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Epilepsy following cortical injury: Cellular and molecular mechanisms as targets for potential prophylaxis

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Summary

The sequelae of traumatic brain injury, including posttraumatic epilepsy, represent a major societal problem. Significant resources are required to develop a better understanding of the underlying pathophysiologic mechanisms as targets for potential prophylactic therapies. Posttraumatic epilepsy undoubtedly involves numerous pathogenic factors that develop more or less in parallel. We have highlighted two potential “prime movers”: disinhibition and development of new functional excitatory connectivity, which occur in a number of animal models and some forms of epilepsy in humans. Previous experiments have shown that tetrodotoxin (TTX) applied to injured cortex during a critical period early after lesion placement can prevent epileptogenesis in the partial cortical (“undercut”) model of posttraumatic epilepsy. Here we show that such treatment markedly attenuates histologic indices of axonal and terminal sprouting and presumably associated aberrant excitatory connectivity. A second finding in the undercut model is a decrease in spontaneous inhibitory events. Current experiments show that this is accompanied by regressive alterations in fast-spiking γ-aminobutyric acid (GABA)ergic interneurons, including shrinkage of dendrites, marked decreases in axonal length, structural changes in inhibitory boutons, and loss of inhibitory synapses on pyramidal cells. Other data support the hypothesis that these anatomic abnormalities may result from loss of trophic support normally provided to interneurons by brain-derived neurotrophic factor (BDNF).

Approaches that prevent these two pathophysiologic mechanisms may offer avenues for prophylaxis for posttraumatic epilepsy. However, major issues such as the role of these processes in functional recovery from injury and the timing of the critical period(s) for application of potential therapies in humans are critical and need to be resolved.

Keywords

Posttraumatic epilepsy; Interneurons; Fast-spiking; Sprouting; GAP43; Neurofilaments; Laser uncaging; Caged glutamate; Brain-derived neurotrophic factor; TrkB; Cortical isolation; Undercut; Tetrodotoxin

Introduction

Critical issues

The importance of brain trauma as a risk factor for the development of epilepsy is well established (Salazar et al., 1985; Annegers et al., 1998). The high incidence of epileptogenesis

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following severe injury such as penetrating brain wounds in war time (Salazar et al., 1985) and the expected marked increase due to the current conflict in Iraq, emphasize the importance of developing prophylactic strategies that might be applied during the latent period between injury and the development of seizures. Unfortunately, multiple trials with anticonvulsant drugs have been ineffective (Temkin et al., 2001).

There are several important unknowns with respect to development of such prophylactic therapies that should be recognized as part of this effort. First, we need to know which pathophysiologic processes to target. A large number of mRNAs are activated in brain by trauma or seizures per se (Raghavendra et al., 2003; Lukasiuk & Pitkanen, 2004). Many abnormalities, including alterations in ion channels (Mody, 1998; Chen et al., 2002), transporters (Tanaka et al., 1997; Jin et al., 2005; Huberfeld et al., 2007), transmitter receptors (Chuang et al., 2001; Furtinger et al., 2001); glial cells (Ding et al., 2007), and development of aberrant connectivity (Tauck & Nadler, 1985; Salin et al., 1995; Jacobs et al., 1999; Wuarin & Dudek, 2001; Jin et al., 2006) have been found in various models of epileptogenesis. Therefore, multiple pathophysiologic epileptogenic processes are likely activated simultaneously or sequentially by brain trauma. Will targeting any one of these be adequate to provide antiepileptogenesis, or are there some “prime movers” that should receive the most focused investigative attention, given the urgency of the problem (Garga & Lowenstein, 2006) and finite resources and manpower?

A second important issue is the relationship between the latent period from injury to onset of epilepsy and the critical period, that is, the time over which a treatment must be administered to abort epileptogenesis. It is encouraging that in the only animal model in which prophylaxis of posttraumatic epileptogenesis has been achieved, the critical period is ~3 days, much shorter than the latency at which epileptogenesis can be detected in slices in vitro–10–14 days or longer (Graber & Prince, 1999, 2004). It seems likely that these important time intervals will vary in different animal models, in different cortical areas (e.g., neocortex vs. hippocampus), and with different degrees and types of injury (e.g., cf. Hoffman et al., 1994; Kelly et al., 2001; and D’Ambrosio et al., 2005). In humans, the latent period may be as long as years (Salazar et al., 1985); however, the critical period is unknown. This makes the timing for onset and duration of prophylactic treatment a critical and unknown variable.

In considering prophylactic approaches, a third critical problem emerges. Both adaptive and maladaptive processes are activated in brain by injury. The former can contribute to functional recovery (e.g., Hurwitz et al., 1990; Chollet et al., 1991), whereas the latter may lead to epileptogenesis. Are these similar processes that differ only quantitatively and in their intensity or timing with respect to injury, or are there distinctly different types of plasticity involved in epileptogenesis versus functional recovery? A prophylactic treatment for epileptogenesis that interfered with motor, sensory, or cognitive recovery following brain injury would not be useful. The answers to these questions are critical if we are to make long-term progress to prevent the occurrence of epileptogenesis after head trauma.

Which model(s)?

The model employed should ideally depend entirely on the question being addressed, and the advantages and disadvantages of the available choices (reviewed in Pitkanen et al., 2006). Certain basic features are desirable including the ease with which a traumatic injury can be applied, reproducibility from animal to animal, a latent period to epileptogenesis that is reasonably short, and, if detailed electrophysiologic data are needed, the capacity to retain epileptogenic activity in vitro to facilitate a variety of complex experiments. We have used the partially isolated neocortex (“undercut”) model of posttraumatic epileptogenesis, which has these advantages as well as others including the presence of a relatively focal lesion at a known

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neocortical site and relative preservation of adjacent cortex and more remote structures (e.g., hippocampus) Graber & Prince, 2006).

The partially isolated neocortical island with intact pial circulation ("undercuts" below) is an established in vivo and in vitro model for development of chronic posttraumatic hyperexcitability and epileptogenesis (Sharpless & Halpern, 1962; Halpern, 1972; Prince & Tseng, 1993; Hoffman et al., 1994; Salin et al., 1995; reviewed in Graber & Prince, 2006). This model provides a high yield of animals with epileptogenic cortex that may be studied in vitro (Hoffman et al., 1994; Graber & Prince, 2006). Previous results show that this lesion results in epileptogenesis in a variety of species (Graber & Prince, 2006). Isolated islands of neocortical gray matter, with neuropathologic evidence of substantial reorganization, are also present in postmortem specimens from epileptic children who developed extensive underlying white matter lesions as infants (Marin-Padilla, 1997). Seizures were also a frequent occurrence in humans subjected to psychosurgery in which cortical areas were partially isolated (Echlin et al., 1952; Scoville, 1960).

Disinhibition, increases in neuronal membrane excitability, and increases in excitatory synaptic coupling have been suggested as potential mechanisms in this chronic epilepsy model (Purpura & Housepian, 1961; Ribak & Reiffenstein, 1982; Prince & Tseng, 1993; Salin et al., 1995; Bush et al., 1999; Prince, 1999). The undercut cortex becomes progressively more epileptogenic over several weeks (Grafstein & Sastry, 1957; Sharpless & Halpern, 1962), and spontaneous interictal discharges can persist at least 1 year in the monkey (Echlin & Battista, 1963). Interictal epileptiform activity can be recorded within partially isolated cortex of anesthetized rats, and c-fos-immunoreactivity (IR) is increased for weeks in the injured cortex, suggesting ongoing abnormal activity (Jacobs et al., 2001). Clinical seizures have been infrequently observed, perhaps because the partially isolated cortex has limited connections with surrounding or subcortical areas. However, electrographic focal ictal episodes originating in the partial isolation of an implanted rat, associated with subtle behavioral alterations, have been observed during video-EEG (electroencephalography) recording (Graber & Prince, 2006). Spontaneous and evoked interictal activity, and more rarely ictal-like discharges, occurs in vitro in rodent neocortical slices cut through chronic isolations (Prince & Tseng, 1993; Hoffman et al., 1994; Salin et al., 1995).

Methods

Methods for producing partial neocortical isolations have been reviewed in detail recently (Graber & Prince, 2006) and will not be detailed here. Standard techniques for preparing and maintaining in vitro neocortical slices were used (Hoffman et al., 1994; Li & Prince, 2002). About 2–3 weeks after placement of a lesion producing partially isolated cortex, acute slices cut through the injured area and maintained in vitro show abnormal evoked epileptiform potentials consisting of polyphasic field potentials that are all or none in nature, develop with a variable latency following the normal evoked event, and propagate across the cortical structures (Prince & Tseng, 1993; Hoffman et al., 1994; Graber & Prince, 1999). The incidence and duration of spontaneous interictal discharges are markedly increased when slices are perfused with artificial cerebrospinal fluid (ACSF) containing physiologic concentrations of glutamine (Tani et al., 2007). In the experiments using laser scanning photostimulation of caged glutamate described later and illustrated in Fig. 2, glutamate uncaging was accomplished using 300–600-μs UV laser flashes. Cortical layers II–VI were stimulated in grids of 600–650 × 1000~1200 μm with 50-μm spacing. Evoked EPSCs were recorded from layer V pyramidal neurons that were voltage clamped at −70 mV. In these experiments, "composite amplitude" was defined as the sum of the amplitudes of all detected synaptic events during a 200-ms time window beginning 12 ms after the laser flash. "Region-normalized EPSC amplitude" was obtained by dividing the sum of all composite EPSC amplitudes evoked within a given distance

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from the soma. A "hot spot" was defined as an uncaging spot from which an EPSC was evoked. Immunocytochemical procedures were as described previously (Salin et al., 1995; Rosen et al., 1998; Jacobs et al., 1999).

Potential epileptogenic processes during the latent period: Targets for prophylaxis

Increases in excitatory connectivity and sprouting—Immunocytochemical experiments show that there is intense immunoreactivity for 68-kDa and 200-kDa neurofilament proteins in the neuropil as early as 3 days following the partial isolation that persists for weeks following the lesion (I. Parada and D.A. Prince, unpublished data). Both cortical interneurons and pyramidal cells show this increased neurofilament expression (Fig. 5 of Graber & Prince, 2006; Fig. 1D,E). These changes can be taken as a proxy for axonal sprouting within the injured area (Yang et al., 1996; King et al., 2001). Sprouting of axonal terminals has also been demonstrated with immunocytochemistry using an antibody for growth associated protein (GAP) 43, which is prominent both in developing axonal terminals and in those that are reactivated and are sprouting following injury (Fig. 1A,B) (Bendotti et al., 1997; McKinney et al., 1997). Previous studies have shown that there are gross changes in axons of layer V excitatory pyramidal cells following partial isolation, including increases in axonal length, numbers of axon collaterals, and boutons that are presumably sites of synapses (Salin et al., 1995). Electrophysiologic data, including measurements of excitatory currents in layer V pyramidal cells of chronically epileptogenic cortex and field potential recordings of evoked events, support the presence of enhanced excitatory inputs and hyperexcitable circuits (Hoffman et al., 1994; Salin et al., 1995; Li & Prince, 2002).

Recently the use of laser scanning photostimulation of caged glutamate has allowed detailed mapping of synaptic connectivity in the undercut and shown increased excitatory connectivity onto pyramidal cells throughout the cortex in the epileptogenic region (Jin et al., 2006). There is a marked increase in the number of sites in layers II, III, and V from which laser stimulation can evoke EPSCs onto individual recorded neurons in layer V (Fig. 2C), and the evoked events have a larger amplitude than controls (Fig. 2B). Therefore, the electrophysiologic data strongly support the conclusion that the sprouting seen with anatomic studies is associated with new, functional, excessive recurrent excitatory circuits.

Assuming that functionally effective sprouting represents a key pathogenetic mechanism in development of posttraumatic epilepsy, the question arises as to whether this process can be altered by potential therapies applied during the latent period. Previous experiments provide a "proof in principle" that prophylaxis of epileptogenesis is possible in this model. Placement of a sheet of slow release resin (ELVAX®) impregnated with tetrodotoxin (TTX) over the cortical isolation at the time of surgery prevents the occurrence of evoked and spontaneous epileptiform discharges in in vitro slices weeks later, after TTX washout (Graber & Prince, 1999). TTX blocks voltage-dependent sodium channels and presumably eliminates all neurotransmission within the injured tissue. The mechanisms by which blockade of activity interferes with epileptogenesis are unknown. Our results are contrary to the proposal that reduced activity in the isolation underlies epileptogenesis by inducing "homeostatic" upscaling of excitatory synaptic activity (Houweling et al., 2005). There is a critical period for prophylaxis by the TTX in the first 3 days after injury (Graber & Prince, 2006). If the treatment with TTX was delayed for more than 3 days after placement of the undercut, slices were epileptogenic at the end of 2–3 weeks.

How might TTX treatment prevent the development of hyperexcitability? From the preceding, one hypothesis might be that blocking activity with TTX limits the sprouting response and the resulting maladaptive excitatory connectivity. Activity-related wiring of intracortical and interhemispheric connections is known to play a role in cortical ontogenesis and plastic changes in connections in the mature brain following injury (Carmichael & Chesselet, 2002; Mizuno

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et al., 2007; Wang et al., 2007). We, therefore, tested the hypothesis that there is an activity-dependent link between cortical injury and the axonal sprouting response that would be blocked by TTX. Both the upregulation of 68-kDa and 200-kDa neurofilament and GAP43 immunoreactivity in the undercut, indices of axonal and terminal sprouting, were significantly reduced by the TTX treatment (cf. Fig. 1B with C and E with F). Therefore, there is an as yet undefined activity link between injury and the development of new connectivity within injured cortex that may potentially be a target for prophylactic therapies. It remains to be seen whether therapies that curtail sprouting and epileptogenesis will affect recovery of other functions.

Alterations in GABAergic interneurons: A second potential target for prophylactic strategies

γ-aminobutyric acid $(GABA)_A$ receptor-mediated postsynaptic inhibition (termed “inhibition” subsequently) has important roles in normal cortical function (Sillito, 1977; McBain & Fisahn, 2001; Freund, 2003) and in controlling events implicated in epileptogenesis (Prince & Wilder, 1967; Wong & Prince, 1979; Miles & Wong, 1987; Chagnac-Amitai & Connors, 1989; Luhmann & Prince, 1992). Anatomic (Sloper et al., 1980; Ribak et al., 1982; Houser et al., 1986; De Lanerolle et al., 1989; Marco et al., 1996; Rosen et al., 1998; Spreafico et al., 1998; DeFelipe, 1999; Andre et al., 2001) and/or electrophysiologic data (Franck & Schwartzkroin, 1984; Ashwood & Wheal, 1986; Franck et al., 1988; Neumann-Haefelin et al., 1995; Williamson et al., 1999; Zhu & Roper, 2000; Sayin et al., 2003) document decreases in numbers of interneurons and/or postsynaptic inhibition in epileptogenic hippocampus and neocortex. However, other data emphasize the preservation of GABAergic neurons after various types of injury, and the potential for sprouting new inhibitory connections (Nieoullon & Dusticier, 1981; Goldowitz et al., 1982; Westenbroek et al., 1988; Babb et al., 1989a,b; Davenport et al., 1990; Seil et al., 1994; Magloczky & Freund, 2005). Both enhanced inhibitory input (e.g., Gulyas & Freund, 1996; Tamas et al., 1998; Bacci et al., 2003) and decreased excitatory drive onto interneurons (Sloviter, 1991; Lothman et al., 1996; Doherty & Dingledine, 2001) have been proposed as potential epileptogenic mechanisms, (but see Bernard et al., 1998; Jacobs & Prince, 2005). Electrophysiologic data can indicate a reduction in GABAergic inhibition when anatomic indices in the same model do not, presumably because of the limited sensitivity of some techniques for detecting subtle abnormalities (Franck et al., 1988 10636). For example, PV-containing FS cells in the partial cortical isolation are not decreased in number (Graber et al., 1999) and there is also no obvious reduction in calbindin or GAD-containing interneurons (Prince & Jacobs, 1998). However, mIPSC frequency is decreased in layer V P cells of undercut cortex (Li & Prince, 2002), perhaps because of anatomic and electrophysiologic axonal abnormalities not detectable without a more detailed analysis of filled cells. We, therefore, tested the hypothesis that there might be structural abnormalities in GABAergic interneurons in chronic neocortical isolations, using immunocytochemical techniques applied to cortical sections and biocytin-filled layer V FS cells.

Whole cell recordings were obtained from FS interneurons in control cortex and within the partially isolated epileptogenic cortex with biocytin-containing patch pipettes. After processing, a total of 13 cells in control cortex and 11 from the partially isolated cortex were found to have well-filled axonal and dendritic arbors suitable for analysis. These neurons had anatomic characteristics of basket cells (Somogyi et al., 1983), and several in each group that were tested were immunopositive for parvalbumin. Measurements from confocal stacks with Neurolucida software (Methods) showed that there was a very significant reduction in axonal length in the cells of undercut versus control (Fig. 3; 3,429.8 968.1 μm in control, n = 6; 726.9 ± 325.1 μm in undercut, n = 5; $p < 0.001$). The dendrites of undercut FS interneurons were thinner than in controls, and measurements using Volocity software (Methods) confirmed that they were decreased in volume (~1.3 0 ± 0.0.15 $\mu m^3/1$ μm length in control and 0.6 ± 0.39 $\mu m^3/1$ μm length in undercut, $p < 0.05$).

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When axonal segments of these biocytin-filled inhibitory interneurons were examined in confocal images, several abnormalities were noted. Swellings along segments of axons in both control and undercut were identified as presynaptic boutons, as they were often immunoreactive for the vesicular GABA transporter VGAT (Fig. 4B) and could be in close apposition to post-synaptic gephyrin clusters that presumably marked the location of clusters of postsynaptic $GABA_A$ receptors (not shown). The total number of boutons per micrometer of axonal length was not significantly different in FS cells from undercut versus control. However when boutons were classified by size as “big” (>1 μm in diameter; arrows in Fig. 4) and “small” (<1 μm; arrowhead in Fig. 4), a significant reduction in big, and increase in small boutons as a percentage of the total over comparable axonal lengths was found in FS cells from the undercut (Fig. 5). There was also a marked reduction in the percentage of small boutons closely apposed to postsynaptic gephyrin-IR clusters in the undercut (not shown).

The preceding findings suggested that synaptic contacts between FS and P cells in the undercut might be abnormal. Synapses from FS interneurons are predominantly targeted to somata of P cells (Somogyi et al., 1983) and so a quantitative EM analysis of symmetrical (inhibitory) synapses onto layer V pyramidal cell bodies in control and undercut cortex was performed. (J. Wenzel, P. A. Schwartzkroin, and D. A. Prince, unpublished). Results showed that there was a significant decrease in inhibitory synapses on somata of pyramidal cells in undercut cortex compared to naive or contralateral cortex. The numbers of axonal terminals that were in close approximation to somata, were not different in undercut versus control images. Serial EM was not done, so data regarding bouton sizes or vesicular content are not available. These findings indicate that structural alterations occur in FS interneurons of the partially isolated, epileptogenic cortex that would make GABAergic neurotransmission less effective. Selective loss of inhibitory synapses has been previously reported on layer V/VI P neurons at the margins of chronic cortical isolations in cat (Ribak & Reiffenstein, 1982). The loss of inhibitory synapses in the axotomized pyramidal cells of layer V in the undercut cortex is in some respects similar to that found in motoneurons following axotomy where there is “stripping” of inhibitory, more than excitatory synapses (Sumner & Sutherland, 1973; Takata, 1981; Mendell, 1984) and decreased postsynaptic inhibition. This mechanism may also explain the loss of functional inhibition in kainate-induced hippocampal epileptogenesis (Franck et al., 1988). The prevalence of small boutons perisomatically on P cells in the undercut may also be associated with other pre- and postsynaptic alterations that would make inhibitory transmission less effective (Pierce & Lewin, 1994; Harris & Sultan, 1995).

What mechanisms might underlie the presumed atrophic changes in GABAergic interneurons? We speculate that the changes that we see are a reversion to an earlier developmental stage. Compared to interneurons in mature cortex, those examined early in development have reduced axonal arbors, decreased dendritic volumes and lengths, make fewer synapses on P cells, and would be are less effective in releasing GABA and inducing inhibitory events (Bahr & Wolff, 1985; Seress & Ribak, 1990; De Felipe et al., 1997; Jin et al., 2003). One critical factor for growth and development of interneurons in cortex is the availability of brain-derived neurotrophic factor (BDNF) (McAllister et al., 1995; Marty et al., 1997; Aguado et al., 2003; Jin et al., 2003). Inhibitory contacts and the frequency of mIPSCs onto pyramidal cells genetically altered to limit their capacity to produce BDNF are significantly reduced compared to adjacent nonaltered pyramidal cells (Kohara et al., 2003). We speculate that reductions in the numbers of layer V pyramidal cells (Graber et al., 1999) and alterations in surviving ones indicated by decreased somatic areas and abnormal intrinsic membrane properties (Prince & Tseng, 1993), result in reduced availability of BDNF as a retrograde trophic factor for presynaptic interneurons. This may, in turn, result in the observed functional and structural abnormalities in the epileptogenic cortex. Recent results from gene array experiments show that mRNA for both BDNF and its receptor, TrkB, are significantly decreased 3 days after the partial cortical isolation is placed and that BDNF mRNA remains down at 3 weeks while TrkB

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recovers (K. Graber and D. A. Prince, unpublished). Immunocytochemical experiments in undercuts show that BDNF protein is also significantly downregulated in pyramidal cells 3 and 7 days after the undercut (not shown), and that there is also a significant reduction in immunoreactivity for the BDNF receptor, Trk B, on the parvalbumin-containing interneurons and P cells (cf. Fig. 6A,B with C,D).

Discussion

There are many processes initiated by cortical injury that are ongoing in parallel with the ones described earlier. Each of them, or combinations of several, might well be important contributors to epileptogenesis and targets for prophylaxis. As mentioned previously, even if there are multiple mechanisms involved, it may be that intervention to affect particularly critical ones would be sufficient to prevent or markedly reduce subsequent hyperexcitability. However, at least in experimental models and likely also in humans, the timing of the lesion during development, the latency between the occurrence of a lesion and the onset of prophylactic therapy, the structures involved, the genetic background, and other factors will influence the results. Although this is a daunting proposition, it is critically important to begin to address the issue of prophylaxis, particularly because of the specter of increasing numbers of individuals who have sustained significant brain injury in military service (Garga & Lowenstein, 2006).

Here we have highlighted two potentially epileptogenic processes in the chronically injured cortex. The first, development of maladaptive axonal sprouting of excitatory connectivity, appears to depend at least in part on the level of activity within the circuit and may be altered by “ quieting” the involved cells. A similar result has been reported in thermal ischemic lesions of somatosensory cortex (Carmichael & Chesselet, 2002). Neural activity is well established as an important element in normal development of connectivity in cortical and other structures. For example, in neocortex, activity of layer II/III pyramidal cells is important in development of intrinsic and callosal connections (Mizuno et al., 2007; Wang et al., 2007). The molecular link between activity and sprouting in the above-noted experiments is not known.

One major challenge will be to develop experimental protocols that differentially affect maladaptive (i.e., epileptogenic) versus adaptive, or functionally restorative sprouting. It may be possible to accomplish this if the two processes have different time courses or critical periods, or have different cascades of underlying molecular events. A second avenue for investigation will be a search for agents that are more selective and less toxic than TTX, and can be applied directly to injured cortex or administered parenterally to target the events underlying aberrant circuit rewiring.

Structural and functional alterations in interneurons leading to defects in GABAergic neurotransmission represent a second set of abnormalities potentially approachable with new prophylactic strategies. It will be important to determine whether there is also an activity-related link between injury and the events leading to these interneuronal alterations, as suggested for the maladaptive sprouting. If the atrophic changes in FS interneurons are related to de-afferentation and a decrease in BDNF signaling, silencing the cortex would be expected to make matters worse, as both BDNF release and maintenance of interneuronal structure/function are activity dependent (see Marty et al., 1997 for review). Decreased activity as a result of the de-afferentation in the partial isolation and the TTX treatment should *decrease* BDNF release. So block of activity might have opposite effects on sprouting versus maintenance of inhibitory functionality.

If the decreases in BDNF and TrkB in the undercut cortex underlie the anatomic alterations in FS cells, would supplying exogenous BDNF rescue these cells from their presumed reversion to an early developmental stage? Experiments focused on this question are underway, with the

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caveat that BDNF signaling may also enhance excitatory neurotransmission (Kang & Schuman, 1995; Carmignoto et al., 1997; but see Frerking et al., 1998), and under some circumstances, promote epileptogenesis (Kokaia et al., 1995; Binder et al., 1999). Further information regarding the time course of adaptive versus aberrant sprouting and alterations in interneurons in relation to injury will be important in developing effective prophylactic strategies.

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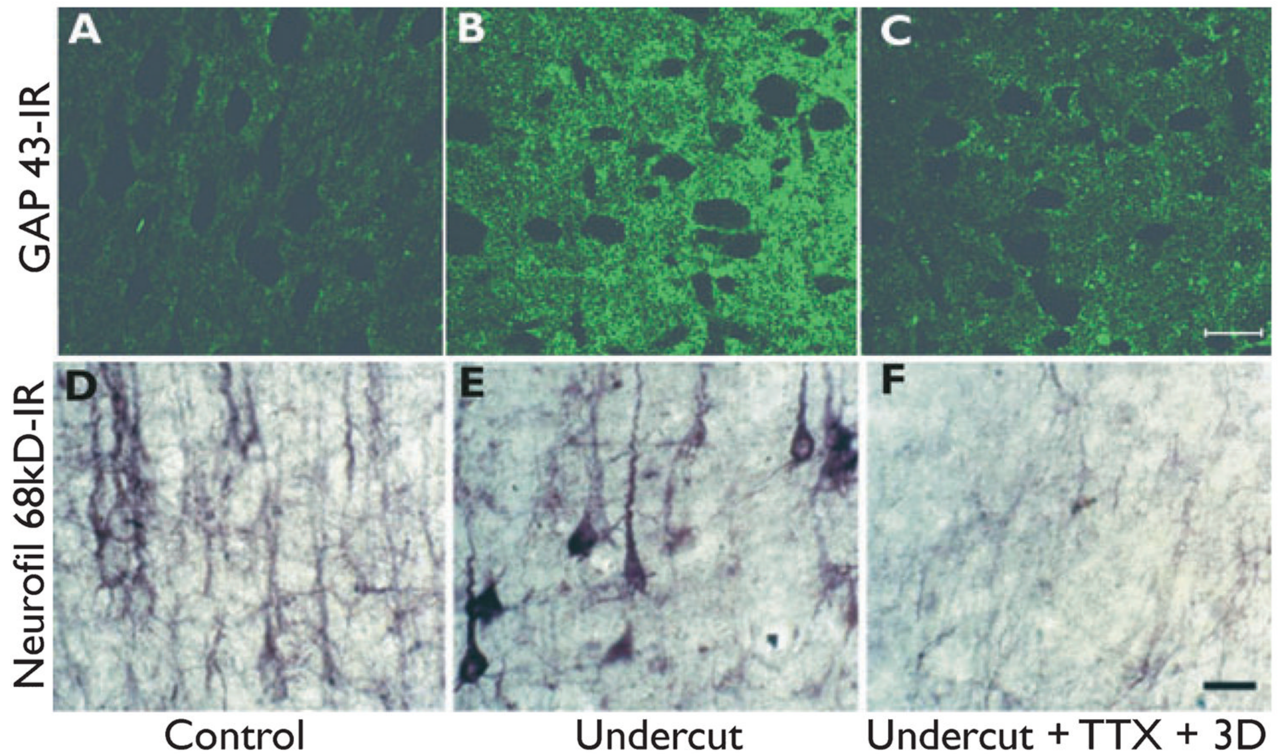

Figure 1.

Immunoreactivity (IR) of axons and terminals in partially isolated neocortex. (**A**–**C**) Sections through layer V of rat sensorimotor cortex reacted with growth-associated protein (GAP) 43 antibody. (**D**–**F**) Comparable sections from another rat reacted with antibody for 68-kDa neurofilaments. (**A**, **D**) Control from layer V of hemisphere contralateral to the undercut. (**B**, **E**). GAP43-IR (**B**) and 68-kDa neurofilament-IR (**E**) in layer V of undercuts made 3 days earlier, contralateral to **A** and **D**, respectively. (**C**, **F**). Representative sections from undercuts of two other rats in which Elvax® impregnated with tetrodotoxin was placed subdurally over the undercut area at the time of surgery. Immunocytochemistry was done after 3 days in **C** and after 3 weeks in **F**. Tetrodotoxin (TTX) treatment reduced IR for both GAP43 and neurofilament in the undercuts. Calibrations in **C** and **F**: 50 μm for **A**–**C** and **D**–**F**, respectively. *Epilepsia* © ILAE

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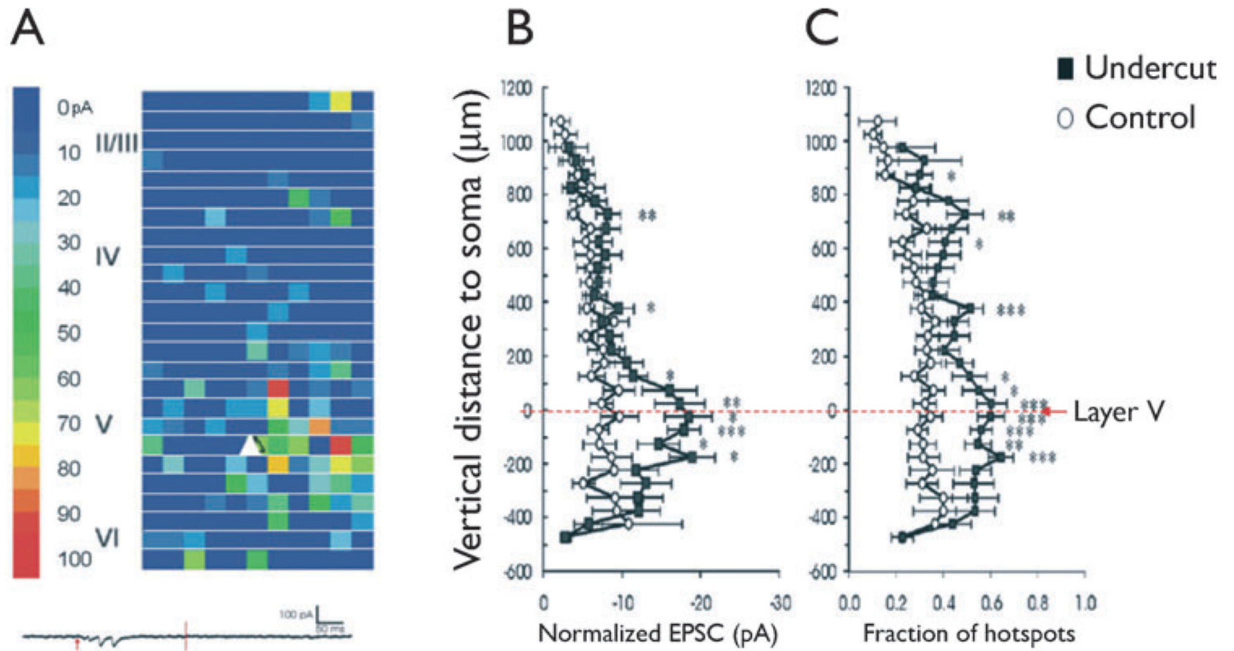

Figure 2.
Laser scanning photostimulation with caged glutamate. (**A**) Representative map of EPSCs onto a pyramidal cell (white triangle) in slice from naive cortex. A 500 × 1200 μm photostimulation grid shows color-coded composite amplitude of EPSCs at each uncaging site detected between 12 and 200 ms of uncaging stimulus onset (scale on left). Roman numerals: cortical lamina. Trace at bottom: Single sweep showing an uncaged glutamate-evoked EPSC, in the absence of direct activation of the neuron, in a slice of control neocortex. Red arrow: time of glutamate uncaging (200 μs UV laser flash); vertical line: time point 200 ms after photostimulation. (**B**, **C**) Layer-specific enhancement of excitatory synaptic input onto layer V pyramidal neurons. (**B**) Comparison of region-normalized EPSC amplitude evoked by uncaging stimuli at various cortical depths. 0: Position of somata. Positive along y-axis: toward pial surface; negative toward white matter in **B** and **C**. (**C**) Mean fraction of hot spots/total uncaging spots at various vertical distances from somata. Asterisks: Two way analyses of variance (ANOVAs) $p < 0.0001$ for all. Open circles: control; black squares: undercut. Modified from Jin et al., 2006 with permission.
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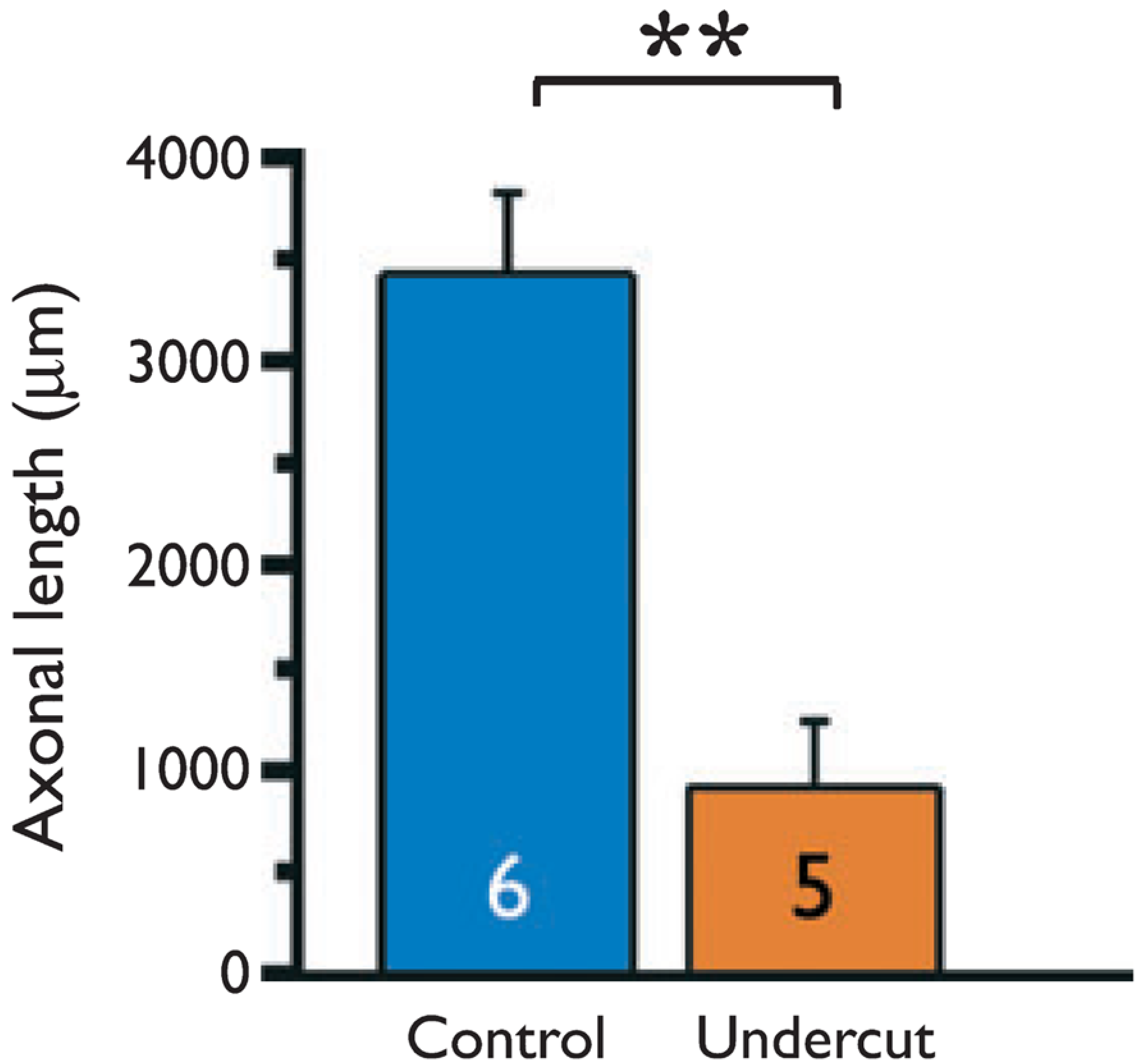

Figure 3.

Graph of axonal lengths for six biocytin-filled FS inter-neurons in control and five in undercut cortex. Measurements obtained from stacks of confocal images. Mean ± SD for control: 3429.8 ± 968.1 μm and for undercut: 726.9 ± 325.1 μm. **$p < 0.001$

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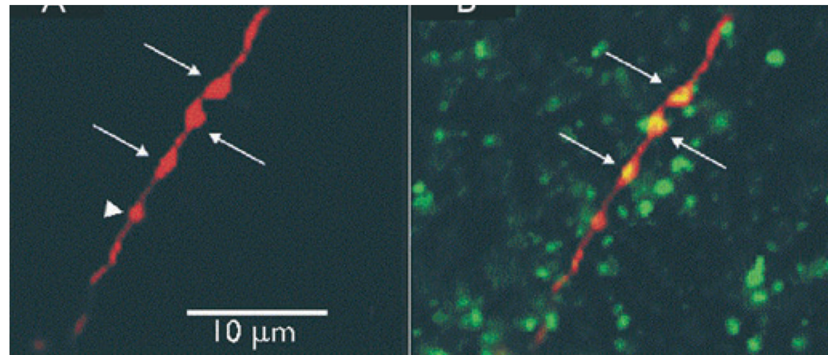

Figure 4.
Confocal images of an axonal segment of biocytin-filled FS interneuron. (**A**) Axonal segment of control layer V FS cell filled with Texas red-biocytin. Arrows point to large (>1 μm) boutons and arrowhead points to a small (<1 μm) bouton. (**B**) Same section double-labeled with Texas red and vesicular γ-aminobutyric acid (GABA) transporter (VGAT) antibody (green). Larger swellings along axon (arrows) contain VGAT in merged image (yellow) and are presumed presynaptic GABAergic boutons. Optical sections in **A**, **B**: 0.5 μm
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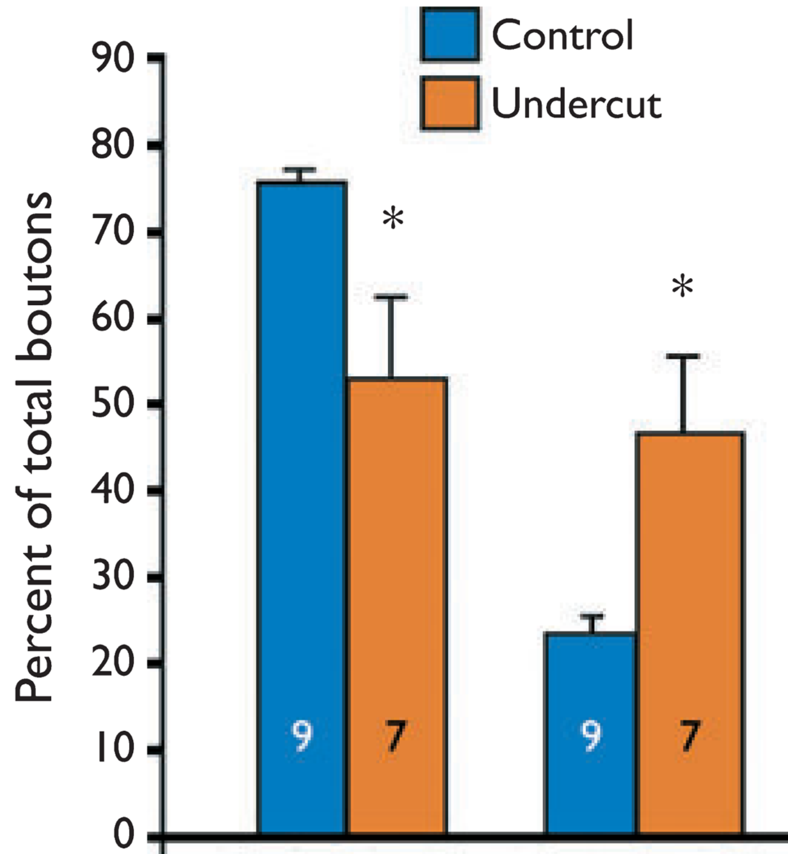

Figure 5.
Graphs of bouton sizes for axons of nine control and seven undercut layer V biocytin-filled FS interneurons. Left graph: big boutons (>1 μm diameter) from control (black) and undercut cortex (gray) as a percentage of total boutons counted. Right graph: Small boutons (<1 μm diameter) as a percentage of total boutons in control and undercut. The total number of boutons in control versus undercut cortex is not significantly different (not shown); however, there are significantly fewer big and many smaller boutons in axons of the undercut FS cells. *$p < 0.05$. Total axon length analyzed from stacks of confocal images was 3,321 μm for nine control cells and 2,528 μm in seven undercut FS cells.
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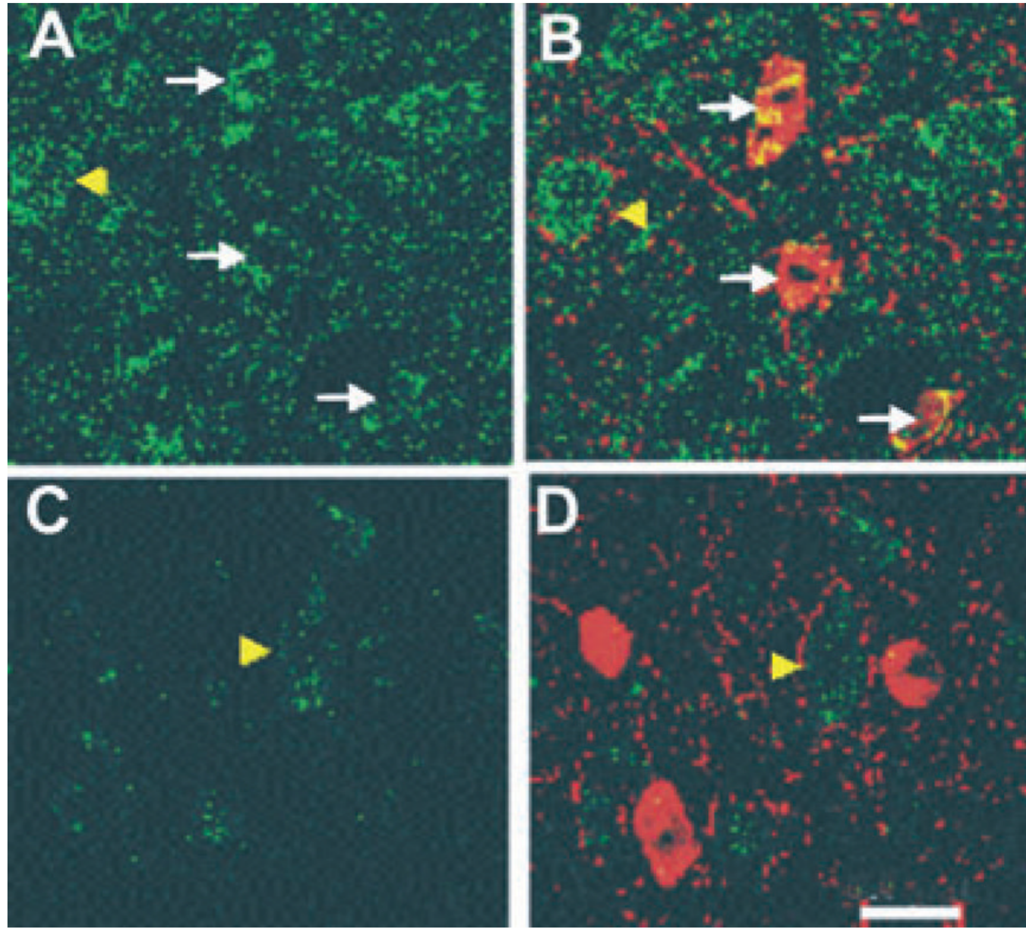

Figure 6.

Decreased TrkB-IR (immunoreactivity) in undercut cortex. (**A**, **B**). Confocal images of same layer V control section contralateral to undercut, reacted for TrkB-IR (green) (**A**) and double labeled with TrkB and parvalbumin antibodies (red) (**B**). TrkB-IR is yellow on the interneurons in merged image. Arrows point to three parvalbumin-containing interneurons. Arrowhead points to probable pyramidal cell also containing TrkB-IR pixels. (**C**, **D**) Section from undercut cortex contralateral to **A**, **B**, reacted with TrkB antibody (**C**) and double labeled with TrkB + parvalbumin antibodies (**D**). Arrowhead in **C**, **D** points to a presumed pyramidal cell. TrkB-IR is decreased on pyramidal cell and interneurons in **C**,**D** compared to **A**, **B** control. Animal perfused 3 days after the partial cortical isolation. Calibration in **D**: 20 μm for all segments. *Epilepsia* © ILAE