Translating regenerative medicine
techniques for the treatment of
epilepsy

Takao Yasuhara, Isao Date, M. Grant Liska, Yuji Kaneko, Fernando L. Vale

Abstract:
Epilepsy is considered a chronic neurological disorder and is accompanied by persistent and diverse disturbances in electrical brain activity. While antiepileptic pharmaceuticals are still the predominant treatment for epilepsy, the advent of numerous surgical interventions has further improved outcomes for patients. Despite these advancements, a subpopulation continues to experience intractable seizures which are resistant to current conventional and nonconventional therapeutic options. In this review, we begin with an introduction to the clinical presentation of epilepsy before discussing the clinically relevant laboratory models of epilepsy. Finally, we explore the implications of regenerative medicine – including cell therapy, neuroprotective agents, and electrical stimulation – for epilepsy, supplemented with our laboratory's data. This paper is a review article. Referred literature in this paper has been listed in the references section. The datasets supporting the conclusions of this article are available online by searching various databases, including PubMed. Some original points in this article come from the laboratory practice in our research center and the authors' experiences.

Keywords:
Central nervous system disorders, electrical stimulation, epilepsy, neurogenesis, neuroprotective agents, regenerative medicine, stem-cell therapy

Introduction

Defined by the existence of various irregularities in brain electrical activity (seizures), epilepsy is a chronic neurological disorder which affects a diverse population of patients. Antiepileptic drugs are the initial treatment option for patients with epilepsy, yet approximately 20%–40% of patients display “refractory” epilepsy and do not respond favorably to these pharmaceuticals. Excision of causative tissue may be a viable treatment modality for patients with temporal lobe epilepsy (TLE) – the most common form of epilepsy – accompanied by mesial temporal sclerosis, or for patients with lesion-induced epilepsy. Alternatively, patients who exhibit refractory epilepsy and are not candidates for surgical intervention may benefit from alternative therapies such as electrical stimulation, magnetic stimulation, adrenocorticotropic or immunoglobulin medications, ketogenic diet, and psychobehavioral therapy. Even with various, patient-specific combinations of the aforementioned treatment modalities, many patients remain encumbered by persistent epileptic seizures, emphasizing the need for innovative therapeutic strategies to treat epilepsy. One such novel intervention strategy which has been proposed is stem-cell therapy. Induced pluripotent stem cells (iPSC) present a promising candidate donor cell for transplantation, yet critical concerns related to their tumorigenicity and irregular electrical activity intrinsic to the epileptic patient-derived cell should first be addressed. Interestingly, iPSCs harvested from epileptic patients may be valuable in identifying new pathological mechanisms and treatment targets for this disease.
Here, we review relevant rodent models of epilepsy and discuss current progress in the treatment of epilepsy, with a focus on regenerative therapies including cell therapy, neuroprotection, and electrical stimulation. Moreover, due to its prevalence, TLE will receive the majority of our attention.

**Murine Modeling of Epilepsy and Epileptogenesis**

Two traditional explanations exist for the development of epilepsy – the recurrent excitation hypothesis and the recurrent inhibition hypothesis. Recurrent excitation hypothesis proposes that seizures originate from abnormal excitatory circuitry mainly induced by mossy fiber sprouting, resulting in hyperexcitability of dentate granule cells. Alternatively, the recurrent inhibition hypothesis holds that epileptic foci in the dentate granule cells result from a lack of inhibitory input. Importantly, both hypotheses have displayed merit and have received support from various studies. In either case, epileptogenesis is thought to involve three progressive stages: an initial insult which precipitates pathological alterations, a latent asymptomatic period, and a chronic symptomatic phase. Rodent models of epilepsy have been widely utilized in the characterization of TLE pathogenesis, and other subcategories of epilepsy. Many of the pathological symptoms which occur in epileptic patients are reproduced in TLE models, such as loss of inhibitory gamma-aminobutyric acid (GABAergic) interneurons, formation of abnormal neuronal circuit, loss of excitatory neurons in discrete hippocampal regions, changes in expression of multiple receptors/ion channels, and a consequential hyperexcitability due to the loss of excitation/inhibition balance.

In the quest to characterize underlying mechanisms of TLE, various techniques have been employed including hyperthermia, trauma, hypoxia for newborn animals, chemo convulsants, electrical stimulation, tetanus toxins, and genetic manipulations. Among the two most established models of TLE are the poststatus epilepticus model and kindling model. Status epilepticus, in humans, describes an acute, prolonged (5+ min) epileptic crisis; this if often accompanied by additional seizures after a latency period. Poststatus epilepticus models use a severe insult, such as those induced by the chemoconvulsants kainic acid (KA) or pilocarpine, to mimic status epilepticus and provoke periodic seizures within the following weeks. Thereafter, the periodic seizures can be tracked, and therapeutic interventions probed for beneficial effects. Both intrahippocampal and intraperitoneal administration of KA have been used to induce TLE models, and pose distinct advantages/disadvantages; systemic administration is convenient but highly toxic, resulting in increased specimen mortality while intrahippocampal delivery requires invasive surgery, yet has a lower mortality rate.

These chemoconvulsant models of TLE are valuable, but kindling models offer a distinct advantage due to their high success rate, lower mortality rate, and recapitulation of clinical symptomology. Kindling models use mild, recurrent electrical stimulation of the perforant pathway to target brain regions commonly responsible for clinical epileptogenesis, including the hippocampus, amygdala, and other causative loci. Important for the ongoing progression of epileptic research is a thorough understanding of the benefits and drawbacks of each model. Indeed, many of the epileptic mechanisms and therapeutic strategies discussed here were uncovered by the appropriate utilization of these models.

**Cell Therapy for Treating Epilepsy**

In an attempt to substitute the damaged/irregular hippocampal neurons, a number of transplantable cell types have been examined in epilepsy models including fetal hippocampal cells, neuronal precursor cells from medial ganglionic eminences, and various neural stem cells (NSCs). Embryonic stem cell-derived GABAergic neurons displayed notable efficacy in replacing GABAergic neurons of epileptic animals. Similarly, GABAergic inhibitory interneurons transplanted into the hippocampus moderated epileptic hyperactivity, seizures, and additional abnormal behavioral metrics.

This was in contrast to transplantation of the same cells into the basolateral amygdala, which rescued the hyperexcitability deficit but afforded no favorable effects on seizure activity. These findings shed light on possible mechanisms of epileptogenesis and also indicate inhibitory interneurons as potential therapeutic targets. In addition to direct cell replacement, stem cell transplantation may mediate functional benefits in epilepsy by targeting various pathogenic mechanisms through antithetical therapeutic pathways – promoting angiogenesis, synaptogenesis, neurogenesis, and anti-inflammatory effects to rescue/preserve damaged hippocampal tissue.

Our laboratory investigated the effects of intrahippocampal transplant of adult-derived NSCs in a KA-induced model of epilepsy. Two weeks following intraventricular KA infusion, transplanted rats displayed a reduction in abnormal electrical activity, as measured by electrophysical recording. Furthermore, graft survival was detected in the CA3 region 5 weeks posttransplantation, with signs of migration into the subgranular zone. The majority of transplant cells expressed GFAP (a marker of astrocytic phenotype), yet a subpopulation of transplant cells expressed Neuronal Nuclei (NeuN) (a phenotypic marker of mature neurons); these
findings were accompanied by immunohistochemical evidence showing normalization of abnormal mossy fiber sprouting and a preservation of GABAergic inhibitory neurons.[27] In other studies, transplanted NSCs have been found to secrete important trophic factors such as stem cell factor which may have further contributed to the therapeutic effects reported.[28] Stem/progenitor cells positive for cellular kit (c-kit), a receptor for stem cell factor, confer neuroprotection by increasing astrocytic glutamate transporter GLT1 and consequently reducing extracellular glutamate.[29] Moreover, NSCs may promote neurogenesis of endogenous stem cells which contribute to the reparative effort.[30] These various therapeutic effects of NSC transplantation may work cooperatively to promote the survival of damaged cells in the epileptic brain and moderate the hyperactive, abnormal neural circuitry.

The secretion of various neurotrophic/neuroprotective factors by stem cells has been recognized as a leading mechanism of transplantation therapy.[31] With this in mind, our laboratory has developed an interest in investigating the effects which encapsulated stem cell transplantation have in the epileptic brain. Beyond allowing researchers to focus on the secretory mechanisms of stem cell therapy, stem cell encapsulation is associated with a number of distinct, clinically-relevant advantages; physical barriers prevent tumorigenesis while still allowing the cells to receive nutrients from the surrounding environment through the semipermeable membrane. The membrane also allows the distribution of secreted trophic factors which can exert therapeutic effects on the surrounding tissue. Cells which are encapsulated can be tailored or modified to secrete specific therapeutic factors or lack replicative senescence. Encapsulation also prevents immunologic rejection by host defenses, as the capsule hinders immunocompetent cells from accessing transplant epitopes. Due to this immune privilege, a more diverse array of transplantable stem cell types are safe and viable, including xenografts and conventionally immunogenic cell types. The secretion of trophic factors directly from encapsulated cells may be superior to direct trophic factor infusion through a mini-pump system, as the degradation of these proteinaceous molecules is reduced and dynamic host-graft interactions are still permitted with encapsulation. Chief among the stem cell donor sources which have been demonstrated to exhibit potent secretory effects are mesenchymal stem cells (MSCs) and umbilical cord blood cells.[32,33]

Nonencapsulated stem cell transplantation has also been tested in epilepsy models; genetically-modified MSCs which were prompted toward an inhibitory GABAergic phenotype amened functional deficits in a pilocarpine model of epilepsy.[34] Another study demonstrated the efficacy of intravenous mononuclear MSCs in ameliorating pilocarpine-induced epileptic activity.[35] In both studies, MSCs transplantation was associated with neuroprotection and neurorestoration mediated by anti-inflammatory effects.[26,34] In rats receiving MSCs 3 weeks following pilocarpine injections, the number of doublecortin + neuronal precursor cells decreased.[34] As an increase in abnormal neurogenesis is associated with acute phase epileptogenesis, this reduction in neurogenesis can be interpreted as a positive outcome. Rats receiving MSCs 10 months after pilocarpine injection, however, displayed an increase in doublecortin + neuronal precursor cells; the neuronal death associated with chronic epilepsy pathology suggests that the increase in neurogenesis at this later time point could be a positive indication for the restorative capacity of MSC therapy. Importantly, the necessity of this dynamic downregulation then upregulation of neurogenesis emphasizes the importance for cellular crosstalk between the epileptic brain and transplanted cells. The details of this relationship between neurogenesis and epilepsy development/progression are still shrouded in controversy regarding its therapeutic and pathological implications.[35] Moreover, the role of environmental queues, the timing of the neurogenesis-to-neurodegeneration transition, and its corresponding processes are yet to be established.

The advent of iPSCs and their potential within nervous system diseases has incited a burgeoning sub-field of research within the regenerative medicine community.[36] Notably, methods have been described for prompting human iPSCs into a primitive neural stem cell phenotype within 7 days.[37] Thereafter, it was shown that these induced NCs could differentiate into specialized subtypes of neurons such as motor neurons, dopaminergic neurons, and GABAergic neurons.[37] Thus, these induced stem cells could present a promising and versatile donor source for the treatment of epilepsy. As mentioned previously, isolating stem cells from diseased patients could be a valuable tool in characterizing the causative pathological mechanisms of epilepsy, and in developing novel drug targets. The potential to develop in vitro epilepsy models amenable to pharmaceutical screening assays has been demonstrated. After developing iPSCs from skin fibroblasts of patients with Dravet syndrome – an infantile-onset epileptic condition – iPSCs were differentiated into neurons which assumed predominantly GABAergic phenotypes, with electrophysiological characteristic consistent with neurons derived from murine epilepsy models.[38] Complimenting this, an in vitro model established by similar protocol was responsive to the common anti-epileptic drug phenytoin (Dilantin), replicating the therapeutic response in vitro which is observed in humans.[39] Together, these studies demonstrate a powerful research opportunity: establishing an in vitro
Neuroprotective Agents for Treating Epilepsy

A variety of neurotrophic factors have been vetted as potential therapeutic options for the treatment of TLE. Overexpression of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor within the hippocampus lessened cell death, increased neurogenesis, and provided anti-inflammatory effects in a pilocarpine-induced status epilepticus model. When insulin-like growth factor-1 (IGF-1) was coadministered with KA in a chemoconvulsant model of TLE, IGF-1 mice displayed a reduction in hippocampal neurogenesis (a favorable outcome, given the acute phase measurement), a decrease in seizure activity, downregulation of cellular-level neurodegenerative markers, and improvement in cognitive metrics. Innovative growth factor-based therapies also include modulating the mammalian target of rapamycin (mTOR) signaling pathway, which has been implicated in pharmacological hindering of epileptogenesis.

Discrepancies exist in the literature regarding the appropriateness of BDNF in treating epileptic conditions; when BDNF interacts with the tropomyosin receptor kinase B (TrkB) receptor, the downstream signaling pathway may promote epileptogenesis. Furthermore, analysis of mossy fiber pathways in the hippocampus reveals that seizures are associated with a drastic upregulation of BDNF and an increase in BDNF-TrkB signaling. Supporting this harmful role, intraventricular administration of BDNF at either 1 or 3 μg/h for 7 days provoked spontaneous seizures while overexpression of BDNF worsened already-present seizure activity. Finally, matrix metalloproteinase-9, which promotes the conversion of pro-BDNF to BNF, has been revealed to facilitate epileptogenesis. Conversely, certain studies have found anti-epileptogenic effects of BDNF treatment. Our investigations have found that continuous low-dose (200–300 pg/h) BDNF administration through encapsulated BDNF-secreting cells exerted anti-epileptic effects. Outcome measures verified behavioral and electrophysiological ameliorations in rats receiving BDNF treatment. Immunohistochemical analysis showed an increase of neuronal precursor cells (doublecortin+) within the dentate gyrus and a preservation of mature neurons (NeuN+) in the CA1 and CA3. Other studies support the notion that continuous low-dose BDNF may attenuate epileptic activity by increasing neuropeptide Y (NPY) expression. Apparent from these studies is the importance of dosing and timing in the therapeutic usage of BDNF, particularly considering the BDNF upregulation seen in epileptic hippocampi.

Erythropoietin (EPO) is a well-characterized and widely-studied hormone which has the capacity for neuroprotection in diverse diseases of the central nervous system, such as ischemic stroke and Parkinson’s disease. A number of studies have evaluated EPO for therapeutic effects in the epileptic brain. EPO conferred anti-epileptic effects in a model of febrile seizures by dampening postseizure inflammation and through molecular regulation, rescuing numerous seizure-induced molecular alterations. Using a KA-induced epilepsy model, our laboratory reported that intraventricular infusion of EPO reduced mortality and improved behavioral metrics. Furthermore, histological data showed a preservation of NeuN+ mature neurons in the CA1 region and a suppression of abnormal neurogenesis. Importantly, administration of an NPY Y2 receptor antagonist negated the therapeutic efficacy of EPO, indicating NPY’s role in the therapeutic effects exerted by EPO. Further, recent evidence was provided demonstrating the neurogenic and neuroprotective effect exerted by intraventricular NPY infusion in an epilepsy model. Our laboratory found that adjunctive treatment of EPO with NSCs in a KA-induced model of epilepsy significantly increased the survival rate of NSCs and drastically decreased mossy fiber sprouting compared to all other groups. Another study by our group found that infusion of carbamylated EPO Fc fusion protein conferred robust neuroprotective effects, yet without hematopoietic effects, in a model of Parkinson’s disease. Our group proposes that EPO may be a novel therapeutic agent for the treatment of epilepsy.

Electrical stimulation for treating epilepsy

Electrical stimulation has a long history within the clinic for treatment of epilepsy and other neurological conditions, yet its use has not been optimized. While the safety of electric stimulation is largely undisputed, with vagus nerve stimulation being employed regularly for refractory epilepsy, treatment is often expensive and the efficacy is variable. Vagus nerve stimulation may exert therapeutic effects to multiple regional structures which surround the stimulated tissue including the locus ceruleus and raphe nuclei. In the kindling model of epilepsy, vagus nerve stimulation slowed the rate of hyperpolarization in cerebral cortex neurons, and elevated the seizure threshold. In addition,
electrical stimulation for two consecutive days caused nearly 50% increase in the number of hippocampal BrdU + cells, whereas stimulation for 1 month incited morphological evidence of newly formed neurons and BDNF upregulation in the CA3. These investigations support the notion that electrical stimulation may counteract epileptic aberrations through cellular reorganization and neurotrophic mechanisms within the causal brain loci. Other stimulation modalities, including epidural stimulation, deep brain stimulation, and transcranial magnetic stimulation, have been explored for the treatment of epilepsy, but with inconsistent or controversial findings. In one of our laboratory studies using an amygdala-kindling model of epilepsy, chemical suppression of the anterior thalamic nucleus was effective in reducing behavioral dysfunction and neurogenic abnormalities of the hippocampus. This implies that the anterior thalamic nucleus may be a beneficial target for electrical stimulation, being that its chemical suppression reduced off-target seizure activity. Brain regions such as the cerebellum, cerebral cortex, substantia nigra pars reticula, and subthalamic nucleus may also be targets responsive to electrical stimulation.

Using a stroke model, we recently provided data for the neuroprotective and neuroregenerative effects of epidural and deep brain stimulation, which prompted angiogenesis, neurogenesis, anti-inflammatory effects, anti-apoptotic effects, and an upregulation of trophic factors. We demonstrated that electrical stimulation, particularly at low frequencies, conferred therapeutic benefits. In line with this, electrical stimulation of the spinal cord in Parkinson’s disease increased neuroplasticity, presenting itself as a possible alternative treatment option. While debate persists as to the efficacy and mechanisms of electrical stimulation, a substantial body of positive preclinical data merits ongoing investigations into its applications in epilepsy. Improving our understanding of the mechanisms which underlie epileptic pathology will compliment and facilitate the search for novel therapeutic regimens.

Conclusions

Current treatment options for epilepsy include anti-epileptic medications and traditional treatment modalities such as surgical intervention and electrical stimulation. However, the need for new treatment options for patients with refractory epilepsy still exists. Critical to the progress of epilepsy research has been the establishment of standardized models, highlighting the importance which basic science research plays in improving patient outcomes. Moreover, translational research efforts are critical in determining safety profiles and efficacy readouts for promising therapeutic options. Looking forward, regenerative medicine is an exciting frontier for epilepsy, providing opportunities for innovative treatment options and new tools for the exploration of disease mechanisms and pathology. Our laboratory endorses the concept that basic and translation research efforts into cell therapies, neuroprotective agents, electrical stimulation, and other regenerative tools are worthy endeavors in the quest to find effect means of managing epilepsy.

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Conflicts of interest

There are no conflicts of interest.

References

1. Hirose G. An overview of epilepsy: Its history, classification, pathophysiology and management. Brain Nerve 2013;65:509-20.
2. Brodie MJ. Road to refractory epilepsy: The glasgow story. Epilepsia 2013;54 Suppl 2:5-8.
3. Health Quality Ontario. Functional brain imaging: An evidence-based analysis. Ont Health Technol Assess Ser 2006;6:1-79.
4. Sørensen AT, Kokaia M. Novel approaches to epilepsy treatment. Epilepsia 2013;54:1-9.
5. Cendes F. Epilepsy in 2011: Insights into epilepsy treatments and biomarkers. Nat Rev Neurol 2012;8:270-1.
6. Jang J, Yoo JE, Lee JA, Lee DR, Kim JY, Huh YJ, et al. Disease-specific induced pluripotent stem cells: A platform for human disease modeling and drug discovery. Exp Mol Med 2012;44:202-13.
7. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-72.
8. Kang HC. Disease-specific pluripotent stem cells. Korean J Pediatr 2010;53:246-9.
9. Sharma AK, Reams YJ, Jordan WH, Miller MA, Thacker HL, Snyder PW, et al. Mesial temporal lobe epilepsy: Pathogenesis, induced rodent models and lesions. Epilepsia 2011;52:984-99.
10. Scharfman HE. The neurobiology of epilepsy. Curr Neurol Neurosci Rep 2007;7:348-54.
11. Maguire J. Epileptogenesis: More than just the latent period. Epilepsy Curr 2016;16:31-3.
12. Shetty AK. Progress in cell grafting therapy for temporal lobe epilepsy. Neurotherapeutics 2011;8:721-35.
13. Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, et al. Animal models of epilepsy: Use and limitations. Neuropsychiatr Dis Treat 2014;10:1693-705.
14. Lowenstein DH, Allredge BK. Status epilepticus. N Engl J Med 1998;338:970-6.
15. Scorza FA, Arida RM, Naffah-Mazzocoratti MdA G, Scerni DA, Calderazzo L, Cavalheiro EA, et al. The pilocarpine model of epilepsy: What have we learned? An Acad Bras Cienc 2009;81:345-65.
16. Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods 2008;172:143-57.
17. Lüscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 2002;50:105-23.
18. Anderson SA, Baraban SC. Cell therapy using GABAergic neural

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progenitors. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies. [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2012.

19. Gallego JM, Sancho FJ, Vidueira S, Ortiz L, Gómez-Pinedo U, Barcia JA, et al. Injection of embryonic median ganglionic eminence cells or fibroblasts within the amygdala in rats kindled from the piriform cortex. Seizure 2010;19:461-6.

20. Calcagnotto ME, Ruiz LP, Blanco MM, Santos-Junior JG, Valente MF, Patti C, et al. Effect of neuronal precursor cells derived from medial ganglionic eminence in an acute epileptic seizure model. Epilepsia 2010;51 Suppl 3:71-7.

21. Calcagnotto ME, Zipanic I, Piquer-Gil M, Mello LE, Alvarez-Dolado M. Grafting of GABAergic precursors rescues deficits in hippocampal inhibition. Epilepsia 2010;51 Suppl 3:66-70.

22. Zipanic I, Calcagnotto ME, Piquer-Gil M, Mello LE, Alvarez-Dolado M. Transplantation of GABAergic precursors restores hippocampal inhibitory function in a mouse model of seizure susceptibility. Cell Transplant 2010;19:549-64.

23. Chen YJ, Vogt D, Wang Y, Visel A, Silverberg SN, Nicholas CR, et al. Use of “MGE enhancers” for labeling and selection of embryonic stem cell-derived medial ganglionic eminence (MGE) progenitors and neurons. PLoS One 2013;8:e61956.

24. Maisano X, Litvina E, Tagliatela S, Aaron GB, Grabel LB, Naegle JR, et al. Differentiation and functional incorporation of embryonic stem cell-derived GABAergic interneurons in the dentate gyrus of mice with temporal lobe epilepsy. J Neurosci 2012;32:46-61.

25. Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. Nat Neurosci 2013;16:692-7.

26. Costa-Ferro ZS, Souza BS, Leal MM, Kaneto CM, Azevedo CM, Calcagnotto ME, et al. Injection of embryonic median ganglionic eminence (MGE) progenitors and neurons. Neurobiol Dis 2012;46:302-13.

27. Jing M, Shingo T, Yasuhara T, Kondo A, Morimoto T, Wang F, et al. The combined therapy of intrahippocampal transplantation of adult neural stem cells and intraventricular erythropoietin-infusion ameliorates spontaneous recurrent seizures by suppression of abnormal mossy fiber sprouting. Brain Res 2009;1295:203-17.

28. Yasuhara T, Matsukawa N, Hara K, Yu G, Xu L, Maki M, et al. Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson’s disease. J Neurosci 2006;26:12497-511.

29. Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Simone C, et al. Systemic transplantation of c-kit+ cells exerts a therapeutic effect in a model of amyotrophic lateral sclerosis. Hum Mol Genet 2010;19:3782-96.

30. Ryu S, Lee SH, Kim SU, Yoon BW. Human neural stem cells promote proliferation of endogenous neural stem cells and enhance angiogenesis in ischemic rat brain. Neural Regen Res 2016;11:298-304.

31. Stoniesifer C, Corey S, Ghanekar S, Diamandis Z, Acosta SA, Borlongan CV, et al. Stem cell therapy for abrogating stroke-induced neuroinflammation and relevant secondary cell death mechanisms. Prog Neurobiol 2017. pii: S0301-0082(17)30082-5.

32. Yasuhara T, Kari K, Maki M, Xu L, Yu G, Ali MM, et al. Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic-ischaemic rats with human umbilical cord blood grafts. J Cell Mol Med 2010;14:914-21.

33. Wang F, Yasuhara T, Shingo T, Kameda M, Tajiri N, Yuan WJ, et al. Intravenous administration of mesenchymal stem cells exerts therapeutic effects on parkinsonian model of rats: Focusing on neuroprotective effects of stromal cell-derived factor-1alpha. BMC Neurosci 2010;11:52.

34. Long Q, Qiu B, Wang K, Yang J, Jia C, Xin W, et al. Genetically engineered bone marrow mesenchymal stem cells improve functional outcome in a rat model of epilepsy. Brain Res 2013;1532:1-3.

35. Kokaia M. Seizure-induced neurogenesis in the adult brain. Eur J Neurosci 2011;33:1133-8.

36. Chailangkarn T, Acab A, Muotri AR. Modeling neurodevelopmental disorders using human neurons. Curr Opin Neurobiol 2012;22:785-90.

37. Yan Y, Shin S, Jha BS, Liu Q, Sheng J, Li F, et al. Efficient and rapid derivation of primitive neural stem cells and generation of brain subtype neurons from human pluripotent stem cells. Stem Cells Transl Med 2013;2:862-70.

38. Higurashi N, Uchida T, Lossin C, Misumi Y, Okada Y, Akamatsu W, et al. A human dravet syndrome model from patient induced pluripotent stem cells. Mol Brain 2013;6:e19.

39. Jiao J, Yang Y, Shi Y, Chen J, Gao R, Fan Y, et al. Modeling dravet syndrome using induced pluripotent stem cells (iPSCs) and directly converted neurons. Hum Mol Genet 2013;22:4241-52.

40. Harnod T, Wang YC, Lin CL, Tseng CH. High risk of developing subsequent epilepsy in patients with sleep-disordered breathing. PLoS One 2017;12:e0173491.

41. Chen JW, Ruff RL, Eavey R, Wasterlain CG. Posttraumatic epilepsy and treatment. J Rehabil Res Dev 2009;46:685-96.

42. Mijnt PK, Staufenberg EF, Sanathan K. Post-stroke seizure and post-stroke epilepsy. Postgrad Med J 2006;82:568-72.

43. Bovolenta R, Zucchini S, Paradiso B, Rodi D, Merigo F, Navarro Mora G, et al. Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. J Neuroinflammation 2010;7:81.

44. Miliadous P, Stamatakis A, Koutsoudaki PN, Tiniakos DG, Stylianopoulou F. IGF-I ameliorates hippocampal neurodegeneration and protects against cognitive deficits in an animal model of temporal lobe epilepsy. Exp Neurol 2011;231:223-35.

45. Russo E, Citarro R, Constanti A, De Sarro G. The mTOR signaling pathway in the brain: Focus on epilepsy and epileptogenesis. Mol Neurobiol 2012;46:662-81.

46. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci 2009;29:6964-72.

47. Heinrich C, Lähteeni S, Suzuki F, Anne-Marie L, Huber S, Häussler U, et al. Increase in BDNF-mediated trkB signaling promotes epileptogenesis in a mouse model of mesial temporal lobe epilepsy. Neurobiol Dis 2011;42:35-47.

48. McNamara JO, Scharfman HE. Temporal lobe epilepsy and the bdnf receptor, trkB. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper’s Basic Mechanisms of the Epilepsies. [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2012.

49. Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol 2002;174:201-14.

50. Croll SD, Suri C, Compton DL, Simmons MV, Yancopoulos GD, Lindsay RM, et al. Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vivo hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 1999;93:1491-506.

51. Mizoguchi H, Nakade J, Tachibana M, Ibi D, Someya E, Koike H, et al. Matrix metalloproteinase-9 contributes to kindled seizure development in pentylenetetrazole-treated mice by converting pro-BDNF to mature BDNF in the hippocampus. J Neurosci 2011;31:12963-71.

52. Kuramoto S, Yasuhara T, Agati T, Kondo A, Jing M, Kikuchi Y, et al. Y.
et al. BDNF-secreting capsule exerts neuroprotective effects on epilepsy model of rats. Brain Res 2011;1368:281-9.
53. Koyama R, Ikegaya Y. To BDNF or not to BDNF: That is the epileptic hippocampus. Neurosci Res 2005;11:282-7.
54. Kadota T, Shingo T, Yasuhara T, Tajiri N, Kondo A, Morimoto T, et al. Continuous intraventricular infusion of erythropoietin exerts neuroprotective/rescue effects upon parkinson's disease model of rats with enhanced neurogenesis. Brain Res 2009;1254:120-7.
55. Maurer MH, Schäbitz WR, Schneider A. Old friends in new constellations – The hematopoetic growth factors G-CSF, GM-CSF, and EPO for the treatment of neurological diseases. Curr Med Chem 2008;15:1407-11.
56. Jung KH, Chu K, Lee ST, Park KI, Kim JH, Kang KM, et al. Molecular alterations underlying epileptogenesis after prolonged febrile seizure and modulation by erythropoietin. Epilepsia 2011;52:541-50.
57. Kondo A, Shingo T, Yasuhara T, Kuramoto S, Kameda M, Kikuchi Y, et al. Erythropoietin exerts anti-epileptic effects with the suppression of aberrant new cell formation in the dentate gyrus and upregulation of neuropeptide Y in seizure model of rats. Brain Res 2009;1296:127-36.
58. Corvino V, Marchese E, Giannetti S, Lattanzi W, Bonvissuto D, Biamonte F, et al. The neuroprotective and neurogenic effects of neuropeptide Y administration in an animal model of hippocampal neurodegeneration and temporal lobe epilepsy induced by trimethyloxanthine. J Neurochem 2012;122:415-26.
59. Thomas Tayra J, Kameda M, Yasuhara T, Agari T, Kadota T, Wang F, et al. The neuroprotective and neurorescue effects of carboxamylated erythropoietin fc fusion protein (CEPO-fc) in a rat model of Parkinson's disease. Brain Res 2013;1502:55-70.
60. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: New advances. Lancet 2015;385:884-98.
61. Kawai K, Shimizu H, Maehara T, Murakami H. Outcome of long-term vagus nerve stimulation for intractable epilepsy. Neurol Med Chir (Tokyo) 2002;42:481-9.
62. Krahl SE, Clark KB. Vagus nerve stimulation for epilepsy: A review of central mechanisms. Surg Neurol Int 2012;3:255-9.
63. Naritoku DK, Terry WJ, Helfert RH. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. Epilepsy Res 1995;22:53-62.
64. Zagon A, Kemeny AA. Slow hyperpolarization in cortical neurons: A possible mechanism behind vagus nerve stimulation therapy for refractory epilepsy? Epilepsia 2000;41:1382-9.
65. Biggio F, Gorini G, Utzner C, Olla P, Marrosu F, Mocchetti I, et al. Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. Int J Neuropsychopharmacol 2009;12:1209-21.
66. Revesz D, Tjernstrom M, Ben-Menachem E, Thorlin T. Effects of vagus nerve stimulation on rat hippocampal progenitor proliferation. Exp Neurol 2008;214:259-65.
67. Kuramoto S, Yasuhara T, Agari T, Kondo A, Matsu T, Miyoshi Y, et al. Injection of muscimol, a GABAa agonist into the anterior thalamic nucleus, suppresses hippocampal neurogenesis in amygdala-kindled rats. Neurol Res 2009;31:407-13.
68. Morace R, DI Gennaro G, Quarato P, D'Aniello A, Amascia A, Grammaldo L, et al. Deep brain stimulation for intractable epilepsy. J Neurosurg Sci 2016;60:189-98.
69. Loddenkemper T, Pan A, Neme S, Baker KB, Rezai AR, Dinner DS, et al. Deep brain stimulation in epilepsy. J Clin Neurophysiol 2001;18:514-32.
70. Paz JT, Chavez M, Saillat S, Deniau JM, Charpier S. Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigrothalamic pathway. J Neurosci 2007;27:929-41.
71. Morimoto T, Yasuhara T, Kameda M, Baba T, Kuramoto S, Kondo A, et al. Striatal stimulation nurtures endogenous neurogenesis and angiogenesis in chronic-phase ischemic stroke rats. Cell Transplant 2011;20:1049-64.
72. Baba T, Kameda M, Yasuhara T, Morimoto T, Kondo A, Shingo T, et al. Electrical stimulation of the cerebral cortex exerts antiapoptotic, angiogenic, and anti-inflammatory effects in ischemic stroke rats through phosphoinositide 3-kinase/Akt signaling pathway. Stroke 2009;40:e598-605.
73. Yadav AP, Nicolelis MAL. Electrical stimulation of the dorsal columns of the spinal cord for Parkinson's disease. Mov Disord 2017;32:820-32.
74. Fuentes R, Petersson P, Siesser WB, Caron MG, Nicolelis MA. Spinal cord stimulation restores locomotion in animal models of Parkinson's disease. Science 2009;323:1578-82.