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Chapter 45

The developing cortex

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INTRODUCTION

There is a general agreement that developing circuits are more readily engaged in seizure manifestations and epilepsies than those in adults. This is validated both in terms of the incidence of seizures and epidemiological studies performed at various developmental stages in a wide range of countries to the extent that epilepsies are considered by some primarily developmental disorders. Yet the agreement appears to stop just there. Why do immature neurons generate seizures? What are the clinical implications? Do seizures lead to long-term sequels and how? Do seizures operate in the developing brain as in the adults or are we dealing with a different event altogether? Is it really justified to use similar therapeutic strategies in children and adults? All these questions are conditioned by our fundamental understanding of the properties of the developing brain. In essence here, perhaps even more than in other domains, the link between basic research on the mechanisms underlying brain development and seizures is all the more instrumental. There have been spectacular advances in developmental neurobiology offering a unique opportunity to develop this field further and correct a number of false assumptions, which has major implications for the above issues; these advances are summarized here. Our message is that early life epilepsies should be examined from a developmental perspective and not from an adult one, taking into account the unique features of the developing brain.

The developing brain is extremely heterogeneous – far more so than in the adult brain – since at any given stage, very immature neurons endowed with little or no synapses can coexist with neurons that already have thousands of synapses. Neurons of the human neocortex divide and become postmitotic in a period extending over 100 days, hence 99-day-old neurons will operate with neurons that formed a couple of hours earlier. Seizures when generated exert very different actions on these neurons. The developing brain operates with signals that even when they are identical to those of adults have a different, sometimes even opposite, action. Thus, seizures are generated by some basic features of immature neurons, which tend to oscillate, and the unique actions of the inhibitory transmitter GABA, which excites immature neurons because of a higher intracellular concentration of chloride. Virtually all voltage and transmitter gated currents differ in young and adult neurons. Immature signals tend be inaccurate and long lasting when compared to adult signals because of a developmental sequence of expression of subunits. This leads to a jittery nonprecise generation of spikes in response to a synapse driven stimulus and this is also known to facilitate the generation of seizures. These observations suggest that drugs used in adult brains can have different, even opposite, actions in young patients. Also, immature networks will react differently to insults and seizures than adult networks: whereas adult circuits operate by means of cell loss and reactive plasticity, immature neurons are far more resistant to damage than adults. However, seizures do lead to long-terms deleterious sequels in young brains, which are mediated by alterations of developmental programs rather than cell loss. The programmatic versus lesion-related response to seizures has major implications in that misplaced, misconnected, or maldeveloped neurons may be a unifying link between seizures of the immature brain that in turn impact the operation of cortical maps and functional units. Hence the importance of examining in more detail the outcome of seizures on developing functional cortical units.

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BACKGROUND

Developing neurons and networks follow a developmental sequence

The formation of the adult networks with their plethora of behaviorally relevant oscillations and patterns takes time and follows a sequence. Ionic channels and network driven patterns do not appear randomly but obey a precisely timed sequence. Thus, at the cellular level, neurons first express calcium currents and a variety of potassium currents followed by sodium currents (Spitzer, 1994; Moody and Bosma, 2005). Furthermore, the subunits that generate these currents also follow a sequence involving sloppy currents with long time courses followed by more accurate currents with faster kinetics. This also applies to transmitter gated currents that are initially long lasting (cf the NMDA receptor-mediated currents that shift from several hundred milliseconds to a few tens of seconds in adults; Monyer et al., 1994). Developing neurons do not generate precise currents early on because this is unnecessary initially. This also applies to pre- and postsynaptic currents, the former usually maturing before the latter, with presynaptic inhibition such as that generated by G proteins acting on GABA, 5HT, or adenosine receptors inhibiting transmitter release prior to the transmitter gated postsynaptic currents (PSCs) (Ben-Ari et al., 2007). Another general trend is the earlier maturation of GABAergic signals that precede the formation of glutamatergic synapses, GABAergic signals being to all intent and purposes a pioneer system that operates by default with its synapses being automatically formed when the pre and postsynaptic elements meet. In contrast, glutamatergic synapses require more elaborate and mature postsynaptic neurons.

This developmental sequence has also been documented at the level of network driven patterns. The neurons in the fetal rodent neocortex and hippocampus *in vitro* generate first randomly occurring nonsynchronized voltage-gated spikes that drive calcium transients and participate in neuronal differentiation (Spitzer et al., 2000). Then a small subset of neurons – primarily GABAergic interneurons – interconnect and generate by means of gap junctions synchronized calcium plateaux in small cell assemblies (SPAs) (Crepel et al., 2007). These are nonsynapse driven events generated by intrinsic voltage-gated calcium currents. Then and only then the first synapse driven pattern appears, the so-called giant depolarizing potentials (GDPs) that are recorded in virtually all brain structures *in vitro* (Ben-Ari, 2001). GDPs are generated by excitatory GABA synapses and glutamatergic synapses and disappear when more elaborate patterns can be generated by a more developed network involving neurons endowed with several thousand synapses. A recent study has shown that GDPs are orchestrated by single highly arborized GABAergic interneurons that cover large parts of the hippocampus, constituting pioneer neurons that modulate the activity of large neuronal populations via their connections (Bonifazi et al., 2009). Using fast dynamic imaging techniques and target patch recordings, Bonifazi et al. (2009) determined the connectivity between hundreds of neurons and showed that they correspond to a scale-free network with specific types of GABAergic "hub" neurons playing a central role in their organization, thus further reinforcing the importance of GABA signals in the operation of early patterns. Determining these sequences is crucial since activity plays a crucial role in brain maturation (Ben-Ari and Spitzer, 2010).

Different actions of signaling molecules in young and adult neurons: the case of GABA

One important message of developmental neurobiology is that proteins and signaling molecules are used during brain maturation but not necessarily with identical modes of operation and mechanisms. Thus, GABA – the principal adult inhibitory transmitter – operates differently in neonatal neurons because of a universal difference in chloride gradient (Ben-Ari et al., 2007). Immature neurons possess higher intracellular concentrations of chloride leading to a different gradient for the major anion involved in mediating the actions of GABA. This is due to early expression of the chloride cotransporter NKCC1, which loads immature neurons with chloride (Yamada et al., 2004), and delayed expression of the chloride extruder KCC2 (Rivera et al., 1999). As a consequence, GABA will depolarize and occasionally excite immature neurons, whereas hyperpolarization and inhibition are more classically associated with the actions of GABA in adults. The consequence of this difference is that a major source of inhibitory drive is lacking in immature neurons and this entrains a lower threshold for seizure generations (Dzhala et al., 2005). Also drugs that act by means of a reinforcement of GABAergic inhibition may be expected to exert proconvulsive effects instead of anticonvulsive actions.

Primitive patterns of electrical activity in the developing cortex *in vivo*

In keeping with the developmental changes in signaling mechanisms observed using *in vitro* models, special organization of neuronal network activity has also been observed in the developing cortex *in vivo*. This organization of the immature brain activity has two distinct features. First, activity in the immature cortex is highly discontinuous, with the bursts of activity separated by long-lasting (up to tens of seconds) periods of virtually complete electrical silence (so-called "tracé discontinue") (Dreyfus-Brisac, 1962; Dreyfus-Brisac and Larroche,

1971; Stockard-Pope et al., 1992; Lamblin et al., 1999) (see Chapter 7). With maturation, this discontinuous temporal organization of immature EEG shifts towards so-called "tracé alternant," with the interburst intervals presenting low amplitude activity, and further evolves to "trace continu," with only the short (hundreds of milliseconds), silent "down-state" periods during slow wave sleep. These developmental changes from "tracé discontinue" to "tracé alternant" and then to "tracé continu" are well demonstrated both in humans and animals, the only difference being that in humans these changes mainly occur during the fetal stages of development, whereas in rodents there is a shift towards the postnatal period, indicating that they are related to brain development rather than to birth. Mechanisms underlying the developmental changes in temporal EEG organization are at present unknown. It has been suggested that several factors are involved, including a progressive increase in the number of synaptic connections and an increase in their strength. Indeed, experimental evidence and computer modeling indicates that maintenance of the depolarized active state of cortical neurons during awake and during activated cortical up-states requires a certain level of mutual excitation in the cortical networks. In keeping with these findings, with a smaller amount of synaptic connections between the cortical neurons, the immature network should spend most of the time in a silent state, similar to the adult sleep down-states that are promoted in experiments in which the number and strength of synaptic connections are reduced. On the other hand, maturation of arousal systems that are involved in tonic cortical activation during awake seems to be another limiting factor. For instance, it has been shown that cholinergic input to the cortex promotes the occurrence of the active cortical states and shortens the silent periods in the neonatal rat visual cortex (Hanganu et al., 2007). Further research on the developmental control of cortical activity by arousal systems will ultimately lead to important insights into the ontogeny of cortical EEG.

The second major developmental difference in EEG organization is expression of the particular patterns of activity during the early developmental stages. In humans, EEG recordings performed in premature newborns revealed a number of primitive activity patterns transiently expressed during development, and we are only just beginning to understand how these patterns are generated and the roles they play in cortical development. Animal models appear to be very useful in this research. In recent studies using neonatal rat as an experimental model, the mechanisms underlying the generation of one of the dominant immature EEG patterns – spindle-bursts – were explored (Khazipov et al., 2004a,b; Hanganu et al., 2006; Minlebaev et al., 2007; Marcano-Reik and Blumberg, 2008; Yang et al., 2009), which are homologous to delta-brushes in preterm human neonates (Lamblin et al., 1999; Khazipov and Luhmann, 2006; Milh et al., 2007) and share common features with "slow activity transients" more recently described using DC-EEG recordings (Vanhatalo et al., 2002; Vanhatalo and Kaila, 2006). Spindle-burst is an intermittent, spatially confined oscillation at alpha-beta-gamma frequency nested in slower delta waves. Spindle-bursts are reminiscent of adult sleep spindles (Steriade, 2001). However, in contrast to sleep spindles, neonatal spindle-bursts are local events with a limited tendency to spread likely due to a delayed development of intracortical connections, which are known to be involved in synchronization of spindles in adult brain (Destexhe et al., 1999). Furthermore, spindle-bursts are present in the waking pup even during walking and feeding, typically triggered by myoclonic twitches of isolated muscles or whole body startles. Myoclonic twitches are one of the most remarkable developmental motor phenomena in the neonatal rat (Blumberg and Lucas, 1994; O'Donovan, 1999; Petersson et al., 2003), human fetus, and premature neonate (Hamburger, 1975; De Vries et al., 1982; Cioni and Prechtl, 1990; Prechtl, 1997). This particular type of motor activity results from the stochastic bursts of activity generated in the spinal cord under brainstem control (Blumberg and Lucas, 1994; Kreider and Blumberg, 2000; Karlsson et al., 2005). Delay between the movements and cortical spindle-bursts and the observation that spindle-bursts can also be induced by direct sensory stimulation indicated that they are triggered by sensory feedback initiated by spontaneous movements. Importantly, spindle-bursts persist after sensory deafferentation (e.g., spinal cord transection), although at a reduced frequency. These results suggested that spindle-bursts are endogenous – probably thalamocortical or intracortical – oscillations. However, external stimuli, e.g., brought about by the thalamocortical afferents, can trigger these oscillations in the somatosensory cortex in a somatotopic manner. This paradigm has also been demonstrated in premature human neonates, in which delta-brushes in C3-C4 electrodes (over hands representation) and in Cz electrodes (over feet representation) are correlated with spontaneous movements of the hands and feet, respectively, and can be triggered by direct sensory stimulation in a somatotopic manner (Milh et al., 2007).

In the visual system, early stages of development are characterized by the presence of spontaneous waves of activity in the retina before it can respond to light. Retinal waves are generated in the network of retinal ganglion and amacrine cells and synchronize most of the retinal activity (Galli and Maffei, 1988; Meister et al.,

1991; Wong et al., 1993; Torborg and Feller, 2005). Retinal waves play an instructive role in the development of retinogeniculate connectivity (Shatz and Stryker, 1988; Penn et al., 1998; Muir-Robinson et al., 2002; Stellwagen and Shatz, 2002; Grubb et al., 2003; McLaughlin et al., 2003; Chandrasekaran et al., 2005; Mrsic-Flogel et al., 2005; Nicol et al., 2007). Evidence also exists for the contribution of retinal waves to cortical development. In monkeys, ocular dominance columns (ODCs) are formed already *in utero* before visual experience (Rakic, 1976). Although enucleation experiments suggest that retinal input may not be required for the formation of ODCs (Crowley and Katz, 1999), complete blockade of retinal activity can disturb segregation of thalamocortical connections in ODCs (Stryker and Harris, 1986). In neonatal mice, suppression of retinal waves during the first postnatal week also results in imprecise geniculocortical mapping, suggesting that spontaneous retinal waves are also involved in the development of thalamic connections to the visual cortex (Cang et al., 2005). These findings suggested the hypothesis that retinal waves are transmitted to and trigger activity in the developing visual cortex. This hypothesis has been tested in neonatal rats (Hanganu et al., 2006). Using extracellular and whole-cell recordings from visual cortex of neonatal rats *in vivo*, it was shown that, similar to the somatosensory cortex, the dominant pattern of activity in the immature visual cortex is a spindle-burst. Simultaneous recordings from the retina and V1 cortex revealed a strong correlation between the spindle-bursts in visual cortex and spontaneous retinal waves. In addition, V1 spindle-bursts could be reliably evoked by direct stimulation of the optic nerve. Pharmacological modulation of retinal activity affected the rate of occurrence of V1 spindle-bursts; for instance, intraocular forskolin injection, known to increase the frequency and amplitude of retinal waves, greatly increased the rate of occurrence of cortical spindle-bursts in the contralateral V1 cortex. On the other hand, blocking the propagation of retinal activity with local application of tetrodotoxin or removing the retina entirely resulted in a twofold reduction of V1 spindle-burst frequency, analogous to the reduction of somatosensory spindles after spinal cord transections. These results, therefore, support the hypothesis that spindle-bursts are self-organized patterns that are triggered by sensory feedback. Similar to neocortical activity in neonatal rats, spindle-bursts continue to be present at later stages of development when the retina becomes responsive to light. In postnatal day [P]22-39 ferrets, V1 multiple unit activity is organized in bursts with about 10 Hz intraburst multiple unit activity, and these bursts can be evoked by retinal light stimulation. Studied with multielectrode arrays, this bursting activity exhibited a patchy structure that reflects ocular dominance columns in visual cortex (Chiu and Weliky, 2001, 2002). Collectively, these observations reflect the importance of centripetal information flow from the periphery to the center and suggest that this may provide the core of activity-dependent modulation of the construction of operative cortical networks.

Intracellular studies using whole-cell recordings from neonatal rat somatosensory (Khazipov et al., 2004a,b; Minlebaev et al., 2007) and visual (Minlebaev et al., 2009) cortex revealed that spindle-bursts are mediated by both glutamatergic and GABAergic synaptic currents in neocortical neurons. Mechanisms of spindle-bursts were investigated in more detail in the neonatal rat barrel cortex (Minlebaev et al., 2007) using a superfused cortex preparation *in vivo*, enabling the application of drugs directly to the cortex. Pharmacological analysis revealed that spindle-bursts are blocked by the AMPA/kainate receptor antagonist CNQX but are little affected by NMDA receptor or gap-junction antagonists. Because the glutamatergic synapses on cortical neurons are mainly provided by the intracortical connections and thalamocortical inputs, two nonexclusive models of spindle-burst oscillations can be proposed: intracortical and thalamocortical. In the intracortical model, the spindle-burst is a local oscillation generated by the network of interconnected cortical glutamatergic neurons. Each cycle of oscillation is set by synchronous excitation of cortical neurons via AMPA/kainate receptors at the cortico-cortical synapses. Because the GABA(A) receptor antagonists did not significantly affect the frequency of spindle-burst oscillations, neuronal inhibition probably comes from an after-hyperpolarization mediated by voltage- and calcium-dependent potassium channels. In the thalamocortical model, spindle-bursts are generated by a rhythmic thalamocortical input provided by oscillations in thalamic neurons, similar to the mechanism of adult sleep spindles (Steriade et al., 1993). The spatial confinement of spindle-bursts in the developing brain can be explained by the relative scarcity of long-range intracortical and corticothalamic connections early in development. Long-range intracortical projections have been shown to be critical for synchronization of sleep spindles over the entire cortex in adult cats (Contreras et al., 1996). Locally emerging spindles in newborn rats may be aided by shunting surround inhibition of depolarizing GABA. These two models are not incompatible: thalamic and cortical mechanisms may cooperate in the induction of spindle oscillations.

WHY DO IMMATURE NETWORKS GENERATE SEIZURES MORE READILY?

There are several reasons why immature networks are intrinsically more prone to generate oscillations and

seizures than adults. Immature neurons are electrically compact, have a higher input resistance and small capacitance, and generate larger membrane potential responses to smaller currents. As a consequence, even with a smaller number of connections, immature neurons more readily generate synchronized activities. Also, because of their sloppy ionic currents characterized by long-lasting kinetics, activation of their synaptic inputs will generate a jittery response compared with the high-fidelity spike timing response of many adult synapses, known to be associated with bursts and seizures. Thus adult epileptic neurons have a low-fidelity spike response instead of the usual high fidelity response and this has been shown to promote seizures (Foffani et al., 2007).

The higher intracellular concentrations of chloride and associated excitatory actions of GABA will also facilitate the generation of seizures (Dzhala and Staley, 2003; Khazipov et al., 2004a,b; Dzhala et al., 2005). The lack of an efficient postsynaptic G protein-mediated inhibitory drive – GABA(B), adenosine, or 5 HT – is also likely to contribute to this trend.

SEIZURES BEGET SEIZURES IN THE DEVELOPING BRAIN

To determine the effects of seizures on immature networks, we developed an intact *in vitro* preparation consisting of the two intact interconnected hippocampi placed in a chamber with three compartments (Khazipov et al., 1999). Each hippocampus is placed in a separate compartment and the connecting commissures in the third chamber. With this arrangement, each compartment can be perfused with different liquids and their effects investigated. Thus, kainate or another convulsive agent is applied to one hippocampus and the effects of propagated seizures on the other untreated hippocampus investigated. Recordings of neurons and neuronal populations from both hippocampi show the effects of kainite-generated propagated seizures, and the electrical connections between both sides can be investigated by interrupting the flow of activity from the kainite-treated hippocampus to the untreated hippocampus after a desired number of seizures have been allowed to take place (Khalilov et al., 1997; Khalilov et al., 1999). Using this preparation, several important observations were made including:

(1) Single or a few episodes of propagated ictal events do not transform the naïve hippocampus to one that seizes spontaneously. In contrast, a series of 10 or more seizures leads to longterm consequences with the naïve hippocampus generating ongoing seizures once disconnected from the kainite-treated side, i.e., the propagated seizures convert the naive hippocampus into an epileptic one. The newly formed epileptogenic focus can generate seizures for several days, confirming its permanent basis (Khalilov et al., 1999).

(2) Investigation of the sequence of events in more detail revealed that the seizures that initiated on the kainite-treated side tended to back propagate from the naïve side, which progressively took over the lead.

(3) Several conditions must be met for seizures to beget seizures including the propagation of high-frequency oscillations (HFOs, >40 Hz), since lower frequency oscillations do not transform a naïve network to one that seizes. HFOs are in turn only produced when GABA and NMDA receptors are operative. Application of a GABA receptor antagonist to one or both hippocampi generates seizures, but with only low-frequency components that fail to transform either side to an epileptic focus. The GABAergic network is instrumental in enabling the formation of an epileptogenic mirror focus by means of the generation of HFOs (Khalilov et al., 2003, 2005).

(4) In the epileptogenic focus, single channel recordings reveal a chronic accumulation of chloride in neurons associated with a permanent change in the actions of GABA, which now excites neurons and further contributes to the generation of further seizures. This chronic transformation is mediated in part by a loss of chloride exporter device (KCC2) and is partly blocked by a diuretic agent that also prevents the actions of the NKCC1 transporter (Nardou et al., 2009).

To sum up, the profound changes in chloride concentrations and the consequent change in the actions of GABA, which becomes excitatory, are key events in early seizures. Interestingly, similar changes have been reported in adult human temporal lobe epilepsy (TLE) neurons (Cohen et al., 2002) but appear to be present in some but not all recordings made from immature, surgically removed infantile epileptic material (Cepeda et al., 2007; Tyzio et al., 2009). Other observations suggest that an earlier maturation of thalamic neurons versus neocortical ones underlies the well-known electroclinical dissociation with seizures present in the EEG but with no clinical manifestations as well as some paradoxical actions of GABA-acting antiepileptic agents that will block thalamic seizures but not neocortical ones (Glykys et al., 2009).

DEVELOPING NETWORKS ARE MORE RESISTANT THAN ADULT NETWORKS TO SEIZURE-INDUCED DAMAGE

The reactive plasticity sequence of events generated by seizures

In adults: Extensive investigations in adults show that recurrent seizures generate a brain damage syndrome with

a pattern of cell loss that is reminiscent of that observed in TLE. Thus, administration of the powerful excitotoxic agent kainate generates seizures with electrographic and clinical signs that are of typical limbic symptomatology followed by damage in vulnerable zones that include the CA3 regions of the hippocampus and various neuronal populations in the limbic system (Ben-Ari, 1985). The kainate model has been extensively investigated (over 2332 references) with essentially similar results in a wide range of experimental conditions and animal species. The seizures are generated in neurons (the CA3 pyramidal neurons) that have a dense network of kainatergic synapses notably in mossy fiber terminals. After a series of recurrent seizures, there is an extensive pattern of cell loss – caused by the excessive neuronal activity – and this is followed by a phenomenon of reactive plasticity associated with sprouting of mossy fibers and formation of novel aberrant synapses that contribute to generate more seizures as they enhance network excitability. The sequence leading to this outcome is now relatively well understood and it includes the activation by seizures of thousands of genes including immediate early genes, growth factors, cytoskeletal proteins, and various guiding factors that will orient new fibers to their destination. The most spectacular illustration of the importance and precision of this sequence is illustrated by a recent study showing that novel synapses formed after seizures use a novel receptor-mediated signal that is not present in naïve neurons (Epsztein et al., 2005). Indeed, whereas naïve granule cells of the fascia dentate use fast-acting AMPA receptor-mediated synapses, newly formed mossy fiber terminals established on granule cells after seizures operate by means of slow kinetics kainate receptor-mediated synapses. This dramatic shift of operation is instrumental in seizure generation since specific kainate receptor-mediated antagonists block seizures in chronic TLE animals (Epsztein et al., 2009). Therefore, the processes of cell loss and reactive plasticity, which occur also in humans, are nodal features of adult TLE.

In developing brains: In the developing brain in contrast, this process does not take place during the first 2 weeks after birth in rodents (Nitecka et al., 1984; Tremblay et al., 1984). Administration of high doses of kainate generates seizures with a wide range of clinical manifestations, but there is no cell loss at least until the end of the second week after birth. Even very high concentrations of convulsive agents fail to produce cell loss indicating that neurons are very resistant to kainate and to seizures at an early developmental stage. This has also been confirmed with pilocarpine and other epilepsy models. Interestingly, the animals can be kindled readily at an early stage suggesting that recurrent seizures can transform networks to epileptic ones capable of generating seizures spontaneously (see above). But why don't seizures produce cell loss? The answer to this question is still not clear although classical markers of activity are present in immature neurons including c fos and other stress molecules. One possibility is that the limbic synaptic connections are not fully mature at an early stage – notably the inputs of the hippocampal formation and in particular the perforant pathway consequent to the delayed maturation of the granule cell of the fascia dentate, which matures late in all animal species investigated. Therefore, the hippocampus – a gateway to seizures in the limbic system – is delayed in its maturation and the lack of well-developed mossy fiber terminals may be a major cause for the lack of cell loss at an early age. In addition, reactive plasticity also fails to play an important role at that stage. Clearly the developmental sequence is altered permanently leading to aberrant features in adult networks with some neurons having immature features.

Yet, seizures early in life can lead to severe life-alterinqing neurological sequelae including in animal models. Thus, early recurrent seizures produced by kainate or other convulsive agents produce long-lasting effects in behavioral terms and in seizure susceptibility in adulthood. How are these effects produced? The most reasonable assumption is that seizures interrupt one or more developmental sequences leading to malformed network(s) with possibly misplaced/misconnected neurons leading to long-term sequelae. The evidence for this scenario comes from experiments that reflect both the importance of early activity and receptors in essential developmental procedures including migration, proliferation, etc., and from studies designed to investigate how genetic mutations alter brain development leading to neurological sequelae and seizures.

ALL DEVELOPMENTAL STEPS ARE ACTIVITY AND RECEPTOR DEPENDENT

The formation of an operative brain with a plethora of behaviorally relevant oscillations and integrative functional units and ensembles is organized around a series of steps including cell proliferation, neuronal migration, neuronal differentiation, and synapse formation followed by the construction of functional units and the elimination of superfluous neurons and connections. All these steps are modulated by electrical activity and the environment. Thus, activation of GABA receptors controls cell proliferation and neuronal migration. Blocking GABA receptors, which are present on migrating neurons where they express synapses, retards or even blocks migration. Similarly, neuronal differentiation and synapse formation are activity dependent and perturbed activity can lead to malformations and inadequate connections. In fact even axonal branching and

targeting are altered by the activity of the parent cell, illustrating the major impact of activity and calcium spikes on growth and network construction (Zheng and Poo, 2007). The phenotype of neurons and their expression of functional GABAergic machinery depend on a series of calcium spikes generated during maturation by the neuron (Spitzer, 2006). Formation of neuronal networks is also an activity-dependent process, with a combination of spontaneous and sensory-driven activity patterns that participate in the formation of cortical circuits. Epileptiform events evidently provide very different conditions for synaptic plasticity. It is therefore not surprising that enhanced or perturbed activity will alter the developmental sequence and lead to malformed circuits and associated sequelae.

MECHANISMS UNDERLYING THE GENERATION OF SEIZURES BY GENETIC MIGRATION DISORDERS: DEVELOPMENTAL SEQUENCES AND CORTICAL MAPS

A wide range of genetic mutations are associated with migration disorders and other developmental malformations and include severe intractable seizures amongst the neurological sequelae. These mutations implicate a wide range of different proteins including cytoskeletal proteins but also transmitter gated receptors and other important signaling molecules. These disorders offer interesting possibilities for investigating not only the mechanisms underlying seizure generation in these tissues, which are often operated on to remove the epileptic focus in an effort to stop the seizures, but also in animal models in order to investigate the links between the morphological alterations and the seizures. What are the features of misplaced neurons and connections and how do these interfere with cortical operation leading to neurological sequelae and seizures?

Using the sRNAi technique *in utero* to mimic the human manifestation by invalidating a small neuronal population and determining how this in turn interferes with the formation of cortical maps has ecently revealed several important features of the events that occur after invalidating a protein (LoTurco and Bai, 2006). This technique turned out to be advantageous compared with conventional knock outs (Kos), which are associated with complicated repair and replacement strategies leading to little or no morphological abnormalities. Thus, whereas the doublecortin Ko leads to no morphological abnormality in the neocortex, *in utero* sRNAi of double cortin gene (DCX) leads to the formation of a small double cortex with neurons in which the DCX protein has been invalidated remaining in the deep layers of the cortex (Bai et al., 2003). This enables the electrical properties of these misplaced neurons to be investigated and the determination of how they alter the operation of the overlying cortex.

In animals in which DCX has been invalidated in small neuronal populations, while the rest of the cortex remains normal, several important features are apparent (Ackman et al., 2009):

(1) Misplaced DCX invalidated neurons remain immature with a silent behavior and somewhat sloppy currents and astonishingly few synapses. These neurons remain in the vicinity of the corpus callosum and have axonal arbors that extend over great distances to regions that they do not innervate normally.
(2) The overlying layer 2/3 neurons to which misplaced neurones were programed to go react to the absence of this population by extensive sprouting and formation of aberrant glutamatergic synapses. Consequently these neurons have a fivefold increase in glutamatergic PSCs and a tendency to generate synchronized activities. These neurons project to the misplaced neurons with which they were programed to interconnect.
(3) Together these misplaced neurons and the overlying cortex generate far greater oscillatory behavior than the adjacent normal cortex. This suggests that the defect is both in the misplaced neurons and overlying cortex.

Interestingly, other models that produce misplaced neurons, notably the MAM injection model, also produce neurons that interact both with their aberrant host regions (the hippocampus for these neocortical neurons) and their programed targets. This leads to the formation of an aberrant bridge between neocortex and hippocampus that underlies the ease with which seizures are generated and propagated readily between hippocampus and neocortex (Chevassus et al., 1999). Again the issue here is not cell loss but aberrant connectivity.

CONCLUSIONS

Understanding and curing developmental insults requires consideration of the unique sequences of brain development as well as the inherent heterogeneity that characterizes the immature brain. Indeed, the large repertoire of currents and signaling cascades in immature neurons leads to a wide range of possible sequelae to genetic and environmental insults associated with seizures. Signals that alter developmental patterns and retard or block developmental sequences lead to programatic sequelae that will be manifested because of the wrong connections and messages that they establish consequently. These issues should be investigated at length and developmental neurobiology incorporated in the analysis of

infantile neurological disorders. Only this approach will lead to the development of novel therapeutic avenues for disorders that have remained largely resistant to treatment. The neuroarchaeology concept that I have suggested elsewhere is fundamental in that it suggests architectural and electrical signatures of brain disorders including epilepsies and brain malformations (Ben-Ari, 2008). Several studies confirm the immaturity of misplaced and misconnected neurons. If validated this concept will pave the way to develop novel therapeutic approaches centered on the use of specific blockers of immature currents that will antagonize these misplaced cells only.

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