, it is one of the consequences of activity-dependent plasticity that occurs during the critical period.

Elimination of synapses

In all mammalian species, the rapid increase in synaptic number is followed by a regression. In humans and nonhuman primates, about 40% of synapses in the brain are eliminated before the end of adolescence.[124-126] This process, termed pruning, occurs without significant loss of neurons. Synapse elimination also occurs outside the brain. For example, each striate muscle fiber at birth receives multiple innervations from motor neurons—all but one synapse is eliminated during postnatal development. These and many other examples suggest pruning as a general process that is essential for the establishment of precise synaptic connections.

Mechanisms involved in synapse elimination have been examined extensively at the vertebrate neuromuscular junction.[127] When action potentials in the nerve are chronically blocked by tetrodotoxin during the normal period of synapse elimination, muscle fibers remain innervated by multiple neurons. On the other hand, when activities of muscle fibers are boosted through direct electrical stimulations of the muscle, synapse elimination is accelerated. Therefore, synaptic elimination at the neuromuscular junction is clearly activity-dependent. How does this process work? One possibility is a competitive mechanism in which the synapse that produces the strongest response in the muscle cell survives, while the others are eliminated. Using co-culture of muscle cells and spinal cord neurons, Lo and Poo[128] showed that a brief high frequency stimulation of one nerve-muscle synapse causes long-term depression of the neighboring synapse formed by another neuron, and the depression is more pronounced at the weaker synapse. This activity-dependent heterosynaptic action provides a mechanism for synapse elimination at the neuromuscular junction. Among the initial synapses formed by several neurons on a muscle cell, one is stronger than the others presumably due to genetic predisposition; under normal condition, this strong synapse expands and grows even stronger, while the others are weakened through heterosynaptic depression, and are eventually eliminated. This hypothesis has been recently examined in vivo by Sanes and colleagues.[129] Using a genetic method to selectively inhibit synaptic transmission from one of the two motor neurons that innervate a muscle cell, they showed that stronger inputs are heavily favored in the process of synapse elimination.

In the cerebellum, the connection between climbing fibers and Purkinje cells also undergoes extensive regression. In neonatal rats and mice, each Purkinje cell is innervated by several climbing fibers, but by P21, most Purkinje cells receive a single climbing fiber.[130,131] When NMDA receptors are blocked chronically between the second and third week, Purkinje cells remain innervated by multiple climbing fibers.[132] This seems to suggest a critical period during which synaptic activation of NMDA receptors are required. However, the climbing fiber-Purkinje cell (CF-PC) synapse has only AMPA receptors, and no NMDA receptors. So far, there is no direct evidence implicating synaptic transmission at the CF-PC synapse in the process of synapse elimination. Thus, whether activity has an instructive role in the regression of the CF-PC synapse remains unknown.

Little is known about the mechanisms involved in the pruning process that takes place in the neocortex. The formation of ocular dominance columns has been long used as a model for the study of activity-dependent synapse elimination in the neocortex. As discussed earlier however, recent studies have questioned the role of synapse elimination in this process. Nevertheless, the fact that the density of cortical synapses decreases substantially

during postnatal development suggests that, like other brain structures, pruning is a key event in the maturation of the neocortex. Unlike the neuromuscular junction and the CF-PC synapse, the process is less dramatic in the cortex: each neuron in adult cortex still receives innervations from many neurons, making it difficult to quantitatively study synapse elimination at the single-cell level. Recent studies combining in vivo multi-photon imaging with mouse genetics provided a solution for this problem. Gan and colleagues[133] used two-photon microscopy to visualize dendritic spines in layer 1 of the somatosensory cortex in transgenic mice expressing yellow fluorescent protein in a subpopulation of pyramidal neurons. By repeated imaging of the same group of dendritic branches over several weeks, they showed that about 15% of spines are eliminated between four to six weeks of age, whereas in adult, the rate of spine elimination is much lower (5% over two weeks). In addition, sensory deprivation through whisker trimming reduces spine elimination; and this effect is more pronounced in young mice. These observations are consistent with the results obtained at the neuromuscular junction, suggesting a role for sensory experience in synapse elimination in the developing cortex. For technical reasons, only mice older than four weeks have been examined in this study. This is somewhat regrettable since the most important phase of synapse elimination probably occurs between two and three weeks in the mouse brain. How sensory experience regulates this early phase of pruning will require further studies.

Conclusions

The role of the environment in brain development has long been the focus of the nature-nurture debate. Although there is a clear consensus that early life experience is critical for the development of the brain, the exact role of experience in the formation of neuronal circuits is still controversial. The model established by early studies in the visual cortex suggests that the initial pattern of synapse formation lacks precision, and through activity-dependent synapse elimination and strengthening, a highly precise neuronal circuit emerges during a critical period of development. Recent studies have shown, however, that relatively precise patterns of synapses are already present in the neocortex before the onset of sensory experience. The recent data suggest that the formation and the plasticity of neuronal circuits should be regarded as two distinct processes. Neurons, guided by molecular cues, connect specifically with each other to form networks that are structurally similar to those present in the adult brain. The newly formed neural networks are then subjected to environmental modifications through activity-dependent mechanisms. Normal experience consolidates existing connections, whereas abnormal experience cause the elimination of some connections and the expansion of others.

In this two-step model, the role of activity is more complex. As in the classic model, experience-related activity plays an instructive role during the plasticity phase through use-dependent mechanisms. On the other hand, activity is also required for the formation phase, although its role here is likely to be a permissive one. Since the formation phase usually takes place before the onset of sensory experience, spontaneous activity has therefore an essential role. A major challenge is to understand how spontaneous activity is regulated in the developing brain. Neurotransmitters such as acetylcholine, 5-HT, and GABA, may have important roles. Although some aspects of this question can be examined in reduced systems such as cell culture and brain slices, the definitive answer can only be obtained in vivo under physiological conditions. The development of high resolution, non-invasive, imaging techniques seems to be a critical step.

The suggestion that molecular cues may play instructive roles in circuitry formation further provides the rationale for the search for patterning molecules. It seems reasonable to speculate that for any given structure, a myriad of molecules are involved in circuitry formation. Although some of the molecules have been identified, our knowledge is still very limited. The rapid progress of molecular biology and genomics will undoubtedly accelerate the pace of discovery in this area. As various key players start to emerge, the next, and perhaps more challenging, question is to understand the complex interactions that constitute various signaling pathways.

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