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CHAPTER 27 ■ NEUROMODULATION OF SEIZURES, EPILEPTOGENESIS, AND EPILEPSY

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Refs 37, 72, 129 not cited in text.

INTRODUCTION

Epilepsy is a complex disease that is influenced by a large number of diverse variables. Some of these factors, such as genes that influence seizure susceptibility, are internal. Others, which alter normal brain excitability, such as traumatic brain injury, are external.

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As described elsewhere in this volume, these variables can have different and distinct effects. For example, genes may influence both the development of the disorder (the epileptogenesis), or the frequency and severity of seizures after epilepsy is established. Furthermore, these same factors may also influence treatment, because they can alter the efficacy of antiepileptic drugs.

A broad range of endogenous factors include a subset that can be termed "neuromodulators". These factors are essential components of the normal central nervous system (CNS) and play an important role in the balance of excitation and inhibition in the normal brain. Here, we review neuromodulatory compounds and principles that best exemplify those that affect seizure susceptibility, epileptogenesis, and epilepsy.

In this chapter, neuromodulators are categorized into proteins and small molecules that are (a) primarily expressed in, or released from, neurons; (b) present in the extracellular space as part of the milieu that is commonly referred to as the extracellular matrix (ECM); and (c) primarily associated with astrocytes.

This chapter emphasizes neuromodulators that influence excitability in the hippocampus and the adjacent parahippocampal region (including the entorhinal cortex), two brain regions known to be centrally involved in limbic seizure activity. This focus does not imply that neuromodulation is most robust in those areas, but rather reflects the fact that the majority of information in the field of epilepsy research derives from these limbic regions. This includes studies in humans, in which neuromodulation has been mostly examined using surgically resected hippocampal and parahippocampal tissue from patients with intractable temporal lobe epilepsy (TLE).

In view of the complexity of the subject matter, often involving multiple receptors as well as intricate modes of regulation of expression and release for any given neuromodulator, only specific examples will be discussed in detail in each category. Furthermore, a major message of this overview is that no single neuromodulator influences excitability in a simple manner, and that cross-talk between modulators is likely to be associated with seizure activity.

Tables 1 through 4 provide bulleted lists of the neuromodulator categories that have been associated with pathophysiologically or therapeutically relevant aspects of seizures or epilepsy. Several other relevant categories, such as the neurosteroids, are discussed elsewhere in this volume. The selection included here should be viewed merely as a snapshot of current knowledge. There can be little doubt that additional members of these families will become relevant as well, and that novel, important neuromodulators will be identified in the future.

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NEURON-DERIVED NEUROMODULATORS

Neuropeptides

Neuropeptides comprise a variety of small proteins that were originally shown to be coexpressed with classical neurotransmitters in neurons; it was assumed that this coexpression indicated a role in synaptic transmission. Accordingly, many studies focused on the mechanism(s) by which peptides might exert their effects in concert with colocalized classical transmitters such as γ-aminobutyric acid (GABA) or glutamate. It is widely recognized, however, that neuropeptides have effects independent of classical transmitters. These effects include an influence on ion channels, synaptic transmission, and hence excitability. Neuropeptides also affect growth, proliferation, vasculature, and neurogenesis, although these effects may only occur at a certain time during development or after an injurious insult.

A discussion of neuropeptides is particularly germane to epilepsy because many of these have robust effects on seizure threshold, seizure susceptibility, epileptogenesis, and epilepsy (i.e., the state of spontaneous, recurrent seizures). However, the concept that neuropeptides modulate seizures—and potentially epilepsy—did not arise from studying their basic, fundamental properties as cotransmitters. Instead, it was initiated by reports that showed that seizures alter the expression of neuropeptides and their receptors. This was first demonstrated in laboratory animals,[80,138] although seizure-induced alterations in neuropeptides have also been found in surgically removed brain tissue from patients with intractable TLE.[50]

To date, it is still widely debated whether these changes in neuropeptide expression reflect a mechanism to repair the brain after seizure-induced neuronal damage or whether altered levels of neuropeptides indicate a compensatory, anticonvulsant response of the brain to prevent further seizure activity. An intriguing suggestion is that these changes are due to a pattern of genomic responses set into play to recapitulate developmental patterns.

Table 1 lists a large number of neuropeptides, organized according to the initial studies describing their expression and function throughout the body. Notably, these perspectives have evolved over time, because many of these peptides were identified in more than one brain area and found to have multiple functions.[204] Table 1

Figure 1 illustrates a focused view of these neuropeptides, using the dentate gyrus as an example. The dentate gyrus harbors a variety of GABAergic interneurons, which normally play an important role in preventing abnormal excitability. These neurons also contain one or more neuropeptides. In addition, Fig. 1

TABLE 1

NEUROPEPTIDES

Family Member	Amino Acid #	Receptors	Convulsant Pro (+) or Anti (–)	References
POMC-derived neuropeptides				
Adrenocorticotropin (ACTH)	39	MCR2	(–)	28
Melanocyte stimulating hormone (MSH)	13	MCR1	(–)	1
β-Lipotropin (β-LPH)	89			
Met-enkephalin	5	μ,δ,κ	μ: (+)	171,196
Leu-enkephalin	5	μ,δ,κ	μ: (+)	171,196
β-Endorphin	30	μ,δ,κ	(–)	229
Dynorphin	17	μ,δ,κ	(–)	196,229
Nociceptin (Orphanin FQ)	17	ORL-1	(–)	170
Tachykinins				
Substance P	11	NK1	(+)	229
Neurokinin-α	10	NK2		
Neurokinin-β	10	NK3	(+)	229
Bradykinin	9	B1, B2	(+/–)	2
Hypothalamic peptides				
Hormones				
Thyrotropin stimulating hormone (TSH)	201			
Oxytocin (OT)	9		(–)	1
Luteinizing hormone (LH)	204			
Follicle stimulating hormone (FSH)	204			
Vasopressin (AVP) or antidiuretic hormone (ADH)	9	V1A, V1b, V2	(+)	1, 45
Growth hormone	191			
Releasing and inhibiting factors				
Corticotropin releasing hormone (CRH or CRF)	41	CRF1,2	(+)	
Thyrotropin releasing hormone (TRH)	3	TRH1,2	(–)	118
Growth hormone releasing hormone (GnRH)	44			
Luteinizing hormone releasing hormone (LHRH or GHRH)	10			
Somatostatin growth hormone release inhibiting hormone	14 or 28	SST (1-5)	(–)	20, 223
Gut peptides				
Motilin	22			
Cholecystokinin (CCK)	8	CCK1,2	(–)	244
Vasoactive intestinal polypeptide (VIP)-glucagon family				216
Secretin	27			
VIP	28	PACAPR type II	(+)?	39, 48
Pituitary adenylate cyclase activating peptide (PACAP)	27 or 38	PACAPR type I	(+)	39
Glucagon-like peptide (GLP)-1	36	GLP-1R, rGLP-1R	(–)	58
Neuropeptide tyrosine			(–)	227
Neuropeptide tyrosine (NPY)	36	YR (1-5)		
Pancreatic polypeptide (PP)	36			
Peptide tyrosine-tyrosine (PYY)	36			
Bombesin peptides				
Bombesin (Gastrin-releasing peptide; GRP)	27	GRP R1,2	(–)	4
Gastrin	17			
Neuromedin B	10			
Galanin	29 or 30	GAL (1-3)	(–)	229
Neurotensin	13	NTSR1,2		
Calcitonin gene-related peptide (CGRP)				115
Vascular peptides				
Natriuretic hormone family				
Atrionatriuretic hormone (ANH) or atriopeptin	28	NPRA, B, C	(+)	136
Brain natriuretic hormone (BNP)	32		(+)	136
C-type natriuretic hormone (CNP)	22		(+)	136
Angiotensins I–IV	6–10	AT (1-4)	(–)	211
Placental peptides				
Prolactin	198			16, 123,124
Chorionic gonadotropin				
Placental lactogen (choriomammotropin)				

The major categories of neuropeptides are listed, with emphasis on those that have been associated with seizures or epilepsy. Peptide length refers to the number of amino acids. Receptor subtypes are shown for examples with multiple receptors. Peptides that have been shown to exert proconvulsant (+) or anticonvulsant (–) activity are indicated; mixed effects are denoted by +/–; a question mark indicates effects that are not clearly proconvulsant or anticonvulsant. References are primarily reviews, but specific citations are provided when reviews are unavailable.

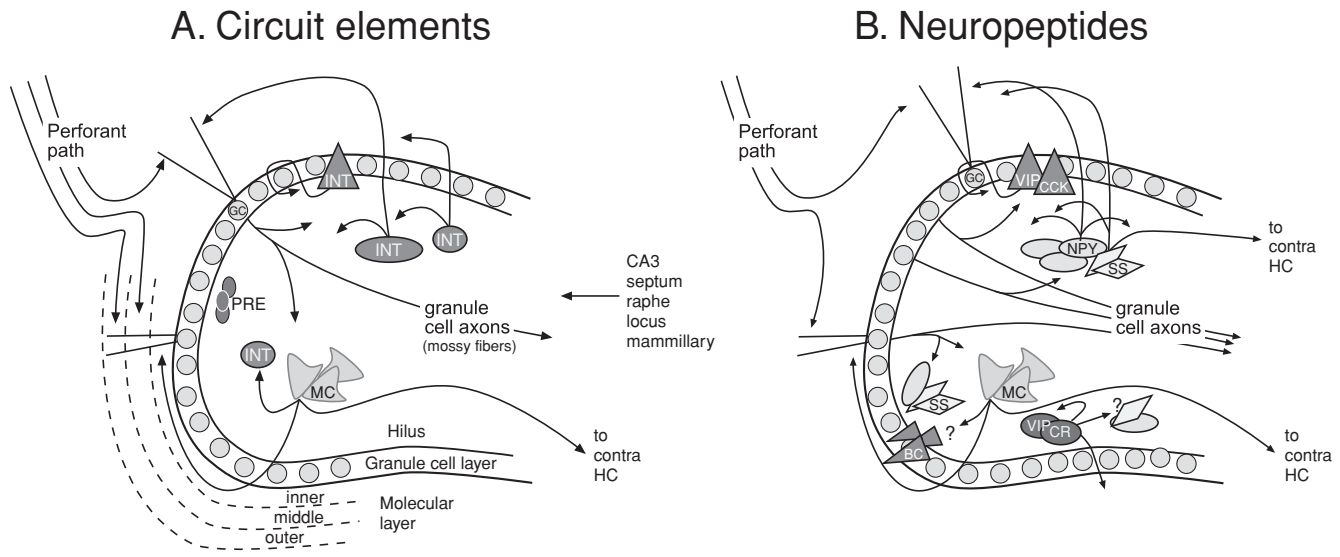

FIGURE 1. Circuit elements and neuropeptides in the rat dentate gyrus under normal conditions. **A:** Major components of dentate gyrus circuitry. These include the dense layer of granule cells (*GC*); diverse GABAergic "interneurons" (*INT*), hilar mossy cells (*MC*), as well as precursors to granule cells (*PRE*) that are located in the subgranular zone. The major afferent input to the dentate gyrus is the perforant pathway, which contains the axons of neurons in the entorhinal cortex. Lateral entorhinal neurons innervate the outer molecular layer (*outer*) and medial entorhinal neurons innervate the medial molecular layer (*medial*). Mossy cells and other fibers innervate the inner molecular layer (*inner*). In addition, inputs to the dentate gyrus also arise from area CA3 pyramidal cells, the septum, dorsal raphe, locus coeruleus, mammillary bodies, and other areas. The main projections from the dentate gyrus are from the granule cell axons (the mossy fibers), which terminate locally on hilar processes and the layer containing the proximal dendrites of CA3 pyramidal cells (stratum lucidum; not shown). In addition, mossy cells project both ipsilaterally and contralaterally to the dentate gyrus. Some GABAergic neurons also project contralaterally. Reproduced with permission from Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6:347–470; and Scharfman HE. The role of nonprincipal cells in dentate gyrus excitability and its relevance to animal models of epilepsy and TLE. In: Delgado-Esqueta AV, Wilson W, Olsen RW, Porter RJ, editors. *Basic mechanisms of the epilepsies: molecular and cellular approaches*, 3rd ed. New York: Lippincott-Raven; 1999:805-820.[77,179] **B:** Many neuropeptides are expressed in the normal dentate gyrus, and some of the most well-studied examples are shown.[77] Opiates are mainly expressed in granule cells. In contrast, other neuropeptides are primarily present in interneurons. Basket cells contain both vasoactive intestinal polypeptide and cholecystokinin; hilar neurons that innervate the outer molecular layer (as well as other targets) express neuropeptide Y and/or somatostatin; vasoactive intestinal polypeptide and calretinin that define a population that innervates the inner molecular layer, as well as other interneurons; in addition, other vasoactive intestinal polypeptide-containing cells exist and may be coupled by gap junctions.[90] Recent studies have indicated that substance P has widespread expression in interneurons.[197]

two principal cells—the abundant granular cells and the less numerous hilar "mossy cells"—use glutamate as a neurotransmitter and also express various peptides, as well as other neuromodulators. This subregion of the hippocampus plays a central role in seizure generation and propagation in animals and humans, and has been studied extensively with regard to the neuromodulatory effects of peptides in epilepsy.

Neuropeptide Y

Fig. 2

Neuropeptide Y is the first example. This peptide is normally expressed in a subset of GABAergic neurons in the dentate gyrus. The axons of these cells project to numerous areas of the region and thus exert multiple effects (Fig. 2A). A primary effect of neuropeptide Y is to reduce excitatory transmission from granule cells to their targets, thereby decreasing the excitatory output of the dentate gyrus to hippocampal pyramidal cells. Thus, when synthetic neuropeptide Y is applied to slices of rodent dentate gyrus, it inhibits the excitatory output of granule cells by acting on neuropeptide Y receptors on the terminals of granule cell axons. This function is likely to be important in the control of seizure activity in the hippocampus since overexpression of neuropeptide Y is anticonvulsant [227] and because decreased neuropeptide Y expression leads to increased seizure susceptibility.[13]

Neuropeptide Y also has other actions in the dentate gyrus. For example, it modulates calcium entry into granule cells, [139] and also affects a specific potassium ion (K^+) channel on GABAergic neurons in the dentate gyrus.[156] The latter could influence neuronal firing and GABA release and therefore modulate the targets of GABAergic neurons.

Neuropeptide Y also facilitates the proliferation of new granule cells in the adult brain, and thus influences adult neurogenesis.[88] These actions appear to be mediated by the Y1 subtype of neuropeptide Y receptors, which are situated on the proliferating cells. The source of neuropeptide Y may be the GABA/neuropeptide Y coexpressing interneurons, which have axon terminals in the subgranular zone in which the proliferating cells are located.[52]

Substantial evidence suggests that neuropeptide Y function may change after seizures, as initially suggested by studies showing that seizures affect the expression of neuropeptide Y in granule cells. Particularly after chronic seizures, the axons of the granule cells strongly express neuropeptide Y (Fig. 2B).[11]

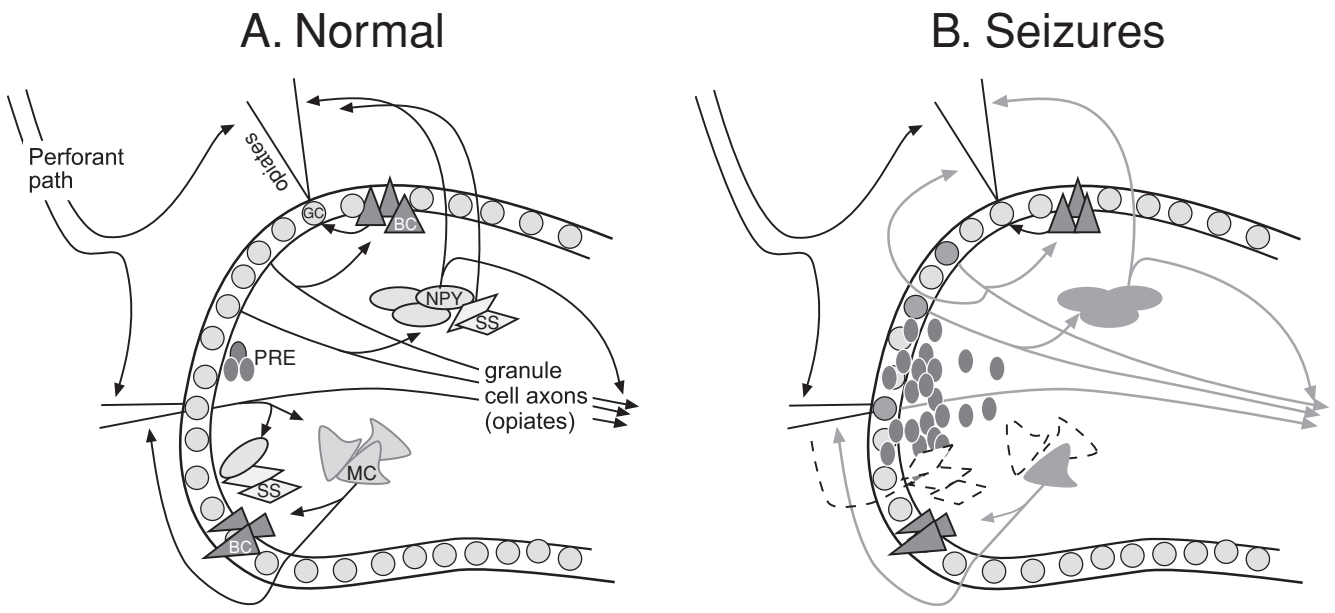

FIGURE 2. Changes in neuropeptide Y and somatostatin in the dentate gyrus after seizures. **A:** Normal circuitry and expression of neuropeptide Y and somatostatin in the rodent dentate gyrus. **B:** Alterations in neuropeptide Y and somatostatin in animal models of epilepsy are illustrated schematically. For the neuropeptide Y system, these changes include: (a) new expression in granule cells and their axons, the mossy fibers; (b) increased expression in interneurons that normally express neuropeptide Y—this effect appears to develop mostly after acute rather than chronic seizures; (c) novel expression of neuropeptide Y in some mossy cells[88]; and (d) expression in newly formed granule cells, although the extent that this occurs is unclear. In addition, changes in receptors occur (not shown). For the somatostatin system, the best documented alteration is seizure-induced cell death of those interneurons that normally express this peptide.

This phenomenon may be functionally significant for two reasons: First, neuropeptide Y may exert more robust effects on the normal targets of granule cell axons; second, neuropeptide Y may have additional effects at new locations, because the axons of granule cells (mossy fibers) make new connections after chronic seizures ("mossy fiber sprouting"). Indeed, neuropeptide Y inhibits the effects of glutamate released from the sprouted axons of granule cells in epileptic rodents.[218] These data support the concept that neuropeptide Y is "anticonvulsant" and its upregulation after seizures is "compensatory" in nature. Interestingly, acute seizures, as opposed to chronic seizures, appear to have different effects on neuropeptide Y expression. Thus, a brief seizure results in a preferential, transient increase in neuropeptide Y expression in GABAergic neurons,[200] but does not cause robust changes in neuropeptide Y expression in granule cells. The functional role of the increase in neuropeptide Y within GABAergic neurons is still not clear, but it could be a way to inhibit glutamate release, which could prevent another seizure. It is important to add that this transient increase of neuropeptide Y in GABAergic neurons may be underestimated, because seizures may injure neuropeptide Y–containing neurons.[29,49,212]

Additional complications arise from the fact that some, but not all, neuropeptide Y receptors change as a consequence of seizures. It appears that the predominant change is an increase in Y2 receptors. This would be likely to enhance the ability of neuropeptide Y to inhibit synaptic transmission, because Y2 receptors are normally responsible for this effect.[65,226]

However, other receptors do not necessarily show robust changes, or the studies to date are not in complete agreement.[12] An increase in receptors may not lead to a change in effect if neuropeptide Y is not released in sufficient concentration to activate the new receptors.

To add to the complex picture, granule cells formed after seizures, although likely to express neuropeptide Y, have distinct physiologic properties and may therefore release neuropeptide Y differently. On the other hand, neurons in the epileptic brain likely release more neuropeptide Y. This may be the case for newly formed granule cells, which typically exhibit burst discharges[181] and therefore may release neuropeptide Y.

In summary, a series of experimental studies have documented the robust influence of neuropeptide Y in the normal dentate gyrus and suggested that this peptide be considered an endogenous anticonvulsant. Moreover, neuropeptide Y expression is highly plastic and altered by seizures. These changes appear to indicate anticonvulsant properties and a compensatory inhibitory role for neuropeptide Y in epilepsy. However, the complexities of neuropeptide Y changes after seizures, and the lack of a detailed understanding of several of these changes, suggest that firm conclusions are still premature.

Somatostatin

Somatostatin is another example of a neuropeptide with robust actions in the dentate gyrus, making it another candidate to serve an endogenous anticonvulsant role.[20,223] Normally, somatostatin is preferentially expressed in a subset of GABAergic neurons in the dentate gyrus, which innervate the outer molecular layer and have collaterals in the hilus (Fig. 2A). The axonal projection to the outer molecular layer has received greatest attention because it is most dense, and because of the potential for selective modulation of the lateral perforant path, a major cortical input to the dentate gyrus, which is selective for the outer molecular layer.

In most but not all cases, somatostatin appears to depress this input when single afferent stimuli are tested.[14,178] More robust effects are observed after tetanization, which would

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normally lead to long-term potentiation (LTP); somatostatin blunts this effect.[14] These data suggest that somatostatin depresses the glutamatergic activation of granule cells by an action at the site of synaptic input, and it does so preferentially after high-frequency input. In addition, somatostatin depresses calcium-mediated action potentials (calcium "spikes") in granule cells by inhibiting N-type calcium channels. The net effect could contribute to the depression of LTP in the lateral perforant path.[14] In light of these results, it is no surprise that somatostatin inhibits seizures in animal models of epilepsy.[20] Likewise, in somatostatin knockout mice, seizures elicited by chemoconvulsants are more severe, and after-discharges are longer in animals that are kindled by stimulation of the perforant path.[29]

Somatostatin may also have other actions, however. Thus, in other areas of the hippocampus, exposure to somatostatin leads to additional effects, such as modulation of the M-current.[188] This action may have numerous consequences, because M channels are located in diverse areas of the dentate gyrus—not only on neuronal somata, but also on their axons.

As is the case of neuropeptide Y, somatostatin expression is dramatically changed after seizures. The decrease in somatostatin is due to the fact that somatostatin-containing neurons are extremely vulnerable to seizure-induced neuronal death (Fig. 2B). The loss of somatostatin may contribute to the change in hippocampal excitability following seizures.

However, somatostatin cell loss appears to be selective for animal models of epilepsy in which seizures are severe (i.e., models that use an initial period of status epilepticus to induce chronic seizures). Somatostatin cell loss is also evident in hippocampal tissue resected from patients with intractable TLE.[167] In contrast, animal models in which seizures are milder, such as electroconvulsive shock or kindling, do not necessarily result in the loss of somatostatinergic neurons, but lead to an *increase* in somatostatin expression within those neurons that normally express this neuropeptide.[153,186]

It is also necessary to consider that somatostatin receptors may be altered in epilepsy. In tissue resected from patients with pharmacoresistant TLE, only type 2 (i.e., the receptor known to mediate anticonvulsant effects) is altered in the dentate gyrus.[47] It appears that both the mRNA for the type 2 receptor and receptor binding increase in the granule cell layer, remain unaltered in the inner molecular layer, and decrease in the outer molecular layer. The latter changes may reflect a compensation for the loss of afferents, which degenerate due to the vulnerability of hilar somatostatin neurons. Other somatostatin receptors (types 1–4) in the dentate gyrus remain remarkably normal after kainic acid (KA)-induced status epilepticus, although changes occur elsewhere in the hippocampus.[162]

Taken together, the source of somatostatin and its receptor-mediated actions differ substantially between the normal and the epileptic brain. These differences are likely to alter the peptide's effects in a pathologic situation. This implies, for example, that synthetic somatostatin analogs may not be able to depress perforant path transmission in the epileptic brain because of the lack of appropriate receptor targets (see earlier discussion), and that it may be possible to design somatostatin-related therapeutic interventions that are geared specifically to the epileptic condition.

The changes in neuropeptide Y and somatostatin in the rat dentate gyrus after chronic seizures are illustrated schematically in Figure 2. As noted earlier, neuropeptide Y and somatostatin are just two of a long list of neuropeptides that have been studied intensively in the dentate gyrus in the context of seizures (Table 1). Thus, many neuropeptides appear to modulate excitability normally and specifically influence seizures/epilepsy in animal models of epilepsy. Most of them also have the same dual relationship with seizures as neuropeptide Y and somatostatin (i.e., they are both regulated by seizures and capable of modulating seizures). Finally, it should also be noted that there appear to be significant species differences in neuropeptide expression in the dentate gyrus. However, despite the fact that some of the peptides present in humans are not identical to those in rats or mice, the relationship of peptidergic neurons to epilepsy appears to be essentially similar in rodents and humans.

Calcium-Binding Proteins

Calcium-binding proteins have also received considerable attention with respect to their influence on the function of neurons in the dentate gyrus. Strictly speaking, this group has many members (Table 2), but those in the dentate gyrus that have received most attention are those that have the highest levels of expression. One example is calbindin D28K, which is primarily expressed in granule cells, although studies have also shown immunoreactivity in selected GABAergic neurons.[193] Other examples of calcium-binding proteins include parvalbumin and calretinin, which have been selectively localized to GABAergic neurons.[198]

Table 2

The distinct distribution of these proteins naturally raises questions about their specific functions in various cell types. Interest grew after it was shown that those neurons in the dentate gyrus that appear most prone to seizure-induced damage (i.e., somatostatin-containing neurons and the glutamatergic mossy cells) lacked either calbindin or parvalbumin. However, later studies suggested a more complex relationship between calcium-binding proteins and vulnerability, because calcium-binding proteins such as calretinin were also found in susceptible neurons.[78]

Functional studies revealed that calbindin and parvalbumin have important roles in the regulation of calcium within the cell. In the granule cell, calbindin modulates calcium levels and therefore has several potential functions, including an influence on transmitter release. Indeed, overexpression of calbindin leads to alteration in granule cell transmission to pyramidal cells of CA3 due to a presynaptic mechanism at granule cell boutons. These changes are clearly important because of the dramatic alteration in hippocampal function in vivo after overexpression of the protein only in granule cells.[57] Parvalbumin also appears to have important functional roles in the regulation of calcium entry, primarily presynaptically.[40] These are likely to be substantial in their net effect in vivo, given that parvalbumin knockout mice have altered seizure susceptibility.[185] It is not clear, however, whether this is a specific defect in normal parvalbumin function or a compensatory effect.

In human tissue derived from patients with intractable TLE, calbindin expression in granule cells is reduced,[131] and the expression of parvalbumin immunoreactivity undergoes complex changes depending on hippocampal subregion and clinical features.[198,233] Some argue that these changes may be neuroprotective if intracellular calcium levels were effectively lowered by calcium-binding proteins, but others would suggest that this might not necessarily be the case.[219] In summary, the functional significance of neuromodulation by calbindin, parvalbumin, and many other calcium-binding proteins in epilepsy is still controversial and requires careful additional investigation and analysis.[35]

Endogenous Trace Metals

Trace metals exert a number of biologic effects throughout the body that are caused, among other mechanisms, by the metals' ability to serve as cofactors to a large number of enzymes. They also interact directly with cell membrane and intracellular receptors, and regulate oxidation/reduction processes within

TABLE 2

CALCIUM-BINDING PROTEINS

I. EF hand calcium-binding proteins
α-Actinin
Calbindin D28K
  Calbindin D28k
  Calretinin 20k, -22k
Calcyphosine (p24)
Calmodulin
  Calmodulin
  Calcineurin
  Caltractin
Calpain
  μ-Calpain I, II
  Grancalcin
  Sorcin
Centrin
Neuronal calcium sensors
  Frequenin
  Hippocalcin
  Neuronal calcium sensor-1
  Neurocalcin
  Recoverin
  S-modulin
  Vilip-1,2,3
  Visinin
Parvalbumin
  Parvalbumin
  Oncomodulin
Spectrin
S100 family
  Calbindin D9k
  S100A
  S100L/S100A2
  S100E/S100A3
  Placental calcium-binding protein /S100A4
  S100D/S100A5
  Calcyclin/S100A6
  S100A7
  MRP-8/S100A8
  MRP-14/S100 A9
  p11/S100A10
  Calgranulin C/S100A12
  S100β
  S100C
  S100P
  Profilaggrin
  Trichohyalin
Sorcin
SPARC (osteonectin)
Troponin
  Troponin C
  Tn l
  Tn T

II. Annexins (I–XI)
III. Other
  Calmegin
  Calnexin
  Calreticulin
  Calsequestrin
  Crystallins

cells. Several of these properties play a role in the metals' effects on cellular excitability in the CNS. It is therefore not surprising that metals such as iron, manganese, and selenium, acting in a neuromodulatory role, may influence the development or termination of seizure activity.[85,189,208]

The endogenous trace metal zinc has been most frequently associated with seizures. This is primarily due to the observation that zinc is localized in, and can be released from, many glutamatergic neurons throughout the limbic system. Granule cells in the dentate gyrus, and especially their axon terminals, contain a particularly high concentration of zinc. Within the cell, zinc is bound to specific proteins, such as metallothioneins, or exists in presynaptic vesicles and can be released into the extracellular compartment. Extracellularly, zinc can have quite diverse effects, making it difficult to predict its net function. Thus, zinc can depress (e.g., by reducing NMDA receptor function) or enhance (e.g., by interfering with $GABA_A$ receptor-mediated inhibition) excitability. The reader is directed to an excellent review of this topic.[76]

The inhibition of $GABA_A$ receptor function by zinc is particularly interesting in the context of epilepsy. The metal normally has little effect on $GABA_A$ receptors on granule cells, because the receptor subunits are not assembled in a combination that optimizes zinc sensitivity. However, these subunits change their expression patterns under epileptic conditions, resulting in reductions in the α1- and γ2-subunits and an increase in the δ-subunit; these changes greatly enhance zinc sensitivity of the receptor.[3,42,174]

Zinc may also show increased effects on $GABA_A$ receptors in the epileptic brain, because zinc-rich mossy fibers develop collaterals that innervate the proximal dendritic region of granule cells (mossy fiber sprouting). These new collaterals constitute an increased source of zinc, which may be of functional significance, because enhanced zinc release from sprouted mossy fibers may further decrease the inhibition of granule cells. In addition, the release of zinc may be greater under conditions of chronic epilepsy, given the predisposition for burst discharges.[30] Such a dampening of the normal inhibitory "gate" function of the dentate gyrus might facilitate seizure activity in limbic circuits. Thus, zinc appears to play a critical role in the mechanisms that link changes in $GABA_A$ receptor subunits and mossy fiber reorganization in the epileptic brain to the epileptic state.

Cytoskeletal Proteins

Cytoskeletal elements are a fundamental component of nerve cells, and recent studies suggest relevance to epilepsy, particularly for the filamentous proteins. This group includes actin filaments, intermediate filaments (e.g., neurofilaments), and microtubules (such as α- and β- tubulin). In addition to this group, proteins such as clathrin and stathmin are important to consider, because they are critical to endocytosis.

Recent evidence suggests that several of these proteins may also be involved in epileptogenesis, in the response of the nervous system to seizures and, in the developing brain, in the resistance to seizure-induced neuronal damage.[126] Notably, some of the seizure-related changes in the expression pattern of cytoskeletal proteins, which may in part be due to cell swelling, have been revealed using gene profiling techniques.[127]

The involvement of cytoskeletal elements in epilepsy is probably related to their role in the intracellular movement of proteins into different cellular compartments, which, in turn, can modify neuronal excitability. Thus, cytoskeletal proteins are involved in the trafficking of neurotransmitter receptors. Proteins such as clathrin may alter excitability by changing the concentration of molecules available to the extracellular milieu. In addition, the cytoskeleton may be causally involved

in the dendritic deformation (i.e., beading) that has been described in animals with chronic seizures and in humans with TLE. For example, electron microscopic analysis indicates that cytoskeletal changes may be responsible for the unique beading of dendrites in epileptic tissue,[222] although other hypotheses that are independent of the cytoskeleton have also been suggested.[207]

Growth Factors

The term "growth factor" is defined loosely here to refer to several protein families, expressed either in neurons or glia, that were originally identified for their roles in CNS growth and development (Table 3). Subsequent studies revealed that these same proteins influence neurons after maturity, and, interestingly, have striking effects in the context of epilepsy.

Table 3

Growth factors influence excitability in the adult CNS both directly and indirectly. The reader is directed to a review of this topic.[180] Many of the effects are due to modulation by transcription factors, which in turn affect the expression of proteins that can alter excitability. Their importance to epilepsy is supported by studies showing that the expression of growth factors and their receptors is dramatically altered by seizures, both in animal models of epilepsy and in TLE (a review of this topic is available[180]). Similar to the neuropeptide changes observed after seizures (see previous section), these effects may be compensatory in nature and may recapitulate developmental programs—a plausible interpretation in light of the prominent role of growth factors in brain development.

Experimental interference with growth factor function can result in the withering or retraction of axonal pathways, suggesting that these proteins regulate axonal growth and maintain neuronal integrity.[206] Furthermore, growth factors influence the morphology and density of dendritic spines,[67,111] and also cause additional structural alterations of synapses.[228] All these effects may be critical in epilepsy, in which dramatic spine changes occur as a consequence of seizures, [213,235] and the axons of injured neurons degenerate. In addition, growth factors may contribute to circuit rearrangements after seizures, including axonal sprouting, formation of new synapses, and other structural alterations.[100]

AQ? Spine? OK?

Several families of growth factors can be categorized in many ways. in table 3, the classic growth factor families are organized according to their receptors, which are primarily receptor tyrosine kinases or serine threonine kinases. These include the tyrosine kinase receptor superfamily (ephrins, epidermal growth factor [EGF] family, fibroblast growth factor [FGF] family, insulin growth factor [IGF] family, neurotrophins, and vascular endothelial growth factor [VEGF] family), and the serine threonine kinase receptor superfamily (including transforming growth factor [TGF}-β family). In addition, Table 3 includes two other categories critical to normal growth and development. These include axon guidance molecules (netrins, the reticulon family, semaphorins, and slit proteins) and morphogens (bone-morphogenic proteins, the hedgehog family, the wnt family). Inflammatory cytokines (the interleukins [IL] and the tumor necrosis factor [TNF] family) are discussed elsewhere in this volume (see Vezzani and Janigro). Chemotactic cytokines (chemokines) are also relevant, particularly in relation to the mechanisms that control axon guidance, but to date limited evidence points to their role in seizures, excitability, and epilepsy.

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Neurotrophins

The neurotrophins are a family of growth factors that show robust expression in the adult CNS and are known to influence a wide variety of normal functions. They also provide some of the best examples of growth factors that have been shown to influence seizures. The dentate gyrus is useful as an example of a site in which neurotrophins are likely to affect seizure activity, because of the evidence that this region shows robust neurotrophin expression and action. Most of these studies are focused on brain-derived neurotrophic factor (BDNF) or NT-3, and much less is known about the other neurotrophins, (e.g., the prototypic member of the neurotrophin family, NGF, and the fourth major member, NT-4/5).

Figure 3 depicts the normal expression pattern of BDNF in the rodent dentate gyrus. The same pattern appears to be present in humans.[148] Thus, BDNF is mainly localized in granule cells, although a small proportion may also be contained in nongranule cells and in afferents from the entorhinal cortex (i.e., the perforant path).[41,231,242] Notably, BDNF enhances the expression of neuropeptides such as neuropeptide Y in GABAergic neurons, indicating potentially significant interactions between hippocampal neuromodulators.[134] Simply viewed, neuropeptide Y induction may limit excessive excitation by BDNF and thus prevent the development of seizures.

Fig. 3

BDNF not only supports dendritic structure and plasticity in the dentate gyrus, but also stimulates the proliferation of cells in the subgranular zone, a major source of newly generated dentate granule cells in the adult brain.[175] Moreover, BDNF has robust effects on the physiology of granule cells and their targets, influencing glutamatergic and GABAergic circuits.[24,116,151] These effects often involve changes in transmitter release and depend on protein synthesis.[24] In addition, BDNF depolarizes granule cells by an effect on the Nav 1.9 sodium channel.[116] Finally, BDNF may also signal via glial cells.[168]

After seizures, BDNF levels rapidly increase in granule cells.[81] Judged from studies in both experimental animals and patients with intractable TLE, this effect appears to persist for weeks if the initial seizures are severe (e.g., status epilepticus).[180,182] This, and the fact that BDNF protein is also expressed in sprouted axons,[182] supports the pathophysiologic relevance of BDNF in chronic epilepsy.

The neuromodulatory effects of BDNF are probably also influenced by its precursor, proBDNF. proBDNF exerts its own physiologic function—for example, the regulation of cell death—through the p75 receptor.[94] This function is increased after seizures. These increases, together with elevations in mature BDNF, might contribute to seizure-induced neuronal loss. To add to the complexity, however, seizures also increase the activity of matrix metalloproteases (MMPs),[132] which are able to cleave proBDNF into BDNF.[119] This could generate BDNF at extrasynaptic locations where proBDNF, but not BDNF, is likely to be released. Extrasynaptic release of this newly formed BDNF may then influence epileptic phenomena by targeting novel receptor sites.

Because of the multiple proexcitatory roles of BDNF in the dentate gyrus in physiology and pathology, it is not surprising that BDNF infusion can cause seizures, that BDNF overexpression increases seizure susceptibility, and that deficits in trkB receptors can block kindling epileptogenesis.[21] Furthermore, BDNF polymorphisms have been linked to febrile seizures,[21,176] and BDNF or trkB are frequently identified in microarray studies of genes linked to epilepsy.[128] However, analogous to the caution suggested for neuropeptide Y as an "anticonvulsant" (see the earlier discussion), more information is needed before considering BDNF an endogenous "convulsant." This caveat is supported by studies that demonstrate complex and unexpected effects of trkB transgenic and conditional BDNF knockout mice.[176] In vivoinfusions of BDNF, too, yield somewhat conflicting results,[176] possibly due to the fact that chronic BDNF treatment downregulates its own receptor.[240] These and related issues must be resolved before BDNF manipulation can

TABLE 3

GROWTH FACTORS AND CYTOKINES

			References	
Receptor tyrosine kinases	**Ligand**	**Receptor**	**Animal**	**Human**
Ephrins			239	
Ephrin A (1-8)	Ephrin A (1-8)	EphA (1-5)		
Eprin B (1-6)	Ephrin B (1-6)	EphB (1-3)		
Epidermal growth factor family			152	
Epidermal growth factor	EGF	erbB1(HER 1)		71
Heparin-binding epidermal growth factor	HB-EGF	erbB2 (Neu, HER2)	64	51
Transforming growth factor α	TGFα	erbB3(HER31)		
Neuregulins		erbB4(HER 4)	64	51
Neu differentiation factor	Heregulin			
ACh receptor inducting activity	ARIA			
Glial growth factor (GGF)	Neuregulin 1			
Neuregulin 2-4				
Vaccinia growth factor	VGF			
Amphiregulin	AR			
Fibroblast growth factor family (FGF)-1–23			79, 245	
Fibroblast growth factor 1 (acidic)	aFGF, FGF1	FGFRI (flg)		
Fibroblast growth factor 2 (basic)	bFGF, FGF2	FGFRII, III, IV		
Insulin growth factor family			104, 107	
Insulin	Ins	IR		
Insulin-like growth factor 1	IGF-1	IGFR II(M6P)		
Insulin-like growth factor 2	IGF-2	IGFR II(M6P)		
Neurotrophin family			82	
Nerve growth factor	NGF	TrkA		
VGF (nonacronymic)			172	
Brain-derived neurotrophic factor	BDNF	TrkB	21, 176	21, 176
Neurotrophin-3	NT-3	TrkC		
Neurotrophin-4/5	NT-4/5	p75		
Vascular endothelial growth factor			46	
Vascular endothelial growth factor	VEGFA	VEGFR1 (flt-1)		
	VEGFB	VEGFR2 (flk-1)		
	VEGFC	VEGFR3 (flt-4)		
	VEGFD	neuropilin 1		
	VEGFE	neuropilin 2		
Placental growth factor	PlGF			
Platelet-derived growth factor	PDGFA,B,C,D, AB	PDGFR-α or-β	135	
Serine-threonine kinases				
Transforming growth factor superfamily				
Transforming growth factor-β	TGF-β (1–3)	TGF-β (I-III)	145	
Glia-derived neurotrophic factor family			113	113
Glia-derived neurotrophic factor	GDNF	c-Ret + GFRa1		
Neurturin		c-Ret + GFRa2		
Artemin		c-Ret + GFRa3		
Persephin				
Bone-morphogenic proteins (BMP)-1–20	BMPs	BMPRI(A, B), II		
Growth/differentiation factors (GDF)-1–15				
Growth/differentiation factors	GDFs			
Activtins/Inhibins		Act RI, Act RII	217	
Axon guidance molecules				
Netrins				
Netrin 1,2		DCC/frazzled/UNC-40, UNC-5		
Reticulon family				
Reticulon	Rtn			
Nogo (A, B, C)	Nogo A-C	Nogo receptor (Ngr 1–3)	140	10
Semaphorins				
Semaphorin family (1–8)	sema 3A,3C,3F	Neuropilins 1,2	15	97
	sema 1,4D,5,7A	Plexins A–D		
Slits (1–3)	Slit (1–3)	Robo (1–2)		

(continued)

TABLE 3

CONTINUED

Receptor tyrosine kinases	References: Ligand	Receptor	Animal	Human
Morphogens				
Bone-morphogenic proteins				
Hedgehog family				
Desert Hedgehog	Dhh	Patched 1		
Sonic Hedgehog	Shh	Patched 1	11	
Indian Hedgehog	Ihh	Patched 1		
Wnt (Wingless/Int-1) family (1–15)	Wnts 1-15	Frizzled 3	130	44
Cytokines				
Interleukins	IL-1, 2, etc.	IL-1R, IL-2R, etc.	99, 224	
gp130/Interleukin-6 family			99	
Ciliary neurotrophic factor	CNTFR	gp130/LIFR + CNTFR-α		
Leukemia inhibitory factor	LIF	gp130/LIFR + LIFR	143	
Oncostatin-M	OSM	gp130/OSMR + OSMR		
Cardiotropin-1	CT-1	gp130/LIFR + CT-1R		
Interleukin-6	IL-6	gp130 + IL-6R		
Interleukin-11	IL-11	gp130 + IL-11R		
Tumor necrosis factor superfamily				
Tumor necrosis factor -α, -β	TNF-α, -β	TNFR I, II, p55, p75	9, 194	

The major families of growth factors and cytokines are listed, including axon guidance molecules and other compounds that influence the growth-associated processes that accompany epileptogenesis. References document the influence of growth factors on seizures or epilepsy, and are divided between studies in laboratory animals (Animals) or clinical research (Human). References listed to the right of a category review members of that category. When placed adjacent to a select example, they apply only to studies of that particular growth factor or receptor.

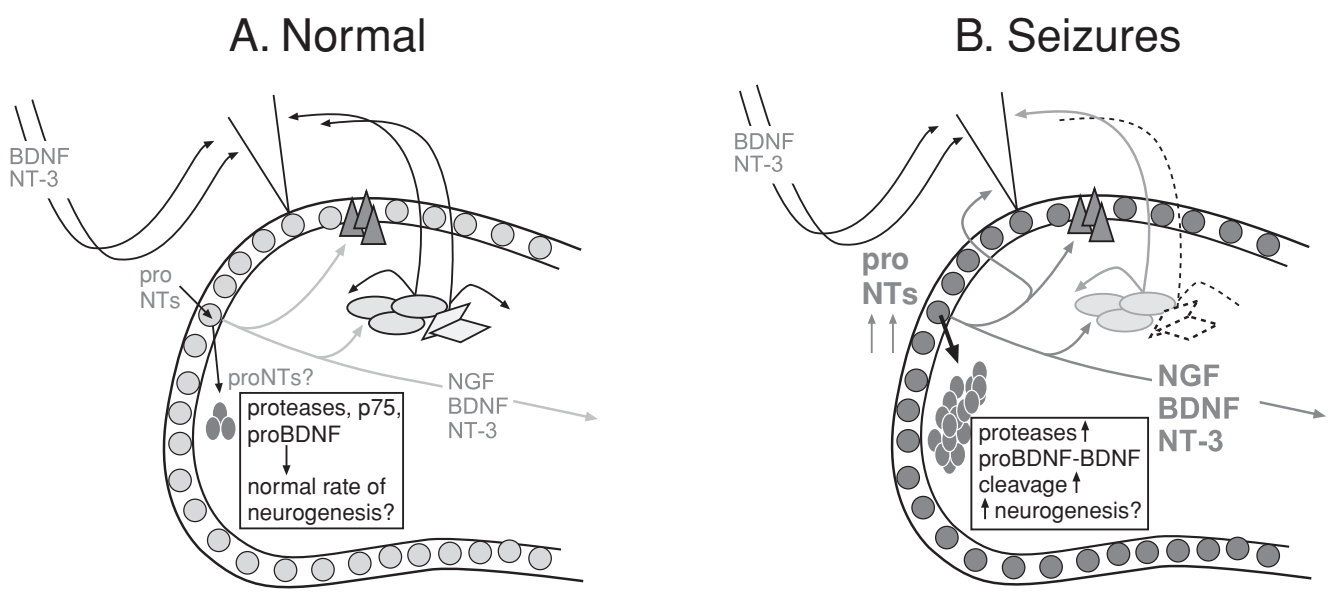

FIGURE 3. Changes in neurotrophins in the dentate gyrus after seizures. **A:** Normal pattern of expression of the neurotrophins NGF, BDNF, and NT-3. The predominant cell type expressing neurotrophins normally is the granule cell. In addition, lower levels of BDNF and NT-3 are present in perforant path axons. Proneurotrophins are present in the soma and are thought to be released in the vicinity of the soma. Proneurotrophins are typically cleaved by extracellular proteases, but can also act directly on p75 receptors to modulate survival. **B:** Changes in neurotrophins associated with chronic seizures are schematically illustrated. NGF and BDNF expression is increased in granule cells, and NT-3 levels decline; BDNF and NT-3 decline in the perforant path when seizure-induced entorhinal cell loss occurs. The altered levels of neurotrophins are likely to have diverse effects on granule cell structure and function. In addition, indirect effects may occur, such as induction of neuropeptide Y by BDNF, either in granule cells or adjacent interneurons (cf. Figure 2). Upregulation of proneurotrophins are also likely and, in the case of proBDNF, increased synthesis may influence seizure-induced cell death by increased activation of the p75 neurotrophin receptor, or seizure-induced neurogenesis by the increase in BDNF that would result from proBDNF cleavage. These changes, as well as alterations in receptors for neurotrophins, are likely to have a complex net effect on the excitability of the dentate gyrus.

be viewed as a bona fide approach to ameliorate seizure disorders clinically.

Vascular Endothelial Growth Factor

VEGF was initially discovered as a protein that has robust effects on the vasculature, altering permeability and also enhancing angiogenesis.[32] Although it was not originally anticipated to have an influence in the CNS, recent studies have demonstrated that VEGF expression occurs throughout the brain, and that neurons are influenced by exposure to VEGF.

VEGF exists in more than one isoform (VEGFA through -E) and has numerous receptors (VEGFR1, VEGFR2, neuropilins), leading to the potential for a variety of distinct effects. Normally, VEGF is primarily associated with glia and endothelial cells, so that many of its effects are likely to be directed at glia or blood vessels under physiologic conditions. However, in animal models of injury, VEGF expression appears to increase in various areas of the brain.[46] Interestingly, after seizures, VEGF expression also increases in neurons, and some of these changes occur in brain areas that are highly susceptible to seizures, such as the hippocampus. These findings have led to the suggestion that VEGF may have a role in epilepsy.

More evidence of a link between VEGF and epilepsy has come from functional studies. Thus, VEGF alters potassium channel function and synaptic transmission, although it is not clear whether these effects are direct or indirect[137,241] Perhaps the most relevant of these functional studies as it pertains to epilepsy is that VEGF depresses synaptic transmission and reduces epileptiform activity in vitro.[137] The reduction in activity appears to be greater if epileptiform discharges are examined in slices from an animal with recurrent spontaneous seizures (i.e., epilepsy), as compared to slices from a normal animal that are acutely exposed to a disinhibitory agent. The results suggest potent effects in the epileptic brain relative to normal brain, possibly due to altered expression of VEGF and its receptors after recurrent seizures.

INTERCELLULAR MODULATORS

Components of the ECM, a scaffolding system in the interstitial space made up of glycoproteins, proteoglycans, and other molecules such as hyaluronic acid (Table 4), have recently begun to attract attention as modulators of neuronal function with possible links to epileptogenesis. This is in part based on the fact that the expression of several matrix molecules, such as neural cell adhesion molecule (NCAM),[149] tenascins,[25,150] and chondroitin sulfate proteoglycans such as phosphocan and neurocan, is altered in animal models of TLE. These changes are chronologically and topographically associated with the development of granule cell dispersion and mossy fiber sprouting in epileptic animals.[25,92] Gene microarray studies have confirmed and expanded the correlation between extracellular matrix components and seizures, adding cell adhesion molecules to the list of neuromodulatory proteins.[128] Notably, elevations of glycosaminoglycans and tenascins are also seen in surgical brain tissue obtained from patients with pharmacoresistant TLE. Jointly, these studies therefore raise the possibility that changes in the extracellular matrix may be *causally* involved in the cellular and synaptic reorganization seen in TLE.[7,38,92,96]

Another important aspect of the ECM that is relevant to axonal and cellular reorganization in epilepsy are changes in MMPs after seizures. These enzymes are notable because they normally degrade the ECM and appear to be altered in their expression after seizures.[83] Again the question of whether neuromodulators interact is raised, because the MMPs also cleave proBDNF to BDNF. Thus, degradation of the ECM and elevated BDNF may work in concert to facilitate changes in neural circuits, including mossy fiber sprouting. Finally, the ECM may also modulate excitability through intercellular and transmembrane proteins, like the integrins. These proteins bind both to the ECM and to neuronal plasma membranes and can therefore activate signaling cascades that regulate neural activity.[163] Taken together, these studies suggest that the ECM, both directly and indirectly, could modulate excitability in the context of epilepsy.

TABLE 4

ECM AND OTHER STRUCTURAL PROTEINS

ECM
Collagen
Fibronectin
Laminin
Elastin
Proteoglycans
Chondroitin
Heparan
Keratan
Hyaluronic acid
Syndecan
Transmembrane glycoproteins
Integrins
Cytoskeleton
Actin filaments
Microfilaments
Intermediate filaments
Neurofilaments (NF)
Microtubules
α and β-Tubulin assemblies
Microtubule motors dynein and kinesins
Cell adhesion molecules
Calcium-independent
Neural cell adhesion molecules (NCAMs)
Highly polysialylated CAM (PSA NCAM)
L1
Calcium-dependent
N-cadherins
Integrins

NEUROMODULATION BY ASTROCYTES

The abnormal appearance of non-neuronal cells in the epileptic brain has long been appreciated.[163] Until relatively recently, however, gliosis was viewed simply as a reaction to seizure-related neuronal injury or degeneration, without major functional consequences for the disease process. With a few exceptions, the conceptual and experimental approach to the primary goal of epilepsy research—that is, the elucidation of the cellular mechanisms underlying human epilepsies—was decidedly neurocentric. In functional terms, glial cells (including "reactive" glia) were at best regarded as sinks to buffer the abnormal, proconvulsant rises in extracellular K^+ concentrations that were known to accompany seizure activity.[98,199]

Not until the late 1970s (first articulated comprehensively in an influential monograph of Brotchi),[27] that glial cells began to be considered as significant pathogenic factors in chronic epilepsy. The contributions of these cells to *epileptogenesis* were recognized even later. Noninvasive imaging methods, modern electrophysiologic approaches in animals and humans, the revolution in molecular biology and genetics, and the

recent enthusiastic embrace of glial biology by the neuroscience community, have jointly focused the attention of epilepsy research on the pathophysiologic role of glia. It is clear that glial cells participate actively in the development and maintenance of chronic epilepsy, although the involvement differs between the three major types of glial cells (microglia, oligodendrocytes, and astrocytes). Moreover, accumulating evidence suggests that abnormal glial function may also be a determining factor in the very early stages of epileptic disorders.

The role of resident and, in particular, activated microglial cells in epilepsy appears to be closely linked to the cells' function as the resident immune cells of the brain. As a major source of paracrine signals releasing pro- and anticonvulsive cytokines, growth factors and other neuroactive peptides, and proteins, the agile microglia are increasingly thought to influence not only neuronal communication in the normal brain, but also to facilitate the excessive electrical discharges characteristic of epileptic conditions (see Vezzani and Janigro, this volume).

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Very few studies indicate an involvement of oligodendrocytes, the second major classes of glial cells in the brain, in seizure activity.[146,234] Normal or seizure-induced changes in oligodendrocyte function are therefore not likely to be a major, consistent feature of epileptogenesis or epilepsy. However, this conclusion is still tentative and may have to be revisited in view of recent evidence linking oligodendrocyte function to neurogenesis, a likely factor in the pathophysiology of TLE.[157,177]

Astrocytes are intimately related to neurons in terms of lineage, anatomic arrangements, and function. As indicated by the recently coined term "tripartite synapse,"[5] astrocytes are now in fact considered integral players in neurotransmission. This notion, in turn, prompted a closer look into the function of these glial cells in epilepsy. It is clear that astrocytes, and astrocyte-derived factors, play a critical role in the modulation of acute and chronic seizure activity.

Astrocyte Changes in Epilepsy

Although the "reactive gliosis" seen in the brain of epileptic patients was long suspected to signify disease-related morphologic changes in astrocytes, definitive proof for the identity of the abnormal glial cells was unavailable until relatively recently. This changed with the discovery of glial fibrillary acidic protein (GFAP), a 55-kDa member of the intermediate filament protein family, which is the major fibrous protein of astrocytes.[19] Using anti-GFAP antibodies, hypertrophied astrocytes were soon visualized immunohistochemically in excised tissue specimens obtained during epilepsy surgery[103] and in several animal models of chronic epilepsy. GFAP immunostaining labels pathologic astrocytes primarily in limbic brain areas such as the hippocampus and the parahippocampal region, which are also preferentially sensitive to seizure-induced neurodegeneration.[87,165] GFAP-positive astrocytes were therefore originally believed to be a highly selective tool to identify epileptic lesions. It turns out, however, that abnormal, GFAP-immunoreactive astrocytes are also seen in anatomic sites distant from the seizure focus.[33] Further analyses of the effects of the nature, frequency, and duration of the epileptogenic stimulus on GFAP-positive cells, the use of additional structural astrocyte markers such as vimentin and laminin, and the use of various distinct animal models, also revealed a more complex picture of astrocytic involvement in epilepsy.

Astrocytes react rapidly (within 1 hour) to strong convulsive stimuli through upregulation of glial protein synthesis,[203] but change more slowly in response to moderate seizure activity.[117,205] Although the duration of these and other seizure-related changes (see later discussion) has so far not been studied methodically, they are likely to persist in chronically epileptic animals.[84] Surprisingly, prominent brain region–specific astrocytic hypertrophy can sometimes be detected in the absence of substantive neurodegeneration. This includes in kindled animals, in which the glial changes are accompanied by a reorganization of the cytoskeleton and persist for weeks after the last seizure,[108,109] and in the EL mouse, in which astrocytes proliferate in the hippocampus.[55] These and a considerable number of other supportive examples indicate that a variety of seizures, ranging from single, intense convulsive insults to repetitive, mild episodes, cause regionally distinct structural astrocytic changes that are not necessarily associated with overt neuronal loss or injury. However, these microscopic data do not consider the molecular events(s) linking seizure activity to the glial reaction. They also do not address the presumed functional significance of astrocytic hypertrophy and/or proliferation, including the possible role of these reactive processes in the development and maintenance of chronic epilepsy.[7,144,166]

Astrocytes as Modulators of Extracellular Glutamate

The fact that astrocytes undergo physical changes in response to seizure activity led many investigators to examine the fate of astrocytic neurochemistry in epilepsy. The most obvious molecule of interest in this regard was glutamate, which had been speculatively associated with the pathophysiology of epilepsy as early as 1954.[91] The realization, two decades later, that glutamate is the major excitatory neurotransmitter in the brain initially suggested that enhanced *neuronal* glutamate release might be critically involved in the precipitation of epileptic discharges.[54] Although neuron-derived glutamate is still believed to play a major role in seizure activity, attention has increasingly shifted to astrocytes as the major determinants and sources of extracellular glutamate levels in both the normal and epileptic brain. Thus, the physiologic concentration of glutamate in the extracellular milieu, which is in the low micromolar range, is controlled mainly by two highly efficient astrocytic glutamate transporters (GLT)-1 and GLAST. These uptake sites also prevent the accumulation of convulsant, excitotoxic concentrations of the amino acid, as demonstrated in animals with a genetic deletion in either one of these proteins.[169,209,230] These studies, as well as a report showing substantial reductions in GLT-1 and GLAST expression in epilepsy-prone rats prior to seizure onset,[60] are particularly important because they provide evidence that malfunctioning astrocytes can *cause* seizure activity by elevating extracellular glutamate to pathologic levels. Interestingly, GLT-1 is also reduced as a sequela of epilepsy, thus potentially exacerbating the clinical condition.[173]

Selective transporters are not the only determinants of extracellular glutamate linked to astrocyte function. As shown originally by Haydon et al., neuronal stimulation induces a frequency-dependent calcium response in closely apposed astrocytes, which then causes these cells to release glutamate. This astrocyte-derived glutamate, in turn, modulates glutamatergic neuronal transmission.[5] It follows that any breakdown of this finely tuned interplay between neurons and astrocytes may result in enhanced glutamate release and seizure activity.[70] This hypothesis, which again indicates that astrocytes can be *causally*involved in epileptogenesis, has recently been supported by experimental manipulation of intracellular astrocytic calcium (Ca^{2+}).[214]

Within astrocytes, glutamate serves as a substrate of glutamine synthetase, an essential component of the glutamine–glutamate shuttle between astrocytes and neurons.[95] This enzyme can be viewed as a precursor of potentially neurotoxic neuronal glutamate.[8] Alternatively, glutamine synthetase may function as a guardian against the accumulation of convulsive

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and neurotoxic concentrations of glutamate in large astrocytic vesicles, which release their content via SNARE-dependent exocytosis to cause excitation in neighboring neurons.[105] It is therefore unclear whether inhibition of glutamine synthetase, which normally causes a *decline* in extracellular glutamate levels in the brain,[23] has pro- or anticonvulsant consequences under epileptic conditions. Measurements of glutamine formation in animal models of epilepsy[59,215] and in the brain of patients with TLE[63,201] have produced equivocal results and have so far failed to clearly define its role in seizures. Similarly inconclusive data have been obtained in nuclear magnetic resonance (NMR) spectroscopic studies in epileptic tissue, which examined the astrocyte-specific incorporation of ^{13}C-acetate into ^{13}C-glutamate and ^{13}C-glutamine.[114,141,147,155]

The external mechanisms controlling astrocytic glutamate disposition are currently only poorly understood, but are of obvious relevance for epilepsy, because unchecked glutamate release is likely to intensify both acute and chronic seizure activity. It is therefore noteworthy that hemichannels and multidrug resistance proteins[68] (i.e., proteins that regulate glutamate exocytosis from astrocytes) are upregulated in astrocytes in chronic epilepsy.[120,133,221] Notably, several subtypes of metabotropic[6,210,220] or ionotropic[192] glutamate receptors are also overexpressed in astrocytes in the epileptic brain, although the functional significance of these receptors has not been clarified so far.

Taken together, these studies leave little doubt that astrocytes, rather than neurons or other brain cells, hold the key to the role of glutamate in epileptic phenomena. Conceptually, glutamate may therefore be assigned the somewhat unorthodox function of an astrocyte-derived or astrocyte-controlled *neuromodulator*. However, because of the large number of functional proteins serving as its intra- and extracellular targets, and due to its multiple roles in cytosolic and mitochondrial energy flux and intermediary metabolism, the participation of astrocytic glutamate in epileptogenesis and chronic epilepsy is highly complex and requires considerable additional scrutiny.

Neuromodulation by Other Astrocytic Factors and Mechanisms

The role of astrocytes in seizure generation and maintenance is not only defined by the complex fate of glutamate itself but also involves several other glial products, which indirectly affect glutamate metabolism and function. This growing list of endogenous factors includes nitric oxide (NO), which is produced by nitric oxide synthase (NOS). At least two isoforms of this enzyme (neural NOS [nNOS] and inducible NOS [iNOS]) are upregulated in reactive astrocytes in response to seizures,[34,102] leading to increased NO formation, which in turn stimulates glutamatergic neurotransmission and may thus affect hyperexcitability.[71] The neuroexcitatory glutamate homolog aspartate, too, is formed in astrocytes, although it is still unclear whether glial release of this amino acid can augment or substitute for the neuronal effects of glutamate under physiologic and pathologic conditions. Resolution of this issue seems important in view of the fact that extracellular aspartate levels are significantly enhanced in TLE[110,195] and in animal models of epilepsy.[66,142,161] The activity of its degradative enzyme, aspartate aminotransferase, is also increased in actively spiking human epileptic cortex[110]; however, and the de novo synthesis of^{13}C-aspartate from ^{13}C-acetate is unchanged in chronically epileptic rats.[141] Notably, astrocytes also play a major role in the generation and disposition of other endogenous amino acids such as glycine and D-serine, or dipeptides like N-acetylaspartylglutamate, all of which profoundly influence glutamate function in the normal brain. These agents, sometimes cumulatively termed "gliotransmitters," are therefore increasingly studied for their possible role in the pathophysiology of various brain diseases, including epilepsy.[43,101]

Another astrocytic product with links to glutamatergic neurotransmission is kynurenic acid (KYNA), a neuroinhibitory metabolite of the kynurenine pathway of tryptophan degradation. KYNA's preferential blockade of *N*-methyl-D-aspartic acid (NMDA) receptor function[158] probably accounts for its potent anticonvulsant and antiexcitotoxic properties.[74] Interestingly, the extracellular levels of endogenous KYNA are acutely elevated following the administration of convulsive agents.[237] Moreover, KYNA-forming astrocytes are hypertrophic, and KYNA synthesis is enhanced in the limbic brain areas of chronically epileptic animals.[56,236] Interpreted teleologically, enhanced KYNA production and release can therefore be viewed as an endogenous attempt to mobilize astrocytes for antiepileptogenic, anticonvulsant, and neuroprotective purposes. Related to this role of KYNA, attention must also be paid to the lysine metabolite a-aminoadipate, which is present in the mammalian brain in micromolar concentrations and is avidly accumulated by astrocytes.[89,164] Within astrocytes, α-aminoadipate inhibits the biosynthesis of KYNA and may thus indirectly facilitate seizure activity.[191,238]

The active participation of astrocytes in seizure activity is not necessarily limited to molecules and mechanisms that affect glutamatergic neurotransmission directly. Thus, astrocytes express a large number of proteins that cause structural changes in the cell, alter extracellular ion concentrations, or control intra- and intercellular signalling through an array of messenger molecules. Seizure-related changes in several of these proteins have been reported in TLE and in both acute and chronic animal models of epilepsy, and may contribute to—or protect against—pathology. Examples include the embryonic intermediate filament component nestin, which may remodel the glial cytoskeleton in the epileptic brain[190]; S100β, which may be involved in structural reorganization in association with chronic seizure activity[17]; and the small heat-shock protein 27, which may actively participate in seizure-induced neurodegeneration.[18] Seizures also cause abnormal expression patterns and changes in the biophysical properties of astrocytic K^+ and Na^+ channels,[22,86,202] increase astrocytic communication by upregulating the connexin 43 gap junction protein,[73] and reduce the density of the water channel aquaporin 4, which regulates the clearance of extracellular K^+along the perivascular membrane domain of astrocytes.[62] These changes are accompanied by dysfunctional astrocytic enzymes, receptors, and transporters, which influence seizure activity through altering the metabolic fate and biologic effects of important chemical messengers such as GABA,[121,183,187] adenosine,[69] and prostaglandins.[53] Future research will need to dissect the respective contributions of these diverse impairments to disease manifestation. In addition, we must consider that the pathogenic role of microglia-derived cytokines in epileptogenesis and epilepsy, too, is at least in part dependent on the presence of astrocytes.[125,225]

Astrocyte Dysfunction: Implications for Pathogenesis and Therapy

Despite the large number of studies demonstrating astrocytic abnormalities, most of the information accumulated so far is correlative in nature and does not clarify if astrocyte impairment can in fact *cause*, rather than play an adjuvant role in, the epileptic condition.[26,93] Causality is especially difficult to establish in the chronically epileptic brain, in which astrocytes have already undergone structural and functional changes as a consequence of seizure activity. This distinction between a

primary and secondary role of astrocytes in epileptic disorders is of more than theoretical interest because it has implications for the design of therapeutic strategies. Experimentally, the question can be most unequivocally addressed by selectively manipulating astrocyte function in the normal brain and assessing the effects of the intervention on various seizure parameters. Approaches include direct or indirect interference with astrocytic glutamate function (see the earlier discussion) and the specific elimination of astrocytic proteins such as S100β[61] or GFAP.[154]

Other studies have successfully used fluorocitrate, an aconitase inhibitor that selectively incapacitates astrocytes by reversibly blocking tricarboxylic acid cycle activity. These experiments, which are especially instructive in view of the established dysfunction of cellular energy metabolism in epilepsy,[106] showed that transient astrocyte poisoning causes epileptiform discharges and even convulsive seizures[232] and also lowers the seizure threshold to systemically administered chemoconvulsants.[122] Taken together, all these studies provide convincing evidence that astrocyte dysfunction can singularly trigger epileptogenic events in the normal brain.

Each of the many changes in astrocytic biochemistry seen in epileptic animals and humans may play an active role in pathophysiology. As described earlier for some of the most prominent examples elaborated to date, these changes can be simplistically categorized into being either facilitatory or inhibitory of the clinical condition. It follows that it may be possible to develop novel therapeutic agents by targeting astrocytes to specifically interfere with proconvulsive mechanisms or to boost endogenous anticonvulsive principles. Exploitation of this concept—the pharmacologic targeting of glial neuromodulators for the treatment of epilepsy—is still in its infancy,[236] although the use of astrocytes for therapeutic purposes has received considerable attention, for example, in the area of Alzheimer disease and Parkinson disease.[36,184] Notably, this approach is not limited to those glial mechanisms known to be chronically impaired in the disease, but could also be advantageously used to influence physiologic signalling processes that normally link astrocytes to neuronal and cerebrovascular function.[112,159] Any of these manipulations of astrocyte function may, in fact, play a role in the clinical efficacy of a number of currently used anticonvulsant drugs and therapies.[31,75,160,243]

SUMMARY AND CONCLUSIONS

Although often considered relatively minor role players, neuromodulators are increasingly recognized as critical factors in brain physiology. As reviewed here, their ability to modulate neuronal excitability provides a logical link to seizure activity. Indeed, studies in animals have demonstrated that virtually all neuromodulators examined so far are capable of enhancing or reducing seizure susceptibility in the normal brain either directly or indirectly. Alone or in concert, they may therefore play a significant role in seizure initiation under otherwise physiologic conditions.

Perhaps more intriguing, neuromodulators appear to be critically involved in processes that are relevant to the development of epilepsy, such as neurogenesis, axonal sprouting and, more generally, the altered expression of genes that are causally related to epileptogenesis. In turn, neuromodulators—and neuromodulation—are themselves altered once the state of chronic epilepsy is established. This and concomitant persistent alterations in receptors for neuropeptides and growth factors, as well as changes in neural circuitry, glia, and extracellular milieu, explain why the same neuromodulator may have quantitatively and qualitatively different effects in the normal and epileptic brain. In addition to being relevant for pathophysiologic considerations, these differences also highlight the importance of studying animal models that optimally approximate the epileptic condition in humans.

Despite their complexity, the neuromodulatory mechanisms summarized here provide a vast, varied, and rich resource for new potential therapeutic targets. Indeed, one could argue that a focus on modulators would constitute a superior strategy for anticonvulsant drug development, because it may minimize the side effects associated with conventional targets such as classic neurotransmitters systems (glutamate, GABA) or ion channels (sodium channels, potassium channels). Given the rapid progress in our understanding of the role of neuromodulation in the pathophysiology of epilepsy, this concept could be evaluated clinically in the not too distant future.

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References

1. Abood LG, Knapp R, Mitchell T, et al. Chemical requirements of vasopressins for barrel rotation convulsions and reversal by oxytocin. *J Neurosci Res*. 1980;5:191–199.
2. Adolfo Arganaraz G, Regina Perosa S, et al. Role of kinin B1 and B2 receptors in the development of pilocarpine model of epilepsy. *Brain Res*. 2004;1013:30–39.
3. Alsbo CW, Kristiansen U, Moller F, et al. $GABA_A$ receptor subunit interactions important for benzodiazepine and zinc modulation: a patch-clamp and single cell RT-PCR study. *Eur J Neurosci*. 2001;13:1673–1682.
4. Andrews N, Davis B, Gonzalez MI, et al. Effect of gastrin-releasing peptide on rat hippocampal extracellular GABA levels and seizures in the audiogenic seizure-prone DBA/2 mouse. *Brain Res*. 2000;859:386–389.
5. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci*. 1999;22:208–215.
6. Aronica E, van Vliet EA, Mayboroda OA, et al. Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial TLE. *Eur J Neurosci*. 2000;12:2333–2344.
7. Babb TL, Mathern GW, Pretorius JK, Cifuentes F. Astrocytes may contribute to the latent period in progressive neuron loss, axon sprouting, and chronic seizures in rat kainate hippocampal epilepsy. *Epilepsy Res Suppl*. 1996;12:343–354.
8. Bacci A, Sancini G, Verderio C, et al. Block of glutamate-glutamine cycle between astrocytes and neurons inhibits epileptiform activity in hippocampus. *J Neurophysiol*. 2002;88:2302–2310.
9. Balosso S, Ravizza T, Perego C, et al. Tumor necrosis factor-α inhibits seizures in mice via p75 receptors. *Ann Neurol*. 2005;57:804–812.
10. Bandtlow CE, Dlaska M, Pirker S, et al. Increased expression of Nogo-A in hippocampal neurons of patients with TLE. *Eur J Neurosci*. 2004;20:195–206.
11. Banerjee SB, Rajendran R, Dias BG, et al. Recruitment of the Sonic hedgehog signalling cascade in electroconvulsive seizure-mediated regulation of adult rat hippocampal neurogenesis. *Eur J Neurosci*. 2005;22:1570–1580.
12. Baraban SC. Neuropeptide Y and epilepsy: recent progress, prospects and controversies. *Neuropeptides*. 2004;38:261–265.
13. Baraban SC, Hollopeter G, Erickson JC, et al. Knockout mice reveal a critical antiepileptic role for neuropeptide Y. *J Neurosci*. 1997;17:8927–8936.
14. Baratta MV, Lamp T, Tallent MK. Somatostatin depresses long-term potentiation and Ca^{2+} signaling in mouse dentate gyrus. *J Neurophysiol*. 2002;88:3078–3086.
15. Barnes G, Puranam RS, Luo Y, McNamara JO. Temporal specific patterns of semaphorin gene expression in rat brain after kainic acid-induced status epilepticus. *Hippocampus*. 2003;13:1–20.
16. Bauer J. Epilepsy and prolactin in adults: a clinical review. *Epilepsy Res*. 1996;24:1–7.
17. Bendotti C, Guglielmetti F, Tortarolo M, et al. Differential expression of S100β and glial fibrillary acidic protein in the hippocampus after kainic acid-induced lesions and mossy fiber sprouting in adult rat. *Exp Neurol*. 2000;161:317–329.
18. Bidmon HJ, Gorg B, Palomero-Gallagher N, et al. Bilateral, vascular and perivascular glial upregulation of heat shock protein-27 after repeated epileptic seizures. *J Chem Neuroanat*. 2005;30:1–16.
19. Bignami A, Eng LF, Dahl D, Uyeda CT. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Res*. 1972;43:429–435.
20. Binaschi A, Bregola G, Simonato M. On the role of somatostatin in seizure control: clues from the hippocampus. *Rev Neurosci*. 2003;14:285–301.

21. Binder DK. The role of BDNF in epilepsy and other diseases of the mature nervous system. *Adv Exp Med Biol*. 2004;548:34–56.
22. Bordey A, Spencer DD. Distinct electrophysiologic alterations in dentate gyrus versus CA1 glial cells from epileptic humans with temporal lobe sclerosis. *Epilepsy Res*. 2004;59:107–22.
23. Bottcher T, Goiny M, Bering J, et al. Regional differences in glutamine synthetase inhibition by L-methionine sulfoximine: a microdialysis study in the rabbit brain. *Exp Brain Res*. 2003;150:194–200.
24. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol*. 2005;76:99–125.
25. Brenneke F, Schachner M, Elger CE, Lie AA. Upregulation of the ECM glycoprotein tenascin-R during axonal reorganization and astrogliosis in the adult rat hippocampus. *Epilepsy Res*. 2004;58:133–143.
26. Briellmann RS, Kalnins RM, Berkovic SF, Jackson GD. Hippocampal pathology in refractory TLE: T2-weighted signal change reflects dentate gliosis. *Neurology*. 2002;58:265–271.
27. Brotchi J. Activated astrocytes and epileptogenic focus. Histoenzymology. *Acta Neurol Belg*. 1979;79:137–304.
28. 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.
29. Buckmaster PS, Tam E, Schwartzkroin PA. Electrophysiologic correlates of seizure sensitivity in the dentate gyrus of epileptic juvenile and adult gerbils. *J Neurophysiol*. 1996;76:2169–2180.
30. Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a TLE model. *Science*. 1996;271:369–373.
31. Cardile V, Pavone A, Gulino R, et al. Expression of brain-derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS) in rat astrocyte cultures treated with levetiracetam. *Brain Res*. 2003;976:227–233.
32. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. *Semin Cell Dev Biol*. 2002;13:39–53.
33. Castiglioni AJ, Peterson SL, Sanabria EL, et al. Structural changes in astrocytes induced by seizures in a mode of TLE. *J Neurosci Res*. 1990;26:334–341.
34. Catania MV, Giuffrida R, Seminara G, et al. Upregulation of neuronal nitric oxide synthase in in vitro stellate astrocytes and in vivo reactive astrocytes after electrically induced status epilepticus. *Neurochem Res*. 2003;28:607–615.
35. Celio MR, Pauls T, Schwaller B, eds. *Guidebook to calcium binding proteins*. Oxford: Oxford University Press; 1996.
36. Chen LW, Yung KL, Chan YS. Reactive astrocytes as potential manipulation targets in novel cell replacement therapy of Parkinson's disease. *Curr Drug Targets*. 2005;6:821–833.
37. Chepurnova NE, Ponomarenko AA, Chepurnov SA. Peptidergic mechanisms of hyperthermia-evoked convulsions in rats in early postnatal ontogenesis. *Neurosci Behav Physiol*. 2002;32:505–511.

Ref 37 is not cited in text?

38. Chevassus-Au-Lois N, Niquet J, Ben-Ari Y, Represa A. Cellular plasticity. In: Engel J, Pedley T, eds. *Epilepsy: A comprehensive textbook*. Philadelphia: Lippincott-Raven; 1997: 387–396.
39. Ciranna L, Cavallaro S. Opposing effects by pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide on hippocampal synaptic transmission. *Exp Neurol*. 2003;184:778–784.
40. Collin T, Chat M, Lucas MG, et al. Developmental changes in parvalbumin regulate presynaptic Ca^{2+} signaling. *J Neurosci*. 2005;25:96–107.
41. Conner JM, Lauterborn JC, Yan Q, et al. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J Neurosci*. 1997;17:2295–2313.
42. Coulter DA. Mossy fiber zinc and TLE: pathologic association with altered "epileptic" gamma-aminobutyric acid A receptors in dentate granule cells. *Epilepsia*. 2000;41 Suppl 6:S96–99.
43. Coyle JT. The nagging question of the function of N-acetylaspartylglutamate. *Neurobiol Dis*. 1997;4:231–238.
44. Crino PB. Molecular pathogenesis of focal cortical dysplasia and hemimegalencephaly. *J Child Neurol*. 2005;20:330–336.
45. Croiset G, De Wied D. Proconvulsive effect of vasopressin; mediation by a putative V2 receptor subtype in the central nervous system. *Brain Res*. 1997;759:18–23.
46. 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.
47. Csaba Z, Pirker S, Lelouvier B, et al. Somatostatin receptor type 2 undergoes plastic changes in the human epileptic dentate gyrus. *J Neuropathol Exp Neurol*. 2005;64:956–969.
48. Cunha-Reis D, Sebastiao AM, Wirkner K, et al. VIP enhances both pre- and postsynaptic GABAergic transmission to hippocampal interneurones leading to increased excitatory synaptic transmission to CA1 pyramidal cells. *Br J Pharmacol*. 2004;143:733–744.
49. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human TLE. *Brain Res*. 1989;495:387–395.
50. de Lanerolle NC, Kim JH, Williamson A, et al. A retrospective analysis of hippocampal pathology in human TLE: evidence for distinctive patient subcategories. *Epilepsia*. 2003;44:677–687.
51. Deadwyler GD, Pouly S, Antel JP, Devries GH. Neuregulins and erbB receptor expression in adult human oligodendrocytes. *Glia*. 2000;32:304–312.
52. Deller T, Leranth C. Synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. *J Comp Neurol*. 1990;300:433–447.
53. Desjardins P, Sauvageau A, Bouthillier A, et al. Induction of astrocytic cyclooxygenase-2 in epileptic patients with hippocampal sclerosis. *Neurochem Int*. 2003;42:299–303.
54. Dodd PR, Bradford HF. Release of amino acids from the maturing cobalt-induced epileptic focus. *Brain Res*. 1976;111:377–388.
55. Drage MG, Holmes GL, Seyfried TN. Hippocampal neurons and glia in epileptic EL mice. *J Neurocytol*. 2002;31:681–692.
56. Du F, Williamson J, Bertram E, Lothman E, et al. Kynurenine pathway enzymes in a rat model of chronic epilepsy: immunohistochemical study of activated glial cells. *Neuroscience*. 1993;55:975–989.
57. Dumas TC, Powers EC, Tarapore PE, Sapolsky RM. Overexpression of calbindin D(28k) in dentate gyrus granule cells alters mossy fiber presynaptic function and impairs hippocampal-dependent memory. *Hippocampus*. 2004;14:701–709.
58. During MJ, Cao L, Zuzga DS, et al. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med*. 2003;9:1173–1179.
59. Dutuit M, Didier-Bazes M, Vergnes M, et al. Specific alteration in the expression of glial fibrillary acidic protein, glutamate dehydrogenase, and glutamine synthetase in rats with genetic absence epilepsy. *Glia*. 2000;32:15–24.
60. Dutuit M, Touret M, Szymocha R, et al. Decreased expression of glutamate transporters in genetic absence epilepsy rats before seizure occurrence. *J Neurochem*. 2002;80:1029–1038.
61. Dyck RH, Bogoch II, Marks A, et al. Enhanced epileptogenesis in S100B knockout mice. *Mol Brain Res*. 2002;106:22–29.
62. Eid T, Lee TS, Thomas MJ, et al. Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc Natl Acad Sci U S A*. 2005;102:1193–1198.
63. Eid T, Thomas MJ, Spencer DD, et al. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial TLE. *Lancet*. 2004;363:28–37.
64. Eilam R, Pinkas-Kramarski R, Ratzkin BJ, et al. Activity-dependent regulation of Neu differentiation factor/neuregulin expression in rat brain. *Proc Natl Acad Sci U S A*. 1998;95:1888–1893.
65. El Bahh B, Balosso S, Hamilton T, et al. The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y2 and not Y5 receptors. *Eur J Neurosci*. 2005;22:1417–1430.
66. Engstrom ER, Hillered L, Flink R, et al. Extracellular amino acid levels measured with intracerebral microdialysis in the model of posttraumatic epilepsy induced by intracortical iron injection. *Epilepsy Res*. 2001;43:135–144.
67. Ethell IM, Pasquale EB. Molecular mechanisms of dendritic spine development and remodeling. *Prog Neurobiol*. 2005;75:161–205.
68. Evanko DS, Zhang Q, Zorec R, Haydon PG. Defining pathways of loss and secretion of chemical messengers from astrocytes. *Glia*. 2004;47:233–240.
69. Fedele DE, Gouder N, Guttinger M, et al. Astrogliosis in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation. *Brain*. 2005;128:2383–2395.
70. Fellin T, Haydon PG. Do astrocytes contribute to excitation underlying seizures?. *Trends Mol Med*. 2005;11:530–533.
71. Ferraro G, Sardo P. Nitric oxide and brain hyperexcitability. *In Vivo*. 2004;18:357–366.
72. Ferrer I, Alcantara S, Ballabriga J, et al. Transforming growth factor-alpha (TGF-α) and epidermal growth factor-receptor (EGF-R) immunoreactivity in normal and pathologic brain. *Prog Neurobiol*. 1996;49:99–123.
73. Fonseca CG, Green CR, Nicholson LF. Upregulation in astrocytic connexin 43 gap junction levels may exacerbate generalized seizures in mesial TLE. *Brain Res*. 2002;929:105–116.
74. Foster AC, Vezzani A, French ED, Schwarcz R. Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neurosci Lett*. 1984;48:273–278.
75. Fraser CM, Sills GJ, Butler E, et al. Effects of valproate, vigabatrin and tiagabine on GABA uptake into human astrocytes cultured from foetal and adult brain tissue. *Epileptic Disord*. 1999;1:153–157.
76. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci*. 2005;6:449–462.
77. Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6:347–470.
78. Freund TF, Magloczky Z. Early degeneration of calretinin-containing neurons in the rat hippocampus after ischemia. *Neuroscience*. 1993;56:581–596.
79. Gale K. Fibroblast growth factors and seizure-induced neuroprotection In: Binder DK, Scharfman HE, eds. *Growth factors and epilepsy*. Novasciences; in press.
80. Gall CM. Localization and seizure-induced alterations of opioid peptides and CCK in the hippocampus. *NIDA Res Monogr*. 1988;82:12–32.
81. Gall CM. Regulation of brain neurotrophin expression by physiologic activity. *Trends Pharmacol Sci*. 1992;13:401–403.
82. Gall CM. Seizure-induced changes in neurotrophin expression: implications for epilepsy. *Exp Neurol*. 1993;124:150–166.

83. Gall CM, Lynch G. Integrins, synaptic plasticity and epileptogenesis. *Adv Exp Med Biol*. 2004;548:12–33.
84. Garzillo CL, Mello LE. Characterization of reactive astrocytes in the chronic phase of the pilocarpine model of epilepsy. *Epilepsia*. 2002;43 Suppl 5:107–109.
85. Gorter JA, Mesquita AR, van Vliet EA, et al. Increased expression of ferritin, an iron-storage protein, in specific regions of the parahippocampal cortex of epileptic rats. *Epilepsia*. 2005;46:1371–1379.
86. Gorter JA, van Vliet EA, Lopes da Silva FH, et al. Sodium channel β1-subunit expression is increased in reactive astrocytes in a rat model for mesial TLE. *Eur J Neurosci*. 2002;16:360–364.
87. Gramsbergen JB, van den Berg KJ. Regional and temporal profiles of calcium accumulation and glial fibrillary acidic protein levels in rat brain after systemic injection of kainic acid. *Brain Res*. 1994;667:216–228.
88. Gray W, Scharfman HE. Neuropeptide Y and hippocampal neurogenesis. In: Zukowska Z, Feuerstein G, eds. *NPY family of peptides in inflammation, immune disorders, angiogenesis, and cancer*. Switzerland: Birkäuser in press.
89. Guidetti P, Schwarcz R. Determination of α-aminoadipic acid in brain, peripheral tissues, and body fluids using GC/MS with negative chemical ionization. *Mol Brain Res*. 2003;118:132–139.
90. Hajos N, Acsady L, Freund TF. Target selectivity and neurochemical characteristics of VIP-immunoreactive interneurons in the rat dentate gyrus. *Eur J Neurosci*. 1996;8:1415–1431.
91. Hayashi T. Effects of sodium glutamate on the nervous system. *Keio J Med*. 1954;3:183–192.
92. Heck N, Garwood J, Loeffler JP, et al. Differential upregulation of ECM molecules associated with the appearance of granule cell dispersion and mossy fiber sprouting during epileptogenesis in a murine model of TLE. *Neuroscience*. 2004;129:309–324.
93. Heinemann U, Gabriel S, Schuchmann S, Eder C. Contribution of astrocytes to seizure activity. *Adv Neurol*. 1999;79:583–590.
94. Hempstead BL. Dissecting the diverse actions of pro- and mature neurotrophins. *Curr Alzheimer Res*. 2006;3:19–24.
95. Hertz L, Zielke HR. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci*. 2004;27:735–743.
96. Hoke A, Silver J. Proteoglycans and other repulsive molecules in glial boundaries during development and regeneration of the nervous system. *Prog Brain Res*. 1996;108:149–163.
97. Holtmaat AJ, Gorter JA, De Wit J, et al. Transient downregulation of Sema3A mRNA in a rat model for TLE. A novel molecular event potentially contributing to mossy fiber sprouting. *Exp Neurol*. 2003;182:142–150.
98. Hotson JR, Sypert GW, Ward AA, Jr. Extracellular potassium concentration changes during propagated seizures in neocortex. *Exp Neurol*. 1973;38:20–26.
99. Jankowsky JL, Patterson PH. Differential regulation of cytokine expression following pilocarpine-induced seizure. *Exp Neurol*. 1999;159:333–346.
100. Jankowsky JL, Patterson PH. The role of cytokines and growth factors in seizures and their sequelae. *Prog Neurobiol*. 2001;63:125–149.
101. Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry*. 2004;9:984–979.
102. Kalinichenko SG, Dudina YV, Dyuizen IV, Motavkin PA. Induction of NO synthase and glial acidic fibrillary protein in astrocytes in the temporal cortex of the rat with audiogenic epileptiform reactions. *Neurosci Behav Physiol*. 2005;35:629–634.
103. Kallioinen MJ, Heikkinen ER, Nystrom S. Histopathologic and immunohistochemical changes in neurosurgically resected epileptic foci. *Acta Neurochir*. 1987;89:122–129.
104. Kalynchuk LE, Meaney MJ, Kar S. Amygdala kindling decreases insulin-like growth factor-I receptor binding sites in the rat hippocampus. *Brain Res*. 2002;935:118–123.
105. Kang N, Xu J, Xu Q, et al. Astrocytic glutamate release-induced transient depolarization and epileptiform discharges in hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 2005;94:4121–4130.
106. Kann O, Kovacs R, Njunting M, et al. Metabolic dysfunction during neuronal activation in the ex vivo hippocampus from chronic epileptic rats and humans. *Brain*. 2005;128:2396–2407.
107. Kar S, Seto D, Dore S, et al. Systemic administration of kainic acid induces selective time dependent decrease in [125I]insulin-like growth factor I, [125I]insulin-like growth factor II and [125I]insulin receptor binding sites in adult rat hippocampal formation. *Neuroscience*. 1997;80:1041–1055.
108. Khurgel M, Ivy GO. Astrocytes in kindling: relevance to epileptogenesis. *Epilepsy Res*. 1996;26:163–175.
109. Khurgel M, Switzer RC III, Teskey GC, et al. Activation of astrocytes during epileptogenesis in the absence of neuronal degeneration. *Neurobiol Dis*. 1995;2:23–35.
110. Kish SJ, Dixon LM, Sherwin AL. Aspartic acid aminotransferase activity is increased in actively spiking compared with non-spiking human epileptic cortex. *J Neurol Neurosurg Psychiatry*. 1988;51:552–556.
111. Klein R. Eph/ephrin signaling in morphogenesis, neural development and plasticity. *Curr Opin Cell Biol*. 2004;16:580–589.
112. Koehler RC, Gebremedhin D, Harder DR. Role of astrocytes in cerebrovascular regulation. *J Appl Physiol*. 2006;100:307–317.
113. Kokaia M, Lindvall O. The GDNF family of neurotrophic factors and epilepsy. In: Binder DK, Scharfman HE, eds. *Growth factors and epilepsy*. Novasciences; in press.
114. Kondziella D, Hammer J, Sletvold O, Sonnewald U. The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: glial-neuronal metabolic interactions. *Neurochem Int*. 2003;43:629–637.
115. Kovacs A, Telegdy G. The effects of calcitonin-gene related peptide (CGRP) on strychnine-induced seizures. *Neurobiology*. 1997;5:75–77.
116. Kovalchuk Y, Holthoff K, Konnerth A. Neurotrophin action on a rapid timescale. *Curr Opin Neurobiol*. 2004;14:558–563.
117. Kragh J, Bolwig TG, Woldbye DP, Jorgensen OS. Electroconvulsive shock and lidocaine-induced seizures in the rat activate astrocytes as measured by glial fibrillary acidic protein. *Biol Psychiatry*. 1993;33:794–800.
118. Kubek MJ, Garg BP. Thyrotropin-releasing hormone in the treatment of intractable epilepsy. *Pediatr Neurol*. 2002;26:9–17.
119. Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. *Science*. 2001;294:1945–1948.
120. Lee SH, Magge S, Spencer DD, et al. Human epileptic astrocytes exhibit increased gap junction coupling. *Glia*. 1995;15:195–202.
121. Lee TS, Bjornsen LP, Paz C, et al. GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. *Acta Neuropathol (Berl)*. 2006: 1–13.
122. Lian XY, Stringer JL. Inhibition of aconitase in astrocytes increases the sensitivity to chemical convulsants. *Epilepsy Res*. 2004;60:41–52.
123. Lin YY, Su MS, Yiu CH, et al. Relationship between mesial temporal seizure focus and elevated serum prolactin in TLE. *Neurology*.1997;49:528–532.
124. Lin YY, Yen SH, Pan JT, et al. Transient elevation in plasma prolactin level in rats with temporal lobe status epilepticus. *Neurology*. 1999;53:885–887.
125. Liu H, Prayson RA, Estes ML, et al. In vivo expression of the interleukin 4 receptor α by astrocytes in epilepsy cerebral cortex. *Cytokine*. 2000;12:1656–1661.
126. Lopez-Picon F, Puustinen N, Kukko-Lukjanov TK, Holopainen IE. Resistance of neurofilaments to degradation, and lack of neuronal death and mossy fiber sprouting after kainic acid-induced status epilepticus in the developing rat hippocampus. *Neurobiol Dis*. 2004;17:415–426.
127. Lukasiuk K, Kontula L, Pitkanen A. cDNA profiling of epileptogenesis in the rat brain. *Eur J Neurosci*. 2003;17:271–279.
128. Lukasiuk K, Pitkanen A. Large-scale analysis of gene expression in epilepsy research: is synthesis already possible?. *Neurochem Res*. 2004;29:1169–1178.
129. Lurton D, Cavalheiro EA. Neuropeptide-Y immunoreactivity in the pilocarpine model of TLE. *Exp Brain Res*. 1997;116:186–190.
130. Madsen TM, Newton SS, Eaton ME, et al. Chronic electroconvulsive seizure upregulates beta-catenin expression in rat hippocampus: role in adult neurogenesis. *Biol Psychiatry*. 2003;54:1006–1014.
131. Magloczky Z, Halasz P, Vajda J, et al. Loss of Calbindin-D28K immunoreactivity from dentate granule cells in human TLE. *Neuroscience*. 1997;76:377–385.
132. Marcinkiewicz M, Nagao T, Day R, et al. Pilocarpine-induced seizures are accompanied by a transient elevation in the messenger RNA expression of the prohormone convertase PC1 in rat hippocampus: comparison with nerve growth factor and brain-derived neurotrophic factor expression. *Neuroscience*. 1997;76:425–439.
133. Marroni M, Agrawal ML, Kight K, et al. Relationship between expression of multiple drug resistance proteins and p53 tumor suppressor gene proteins in human brain astrocytes. *Neuroscience*. 2003;121:605–617.
134. Marty S. Differences in the regulation of neuropeptide Y, somatostatin and parvalbumin levels in hippocampal interneurons by neuronal activity and BDNF. *Prog Brain Res*. 2000;128:193–202.
135. Masuda Y, Miura N, Kawarada Y, et al. Platelet-derived growth factor B-chain homodimer suppressing a convulsion of epilepsy model mouse El. *Biochem Biophys Res Commun*. 1996;223:60–63.
136. Mazarati AM, Halaszi E, Telegdy G, et al. ANP(1-28), BNP(1-32) and CNP(1-22) increase the severity of picrotoxin-kindled seizure syndrome in rats. *Life Sci*. 1993;52:PL19–24.
137. 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;25:8889–8897.
138. McGinty JF, Henriksen SJ, Goldstein A, et al. Dynorphin is contained within hippocampal mossy fibers: immunochemical alterations after kainic acid administration and colchicine-induced neurotoxicity. *Proc Natl Acad Sci U S A*. 1983;80:589–593.
139. McQuiston AR, Petrozzino JJ, Connor JA, Colmers WF. Neuropeptide Y1 receptors inhibit N-type calcium currents and reduce transient calcium increases in rat dentate granule cells. *J Neurosci*. 1996;16:1422–1429.
140. Meier S, Brauer AU, Heimrich B, et al. Molecular analysis of Nogo expression in the hippocampus during development and following lesion and seizure. *FASEB J*. 2003;17:1153–1155.
141. Melo TM, Nehlig A, Sonnewald U. Metabolism is normal in astrocytes in chronically epileptic rats: a (13)C NMR study of neuronal-glial interactions in a model of TLE. *J Cereb Blood Flow Metab*. 2005;25:1254–1264.
142. Millan MH, Chapman AG, Meldrum BS. Extracellular amino acid levels in hippocampus during pilocarpine-induced seizures. *Epilepsy Res*. 1993;14:139–148.
143. Minami M, Maekawa K, Yamakuni H, et al. Kainic acid induces leukemia inhibitory factor mRNA expression in the rat brain: differences in the time course of mRNA expression between the dentate gyrus and hippocampal CA1/CA3 subfields. *Mol Brain Res*. 2002;107:39–46.

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144. Miyazaki T, Miyamoto O, Janjua NA, et al. Reactive gliosis in areas around third ventricle in association with epileptogenesis in amygdaloid-kindled rat. *Epilepsy Res*. 2003;56:5–15.
145. Morgan TE, Nichols NR, Pasinetti GM, Finch CE. TGF-β1 mRNA increases in macrophage/microglial cells of the hippocampus in response to deafferentation and kainic acid-induced neurodegeneration. *Exp Neurol*. 1993;120:291–301.
146. Morita T, Shimada A, Ohama E, et al. Oligodendroglial vacuolar degeneration in the bilateral motor cortices and astrocytosis in epileptic beagle dogs. *J Vet Med Sci*. 1999;61:107–111.
147. Muller B, Qu H, Garseth M, et al. Amino acid neurotransmitter metabolism in neurones and glia following kainate injection in rats. *Neurosci Lett*. 2000;279:169–172.
148. Murray KD, Isackson PJ, Eskin TA, et al. Altered mRNA expression for brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase in the hippocampus of patients with intractable TLE. *J Comp Neurol*. 2000;418:411–422.
149. Niquet J, Jorquera I, Ben-Ari Y, Represa A. NCAM immunoreactivity on mossy fibers and reactive astrocytes in the hippocampus of epileptic rats. *Brain Res*. 1993;626:106–116.
150. Niquet J, Jorquera I, Faissner A, Ben-Ari Y, Represa A. Gliosis and axonal sprouting in the hippocampus of epileptic rats are associated with an increase of tenascin-C immunoreactivity. *J Neurocytol*. 1995;24:611–624.
151. Olofsdotter K, Lindvall O, Asztely F. Increased synaptic inhibition in dentate gyrus of mice with reduced levels of endogenous brain-derived neurotrophic factor. *Neuroscience*. 2000;101:531–539.
152. Opanashuk LA, Mark RJ, Porter J, et al. Heparin-binding epidermal growth factor-like growth factor in hippocampus: modulation of expression by seizures and anti-excitotoxic action. *J Neurosci*. 1999;19:133–146.
153. Orzi F, Zoli M, Passarelli F, et al. Repeated electroconvulsive shock increases glial fibrillary acidic protein, ornithine decarboxylase, somatostatin and cholecystokinin immunoreactivities in the hippocampal formation of the rat. *Brain Res*. 1990;533:223–231.
154. Otani N, Nawashiro H, Nomura N, et al. A role of glial fibrillary acidic protein in hippocampal degeneration after cerebral trauma or kainate-induced seizure. *Acta Neurochir Suppl*. 2003;86:267–269.
155. Otsuki T, Nakama H, Kanamatsu T, Tsukada Y. Glutamate metabolism in epilepsy: 13C-magnetic resonance spectroscopy observation in the human brain. *Neuroreport*. 2005;16:2057–2060.
156. Paredes MF, Greenwood J, Baraban SC. Neuropeptide Y modulates a G protein-coupled inwardly rectifying potassium current in the mouse hippocampus. *Neurosci Lett*. 2003;340:9–12.
157. Parent JM. The role of seizure-induced neurogenesis in epileptogenesis and brain repair. *Epilepsy Res*. 2002;50:179–189.
158. Parsons CG, Danysz W, Quack G, et al. Novel systemically active antagonists of the glycine site of the N-methyl-D-aspartate receptor: electrophysiologic, biochemical and behavioral characterization. *J Pharmacol Exp Ther*. 1997;283:1264–1275.
159. Pascual O, Casper KB, Kubera C, et al. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005;310:113–116.
160. Pavone A, Cardile V. An in vitro study of new antiepileptic drugs and astrocytes. *Epilepsia*. 2003;44(Suppl 10):34–39.
161. Pena F, Tapia R. Relationships among seizures, extracellular amino acid changes, and neurodegeneration induced by 4-aminopyridine in rat hippocampus: a microdialysis and electroencephalographic study. *J Neurochem*. 1999;72:2006–2014.
162. Perez J, Vezzani A, Civenni G, et al. Functional effects of D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp-NH_2 and differential changes in somatostatin receptor messenger RNAs, binding sites and somatostatin release in kainic acid-treated rats. *Neuroscience*. 1995;65:1087–1097.
163. Pollen DA, Trachtenberg MC. Neuroglia: gliosis and focal epilepsy. *Science*. 1970;167:1252–1253.
164. Pow DV. Visualising the activity of the cystine-glutamate antiporter in glial cells using antibodies to aminoadipic acid, a selectively transported substrate. *Glia*. 2001;34:27–38.
165. Represa A, Niquet J, Charriaut-Marlangue C, Ben-Ari Y. Reactive astrocytes in the kainic acid-damage hippocampus have the phenotypic features of type-2 astrocytes. *J Neurocytol*. 1993;22:299–310.
166. Represa A, Niquet J, Pollard H, Ben-Ari Y. Cell death, gliosis, and synaptic remodeling in the hippocampus of epileptic rats. *J Neurobiol*. 1995;26:413–425.
167. Robbins RJ, Brines ML, Kim JH, et al. A selective loss of somatostatin in the hippocampus of patients with TLE. *Ann Neurol*. 1991;29:325–332.
168. Rose CR, Blum R, Pichler B, et al. Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. *Nature*. 2003;426:74–78.
169. Rothstein JD, Dykes-Hoberg M, Pardo CA, et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*. 1996;16:675–686.
170. Rubaj A, Zgodzinski W, Gustaw K, Sieklucka-Dziuba M. Nociceptin, OP4 receptor ligand in different models of experimental epilepsy. *Peptides*. 2002;23:497–505.
171. Sagratella S, Scotti de Carolis A. In vivo and in vitro epileptogenic effects of the enkephalinergic system. *Ann Ist Super Sanita*. 1993;29:413–418.
172. Salton SR, Ferri GL, Hahm S, et al. VGF: a novel role for this neuronal and neuroendocrine polypeptide in the regulation of energy balance. *Front Neuroendocrinol*. 2000;21:199–219.
173. Samuelsson C, Kumlien E, Flink R, et al. Decreased cortical levels of astrocytic glutamate transport protein GLT-1 in a rat model of posttraumatic epilepsy. *Neurosci Lett*. 2000;289:185–188.
174. Saxena NC, Macdonald RL. Assembly of $GABA_A$ receptor subunits: role of the δ subunit. *J Neurosci*. 1994;14:7077–7086.
175. Scharfman H, Goodman J, Macleod A, et al. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Exp Neurol*. 2005;192:348–356.
176. Scharfman HE. Brain-derived neurotrophic factor and epilepsy - a missing link?. *Epilepsy Curr*. 2005;5:83–88.
177. Scharfman HE. Functional implications of seizure-induced neurogenesis. *Adv Exp Med Biol*. 2004;548:192–212.
178. Scharfman HE. Pre- and postsynaptic actions of somatostatin in rat and rabbit hippocampal slices. In: Dunwiddie T, Lovinger D, eds. *Presynaptic receptors in the CNS: physiology and pharmacology*. Boston: Birkäuser; 1993: 42–70.
179. Scharfman HE. The role of nonprincipal cells in dentate gyrus excitability and its relevance to animal models of epilepsy and TLE. In: Delgado-Esqueta AV, Wilson W, Olsen RW, Porter RJ, eds. *Basic mechanisms of the epilepsies: molecular and cellular approaches*, 3rd ed. New York: Lippincott-Raven; 1999: 805–820.
180. Scharfman HE. Seizure-induced neurogenesis in the dentate gyrus and its dependence on growth factors and cytokines. In: Binder DK, Scharfman HE, eds. *Growth factors and epilepsy*. Novasciences; in press.
181. Scharfman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *J Neurosci*. 2000;20:6144–6158.
182. Scharfman HE, Sollas AL, Smith KL, et al. Structural and functional asymmetry in the normal and epileptic rat dentate gyrus. *J Comp Neurol*. 2002;454:424–439.
183. Schousboe A. Pharmacological and functional characterization of astrocytic GABA transport: a short review. *Neurochem Res*. 2000;25:1241–1244.
184. Schubert P, Ogata T, Marchini C, Ferroni S. Glia-related pathomechanisms in Alzheimer's disease: a therapeutic target?. *Mech Ageing Dev*. 2001;123:47–57.
185. Schwaller B, Tetko IV, Tandon P, et al. Parvalbumin deficiency affects network properties resulting in increased susceptibility to epileptic seizures. *Mol Cell Neurosci*. 2004;25:650–663.
186. Schwarzer C, Sperk G, Samanin R, et al. Neuropeptides-immunoreactivity and their mRNA expression in kindling: functional implications for limbic epileptogenesis. *Brain Res Rev*. 1996;22:27–50.
187. Schwarzer C, Tsunashima K, Wanzenbock C, et al. GABA(A) receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced TLE. *Neuroscience*. 1997;80:1001–1017.
188. Schweitzer P, Madamba SG, Siggins GR. Somatostatin increases a voltage-insensitive K^+ conductance in rat CA1 hippocampal neurons. *J Neurophysiol*. 1998;79:1230–1238.
189. Schweizer U, Brauer AU, Kohrle J, et al. Selenium and brain function: a poorly recognized liaison. *Brain Res Rev*. 2004;45:164–178.
190. Scorza CA, Arida RM, Cavalheiro EA, et al. Expression of nestin in the hippocampal formation of rats submitted to the pilocarpine model of epilepsy. *Neurosci Res*. 2005;51:285–291.
191. Sechi G, Rosati G, Deiana GA, et al. Co-variation of free amino acids in brain interstitial fluid during pentylenetetrazole-induced convulsive status epilepticus. *Brain Res*. 1997;764:230–236.
192. Seifert G, Huttmann K, Schramm J, Steinhauser C. Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. *J Neurosci*. 2004;24:1996–2003.
193. Seress L, Gulyas AI, Freund TF. Parvalbumin- and calbindin D28k-immunoreactive neurons in the hippocampal formation of the macaque monkey. *J Comp Neurol*. 1991;313:162–177.
194. Shandra AA, Godlevsky LS, Vastyanov RS, et al. The role of TNF-α in amygdala kindled rats. *Neurosci Res*. 2002;42:147–153.
195. Sherwin AL. Neuroactive amino acids in focally epileptic human brain: a review. *Neurochem Res*. 1999;24:1387–1395.
196. Simmons ML, Chavkin C. Endogenous opioid regulation of hippocampal function. *Int Rev Neurobiol*. 1996;39:145–196.
197. Sloviter RS, Ali-Akbarian L, Horvath KD, Menkens KA. Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. *J Comp Neurol*. 2001;430:283–305.
198. Sloviter RS, Sollas AL, Barbaro NM, Laxer KD. Calcium-binding protein (calbindin-D28K) and parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. *J Comp Neurol*. 1991;308:381–396.
199. Somjen GG, Rosenthal M, Cordingley G, et al. Potassium, neuroglia, and oxidative metabolism in central gray matter. *Fed Proc*. 1976;35:1266–1271.
200. Sperk G, Bellmann R, Gruber B, et al. Neuropeptide Y expression in animal models of TLE. *Epilepsy Res Suppl*. 1996;12:197–203.

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201. Steffens M, Huppertz HJ, Zentner J, et al. Unchanged glutamine synthetase activity and increased NMDA receptor density in epileptic human neocortex: implications for the pathophysiology of epilepsy. *Neurochem Int*. 2005;47:379–384.
202. Steinhauser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol*. 2002;447:227–237.
203. Steward O, Kelley MS, Schauwecker PE. Signals that regulate astroglial gene expression: induction of GFAP mRNA following seizures or injury is blocked by protein synthesis inhibitors. *Exp Neurol*. 1997;148:100–109.
204. Strand F. *Neuropeptides: regulators of physiologic processes*. Cambridge: The MIT Press; 1999.
205. Stringer JL. Repeated seizures increase GFAP and vimentin in the hippocampus. *Brain Res*. 1996;717:147–153.
206. Svendsen CN, Sofroniew MV. Do central nervous system neurons require target-derived neurotrophic support for survival throughout adult life and aging?. *Perspect Dev Neurobiol*. 1996;3:133–142.
207. Swann JW, Al-Noori S, Jiang M, Lee CL. Spine loss and other dendritic abnormalities in epilepsy. *Hippocampus*. 2000;10:617–625.
208. Takeda A. Manganese action in brain function. *Brain Res Rev*. 2003;41:79–87.
209. Tanaka K, Watase K, Manabe T, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*. 1997;276:1699–1702.
210. Tang FR, Lee WL. Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial TLE. *J Neurocytol*. 2001;30:137–143.
211. Tchekalarova J, Georgiev V. Angiotensin peptides modulatory system: how is it implicated in the control of seizure susceptibility?. *Life Sci*. 2005;76:955–970.
212. Thompson K, Holm AM, Schousboe A, et al. Hippocampal stimulation produces neuronal death in the immature brain. *Neuroscience*. 1998;82:337–348.
213. Thompson SM. Consequence of epileptic activity in vitro. *Brain Pathol*. 1993;3:413–419.
214. Tian GF, Azmi H, Takano T, et al. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
215. Tiffany-Castiglioni EC, Peterson SL, Castiglioni AJ. Alterations in glutamine synthetase activity by $FeCl_2$-induced focal and kindled amygdaloid seizures. *J Neurosci Res*. 1990;25:223–228.
216. Tirassa P, Costa N, Aloe L. CCK-8 prevents the development of kindling and regulates the GABA and NPY expression in the hippocampus of pentylenetetrazole (PTZ)-treated adult rats. *Neuropharmacology*. 2005;48:732–742.
217. Tretter YP, Munz B, Hubner G, et al. Strong induction of activin expression after hippocampal lesion. *Neuroreport*. 1996;7:1819–23.
218. Tu B, Timofeeva O, Jiao Y, Nadler JV. Spontaneous release of neuropeptide Y tonically inhibits recurrent mossy fiber synaptic transmission in epileptic brain. *J Neurosci*. 2005;25:1718–1729.
219. Tymianski M, Spigelman I, Zhang L, et al. Mechanism of action and persistence of neuroprotection by cell-permeant Ca^{2+} chelators. *J Cereb Blood Flow Metab*. 1994;14:911–923.
220. Ulas J, Satou T, Ivins KJ, et al. Expression of metabotropic glutamate receptor 5 is increased in astrocytes after kainate-induced epileptic seizures. *Glia*. 2000;30:352–361.
221. van Vliet EA, Redeker S, Aronica E, et al. Expression of multidrug transporters MRP1, MRP2, and BCRP shortly after status epilepticus, during the latent period, and in chronic epileptic rats. *Epilepsia*. 2005;46:1569–1580.
222. Vaquero J, Oya S, Cabezudo JM, Bravo G. Morphologic study of human epileptic dendrites. *Neurosurgery*. 1982;10:720–724.
223. Vezzani A, Hoyer D. Brain somatostatin: a candidate inhibitory role in seizures and epileptogenesis. *Eur J Neurosci*. 1999;11:3767–3776.
224. Vezzani A, Moneta D, Richichi C, et al. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. *Epilepsia*. 2002;43(Suppl 5):30–35.
225. Vezzani A, Moneta D, Richichi C, et al. Functional role of proinflammatory and anti-inflammatory cytokines in seizures. *Adv Exp Med Biol*. 2004;548:123–133.
226. Vezzani A, Sperk G. Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in TLE?. *Neuropeptides*. 2004;38:245–252.
227. Vezzani A, Sperk G, Colmers WF. Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends Neurosci*. 1999;22:25–30.
228. Vicario-Abejon C, Owens D, McKay R, Segal M. Role of neurotrophins in central synapse formation and stabilization. *Nat Rev Neurosci*. 2002;3:965–974.
229. Wasterlain CG, Mazarati AM, Naylor D, et al. Short-term plasticity of hippocampal neuropeptides and neuronal circuitry in experimental status epilepticus. *Epilepsia*. 2002;43(Suppl 5):20–29.
230. Watanabe T, Morimoto K, Hirao T, et al. Amygdala-kindled and pentylenetetrazole-induced seizures in glutamate transporter GLAST-deficient mice. *Brain Res*. 1999;845:92–96.
231. Wetmore C, Ernfors P, Persson H, Olson L. Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. *Exp Neurol*. 1990;109:141–152.
232. Willoughby JO, Mackenzie L, Broberg M, et al. Fluorocitrate-mediated astroglial dysfunction causes seizures. *J Neurosci Res*. 2003;74:160–166.
233. Wittner L, Eross L, Czirjak S, et al. Surviving CA1 pyramidal cells receive intact perisomatic inhibitory input in the human epileptic hippocampus. *Brain*. 2005;128:138–152.
234. Wolf HK, Wellmer J, Muller MB, et al. Glioneuronal malformative lesions and dysembryoplastic neuroepithelial tumors in patients with chronic pharmacoresistant epilepsies. *J Neuropathol Exp Neurol*. 1995;54:245–254.
235. Wong M. Modulation of dendritic spines in epilepsy: cellular mechanisms and functional implications. *Epilepsy Behav*. 2005;7:569–577.
236. Wu HQ, Rassoulpour A, Goodman JH, et al. Kynurenate and 7-chlorokynurenate formation in chronically epileptic rats. *Epilepsia*. 2005;46:1010–106.
237. Wu HQ, Schwarcz R. Seizure activity causes elevation of endogenous extracellular kynurenic acid in the rat brain. *Brain Res Bull*. 1996;39:155–162.
238. Wu HQ, Ungerstedt U, Schwarcz R. L-α-aminoadipic acid as a regulator of kynurenic acid production in the hippocampus: a microdialysis study in freely moving rats. *Eur J Pharmacol*. 1995;281:55–61.
239. Xu B, Li S, Brown A, et al. EphA/ephrin-A interactions regulate epileptogenesis and activity-dependent axonal sprouting in adult rats. *Mol Cell Neurosci*. 2003;24:984–999.
240. Xu B, Michalski B, Racine RJ, Fahnestock M. The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphologic changes. *Neuroscience*. 2004;126:521–531.
241. Xu JY, Zheng P, Shen DH, et al. Vascular endothelial growth factor inhibits outward delayed-rectifier potassium currents in acutely isolated hippocampal neurons. *Neuroscience*. 2003;118:59–67.
242. Yan Q, Rosenfeld RD, Matheson CR, et al. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience*. 1997;78:431–448.
243. Yudkoff M, Daikhin Y, Nissim I, et al. Ketogenic diet, brain glutamate metabolism and seizure control. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:277–285.
244. Zhang LX, Wu M, Han JS. Suppression of audiogenic epileptic seizures by intracerebral injection of a CCK gene vector. *Neuroreport*. 1992;3:700–702.
245. Zucchini S, Barbieri M, Simonato M. Alterations in seizure susceptibility and in seizure-induced plasticity after pharmacologic and genetic manipulation of the fibroblast growth factor-2 system. *Epilepsia*. 2005;46(Suppl 5):52–58.