Seizure-Induced Axonal Sprouting: Assessing Connections Between Injury, Local Circuits, and Epileptogenesis

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Neurons and neural circuits undergo extensive structural and functional remodeling in response to seizures. Sprouting of axons in the mossy fiber pathway of the hippocampus is a prominent example of a seizure-induced structural alteration which has received particular attention because it is easily detected, is induced by intense or repeated brief seizures in focal chronic models of epilepsy, and is also observed in the human epileptic hippocampus. During the last decade the association of mossy fiber sprouting with seizures and epilepsy has been firmly established. Many anatomical features of mossy fiber sprouting have been described in considerable detail, and there is evidence that sprouting occurs in a variety of other pathways in association with seizures and injury. There is uncertainty, however, about how or when mossy fiber sprouting may contribute to hippocampal dysfunction and generation of seizures. Study of mossy fiber sprouting has provided a strong theoretical and conceptual framework for efforts to understand how seizures and injury may contribute to epileptogenesis and its consequences. It is likely that investigation of mossy fiber sprouting will continure to offer significant opportunities for insights into seizure-induced plasticity of neural circuits at molecular, cellular, and systems levels.

Axonal sprouting is a prominent feature in brain development and is an essential cellular process in the establishment of neural connections and formation of neural circuits. The formation of neural circuits and their organization into complex networks involves a coordinated sequence of overlapping cellular events that include cell birth, differentiation, migration, neurite outgrowth or sprouting, synapse formation, programmed cell death, and activity-dependent pruning that refines neural connections. It is now recognized that neural circuits continue to undergo neurite outgrowth and axonal sprouting in response to seizures. This review focuses on anatomic and physiologic aspects of axonal sprouting, its association with injury and seizures in neural circuits, and issues related to the contribution of sprouting to epilepsy and its consequences, but does not address the molecular aspects of sprouting in development and circuit remodeling.

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Axonal Sprouting in Response to Injury and Damage

Axonal sprouting was long regarded as an essential event in the formation of neural circuits during development, but it was the prevailing view that sprouting did not continue in the adult nervous system. This viewpoint was challenged by a series of experimental observations by Steward, Cotman, and Lynch (1,2), who demonstrated that pathways in the hippocampal formation of the adult possessed a capacity to undergo sprouting and rearrangement of synaptic connectivity in response to injury and damage. In pioneering studies, unilateral electrolytic lesions of the entorhinal cortex denervated the ipsilateral dentate gyrus, and induced sprouting and reinnervation by surviving axons of the normally sparse crossed pathway from the contralateral entorhinal cortex. The sprouting axons of the crossed pathway formed synapses with granule cells in the denervated dentate gyrus which not only supported functional synaptic transmission (3–5), but also had the capacity to undergo synaptic plasticity and long-term potentiation (LTP) (6). Lesion-induced sprouting was also observed in the septohippocampal, associational, and mossy fiber pathways within the dentate gyrus and hippocampal formation (7–9) in response to electrolytic damage, axotomy, and chemical toxins (10–12). A potential connection of lesion-induced sprouting to phenomena of epilepsy was initially suggested by Messenheimer and Steward (13), who observed that sprouted axons of the crossed entorhinal pathway to the dentate gyrus gained access to hippocampal networks modified by kindling. This observation suggested that sprouting might be linked to seizure-induced transformation of neural circuits.

Seizure-Induced Axonal Sprouting

Further evidence suggesting links between seizures, neuronal damage, and sprouting was provided by studies of Nadler, Ben-

Ari, et al. (14–16) using the glutamate analogue kainic acid, which induced intense status epilepticus and macroscopic damage in the hilus of the dentate gyrus, subfields of the hippocampus, and a variety of extrahippocampal regions, accompanied by reactive sprouting of the mossy fiber axons in the dentate gyrus (10). In these initial studies, it could not be determined whether the sprouting induced by kainic acid was a consequence of direct neurotoxic damage, excitotoxic damage as a consequence of status epilepticus, or both factors. Experiments combining kainic acid administration with anticonvulsant treatment supported the viewpoint that at least some component of the damage and accompanying axon sprouting might be caused by seizures (17–19). With the massive and macroscopic damage associated with seizures in these models, however, the specific contributions of lesion-induced deafferentation and seizures to induction of axonal sprouting were difficult to distinguish.

Axonal sprouting was first definitively associated with seizures by the observation in kindled rats that mossy fiber axons labeled by Timm histochemistry expanded their terminal field to the supragranular region of the dentate gyrus, where such terminals are not normally found (20,21). This reorganization of the mossy fiber terminal field was not associated with macroscopic damage in rats experiencing repeated brief seizures induced by kindling. Mossy fiber sprouting develops after only a few brief seizures, progresses with repeated seizures, and is permanent (22). Sprouting is induced not only by seizures evoked by direct stimulation of hippocampal afferents, but also by seizures that propagate into the hippocampus from remote regions (23). Repeated brief seizures (e.g., only a few partial seizures evoked by kindling) are sufficient to induce mossy fiber sprouting in the absence of extensive hippocampal damage (22).

Seizure-induced sprouting has been observed in numerous chronic models of epilepsy, including genetic mouse models such as the tottering and stargazer strains (24,25), after eight to 10 electroconvulsive (ECT) seizures (26,27), and after seizures evoked by flurothyl, pentylenetetrazol, and hyperthermia in immature rats (28–30). Although seizures in developing animals appear to induce less damage and less sprouting than those in adults (31), pathways in the developing brain undergo sprouting and other structural/functional alterations in response to both injury and seizures (8,29), which has implications for the consequences of seizures during development. As mossy fiber sprouting has been observed in the human epileptic temporal lobe (32–35) and in chronic epileptic models in a variety of species, sprouting can be regarded as a common seizure-induced cellular phenomenon of epilepsy. Because mossy fiber sprouting is progressively induced by kindling and also is observed in dentate gyrus and hippocampus of humans with temporal lobe epilepsy, poorly controlled seizures in humans may induce progressive sprouting and synaptic reorganization. The question of whether seizures are sufficient to induce sprouting in the absence of neuronal damage and deafferentation has not been definitely resolved (20,36), as a continuing series of studies have demonstrated that seizures evoked by kindling, despite the absence of macroscopic damage, also induce topographically specific and cumulative patterns of neuronal damage in a variety hippocampal and limbic areas (37–41).

Features of Mossy Fiber Axonal Sprouting

Detailed anatomic studies have demonstrated that seizure-induced mossy fiber reorganization is not limited to the supragranular region of the dentate gyrus, but also includes axonal growth in the hilus, development of infrapyramidal to suprapyamidal (interblade) connectivity not observed in normal rats, and expansion of the terminal field of the mossy fiber pathway in CA3 and along the septotemporal axis of the hippocampus over distances as long as 700–800 microns (29,42,43). Seizure-induced reorganization along the septotemporal axis is of particular interest from the point of view of possible functional effects of sprouting, as physiologic studies have demonstrated right- and left-specific place cells organized in lamellar patterns along the septotemporal axis (44,45). Anatomic studies also revealed specificity of afferent projections along the septotemporal axis in the rat, with amygdala and limbic inputs projecting to the temporal region and neocortical inputs projecting to septal and more distributed areas along the axis (46). With the perspective of these anatomic and physiologic observations, functional activity in the reorganized sprouted projections along the septotemporal axis would thus potentially disrupt the topographic organization of afferent inputs undergoing processing in hippocampal circuitry and could produce paradigm-specific behavioral and cognitive dysfunction. In addition to axonal sprouting, the dendrites of granule cells undergo sprouting in association with seizures (43,47,48). Although granule cells appear to possess a robust capacity for seizure-induced process formation and structural reorganization in adulthood, sprouting also has been observed in other systems such as CA1 and neocortex (49–52), which indicates that seizure-induced sprouting is most likely a general property of neurons and circuits in a variety of neural networks.

Assessing the Functional Effects of Mossy Fiber Sprouting

The spatial, temporal, and morphologic features of seizure-induced sprouting have been relatively well characterized, but understanding of the effects of sprouting on the functional properties of hippocampal circuitry is more limited. With the evidence that lesion-induced sprouting in the entorhinal den-

tate pathways supported functional synaptic transmission, pathways reorganized by seizure-induced mossy fiber sprouting might potentially modify properties of hippocampal circuits. The functional effects of mossy fiber sprouting will depend on the type and numbers of postsynaptic targets contacted by the sprouted axons. Synapses formed by sprouted mossy fibers on dendrites of granule cells would result in recurrent excitatory circuits and would be expected to increase excitatory drive, potentially promoting epileptogenesis. Conversely, synapses formed by sprouted mossy fibers on inhibitory interneurons would enhance inhibition. Histologic and ultrastructural evidence in several experimental models suggested that sprouted mossy fibers form both recurrent excitatory and recurrent inhibitory circuits (43,53–60), but definitive quantitative analyses of their relative numbers are not available. Mossy fiber terminals may form synapses with dendrites of inhibitory interneurons in normal animals (55,56), but it appears that many sprouted mossy fiber terminals form synapses on granule cells, and therefore are likely to increase recurrent excitation in the dentate gyrus. Although seizures induce expression of the 67-kDa isoform of γ-aminobutyric acid (GABA) synthetic enzyme glutamic acid decarboxylase (GAD67) in mossy fiber terminals (61,62), the overwhelming majority of terminals formed by sprouted fibers are asymmetric (Gray type), and therefore excitatory.

In 1985 Tauck and Nadler (63) analyzed evoked field potentials in the dentate gyrus of hippocampal slices from kainic acid–treated rats, and demonstrated an association between the duration and complexity of multispike field potentials and the extent of mossy fiber sprouting examined by the Timm method. This association was consistent with recurrent excitation as a consequence of sprouting, but was a relatively limited, indirect test of the hypothesis that mossy fiber sprouting formed new recurrent excitatory circuits and increased recurrent excitation. Definitive evidence for recurrent excitatory circuits would include evidence of monosynaptic excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) evoked by current injection in simultaneously recorded pairs of granule cells, which normally show no physiologic or anatomic evidence of recurrent connections. The extensive neuronal loss in the kainic acid–treated dentate gyrus potentially confounded the interpretation of abnormalities observed in population field potentials.

Other evidence suggesting a relation between mossy fiber sprouting and increased excitation in the dentate gyrus included observations that evoked EPSCs, spontaneous EPSCs, and spontaneous burst discharges develop after kainic acid- or pilocarpine-induced status epilepticus in association with development of mossy fiber sprouting (64–70). These studies revealed little or no change in apparent excitability in normal medium with inhibition intact, but with decreased inhibition or elevated $[K^+]_o$ consistently demonstrated evidence of increased local circuit excitation and epileptiform activity not observed in normal control preparations. Support for functional synaptic transmission in the sprouted mossy fiber pathway was provided by in vivo current source density analysis in kindled rats (71). In these studies, an inward current (sink) spatially corresponding to the terminal field of the sprouted mossy fiber terminals in the inner molecular layer of the dentate gyrus developed at a latency consistent with disynaptic transmission in response to perforant path stimulation (71).

More direct evidence for development of recurrent excitation in association with mossy fiber sprouting was obtained by Wuarin and Dudek (72) in hippocampal slices from kainic acid–treated rats. Focal application of glutamate microdrops to dendrites and cell bodies of granule cells remote from the recorded granule cell normally evokes no responses, but in hippocampal slices with sprouting, microdrops evoked EPSPs at long and variable latency when inhibition was blocked. Although supporting the formation of recurrent excitatory circuits, the long and variable latency of these responses suggested that recurrent excitation was generated by multisynaptic circuits rather than monosynaptically. Using similar techniques in kindled rats with mossy fiber sprouting, Lynch and Sutula (69) also demonstrated trains of EPSPs and population discharges evoked by glutamate microstimulation remote from the recording site at 1 week after induction of kindled seizures when sprouting is first detectable by Timm histochemistry. EPSPs were not evoked in hippocampal slices from kindled rats examined at 24 h after a single afterdischarge before the development of sprouting (69). Monosynaptic EPSPs evoked at short latency (2.6 ± 0.36 ms) between blades of the dentate gyrus were observed under conditions in which recurrent inhibitory circuits were blocked by bicuculline, polysynaptic activity was suppressed by 10 mM Ca^{2+} in the bathing medium, and perforant path activation was prevented by knife cuts (69). Recent studies by Waurin and Dudek also showed monosynaptic EPSCs in granule cells in response to stimulation by flash photolysis of caged glutamate at sites remote from the recording site (67). Although still falling short of conclusive evidence for the formation of recurrent excitatory circuits that would require dual recordings from granule cells, these studies from multiple laboratories support the viewpoint that seizures induce recurrent excitatory connectivity in the dentate gyrus.

Despite the presence of extensive mossy fiber sprouting after status epilepticus in kainic acid- and pilocarpine-treated rats, most measures of spontaneous and evoked physiologic activity appear normal unless inhibition is reduced (64,69,72). This observation is consistent with the clinical fact that patients with frequent and intractable seizures still function relatively normally and manifest epileptic behaviors only briefly and sporadically. In the presence of extensive seizure-induced sprouting, physiologic evidence of abnormality such as recurrent excitation

and excitatory connectivity may be variably expressed and detected in patchy distribution, as might be expected from the spatially delimited projections of sprouted mossy fiber collaterals. The variable spatial and temporal expression of physiologic abnormalities is consistent with the anatomic irregularity of mossy fiber connections in the dentate gyrus reorganized by seizure-induced sprouting, and with a stochastic process of synchronization in a complex neural network. Supporting this viewpoint, physiologic abnormality in association with sprouting also emerges when $[K^+]_o$ is elevated (65,73), as occurs during seizures. The expression of abnormal recurrent excitation by recurrent excitatory circuits formed by sprouted mossy fibers is likely to be dependent on the level of activity in inhibitory pathways, the extracellular ionic milieu, and metabolic conditions.

Conclusions from Experimental Studies of Sprouting in Neural Circuitry

Anatomic and physiologic studies investigating the effects of seizure-induced mossy fiber sprouting have revealed two major findings pertinent to assessing the role of mossy fiber sprouting in development of recurrent excitation, abnormal hippocampal function, and epileptogenesis:

1. There is a correlation between the development of indirect measures of recurrent excitation and anatomic evidence for mossy fiber sprouting in multiple experimental models, and
2. The evidence for abnormal recurrent excitation, including monosynaptic excitatory connections, becomes apparent only conditionally when inhibition is reduced or $[K^+]_o$ is elevated, and is therefore dependent on multiple factors including the strength of inhibition, the extracellular ionic milieu, and possibly metabolic conditions.

The Challenges of Assessing Functional Effects of Sprouting in a "Complex System"

What are the implications of the variable and conditional expression of functional abnormality associated with seizure-induced mossy fiber sprouting in chronic models of epilepsy? Some skepticism about the potential importance of mossy fiber sprouting has emerged, as several studies have not detected simple or straightforward relations between sprouting in the dentate gyrus and outcome variables such as spontaneous seizure frequency (74–77). These findings should come as no surprise, as previous studies have demonstrated that the dentate gyrus, and therefore mossy fiber sprouting, is not required for the development or expression of seizures arising from hippocampal circuitry (78–80).

Failure to appreciate the implications of the conditional expression of functional abnormalities, as discussed earlier, and the challenges presented in attempting to identify "causal" processes and mechanisms of abnormality in "complex systems" such as neural circuitry are potentially significant interpretive flaws. Given the conditional expression of functional abnormality in association with sprouting, it is not surprising that robust and dramatic emergent properties may be observed in response to incremental changes in a variety of the components of a "complex system" such as hippocampal circuitry. These components may include inhibitory interneurons, $[K^+]_o$, pH, or other unrecognized factors. In such systems, relations between abnormality in a single component (e.g., sprouting) and the emergent abnormal functional property may be highly nonlinear and pose significant challenges for experimentalists.

Periodic spontaneous seizures, the hallmark of epilepsy, are likely to be caused by a constellation of abnormalities in the complex distributed systems of neural circuitry in the hippocampus and elsewhere. From the perspective of hippocampal circuitry as a complex system, the difficulty of detecting linear relations between seizure frequency and sprouting should come as no surprise. At best, the finding of a correlation is only suggestive that mossy fiber sprouting may contribute to seizures. The finding of a lack of correlation is not strong evidence against the importance of seizure-induced sprouting and synaptic reorganization, but points out that other alterations also may contribute to epileptogenesis and its consequences (81–83).

Emerging Viewpoints

Axonal sprouting, a prominent cellular event in development of neural circuits, is now firmly established as a common feature of neural circuit remodeling in response to seizures. Efforts to assess the functional effects of sprouting present considerable challenges requiring not only sophisticated experimental approaches, but also appreciation of the difficulties in establishing cause in nonlinear complex systems such as neural networks. The emerging physiologic evidence supports the view that recurrent excitatory circuits formed by sprouted mossy fibers contribute to increases in recurrent excitation under certain conditions in the complex system of hippocampal circuitry, and may be revealed only in settings where one or more other alterations may be occurring. Although analysis of complex systems such as neural circuitry is challenging, understanding how and when seizure-induced sprouting alters function is likely to provide therapeutic opportunities and insights into epileptogenesis and the consequences of poorly controlled epilepsy, including memory and behavioral dysfunction.

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