



# Aspects of epileptogenesis: Maturation of neuronal circuits

W. DOUGLAS KNOWLES, PHD

**S**USCEPTIBILITY to epileptogenesis and the propensity for acquiring particular types of epilepsies are related to developmental age. Changes in the brain's organization, connectivity, and functional mechanisms alter the susceptibility to and the expression of epileptic activity. From clinical observation, we know that overall susceptibility to epilepsy varies greatly with age. The highest age-specific incidence of epilepsy occurs during the first year of life, declining steadily thereafter until age 60, when a second rise in incidence occurs.<sup>1,2</sup> Different epileptic syndromes tend to occur at different developmental ages. During the neonatal period, seizures are most often the result of hypoxic-ischemic encephalopathy or intraventricular hemorrhage. While generalized tonic seizures are relatively common, they may be poorly organized, particularly in premature infants.<sup>3</sup> The generalized syndromes tend to occur earlier in life, with more focal syndromes occurring later.<sup>2</sup>

## ANIMAL MODELS OF EPILEPSY

In interpreting the implications of developmental experimental models of epilepsy, it is necessary to be able to correlate the developmental stage of the animals with the developmental age of the human patients. This is a difficult task which is often neglected, but some generalizations are possible. In common experimental animals, the brain is at various stages of

development at the time of birth. Rats, mice, and rabbits are born before the main period of neuroblast differentiation. Many types of neurons, particularly local interneurons, have not yet fully differentiated. Synapse formation and axon myelination occur to a large extent after birth. Cat, guinea pig, and sheep brains are more nearly mature at birth. Human brains are at a somewhat intermediate stage at birth and have been compared to the rodent brain at five to eight days of age.<sup>4-9</sup> Obviously, the rate of maturation is quite different in experimental animals and humans.

It is a common finding in experimental animals, as in humans, that immature animals are more susceptible to epileptogenesis. For example, convulsant thresholds to drugs are much lower in rat pups than in adults.<sup>4,10,11</sup> The age-related expression of various epileptogenic models and the animal's relative susceptibility to them depends on the exact epileptogenic model used. For example, in vitro hippocampal slices from 1- to 2-week-old rats and rabbits display a relatively high degree of convulsant-induced epileptogenicity and are susceptible to spontaneous episodes of spreading depression. After about 2 weeks, hippocampal epileptogenesis declines. On the other hand, neocortical slices are relatively resistant to convulsant-induced epileptogenesis until after 1 to 2 weeks. Kittens, because of their earlier maturation, are closer at birth to the adult pattern of epileptogenesis.<sup>4,12-14</sup>

It is not clear, however, that one can consider epileptogenicity as a simple function of maturational age. Many interacting mechanisms are involved in the generation of epileptic activity and its spread, and the exact maturational sequence of each of these mechanisms may be important. Some of these mechanisms are discussed below.

From the Departments of Brain and Vascular Research and Neurology, The Cleveland Clinic Foundation Research Institute.

This work was supported by Grant NS 24956 from NIH.

## BURSTING CELLS

The hallmark of epileptogenesis has long been considered the bursting neuron. Intracellular recordings from epileptogenic models demonstrate that most of the neurons fire in a short, high-frequency burst of action potentials superimposed on a slower underlying depolarization (the paroxysmal depolarization shift, or PDS). Further, epileptogenesis occurs preferentially in brain regions or subregions containing neurons which normally fire (asynchronously) in this pattern. The normal generation of asynchronous bursts is the result of ionic currents intrinsic to the membrane of these neurons. The abnormal synchronization of these bursts is critical in epileptogenesis, and is primarily mediated by chemical synapses. Thus, the importance of the maturation of intrinsic burst-generating currents and synaptic function is clear.

The PDS is produced by a combination of sodium and calcium currents. While normal action potentials are generated by voltage-dependent sodium channels, the ability of a neuron to support bursting patterns of activity probably most specifically depends on the presence of a sufficient density of voltage-dependent calcium channels in that neuron's membrane. When sufficiently depolarized, these channels can produce regenerative calcium currents which generate a major portion of the PDS. Both sodium and calcium channels must be functional before epileptogenesis can occur.

Voltage-dependent sodium channels are apparently functional very early, while calcium channels may mature later. Even fetal rabbit hippocampal neurons produce action potentials.<sup>15</sup> In rats the number and density of functional sodium channels increase to adult levels during the first 2 postnatal weeks.<sup>13</sup> Calcium channels may be somewhat slower to develop, probably becoming functional during the first week in rat neocortex<sup>13</sup> and kitten and rabbit hippocampus.<sup>7,8</sup>

## EXCITATORY SYNAPTIC MECHANISMS

Epileptogenesis is dependent on functional excitatory synapses to synchronize the activity of neurons in the affected region. Recent evidence has indicated that N-methyl-D-aspartate-type excitatory amino acid neurotransmission plays a particularly important role in epileptogenesis.<sup>16</sup> The development of excitatory amino acid neurotransmission is thus an important facet of the development of epileptogenesis. Physiological studies have demonstrated that development of

excitatory synaptic transmission occurs early and can be observed in fetal rabbits.<sup>15</sup> Cerebellar excitatory amino acid receptor binding is low in rats during the first week of life but increases markedly during the second and third weeks.<sup>17</sup> Similarly, the neurotoxic effects of kainic acid, which are presumably mediated by excitatory amino acid receptors, develop in rats and rabbits during the second to fourth postnatal week.<sup>18-21</sup> However, the pattern of excitatory amino acid receptor development is not simple. Glutamate and kainate binding develop differently,<sup>17</sup> different brain regions develop receptors at different rates,<sup>18,21</sup> and there may be differential development of different subtypes of the kainate receptor.<sup>19</sup>

Epileptogenesis further requires that excitatory synaptic function be appropriately organized. In particular, excitatory synaptic feedback loops, or recurrent excitation, are required to produce synchronized epileptic activity.<sup>22</sup> Thus, in addition to the existence of appropriate receptors, an excitatory synaptic network must mature sufficiently before epileptogenesis can occur.

Obviously, epileptogenesis depends on the growth and arborization of neuronal axons. This occurs at different rates in different regions of the brain, and differs among species. In the later maturing rodents, long axon neurons are present at birth, but the short axon local interneurons develop after birth. Arborization of both types of neurons continues during the first few weeks after birth. In earlier maturing kittens, guinea pigs and humans, axonal branching patterns are largely mature at birth. It would appear that axonal arborization is a maturational event sufficiently early that it does not delay the development of epileptogenesis. However, myelination of axons with its associated increase in conduction velocity continues through the first month in kittens,<sup>4,23,24</sup> so that the spread of epileptic activity and the resultant expression of seizure types may depend on the maturation of myelination.

While axonal arborization occurs relatively early, functional development of synapses is slower to develop. Excitatory and inhibitory synaptic function develop at different rates. In the early maturing kitten, both symmetric and asymmetric synapses (presumed inhibitory and excitatory, respectively) are seen at 1 week, and increase in number with age.<sup>7</sup> In the more slowly developing rabbit, however, only dendritic asymmetric (excitatory) synapses are seen in the first week, and axosomatic symmetric (inhibitory) synapses develop by 4 weeks.<sup>9</sup> These anatomic results suggest that excitatory synaptic function matures earlier than inhibitory function. The physiological studies de-

scribed below support this hypothesis.

#### INHIBITORY MECHANISMS

The most easily created and most reliable experimental models of epileptogenesis are produced by blockade of gamma aminobutyric acid (GABAergic) synaptic transmission. Thus the concept that GABAergic mechanisms are crucial for prevention of epileptic activity has arisen. However, many other inhibitory mechanisms exist in cortical neurons, and disruption of some of these mechanisms can also produce epileptiform activity.

One inhibitory mechanism which participates in termination of cellular bursts is the calcium-dependent potassium current. This current hyperpolarizes the cell following the depolarization underlying the burst. This current matures early, being seen in even perinatal rabbits.<sup>8</sup> Thus, it is probably not critical in the development of epileptogenesis.

The appearance of excitatory synapses before inhibitory synapses suggests that there is a period when a predominance of excitatory mechanisms with a deficiency of inhibitory control may explain the high incidence of epileptogenesis in neonates and infants. It is thought that in cortex, inhibition is mediated largely by local interneurons. The observation that in rat forebrain, short axon interneurons appear after birth<sup>4,5</sup> and the finding that prior to the age of 2 weeks, only pyramidal cell type neurons can be stained by intracellular injection of fluorescent dye in *in vitro* slices support the hypothesis of late development of inhibitory mechanisms. Note, however, that in the earlier maturing kitten, interneurons are present much earlier.<sup>7,25</sup>

The anatomic presence of interneurons and synaptic profiles suggests the establishment of functional synaptic transmission. Physiological recordings from brain slices have confirmed the later establishment of inhibitory synaptic function as compared to excitatory synaptic function, at least in the more slowly maturing rabbit and rat. Excitatory synaptic function is present at birth in rabbit and rat, but inhibitory synaptic function does not develop until 2 weeks in rabbit<sup>8</sup> and 1 week in rat.<sup>13</sup> Again, these results may explain the early period of high epileptogenicity.

The development of synaptic function has been studied in some detail with the aim of understanding whether maturation of presynaptic or postsynaptic elements occurs first. In their anatomic study of develop-

ing rabbit, Schwartzkroin et al<sup>9</sup> report that very immature animals have smaller synapses with smaller area of contact and fewer synaptic vesicles in the presynaptic terminal than animals older than 2 weeks. Interneurons containing the GABA synthetic enzyme glutamic acid decarboxylase (GAD) are seen in rabbits as young as eight days, but the GAD often does not appear in specialized synaptic terminals.<sup>26</sup> Thus, development of synaptic function is probably at least partially dependent on maturation of the presynaptic element.

The postsynaptic neuron matures during this period as well. Perinatally, the asymmetric (excitatory) synapses are located mainly on the soma. During the first 2 weeks, the asymmetric synapses increase mostly on the dendrites, and symmetric synapses increase on the soma.<sup>9</sup> The responses of the postsynaptic neuron to exogenously applied GABA and the GABA<sub>B</sub> agonist, baclofen, are not mature in the CA1 region of hippocampus of 8- to 12-day-old rabbits.<sup>27</sup> Thus, in this region, there is postnatal maturation of the postsynaptic neurons. Interestingly, area CA3 is much more mature in its GABA and baclofen responses at the same age.<sup>27</sup> This further supports the idea of differential maturation of different brain regions.

The conclusion to be drawn from these data is that excitatory synaptic function is established at birth in rabbits and rats, and continues to mature during the first few weeks. Inhibitory synaptic function probably matures somewhat later, being absent during the first week or so and developing during the next several weeks. Maturation of inhibitory synaptic function is probably critically dependent on the maturation of both synaptic junctions and postsynaptic receptor function. The result is that there may be a developmental window of increased epileptogenic susceptibility during the first 2 weeks when excitatory synaptic function is sufficiently organized, but inhibitory synaptic function is still relatively ineffective.

#### IONIC ENVIRONMENT

Extracellular potassium ion concentration is a significant determinant of neuronal excitability and thus of epileptic susceptibility. Spontaneous and evoked episodes of seizure-like spreading depression associated with large increases in extracellular potassium are seen in hippocampal slices from immature rabbits much more frequently than in adult tissue.<sup>14</sup> The increased susceptibility of the younger animals is probably related to two factors. The Na-K adenosinetriphosphatase

(ATPase) activity is low in neonates and increases during the first 2 weeks.<sup>28</sup> As discussed above, this is also the period during which inhibitory synaptic function matures. The Na-K ATPase activity is a measure of cellular potassium pumping, which affects extracellular potassium levels. Poor regulation of extracellular potassium, combined with immature inhibitory synaptic function, could clearly combine to increase epileptogenesis. Once again, there are different rates of maturation in different regions. CA1 matures later than CA3, both in terms of inhibitory synaptic function and Na-K ATPase activity. This is reflected in the increased susceptibility of CA1 (relative to CA3) to epileptic-like activity in the newborn rabbit.<sup>14,28</sup>

## CONCLUSIONS

Infants and neonatal animals are susceptible to epileptic seizures, and experimental evidence suggests that the differential development of several neuronal mechanisms can explain this susceptibility. The primarily generalized seizure types are more common in younger organisms than in adults, probably because of the less mature organization and functional status of neuronal tissue which limits the spread of initially focal

types of epileptic activity. It seems that the window of increased susceptibility can be explained by an earlier maturation of excitatory synaptic function which is followed by a later maturation of inhibitory synaptic function. The delayed maturation of control of extracellular potassium concentration by the Na-K ATPase pump may also play a role.

These observations certainly do not fully explain clinical experiences with the development of epileptogenesis. A complete explanation should also take into account the particular risks involved with perinatal and infant life. Perinatal anoxia, for instance, is a unique risk that probably contributes to the occurrence of seizure in a certain group. The experimental data do not tell us much about the specific reasons for the high incidence of specific types of epilepsy, such as febrile seizures, infantile spasms, or Lennox-Gastaut syndrome, at different periods of development.

W. DOUGLAS KNOWLES, PHD  
Departments of Brain and Vascular Research  
and Neurology  
The Cleveland Clinic Foundation Research  
Institute  
One Clinic Center  
9500 Euclid Avenue  
Cleveland, Ohio 44195

## REFERENCES

- Hauser WA, Kurland LT. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia* 1975; 16:1-66.
- Gomez MR, Klaus DW. Epilepsies of infancy and childhood. *Ann Neurol* 1983; 13:113-124.
- Hill A, Volpe JJ. Seizures, hypoxic-ischemic brain injury, and intraventricular hemorrhage in the newborn. *Ann Neurol* 1981; 10:109-121.
- Moshé SL. Epileptogenesis and the immature brain. *Epilepsia* 1987; 28(suppl 1):S3-S15.
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev* 1979; 3:79-83.
- Gottlieb A, Keydor I, Epstein HT. Rodent brain growth stages. An analytical review. *Biol Neonate* 1977; 32:166-176.
- Schwartzkroin PA, Altschuler RJ. Development of kitten hippocampal neurons. *Brain Res* 1977; 134:429-444.
- Schwartzkroin PA. Development of rabbit hippocampus: Physiology. *Dev Brain Res* 1982; 2:469-486.
- Schwartzkroin PA, Kunkel DD, Mathers LH. Development of rabbit hippocampus: Anatomy. *Dev Brain Res* 1982; 2:453-468.
- Vernadakis A, Woodbury DM. The developing animal as a model. *Epilepsia* 1969; 10:163-178.
- Albala BJ, Moshé SL, Okada R. Kainic acid induced seizures: A developmental study. *Dev Brain Res* 1984; 13:139-148.
- Prince DA, Gutnick MJ. Neuronal activities in epileptogenic foci of immature cortex. *Brain Res* 1972; 45:455-468.
- Kriegstein AR, Suppes T, Prince DA. Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons in vitro. *Dev Brain Res* 1987; 34:161-171.
- Haglund MM, Schwartzkroin PA. Seizure-like spreading depression in immature rabbit hippocampus in vitro. *Dev Brain Res* 1984; 14:51-59.
- Schwartzkroin PA, Kunkel DD. Electrophysiology and morphology of the developing hippocampus of fetal rabbits. *J Neurosci* 1982; 2(4):448-462.
- Meldrum B. Excitatory amino acids and epilepsy. [In] Hicks TP, Lodge D, McLennan H, eds. *Excitatory Amino Acid Transmission*. New York, Alan R Liss Inc, 1987, pp 189-196.
- Slevin JT, Coyle JT. Ontogeny of receptor binding sites for (<sup>3</sup>H) glutamic acid and (<sup>3</sup>H) kainic acid in the rat cerebellum. *J Neurochem* 1981; 37:531-533.
- Nitecka L, Tremblay D, Charton G, Bouillot JP, Berger ML. Maturation of kainic acid seizure-brain damage syndrome in the rat. *Neuroscience* 1984; 13(4):1073-1094.
- Garthwaite J, Wilkin GP. Kainic acid receptors and neurotoxicity in adult and immature rat cerebellar slices. *Neuroscience* 1982; 7:2499-2514.
- Gaddy J, Britt M, Neill D, Haigler H. Susceptibility of rat neostriatum to damage by kainic acid: age dependence. *Brain Res* 1979; 176:192-196.
- Franck JE, Schwartzkroin PA. Immature rabbit hippocampus is damaged by systemic but not intraventricular kainic acid. *Dev Brain Res* 1984; 13:219-227.
- Knowles WD, Traub RD, Wong RKS, Miles R. Properties of neural networks: Experimentation and modeling of the epileptic hippocampal slice. *Trends Neurosci* 1985; 8:73-79.
- Purpura DP. Ontogenetic models in studies of cortical seizure activities. [In] Purpura DP, Penry JK, Tower D, Woodbury DM, Walter R, eds. *Experimental Models of Epilepsy*. New York, Raven Press,

- 1972, pp 531–556.
24. Oka H, Samejima A, Yamamoto T. Post-natal development of pyramidal tract neurons in kittens. *J Physiol* 1985; **363**:481–499.
25. Purpura DP, Pappas GD. Structural characteristics of neurons in the feline hippocampus during postnatal ontogenesis. *Exp Neurol* 1968; **22**:379–393.
26. Kunkel DD, Hendrickson AE, Wu JY, Schwartzkroin PA. Glutamic acid decarboxylase (GAD) immunocytochemistry of developing rabbit hippocampus. *J Neurosci* 1986; **6**(2):541–552.
27. Janigro D, Schwartzkroin PA. Effects of GABA and baclofen on pyramidal cells in the developing rabbit hippocampus: An “in vitro” study. *Brain Res* 1988; **469**(1-2):171–184.
28. Haglund MM, Stahl WL, Kunkel DD, Schwartzkroin PA. Developmental and regional differences in the localization of Na,K-ATPase activity in the rabbit hippocampus. *Brain Res* 1985; **343**:198–203.