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Concise Review: Prospects of Stem Cell Therapy for Temporal Lobe Epilepsy

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Abstract

Certain regions of the adult brain have the ability for partial self-repair after injury through production of new neurons via activation of neural stem/progenitor cells (NSCs). Nonetheless, there is no evidence yet for pervasive spontaneous replacement of dead neurons by newly formed neurons leading to functional recovery in the injured brain. Consequently, there is enormous interest for stimulating endogenous NSCs in the brain to produce new neurons or for grafting of NSCs isolated and expanded from different brain regions or embryonic stem cells into the injured brain. Temporal lobe epilepsy (TLE), characterized by hyperexcitability in the hippocampus and spontaneous seizures, is a possible clinical target for stem cell-based therapies. This is because these approaches have the potential to curb epileptogenesis and prevent chronic epilepsy development and learning and memory dysfunction after hippocampal damage related to status epilepticus or head injury. Grafting of NSCs may also be useful for restraining seizures during chronic epilepsy. The aim of this review is to evaluate current knowledge and outlook pertaining to stem cell-based therapies for TLE. The first section discusses the behavior of endogenous hippocampal NSCs in human TLE and animal models of TLE and evaluates the role of hippocampal neurogenesis in the pathophysiology and treatment of TLE. The second segment considers the prospects for preventing or suppressing seizures in TLE using exogenously applied stem cells. The final part analyzes problems that remain to be resolved before initiating clinical application of stem cell-based therapies for TLE.

Keywords

Temporal lobe epilepsy; Adult neurogenesis; Dentate gyrus; Epilepsy; Hippocampal stem cells Hippocampal progenitors; Neural stem cells; Stem cell grafts

Introduction

Hippocampal lesions inflicted by acute seizures or head injury initially lead to epileptogenic structural changes and then progress into hippocampal dysfunction exemplified by chronic epilepsy, learning and memory impairments, and dramatically declined dentate neurogenesis. Approximately 50 million people suffer from epilepsy and approximately

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40% of patients have temporal lobe epilepsy (TLE). TLE is typified by the progressive expansion of spontaneous recurrent motor seizures (SRMS; typically described as complex partial seizures in humans) stemming from the limbic system regions, especially the hippocampus [1, 2]. The TLE with hippocampal sclerosis, one of the most prevailing types of partial seizure disorders [3–5], is often allied with an initial precipitating event such as febrile convulsions, trauma, status epilepticus (SE), or encephalitis [6–8]. The hippocampal sclerosis is characterized by widespread hippocampal neuronal loss and aberrant mossy fiber sprouting [9–11]. Thirty-five percent of the people with TLE have chronic seizures that are resistant to antiepileptic drugs [12–14], and most TLE patients have learning and memory impairments and depression [15–18]. Moreover, antiepileptic drugs merely provide symptomatic treatment without influencing the course of the disease. Thus, there is a pressing need to develop alternative therapeutic approaches that suppress the evolution of both chronic epilepsy and learning and memory dysfunction after the initial precipitating injury (IPI).

The onset of chronic epilepsy following the IPI usually occurs after a latent period [2, 19–21]. Hence, testing the efficacy of promising intervention strategies applied shortly after the injury has immense value. Particularly, grafting of neural stem/progenitor cells (NSCs) expanded from different brain regions has significance, as this strategy may curb epileptogenesis and prevent the development of chronic epilepsy and learning and memory dysfunction after an IPI. Furthermore, grafting of NSCs into the hippocampus and/or application of strategies that activate endogenous NSCs to produce new neurons in the hippocampus during chronic epilepsy may be useful for easing both chronic seizures and learning and memory impairments. In this review, we will discuss the current knowledge and prospects pertaining to stem cell-based therapies for TLE.

Behavior of Endogenous Hippocampal Stem Cells in TLE

The concept of neurogenesis in the adult brain is now widely accepted, particularly in the two neurogenic regions, the subventricular zone of the forebrain and the subgranular zone (SGZ) of the dentate gyrus (DG). Neurogenesis in the DG of the hippocampus occurs in a wide variety of species, which includes rodents [22, 23], tree shrews [24, 25], monkeys [25, 26], and humans [27]. Interestingly, a vast majority of proliferating cells in the hippocampus differentiate into neurons [23, 26, 28–30]. It is believed that a subset of glial-fibrillary acidic protein-expressing cells located in the SGZ and granule cell layer (GCL) are likely NSCs in this region [31–33]. Slow proliferation of these NSCs produces a pool of transit amplifying cells, which proliferate further and give rise to new neurons and glia. Newly differentiated granule cells migrate into the GCL, express the mature neuronal marker neuron-specific nuclear antigen (NeuN), grow dendrites into the molecular layer, and send axons into the CA3 region [32, 34, 35]. During this stage, immature neurons likely receive γ-amino butyric acid (GABA) mediated excitatory synaptic inputs [36–38]. However, major glutamatergic synaptic activation from perforant path afferents does not occur until new neurons are 2 or more weeks old [36, 37], which coincides with appearance of spines on dendrites of newly born neurons [39]. Thus, incorporation of newly born neurons in the DG to the functional hippocampal circuitry takes over 2 weeks after their birth. Over the last decade, there has been a lot of interest in identifying the contribution of dentate neurogenesis to the pathophysiology of TLE.

Extent of Dentate Neurogenesis During the Early Phases of TLE

Blumcke and colleagues [40], by evaluating the expression of nestin in the DG of young (<2 years old) TLE patients, reported more nestin$^+$ cells in the DG. An increased Ki-67 proliferation index and clusters of supragranular nestin$^+$ cells within the molecular layer of the DG were also observed. Furthermore, confocal studies revealed colocalization of nestin

with β-III tubulin, suggesting a neuronal fate for some of these nestin$^+$ cells. Thus, early onset of TLE in pediatric patients is likely associated with increased neurogenesis in the DG, which is consistent with a number of studies in animal models of TLE where SE was found to increase NSC proliferation and neurogenesis in the SGZ of the DG ([41–46]; Figure 1). Hippocampal injury inflicted by excitotoxins such as kainic acid also increases neurogenesis in the DG [47]. Hippocampal injury or SE induces an initial, transitory proliferative surge in the SGZ with the number of new neurons increasing several folds during the first few weeks after injury [44, 45, 48]. This may be due to the release of mitogenic factors from dying neurons, deafferented granule cells, and reactive glia, as several neurotrophic factors are upregulated in the hippocampus after seizures or excitotoxic injury [49–51]. It may also be due to increased levels of neuropeptide Y (NPY), because acute seizures or SE increase NPY expression, and NSCs responsive to NPY exist in the hippocampus [52–56]. Thus, acute seizures or any other IPIs in the hippocampus considerably increase dentate neurogenesis likely through a surge in proliferation of NSCs. However, some of the increase may also be due to an enhanced survival of newly formed granule cells.

Aberrant Migration of Newly Born Granule Cells After Status Epilepticus

In normal conditions, a vast majority of newly born neurons in the SGZ migrates into the GCL. However, in conditions such as SE, a large number of newly born neurons (i.e., granule cells) migrate away from the GCL into the dentate hilus ([45, 48, 57–63]; Figure 1). An elegant study by Parent and associates [58] reports appearance of chain-like progenitor cell formations extending into the hilus and molecular layer after the SE, suggesting that seizures alter migratory behavior of dentate granule cell precursors. Likewise, ectopic dentate granule cells were also found in the hilus and molecular layer of epileptic human DG [58]. Interestingly, ectopic granule cells in the dentate hilus exhibit several features of normal granule cells in the GCL, which comprise outgrowth of mossy fiber axons and incorporation of these axons into the pre-existing hippocampal circuitry [57, 60, 64] and standard granule cell membrane properties and firing behavior [60]. Nevertheless, extensive studies by Scharfman and colleagues have shown that ectopic granule cells exhibit some features that are inconsistent with normal granule cells in the GCL. These include an increased proportion of somatic and dendritic asymmetric (presumably excitatory) synapses, enhanced mossy fiber innervation, a distinct pattern of activation during spontaneous seizures, and the occurrence of spontaneous epileptiform bursts [57, 60, 64–66]. Recently, McCloskey and colleagues [65], by quantifying the population of ectopic granule cells at different times after pilocarpine-induced SE using immunostaining for Prox-1 (a marker of dentate granule cells [67]), demonstrated that the size of the hilar ectopic granule cell population after SE is substantial and stable over time. Additionally, correlation was found between the size of ectopic granule cell population and the frequency of behavioral seizures.

Thus, it appears that newly born granule cells that migrate into the hilus after the SE contribute to the development of chronic epilepsy. From this perspective, blocking the generation of ectopic granule cells following SE might be useful for thwarting the progression of SE into chronic epilepsy. Indeed, a study has tried to suppress the SE induced NSC proliferation in the DG with the antimitotic agent cytosine-β-D-arabinofuranoside (Ara-C) and evaluated the frequency of SRMS [68]. In this study, rats received continuous intracerebroventricular infusions of Ara-C or vehicle for 14 days from one day before the onset of SE. Rats were video monitored for ~12 hours per day from day 28 to day 34 after the SE. During this period, SRMS were observed in a majority of both vehicle-treated and Ara-C-treated rats; however, there was ~70% reduction in the frequency of SRMS and ~34% decrease in the duration of individual seizures in the Ara-C group. Interestingly, milder chronic epilepsy in Ara-C treated rats was associated with reduced density of ectopic granule cells in the hilus; however, the aberrant mossy fiber sprouting was unaffected [68].

These results support the idea that newly born granule cells that migrate into the hilus shortly after the SE contribute to the development of chronic epilepsy. However, there are some concerns. First, as analyses of SRMS were done very early (28 –34 days) after the SE, it is unknown whether beneficial effects of Ara-C would persist at later time points after the SE. Because in animal models of epilepsy SRMS are generally very robust at 2– 4 months post-SE [69], it is necessary to analyze the effects of Ara-C exposure during the early post-SE period on SRMS occurring at extended time points after the SE in future studies. Second, examining seizure frequency or duration in the pilocarpine model as an endpoint is difficult because these animals show rather diffuse damage and the SRMS may not arise from the hippocampus. Third, it is not clear whether the positive effects of Ara-C exposure are a result of decreased number of ectopic granule cells or decreased proliferation of glia after the SE. Fourth, it is unknown whether Ara-C treatment would block other epileptogenic changes that promote the development of chronic seizures. Fifth, Ara-C treatment may also suppress the compensatory neurogenesis in the GCL that might otherwise improve inhibition [70]. Thus, it remains to be seen whether complete elimination of aberrant neurogenesis after the SE would prevent the evolution of SE into chronic epilepsy.

Status of Dentate Neurogenesis During Chronic Epilepsy

In contrast to the increased neurogenesis observed in DG following SE or hippocampal injury, in conditions such as chronic epilepsy, DG neurogenesis declines considerably. This was evidenced by the following studies. First, Mathern and associates [71] showed that surgically resected hippocampi from children with frequent seizures exhibit decreased density of cells positive for polysialic acid neural cell adhesion molecule (a marker of newborn neurons) in the DG in comparison with age-matched autopsy cases. This suggested that severe seizures during early childhood are associated with decreased dentate neurogenesis. Second, Pirttila and colleagues [72, 73] report diminished dentate neurogenesis in a group of TLE patients with frequent seizures in comparison with control brains. In addition to seizures, decreased neurogenesis in these patients appeared to be associated with severe hippocampal damage, as also seen in an earlier study [74]. Third, a recent study by Fahrner et al. [75] suggests decreased dentate neurogenesis in the hippocampus of TLE patients based on decreased synthesis of mRNA for doublecortin (a marker of newly born neurons) and absence of cells positive for Ki-67 (a marker of proliferating cells).

Thus, chronic TLE in humans is associated with decreased DG neurogenesis. These results are supported by studies in kainate models of TLE [48]. By analyzing neurogenesis in two different kainate models of rat TLE at both early and delayed time points after SE and/or hippocampal injury, Hattiangady and colleagues [48] demonstrate that chronic TLE is associated with severely diminished addition of new neurons to the adult DG (Fig. 2). The overall reductions in the addition of new neurons to the chronically epileptic hippocampus ranged from 64% to 81% in comparison with the age-matched intact hippocampus. Interestingly, the overall decrease in neurogenesis was considerably greater in rats exhibiting more SRMS, suggesting that greater frequency of SRMS during chronic epilepsy is more detrimental to DG neurogenesis.

Potential Mechanisms of Decreased Neurogenesis During Chronic Epilepsy

It is generally perceived that increases in neurogenesis after SE or acute seizures are a result of neuronal activity-related enhancements in NSC proliferation [44, 45]. Consistent with this idea, a study by Deisseroth et al. [76] shows that increased excitatory stimuli act directly on hippocampal NSCs to favor neuron production. Likewise, a mild reduction in net hippocampal excitatory activity via diazepam administration reduces neuronal fate-choice of newly born cells, suggesting that excitation-neurogenesis coupling exists in adult

hippocampal NSCs [76]. However, the above mechanism is inconsistent with the decreased neurogenesis observed during chronic epilepsy. Since some studies link decreased neurogenesis in certain hippocampal injury models to inflammation [77, 78], one may presume that decreased dentate neurogenesis is linked to the increased number of activated microglia in the chronically epileptic hippocampus. Evaluation of microglia, however, reveals a minimal number of activated microglia during chronic epilepsy, underscoring that dramatically decreased neurogenesis during chronic epilepsy is not due to chronic inflammation [48]. Considering this, decreased neurogenesis during chronic epilepsy may be due to the following. First, it is likely that DG milieu during chronic epilepsy is not conducive for increased NSC proliferation. Indeed, the concentration of several NSC proliferation factors such as fibroblast growth factor (FGF)-2, insulin-like growth factor-1, and brain-derived neurotrophic factor (BDNF) [79, 80] decreases during chronic epilepsy [48, 51]. Second, it is possible that chronic epilepsy depletes the number of NSCs. However, a human study reports an increased expression of NSC marker Musashi-1 in adult epileptic hippocampal tissues obtained from TLE patients [81], suggesting that NSCs persist during chronic epilepsy; however, it is unknown whether these cells exhibit significant proliferation or progeny of these cells differentiate into mature neuronal phenotypes. Detailed studies in future on proliferation of NSCs, neuronal differentiation of newly born cells, and long-term survival of newly differentiated neurons during chronic epilepsy may address these questions.

Is There a Link Between Decreased Neurogenesis and the Pathophysiology of TLE?

Diminished dentate neurogenesis during chronic epilepsy might contribute to the persistence of seizures and learning and memory impairments. This is because one previous study suggests that ~14% of newborn neurons in the DG of young rats differentiate into inhibitory GABA-ergic basket cells [82]. If this finding were valid for new neurons born throughout adulthood, dramatically decreased neurogenesis during chronic epilepsy would result in minimal addition of new GABA-ergic basket cells to the DG circuitry, which may contribute to persistence of reduced inhibitory neurotransmission. This idea is consistent with the observations in animal models of TLE that chronic epilepsy is associated with both reduced inhibition of granule cells and reduced number of GABA-ergic basket cells in the DG [83–87]. In addition, a recent report by Jakubs and coworkers [70] reports that adult-generated hippocampal granule cells in a rat model of epilepsy display reduced excitatory synaptic input and decreased excitability, implying that their functional integration was adjusted to the prevailing functional state in the hippocampal network [88]. From this viewpoint, it appears that reduced neurogenesis during chronic epilepsy contributes to maintenance of DG hyperexcitability. Furthermore, as newly added granule cells get incorporated into circuits supporting spatial memory as they mature, it has been suggested that newly born neurons make a unique contribution to memory processing in the dentate gyrus and formation of the temporal clusters of long-term episodic memories [89–95]. In addition, animal models of depression evince that dentate neurogenesis responds to factors modulating depression such as stress or antidepressant treatment [96, 97], implying some rapport between extent of neurogenesis and mood. Therefore, it is possible that hippocampal-dependent cognitive deficits and depression observed during chronic epilepsy [98, 99] are linked at least partially to diminished dentate neurogenesis.

Potential of Endogenous Hippocampal Stem Cells for Treating TLE

Increased neurogenesis following SE leads to abnormal migration of a substantial number of newly born granule cells into the dentate hilus, where they exhibit spontaneous epileptiform bursts and a distinct pattern of activation during SRMS. There is also evidence that a greater number of ectopic granule cells in the hilus is associated with increased frequency of SRMS, and blocking the ectopic migration of granule cells shortly after the SE results in much

milder chronic epilepsy. However, rigorous and long-term studies are needed to ascertain whether complete elimination of aberrant neurogenesis after the SE would prevent the evolution of SE into chronic epilepsy. If successful, a new therapy for prevention of chronic epilepsy that ensues after SE or brain injury may be developed. In contrast to the scenario at early post-SE, chronic TLE is associated with dramatically declined production of new neurons in the adult DG. Because decreased neurogenesis may contribute to the persistence of seizures and impairments in learning and memory during chronic epilepsy, development of strategies that enhance the production of new neurons in the chronically epileptic hippocampus may be useful for treating chronic TLE. Nevertheless, it should be noted that chronic epilepsy is associated with persistent loss of significant fractions of several hippocampal cell types in addition to decreased neurogenesis, which may also affect learning ability. Additionally, a dramatic increase in the production of new neurons in conditions such as chronic epilepsy may be detrimental, as substantially increased neurogenesis observed after the initial SE (i.e., during the acute injury phase) has been found to be pathological and to promote abnormal hyperexcitability [60, 100–102]. Rigorous studies on animal models are needed to comprehend these issues and to develop potential neurogenesis-related therapies for chronic TLE.

Potential of Stem Cell Grafts for Treating TLE

Although the effects of fetal cell grafts have been studied extensively in animal models of TLE [85, 103–109], studies on the efficacy of stem cell grafts for treating TLE are very few hitherto. In the last few years, adult stem cell-, NSC-, and embryonic stem cell-based therapies have received considerable interest for treating a variety of neurodegenerative diseases [110–115]. Both NSC- and ESC-based therapies may be useful for treating chronic epilepsy because of their potential ability to replace neuronal populations that are lost during the course of the disease and to repair the disrupted circuitry. Alternatively, if these cells produce a large number of GABA-ergic inhibitory interneurons, they may be efficient for inhibiting seizure activity. Stem cell grafting may also be useful for cell-based delivery of antiepileptic or neuroprotective factors.

Prospective Donor Stem Cells for Grafting into the Hippocampus in TLE

Neural Stem/Progenitor Cells—Multipotent NSCs present in the developing and adult central nervous system (CNS) can be expanded in culture using mitogens of NSCs such as FGF-2 and epidermal growth factor (EGF). These cells can be maintained in an undifferentiated state or can be induced to differentiate into neurons, astrocytes, and oligodendrocytes [116–122]. Studies on FGF-2- and EGF-responsive NSCs have shown that these cells satisfy the criteria of regional NSCs [123, 124]. Thus, NSCs from different areas of the human CNS could potentially be harvested in culture for prolonged periods and used as a source of donor tissue for grafting in neurodegenerative diseases [125–128]. NSCs may also serve as donor cells for grafting in TLE. However, the functional recovery after grafting of NSCs into the injured hippocampus will depend on the behavior of grafted NSCs. It will be helpful if they differentiate into a large number of neurons specific to the lesioned site of grafting and facilitate the reconstruction of the disrupted circuitry. Alternatively, it may also be useful if they differentiate into GABA-ergic interneurons and establish connectivity with host hippocampal neurons exhibiting hyperexcitability or simply synthesize and secrete antiepileptic factors on a long-term basis.

In vitro proliferation and differentiation of NSCs from embryonic day 19 (E19) and postnatal hippocampi have been analyzed [120, 121]. Hippocampal NSCs proliferate in the presence of EGF and/or FGF-2 and form neurospheres in vitro. Proliferating cells within neurospheres are negative for markers of neurons and glia but express various markers of NSCs. Furthermore, some cells within neurospheres have self-renewal ability. NSCs from

hippocampus are multipotent, as they can give rise to neurons, astrocytes, and oligodendrocytes. Moreover, the progeny of hippocampal NSCs give rise to larger pyramidal-shaped neurons [120, 121], some of which resemble hippocampal CA3 pyramidal neurons. Only a fraction (~20%) of neurons in neurosphere cultures of E19 hippocampus are $GABA^+$, and CA3 pyramidal-like neurons lack GABA [120] (Fig. 3). Thus, NSCs from E19 hippocampus have the ability to give rise to both hippocampal pyramidal-like neurons and interneurons and hence appear ideal for grafting into the hippocampus in conditions such as TLE. On the other hand, analyses of NSCs isolated from anterior subventricular zone (aSVZ) reveal their preference for differentiating into GABA-ergic neurons (Fig. 3). Grafting of NSCs from aSVZ may also be useful for treating TLE, as depletion of GABA-ergic interneurons appears to be one of the reasons for hippocampal hyperexcitability in TLE, and replenishment of GABA-ergic interneurons through grafting likely strengthens hippocampal inhibition [83–87, 129]. However, considering the ineffectiveness of GABA mimetic drugs for controlling seizures in pharmacoresistant epilepsy [130], it remains to be seen whether furnishing GABA through addition of new inhibitory neurons to the hippocampal circuitry would be useful for restraining seizures on a long-term basis.

Embryonic Stem Cells—Unlike neural precursors and NSCs, embryonic stem cells are very promising in terms of providing an infinite supply of donor cells for grafting in neurodegenerative diseases. ESCs are derived from the inner cell mass of the developing blastocyst and hence are capable of generating every cell type present in the body. ESCs exhibit high levels of telomerase activity [131] and a short G_1 cell cycle checkpoint [132] and therefore exhibit proliferation ceaselessly in culture without losing their pluripotency [133]. However, ESCs may not be ideal for direct transplantation in an undifferentiated state into the brain because they are prone to uncontrolled proliferation and teratoma formation [134]. Therefore, it is necessary to generate cell lines that are committed to specific neural lineages from ESCs in culture prior to grafting. In this way, it is possible to tailor cell therapy from ESCs for specific neurodegenerative disorders. Indeed, recent in vitro studies have reported success in pushing the progeny of ESCs to differentiate into dopaminergic neurons [135–138], spinal motor neurons [139, 140], and oligodendrocytes [141]. Thus, ESCs are very promising as a source of initial donor tissue for generating a variety of neural precursors in culture for grafting in neurodegenerative conditions. However, it remains to be validated whether different types of hippocampal neurons could be generated from ESCs.

Effects of Grafting Human NSCs in a Pilocarpine Model of TLE

Chu and colleagues [142] (Table 1) examined the effects of grafting NSCs on SRMS in rats that underwent SE. Donor cells were β-galactosidase (β-gal) encoded human NSCs, and the grafting was performed just a day after the induction of SE via i.v. injection. Between 28 and 35 days after the SE, 87% of animals receiving no NSCs (i.e., epilepsy-only group) showed SRMS. In contrast, only 13% of animals receiving NSCs displayed SRMS. Furthermore, in comparison with the epilepsy-only group, animals receiving NSCs exhibited milder SRMS and smaller field excitatory postsynaptic potential amplitudes in the CA1 region following stimulation of Schaffer collaterals. Grafted cells positive for β-gal were observed in multiple regions of the brain, including the hippocampus. Investigation of phenotypes revealed that only a few cells derived from NSCs expressed markers of mature neurons. Even the cells that expressed these markers did not morphologically resemble any of the major hippocampal neuronal types. However, a fraction of β-gal^+ grafted cells coexpressed markers of interneurons such as GABA and parvalbumin (PV) in the hippocampus and the piriform cortex, suggesting that cells derived from NSCs differentiate into GABA-synthesizing cells.

Thus, suppression of chronic SRMS in animals receiving NSCs is not due to NSC-mediated replenishment of hippocampal cell layers. However, the results suggest that NSCs have the ability to differentiate into GABA-synthesizing cells following engrafting into the injured hippocampus. It is possible that introduction of new GABA-synthesizing cells decreases neuronal excitability in the injured hippocampus and thereby suppresses SRMS. Nevertheless, several issues are obscure. First, it is unclear whether beneficial effects mediated by NSCs at 28–35 days post SE would persist at extended time points after the SE. This is important because, in animal models of epilepsy, SRMS are generally very robust at 2–4 months after the SE [69]. Hence, to fully ascertain the efficacy of NSC administration for blocking the evolution of TLE after the SE, it is necessary to analyze the effects of NSC administration on a long-term basis using both behavioral measures as well as extensive electroencephalographic recordings. Second, it is imperative to examine whether NSC-derived cells expressing GABA and PV are indeed interneurons and whether these cells establish synaptic connectivity with hippocampal neurons exhibiting hyperexcitability. Additionally, it is uncertain whether cells coexpressing β-gal and markers of GABA are newly differentiated interneurons or represent existing host neurons that are fused with NSC-derived β-gal$^+$ cells. Thus, detailed long-term experiments are critical prior to the clinical application of NSC therapy for TLE.

Properties of Embryonic Stem Cell Grafts in the Epileptic Hippocampus

Recently, Ruschenschmidt and colleagues [143] (Table 1) examined characteristics of mouse embryonic stem cell-derived neural precursors (ESNs) transplanted into the hippocampi of chronically epileptic and sham-control rats. Most ESNs were found in clusters at the transplant site, although individual ESNs exhibited short-distance migration into the host tissue. However, grafted ESNs extended processes into the surrounding host brain tissue. Electrophysiological analyses of ESNs revealed their ability to generate action potentials and express voltage-gated Na+ and K+ currents, as well as hyperpolarization-activated currents. Furthermore, most ESNs received non-*N*-methyl-D-aspartate and $GABA_A$ receptor-mediated synaptic input. Interestingly, no obvious differences were found in the functional properties of ESNs between sham-control and pilocarpine-treated rats. Thus, after transplantation into the hippocampus of sham-control and chronically epileptic rats, ESNs display synaptic properties characteristic of neurons. These results are promising in terms of achieving appropriate survival of grafted ESNs in the chronically epileptic hippocampus. Striking invasion of host tissue by a dense plexus of graft-derived fibers appears useful for widespread delivery of inhibitory mediators to the host brain through grafts of ESNs. However, several issues remain to be addressed in future studies. These include whether or not the grafted ESNs have the ability (a) to survive for prolonged periods in the epileptic hippocampus, (b) to differentiate into functional principal hippocampal neurons and/or GABA-ergic interneurons, and (c) to suppress SRMS and learning and memory deficits observed during chronic epilepsy.

Survival and Differentiation of Hippocampal NSCs in Rat Models of TLE

Grafting studies were conducted to determine whether hippocampal NSCs could survive grafting into the lesioned hippocampus and give rise to multiple cell types [144] (Table 1). Proliferating NSCs in neurospheres were labeled with 5′-bromodeoxyuridine (BrdU), treated with BDNF (20 g/ml), and grafted stereotaxically into the lesioned CA3 region of the hippocampus at 4 days after kainic acid-induced injury, a model of TLE. Grafts were analyzed with immunostaining for BrdU, NeuN, GABA (a marker of inhibitory neurons), and S-100β (a marker of mature astrocytes). All grafts showed widespread dispersion of BrdU-labeled cells away from the transplant core (Fig. 4). Although most of the grafted cells were restricted to the lesioned CA3 region, some cells migrated into the dentate SGZ and GCL and differentiated predominantly into neurons (Fig. 4). Although a fraction of grafted

cells differentiated into neurons in the graft core (Fig. 4), a vast majority of graft-derived cells differentiated into S-100β^+ astrocytes (Fig. 5). Some of the graft-derived neurons expressed the inhibitory neurotransmitter GABA (Fig. 5). Thus, hippocampal NSCs placed into the hippocampus shortly after injury survive grafting and give rise to both neurons (including GABA-ergic neurons) and astrocytes. However, detailed long-term studies are necessary to assess their ability to prevent chronic epilepsy development after SE or brain injury. Recently, the efficacy of hippocampal NSCs for suppressing seizures was examined by grafting these cells into the hippocampi of rats exhibiting chronic TLE [145]. The results suggest that hippocampal NSC grafting is efficacious for reducing both frequency and intensity of SRMS in rats exhibiting chronic TLE (Table 1).

Overall Conclusions

There is no conclusive evidence in support of using stem cells for treating TLE hitherto. However, it is important to acknowledge that this field is still in infancy, and the initial studies are promising. Usefulness of four distinct stem cell-based approaches needs to be assessed rigorously in future using animal models of TLE for further advances in this field. The first approach should involve development of methods for testing whether inhibiting increased proliferation of hippocampal NSCs during the first few weeks following the SE would prevent the development of chronic epilepsy. Addressing this issue is important in light of studies suggesting that epileptic seizures such as SE not only increase dentate neurogenesis but also lead to abnormal migration of newly born granule cells into the dentate hilus, where they exhibit spontaneous epileptiform bursts and may contribute to the development of chronic epilepsy [57, 58, 60, 64, 65, 146]. However, it should be recognized that although the above approach is interesting to pursue, clinical application of this antiepileptogenic therapy for preventing TLE after IPI is complicated. This is because many patients with TLE do not seem to have any IPI, and many patients with IPIs (e.g., prolonged febrile seizures) do not progress into TLE [147–149]. Thus, predicting those that will benefit from early therapeutic interventions will be difficult.

The second approach should focus on developing strategies that activate endogenous NSCs in the chronically epileptic hippocampus to produce a large number of new neurons including GABA-ergic interneurons. This approach has significance because studies in both TLE and animals models of TLE suggest that chronic TLE is associated with dramatically declined production of new neurons in the adult DG. Decreased neurogenesis during chronic epilepsy may contribute to the persistence of seizures possibly due to decreased addition of new GABA-ergic interneurons. Likewise, impairments in learning and memory during chronic epilepsy may be due to participation of minimal or no new neurons in learning processes. Considering this, development of strategies that enhance the production of new neurons and facilitate the differentiation of new neurons into GABA-ergic neurons in the chronically epileptic hippocampus may be useful for treating seizures as well as learning and memory deficits in chronic TLE.

The third strategy should comprise rigorous analyses of the efficacy of grafts of NSCs or ESNs placed into the hippocampus after the onset of chronic epilepsy for suppressing seizures and learning and memory deficits. This is because the initial results of stem cell grafting studies in TLE models reported so far [142–145] are promising in terms of their short term survival and their effectiveness for reducing the frequency of seizures. However, it is important to critically examine whether grafting of NSCs or ESNs into the hippocampus during chronic epilepsy would lead to long-term survival of graft-derived cells, differentiation of graft-derived cells into functional principal hippocampal neurons and/or GABA-ergic interneurons, long-term suppression of SRMS, and improvements in learning and memory deficits. In view of findings that delivery of anticonvulsant compounds such as

NPY, glial-derived neurotrophic factor, and adenosine is efficacious for reducing seizures in animal models of TLE [150–152], a fourth approach would be to try a combination therapy comprising NSC/ESC cell transplants and cell or recombinant viral vector-based delivery of anticonvulsant compounds into the hippocampus during chronic epilepsy. This strategy might turn out to be very effective, as seizure control would likely be mediated by both GABA-ergic interneurons derived from NSC/ESC transplants and anti-convulsant compounds released by genetically engineered cells or host cells infected with viral vectors encoding these compounds.

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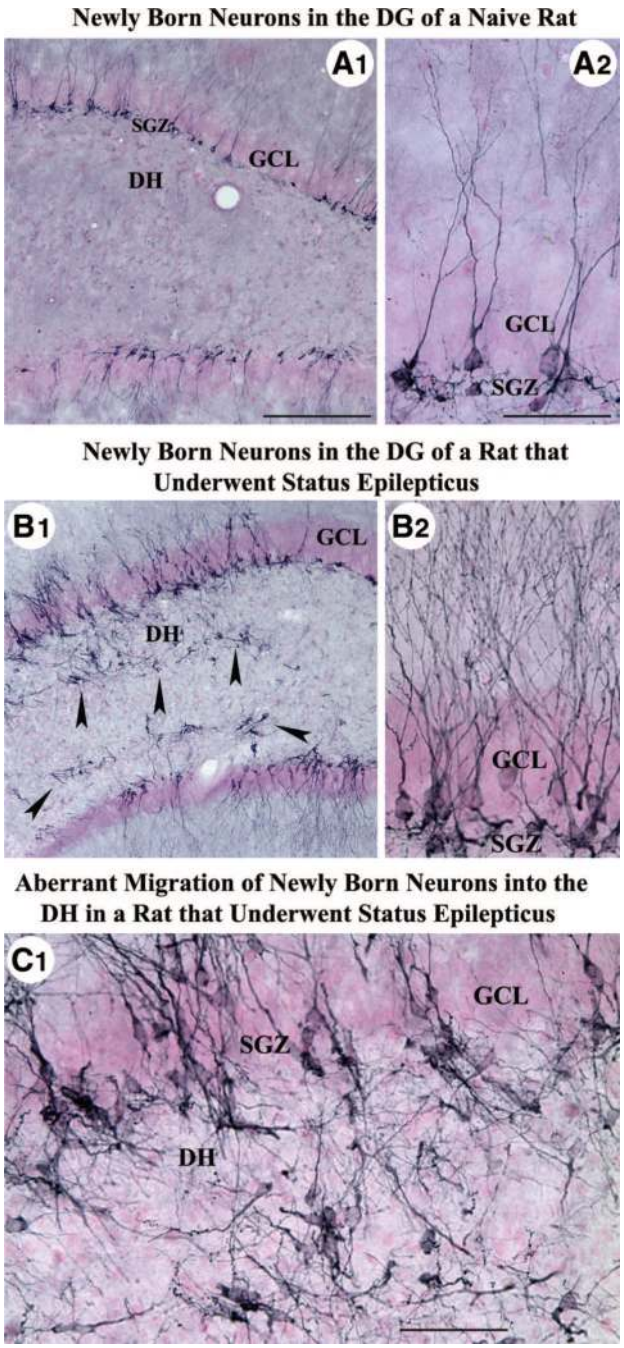

Figure 1.

Changes in dentate neurogenesis following kainic acid-induced status epilepticus. Newly born neurons in the DG of a naïve adult rat **(A1)** and an adult rat that underwent status epilepticus 12 days prior to euthanasia **(B1)** were visualized with immunostaining for doublecortin, a marker of newly born neurons. **(A2)** and **(B2)** show magnified views of regions of dentate gyrus from **(A1)** and **(B1)**, respectively. Note that, in comparison with the dentate gyrus of a control rat **(A1, A2)**, a rat that underwent status epilepticus **(B1, B2)** exhibits considerably increased density of doublecortin$^+$ new neurons and abnormal migration of newly born neurons into the dentate hilus (indicated by arrowheads in **[B1]**). **(C1)** is a magnified view of a region from **(B1)** showing aberrantly migrated newly born

neurons in the dentate hilus. Scale bar **(A1, B1)** = 200 μm; **(A2, B2, C1)** = 50 μm. Abbreviations: DG, dentate gyrus; DH, dentate hilus; GCL, granule cell layer; SGZ, subgranular zone.

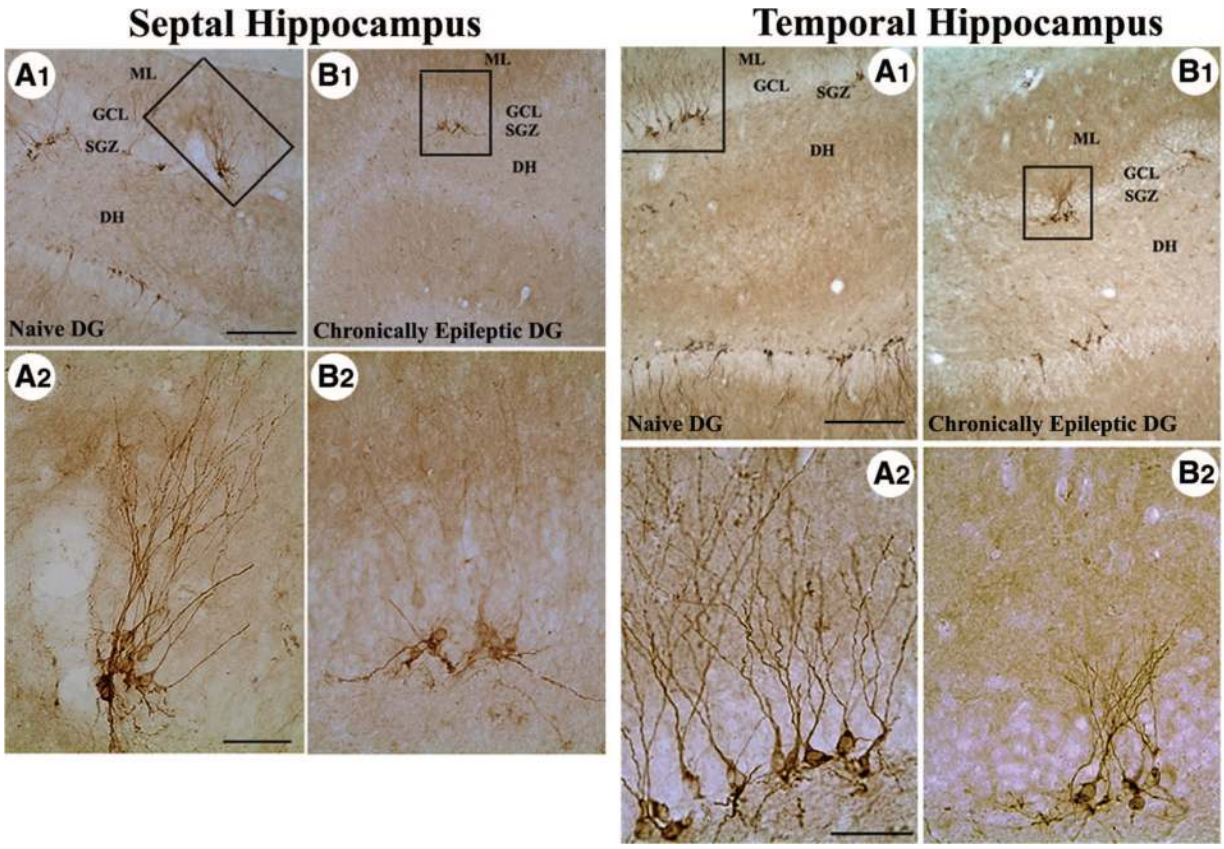

Figure 2.

Distribution of doublecortin-immunopositive (newly formed) neurons in the septal and temporal regions of the chronically epileptic hippocampus. (**A1):** Dentate gyrus of a naïve, age-matched control rat. **(B1):** Dentate gyrus of a chronically epileptic rat at 5 months post-kainic acid administration. Left panel, septal hippocampus; right panel, temporal hippocampus. **(A2)** and **(B2)** are magnified views of boxed regions in **(A1)** and **(B1)**. Note that the number of newly formed neurons is much less in the chronically epileptic dentate gyrus in comparison with the age-matched naïve dentate gyrus. In addition, unlike the newly formed neurons with extensive vertically oriented dendrites in the naïve dentate gyrus, newly formed neurons in the chronically epileptic dentate gyrus predominantly exhibit immature horizontally oriented or basal dendrites and less extensive vertical dendrites. Scale bar **(A1, B1)** = 200 μm; **(A2, B2)** = 50 μm. Figure reproduced from Hattiangady et al. [48]. Abbreviations: DG, dentate gyrus; DH, dentate hilus; GCL, granule cell layer; ML, molecular layer; SGZ, subgranular zone.

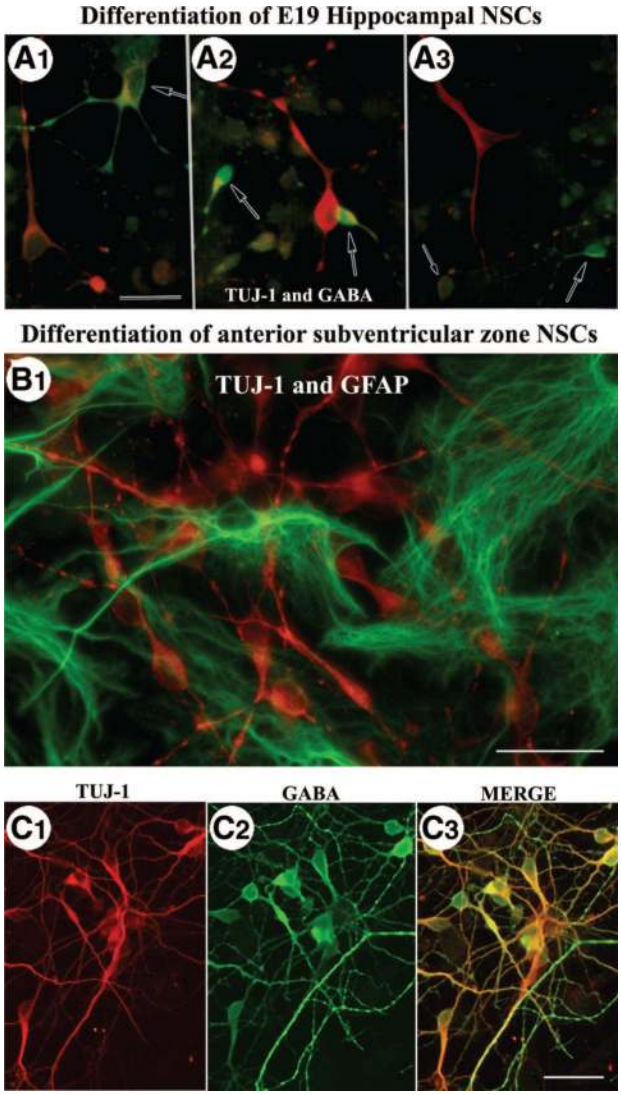

Figure 3.

NSCs isolated from embryonic day 19 hippocampus differentiate into both larger pyramidal shaped cells that are immunopositive for TuJ-1 but lack GABA (presumably hippocampal pyramidal neurons) and interneuron-like cells that are immunopositive for both TuJ-1 and GABA (presumably GABA-ergic interneurons). **(A1)** shows a larger multipolar GABA immunopositive neuron (yellowish green, indicated by arrow), whereas **(A2)** and **(A3)** show smaller GABA immunopositive neurons (arrows). Note that larger neurons resembling CA3 pyramidal neurons are immunopositive for TuJ1 (red) but negative for GABA. In contrast, GABA-ergic neurons are positive for both GABA and TuJ1 and hence exhibit yellowish green color in double exposure photography. Figure reproduced from Shetty [120]. Middle panel: **(B1)** shows differentiation of NSCs isolated from the anterior subventricular zone into TuJ-1 positive neurons (red) and astrocytes (green). Lower panel: **(C1–C3)** illustrate differentiation of neurons derived from anterior subventricular zone NSCs into GABA-ergic cells. Scale bar = 25 μm. Abbreviations: E19, embryonic day 19; GABA, γ-amino butyric acid; GFAP, glial-fibrillary acidic protein; NSCs, neural stem/progenitor cells.

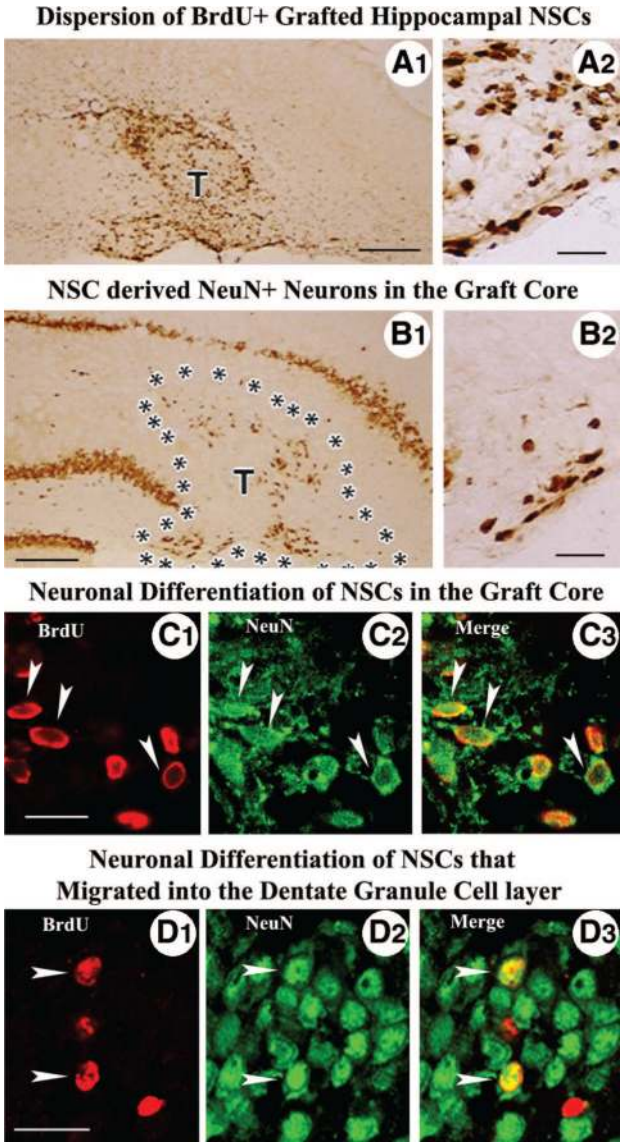

Figure 4.

Dispersion and differentiation of BrdU-labeled NSCs isolated from the embryonic day 19 hippocampus following grafting into the lesioned hippocampal CA3 region of a kainic acid-treated rat. Note that cells derived from NSC grafts disperse extensively in the hippocampus **(A1)**, and the graft core contains a significant number of NeuN immunopositive neurons **(B1)**. **(A2)** and **(B2)** are magnified views of graft regions from **(A1)** and **(B1)**. Panels **(C1–C3)** illustrate differentiation of $BrdU^+$ NSCs into NeuN immunopositive neurons in the graft core (arrowheads), whereas panels **(D1–D3)** demonstrate neuronal differentiation of $BrdU^+$ NSCs that migrated into the dentate granule cell layer (arrowheads). Scale bar **(A1, B1)** = 200 μm; **(A2, B2)** = 30 μm; **(C1–D3)** = 20 μm. Abbreviations: BrdU, 5′-bromodeoxyuridine; NeuN, neuron-specific nuclear antigen; NSCs, neural stem/progenitor cells; T, transplant.

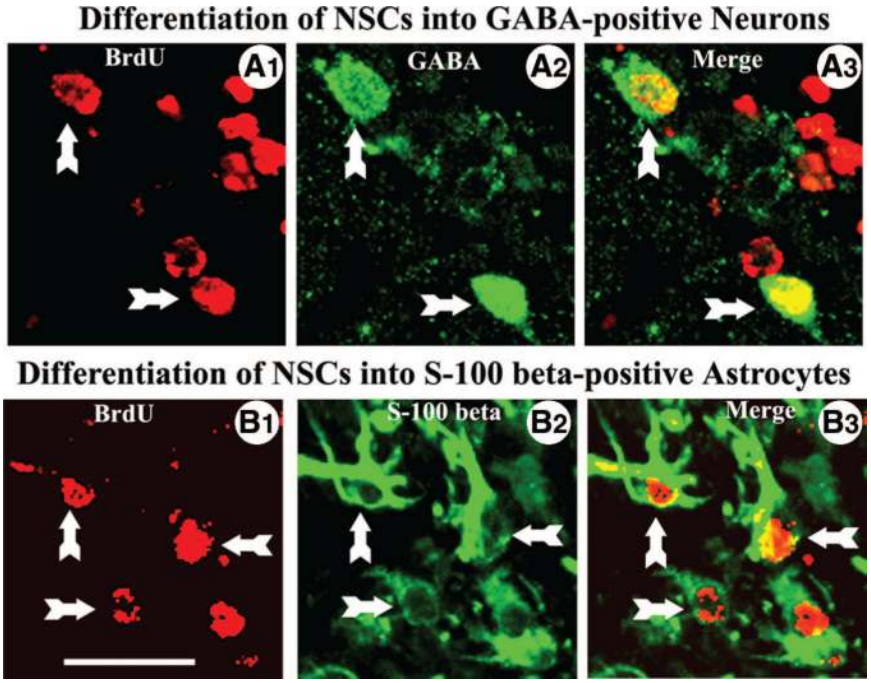

Figure 5.

Differentiation of grafted BrdU-labeled hippocampal neural stem/progenitor cells into GABA$^+$ neurons and S-100β^+ mature astrocytes following grafting into the lesioned hippocampal CA3 region of a kainic acid-treated rat. **(A1–A3)** demonstrate GABA$^+$ neurons derived from grafted NSCs and **(B1–B3)** show S-100β^+ astrocytes derived from grafted NSCs. Scale bar = 20 μm. Abbreviations: BrdU, 5′-bromodeoxyuridine; GABA, γ-amino butyric acid; NSCs, neural stem/progenitor cells.

Table 1

Stem cell transplantation studies in epilepsy models

Study	Cell type and source	Animal model	Timing of grafting	Route/site of grafting	Therapeutic outcome	Survival and differentiation
Chu et al. [142]	NSCs from VZ of 15 weeks gestation human brain	Pilocarpine model of rat TLE	1 day after SE	Intravenous injection of NSCs into tail vein	Reduced frequency of SRMS at 28-35 days post-SE	Extent of survival unknown Some cells differentiated into GABA-ergic neurons in the hippocampus, amygdala, and piriform cortex
Ruschenschmidt et al. [143]	Mouse embryonic stem cell-derived neuronal precursors[a]	Pilocarpine model of rat TLE	1 month after SE	Intracerebral grafting into hippocampi	Not analyzed	Survival not assessed quantitatively Functional properties of grafted cells were similar to grafted cells placed into the hippocampus of sham-control rats
Shetty and Hattiangady [144]	Hippocampal NSCs from rat pups	ICV KA model of rat TLE	4 days after KA lesion	Intracerebral grafting into hippocampi	Not analyzed	Survival excellent Differentiation into neurons and astrocytes observed. Some grafted cells differentiated into GABA-ergic neurons
Acharya et al. [145]	Hippocampal NSCs from rat pups	IP KA model of rat TLE	4 months after KA induced SE	Intracerebral grafting into hippocampi	~50% reduction in the frequency of spontaneous seizures	Survival excellent Differentiation into neurons rarely observed. Majority of grafted cells differentiated into astrocytes

Abbreviations: GABA, gamma-amino butyric acid; ICV, intracerebroventricular; IP, intraperitoneal; KA, kainic acid; NSCs, neural stem cells; SE, status epilepticus; SRMS, spontaneous recurrent motor seizures; TLE, temporal lobe epilepsy; VZ, ventricular zone.

[a]Embryonic stem cell-derived neuronal precursors were genetically engineered to express enhanced green fluorescent protein under control of the tau promoter.