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REVIEW

Cell therapy in models for temporal lobe epilepsy

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Summary For patients with refractory epilepsy it is important to search for alternative treatments. One of these potential treatments could be introducing new cells or modulating endogenous neurogenesis to reconstruct damaged epileptic circuits or to bring neurotransmitter function back into balance. In this review the scientific basis of these cell therapy strategies is discussed and the results are critically evaluated. Research on cell transplantation strategies has mainly been performed in animal models for temporal lobe epilepsy, in which seizure foci or seizure propagation pathways are targeted. Promising results have been obtained, although there remains a lot of debate about the relevance of the animal models, the appropriate target for transplantation, the suitable cell source and the proper time point for transplantation. From the presented studies it should be evident that transplanted cells can survive and sometimes even integrate in an epileptic brain and in a brain that is subjected to epileptogenic interventions. There is evidence that transplanted cells can partially restore damaged structures and/or release substances that modulate existent or induced hyperexcitability. Even though several studies show encouraging results, more studies need to be done in animal models with spontaneous seizures in order to have a better comparison to the human situation.

Contents

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Introduction

Epilepsy is characterized by recurrent unprovoked seizures and affects 0.5–1% of the population.[1,2] More than 30% of the epilepsy patients have uncontrolled seizures or unacceptable medication-related side effects despite adequate pharmacological treatment.[2] These patients have 'refractory epilepsy'. The underlying pathophysiological process that transforms a normal brain into an epileptic brain is termed epileptogenesis.

Epilepsy surgery is an invasive but often curative treatment option that aims at removing the ictal onset zone, believed to be responsible for seizure occurrence.[3] For patients in whom the ictal onset zone is not well circumscribed or localized in functional brain tissue, few treatment options are left. The inability to adequately treat all patients with refractory epilepsy provides a continuous impetus to investigate novel forms of treatment.

A possible alternative way of treating refractory patients involves neuromodulation through neurostimulation. In our group, we have demonstrated the efficacy and safety of vagus nerve stimulation (VNS) and deep brain stimulation (DBS) in both patients and experimental animal models for epilepsy.[4–8] Other possible alternative treatments are newly developed AEDs,[9] the ketogenic diet[10,11] and transcranial magnetic stimulation.[12] In spite of all these developments, a significant number of patients continue to have uncontrolled seizures which makes a further search for alternative treatments mandatory.[13]

A promising treatment option that also receives considerable attention in other neurodegenerative diseases (e.g. Parkinson's Disease) is cell therapy.[14,15] In general, there are two main strategies that involve the use of cells for the treatment of brain disorders. Firstly, cells can be transplanted to replace lost neurons and/or to release disease modifying substances. Secondly, endogenous cells can be manipulated to affect and modify the disease process.

Temporal lobe epilepsy (TLE) is the most prevalent form of refractory symptomatic epilepsy.[16] Because of its focal nature and the associated cellular defects this epilepsy syndrome is highly attractive to be treated with cell therapy. This review will highlight the most typical cellular alterations in TLE and then discuss various cell therapy strategies including neural grafting to reconstruct damaged epileptic networks, stimulation of endogenous repair and cell transplantation for local delivery of seizure suppressing substances.

Cellular basis and animal models of temporal lobe epilepsy

Although the exact cause for the development of seizures in TLE is still under debate, human TLE is very frequently associated with specific pathophysiological changes that are believed to play an important role in the generation or intensification of the epileptic state.

The most frequent lesion in human TLE and status epilepticus (SE) models is hippocampal sclerosis, evident in up to 90% of surgically resected hippocampi.[17] Hippocampal sclerosis is characterized by extensive gliosis combined with a selective loss of neurons in the dentate gyrus and the hippocampus proper. Neuronal loss involves both glutamatergic neurons (granule cells in dentate gyrus, pyramidal neurons in hippocampus proper) and inhibitory interneurons in dentate hilus and CA1 region. Neuronal cell loss and gliosis can extend to other mesiotemporal regions such as amygdala, entorhinal, perirhinal and temporopolar cortex.[18–22] Most affected neurons are pyramidal neurons of CA1 and CA3 region, excitatory mossy cells in the hilus, and GABAergic inhibitory interneurons also expressing somatostatin, parvalbumin, or neuropeptide Y. Especially the loss of inhibitory interneurons is believed to be a key factor underlying the increased excitability of the epileptic hippocampus.[23,24]

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Mossy fiber sprouting is the growth of aberrant collaterals of granule cell axons (also called mossy fibers) into the inner molecular layer of the dentate gyrus where they preferentially make synaptic connections with dendrites of other granule neurons, forming excitatory feedback loops.[25,26] Mossy fiber sprouting is presumably caused by the loss of normal postsynaptic targets of the granule neurons. One of the arguments for this hypothesis is that the degree of mossy fiber sprouting is correlated to the degree of neuronal loss in hippocampal sclerosis.[27] Electrophysiological studies on human hippocampal slices and experimental studies in animal models for TLE have shown that the extend of mossy fiber sprouting is correlated with excitability of the hippocampus.[28,29]

By far the most used animal model for TLE demonstrating spontaneous seizures and typical brain damage is the status epilepticus (SE) model. In this model a SE is evoked by systemic or intracerebral injection of excitotoxins (kainic acid or pilocarpine) or by tetanic electrical stimulation of temporal lobe structures. After a latent period, during which epileptogenesis is occurring, spontaneous seizures are displayed.[30]

Another commonly used model for TLE is the kindling model. In this model temporal lobe structures are repeatedly stimulated by short electrical pulse trains. The animals respond to the stimulation by displaying an electrical discharge on the EEG (afterdischarge) and abnormal behaviour. With increasing number of stimulations rats display more complex afterdischarges or more severe seizures. Rats consistently displaying tonic–clonic seizures are termed fully kindled. Spontaneous seizures are only seen after a large amount of stimulations but even then gross morphological damage, seen in the status epilepticus model, are not or only moderately evident. Based on the controlled induction of epileptogenesis, the kindling model is a very interesting tool to study the role of events associated with epileptogenesis.[31] Frequently used parameters to assess efficacy of treatments in this model are the current intensity needed to evoke an afterdischarge, termed afterdischarge threshold (ADT), and the number of stimulations needed to fully kindle the rats, termed kindling rate.

Structural repair of damaged epileptic networks

Cell transplantation for repair of hippocampal circuitry

In case of TLE, the sclerotic hippocampus is the most obvious target for circuitry reconstruction given its presumed role in the TLE.[21] However, mediating structural repair of damaged hippocampal networks to restore balance between excitation and inhibition is without doubt an enormous challenge. As already described higher, hippocampal sclerosis involves the loss of different types of both excitatory and inhibitory neurons in different regions throughout the hippocampal structure. Therefore, cellular repair of hippocampal sclerosis will probably require multiple grafts of different cell types throughout the hippocampal structure.

Successful reestablishment of balanced excitatory drive and inhibitory input will demand a great deal of the transplanted cells. They will need to: (1) survive; (2) disperse and/or migrate to appropriate cell layers; (3) generate appropriate phenotypes in correct relative numbers and at the proper locations in the hippocampus; (4) attract suitable afferent input and (5) establish appropriate local and long distance connections with the proper target host and grafted neurons.

The need for proper integration is evident from electrophysiological studies in sclerotic hippocampi which showed that endogenous neurons which display inappropriate synaptic connectivity[25,32] and/or integrate at ectopic locations in the hippocampus[33] are part of hyperexcitable networks. It is therefore very likely that grafted neurons which do not integrate properly could enhance excitability rather than suppressing it.

Indeed, there have been reports which demonstrated pro-epileptic effects of hippocampal transplantation. In a series of experiments, Buzsaki et al. transplanted foetal hippocampal tissue chunks or dissociated foetal hippocampal tissue into intact[34] or fimbria–fornix lesioned, seizure-prone hippocampus.[35–40] By performing electrophysiological recordings the authors found that reciprocal electrophysiological connectivity was established between the graft and the intact or lesioned host brain. However, they found that the most typical EEG pattern in the transplant was highly synchronous bursting behaviour with concurrent large amplitude EEG spikes. Spontaneous EEG seizures were also frequently recorded from the graft which spread into the host brain.[35,36] Moreover, spontaneous behavioural convulsions were detected in a high fraction of the transplanted rats.[34,37,39]

Although the exact mechanism for this transplantation-induced epileptiform activity was not unravelled, the authors suggested that the hyperexcitability of the graft was caused by a lack of afferent control, extensive formation of recurrent excitatory circuitry and insufficient GABAergic inhibition within the graft.[37] Buzsaki and colleagues hypothesized that the grafted hippocampal

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cells served as an epileptic focus that kindled the host brain by repeated seizure induction.[34,39]

Foetal hippocampal cell transplantation

In spite of the hurdles, described in the previous chapter, foetal hippocampal neurons have been transplanted into the hippocampus of the intraventricular kainic acid model.[41–48] In this model there is selective loss of CA3 pyramidal neurons[41] and subsequent expression of spontaneous limbic seizures.[49,50]

A considerable fraction of the transplanted foetal hippocampal cells was able to survive upon transplantation in the damaged CA3 region. However, this survival was severely influenced by postlesion delay (PLD), age of the rats and the type of transplanted cells. Highest survival rates (77%) were seen when foetal CA3 cells were transplanted in young mature rats with a PLD of 4 days.[41] However if the PLD was longer, the fraction of surviving cells was much lower (e.g. 21–31% if PLD was 45 days).[51] Survival in case of transplantation with longer PLD could be dramatically enhanced (up to 99%) by pre-treating the cells with a cocktail of growth factors and an anti-apoptotic factor.[52] Survival rates of transplanted cells also strongly depended on cell specificity. Survival of CA1 cells, transplanted into the damaged CA3 region, was much lower compared to that of CA3 cells after a PLD of 4 days (respectively 42% and 77%). When fetal striatal cells were transplanted survival was even worse (only 4 to 12%).[46]

The migration of foetal hippocampal cells upon transplantation was minimal with the cells remaining clumped at the grafting site.[54,55] By using retrograde tracing, Shetty and Turner, showed that grafted foetal hippocampal CA3 neurons formed short-distance efferent projections to ipsilateral CA1 region and entorhinal cortex and long-distance efferent projections to septum and contralateral hippocampus.[41] However, efferent projections to contralateral hippocampus were only seen in case of homotopic grafting, which means grafting of CA3 neurons in proximity of the damaged CA3 region. If CA3 neurons were transplanted in the CA1 regions or CA1 neurons in the damaged CA3 region no long distance projections towards contralateral hippocampus were found.[42,44] Growth of host afferent projections into the cluster of grafted cells was also demonstrated. Using histochemical staining and anterograde labelling, afferent cholinergic fibers, mossy fibers and commissural fibers of contralateral CA3 neurons were found in the transplantation area.[41] Highest density of afferent fibers in the transplant was seen in case of homotopic grafting. The authors hypothesized that the need for homotopic grafting could be due to the fact that axon guidance pathways in the host may be highly specific, requiring accurate placements of the grafts to achieve access.[56] Both the limited migration and the need for homotopic grafting are very important disadvantages for potential repair of damaged hippocampal circuitry using foetal hippocampal neurons.

Nevertheless, if foetal CA3 neurons were transplanted homotopically, transplantation could result in partial reversal of secondary pathological alterations including aberrant mossy fiber sprouting, possibly by providing an appropriate target.[43,52] Additionally, loss of glutamic acid decarboxylase (GAD) positive interneurons could be reversed. As the graft did not seem to donate the GAD-positive cells, the authors hypothesized that the loss of CA3 afferents led to a downregulation of GAD protein expression, which was reversed by replacing CA3 cells.[45]

Unfortunately in their series of experiments the authors did not perform electrophysiological analysis of connectivity, so it remains uncertain whether the transplanted cells also functionally integrated. They also did not monitor for epileptic activity so it is not clear whether grafting of foetal CA3 neurons and the reversal of some pathological secondary changes resulted into a normalization of the imbalance between excitation and inhibition or a dampening of the epileptic activity.

Neural stem/progenitor cell transplantation

Transplantation of foetal brain tissue has important limitations which will probably always limit its application on a large clinical scale. These limitations include the inability to expand or store foetal cells, resulting in a high number of foetuses needed for one single transplantation (e.g. 6–8 fetal donors to tread one PD patient).[57] Another limitation is that purity and viability of the transplant is difficult to control so that outcome of transplantation is highly variable.[58] Moreover, as already described in the previous chapter, transplanted foetal cells have very limited migratory capabilities and homotopic grafting seems to be required.

Because neural stem/progenitor cells are self-renewing cells which can migrate throughout the brain and are able to generate different neuronal progeny, they could, at least in theory, overcome the limitations of foetal tissue and be a promising alternative cell source. Transplantable neural stem/progenitor cells can be derived in several ways from different sources. They can be produced almost completely in vitro starting from embryonic stem

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cells (ESC) using specific differentiation protocols.[59–68] Expandable neural progenitor cells can also be generated by immortalizing neuroepithelial precursor cells, derived from defined embryonic regions, prior to their terminal mitosis. This can be done by transfecting the cells with a vector containing a transcript encoding for a (temperature sensitive) immortalizing oncogene.[69,70] Neural stem/progenitor cells can also be isolated directly from different regions of the embryonic central nervous system (CNS) but also from restricted areas in the adult brain such as hippocampus, SVZ, striatum, substantia nigra, cortex, spinal cord, septum and optic nerve.[71–78]

Following transplantation in the brain, neural stem/progenitor cells seem to be able to functionally integrate into neural networks.[79,80] However, there are only few reports demonstrating the ability of neural stem/progenitor cells to functionally replace lost neurons and reconstruct damaged circuitry.[81] Transplantation for stroke seems to be most successful with reports on migration of neural progenitor cells towards the lesion with formation of new neurons[82] and reestablishment of neural connections with functional recovery.[83,84] Systemically injected neural stem cells, in a model for multiple sclerosis, migrate to inflammatory demyelinating lesions, where they can remyelinate axons.[85]

To our knowledge, studies showing structural repair of damaged circuitry in sclerotic hippocampus by transplantation of neural stem/progenitor cells are unavailable. In one study, neural stem cells, isolated from human embryos, have been injected systemically one day after induction of SE with pilocarpine. Transplanted cells were found in the hippocampus, amygdala and pyriform cortex 6 weeks after transplantation. About 30% of the cells were immunopositive for GABA and parvalbumin, two proteins also expressed by inhibitory interneurons. Surprisingly, the grafted cells did not express the panneural markers Neuronal Nuclei (NeuN) or β-III-tubulin (β-III-tubulin), indicating that they most probably were not neurons. Transplantation did however result in a significant decrease in daily seizure frequency, seizure severity and the number of rats that displayed spontaneous seizures. It was demonstrated that field excitatory postsynaptic potentials (fEPSP) in the CA1 region were decreased.[86] This indicates that transplantation could have anti-epileptogenic effects without evidence of structural repair by the transplanted cells.

A conditionally immortalized neural progenitor cell line, called MHP36, has been developed and has shown potential to replace, at least in part, CA1 pyramidal neurons in models where the CA1 region was specifically damaged, either by excitotoxic lesioning[87] or ischemia.[88] These MHP36 cells were also transplanted into four sites of the rat brain, extending from the anterior to the posterior pyriform cortex, 3 weeks after SE. However, in this study no replacement of lost pyramidal CA1 or CA3 neurons was reported but a significant augmentation in the number of seizures was found after transplantation.[89]

In a study, performed at the Ghent University Hospital, adult SVZ-derived neural stem cells were transplanted in the lesioned hippocampus of the intrahippocampal kainic acid SE model.[90] Adult neural stem cells were transplanted 3 days or 3 weeks after the kainic acid lesion. This resulted in a low (about 1%) but robust survival of the cells for at least 6 weeks after transplantation. However, only a fraction of the cells differentiated towards neurons while the majority of the cells generated astrocyte-like cells probably contributing to gliosis in response to the lesion.

Stimulating endogenous repair as a strategy?

In ischemia models neural replacement by endogenous neural precursors has been demonstrated in both the striatum and the hippocampus. In the permanent middle cerebral artery occlusion model a small number of lost medium spiny interneurons in the striatum were replaced by endogenous SVZ-derived progenitor cells.[91–93] In the four vessel occlusion model, transient induction of global ischemia leads to selective degeneration of CA1 pyramidal neurons. In this model a fraction of the lost CA1 neurons were replaced by endogenous neural progenitors, migrating from the posterior periventricular region (PPV) to the damaged CA1 region.[94,95] Brief intraventricular infusion of growth factors in the first week after stroke markedly increased reconstitution of CA1.[94]

Recently, enhanced proliferation and migration of neural precursor cells from the PPV towards damaged hippocampal CA1 and CA3 regions has been reported in the pilocarpine SE model for TLE. However, these neural precursor cells exclusively generated glial cells in the damaged hippocampal regions without any indication of neuronal replacement.[96] One strategy that could lead to promoting endogenous repair of sclerotic hippocampus could be to identify the factors which promote neuronal replacement in ischemically damaged hippocampus and/or block neuronal replacement in the sclerotic hippocampus.

In the pilocarpine SE model but also in other models for TLE, seizure-activity stimulates neurogenesis in the granule cell layer of the

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hippocampus.[97–100] However, as the granule cell layer is relatively spared in case of TLE, it is not yet clear whether this enhanced neurogenesis is an attempt of the brain to repair damage or whether it is a part of the epileptogenic process. It seems that a fraction of the newborn granule cells contributes to the formation of abnormal circuitry by migrating towards ectopic locations in the hippocampus,[33] contributing to mossy fiber sprouting[99] or generating a persistent basal dendrite which projects into the hilus and receives synaptic input from sprouted mossy fibers.[101] However, the great majority of the newborn neurons generated in response to seizures, form granule cells which normally integrate into the granule cell layer.

In order to further elaborate on the role of enhanced neurogenesis in response to seizures we recently performed a study, in collaboration with the University of Goteborg, in which we blocked seizure-induced neurogenesis in rats by using low-dose brain radiation one day before hippocampal kindling. We found that suppression of seizure-induced neurogenesis did not slow down of prevent kindling, indicating that new neurons generated in response to seizures play no major role in kindling epileptogenesis.[102] We believe that further studies are needed in other models to further unravel the role of granule cell neurogenesis in TLE before strategies for suppressing or enhancing endogenous hippocampal neurogenesis could be developed.

Cell grafting for local delivery of seizure suppressing substances

Several neurotransmitters and neuromodulators have anticonvulsant effects. Because they are synthesized and secreted by brain derived cells in normal physiological conditions, they are suitable candidates for cell based delivery therapy. According

Table 1 Overview of the transplantation studies with noradrenaline releasing cells

Cell source	Model	Time point of transplantation	Target	Effect on seizures	References
Fetal LC tissue	Kindling after NA-depletion	2 weeks after depletion 6–11 months before kindling	Hippocampus	Lower kindling rate	Barry et al.[106]
Fetal LC tissue	Kindling after NA-depletion and intact rats	Fully kindled	Hippocampus	No effect on seizure rate	Bengzon et al.[108]
Fetal LC tissue	Picrotoxin after NA-depletion	10 days after depletion 5 months before picrotoxin injection	Hippocampus	Protection against picrotoxin induced seizures, less interictal spikes	Bus et al.[37]
Fetal LC tissue	Subcortically denervated, epilepsy-prone hippocampus	Chronic epilepsy state	Hippocampus	Reduction in spontaneous seizures	Bortolotto et al.[111]
Fetal LC tissue	Pilocarpine SE + kindling	2 weeks after SE 6 to 11 months before kindling	Amygdala or pyriform cortex	Decrease of kindling rate	Barry et al.[109]
Fetal LC tissue	KA SE + kindling	10 days after SE 230 days before kindling	Intracerebro-ventricular	Same fraction of epileptic rats; lower seizure frequency; no effect on kindling rate	Holmes et al.[112]
Fetal LC or SCG tissue	Kindling after NA-depletion and aspirative lesion of the fimbria–fornix	4 weeks after depletion 2 weeks after lesion 8 months prekindling	Fimbria–fornix lesion cavity	Lower (LC tissue) or no effect (SCG tissue) on kindling rate	Kokaia et al.[105]

KA: kainic acid; LC: locus coeruleus; TLE: temporal lobe epilepsy; NA: noradrenaline; SCG: superior cervical ganglion; SE: status epilepticus.

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Table 2 Overview of the transplantation studies with acetylcholine releasing cells

Cell source	Model	Time point of transplantation	Target	Effect on seizures	References
Fetal basal forebrain tissue	PTZ and audiogenic stimulation after lesion of the fimbria-fornix	10 days after lesion 1 year before epilepsy induction (PTZ, sound)	Hippocampus	More reactive to PTZ; Less reactive to sound	Cassel et al.[114]
Fetal basal forebrain tissue	PTZ and audiogenic stimulation after lesion of the fimbria-fornix	8—9 days after lesion 3, 7 or 12 months before epilepsy induction (PTZ, sound)	Hippocampus	Less reactive to PTZ; More reactive to sound	Cassel et al., 1991[118]
Septal-diagonal band tissue	Picrotoxin after 192 IgG-saporin lesioned basal forebrain system	10 days after lesion 5 months before picrotoxin injection	Hippocampus	Decrease in kindling rate	Ferencz et al.[119]

PTZ: pentylenetetrazol.

to this strategy noradrenaline (NA, Table 1), acetylcholine (AchE; Table 2), GABA (Table 3) and adenosine secreting cells (Table 4) have been investigated in transplantation studies.

Noradrenaline secreting cells (Table 1)

The inhibitory effects of NA were reported in temporal lobe epileptogenesis. When extracellular NA levels are artificially augmented by blocking its uptake or by electric stimulation of the noradrenaline rich locus coeruleus (LC), there is a significant attenuation of the kindling rate.[103] On the other hand depletion of the noradrenergic system by injecting 6-OHDA has facilitating effects on kindling.[104] Transplantation of NA-rich foetal LC cells in the hippocampus of NA depleted rats reversed the facilitating effect of the lesion but only when NA release by the graft is under control of the host brain and sufficient in response to kindling stimulation.[105—107] Grafting of NA neurons only affected kindling epileptogenesis but not fully kindled seizures. Also no anti-epileptic effects could be demonstrated if the LC tissue was transplanted into intact hippocampus.[108] NA-rich neurons have also been transplanted in NA depleted rats into extrahippocampal regions, such as the amygdala—piriform cortex. In this experiment grafting only affected seizure development if the transplanted LC neurons re-innervated the host hippocampi bilaterally.[109] Compared to foetal LC neurons, NA-rich superior cervival ganglion (SCG) neurons demonstrated less survival, integration and NA release.[110] Therefore transplantation of SCG neurons had little

Table 3 Overview of the transplantation studies with GABA releasing cells

Cell source	Model	Time point of transplantation	Target	Effect on seizures	References
Fetal striatal tissue	Amygdala kindling	Fully kindled	SN	Transient higher ADT Less severe seizures	Löscher et al.[125]
GABA secreting cortical neural cell line	Entorhinal cortex kindling	10 days before kindling	SN	Posterior SN: higher kindling rate Anterior SN: lower kindling rate	Thompson et al.[127]
GABA secreting cortical neural cell line	Entorhinal cortex kindling	12 days before kindling	Pyriform cortex	Increase in ADT No difference in kindling rate	Gernert et al.[128]
GABA secreting cortical neural cell line	Entorhinal cortex kindling	7—10 days before kindling	Hippocampus	Higher ADT Lower ADD Lower kindling rate	Thompson et al.[129]
GABA secreting cortical neural cell line	Pilocarpine SE	45—65 days after SE	Anterior SN	Seizure-suppression up to 13 days after transplantation	Thompson et al.[130]

ADD: afterdischarge duration; ADT: afterdischarge threshold; SE: status epilepticus; SN: substantia nigra.

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Table 4 Overview of the transplantation studies with adenosine releasing cells

Cell source	Model	Time point of transplantation	Target	Effect on seizures	References
Encapsulated, engineered baby hamster kidney cells	Hippocampal kindling	Fully kindled	Lateral ventricle	Day 1–14: almost complete suppression of seizures Day 14–24: gradual loss of seizure protection	Huber et al.[137]
Encapsulated, engineered adult mouse myoblasts	Hippocampal kindling	Fully kindled	Lateral ventricle	Day 1–7: complete suppression of seizures in all rats Day 7-week 8: gradual loss of seizure protection	Güttinger et al.[138]
Encapsulated, engineered embryonic stem cells derived glia	Hippocampal kindling	Fully kindled	Lateral ventricle	Day 3: complete suppression of seizures in all rats Day 7: no seizure suppression	Güttinger et al.[140]

or no effects on kindling rate in NA-depleted rats.[105] Transplantation of foetal LC neurons in a hippocampus, which is epilepsy prone due to subcortical denervation, gave some protection against picrotoxin-induced behavioural seizures and resulted in less interictal spikes.[37]

Foetal LC tissue was also transplanted in the hippocampus of the pilocarpine-induced SE model. After transplantation the number of spontaneous seizures was reduced from approximately 11 per week to less than 1 per week, with the effect starting between 5 and 6 weeks after grafting surgery and being maximal at about 9 weeks. However, no appropriate control groups were used in this study.[111] Milder effects were seen when LC cells were transplanted in immature rats after kainic acid-induced SE. In this study, there was no reduction in the percentage of rats that developed spontaneous seizures, but the transplanted rats displayed fewer spontaneous seizures than sham-transplanted controls. No difference in susceptibility to kindling induced seizures was seen eight months after grafting.[112]

We can conclude from these studies that NA-releasing cells can have inhibitory effects on epileptogenesis in specific animal models where the hippocampus is rendered epilepsy prone. NA releasing grafts do not seem to have large effects on established epilepsy. This limits suitability of NA releasing cells for possible clinical applications in TLE.

Acetylcholine secreting cells (Table 2)

The septohippocampal system is known to play a role in the regulation of hippocampal excitability. It comprises the medial septum (MS) and the vertical limb of the diagonal band of Broca (VDB) which are connected with the hippocampus via the fimbria–fornix (FF). These projections are predominantly cholinergic and GABAergic.[113] Lesioning the FF increases susceptibility for seizure development[114] and produces chronic epileptiform activity (interictal spiking and decreased afterdischarge thresholds).[115] These epileptogenic effects are predominantly due to loss of cholinergic afferents, since selective immunolesioning of these afferents with 192 IgG-saporin, induces comparable effects.[116,117]

Where embryonic, acetylcholine (AchE)-rich basal forebrain tissue was transplanted in the hippocampus of fimbria–fornix lesioned rats, contradictory results were found. Implantation of embryonic basal forebrain tissue into the hippocampus of fimbria–fornix lesioned rats showed that the grafted rats displayed more severe convulsions in response to the proconvulsant pentylenetetrazol (PTZ), but were less reactive to audiogenic stimulation. In this study there was only a poor re-innervation of the deafferented hippocampus.[114] In a subsequent study opposite effects were found, with a reduction of the reactivity to PTZ and an increase of the reactivity to sound. In this study the authors reported an improved integration of the graft.[118] Due to these conflicting results, conclusions about grafting AChE neurons and seizure susceptibility can not be drawn.

Intrahippocampal transplantation of AChE rich foetal septal tissue after saporin-induced lesioning of the forebrain cholinergic system reversed the lesion-induced facilitation of the kindling rate.[119] The authors propose that the suppression of

epileptogenesis in the grafted animals may be due to restoration of the cholinergic activation of inhibitory GABAergic interneurons, although there was no direct proof for this assumption.

In all these studies cholinergic grafts were implanted before induction of epileptogenesis. Whether or not grafts of cholinergic-rich neurons can have anticonvulsive effects after the epileptic syndrome has been established is still unclear.

GABA releasing cells (Table 3)

Initial evidence that administration of GABA inhibits seizure activity was reported in studies using neurotransmitter application in dogs.[120] Peripheral administration of GABA or GABA agonists has several limitations, such as limited capacity to cross the blood–brain barrier, undesirable side effects and proconvulsant activity in primates and humans,[120] due to the diffuse stimulation of $GABA_A$ receptors within the brain. An alternative to increase GABA release at the epileptic focus is to implant GABA-releasing cells.

In different regions of the brain, contributing to the manifestation of seizures, a consistent loss of glutamic acid decarboxylase (GAD)-positive interneurons has been demonstrated. These regions are the substantia nigra pars reticulata (SNr),[121,122] the basolateral amygdala (BLA),[123] the striatum,[124] the pyriform cortex (PC),[123] and the hippocampus.[23,45] GABA releasing cells have been grafted in some of these structures in different models for TLE.

GABA-rich foetal striatal tissue has been transplanted into the SNr of fully amygdala kindled rats.[125] SNr was chosen because of its presumed role in the spreading of seizure acitivity.[126] After transplantation a significant increase in ADT was seen. Also a significant decrease in seizure severity was evident. These seizure-suppressing effects were transient and disappeared in the weeks following transplantation.

As an alternative for foetal GABAergic cells, Thompson et al. have engineered conditionally immortalized mouse neurons to deliver GABA by driving GAD_{65} expression that could be shut down by the administration of doxycycline. This cell line has been transplanted into the SNr,[127] the pyriform cortex[128] and the dentate gyrus of the hippocampus[129] of rats prior to kindling. The effect of transplantation in the SNr was dependent on the location within the SNr. Transplantation in the posterior SNr significantly facilitated kindling development. When cells were transplanted in the anterior SNr kindling rate increased but not significantly.[127] Transplantation of the cells in the pyriform cortex caused a temporary increase in ADT and did not effect kindling rate.[128] The reason why this anticonvulsant effect was partial and transient could have several explanations such as a decrease of in vivo GABA-release, progressive cell death of transplanted cells or down regulation of GABA receptors in the host tissue. Transplantation of the cells in the hippocampus improved the results. After transplantation an elevation of ADT, a slower entorhinal kindling rate, a longer latency between entorhinal stimulation and behavioural seizures was found. The transplanted cells showed limited survival after transplantation and were detected only in 30% of the transplanted animals 3 weeks after grafting.[129] These GABA releasing cells have also been transplanted into the anterior substantia nigra 45–65 days after pilocarpine induced SE.[130] Seven to ten days following transplantation there was a robust suppression of behavioural seizures and a reduction in interictal spikes. The evaluation of the seizure suppressing effect of GABA releasing transplants ended 13 days after transplantation, while it would have been interesting to investigate whether this anticonvulsant effect was long lasting.

The effect of GABA releasing cells on seizures are more convincing than earlier studies with NA and AchE releasing cells. Long-term cell survival and video-EEG monitoring is required in animal models with spontaneous seizures in order to evaluate the duration of the anticonvulsant effect and possible side effects.

Adenosine releasing cells (Table 4)

Adenosine and its analogues have powerful antiseizure and neuroprotective activities.[131,132] During epileptic seizures or status epilepticus, extracellular adenosine concentrations are elevated. This is considered to be an endogenous protective mechanism in order to control the ongoing seizure. Unfortunately, during the process of epileptogenesis the tonic inhibition of adenosine decreases due to down-regulation of its A1-receptors and increased break down of adenosine by adenosine kinase.[133,134] Because of its great potential to control seizures, adenosine might be a good alternative substance for treating epilepsy.

When administered systemically, adenosine and adenosine agonists cause adverse effects which have prevented its therapeutic use.[135] Therefore experiments have been set up in which adenosine was released locally in the brain of kindled rats by synthetic polymers.[136] Analysis of adenosine release revealed that a release of 20–50 ng/day by the polymer is sufficient to provide protection against seizures. The anticonvulsant effects lasted up to fourteen days after transplantation of the polymer.

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At this moment adenosine release was reduced to less than 10 ng per day. These experiments showed that the amounts of adenosine required to locally suppress seizure activity were in the range, achievable for adenosine released from cell sources. Transplantation of cells that have the capacity to survive and release adenosine permanently is a promising tool to achieve a more sustained suppression of seizure activity. Baby hamster kidney fibroblasts,[137] mouse myoblasts[138] and mouse embryonic stem cell derived glia,[139,140] all genetically engineered to release adenosine, have been transplanted in fully kindled rats. Transplantation of engineered hamster kidney fibroblasts and mouse myoblasts resulted in an almost complete suppression of kindled seizures up to 14 days after transplantation. After 14 days there was a gradual loss of seizure protection which could be attributed to a limited survival of the cells.[137,138] This survival dependant effect was even more evident from the experiments where mouse embryonic stem cell-derived glial cells were transplanted into fully kindled rats. Three days after transplantation complete suppression of seizures in 100% of the animals and 90% cell viability was found. Seven days after transplantation, seizure suppression was lost and viable cells no longer detectable.

In all studies seizure-suppressant effects could be contributed to the adenosine release since injection of the A1 receptor antagonist DPCPX (8-cyclopentyl-1,3-dipropyl-xanthine) abolished the adenosine-induced seizure suppressing effects. From these studies it is evident that the search for a cell source, which is able to survive for prolonged time in the brain while continuously secreting anti-seizure substances, is of major importance to develop cell therapies for the treatment of refractory TLE patients.

Conclusion

In TLE, structural changes in the hippocampus are believed to play a key role in the generation of epileptic seizures. However, given the complexity of hippocampal circuitry and cell damage in case of hippocampal sclerosis, structural repair of epileptic hippocampal networks will require complex transplantation strategies in which proper integration and rewiring of the implanted neurons will be of crucial importance. Foetal hippocampal transplantation has been successful in reversing some pathological changes but important disadvantages, such as limited migration and the need for homotopic transplantation, have urged the search for alternative cell types. Exogenous and endogenous neural progenitor cells could be used for the repair hippocampal damage. However, increased knowledge about injury-induced neurogenesis and differentiation pathways will be necessary in order to guide the cells towards the cell type they have to replace and prevent them from contributing to pathological processes such as gliosis.

In another strategy cells are transplanted for the release of neurotransmitters or neuromodulatory agents. Transplantation studies for epilepsy have mainly grafted therapeutic cells before the epileptogenesis induction. Although there are already promising results with certain substances, such as GABA and adenosine, further in vivo studies in animal models with spontaneous seizures are mandatory to initiate extrapolations to the human situation.

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