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Hippocampal Granule cell Pathology in Epilepsy – a Possible Structural Basis for Epileptic Co-Morbidities?

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Abstract

Temporal lobe epilepsy in both animals and humans is characterized by abnormally integrated hippocampal dentate granule cells. Among other abnormalities, these cells make axonal connections with inappropriate targets, grow dendrites in the wrong direction and migrate to ectopic locations. These changes promote the formation of recurrent excitatory circuits, leading to the appealing hypothesis that these abnormal cells may be epileptogenic. While this hypothesis has been the subject of intense study, less attention has been paid to the possibility that abnormal granule cells in the epileptic brain may also contribute to co-morbidities associated with the disease. Epilepsy is associated with a variety of general findings, such as memory disturbances and cognitive dysfunction, and is often co-morbid with a number of other conditions, including schizophrenia and autism. Interestingly, recent studies implicate disruption of common genes and gene pathways in all three diseases. Moreover, while neuropsychiatric conditions are associated with changes in a variety of brain regions, granule cell abnormalities in temporal lobe epilepsy phenocopy granule cell deficits produced by genetic mouse models of autism and schizophrenia, suggesting that granule cell dysmorphogenesis may be a common factor uniting these seemingly diverse diseases. Disruption of common signaling pathways regulating granule cell neurogenesis may begin to provide mechanistic insight into the co-occurrence of temporal lobe epilepsy and cognitive and behavioral disorders.

1. Adult neurogenesis in the hippocampal dentate gyrus

The hippocampal dentate gyrus is important for memory formation and more recent studies suggest it may play an important role in pattern separation [1–3]. During pattern separation, similar inputs are converted to less similar outputs, facilitating the brains ability to

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distinguish between similar stimuli, cues or environments [4]. In addition to its role in memory, lesion studies in humans suggest the hippocampus plays an important role in imagination [5, 6]. Furthermore, while the former functions are typically attributed to dorsal hippocampus (posterior in humans), a variety of studies suggest ventral hippocampus (anterior in humans) may play an important role in regulating mood and affect [7].

The hippocampal dentate gyrus is unusual in that new neurons, known as dentate granule cells, are added to it throughout adulthood in both animals [8–10] and humans [11, 12]. The function of these new neurons is the subject of intense investigation, and has been the topic of numerous recent review articles [13–15]. Briefly, adult-born granule cells are produced by neural progenitor cells located in the sub-granular zone, which is located immediately below the granule cell layer. New cells migrate into the granule cell layer after birth, project axons into the dentate hilus and CA3 stratum lucidum, and dendrites into the molecular layer (Fig. 1). Following maturation, the new cells appear morphologically and physiologically equivalent to cells generated during development *in utero* [16–19].

Adult-generated granule cells do, however, have a transient stage during their development when they exhibit properties not present among older cells. Specifically, immature (< 5 weeks old) granule cells exhibit 1) increased excitability compared to their mature counterparts [20, 21], and 2) enhanced synaptic plasticity and associative LTP induction [22–24]. The distinctive physiological characteristics of immature granule cells may be important for learning and memory. Although there is still significant controversy in the field, anti-neurogenic, cell ablation and selective cell inhibition strategies have implicated newborn granule cells as playing roles in spatial memory, fear conditioning and pattern separation [25–28].

2. Temporal lobe epilepsy is characterized by increased production and aberrant integration of hippocampal dentate granule cells

During the development of epilepsy in animals, the rate of granule cell neurogenesis increases [29], and perhaps more significantly, a large percentage of the newborn dentate granule cells integrate abnormally. Neuronal birthdating studies have demonstrated a large increase in granule cell neurogenesis following a variety of epileptogenic brain insults, including kindling epileptogenesis [30]. Neuronal fate-mapping studies, on the other hand, demonstrate that more than 50% of the new cells can show structural abnormalities, including aberrant basal dendrites, ectopically located somata and granule cell mossy fiber axon sprouting into the dentate inner molecular layer (Fig. 2). Spine density changes are also evident among new cells during epileptogenesis, although the findings are complex: both spine density decreases [31–33] and increases [34, 35] have been observed. Interestingly, there appear to be critical periods during granule cell development when epileptogenic brain injuries can induce particular abnormalities. Once granule cells reach maturity, however, they are comparatively insensitive to developing these changes [35–39].

3. Aberrantly integrated granule cells form recurrent excitatory connections

Abnormally integrated granule cells commonly form excitatory synapses with neighboring granule cells, creating recurrent connections that are hypothesized to be pro-epileptogenic [40–43]. Recurrent circuits created by granule cell mossy fiber axon sprouting, which is characteristic of temporal lobe epilepsy in both animals and humans, are suspected of contributing to dentate hyperexcitability [44–47], although the exact function of this pathology remains uncertain [48]. Formation of recurrent circuits can also occur when granule cells extend basal dendrites into the dentate hilus, where they are subsequently innervated by mossy fiber axons [49–52]. Perhaps the most attractive phenomenon for promoting hyperexcitability, however, occurs when granule cells migrate to ectopic locations in the hilus. Hilar ectopic granule cells have been observed to produce spontaneous epileptiform bursts, and receive more excitatory input from other granule cells [53–55]. These cells have been proposed to be seizure-inducing “hub cells,” which may be able to synchronize network activity by receiving input from unusually large populations of neurons and then exciting an aberrantly large target network [56, 57].

4. Seizure frequency is correlated with abnormal granule cell “load”

If abnormal granule cells truly drive seizures, then a logical prediction would be that animals with large numbers of these abnormal cells would exhibit more seizures than animals with only a few abnormal cells. Several studies utilizing various epilepsy models have shown such a correlation [58–60], although many studies found no correlation [61–63]. To address this issue more decisively, we recently took advantage of new cell labeling techniques and new technologies for conducting 24/7 video-EEG monitoring to explore the relationship between multiple granule cell abnormalities in epileptic animals and seizure frequency. We found significant correlations between seizure frequency and 1) mossy fiber sprouting severity and 2) the frequency of hilar ectopic granule cells [64] (Fig. 3). We also observed a trend between seizure severity and basal dendrite frequency. Interestingly, no single granule cell abnormality consistently increased with seizure frequency. Rather, when all types of granule cell abnormalities were examined together, animals with more seizures tended to have more abnormal cells. The pattern of abnormalities, however, differed among animals, with some having more basal dendrites, for example, while others had more ectopic cells. This observation suggests that abnormal cells of different types may have additive effects on seizure frequency. Finally, in addition to seizure frequency, we also found evidence that mossy fiber sprouting may contribute to seizure duration, further emphasizing the potential pathological effects of abnormal granule cells on network activity.

5. Disrupted production and integration of adult-generated granule cells may contribute to behavioral deficits in epilepsy

Temporal lobe epilepsy is associated with a number of co-morbidities, including cognitive and memory disturbances [65], autism [66, 67], depression [68–70] and psychosis [71–73]. Behavioral deficits are also common in animal models of epilepsy [74–82]. While the mechanisms underlying behavioral deficits in temporal lobe epilepsy are complex, diverse, and involve many brain regions [65], disrupted granule cell neurogenesis could be an

important factor. Indeed, it is difficult to imagine that the hippocampus can function normally in the presence of abnormalities such as those shown in figures 2 and 3. In support of the idea that abnormal granule cells might disrupt behavior, we and others have demonstrated that substantial numbers of newborn cells integrate abnormally in ventral hippocampus. Indeed, both dorsal and ventral hippocampus appear similarly affected [64, 83] (Fig. 4). The presence of abnormal cells in the ventral hippocampus is significant because this region is implicated in regulating mood and affect [7], and correspondingly, disruption of this region would be predicted to impact these functions.

6. Adult neurogenesis is a common target of genes linked to epilepsy and neuropsychiatric disease

In recent years, a number of genes have been identified that are associated with both neuropsychiatric disease and epilepsy. Intriguingly, several of these genes have also emerged as important regulators of granule cell neurogenesis and integration. Several excellent review articles have focused on the relationship between neurogenesis rates and disease [84–86], so the present study will more closely examine common pathologies of granule cell integration. These common pathologies may not be coincidental, raising the possibility that disrupted granule cell integration might be a shared factor linking epileptic co-morbidity and neuropsychiatric illness. In the following sections, we will review three of the most notable genes and gene pathways, and their common effects on granule cell development.

6.1 The mTOR signaling pathway

Disrupted mammalian target of rapamycin (mTOR) signaling appears to play a role in neuropsychiatric disease and epilepsy. mTOR is a serine/threonine kinase involved in the highly conserved PI3K-AKT signaling pathway, and acts as a key regulator of translation, cell growth and proliferation [87]. In the brain, mTOR plays roles in neuronal development, synaptic plasticity and maintenance of LTP [88]. Consequently, mTOR signaling is important for several neurological processes, including learning, memory and social behavior [89, 90]. Genetic studies in humans implicate the mTOR signaling pathway in the development of epilepsy [91–93] autism [94–96] and schizophrenia [97]. In addition, evidence of increased mTOR signaling has been found in sclerotic tissue from human epilepsy patients [98] and clinical trials using mTOR antagonists in tuberous sclerosis complex (TSC) patients with epilepsy have produced promising results [99–102]. Animal studies also support a role for disrupted mTOR signaling in epilepsy and behavior. Markers of mTOR pathway activation are increased during epileptogenesis in several chemically-induced animal models of epilepsy [103–105], and treatment with the mTOR antagonist rapamycin reduces the frequency of seizures in some of these models [103, 104], but see also [106]. Finally, mouse models in which the mTOR pathway inhibitors PTEN [107–111], TSC1 [112, 113] or TSC2 [114] have been deleted lead to hyperactivation of the mTOR pathway and produce impaired synaptic plasticity, behavioral deficits with autistic features and profound epilepsy syndromes.

The mTOR signaling pathway regulates both the rate of adult neurogenesis [115], and the development and integration of the new cells. Manipulations that enhance mTOR signaling, such as deleting TSC1, TSC2 or PTEN, produce dramatic changes in granule cell morphology [109, 110, 116, 117]. Granule cells exhibit a pronounced hypertrophy, develop larger and thicker dendrites, show an increased density of dendritic spines, form hilar basal dendrites and migrate to ectopic locations in the dentate hilus (Fig. 5, A,B,G). The granule cell changes induced by mTOR hyperactivation recapitulate those observed in a variety of epilepsy models [35, 98, 103, 118] (Fig. 5, C–E,H) as well as tissue from epileptic patients [119]. Indeed, intrahippocampal injection of kainic acid to induce epileptogenesis in rodents induces a pronounced activation of the mTOR pathway among dentate granule cells, evident by increased phosphorylation of the downstream protein S6, and profound neuronal hypertrophy (Fig. 6). Other granule cell pathologies in epilepsy also appear to be mediated by mTOR. Treatment with mTOR antagonist rapamycin, for example, can prevent mossy fiber sprouting in the kainic acid and pilocarpine models of epilepsy [103, 106], strongly implicating excess mTOR signaling in the process; although indirect effects of the drug have yet to be excluded.

6.2 Disrupted in schizophrenia 1 (Disc1)

Over two decades ago, Disc1 was identified in a family with recurrent major depression, bipolar disorder and schizophrenia [120–122]. Disc1 encodes a molecule with multiple coiled coil motifs that interacts with numerous proteins, including girdin [123], GSK3 β [124] and Lis1 [125]. Notably, Disc1 has been shown to regulate mTOR signaling via the AKT binding protein KIAA1212 [97] and mTOR inhibition with rapamycin can mitigate behavioral deficits caused by Disc1 knockdown [126]. Disc1 has emerged as an important regulator of adult granule cell neurogenesis and integration [124, 127, 128]. Disc1 knockdown and dominant negative approaches have revealed increases in granule cell soma size, increased primary dendrite number, increased spine density, expansion of the granule cell layer with granule cell somata dislocated to the dentate molecular layer, and granule cells with recurrent basal dendrites [129, 130]. Recurrent basal dendrites project off the basal pole of the cell, but then turn 180° and correctly enter the dentate molecular layer (Fig. 7). In 2008, Kvaajo and colleagues made a mouse carrying a truncating lesion in the endogenous Disc1 orthologue modeling the human mutation [131]. Changes in neuronal structure in these animals were most prominent in the prefrontal cortex and dentate gyrus, and in the dentate included misplaced granule cells and granule cells with misoriented dendrites. Morphological changes were less pronounced than those observed in studies using knockdown and dominant negative approaches, however, perhaps reflecting differences in the strategy used to manipulate Disc1 expression [132].

The abnormalities exhibited by Disc1 knockout granule cells are similar to changes described in several status epilepticus models of epilepsy [35, 39, 133, 134], including ectopic granule cells and granule cells with misoriented dendrites (Fig. 7). Changes we have observed in the intrahippocampal kainic acid (IHpKA) model of epilepsy, which is associated with profound granule cell dispersion, are also notably similar [118, 135] (Figs. 6 and 7). Real-time live imaging of YFP-expressing granule cells in tissue culture led us to propose that the increase in primary dendrites and formation of recurrent basal dendrites

evident in the IHPKA model of epilepsy is a consequence of somatic translocation, whereby the granule cell soma migrates up a primary dendrite, converting dendritic branches to primary dendrites as branch points are “absorbed” by the soma, and shifting the origin of adjacent dendrites from the apical to the basal pole of the cell (Fig. 8). Pronounced migration of newborn granule cells into the molecular layer following *Disc1* KO or KD suggest a similar phenomenon could be occurring in these animals [136–138]. Finally, reduced *Disc1* expression is correlated with expansion of the granule cell layer in the kindling model of epilepsy [139, 140], supporting the hypothesis that changes in *Disc1* expression may mediate some of the granule cell abnormalities observed in epilepsy.

6.3 Reelin

Reelin is an extracellular glycoprotein which regulates cell migration in partnership with its receptors, the very low density lipoprotein receptor (Vldlr) and the apolipoprotein E receptor 2 (ApoER2). Reelin mutations are implicated in epilepsy, autism, bipolar disorder, depression and schizophrenia [141–144]. The impact of the reeler gene on mouse brain development has been extensively studied thanks to the spontaneous appearance of a mutant animal in the early 50s [145]. These so-called “reeler” mice exhibit a variety of behavioral deficits and enhanced sensitivity to seizures [146, 147]. Reduced reelin expression is implicated in driving granule cell dispersion in epilepsy [148], perhaps acting in concert with *Disc1* [140]. Reeler mutant mice exhibit ectopic granule cells in the hilus and molecular layer. Reeler mice also develop mossy fiber sprouting [149], and granule cells with hilar and recurrent basal dendrites are common [150, 151]. Again, disorganization of the dentate is similar between reeler mutants and other models of epilepsy (Fig. 9).

7. Morphologically abnormal granule cells have been identified in non-epileptic neurological conditions

If abnormal granule cells contribute to neuropsychiatric illness, it follows that these abnormalities should be present in tissue from patients. Unfortunately, well-controlled studies of human tissue are difficult to conduct, and therefore data is limited. A small number of studies, however, provide some insight. The presence of abnormal granule cells in patients with temporal lobe epilepsy has been known for some time, because the tissue can be obtained from hippocampal resection surgeries to treat intractable epilepsy. Mossy fiber sprouting is the most common finding in tissue from epileptic patients, in part due to the ease of revealing this abnormality using histochemical stains for zinc [152]. Dentate gyri with misplaced granule cells, granule cells with recurrent and hilar basal dendrites, and granule cells with disorganized dendritic trees are also common findings [119, 153–158]. Few studies have examined patients with schizophrenia; however, in 2003 Senitz and Beckmann examined rapid autopsy material and found an increase in the percentage of granule cells with basal dendrites in tissue from patients with the disease [159]. Studies of granule cell basal dendrites in humans are more complicated than in rodents, since rodent granule cells typically lack basal dendrites, while human granule cells frequently possess basal dendrites under normal conditions [160, 161]. The increase from 28% of cells with basal dendrites in healthy human controls to 46% in patients with schizophrenia, however, is suggestive. Evidence for decreased numbers of granule cell mossy fiber terminals in stratum

lucidum, where they normally contact CA3 pyramidal cells, has been observed in tissue from schizophrenic patients [162–166]. Interestingly, decreased numbers of mossy fiber giant bouton terminals, which contact CA3 pyramidal cells, have also been observed in the pilocarpine model of epilepsy in animals with pyramidal cell loss [167]. Finally, while a number of imaging studies have found reduced hippocampal volume in schizophrenic patients, resolution limits usually preclude identification of the affected hippocampal subregions [168–170]. A recent 7 Tesla MRI study of 16 schizophrenic patients and 15 controls, however, was able to overcome this technological limit. The investigators found that decreased contrast in the dentate granule cell layer was predictive of diagnosis [171], supporting the conclusion that granule cell abnormalities may be a feature of the disease. Finally, Dowlatshahi and colleagues (2000) found evidence for mossy fiber sprouting in tissue from 13 patients with bipolar disorder relative to 15 controls using the zinc stain for mossy fiber axons [172]. Nonetheless, while these observations are intriguing, the incidence of abnormal granule cells in neuropsychiatric illness remains uncertain. Additional high-quality studies of human hippocampi are sorely needed.

8. Selective disruption of adult-generated granule cells can produce behavioral deficits in animals

The best evidence in support of the idea that abnormal granule cells may contribute to neuropsychiatric illness comes from animal models in which adult-generated granule cells were selectively manipulated. Pharmacological treatments to inhibit neurogenesis following epileptogenic brain insults can reduce the number of abnormal granule cells added to the hippocampus, and have been shown to mitigate cognitive impairments [173, 174]. Even more selective manipulations can be achieved using conditional, inducible transgenic mice lines in which genes of interest can be selectively manipulated with great cellular specificity using promoter sequences specific to progenitor cells. Studies of this nature provide an opportunity to generate “proof of principle” data to determine whether abnormal granule cells are capable of causing disease. Bergami and colleagues (2008) were able to achieve such a disruption using GLAST-CreER^{T2} transgenic mice to delete the brain-derived neurotrophic factor receptor TrkB from adult-granule cell progenitors [175]. Adult-generated TrkB knockout granule cells exhibited a number of morphological and physiological defects, including reduced dendritic complexity, reduced spine density and impaired LTP. Animals exhibited increased anxiety-like behavior, evident as decreased spontaneous activity in the open-field test, and fewer entries into the open arms in the elevated plus maze. In 2009, Revest and colleagues overexpressed the pro-apoptotic protein BAX in granule cell progenitors, reducing adult neurogenesis and again increasing the incidence of anxiety-like behaviors in the mice [176]. Alternatively, when Li and colleagues deleted the Ras suppressor neurofibromin from adult-generated granule cells, mice exhibited an enhanced responsiveness to antidepressant treatment, showing positive effects to doses that were ineffective in control animals [177]. This response was associated with an increase in both the production and dendritic complexity of newborn granule cells. Finally, Pun and colleagues selectively deleted PTEN from 10–25% of granule cells, and demonstrated that this manipulation was sufficient to cause epilepsy [110]. Studies examining the behavioral phenotype in these animals are ongoing. Together, these findings demonstrate in principle

that disruption of adult-generated granule cells can cause behavioral deficits and epilepsy [109].

9. Late-generation of granule cells may make them selectively vulnerable to late-occurring insults

Congenital gene mutations have the potential to affect the development of the entire brain, raising the question as to how such a mutation might produce a relatively selective disruption of the dentate gyrus. Regional and cell-specific patterns of gene expression will certainly be important, but one feature of granule cells could confer additional selective vulnerability: In addition to being generated throughout life, hippocampal dentate granule cells are unusual in that their peak period of production during development occurs after almost all other neurons. While most neurons are generated prior to birth, 30% of dentate granule cells are generated in infancy in non-human primates [178]. Human brain development is believed to follow a similar pattern. The temporal dissociation between the production of granule cells and most other neurons creates a critical developmental window during which granule cells can be selectively disrupted. It is well established that developing neurons are more vulnerable to disruption than mature neurons, as was described in relation to epileptogenic insults [179]. This makes sense, as developing neurons must navigate a complex environment and make numerous critical “decisions” about whether and how to integrate into the brain; including migrational routes, axonal trajectories, postsynaptic target identification and survival or apoptosis choices. These decisions are regulated by a host of internal and extrinsic cues which are exquisitely sensitive to disruption. By contrast, mature neurons, which have already made these choices, appear to be much more resistant to insult. Insults occurring late in development, therefore, may selectively impair late-generated granule cells, while leaving earlier-generated cortical and subcortical neurons comparatively unaffected.

An example of the selective-vulnerability of late-generated granule cells comes from studies of anesthesia-induced neurotoxicity. Ten years ago, Jevtovic-Todorovic discovered that exposing developing rodents to clinically relevant doses of commonly-used anesthetics dramatically increased neuronal apoptosis and led to long-lasting cognitive deficits [180]. Naturally, this raised concerns about the safety of anesthetic use in children. A series of follow-up studies confirmed the basic findings (for review see [181]) and extended observations to guinea pigs [182], piglets [183] and non-human primates [184]. Early work examining anesthesia-induced cell death revealed a pattern of neuronal loss that was difficult to explain, with widespread, but scattered, cell loss throughout the brain. Dying neurons were surrounded by similar but seemingly unaffected neurons. Recent work by Hofacer and colleagues (2013), in which mice were treated with anesthetics on P7, P21 and P49, revealed a surprising shift in the pattern of cell loss as the animals aged [185]. At P7, dying cells were found throughout cortex and thalamus, and within the CA1 and CA3 pyramidal cell layers of the hippocampus (all generated embryonically). Few dentate granule cells, however, succumbed to apoptosis at P7. Shift forward to P21 and P49, however, and the pattern reversed. In these older animals dentate granule cells were the most vulnerable population, while the brains regions vulnerable at P7 showed little cell loss. BrdU

birthdating studies confirmed that the dying granule cells were newborn, with a peak period of vulnerability beginning around two weeks after cell birth in rodents. These findings suggest that anesthetic exposure may interfere with newborn granule cell integration into the hippocampus, causing the cells to undergo apoptosis instead. Whether anesthetics also increase apoptosis in humans – and if so, whether this has any long-term negative impacts on child development – remains controversial. Recent retrospective studies, however, have found that children exposed to surgical anesthesia performed more poorly on cognitive tests and/or developed more behavioral problems than children with no exposure [186–192].

Importantly, studies of seizure-induced granule cell disruption and anesthetic neurotoxicity demonstrate in principle that exogenous insults can selectively injure newborn granule cells. Animal work suggests it is a formal possibility that such a mechanism contributes to the development of neuropsychiatric disease. Consistent with this idea, anti-epileptic drugs, which often have similar mechanisms of action to anesthetics, also increase neuroapoptosis in developing brains [193]. Recent work by Bhardwaj and colleagues (2012) found that exposing neonatal rats to phenobarbital increased the incidence of schizophrenia-like behaviors in adulthood [194]. These findings raise the possibility that anti-epileptic drugs themselves could contribute to epileptic co-morbidities.

10. Limitations to the impact of abnormal granule cells

Studies of epilepsy and neuropsychiatric disease have revealed changes in numerous brain regions outside the hippocampus. Disruption of entorhinal cortex, for example, may be an important feature of temporal lobe epilepsy [195, 196]. Changes in prefrontal cortex have been consistently found in patients with schizophrenia [197]. Cerebellar changes are a common finding in imaging and histological studies of autistic brains, and recent studies have found the deletion of TSC1 from Purkinje cells in mouse cerebellum can reproduce features of autism [198]. Granule cell abnormalities may not account for all or even most features of these disparate diseases. Nonetheless, the robust connectivity between the hippocampus and other brain regions – and the potentially critical role of the dentate in regulating information flow through the hippocampus – implies that disrupted granule cell function will have wide-ranging effects [199].

The functional impact of abnormal granule cells remains unclear. Abnormal granule cells examined in epilepsy models exhibit recurrent excitatory connections [200] and the dentate gyrus exhibits increased excitability [45], consistent with the hypothesis that these cells might contribute to seizures. PTEN-knockout granule cells and granule cells from reeler mice also exhibit recurrent connections and increased excitability [110, 149, 201]. Whether and how abnormal cells act to disrupt cognition and behavior remains largely unexplored; however, schizophrenia is associated with hippocampal hypermetabolism in humans [202, 203], and excess hippocampal outflow may drive psychotic and affective symptoms [199]. The addition of hyperexcitable granule cells to the dentate could contribute to these phenomena. Nevertheless, more work is needed to better establish the physiological properties of granule cells in different disease models and following different genetic manipulations. Kvaajo and colleagues (2011), for example, found reduced granule cell excitability in mice in which the *Disc1* gene was truncated [132], and studies of newborn

granule cells in different epilepsy models have found both decreased [204] and increased [205] excitability. Whether additional studies will reveal an excitability pattern most likely to be associated with behavioral deficits remains to be determined. Alternatively, both increased and decreased activity may be pathological, as recently suggested by work from Auerbach and colleagues (2011), in which TSC2 knockout mice were crossed to mice with a deletion of the fragile X mental retardation protein (Fmr1) [206]. TSC2 mice exhibit increased metabotropic glutamate receptor 5 (mGluR5) activity, while Fmr1 mice exhibit reduced activity. Animals with mutations in either gene exhibit behavioral deficits, but crossing the two animals together rescued the phenotype, suggesting the mGluR5 activity must be kept in an optimal range for proper brain function.

11. Conclusions

Temporal lobe epilepsy occurs in association with a number of neuropsychiatric disorders, including autism, bipolar disorder and schizophrenia. The mechanisms underlying these associations remain uncertain; however, recent work identifying human gene mutations associated with both epilepsy and neuropsychiatric disease are beginning to provide some new insights. Here, we observe that several of these genes have emerged as important regulators of hippocampal dentate granule cell integration, and disruption of these genes produces a characteristic pattern of granule cell abnormalities in animal models, including enlarged granule cells with disrupted dendritic structure and migration defects. Significantly, these abnormalities phenocopy granule cell abnormalities characteristic of both genetic and acquired models of temporal lobe epilepsy, in which animals also exhibit behavioral deficits. Correspondingly, we suggest that abnormal granule cells could reflect a common pathology accounting for some of the shared deficits between epilepsy and neuropsychiatric disease.

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Highlights

- Newborn hippocampal granule cells develop abnormally during epileptogenesis
- Aberrant granule cells are hyperexcitable and are hypothesized to drive seizures
- Disproportionate mTOR signaling may underlie aberrant granule cell development
- Granule cell abnormalities are also common in other neurological diseases
- These diseases are often comorbid with epilepsy, suggesting a common etiology

Figure 1.

Photomontage of confocal maximum projections showing granule cell progenitors and granule cells at different maturational stages. To label granule cell progenitors, Gli1-CreER^{T2} mice were crossed with CAG-CAT-EGFP reporter mice. Gli1, a member of the sonic hedgehog family, drives Cre recombinase expression in granule cell progenitors. Activation of Cre recombinase by treating postnatal animals with tamoxifen leads to the persistent expression of green fluorescent protein (GFP) in recombined cells. Animals were killed and immunostained for GFP one week (progenitors), two weeks (immature), and two months (mature) after tamoxifen treatment. Mice were donated courtesy of Dr. Alexandra Joyner (Gli1-CreER^{T2}; [207, 208]) and Dr. Jeffrey Robbins (CAG-CAT-EGFP reporter mice; [209]). Asterisk denotes basal dendrites on immature granule cells. Scale bar = 30 μ m. Figure reprinted with permission from Danzer, 2008 [179] (Copyright © 2008 Sage Publications).

Figure 2.

Normal and abnormal hippocampal dentate granule cells of pilocarpine-treated Gli1-CreER^{T2} x GFP-reporter mice. Typical granule cell morphology (A) consists of the cell body in the granule cell layer, apical dendrites extending towards the molecular layers, and a basal axon in the hilus (arrowhead). Common structural abnormalities develop in granule cells following status epilepticus, including cells with hilar basal dendrites (B), ectopically migrated cells (C), and axonal sprouting in the inner molecular layer (D, arrowheads). Scale bars = 10 μ m.

Figure 3.

Ectopically located newborn granule cells and the amount of mossy fiber sprouting correlate with seizure frequency. Newborn granule cells in the dorsal hippocampus of pilocarpine-treated Gli1-CreER^{T2} x GFP-reporter mice often migrate ectopically into the hilus (A,B). The percentage of newborn granule cells located in the hilus positively correlates to seizure frequency (C). Zinc transporter 3 (ZnT3) and GFP labeling show robust mossy fiber sprouting in the inner molecular layer (D,E). Arrowheads (D) highlight sprouted granule cell mossy fiber axons. The amount of mossy fiber sprouting positively correlates to seizure frequency (F). iml, inner molecular layer; gcl, granule cell layer; h, hilus. Scale bars: A and D 250 μ m; B 20 μ m; E 50 μ m. Figure reprinted with permission from Hester and Danzer, 2013 [64].

Figure 4.

Dorsal and ventral hippocampus are affected similarly following status epilepticus. Ectopic granule cells are largely absent from a Gli1-CreER^{T2} x GFP-reporter mouse that did not develop epilepsy following pilocarpine treatment (A,C) while an animal experiencing 3 seizures per day (B,D) exhibited frequent ectopic granule cells. Within animals, the number of abnormal granule cells is similar in dorsal (top) and ventral (bottom) hippocampus. Scale bars = 250 μm.

Figure 5.

Hippocampal dentate granule cells from PTEN knockout mice (A,B,G) and mice rendered epileptic by inducing status epilepticus with pilocarpine (C–E, H). Note the hypertrophied soma of the PTEN knockout cell (A) relative to the hypertrophied somata of the granule cells from pilocarpine treated mice (C–E, blue arrowheads). PTEN deletion can also alter granule cell migration, leading to the appearance of ectopic cells in the dentate hilus (B, blue arrowhead; compare to figure 2, C for the pilocarpine model). Both PTEN deletion (G) and pilocarpine treatment (H) can lead to increases in dendritic spine density relative to control cells (F). Note: pilocarpine treatment can lead to increased spine density on some cells, and reduced density on others [39]. Scale bars = 30 μ m (A), 100 μ m (B), 30 μ m (C–E) and 10 μ m (F–H). Panels C–E reprinted with permission from Murphy et al., 2011 [35]. Panels A, F and G reprinted with permission from Pun et al., 2012 [110].

Figure 6.

Unilateral injection of kainic acid into the hippocampus increases phosphorylation of the downstream mTOR target S6 (blue) in the injected hemisphere, but not the contralateral (non-injected) hemisphere of thy1-YFP expressing (yellow) mice. Increased activation of the mTOR pathway is associated with a profound neuronal hypertrophy on the injected side. Scale bar = 40 μ m.

Figure 7.

Disc1 knockout and epileptic mice exhibit similar granule cell abnormalities. A: Disc1 knockout granule cell with an elongated, enlarged soma (blue asterisk) and a recurrent basal dendrite (white arrow; used with permission from Duan et al., 2007 [129]). B: YFP-expressing ectopic granule cells in the dentate molecular layer with recurrent basal dendrites (white arrows) in the intrahippocampal kainic acid (IHpKA) model of epilepsy. C: GFP-expressing granule cell with a recurrent basal dendrite (white arrow) in the pilocarpine model of epilepsy. D–F: GFP-expressing control granule cells (D) and granule cells with misoriented dendrites from animals treated with pilocarpine (E,F, blue arrowheads). A granule cell with an enlarged and elongated soma (blue asterisk) is also shown. G–J: Time-lapse imaging of a granule cell in an organotypic hippocampal explant culture, which models epileptogenesis *in vitro*. Images over 6 days *in vitro* (DIV) show elongation of the soma and the shifting of an apical dendrite to the basal pole of the cell, creating a recurrent basal dendrite (blue arrows) (Images used with permission from Murphy and Danzer, 2011 [135]). Scale bars = 30 μ m (A), 30 μ m (B), 15 μ m (C), 30 μ m (D–F), 25 μ m (G–J).

Figure 8.

Model depicting granule cell dysmorphogenesis following somatic translocation into an apical dendrite. (a) As the soma moves into the initial segment of the left apical dendrite (1), the right apical dendrite is “left behind”, gradually shifting its position from the apical to the basal pole of the cell (2). Continuation of this process leads to a shortening of the initial dendritic segment on the left (3), and conversion of the right apical dendrite into a recurrent basal dendrite (4). In extreme cases, further migration of the soma leads to the absorption of branch points, converting these structures into primary dendrites (5), and the formation of dendritic loops projecting towards the hilus (6). (b) An example of a granule cell exhibiting a recurrent basal dendrite with a pronounced dendritic loop and an unusually large number of apical dendrites; the putative end product of the processes outlined in a. Note that the axon (white arrowhead) projects off the base of the dendritic loop, suggesting that this was the original location of the cell body. Scale bar = 50 μm. Images used with permission from Murphy and Danzer, 2011 [135].

Figure 9.

Reeler mutant mice and epileptic mice exhibit similar granule cell abnormalities. Left panel: GFP-expressing granule cells from a Gli1-CreER^{T2}::GFP reporter mouse that exhibited no epileptic seizures (control). Note the normal organization of the granule cell blades (GC) and the absence of ectopic cells in the hilus (H). Middle panel: Gli1-CreER^{T2}::GFP reporter mouse treated with pilocarpine to induce epilepsy. In this animal, the granule cell layer is disorganized and numerous labeled cells are present in the hilus. Right panel: Golgi staining of granule cells in a reeler mutant mouse (used with permission from Drakew et al., 2002 [151]). Similar to the pilocarpine treated animal, the granule cell layer is disorganized and numerous ectopic cells are present in the hilus. Scale bars = 100 μ m.