ANIMAL MODELS OF INFANTILE SPASMS: IS THE HOLY GRAIL FINALLY IN SIGHT?

A New Animal Model of Infantile Spasms with Unprovoked Persistent Seizures. Lee CL, Frost JD Jr, Swann JW, Hrachovy RA. *Epilepsia* 2008;49(2):298–307. PURPOSE: Infantile spasms is one of the most severe epileptic syndromes of infancy and early childhood. Progress toward understanding the pathophysiology of this disorder and the development of effective therapies has been hindered by the lack of a relevant animal model. We report here the creation of such a model. METHODS: The sodium channel blocker, tetrodotoxin (TTX), was chronically infused into the developing neocortex or hippocampus of infant rats by way of an osmotic minipump starting on postnatal day 10–12. RESULTS: After a minimum of 10 days of infusion, approximately one-third of these rats began to display very brief (1–2 s) spasms, which consisted of symmetric or asymmetric flexion or extension of the trunk and sometimes involvement of one or both forelimbs. The typical ictal EEG pattern associated with the behavioral spasms consisted of an initial generalized, high amplitude, slow wave followed by an electrodecrement with superimposed fast activity. The interictal EEG revealed multifocal spikes and sharp waves, and in most animals that had spasms a hypsarrhythmic pattern was seen, at least intermittently, during NREM sleep. Like in humans, the spasms in the rat often occurred in clusters especially during sleep–wake transitions. Comparison of the ictal and interictal EEGs recorded in this model and those from humans with infantile spasms revealed that the patterns and the frequency components of both the ictal events and hypsarrhythmia were very similar. DISCUSSION: The TTX model of infantile spasms should be of value in furthering an understanding of the pathophysiology of this seizure disorder.

COMMENTARY

Infantile spasms is a severe developmental epilepsy syndrome, with several unique clinical features (1). First, the disorder occurs during a specific window in the first year of life, commonly starting between 3 and 8 months of age. Second, infantile spasms can be caused by numerous etiologies, either acquired or congenital, which typically begin weeks-to-months prior to the onset of spasms (2). Therefore, a latent period exists prior to infantile spasms during which neural circuits become epileptogenic in an as yet unknown manner (3). Third, infantile spasms is associated with very specific and unique encephalographic findings consisting of interictal hypsarrhythmia (chaotic high-voltage slow waves, intermixed with multifocal spikes) and ictal electrodecrement (generalized attenuation of waveforms). Fourth, most anticonvulsants are not effective for infantile spasms; treatments that are sometimes effective include glucocorticoids, adrenocorticotrophic hormone (ACTH), and vigabatrin (4). Fifth, the outcome of infantile spasms is often poor, especially when the spasms do not improve with therapy and when neurological development is abnormal prior to the onset of spasms. In summary, the lack of a detailed pathophysiological explanation for many aspects of infantile spasms makes this epilepsy syndrome (sometimes referred to as a "catastrophic" epilepsy) a frustrating clinical enigma.

Progress in understanding and treating infantile spasms has been limited in part by the lack of an appropriate animal model in which to study the underlying neurobiological mechanisms. In fact, an animal model for infantile spasms has been considered to be a "holy grail" of epilepsy research (5). Ideal and minimal criteria for an animal model of infantile spasms have been discussed (6,7). Optimally, the model would mimic the human disorder, including a specific developmental window during which spasm-type seizures begin, maximal spasm occurrence in relationship to the sleep–wake cycle (especially shortly after arousal from sleep), and prevalence of spasms in clusters. The validity of an experimental model would be strengthened if EEG findings in the animal resemble interictal hypsarrhythmia and ictal electrodecrement. An ideal model also would include responsiveness to anticonvulsants that ameliorate spasms in human infants. Humans and other animal species differ markedly in the trajectory of their brain development and behavioral neurological repertoire, so it is unlikely that a single animal model will fulfill all of these criteria. Thus, a model should not necessarily be excluded if it does not meet all criteria, as long as it is relevant to understanding the pathophysiology of infantile spasms.

The developmental epilepsy model presented in the report by Lee et al. is exciting because it demonstrates a heretofore

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elusive criterion: an EEG pattern that closely resembles human hypsarrhythmia. In this model, infant rats develop spasm-type seizures following chronic infusion of the sodium-channel blocker tetrodotoxin (TTX). During the infusion and long after its discontinuation, rats displayed brief spasms involving the trunk and/or limbs. EEG tracings verified an interictal pattern that closely resembles hypsarrhythmia in a human infant. Furthermore, when a spasm occurred, the EEG showed a decremental response following an initial generalized slow wave, again, similar to the human EEG pattern. To support the clinical applicability of the model, the authors present side-by-side examples of human and rat EEGs, including power spectra that exhibited convincing electrophysiological signatures. Therefore, the behavioral interictal- and ictal-EEG patterns as well as temporal features of seizures in this rodent model are reasonably comparable to human infantile spasms and hold promise for the study pathophysiological mechanisms of infantile spasms.

How does chronic infusion of TTX lead to infantile spasms in rats? By blocking neural impulses, TTX depresses neural activity at the infusion site and creates a hyperexcitable condition well beyond the focal administration site. Interestingly, despite the focal nature of the TTX infusions, EEG changes are predominantly generalized, as are the clinical seizures in these rats. Similarly, in humans, focal lesions often lead to symmetric or generalized spasms in EEG findings (8). Somehow, developmentally specific suppression of neural discharges endows the brain with an unusual pattern of hyperexcitability that produces spasms and accompanying EEG patterns (9,10). The mechanism of TTX-induced hyperexcitability requires considerable further investigation.

Given the large number of genetic and acquired etiologies of human infantile spasms, it is likely that several mechanisms interact to cause the clinical syndrome. It has been proposed that all these myriad etiologies converge at a final common pathway to result in the relatively homogeneous entity of infantile spasms (11). This hypothesis states that infantile spasms represent the response of the developing nervous system to any number of stressors that cause the neuronal release of corticotropin-releasing hormone (CRH) (12). CRH is acutely convulsant in the infant brain (but not the adult brain) and is a known endogenous cotransmitter in several brain structures sensitive to seizure generation (e.g., the hippocampus and amygdala). According to this hypothesis, ACTH acts not as an anticonvulsant but by suppressing the stress-provoked, excessive production of CRH. However, seizures following CRH injection lack some of the clinical features of infantile spasms.

Other infantile spasms models are now on the horizon, each presenting a potentially different mechanism of action. One new model involves prenatal administration of the glucocorticoid betamethasone, which causes a prenatal stress, followed by postnatal administration of NMDA, which causes spasm-like seizures in infant rats that are responsive to ACTH administration (13). Another model combines administration of doxorubicin and lipopolysaccharide to produce structural damage and reduce serotonin levels, with resultant spasm-like seizures (14). Finally, administration of a $GABA_B$-receptor agonists in a mouse model of Down syndrome results in extensor spasms and an accompanying electrodecremental EEG response (15). Together, these infantile spasms models hold the promise to increase understanding of the pathophysiology of this devastating developmental epilepsy.

Ideally, the following questions might be answered by an animal model:

- How does specific developmental sensitivity to particular insults, occurring at certain ages of brain maturation, result in such a unique syndrome?
- Can new modes of therapy be devised, based on pathophysiological mechanisms?
- Are there effective nonpharmacological methods of treatment that could be sought?
- Can approaches be developed to prevent the adverse cognitive outcomes frequently associated with infantile spasms?

Research may well be entering a new era of understanding infantile spasms and other catastrophic epilepsies of childhood. The beneficiaries of such knowledge clearly would be affected children and their families, who currently are faced with ineffective or exorbitantly expensive treatment options. In that sense, despite numerous challenges that lie ahead, the Holy Grail may actually be in sight.

by Carl E. Stafstrom, MD, PhD

References

1. Shields WD. Infantile spasms: little seizures, BIG consequences. *Epilepsy Curr* 2006;6:63–69.
2. Guggenheim MA, Frost JD Jr, Hrachovy RA. Time interval from a brain insult to the onset of infantile spasms. *Pediatr Neurol* 2008;38:34–37.
3. Frost JD Jr, Hrachovy RA. Pathogenesis of infantile spasms: a model based on developmental desynchronization. *J Clin Neurophysiol* 2005;22:25–36.
4. Mackay MT, Weiss SK, Adams-Webber T, Ashwal S, Stephens D, Ballaban-Gil K, Baram TZ, Duchowny M, Hirtz D, Pellock JM, Shields WD, Shinnar S, Wyllie E, Snead OC 3rd. Practice parameter: medical treatment of infantile spasms. *Neurology* 2004;62:1668–1681.
5. Baram TZ. Models for infantile spasms: An arduous search to the holy grail. *Ann Neurol* 2007;61:89–91.

6. Stafstrom CE, Holmes GL. Infantile spasms: criteria for an animal model. In: Schwartzkroin PA, Rho JM, eds, *Epilepsy, Infantile Spasms, and Developmental Encephalopathy. Vol. 49*. Boston: Academic Press, 2002:391–411.
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. Acharya JN, Wyllie E, Luders HO, Kotagal P, Lancman M, Coelho M. Seizure symptomatology in infants with localization-related epilepsy. *Neurology* 1997;48:189–196.
9. Galvan CD, Wenzel JH, Dineley KT, Lam TT, Schwartzkroin PA, Sweatt JD, Swann JW. Postsynaptic contributions to hippocampal network hyperexcitability induced by chronic activity blockade in vivo. *Eur J Neurosci* 2003;18:1861–1872.
10. Bausch SB, He S, Petrova Y, Wang XM, McNamara JO. Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. *J Neurophysiol* 2006;96:2151–2167.
11. Brunson KL, Eghbal-Ahmadi M, Baram TZ. How do the many etiologies of West syndrome lead to excitability and seizures? The corticotropin releasing hormone excess hypothesis. *Brain Dev* 2001;23:533–538.
12. Avishai-Eliner S, Brunson KL, Sandman CA, Baram TZ. Stressed-out, or in (utero)? *Trends Neurosci* 2002;25:518–524.
13. Velísek L, Jehle K, Asche S, Velísková J. Model of infantile spasms induced by N-methyl-D-aspartic acid in prenatally impaired brain. Ann Neurol. 2007;61:109–119.
14. Scantlebury MH, Moshe SL. A new model of infantile spasms. *Amer Epil Soc Abstr* 2006.
15. Snead OC 3rd, Stewart L, Aleem I, Shen L, Ashraff A, Cortez MA. A new model of infantile spasms: genotypic, EEG, and behavioral characterization. *Neurology* 2007;68(suppl 1):A211.

Rational Polypharmacy: When Two Old Drugs Are Better Than One

Bumetanide Enhances Phenobarbital Efficacy in a Neonatal Seizure Model. Dzhala VI, Brumback AC, Staley KJ. *Ann Neurol* 2008;63(2):222–235. OBJECTIVES: High levels of expression of the Na^+-K^+-$2Cl^-$ (NKCC1) cotransporter in immature neurons cause the accumulation of intracellular chloride and, therefore, a depolarized Cl^- equilibrium potential (E_{Cl}). This results in the outward flux of Cl^- through $GABA_A$ channels, the opposite direction compared with mature neurons, in which $GABA_A$ receptor activation is inhibitory because Cl^- flows into the cell. This outward flow of Cl^- in neonatal neurons is excitatory and contributes to a greater seizure propensity and poor electroencephalographic response to GABAergic anticonvulsants such as phenobarbital and benzodiazepines. Blocking the NKCC1 transporter with bumetanide prevents outward Cl^- flux and causes a more negative GABA equilibrium potential (E_{GABA}) in immature neurons. We therefore tested whether bumetanide enhances the anticonvulsant action of phenobarbital in the neonatal brain. METHODS: Recurrent seizures were induced in the intact hippocampal preparation in vitro by continuous 5-hour exposure to low-Mg^{2+} solution. The anticonvulsant efficacy of phenobarbital, bumetanide, and the combination of these drugs was studied. RESULTS: Phenobarbital failed to abolish or depress recurrent seizures in 70% of hippocampi. In contrast, phenobarbital in combination with bumetanide abolished seizures in 70% of hippocampi and significantly reduced the frequency, duration, and power of seizures in the remaining 30%. INTERPRETATION: Thus, alteration of Cl^- transport by bumetanide enables the anticonvulsant action of phenobarbital in immature brain. This is a mechanistic demonstration of rational anticonvulsant polypharmacy. The combination of these agents may comprise an effective therapy for early-life seizures.

COMMENTARY

Although traditionally assigned the role as the main inhibitory influence that prevents excessive excitation and seizures in the brain, the neurotransmitter GABA plays a much more complex role than initially imagined. As reviewed in recent *Epilepsy Currents* commentaries (1,2), depending on the intracellular chloride content of postsynaptic neurons, GABA may also be excitatory, even promoting epileptiform activity. The postsynaptic effect of GABA stems from the opening of chloride channels within the $GABA_A$-receptor complex: when intracellular chloride is kept low because of its extrusion by the K^+/Cl^--cotransporter isoform 2, KCC2, $GABA_A$-receptor activation causes the flow of negatively charged chloride ions into the cell. The resulting hyperpolarization of the neuronal membrane is the basis for the inhibitory effect on postsynaptic neurons and for the antiepileptic effects of drugs (e.g., benzodiazepines and barbiturates) that enhance GABAergic neurotransmission. However, when intracellular chloride is high, which occurs when KCC2 expression is reduced or when the $Na^+/K^+/Cl^-$-cotransporter isoform 1 (NKCC1) is expressed, $GABA_A$-activated chloride flow is reversed, and GABA is depolarizing, even surpassing the threshold for action

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potential generation. Because the reversal potential for $GABA_A$-activated chloride currents is never as depolarized as that for the glutamate-receptor activated currents, GABA can still have a "shunting" effect on excitatory potentials and remain a relatively stabilizing influence on excitability.

During the early postnatal period in rodents and likely during the late prenatal and early postnatal period in humans, NKCC1 is expressed highly in the brain, while KCC2 expression is low. Under these conditions, GABA release contributes to the initiation of giant depolarizing potentials (GDPs) in populations of neurons. These GDPs not only serve as a marker of GABAergic excitatory influence but also play a critical role in the development of mature synapses. As the brain matures, NKCC1 expression is downregulated; however, recent evidence points to a recapitulation of this early developmental pattern of chloride transporter expression in neurons exposed to injury and in hippocampal neurons from patients with mesial temporal lobe epilepsy.

Previous studies have shown that the excitatory effect of GABA is at least partially responsible for the increased susceptibility to seizures in neonates (3). Bumetanide, a loop diuretic that potently inhibits NKCC1 transporter, impairs the intracellular accumulation of chloride and thereby, may convert GABA from excitatory to inhibitory. Accordingly, bumetanide abolishes GDPs in neonatal brain and prevents experimental seizures both in vitro and in vivo (3). Not surprisingly, because phenobarbital potentiates the effects of GABA, it is ineffective in reducing seizure activity in the same seizure model.

In clinical practice as well, phenobarbital fails to control acute seizures in more than half of neonates (4). Nevertheless, many physicians continue to choose barbiturates or benzodiazepines as first line agents, perhaps because of their familiarity and lack of compelling data for alternate therapies. In the present study by Dzhala et al., a possible new treatment strategy was tested in the established in vitro low-magnesium seizure model, using intact hippocampi from neonatal rats. This study design may provide a more robust model of hippocampal seizures because the hippocampal circuitry remains intact and the induction of seizures did not involve alteration of ion gradients that may influence chloride homeostasis. The authors hypothesized that if inhibition of NKCC1 with bumetanide alters the chloride gradient of neonatal hippocampal neurons such that GABA becomes more hyperpolarizing, then phenobarbital will gain the seizure suppressive effect that it has in mature brains.

To test their hypothesis, the authors first showed the effects of these drugs on chloride homeostasis and neuronal activity. The reversal potential for $GABA_A$-activated currents in immature pyramidal neurons was –63 mV, compared with a resting potential of –70 mV, indicating that GABA was in fact depolarizing. Consistent with this result, a $GABA_A$-receptor agonist (i.e., isoguvacine) exerted a net excitatory effect, increasing neuronal firing. As expected, although phenobarbital augmented $GABA_A$-activated currents, it did not reduce its excitatory effect. When bumetanide was added, the reversal potential for GABA shifted to –73 mV, suggesting that GABA had become inhibitory. Importantly, the administration of phenobarbital in combination with bumetanide now suppressed neuronal firing.

The authors next asked whether bumetanide could alter the effect of phenobarbital on seizure activity. Bathing intact hippocampi from neonatal rats in a low-magnesium solution induced recurrent seizure-like discharges, which was probably related to hyperexcitability induced when the magnesium block of the NMDA receptor was relieved. In this model, phenobarbital alone stopped seizure-like events in 30% of hippocampi and decreased frequency in others; however, it also increased duration and amplitude of the power spectra of the population activity in those hippocampi, indicating that by some measures the remaining seizures were worsened. Bumetanide alone had some efficacy against seizures: although it abolished seizures in only 20% of hippocampi, it decreased frequency, duration, and amplitude of power spectra in the others. The combination of bumetanide with phenobarbital produced a much stronger effect: seizures were abolished in 70% of hippocampi, and their frequency, duration, and power spectra were reduced in the remaining hippocampi more than with bumetanide alone. These findings support the clinical observation that phenobarbital alone is unlikely to stop seizures in neonates, and bumetanide, although itself weakly anticonvulsant, is also unlikely to produce a clinically satisfactory effect. However, the combination of increasing the inhibitory efficacy of GABA with bumetanide and potentiating its effect with phenobarbital, even when administered simultaneously, has the potential to be a powerful anticonvulsant therapy for neonatal seizures.

While Dzhala et al. used an animal model to demonstrate the efficacy of bumetanide and phenobarbital to decrease artificially induced seizures, the application of these two drugs to the treatment of human epilepsy is logical. Presumably, the strategy will work with benzodiazepines as well. The authors argue that bumetanide has demonstrated safety in neonates, and in vivo studies suggest effective penetration into brain (3); however, Dzhala and colleagues are careful to note the uncertain consequences of altering the delicate balance of inhibitory and excitatory influences in the developing brain using bumetanide. As previously mentioned, depolarizing GABA responses such as GDPs play an important role in the development of mature synapses, therefore long-term administration of bumetanide could have unintended and potentially untoward effects that may manifest later in life. Phenobarbital itself has been implicated in deleterious effects on neuron survival (5) and in persistent cognitive deficits after use during critical developmental stages (6). Therefore, greater

efficacy of a drug combination against typically treatment-resistant neonatal seizures may allow for use of lower doses or shorter duration of phenobarbital therapy. In addition, the benefit of providing earlier control of seizure activity may itself reduce potential long-term consequences of neonatal seizures. Finally, because of mounting evidence for altered chloride homeostasis in adult epileptic brain, the prospective use of this combination therapy may extend beyond neonates to rational combinations of bumetanide and GABA-enhancing medications in treatment-resistant adult epilepsy.

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References

1. Mathews GC. The dual roles of GABA in seizures and epilepsy generate more excitement. *Epilepsy Curr* 2007;7(1):28–30.
2. Kahle KT, Staley K. Altered neuronal chloride homeostasis and excitatory GABAergic signaling in human temporal lobe epilepsy. *Epilepsy Curr* 2008;8(2):51–53.
3. 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 Med* 2005;11(11):1205–1213.
4. Painter MJ, Scher MS, Stein AD, Armatti S, Wang Z, Gardiner JC, Paneth N, Minnigh B, Alvin J. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999;341(7):485–489.
5. Bittigau P, Sifringer M, Genz K, Reith E, Pospischil D, Govindarajalu S, Dzietko M, Pesditschek S, Mai I, Dikranian K, Olney JW, Ikonomidou C. Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. *Proc Natl Acad Sci USA* 2002;99(23):15089–15094.
6. Sulzbacher S, Farwell JR, Temkin N, Lu AS, Hirtz DG. Late cognitive effects of early treatment with phenobarbital. *Clin Pediatr (Phila)* 1999;38(7):387–394.

VEGF As a Target for Neuroprotection

Vascular Endothelial Growth Factor Is Up-Regulated after Status Epilepticus and Protects against Seizure-Induced Neuronal Loss in Hippocampus. Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, Hylton D, Rudge JS, Scharfman HE, Croll SD. *Neuroscienc*e 2008;151(1):232–241. Vascular endothelial growth factor (VEGF) is a protein factor which has been found to play a significant role in both normal and pathological states. Its role as an angiogenic factor is well-established. More recently, VEGF has been shown to protect neurons from cell death both in vivo and in vitro. While VEGF's potential as a protective factor has been demonstrated in hypoxia–ischemia, in vitro excitotoxicity, and motor neuron degeneration, its role in seizure-induced cell loss has received little attention. A potential role in seizures is suggested by Newton et al.'s [Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS (2003) Gene profile of electroconvulsive seizures: Induction of neurotrophic and angiogenic factors. *J Neurosci* 23:10841–10851] finding that VEGF mRNA increases in areas of the brain that are susceptible to cell loss after electroconvulsive-shock induced seizures. Because a linear relationship does not always exist between expression of mRNA and protein, we investigated whether VEGF protein expression increased after pilocarpine-induced status epilepticus. In addition, we administered exogenous VEGF in one experiment and blocked endogenous VEGF in another to determine whether VEGF exerts a neuroprotective effect against status epilepticus-induced cell loss in one vulnerable brain region, the rat hippocampus. Our data revealed that VEGF is dramatically up-regulated in neurons and glia in hippocampus, thalamus, amygdala, and neocortex 24 h after status epilepticus. VEGF induced significant preservation of hippocampal neurons, suggesting that VEGF may play a neuroprotective role following status epilepticus.

COMMENTARY

Vascular endothelial growth factor (VEGF) is a vascular growth factor that induces angiogenesis, increases vascular permeability, and promotes inflammation in the CNS (1,2). VEGF, originally considered as an endothelial-specific growth factor and a potent mitogen for endothelial cells derived from arteries, veins, and lymphatics, has recently been shown to have direct effects on different cell types, including neurons, Schwann cells, astrocytes, neural stem cells, and microglia. Increased levels of VEGF in the brain have been measured after a variety of insults, including hypoxia/ischaemia and seizures. In particular, following seizure induction in experimental models, VEGF was expressed mainly by neurons and astrocytes (3; Nicoletti et al.), while in human temporal lobe epilepsy specimens, prominent VEGF immunostaining was found also in the microvasculature. The identification of VEGF receptors in epileptogenic tissue—not only on endothelial cells of blood vessels (3) but also on astrocytes and neurons (1,2,4)—raised

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the question of the functional consequences of seizure-induced increases in the brain level of VEGF (other than those well described on brain vessels). Neurotrophic and neuroprotective effects of VEGF have been reported in several in vitro and in vivo experimental conditions, suggesting the possibility that this protein may afford neuroprotection by acting directly on neuronal receptors. Indeed, VEGF receptors appear to be inducible in neurons in pathological states, such as after the induction of status epilepticus (4).

Nicoletti et al. provided evidence that VEGF is strongly upregulated in neurons and glia 24 hours after pilocarpine-induced status epilepticus and established (by pharmacological approaches) that VEGF has a neuroprotective potential against status epilepticus-induced cell loss. Using ELISA, these authors demonstrated that the level of this protein doubled in the hippocampus and cortex of rats exposed to status epilepticus; immunocytochemistry clearly showed that VEGF was upregulated in surviving neurons (the activation resolved by 7 days after status epilepticus) and in activated astrocytes. Increased VEGF expression was observed in all brain regions involved in seizure spread as well as in the associated neuronal cell loss and glia activation. Previous work also reported that the seizure-induced neuronal expression of VEGF is transient, while the astrocytic expression is still evident during epileptogenesis preceding the onset of spontaneous seizures and in chronic epileptic tissue (3).

Nicoletti et al. adopted a pharmacological approach to address the functional meaning of VEGF upregulation following seizures: they chronically infused the hippocampus with the VEGF blocker Fit-Fc (an immunoadhesin designed to sequester VEGF) at a dose known to interfere with endogenous VEGF binding or with human recombinant VEGF at a dose below the doses that are optimal for inducing angiogenesis. Control rats also were assessed, using inactivated VEGF or bovine serum albumin to control for protein load or Fc domain of human IgG (hFc), a recombinant human control protein. After 5 days of protein infusion, rats were exposed to status epilepticus and then killed after 24 hours to evaluate the degree of cell loss. Protein infusion was stopped at the time of killing. Stereological estimates of neuronal density in the infused hippocampus showed a significant increase of pyramidal neuron death in the rats receiving the VEGF blocker, while the rats receiving VEGF had less neuronal loss. Interestingly, the levels of VEGF reached in the hippocampus by this pharmacological treatment were almost 200 times higher than the endogenous increase in VEGF induced by seizures, making it unlikely that the much smaller endogenous increases in VEGF are sufficient to mediate neuroprotection. These neuroprotective effects of VEGF were observed at concentrations that were neither associated with increased vascular density or diameter nor with increased vascular permeability.

Available data suggest that VEGF is endowed with anticonvulsant effects, raising the possibility that its neuroprotective action is mediated by antiictal properties (5). Nicoletti et al., however, reported no apparent changes in motor seizure behavior during status epilepticus in rats that received VEGF or its blocker. Nevertheless, this issue requires further investigation, perhaps by using EEG recording of seizures to unequivocally demonstrate that the VEGF neuroprotection is not a consequence of reduced seizure activity. The molecular mechanisms underlying the neuroprotective effect of VEGF are still mostly unexplored; however, there is evidence that the activation of the VEGF receptor, VEGFR2 (which is overexpressed by neurons following seizures), triggers an intracellular phosphatidylinositol 3-kinase/Akt signaling pathway and inhibition of caspase-3 activity that mediate cell survival (6).

In conclusion, Nicoletti et al. envisaged that small molecules, penetrating the blood–brain barrier and mimicking VEGF neuroprotective effects, might be considered a means to provide cell protection in epilepsy. However, this attractive possibility must take into account that the protein can also provoke effects in brain tissue, such as alterations in blood–brain barrier permeability properties, increased vessel density, and inflammation, that have the potential to promote epileptogenesis (3,7–10). Therefore, a major goal would be to learn how to control the detrimental effects of VEGF and to facilitate its brain repair functions.

by Annamaria Vezzani, PhD

References

1. Croll SD, Goodman JH, Scharfman HE. Vascular endothelial growth factor (VEGF) in seizures: a double-edged sword. *Adv Exp Med Biol* 2004;548:57–68.
2. Croll SD, Ransohoff RM, Cai N, Zhang Q, Martin FJ, Wei T, Kasselman LJ, Kintner J, Murphy AJ, Yancopoulos GD, Wiegand SJ. VEGF-mediated inflammation precedes angiogenesis in adult brain. *Exp Neurol* 2004;187:388–402.
3. Rigau V, Morin M, Rousset MC, de Bock F, Lebrun A, Coubes P, Picot MC, Baldy-Moulinier M, Bockaert J, Crespel A, Lerner-Natoli M. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain* 2007;130:1942–1956.
4. Nicoletti JN, Shah SK, Khalid S, Atassi H, Croll SD. VEGFR2 upregulation following pilocarpine-induced status epilepticus in rat. *Abstract Viewer/Itinerary Planner*, Society for Neuroscience, Washington, DC (2005) Program No. 668.12.
5. McCloskey DP, Croll SD, Scharfman HE. Depression of synaptic transmission by vascular endothelial growth factor in adult rat hippocampus and evidence for increased efficacy after chronic seizures. *J Neurosci* 2005;28;8889–8897.
6. Sun FY, Guo X. Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. *J Neurosci Res* 2005;79:180–184.

7. Tomkins O, Friedman O, Ivens S, Reiffurth C, Major S, Dreier JP, Heinemann U, Friedman A. Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. *Neurobiol Dis* 2007;25:367–377.
8. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain* 2007;130:535–547.
9. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia* 2005;46:1724–1743.
10. Vezzani A, Baram TZ. New roles for interleukin-1 beta in the mechanisms of epilepsy. *Epilepsy Curr* 2007;7:45–50.

GABA Regulates Stem Cell Proliferation before Nervous System Formation

Histone H2AX-Dependent $GABA_A$ Receptor Regulation of Stem Cell Proliferation. Andäng M, Hjerling-Leffler J, Moliner A, Lundgren TK, Castelo-Branco G, Nanou E, Pozas E, Bryja V, Halliez S, Nishimaru H, Wilbertz J, Arenas E, Koltzenburg M, Charnay P, El Manira A, Ibañez CF, Ernfors P. *Nature* 2008;451(7177):460–464. Stem cell self-renewal implies proliferation under continued maintenance of multipotency. Small changes in numbers of stem cells may lead to large differences in differentiated cell numbers, resulting in significant physiological consequences. Proliferation is typically regulated in the G1 phase, which is associated with differentiation and cell cycle arrest. However, embryonic stem (ES) cells may lack a G1 checkpoint. Regulaton of proliferation in the "DNA damage" S/G2 cell cycle checkpoint pathway is known for its role in the maintenance of chromatin structural integrity. Here we show that autocrine/paracrine γ-aminobutyric acid (GABA) signalling by means of $GABA_A$ receptors negatively controls ES cell and peripheral neural crest stem (NCS) cell proliferation, preimplantation embryonic growth and proliferation in the boundary-cap stem cell niche, resulting in an attenuation of neuronal progenies from this stem cell niche. Activation of $GABA_A$ receptors leads to hyperpolarization, increased cell volume and accumulation of stem cells in S phase, thereby causing a rapid decrease in cell proliferation. $GABA_A$ receptors signal through S-phase checkpoint kinases of the phosphatidylinositol-3-OH kinase-related kinase family and the histone variant H2AX. This signalling pathway critically regulates proliferation independently of differentiation, apoptosis and overt damage to DNA. These results indicate the presence of a fundamentally different mechanism of proliferation control in these stem cells, in comparison with most somatic cells, involving proteins in the DNA damage checkpoint pathway.

COMMENTARY

GABA, the principal inhibitory neurotransmitter in the brain, is a versatile molecule that not only plays a role in synaptic transmission but also provides important signaling cues in the developing brain. The signaling function occurs through anion-permeable $GABA_A$ receptors whose effects on neuronal excitability are dependent upon the developmental stage: excitatory in immature neurons because of higher intracellular Cl^- concentration ($[Cl^-]_i$) and inhibitory in adults because of a progressive, developmentally programmed loss of $[Cl^-]_i$ (1,2). Recent studies show that GABA stimulates neurons even before synapses have been formed, suggesting that the consumption of GABA-acting drugs, including antiepileptic drugs, may affect neuronal migration and produce misplaced neurons (3). $GABA_A$ receptor-mediated events act to regulate neural stem cell proliferation both in the developing cortex (4) and in the adult subventricular zone (5), supporting the hypothesis that GABA regulates the formation of neurons and cortical units, such as achieving the balance between excitation and inhibition in the neocortex.

As described in the commentary by Mathews in this issue, the $[Cl^-]_i$ gradient is created by the expression of the $Na^+/K^+/Cl^-$-cotransporter isoform 1 (NKCC1) or the reduced expression of the K^+/Cl^- cotransporter (KCC). NKCC1 imports Cl^- and is expressed from the embryonic stage until the first postnatal week, whereas KCC2 exports Cl^- and is weakly expressed at birth and upregulated as the brain matures. These temporal patterns of transporter expression correspond to the switch from GABA being excitatory to inhibitory. In the embryonic cortex and the adult neurogenic regions, GABA depolarizes neuronal stem cells because they have high $[Cl^-]_i$ (4–7).

The Andäng et al. study reviewed here proposes that the GABAergic signaling system plays a role in the generation of embryonic stem cells. Through a variety of in vitro and in vivo studies, the authors demonstrate that mouse embryonic and neural crest stem cells express glutamic acid decarboxylase and functional $GABA_A$ receptors. They also show that stem cells possess the machinery to synthesize and respond to GABA long before the formation of the nervous system: GABA hyperpolarizes embryonic stem cells and decreases proliferation. Two

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important questions raised by this study will be discussed: How can GABA hyperpolarize stem cells and then depolarize immature neurons a few days later? By what mechanism do these actions occur, since the cotransporter that extrudes chloride is not expressed at this early stage? This issue is of importance considering the large number of antiepileptic drugs that exert their action by GABA receptors and are used during pregnancy by women with epilepsy.

Andäng and colleagues note that embryonic stem cells have a relatively depolarized resting membrane potential of −26 mV and a hyperpolarizing $GABA_A$ receptor-mediated response (E_{GABA}) of –78 mV. However, other studies have found depolarizing GABA responses in embryonic neuronal stem cells as well as in the adult hippocampus and subventricular zone (4,5,7). The discrepancy may be explained in several ways. For instance, embryonic stem cells were recorded in cell culture medium, whereas the neuronal stem cells were recorded in standard artificial cerebrospinal fluid. Without knowing the external Cl^- concentration one cannot extrapolate the internal Cl^- concentration from E_{GABA}. Furthermore, the current elicited by application of GABA was not completely blocked by the $GABA_A$-receptor antagonist, bicuculline. The residual current, carried through another channel, may have contributed to the reversal potential seen in this study. While the authors used voltage-sensitive dyes and Ca^{2+} imaging to confirm the nondepolarizing effects of GABA, a direct measurement of the Cl^- gradient would have given an unequivocal determination of the effect of GABA on embryonic stem cell membrane potential. The investigators' use of invasive recording techniques (i.e., intracellular, perforated, or whole cell recordings) may also present problems, as recent studies indicate that basic parameters, particularly the resting membrane potential, cannot be adequately measured in immature neurons by these recording techniques because of their high input resistance that produces large leak currents through the seal between electrode and cell (8). The disparity in outcomes is highly significant (over 30mV) and will lead to major differences in estimations of E_{GABA} and in the resting membrane potential (less than 30mV), which is most likely underestimated. The issue is more than theoretical. Indeed, extensive investigations suggest that the chloride extruder cotransporter KCC2 is functional after delivery and induces the postnatal depolarizing to hyperpolarizing shift in the actions of GABA (1). If GABA hyperpolarizes stem cells and depolarizes immature neurons, a mechanism, such as KCC2 or some other Cl^- transporter, must be present for chloride removal at an early embryonic stage. This putative, undetermined system would have to be eliminated later when neurons develop, migrate, and form coherent patterns.

Andäng et al. suggest that GABA signaling activates the phosphatidylinositol-3-OH-kinase-related kinase (PIKK) family of proteins that phosphorylate histone H2AX—a critical factor in the S/G2 DNA-damage checkpoint complex. Activation of this pathway by GABA hyperpolarization leads to an accumulation of cells in S-phase without influencing cell survival, apoptosis, or overt DNA damage. Their proposed model provides an intriguing mechanism by which GABA can regulate stem cell proliferation, given that a previous study on neuronal stem cells of the developing cortex demonstrated that GABA depolarization was found to inhibit DNA synthesis and cell cycle progression (4). While these two mechanisms are not mutually exclusive, to determine the effect of GABA on cell survival, the authors assayed the amount of cleaved Poly(ADP-ribose) polymerase-1 (PARP-1), an early marker of apoptosis. The use of other more appropriate markers of cell death, such as TUNEL and caspase-3, would reinforce the conclusions and exclude possible negative effects of GABA on survival of embryonic stem cells. Also, the suggestion of a GABA-induced hyperpolarization that (via Cl^- influx) induces cell swelling to activate the DNA-damage checkpoint pathway remains to be directly shown.

The novel finding that GABA can regulate embryonic stem cell proliferation has important implications for studies of development and disease. Controlling embryonic stem cell cycle progression by autocrine/paracrine GABA secretion provides a negative feedback mechanism that regulates embryo growth. GABA levels correlate with the stem cell pool size, and an increase in GABA concentration provides a signal to slow proliferation. The proposed GABA activated DNA-damage checkpoint pathways may shed light on developmental pathological conditions, such as tumor formation. Interestingly, in the developing nervous system, this pathway may be a key regulator for creating a balance between excitatory and inhibitory neuron generation. Inhibitory GABAergic and excitatory glutamatergic neurons arise from two distinct progenitor populations in the brain, the ganglionic eminences and cortical ventricular zone, respectively (9–12). During corticogenesis, the inhibitory interneurons from the ganglionic eminences migrate tangentially to the cortex (9,12) where they may tonically release GABA and provide paracrine GABA signaling of neuronal stem cells that regulate the generation of excitatory neurons (4,13,14). If this GABA signaling is disrupted, increased generation of excitatory neurons might lead to a hyperexcitable cortical circuit, making the brain more prone to epilepsy. The role of GABA signaling is becoming more intriguing. Understanding the diverse developmental roles of GABA may further an understanding of the pathophysiology of a variety of developmental disorders and epilepsy.

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References

1. Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci* 2002;3(9):728–739.

2. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 2007;87:1215.
3. Manent JB, Jorquera I, Mazzucchelli I, Depaulis A, Perucca E, Ben-Ari Y, Represa A. Fetal exposure to GABA-acting antiepileptic drugs generates hippocampal and cortical dysplasias. *Epilepsia* 2007;48(4):684–693.
4. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 1995;15:1287.
5. Liu X, Wang Q, Haydar TF, Bordey A. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* 2005;8: 1179–1187.
6. Plotkin MD, Snyder EY, Hebert SC, Delpire E. Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain. *J Neurobiol* 1997;33:781–795.
7. Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 2006;439:589–593.
8. Tyzio R, Ivanov A, Bernard C, Holmes GL, Ben-Ari Y, Khazipov R. Membrane potential of CA3 hippocampal pyramidal cells during postnatal development. *J Neurophysiol* 2003;90:2964.
9. Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* 1997;278:474–476.
10. Hartfuss E, Galli R, Heins N, Götz M. Characterization of CNS precursor subtypes and radial glia. *Dev Biol* 2001;229:15–30.
11. Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J Neurosci* 2002;22:3161–3173.
12. Anderson SA, Marin O, Horn C, Jennings K, Rubenstein JL. Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development* 2001;128:353–363.
13. Owens DF, Boyce LH, Davis MB, Kriegstein AR. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. *J Neurosci* 1996;16:6414–6423.
14. Owens DF, Liu X, Kriegstein AR. Changing properties of GABA(A) receptor-mediated signaling during early neocortical development. *J Neurophysiol* 1999;82:570–583.

Is Epilepsy a Disease of Synaptic Transmission?

Masking Epilepsy by Combining Two Epilepsy Genes. Glasscock E, Qian J, Yoo JW, Noebels JL. *Nat Neurosci* 2007;10(12):1554–1558. Inherited errors in ion channel genes comprise the largest subset of monogenic causes of idiopathic epilepsy, and pathogenic variants contribute to genetic risk in the complex inheritance of this common disorder. We generated a digenic mouse model of human idiopathic epilepsy by combining two epilepsy-associated ion channel mutations with mutually opposing excitability defects and overlapping subcellular localization. We found that increasing membrane excitability by removing Shaker-like K^+ channels, which are encoded by the *Kcna1* gene, masked the absence epilepsy caused by a P/Q-type Ca^{2+} channelopathy due to a missense mutation in the *Cacna1a* gene. Conversely, decreasing network excitability by impairing Cacna1a Ca^{2+}-channel function attenuated limbic seizures and sudden death in *Kcna1*-null mice. We also identified intermediate excitability phenotypes at the network and axonal levels. Protective interactions between pathogenic ion channel variants may markedly alter the clinical expression of epilepsy, highlighting the need for comprehensive profiling of this candidate gene set to improve the accuracy of genetic risk assessment of this complex disease.

COMMENTARY

Much work has been done in the past 3 decades to support the notion that seizures and interictal spikes result from the combined action of networks of neurons. Alterations in synaptic transmission appear to play a critical role in generating this network activity. Some of the earliest work investigating the nature of paroxysmal depolarizing shifts, which is the intracellular signature of a focal interictal spike, suggested a role for synaptic activity. These studies demonstrated that in the penicillin focus, paroxysmal depolarizing shifts had properties similar to those of an EPSP: they were graded and could be reversed in polarity (1). Follow-up studies have established that postdepolarizing shifts are mediated by glutamatergic neurotransmission.

Additional support for the synaptic transmission hypothesis was provided by studies of acquired epilepsies in experimental animals and in human surgical specimens. One of the most intensely investigated mechanisms of altered synaptic transmission in acquired epilepsies is sprouting of axon collaterals. Sprouting of mossy fibers (i.e., axons) of dentate granule cells of the hippocampus was demonstrated in experimental models and later in human specimens. These studies indicated that formation of new glutamatergic synapses on principal neurons was a key feature of temporal lobe epilepsy (2). Other studies have shown the presence of altered GABAergic synaptic transmission

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in temporal lobe epilepsy and in neurocortical epilepsies. In addition to alterations, both presynaptic input onto postsynaptic neurons and changes in $GABA_A$-receptor structure and function also have been described in various forms of epilepsy.

In contrast to the synaptic transmission theory is the "channelopathy" theory of epileptogenesis, which proposes that defects in ion channels participating in the generation of action potentials lead to epilepsy. Monogenic defects in ion channels, such as mutations of sodium channel or $GABA_A$ receptor subunits, lead to generalize epilepsy febrile seizures plus syndrome or severe myoclonic epilepsy of infancy. Other examples of ion channel defects that result in human epilepsies have been forthcoming, including mutations in potassium channels, which result in benign neonatal febrile seizures, and in calcium channels, which are associated with absence epilepsy.

Theories of altered synaptic transmission and ion channel defect may appear mutually exclusive because ion channels are believed to modulate neuronal excitability by controlling the generation of action potentials, whereas synaptic transmission is largely modulated by neurotransmitter receptors and neurotransmitters. However, a careful analysis of the relationship between ion channels and neurotransmitter release reveals that defects in ion channel function could result in altered synaptic transmission. Furthermore, as the study by Glasscock et al. shows, if the role of ion channels in modulating synaptic transmission and in generating epilepsy is clearly understood, then certain defects in ion channels can be demonstrated to cancel each other and the development of epilepsy is prevented or attenuated.

Invasion of the presynaptic terminal by an action potential causes Ca^{2+} entry, which is typically mediated by the P/Q-type of voltage-gated calcium channels. The amount of calcium entering into the terminal determines the number of synaptic vesicles that fuse and release neurotransmitter from the presynaptic terminal (3). The central pore-forming region of P/Q-type calcium channels is formed by a polypeptide subunit called CACNA1A. Mutations in the gene coding for CACNA1A lead to absence epilepsy in some patients (4). These mutations result in reduced function or loss of function in calcium channels, which is likely to reduce the entry of calcium into presynaptic terminal and depress neurotransmitter release.

The shape of the action potential invading the presynaptic terminal is to a large extent determined by potassium channels. Inhibition of potassium channels at presynaptic terminals leads to prolongation of action potentials (causing activation of more voltage-gated calcium channels) and increases the entry of calcium into the presynaptic terminal (releasing more neurotransmitter) (5). Therefore, inhibition of potassium channels elevates the amount of neurotransmitter released from the presynaptic terminal, whereas inhibition of Ca^{2+} channels diminishes it. Interestingly mutations in the Kv1.1 α subunit of potassium channels lead to seizures of hippocampal onset in patients and mice. Thus, there exist two kinds of ion channel mutations in humans—each having an opposite effect on synaptic transmission, yet both leading to epilepsy.

Glasscok et al. reasoned that if both these mutations were present in a single animal they would cancel out each other's effect. In a series of carefully performed experiments, the investigators demonstrate that the presence of both mutations (i.e., double homozygous) in a single transgenic mouse results in reduced seizures, decreased mortality, and increased long-term survival. In addition, there was marked reduction in seizure activity compared with single homozygous mice, including marked attenuation of spike-wave seizures and diminished frequency and intensity of generalized tonic–clonic seizures. The authors found that changes in excitability occur in the hippocampus: in hippocampal slices treated with elevated potassium, the frequency and duration of epileptiform discharges recorded from double homozygous slices was significantly less than that in slices from single homozygous animals. Furthermore, a direct analysis of synaptic transmission suggested that these two mutations exert opposing effects on presynaptic potentials.

This study has implications for understanding the pathogenesis of both inherited and acquired epilepsies. The authors demonstrate that multiple genes contribute to susceptibility to epilepsy. Some current notions about inherited epilepsies have been derived from Mendelian genetics, however the approach is limited by the fact that many genes expressed in the brain can modify actions of a mutation in a single gene, making the hunt for epilepsy genes extremely complex. It is possible that for each epilepsy mutation, multiple modifying genes are expressed in the brain, which may mask or exaggerate effects of single gene defect.

These studies by Glasscock et al. also suggest a mechanism for more common, acquired epilepsies. Indeed, many examples of acquired alterations in structure and functions of ion channels and receptors have accumulated over the past decade, including changes in $GABA_A$ receptors, chloride tranporters, potassium channels, and sodium channels. The sequence of events that links these acquired defects in ion channels to increased susceptibility to seizures and epilepsy remains unclear. The study by Glasscock and colleagues proposes these defects in the structure and function of ion channels and receptors may alter synaptic transmission to contribute to epileptogenesis.

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References

1. Johnston D, Brown TH. The synaptic nature of the paroxysmal depolarizing shift in hippocampal neurons. *Ann Neurol* 1984;16(suppl):S65–S71.
2. Sutula TP, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res* 2007;163:541–563.
3. Katz B, Miledi R. Further study of the role of calcium in synaptic transmission. *J Physiol* 1970;207(3):789–801.
4. Imbrici P, Jaffe SL, Eunson LH, Davies NP, Herd C, Robertson R, Kullmann DM, Hanna MG. Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia. *Brain* 2004;127(Pt 12):2682–2692.
5. Dodson PD, Forsythe ID. Presynaptic K+ channels: electrifying regulators of synaptic terminal excitability. *Trends Neurosci* 2004;27(4):210–217.