DOES ONE NEONATAL SEIZURE ALTER SYNAPTIC PLASTICITY AND CAUSE LIFELONG COGNITIVE IMPAIRMENT?

A Single Episode of Neonatal Seizures Permanently Alters Glutamatergic Synapses. Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. *Ann Neurol* 2007;61(5):411–426. OBJECTIVE: The contribution of seizures to cognitive changes remains controversial. We tested the hypothesis that a single episode of neonatal seizures (sNS) on rat postnatal day (P) 7 permanently impairs hippocampal-dependent function in mature (P60) rats because of long-lasting changes at the synaptic level. METHODS: sNS was induced with subcutaneously injected kainate on P7. Learning, memory, mossy fiber sprouting, spine density, hippocampal synaptic plasticity, and glutamate receptor expression and subcellular distribution were measured at P60. RESULTS: sNS selectively impaired working memory in a hippocampal-dependent radial arm water-maze task without inducing mossy fiber sprouting or altering spine density. sNS impaired CA1 hippocampal long-term potentiation and enhanced long-term depression. Subcellular fractionation and cross-linking, used to determine whether glutamate receptor trafficking underlies the alterations of memory and synaptic plasticity, demonstrated that sNS induced a selective reduction in the membrane pool of glutamate receptor 1 subunits. sNS induced a decrease in the total amount of *N*-methyl-D-aspartate receptor 2A and an increase in the primary subsynaptic scaffold, PSD-95. INTERPRETATION: These molecular consequences are consistent with the alterations in plasticity and memory caused by sNS at the synaptic level. Our data demonstrate the cognitive impact of sNS and associate memory deficits with specific alterations in glutamatergic synaptic function.

COMMENTARY

There is a burgeoning literature on the effects of seizures, incurred at various ages, on subsequent cognitive and behavioral function (1). The goal of these studies is to understand the mechanisms by which seizure-induced cognitive damage occurs and to prevent such sequelae. Since clinical studies cannot unequivocally separate the effects of seizure type, duration, frequency, and etiology on outcome, researchers have turned to animal models, in which many of those variables can be controlled. The notion that seizures early in life cause minimal cognitive deficits is undergoing revision (2,3). It is now recognized that a seizure at any age can exert an adverse effect on subsequent cognition and behavior, across models and seizure induction techniques. Adverse effects are not limited to prolonged seizures; brief, recurrent seizures also carry an increased risk of later cognitive sequelae (4). Current consensus holds that early life seizures disrupt one or more ongoing developmental processes, resulting in long-term detriment (5).

So far, studies have been largely descriptive. A perplexing question has been the mechanism(s) by which early seizures wreak their havoc. Seizure-induced cell death does not seem to play a major role in the neonatal period. Reorganization of axonal connections (sprouting) occurs in the developing brain, albeit in a different location and pattern than in the mature brain, and is not due to the same initiating mechanism (i.e., neuronal death). Neurogenesis occurs in an age-dependent fashion, but again, the pattern is different than that seen in adult brains after seizures. Alteration of neurotransmitter receptors (e.g., density, stoichiometry, subtypes, sensitivity) likely plays a role in seizure-induced deficits, with both excitatory and inhibitory systems implicated (6,7).

Using correlative physiological, behavioral, cognitive, and molecular techniques, Cornejo et al. describe an informative set of experiments designed to elucidate the mechanism by which neonatal seizures lead to cognitive impairment. Their overall hypothesis was that neonatal seizure-induced alterations in learning and memory are related to altered synaptic plasticity. Each individual seizure was brief (less than 10 minutes), but seizures recurred over periods of up to 3 hours, similar to the pattern seen in many human newborns. When these rats reached adulthood, visuospatial learning and memory were tested using the standard Morris water maze and the 4-trial radial arm water maze, a more challenging test of memory. The authors found that adult rats that had experienced a single episode of seizure in the neonatal period differed from control rats (with no neonatal seizures) in subtle but important ways. Both groups mastered the two mazes similarly, showing that the neonatal

Epilepsy Currents, Vol. 7, No. 6 (November/December) 2007 pp. 163–165
Blackwell Publishing, Inc.

seizure did not impact visuospatial learning, except that the rats with neonatal seizures had more errors on the first day of the 4-trial radial arm water maze test, suggesting a possible subtle learning deficit.

The authors then probed cognitive abilities of the two groups in further detail, using a variation called the 2-trial radial arm water maze, in which rats learn the location of a hidden platform on one trial and then are tested to see if they remember its location 4 hours later in a single additional trial; the test measures episodic-like memory for a single event (8). In this test, rats with neonatal seizures had significantly more errors in finding the platform (showing defective episodic-like memory); they also took longer to find the platform and more often re-entered an incorrect arm (both measuring working memory). The functions assessed by the 2-trial radial arm water maze are highly dependent upon hippocampal NMDA receptors. Therefore, a single episode of neonatal seizure was sufficient to demonstrate robust, lifelong deficiencies in episodic and working memory. The authors found no mossy fiber sprouting or alterations in dendritic spine density or branching to account for the cognitive impairment.

Because of the involvement of glutamate receptors (particularly NMDA subtype) in learning, memory, and synaptic plasticity, the authors then performed extracellular electrophysiology experiments on hippocampal slices using field potential analysis, long-term potentiation (LTP), and long-term depression (LTD). There was no seizure-induced change in paired-pulse facilitation (ruling out a presynaptic effect) or in overall network excitability. However, LTP was decreased and LTD was enhanced after neonatal seizures. Maintenance of the ability to learn visuospatial information, but defective episodic and working memory function, led the authors to hypothesize that differences in glutamate receptor number, type, or distribution could explain the altered neuroplasticity. Hippocampi were fractionated into subregions and subjected to western blot and cross-linking analysis, to examine glutamate receptor expression and distribution. By both methods, intracellular levels of the AMPA receptor subtype GluR1 were increased significantly, while there were no differences in the expression of GluR2/3 or in NMDA receptor subtype 1 (NR1), and the total amount of NR2A was decreased. The increased intracellular GluR1 related to trafficking of the receptor from the membrane pool. An unexpected finding was that PSD-95, a scaffolding protein that interacts with glutamate receptors, was markedly increased after neonatal seizures. Changes in levels of scaffolding proteins may be emerging as a critical factor after seizures though the direction of the change may vary (9). The model used by Cornejo et al.—and expanded upon in an accompanying editorial (10)—proposes how specific receptor alterations could explain the plasticity results.

The permanent nature of all these changes after a single neonatal seizure raises legitimate concern about early seizure effects and their prevention. It is uncertain whether the data described here can be translated into the clinical realm. The experiments of Cornejo et al. are unique in that they present a comprehensive approach, using behavioral, molecular, and electrophysiological techniques, to address the mechanism of cognitive impairment from early life seizures. Methodologically, it would be reassuring to know if the seizures in all rats were similar in frequency, duration, and severity; this information would require electrographic monitoring during kainate treatment, which previously has been demonstrated (11). With such robust effects as a consequence of a single neonatal seizure, it would also be important to know if similar changes occur after multiple seizure episodes or after status epilepticus. The occurrence of spontaneous seizures could influence behavioral performance and plasticity, and these were not reported. If the mechanism proposed here is universal, the results should be verifiable at other ages and in female rats as well. It is also known that $GABA_A$ receptors are chronically altered after neonatal seizures (7) and that altered inhibition has been associated with seizure-induced synaptic plasticity at young ages (12). At the age tested here (P7), GABA is still excitatory (11) and seizures at this age can prevent the transition of GABA's action from excitatory to inhibitory (5,13). These concerns notwithstanding, the authors provide a consistent and exciting model that is one of the first comprehensive explanations for cognitive impairment from early life seizures. There now are additional targets (i.e., glutamate receptor redistribution and trafficking) for further experimental study and eventually, for innovative therapeutic interventions.

by Carl E. Stafstrom MD, PhD

References

1. Haut SR, Veliskova J, Moshe SL. Susceptibility of immature and adult brains to seizure effects. *Lancet Neurol* 2004;3:608–617.
2. Holmes G, Ben-Ari Y. Seizures in the developing brain: perhaps not so benign after all. *Neuron* 1998;21:1231–1234.
3. Sayin U, Sutula TP, Stafstrom CE. Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. *Epilepsia* 2004;45:1539–1548.
4. Holmes GL, Gaiarsa J-L, Chevassus-Au-Louis N, Ben-Ari Y. Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Ann Neurol* 1998;44:845–857.
5. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. *Lancet Neurol* 2006;5:1055–1063.
6. Jensen FE. The role of glutamate receptor maturation in perinatal seizures and brain injury. *Int J Dev Neurosci* 2002;20:339–347.

7. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. Gamma-aminobutyric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem* 2001;77:1266–1278.
8. Stafstrom CE. Behavioral and cognitive testing procedures in animal models of epilepsy. In: Pitkanen A, Schwartzkroin PA, Moshe SL, eds. *Models of Seizures and Epilepsy*. Amsterdam: Elsevier Academic Press, 2006:613–628.
9. Swann JW, Le JT, Lee CL. Recurrent seizures and the molecular maturation of hippocampal and neocortical glutamatergic synapses. *Dev Neurosci* 2007;29:168–178.
10. Holmes GL, Ben-Ari Y. A single episode of neonatal seizures permanently alters glutamatergic synapses. *Ann Neurol* 2007;61: 379–381.
11. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nat Neurosci* 2005;11:1205–1213.
12. Lynch M, Sayin U, Bownds J, Janumpalli S, Sutula T. Long-term consequences of early postnatal seizures on hippocampal learning and plasticity. *Eur J Neurosci* 2000;12:2252–2264.
13. Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben-Ari Y, Holmes GL. Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur J Neurosci* 2004;19:590–600.

Go "West," Young Man. . . The Quest for Animal Models of Infantile Spasms (West Syndrome)

Model of Infantile Spasms Induced by N-methyl-D-aspartic Acid in Prenatally Impaired Brain. Velisek L, Jehle K, Asche S, Veliskova J. *Ann Neurol* 2007;61(2):109–119. OBJECTIVE: Infantile spasms (a catastrophic epileptic syndrome of childhood) are insensitive to classic antiepileptic drugs. New therapies are limited by lack of animal models. Here we develop a new model of flexion spasms based on prenatal exposure to betamethasone combined with postnatal administration of *N*-methyl-D-aspartic acid (NMDA) and determine brain structures involved in the induction of flexion spasms. METHODS: Pregnant rats received two doses of betamethasone on day 15 of gestation. Offspring was injected with NMDA on postnatal day 15. Effects of adrenocorticotropin therapy on the development of age-specific flexion spasms were determined and electroencephalographic correlates recorded. C-fos immunohistochemistry and [^{14}C]2-deoxyglucose imaging identified brain structures involved in the development of flexion spasms. RESULTS: Prenatal betamethasone exposure sensitizes rats to development of NMDA-induced spasms and, most importantly, renders the spasms sensitive to adrenocorticotropin therapy. Ictal electroencephalogram results correspond to human infantile spasms: electrodecrement or afterdischarges were observed. Imaging studies defined three principal regions involved in NMDA spasms: limbic areas (except the dorsal hippocampus), hypothalamus, and the brainstem. INTERPRETATION: Despite certain limitations, our new model correlates well with current infantile spasm hypotheses and opens an opportunity for development and testing of new effective drugs.

COMMENTARY

One hundred sixty years after Dr. West's well-known description of his son's infantile spasms (1), over 50 years after the identification of the classical EEG pattern of this disorder, and 45 years after the term West syndrome was suggested (2), there is little more than a woefully incomplete understanding of this entity. Indeed, there is minimal consensus even about the basic characteristics of this syndrome: is it a generalized seizure disorder or do the spasms emanate from a hidden focus, such as is often seen in infantile spasms associated with tuberous sclerosis? What are the fundamental neuronal circuits supporting spasms and hypsarrhythmia? Specifically, what is the role of the brain stem? What are the relative roles of spasms and hypsarrhythmia in the derangement of normal brain function found in affected infants? Is the hypsarrhythmia interictal or does it represent status epilepticus?

Numerous genetic (3), neurophysiological, and imaging (4) methods have been employed to investigate these and related questions in infants with infantile spasms. However, an understanding of the neurobiology of this syndrome requires an animal model (5–8). The criteria for a suitable model for infantile spasms have been debated (6,7), but no consensus exists on the critical elements that render an animal model ideal or even suitable for studying infantile spasms. For example, the requirement that hypsarrhythmia be included has been questioned, and this pattern, to date, has been reported in an animal model only as an abstract (9).

Epilepsy Currents, Vol. 7, No. 6 (November/December) 2007 pp. 165–167
Blackwell Publishing, Inc.

A second remarkable feature of infantile spasms, their response to adrenocorticotropic hormone (ACTH), also has been considered an element crucial to a useful animal model for infantile spasms. The pioneering work of Sorel and colleagues suggested that the neuropeptide ACTH, acting directly within the brain, might suppress infantile spasms. Early anecdotal success of ACTH therapy was confirmed by blinded controlled studies, although the rate of ACTH efficacy for infantile spasms varied widely, from ~40 to 88% (10,11). While these studies established ACTH as a robust and selective therapy for eradicating infantile spasms and hypsarrhythmia, they failed to explore underlying mechanisms. More recently, a novel mechanism of action for ACTH has been demonstrated; it involves direct action of the hormone on melanocortin receptors, resulting in reduced expression of the excitatory neuropeptide corticotropin-releasing hormone (CRH) within seizure-prone limbic regions (12). This mechanism dissociates the anti-infantile spasms effects of ACTH and its steroid-releasing actions, suggesting that analogs of ACTH that bind to melanocortin receptors (but do not release steroids) may provide effective therapy for infantile spasms, without the multiple systemic side effects of steroids (12,13).

The conceptual framework for the direct effects of ACTH on CNS neurons is built around the hypothesis that ACTH reduces the expression of CRH and that CRH is found in abnormally high levels in patients with infantile spasms. Indeed, infants with infantile spasms have abnormal levels of ACTH and cortisol in their CSF, findings consistent with elevated brain levels of CRH. The expression as well as secretion of CRH (part of the CNS–adrenal stress system) is increased by stress in several brain regions. When released during stress from hippocampal (14), amygdalar (15) and certain brainstem (16) neurons, CRH acts via a G-protein–coupled receptor to excite neurons (17), at least in part, by reducing after-hyperpolarization (18). The stress/CRH hypothesis for the pathophysiology of infantile spasms posits that myriad events that lead to infantile spasms are stressful to the developing brain, resulting in excessive expression and release of CRH in limbic and brainstem regions (8). CRH, in turn, provokes spasms and excessive neuronal synchrony (i.e., hypsarrhythmia). ACTH eliminates infantile spasms not by acting as an anticonvulsant (19), but by reducing expression and secretion of endogenous CRH (12).

The potential role of early life stress in the pathophysiology of infantile spasms is embraced by Velisek et al., in the current work. Previously, Mares et al. described flexor type movements induced by NMDA administration to immature rats (20). These movements resemble the most common variant of infantile spasms, flexion spasms. However, ACTH was not helpful in resolving NMDA-evoked seizures. Therefore, in the current work, Velisek et al. queried whether subjecting rats to prenatal stress followed by the NMDA challenge would render the NMDA-provoked seizures responsive to ACTH. The group injected pregnant rat dams with high doses of a synthetic glucocorticoid to simulate prenatal stress, then administered NMDA on postnatal day 15. This prenatal treatment allowed ACTH to increase the latency for NMDA-provoked seizures. The immediate effect of ACTH in rodents differs from its time course in humans, in whom suppression of infantile spasms and hypsarrhythmia commences after a median of 2 days (11); a finding consistent with transcriptional actions on CRH expression (12). In other immature animal models, such as kindling (21) or CRH-provoked seizures (19), ACTH also failed to demonstrate direct anticonvulsant effects.

In conclusion, the work by Velisek et al. is a valiant effort to create a model for infantile spasms by incorporating three elements: (1) drug-induced seizures that behaviorally resemble spasms; (2) simulated prior stress (although injection of glucocorticoids may not recapitulate the crucial elements by which prenatal stress excites neurons [22]); and (3) a response to ACTH that results in seizure reduction. The model falls short of ideal or suitable models, as delineated by a recent NIH workshop (7). However, because infantile spasms are a common and devastating entity and because optimal models are currently lacking, the efforts of this group offer a useful paradigm for studying certain semiological aspects of infantile spasms.

by Tallie Z. Baram, MD, PhD

References

1. West WJ. On a peculiar form of infantile convulsions. *Lancet* 1841;1:724–725.
2. Eling P, Renier WO, Pomper J, Baram TZ. The mystery of the Doctor's Son, or the riddle of west syndrome. *Neurology* 2002;58:953–955.
3. Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, Lutcherath V, Gedeon AK, Wallace RH, Scheffer IE, Turner G, Partington M, Frints SG, Fryns JP, Sutherland GR, Mulley JC, Gecz J. Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet* 2002;30:441–445.
4. Chugani HT, Chugani DC. Basic mechanisms of childhood epilepsies: studies with positron emission tomography. *Adv Neurol* 1999;79:883–891.
5. Baram TZ, Hirsch E, Snead OC, 3rd, Schultz L. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. *Ann Neurol* 1992;31:488–494.
6. Stafstrom CE, Holmes GL. Infantile spasms: criteria for an animal model. *Int Rev Neurobiol* 2002;49:391–411.
7. Stafstrom CE, Moshe SL, Swann JW, Nehlig A, Jacobs MP, Schwartzkroin PA. Models of pediatric epilepsies: strategies and opportunities. *Epilepsia* 2006;47:1407–1414.
8. Baram TZ. Models for infantile spasms: an arduous journey to the Holy Grai. *Ann Neurol* 2007;61:89–91.

9. Lee CL, Frost JD, Swann JW, Hrachovy RA. Neonatal blockade of cortical hippocampal activity: a possible model of infantile spasms. *Epilepsia* 2006;3.057. Abstract.
10. Hrachovy RA, Frost JD, Glaze DG. High-dose long-duration versus low-dose short duration corticotropin therapy for infantile spasms. *J Pediatr* 1994;124:803–806.
11. Baram TZ, Mitchell WG, Tournay A, Snead OC, Hanson RA, Horton EJ. High-dose corticotropin (ACTH) versus prednisone for infantile spasms: a prospective, randomized, blinded study. *Pediatrics* 1996;97:375–379.
12. Brunson KL, Khan N, Eghbal-Ahmadi M, Baram TZ. ACTH acts directly on amygdala neurons to down-regulate corticotropin releasing hormone gene expression. *Ann Neurol* 2001;49:304–312.
13. Brunson KL, Avishai-Eliner S, Baram TZ. ACTH treatment of infantile spasms: mechanisms of its effects in modulation of neuronal excitability. *Int Rev Neurobiol* 2002;49:185–197.
14. Chen Y, Brunson KL, Adelmann G, Bender RA, Frotscher M, Baram TZ. Hippocampal corticotropin releasing hormone: pre- and postsynaptic location and release by stress. *Neuroscience* 2004;126:533–540.
15. Roozendaal B, Brunson KL, Holloway BL, McGaugh JL, Baram TZ. Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *PNAS* 2002;99:13908–13913.
16. Page ME, Berridge CW, Foote SL, Valentino RJ. Corticotropin-releasing factor in the locus coeruleus mediates EEG activation associated with hypotensive stress. *Neurosci Lett* 1993;164:81–84.
17. Baram TZ, Hatalski CG. Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. *Trends Neurosci* 1998;21:471–476.
18. Aldenhoff JB, Gruol DL, Rivier J, Vale W, Siggins GR. Corticotropin releasingfactor decreases post-burst hyperpolarizations and excites hippocampal neurons. *Science* 1983;221:875–877.
19. Baram TZ, Schultz L. ACTH does not control neonatal seizures induced by administration of exogenous corticotropin-releasing hormone. *Epilepsia* 1995;36:174–178.
20. Mareš P, Velíšek L. N-Methyl-D-aspartate (NMDA)-induced seizures in developing rats. *Dev Brain Res* 1992;65:185–189.
21. Thompson J, Holmes GL. Failure of ACTH to alter transfer kindling in the immature brain. *Epilepsia* 1987;28:17–19.
22. Avishai-Eliner S, Brunson KL, Sandman CA, Baram TZ. Stressed-out, or in (utero)? *Trends Neurosci* 2002;25:518–524.

GABA Excites and Sculpts Immature Neurons Well before Delivery: Modulation by GABA of the Development of Ventricular Progenitor Cells

Excitatory GABA Action Is Essential for Morphological Maturation of Cortical Neurons *in Vivo*. Cancedda L, Fiumelli H, Chen K, Poo MM. *J Neurosci* 2007;27(19):5224–5235. GABA exerts excitatory actions on embryonic and neonatal cortical neurons, but the *in vivo* function of this GABA excitation is essentially unknown. Using *in utero* electroporation, we eliminated the excitatory action of GABA in a subpopulation of rat ventricular progenitors and cortical neurons derived from these progenitors by premature expression of the Cl^- transporter KCC2, as confirmed by the changes in the reversal potential of GABA-induced currents and the resting membrane potential after $GABA_A$ receptor blockade. We found that radial migration to layer II/III of the somatosensory cortex of neurons derived from the transfected progenitors was not significantly affected, but their morphological maturation was markedly impaired. Furthermore, reducing neuronal excitability of cortical neurons *in vivo* by overexpressing an inward-rectifying K^+ channel, which lowered the resting membrane potential, mimicked the effect of premature KCC2 expression. Thus, membrane depolarization caused by early GABA excitation is critical for morphological maturation of neonatal cortical neurons *in vivo*.

COMMENTARY

The fact that GABA excites immature neurons is now almost dogma! There is no exception to the universal rule that developing neurons have a higher internal Cl^- concentration, which roughly shifts from 25 to 30 mM in immature neurons to less than 7 mM in mature neurons. In immature neurons, GABA depolarizes neurons and brings their membrane potential to levels that are sufficient to generate sodium and calcium action potentials. This effect is also sufficient to remove the voltage-dependent Mg^{2+} block from NMDA channels, leading to a large calcium influx; thus, GABA in developing neurons acts in synergy with NMDA signaling (1). The obvious outcome is that GABA potentiating drugs, such as benzodiazepines, will exert opposite actions on the mother's brain from those that it exerts on the fetus.

The reasons underlying the differing actions of immature and mature neurons are thought to be due to an early expression

Epilepsy Currents, Vol. 7, No. 6 (November/December) 2007 pp. 167–169
Blackwell Publishing, Inc.

of the chloride cotransporter KCC2, which acts to import chloride, and a late expression of KCC2, which exports it (2). This finding and other observations indicate that GABA provides most of the excitatory drive in immature neurons by generating a large calcium influx, serving as a trophic factor, and at an appropriate stage following chloride removal, by leading to the excitatory to inhibitory (E to I) shift. Initially discovered with conventional intracellular recording techniques in immature hippocampal neurons almost 2 decades ago, these observations have been confirmed in a wide range of animal species and brain structures, including primate neurons in utero (1). It is not yet completely clear why these properties are needed for developing neurons and what advantages justified their evolutionary conservation. However, a variety of hypotheses can be put forward, with chloride control, water balance, and osmotic pressure all being likely factors.

Although these basic elements have been known for over 2 decades, the implications are just beginning to be understood. Indeed, if GABA excites immature neurons, the effect will be to excite a host of activity-dependent mechanisms, and a plethora of developmental mechanisms are activity dependent, including neuronal migration, differentiation, neuronal growth, synapse, and network formation. The hypothesis that GABA acts as a trophic factor has long been suggested based on in vitro neuronal culture experiments. The recent article by Cancedda and colleagues addresses these issues using a genetic manipulation that imposes an early removal of chloride by expressing KCC2 in immature neurons and thus, instigating an early shift of the actions of GABA and inhibiting these neurons sooner than normally would occur. The investigators used an in utero transfection technique (3) that enables embryos to be transfected with green fluorescent protein (GFP) in addition to the KCC2 construct and then performed assessments during various delays after delivery. To confirm the success of the operation, they measured the GABA reversal potential, which enables estimation of the chloride concentration and the polarity of the actions of GABA. As expected, neurons with the GFP label had more hyperpolarized actions of GABA, and transfected neurons had a lower ongoing activity, confirming the inhibitory actions of GABA.

The authors found, in essence, that migration of neurons, at least to layers 2 to 3 of the cortex, were not affected by the manipulation, suggesting that neurons will migrate to their normal target even with a hyperpolarizing GABA. Also, cortical layering was not affected, indicating that the polarity of the actions of GABA does not play a role in migration. However, the morphology and total length and arbor of dendrites differ significantly between neurons with Excitatory GABA (transfected with EGFP) and those with inhibitory GABA (transfected with KCC2/GFP construct). Interestingly, neurons transfected with an inward-rectifying K^+ channel vector, which also leads to a hyperpolarization, produce the same result. Therefore, imposing an early inhibitory GABA or hyperpolarizing neurons alters their growth.

A previous study, using a similar paradigm, found that an early transfection of KCC2 in neurons in cultures produced (in addition to the expected E to I shift in the actions of GABA) a massive increase of the density of GABAergic synapses (4). The number of GABA, but not glutamate synapses, was increased, as was the frequency of miniature synaptic currents. Therefore, the timely removal of chloride and the E to I shift is a pivotal signal for growth and formation of GABA synapses. This observation, which was not expected a priori, indicates an important biological function in the inhibitory actions of GABA as a major growth and formation signal of development. Interestingly, another recent study showed that shortly before delivery, there is a transient and abrupt E to I shift, which also is mediated by a dramatic reduction in the removal of internal Cl^- concentration such that internal levels drop transiently to values that are lower than those ever observed again in development or in the adult. This shift is triggered by the release of maternal oxytocin, which also induces labor (5). Both observations illustrate the strategic function of the excitatory to inhibitory shift of the actions of GABA.

In summary, the excitatory actions of GABA are now tied to a variety of major developmental issues. The clinical implications of this work are still uncertain. However, the impact of substances that act on GABA systems (including alcohol) and are consumed during gestation—a time when they produce opposite effects on GABAergic transmission in the mother and fetus—may have been underestimated. Recent studies in fact do suggest, somewhat in contrast to the study of Cancedda and colleagues, that migration and network formation are also affected by GABA-acting drugs. Thus, in vitro assays show that GABA receptor antagonists significantly retard neuronal migration and produce small ensembles of displaced neurons (6). The clinical implications of these observations are important particularly in relation to the epilepsies. Indeed, a type of reversed shift of the actions of GABA (i.e., from I to E) appears now to be a basic feature of epileptic networks, including human epileptic neurons (7). Concepts presented in these and similar studies suggest that: epileptogenesis recapitulates ontogenesis. Other studies suggest that GABA-acting drugs may exert deleterious actions on cortical construction. For instance, a recent study shows that some antiepileptic agents given during gestation may produce heterotopic masses in the fetus, most likely by affecting neuronal migration (8). Clearly, this domain of research will fuel a lot of renewed efforts to analyze the actions of GABA-acting drugs on fetal development.

by Yehezkel Ben-Ari, PhD

References

1. Ben Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci* 2002;3:728–739.
2. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Laftinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K+/Cl− co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 1999;397:251–255.
3. Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, Loturco JJ. RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci* 2003;6:1277–1283.
4. Chudotvorova I, Ivanov A, Rama S, Hubner CA, Pellegrino C, Ben-Ari Y, Khazipov R. Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. *J Physiol* 2005;566(Pt 3):671–679.
5. Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hübner CA, Represa A, Ben-Ari Y, Khazipov R. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 2006;314:1788–1792.
6. Manent JB, Jorquera I, Ben-Ari Y, Aniksztejn L, Represa A. Glutamate acting on AMPA but not NMDA receptors modulates the migration of hippocampal interneurons. *J Neurosci* 2006;26:5901–5909.
7. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science* 2002;298:1418–1421.
8. Manent JB, Jorquera I, Mazzucchelli I, Depaulis A, Perruca E, Ben-Ari Y, Represa A. Fetal exposure to GABA-acting antiepileptic drugs generates hippocampal and cortical dysplasias. *Epilepsia* 2007;48:684–693.