Published in final edited form as:
Epilepsia. 2009 January ; 50(1): 24–32. doi:10.1111/j.1528-1167.2008.01743.x.

Gene therapy in epilepsy

Véronique Riban[*], **Helen L. Fitzsimons**[†], and **Matthew J. During**[*]

[*]Neuroscience Program, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Biological Research Tower, Columbus, Ohio, U.S.A.

[†]Neurologix, Inc., Fort Lee, New Jersey, U.S.A.

SUMMARY

Results from animal models suggest gene therapy is a promising new approach for the treatment of epilepsy. Several candidate genes such as neuropeptide Y and galanin have been demonstrated in preclinical studies to have a positive effect on seizure activity. For a successful gene therapy-based treatment, efficient delivery of a transgene to target neurons is also essential. To this end, advances have been made in the areas of cell transplantation and in the development of recombinant viral vectors for gene delivery. Recombinant adeno-associated viral (rAAV) vectors in particular show promise for gene therapy of neurological disorders due to their neuronal tropism, lack of toxicity, and stable persistence in neurons, which results in robust, long-term expression of the transgene. rAAV vectors have been recently used in phase I clinical trials of Parkinson's disease with an excellent safety profile.

Prior to commencement of phase I trials for gene therapy of epilepsy, further preclinical studies are ongoing including evaluation of the therapeutic benefit in chronicmodels of epileptogenesis, as well as assessment of safety intoxicological studies.

Keywords

Seizures; Transplantation; Adeno-associated viral vector; Neuropeptide Y; Galanin

Gene therapy was traditionally defined as an approach to replace the defective copy of a gene with a functional copy and restore normal function in a cell population. It is an elegant therapeutic approach because it derives directly from our knowledge of the molecular biology of a disease, targeting its most upstream level. This approach has proven effective in genetic diseases such as hemophilia (Chuah et al., 2004), X-linked immunodeficiency (Hacein-Bey-Abina et al., 2002), and other metabolic disorders. However, the field of application is indeed broad, including both simple genetic as well as complex acquired disorders, as gene therapy enables either overexpression or knockdown (using interfering RNA, antisense, or ribozymes) of genes within a pathological network and is therefore applicable to any disease for which the cascade of pathophysiological events has been identified. There is a significant unmet need for new therapeutic approaches in epilepsy. About one-third of epileptic patients suffer from pharmacoresistant seizures despite the development of new antiepileptic drugs. For many of

Address correspondence to Matthew J. During, Neuroscience Program, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Biological Research Tower 9th floor, Room 940, 460 W. 12th Ave, Columbus, OH, U.S.A. m.during@aukland.ac.nz.

Conflict of interest: The authors have read the Journal's guidelines on ethical publication, and this review is consistent with those guidelines. M.J.D. is a founder and consultant for Neurologix, Inc., a public company interested in commercializing neurological gene therapy. V.R. has no conflicts. H.L.F. is a paid employee of Neurologix, Inc.

these patients, surgical resection is often the only effective therapeutic approach available. Moreover, antiepileptic drugs do not prevent the progression of the disease, and for epileptic patients, seizure management is often synonymous with lifelong pharmacological treatment, with side effects that can be debilitating, and the risk of increasing refractoriness over time.

WHICH EPILEPSIES ARE GOOD CANDIDATES FOR GENE THERAPY?

Approximately 30% of epilepsies are believed to be idiopathic or of genetic origin (Berkovic et al., 2006). Most of them are complex diseases with both genetic and environmental causation, however autosomal dominant monogenic epilepsies have also been identified, with the majority resulting from polymorphism in ion channels. A mutation in the nicotinic acetylcholine receptor α4 was the first autosomal defect identified in epileptic patients with nocturnal frontal lobe epilepsy (Steinlein et al., 1995). Since then, more than 12 mutations associated with channelopathies have been identified (Berkovic et al., 2006). However, pure monogenic epilepsies are relatively rare, and in complex epilepsies, the impact of environmental influences compared to genetic factors is difficult to assess. In addition, because of the compensatory mechanisms that take place in the brain, the link between the mutation and the hyperexcitability phenotype is sometimes difficult to identify. This may explain why some genetic mouse models reproducing the mutation of a gene sometimes fail to develop spontaneous seizures. Although significant insights into the pathophysiological mechanisms of epilepsy have been gained from these models, the single gene mutations often do not reproduce the full cascade of events that lead to an epileptic phenotype (Noebels, 1996). Finally, it is very difficult to design an approach with gene therapy for a disease that often involves a large area of the brain simply because of the technical limitations of achieving widespread gene transfer. For these reasons, genetic forms of epilepsy are among the most challenging and may not be the most suitable initial targets for development of gene therapy-based treatments. Focal epilepsies, and in particular temporal lobe epilepsy, appear to be better candidates for gene therapy. The physiopathology of temporal lobe epilepsy has been well studied in animal models, as well as from the analysis of surgical resection tissue, and several candidate genes have been identified as potential therapeutic targets (Vezzani, 2004). Furthermore, the epileptogenic area can be well defined by imaging and recording techniques. Gene therapy allows specific targeting of the epileptogenic region, thus sparing the surrounding healthy tissue and minimizing side effects that often go hand in hand with antiepileptic drug treatment.

DELIVERY OF GENES TO THE BRAIN

Route of administration

Delivery of genetic material to the brain is a technical challenge due to the presence of the blood brain barrier, which limits access to the central nervous system (CNS). Intranasal administration is a feasible approach, and transgene expression in neural cells has been achieved with this method of administration. A proof of principle experiment utilizing intranasal delivery of the antiapoptotic gene ICP10PK within a growth-compromised herpes simplex virus vector resulted in transduction of hippocampal neurons, however the level of transgene expression was limited (Laing et al., 2006). Furthermore, this method is not appropriate for therapies in which transduction of only a limited region of the brain is required, unless vectors are developed that target only selected subpopulations of cells (reviewed by Muzyczka & Warrington, 2005).

An invasive approach, such as stereotactic surgery, is a more efficient route for delivery of a therapeutic gene to a specific area of the brain, and high levels of transgene expression can be achieved following injection of a viral vector such as adeno-associated virus (Ruitenberg et al., 2002). To date, this is by far the most commonly used method of gene delivery to the brain.

A major advantage of gene transfer to the brain is the limited immune response induced after intraparenchymal delivery. The cell population within the CNS is devoid of antigen-presenting cells with only a very limited lymphatic system present (Hickey, 2001). However, an invasive surgery induces the breakage of the blood brain barrier and the penetration of activated lymphocytes. Therefore, the notion that the brain is immunologically privileged has been somewhat reevaluated (Barker & Widner, 2004), and while the immune response observed in the brain is generally less pronounced than in other peripheral organs, it remains an important factor in the choice and design of the technique for gene transfer. Other essential factors to be considered are the efficiency of gene delivery, the level and stability of transgene expression, and the ability to regulate transgene expression. Different techniques have been used to express a gene in a specific region of the brain: Cells transplantation in an ex vivo approach (fetal cells, immortalized cells, fibroblasts), nonviral vector delivery, liposomes, and viral vector delivery including herpes simplex virus, retrovirus and lentivirus, adenovirus, and adeno-associated virus.

Gene delivery vehicles

Lipid-based systems of gene delivery are the simplest technique for gene delivery. Their main advantages are a high loading capacity, low immunogenicity, and the transfection of nondividing cells (Ewert et al., 2004; Rettig & Rice, 2007). However, gene expression is inefficient and transient, and they are yet not suitable for gene therapy in neurological disorders. Similarly, nonviral delivery of a nude DNA is not currently feasible due to the low efficiency of transfection and the high level of immune response.

The main gene transfer techniques used in clinical application are cell transplantation and cellular transduction by viral vectors. Cell transplantation approaches currently emphasize the use of stem cells, typically embryonic stem (ES) cells or adult stem cells. Their main advantage is the high compatibility of the transplant with the host. Additionally, ES cells are pluripotent and can differentiate into either glia or different neuronal phenotypes (Rathjen & Rathjen, 2001) and can be transfected in vitro to express a protein of interest. However, the use of human ES cells in the clinic is limited due to ethical debate over destruction of the embryo as well as the potential for generating tumors (Riess et al., 2007). The development of porcine fetal tissue as xenograft material has been proposed to overcome the limitation of stem cells availability. Xenotransplants have been implanted in patients with neurodegenerative diseases (Deacon et al., 1997; Fink et al., 2000). Although the grafts successfully developed synaptic contacts with host cells (Deacon et al., 1997), their use is still limited because they carry an additional risk of infection due to animal pathogens, and the probability that the graft will be rejected is increased compared to allogeneic grafts (Isacson & Breakefield, 1997).

Viral vectors are currently the most promising tools to directly introduce a gene into the brain, in particular herpes simplex virus (HSV), lentivirus, and adeno-associated virus (AAV). Retroviruses are not a suitable gene delivery vehicle for transduction of neuronal cells because they require the cell to undergo mitosis. Furthermore, the use of retrovirus in gene therapy has raised safety issues due to the possibility of insertional mutagenesis (Hacein-Bey-Abina et al., 2003). HSV, AAV, and lentivirus transduce both dividing and nondividing cells, and the use of cell type-specific promoters allows targeted gene transfer to selected populations of neurons. Thus further research to optimize the efficacy of these gene delivery systems is a reasonable approach towards the development of gene-based treatments for neurological disorders.

HSV allows packaging of approximately 20 kb and has strong neuronal tropism. In addition, this vector has the ability to spread through the nervous system, and injection of HSV has resulted in widespread distribution of gene transduction (Berges et al., 2007). The main limitation to the use of HSV is cytotoxicity and elicitation of a cellular immune response (McMenamin et al., 1998). The development of helper virus-free HSV1, in which genes

involved in viral replication have been deleted, decreases the vector neurotoxicity (Krisky et al., 1998). Recently, further development of the vector has included the use of the neuron-specific tyrosine hydroxylase promoter, which effectively restricts the transduction to a subset population of cells (Cao et al., 2008).

Lentiviral vectors hold potential for gene therapy due to their ability to integrate into the host chromosome and transduce most cell types in the brain, which facilitates a high level of sustained transgene expression (Jakobsson & Lundberg, 2006). They also have a relatively large cloning capacity of around nine kilobases (Zhao & Lever, 2007). Lentiviruses are derived from primate or nonprimate immunodeficiency viruses with human immunodeficiency virus (HIV)-based vectors having undergone the most development so far. However, safety concerns arise from the potential for recombination events to occur that may generate a replication-competent virus, and therefore more vigilant safety measures are required compared with other viral vectors. These include removal of the virulence genes from the packaging plasmids and introduction of the genes involved in capsid assembly on two separate plasmids to reduce the chances of recombination (Zufferey et al., 1997). In addition, self-inactivating vectors, with part of the long terminal repeat (LTR) promoter removed, have been developed to abolish transcriptional activity upon vector integration. Various promoters have been evaluated in lentiviral cassettes. When pseudotyped to the glycoprotein of the vesicular stomatitis virus (VSV-G), most promoters displayed a pronounced tropism for neurons, although some pan-specific promoters such as human cytomegalovirus (hCMV) and human CMV/β-actin (CAG) also transduced glia at a lower frequency. On the other hand, the cellular human glial fibrillary acidic promoter (hGFAP) and rat neuron-specific enolase promoter (rNSE) were shown to almost exclusively restrict expression to glia or neurons, respectively (Jakobsson et al., 2003). Lentiviral vectors have been used successfully for therapeutic benefit in animal models of neurological disorders. Lentiviral mediated overexpression of nerve growth factor in cholinergic neurons improved neuron survival following lesion in rats (Blesch et al., 2005), and the introduction of an RNA interference (RNAi) targeting human SOD1 into the muscle of mice overexpressing mutant human SOD1 resulted in increased survival of motor neurons and a substantially extended life span (Ralph et al., 2005).

Particular attention has been drawn to the use of recombinant AAV (rAAV) vectors for delivery of transgenes to the brain after observing a general absence of toxicity, lack of induction of a cellular immune response, and efficient transduction of the brain in animal models (McCown, 2005; Coura Rdos & Nardi, 2007). A large number of serotypes have now been isolated from humans and nonhuman primates (Gao et al., 2005), some of which have been cloned and packaged into recombinant vectors and found to display differing tropism for various neuronal types and brain areas (Burger et al., 2004; Taymans et al., 2007). Methods have also been developed for manufacture of extremely pure, high titer preparations, thus many different rAAV serotypes can now be routinely packaged and purified to this level in the research laboratory. When injected into the brain at moderate to high titers, transgene expression spreading several millimeters can be consistently achieved with some AAV serotypes, including AAV1, AAV5, AAV7, and AAV8 (Burger et al., 2004; Broekman et al., 2006; Taymans et al., 2007). Conversely, precise stereotactic surgery combined with the use of a less efficient serotype (such as rAAV2) now provide the means for targeted transduction of a focal area, such as the hilus or CA1 area of the hippocampus.

The rAAV serotypes that have been characterized to date have primarily neuronal tropism and are therefore not optimal for gene therapy of disorders requiring transduction of glial cells. However it is highly possible that among the large number of serotypes that have been cloned, some with glial tropism will be discovered. Further development of the vector is still needed however, including improvement of expression cassettes, which have a packaging limit of approximately 4.7 kb, and the further characterization of cell-specific promoters for restriction

of expression to particular subclasses of neurons. This is of particular importance for gene therapy of epilepsy, due to the laminar nature of the hippocampus with many layers of neurons in close proximity that have different functions with respect to epileptogenesis. Several promoters have been used with rAAV to restrict expression to a subclass of neuron, such as melanin-concentrating hormone (MCH) neurons in the hypothalamus (Van den Pol et al., 2004), yet promoters have not yet been isolated that restrict expression to neuronal subclasses in the hippocampus, such as GABAergic neurons in the hilus, or principal neurons in the dentate gyrus. This is a particularly difficult challenge for rAAV vectors, due to promoter activity contained within the inverted terminal repeats (Flotte et al., 1993).

Immunization with AAV prior to intracerebral injection generates circulating antibodies that can, in some circumstances, limit the transduction of AAV vector if the titer of neutralizing antibodies is sufficiently high (Peden et al., 2004). Thus, the potential exists in human patients for rAAV to be neutralized by preexisting antibodies. Without postmortem brain analysis, it is difficult to assess the level of transgene expression following rAAV-mediated gene therapy, however in a phase I clinical trial for Parkinson's disease involving intrasubthalamic injection of rAAV-GAD, there was no correlation between the presence of preexisting neutralizing antibodies and improvement in clinical motor scores (Kaplitt et al., 2007).

GENES TARGETED IN EPILEPSY

The goal of gene therapy for epilepsy is to obtain not only a sustained anticonvulsant effect, but also an antiepileptogenic effect that will block the progression of the disease and maintain focalization of the epileptic zone.

One of the first logical targets for gene therapy of epilepsy was the GABAergic system, based on the pharmacologically validated approach that an increase in GABA levels in the epileptogenic area increases the threshold of neuronal excitability, hence decreasing seizure occurrence. Different techniques of in vitro or in vivo transfection of glutamic acid decarboxylase (GAD; the enzyme that catalyzes the synthesis of GABA) were used to increase GABA levels in the tissue of interest (Table 1). Transplantation of fetal GABAergic neurons into the substantia nigra (SN), a structure involved in the propagation of seizures, induced a transient decrease in seizure severity in the kindling model (Loscher et al., 1998). Similarly, transplantation of engineered mouse cortical neurons and glia expressing GAD65 into the SN or piriform cortex showed an anticonvulsant effect (Thompson et al., 2000;Gernert et al., 2002). Viral vector-based approaches have also been used to express GAD in cultured rat hippocampal neurons (Liu et al., 2005), but this technique has not yet been applied in vivo. The different techniques used in these studies were thus able to induce the expression of exogenous GAD in the epileptic tissue and locally increase GABA levels. However, this expression obtained with cell transplantation was only transient. In addition, the effects observed are the consequence of a global increase of GABA levels, and the effect of a strategy targeting a specific cell population are more difficult to predict. Indeed, the loss of interneurons and consecutive feedback inhibition described in epilepsy is restricted to certain population of interneurons. Conversely, some interneurons are preserved and are believed to underlie network synchrony (Bertrand & Lacaille, 2001;Stief et al., 2007). Haberman and colleagues (2002) demonstrated the importance of the preferential transduction of a neuronal population. In their study, the infusion of an rAAV vector coding for a N-methyl D-aspartate receptor 1 (NR1) cDNA fragment in the antisense orientation showed preferential transduction of either inhibitory inter-neurons or primary output neurons depending on the promoter used in the vector construct. Transduction of these two different systems had dramatically opposite effects on focal seizures (Haberman et al., 2002). This study showed the importance of the promoter choice, and more importantly, demonstrated the utility of rAAV vectors in engineering a precise and cell-targeted gene therapy approach to transduce a specific cell population.

Recently, Raol and colleagues (2006) used a different approach targeting the GABA receptor subunits rather than direct modulation of GABA levels. They designed an AAV5 construct coding for the α1 subunit of the GABA receptor under control of the α4 subunit (GABRA4) promoter, which is upregulated after status epilepticus. Intrahippocampal injection of this vector 2 weeks prior to induction of status epilepticus protected against recurrent seizures and demonstrated the importance of GABA receptor composition in the development of epileptic circuits (Raol et al., 2006).

Over the past decade, the roles of two neuropeptides, neuropeptide Y (NPY) (Noe et al., 2006) and galanin (McCown, 2006), as well as the neuromodulator adenosine (Boison, 2007), in the modulation of neuronal excitability have been established. The observation that epileptic seizures induce the release of these neuropeptides led to the hypothesis that they played an important role in epileptic activity. Experimental studies further confirmed their anticonvulsant and neuroprotective role, and suggested that these neuropeptides and their receptors constitute an endogenous system to control epileptic activity. These systems thus appear a promising target for the development of new therapeutics and in particular for gene therapy.

Experimental studies showed that galanin is released during epileptic seizures and has an inhibitory effect on neuronal activity through presynaptic inhibition of glutamatergic transmission, as well as a strong neuroprotective effect (Mazarati & Lu, 2005). Administration of galanin (Mazarati et al., 2000; Kokaia et al., 2001) or nonpeptide ligands (Saar et al., 2002) also induces a robust anticonvulsant effect in animal models of limbic seizures. In a study by Lin and colleagues (2003), an rAAV constitutively overexpressing preprogalanin was injected into the rat hippocampus. Kainic acid-induced seizure activity was significantly decreased, confirming the antiepileptic effect of galanin in vivo (Lin et al., 2003). Interestingly, administration of rAAV-preprogalanin resulted in not only long lasting expression of galanin, but also in the transport of the neuropeptide along the axonal arborization. Haberman et al. (2003) also demonstrated the antiseizure properties of galanin in two rat seizure models. They fused the fibronectin secretory sequence (FIB) onto galanin for constitutive secretion, AAV-FIB-galanin was evaluated in a model of focal seizure genesis, which involves electrical stimulation of the rat inferior collicular column (IC). Preinfusion of AAV-FIB-galanin into the IC increased the threshold for seizures. Moreover, following infusion into the hippocampus, AAV-FIB-galanin also resulted in suppression of electrographic and behavioral seizures induced by kainic acid and also had a neuroprotective effect on the survival of hilar interneurons (Haberman et al., 2003). In a subsequent study, the vector was injected after a series of daily stimulations reached a predetermined threshold of seizure activity, that is, in an already hyperexcitable system, and the sustained anticonvulsant effect observed demonstrated that rAAV-galanin has a robust effect on hippocampal hyperactivity (McCown, 2006).

In several animal models of epilepsy, seizure-induced increases of NPY messenger RNA (mRNA) and protein have been observed in the dentate gyrus of the hippocampus, suggesting a modulatory role of the neuropeptide on neuronal activity (Vezzani et al., 1999). This role was confirmed by in vitro data showing that application of NPY to hippocampal slices reduces glutamatergic synaptic excitation (Klapstein & Colmers, 1997), as well as in vivo studies that showed a strong anticonvulsant effect of NPY mediated by the Y2 and Y5 receptors (Sperk & Herzog, 1997; Reibel et al., 2000). In addition, NPY knockout mice develop spontaneous epileptic seizures, confirming the importance of NPY in controlling neuronal excitability (Baraban et al., 1997; Lin et al., 2006; Morris et al., 2007). In human tissue from temporal lobe resection, NPY-mediated neurotransmission is altered by seizures (Vezzani et al., 1999; Vezzani & Sperk, 2004), and the modulatory role of NPY on epileptic activity has also been validated on hippocampal slices (Patrylo et al., 1999). The effect of chronic overexpression of NPY in the hippocampus was examined in the kainic acid model in rats. rAAV1/2 (a

pseudotyped vector consisting of a 1:1 mixture of AAV1 and AAV2 capsid proteins)-mediated gene transfer of preproNPY to the hippocampus delayed seizure onset and dramatically decreased the occurrence of epileptic seizures (Richichi et al., 2004). In order to more closely approximate the effect of rAAV-NPY on epileptogenesis, the vector was evaluated in a chronic model of spontaneous and progressive temporal lobe epilepsy. In this model, spontaneous seizures develop after recurrent electric stimulation of the hippocampus, and the frequency of seizures increases over time. In rats treated with rAAV1/2-NPY, progression of seizure activity was repressed, and moreover, the frequency of seizures was decreased in some animals (Noe et al., 2008). Together these results show that AAV-mediated overexpression of NPY shows promise for gene therapy of epilepsy.

The inhibitory neuromodulator adenosine has also raised interest as an endogenous anticonvulsant (Lee et al., 1984; Dragunow et al., 1985; Boison et al., 2002). Decreased adenosine levels have been observed in different models of epileptogenesis and epileptic activity (Young & Dragunow, 1994; Gouder et al., 2004; Fedele et al., 2005; Rebola et al., 2005). More recently, adenosine has also been shown to restrict the site of epileptogenesis via activation of A1 receptors (Fedele et al., 2006). Using a different approach of ex vivo gene therapy based on transplantation of cells engineered to release the active modulator, Boison, Huber, and colleagues showed that implantation of encapsulated fibroblasts engineered to release adenosine could protect from seizures in the kindling model (Huber et al., 2001). The antiepileptic effect from released adenosine was however transient due to the short-term survival of the encapsulated fibroblasts. To increase the survival time of the transplant, a recent study was designed with mouse C2C12 myoblasts genetically engineered to release adenosine by genetic inactivation of adenosine kinase (Guttinger et al., 2005). Intra-ventricular graft of the myoblasts induced a short-term antiepileptic effect on kindling seizures and significantly reduced seizures duration for a period of 3 weeks after transplantation.

Neurotrophic factors play an important role in epileptogenesis (Simonato et al., 2006). Whereas neurogenesis is increased after status epilepticus and might contribute to the formation of aberrant circuits, a decrease is observed during the chronic phase. Glial cell line-derived neurotrophic factor (GDNF) administration has been proposed as a neuroprotective and anticonvulsant approach. To examine the role of GDNF as a potential target for gene therapy, rAAV-GDNF was injected in the hippocampus either before or after status epilepticus, which resulted in a decrease in the severity and the number of seizures (Kanter-Schlifke et al., 2007). Similarly, hippocampal fetal cell pretreated and grafted with fibroblast growth factor-2 (FGF2; in addition with a caspase inhibitor) and transplanted in the hippocampus of chronically epileptic rats also decreased the number of recurrent seizures (Rao et al., 2007).

TOWARD THE CLINIC

Currently, more than a thousand clinical trials using gene therapy have been designed, among which 17 target neurological diseases. The clinical outcomes of the phase I to phase III trials are very encouraging and have proven that gene therapy does not present an overall increase in risk factors associated with the technique compared with other surgical approaches. Gene therapy-based treatments for neurological disorders including Alzheimer disease (Tuszynski et al., 2005), late infantile neuronal ceroid lipofuscinosis (Worgall et al., 2008), Canavan disease (McPhee et al., 2006), and Parkinson's disease (Kaplitt et al., 2007; Fiandaca et al., 2008; Marks et al., 2008) have now been tested in human clinical trials with no serious adverse events that were attributed to the gene therapy agent. However, many of the clinical trials did not result in positive results with regard to efficacy.

In the first ex vivo gene therapy trial for epilepsy, a xenograft of GABA-expressing cells in a patient candidate for a temporal lobe resection failed to show an antiepileptic effect (Diacrin

Inc., Charlestown, MA, U.S.A.). Several issues arise from the use of transplant in neurological diseases. In addition to the limited availability of ES cells, the survival of the graft is very variable between patients (Bjorklund, 2000). Experimental results in animal models also tend to show a limited survival time of cell transplants in the epileptic brain. The temporal lobe is a highly heterogeneous region organized into complex layers, and the type of synaptic connections the graft would develop in this multisynaptic circuit is unknown. In addition, the transplanted cells would be subject to recurrent hyperexcitability in the epileptogenic area, which may affect their survival. Data obtained from in vivo experiments using AAV vectors demonstrate that this method of gene delivery may be a more feasible approach for clinical trials. An early proof of principle study demonstrated that gene transfer using adeno-associated vector on human resection slices resulted in an appreciable level of cell transduction of epileptic tissue (O'Connor et al., 1997).

The therapeutic approach in epilepsy targets a disruption of the abnormal epileptic activity rather than reintroducing a cell population that has been lost as has often been the focus for gene therapy of neurodegenerative diseases. The potentiation of an endogenous system of seizure modulation may induce fewer compensatory effects and be more efficient than trying to compensate for a loss of a specific neuronal population. In view of the experimental data on animal models, the modulation of the endogenous system constituted by galanin, NPY, or adenosine appears to be the most likely to translate to clinical trial, and indeed following positive results in preclinical studies, a proposal for the treatment of temporal lobe epilepsy with rAAV-NPY was presented to the Recombinant DNA Advisory Committee of the U.S.A. with favorable review (http://www4.od.nih.gov/oba/RAC/meetings/Sept2004/RACagenda092304.pdf).

In conclusion, the experimental and clinical data obtained from other neurological diseases show the feasibility of gene therapy for epilepsy. However the field of gene therapy is new, and the potential for adverse effects is relatively unknown. As with antiepileptic drugs, there is a possibility of alteration in limbic system function including memory or mood disturbances. Subjects who are good candidates for temporal lobectomy are an ideal population, since the gene transfer would occur in the brain region that has been planned for resection, providing a built in rescue procedure if the gene therapy was ineffective or associated with significant adverse events. An advantage of gene therapy over current drug regimens is the long lasting expression of the therapeutic gene, as well as the ability to target it to only the regions of the brain that it is intended. However the persistence of vector-mediated transgene expression is a double-edged sword; if expression escapes from the targeted area into another brain area, there is a chance of unanticipated negative effects that may not be easily remedied. For this reason, for a gene therapy product to reach phase I clinical trials, it must pass through rigorous animal testing for safety and efficacy at different dose levels, including but not limited to comprehensive assessments of general health, behavior, organ histology, and vector biodistribution.

Importantly, results of human clinical trials of neurological disorders have been very promising with excellent safety profiles (Fiandaca et al., 2008). In the first gene therapy trial for a neurodegenerative disorder, AAV-aspartoacylase was administered intraparenchymally to 10 children with Canavan disease and was well-tolerated with minimal inflammatory or immune response (McPhee et al., 2006). Moreover, in two recently completed clinical trials for AAV-mediated gene therapy of Parkinson's disease, there were no adverse events relating to the gene therapy (Kaplitt et al., 2007; Marks et al., 2008), and improvements in Parkinsonian symptoms were also observed. In the first study, unilateral administration of AAV2-GAD to the subthalamic nucleus of 12 Parkinson's patients resulted in a significant improvement in clinical motor scores up to at least 12 months after surgery (Kaplitt et al., 2007). Fluorodeoxyglucose positron emission photometry also revealed reductions in thalamic motor cortex activity on the

injected side of the brain, which correlated with clinical rating scores (Feigin et al., 2007). Similarly, following bilateral administration of AAV2-neurturin to the putamen of 12 Parkinson's patients, motor function was also improved at 1 year following surgery (Marks et al., 2008). Randomized, controlled phase II trials are now underway for both treatments.

Taken together, the relative low risk associated with gene therapy and the promising preclinical data on both NPY and galanin gene transfer in experimental animal models suggest that temporal lobe epilepsy, a disease clearly refractory to a traditional pharmacological approach, is an ideal candidate with gene therapy likely to have a significant impact on disease management within the coming decade.

Acknowledgments

Research was supported by the National Institutes of Health (NIH).

REFERENCES

Baraban SC, Hollopeter G, Erickson JC, Schwartzkroin PA, Palmiter RD. Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. J Neurosci 1997;17:8927–8936. [PubMed: 9364040]

Barker RA, Widner H. Immune problems in central nervous system cell therapy. NeuroRx 2004;1:472–481. [PubMed: 15717048]

Berges BK, Wolfe JH, Fraser NW. Transduction of brain by herpes simplex virus vectors. Mol Ther 2007;15:20–29. [PubMed: 17164771]

Berkovic SF, Mulley JC, Scheffer IE, Petrou S. Human epilepsies: interaction of genetic and acquired factors. Trends Neurosci 2006;29:391–397. [PubMed: 16769131]

Bertrand S, Lacaille JC. Unitary synaptic currents between lacunosum-moleculare interneurones and pyramidal cells in rat hippocampus. J Physiol 2001;532(Pt 2):369–384. [PubMed: 11306657]

Bjorklund A. Cell replacement strategies for neurodegenerative disorders. Novartis Found Symp 2000;231:7–15. [PubMed: 11131546]

Blesch A, Conner J, Pfeifer A, Gasmi M, Ramirez A, Britton W, Alfa R, Verma I, Tuszynski MH. Regulated lentiviral NGF gene transfer controls rescue of medial septal cholinergic neurons. Mol Ther 2005;11:916–925. [PubMed: 15922962]

Boison D, Huber A, Padrun V, Deglon N, Aebischer P, Mohler H. Seizure suppression by adenosine-releasing cells is independent of seizure frequency. Epilepsia 2002;43:788–796. [PubMed: 12180995]

Boison D. Adenosine-based cell therapy approaches for pharmacoresistant epilepsies. Neurodegener Dis 2007;4:28–33. [PubMed: 17429216]

Broekman ML, Comer LA, Hyman BT, Sena-Esteves M. Adeno-associated virus vectors serotyped with AAV8 capsid are more efficient than AAV-1 or -2 serotypes for widespread gene delivery to the neonatal mouse brain. Neuroscience 2006;138:501–510. [PubMed: 16414198]

Burger C, Gorbatyuk OS, Velardo MJ, Peden CS, Williams P, Zolotukhin S, Reier PJ, Mandel RJ, Muzyczka N. Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2 and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol Ther 2004;10:302–317. [PubMed: 15294177]

Cao HY, Zhang GR, Wang X, Kong L, Geller AI. Enhanced nigrostriatal neuron-specific, long-term expression by using neural-specific promoters in combination with targeted gene transfer by modified helper virus-free HSV-1 vector particles. BMC Neurosci 2008;9:37. [PubMed: 18402684]

Castillo CG, Mendoza S, Freed WJ, Giordano M. Intranigral transplants of immortalized GAB Aergic cells decrease the expression of kainic acid-induced seizures in the rat. Behav Brain Res 2006;171:109–115. [PubMed: 16677720]

Chuah MK, Collen D, Vandendriessche T. Preclinical and clinical gene therapy for haemophilia. Haemophilia 2004;10 Suppl.4:119–125. [PubMed: 15479384]

Coura Rdos S, Nardi NB. The state of the art of adeno-associated virus-based vectors in gene therapy. Virol J 2007;16:99. [PubMed: 17939872]

Deacon T, Schumacher J, Dinsmore J, Thomas C, Palmer P, Kott S, Edge A, Penney D, Kassissieh S, Dempsey P, et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nat Med 1997;3:350–353. [PubMed: 9055867]

Dragunow M, Goddard GV, Laverty R. Is adenosine an endogenous anticonvulsant? Epilepsia 1985;26 480-437.

During MJ, Symes CW, Lawlor PA, Lin J, Dunning J, Fitzsimons HL, Poulsen D, Leone P, Xu R, Dicker BL, et al. An oral vaccine against NMDAR1 with efficacy in experimental stroke and epilepsy. Science 2000;287:1453–1460. [PubMed: 10688787]

Ewert K, Slack NL, Ahmad A, Evans HM, Lin AJ, Samuel CE, Safinya CR. Cationic lipid-DNA complexes for gene therapy: understanding the relationship between complex structure and gene delivery pathways at the molecular level. Curr Med Chem 2004;11:133–149. [PubMed: 14754413]

Fedele DE, Gouder N, Guttinger M, Gabernet L, Scheurer L, Rulicke T, Crestani F, Boison D. Astrogliosis in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation. Brain 2005;128(Pt 10):2383–2395. [PubMed: 15930047]

Fedele DE, Li T, Lan JQ, Fredholm BB, Boison D. Adenosine A1 receptors are crucial in keeping an epileptic focus localized. Exp Neurol 2006;200:184–190. [PubMed: 16750195]

Feigin A, Kaplitt MG, Tang C, Lin T, Mattis P, Dhawan V, During MJ, Eidelberg D. Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. Proc Natl Acad Sci USA 2007;104:19559–19564. [PubMed: 18042721]

Fiandaca M, Forsayeth J, Bankiewicz K. Current status of gene therapy trials for Parkinson's disease. Exp Neurol 2008;209:51–57. [PubMed: 17920059]

Fink JS, Schumacher JM, Ellias SL, Palmer EP, Saint-Hilaire M, Shannon K, Penn R, Starr P, VanHorne C, Kott HS, et al. Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. Cell Transplant 2000;9:273–278. [PubMed: 10811399]

Flotte TR, Afione SA, Solow R, Drumm ML, Markakis D, Guggino WB, Zeitlin PL, Carter BJ. Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. J Biol Chem 1993;268:3781–3790. [PubMed: 7679117]

Gao G, Vandenberghe LH, Wilson JM. New recombinant serotypes of AAV vectors. Curr Gene Ther 2005;5:285–297. [PubMed: 15975006]

Gernert M, Thompson KW, Loscher W, Tobin AJ. Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. Exp Neurol 2002;176:183–192. [PubMed: 12093095]

Gouder N, Scheurer L, Fritschy JM, Boison D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. J Neurosci 2004;24:692–701. [PubMed: 14736855]

Guttinger M, Padrun V, Pralong WF, Boison D. Seizure suppression and lack of adenosine A1 receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. Exp Neurol 2005;193:53–64. [PubMed: 15817264]

Haberman R, Criswell H, Snowdy S, Ming Z, Breese G, Samulski R, McCown T. Therapeutic liabilities of in vivo viral vector tropism: adeno-associated virus vectors, NMDAR1 antisense, and focal seizure sensitivity. Mol Ther 2002;6:495–500. [PubMed: 12377191]

Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. Nat Med 2003;9:1076–1080. [PubMed: 12858168]

Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De Villartay JP, Thrasher AJ, Wulffraat N, Sorensen R, Dupuis-Girod S, Fischer A, Davies EG, Kuis W, Leiva L, Cavazzana-Calvo M. Gene therapy of X-linked severe combined immunodeficiency. N Engl J Med 2002;346:1185–1193. [PubMed: 11961146]

Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302:415–419. [PubMed: 14564000]

Hickey WF. Basic principles of immunological surveillance of the normal central nervous system. Glia 2001;36:118–124. [PubMed: 11596120]

Huber A, Padrun V, Deglon N, Aebischer P, Mohler H, Boison D. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. Proc Natl Acad Sci USA 2001;98:7611–7616. [PubMed: 11404469]

Isacson O, Breakefield XO. Benefits and risks of hosting animal cells in the human brain. Nat Med 1997;3:964–969. [PubMed: 9288721]

Jakobsson J, Ericson C, Jansson M, Bjork E, Lundberg C. Targeted transgene expression in rat brain using lentiviral vectors. J Neurosci Res 2003;73:876–885. [PubMed: 12949915]

Jakobsson J, Lundberg C. Lentiviral vectors for use in the central nervous system. Mol Ther 2006;13:484–493. [PubMed: 16403676]

Kanter-Schlifke I, Georgievska B, Kirik D, Kokaia M. Seizure suppression by GDNF gene therapy in animal models of epilepsy. Mol Ther 2007;15:1106–1113. [PubMed: 17387333]

Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, Bland RJ, Young D, Strybing K, Eidelberg D, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. Lancet 2007;369:2097–2105. [PubMed: 17586305]

Klapstein GJ, Colmers WF. Neuropeptide Y suppresses epileptiform activity in rat hippocampus in vitro. J Neurophysiol 1997;78:1651–1661. [PubMed: 9310450]

Klugmann M, Symes CW, Leichtlein CB, Klaussner BK, Dunning J, Fong D, Young D, During MJ. AAV-mediated hippocampal expression of short and long Homer 1 proteins differentially affect cognition and seizure activity in adult rats. Mol Cell Neurosci 2005;28:347–360. [PubMed: 15691715]

Kokaia M, Holmberg K, Nanobashvili A, Xu ZQ, Kokaia Z, Lendahl U, Hilke S, Theodorsson E, Kahl U, Bartfai T, Lindvall O, Hökfelt T. Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. Proc Natl Acad Sci USA 2001;98:14006–14011. [PubMed: 11698649]

Krisky DM, Wolfe D, Goins WF, Marconi PC, Ramakrishnan R, Mata M, Rouse RJ, Fink DJ, Glorioso JC. Deletion of multiple immediate-early genes from herpes simplex virus reduces cytotoxicity and permits long-term gene expression in neurons. Gene Ther 1998;5:1593–1603. [PubMed: 10023438]

Laing JM, Gober MD, Golembewski EK, Thompson SM, Gyure KA, Yarowsky PJ, Aurelian L. Intranasal administration of the growth-compromised HSV-2 vector DeltaRR prevents kainite-induced seizures and neuronal loss in rats and mice. Mol Ther 2006;13:870–881. [PubMed: 16500153]

Lee KS, Schubert P, Heinemann U. The anticonvulsive action of adenosine: a postsynaptic, dendritic action by a possible endogenous anticonvulsant. Brain Res 1984;321:160–164. [PubMed: 6093928]

Lin EJ, Richichi C, Young D, Baer K, Vezzani A, During MJ. Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. Eur J Neurosci 2003;18:2087–2092. [PubMed: 14622242]

Lin EJ, Young D, Baer K, Herzog H, During MJ. Differential actions of NPY on seizure modulation via Y1 and Y2 receptors: evidence from receptor knockout mice. Epilepsia 2006;47:773–780. [PubMed: 16650144]

Liu W, He X, Cao Z, Sheng J, Liu H, Li Z, Li W. Efficient therapeutic gene expression in cultured rat hippocampal neurons mediated by human foamy virus vectors: a potential for the treatment of neurological diseases. Intervirology 2005;48:329–335. [PubMed: 15956801]

Loscher W, Ebert U, Lehmann H, Rosenthal C, Nikkhah G. Seizure suppression in kindling epilepsy by grafts of fetal GABAergic neurons in rat substantia nigra. J Neurosci Res 1998;51:196–209. [PubMed: 9469573]

Lowenstein PR, Castro MG. Inflammation and adaptive immune responses to adenoviral vectors injected into the brain: peculiarities, mechanisms, and consequences. Gene Ther 2003;10:946–954. [PubMed: 12756415]

Marks WJ Jr, Ostrem JL, Verhagen L, Starr PA, Larson PS, Bakay RA, Taylor R, Cahn-Weiner DA, Stoessl AJ, Olanow CW, Bartus RT. Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. Lancet Neurol 2008;7:400–408. [PubMed: 18387850]

Mazarati AM, Hohmann JG, Bacon A, Liu H, Sankar R, Steiner RA, Wynick D, Wasterlain CG. Modulation of hippocampal excitability and seizures by galanin. J Neurosci 2000;20:6276–6281. [PubMed: 10934278]

Mazarati A, Lu X. Regulation of limbic status epilepticus by hippocampal galanin type 1 and type 2 receptors. Neuropeptides 2005;39:277–280. [PubMed: 15944022]

McCown TJ. Adeno-associated virus (AAV) vectors in the CNS. Curr Gene Ther 2005;5:333–338. [PubMed: 15975010]

McCown TJ. Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. Mol Ther 2006;14:63–68. [PubMed: 16730475]

McLaughlin J, Roozendaal B, Dumas T, Gupta A, Ajilore O, Hsieh J, Ho D, Lawrence M, McGaugh JL, Sapolsky R. Sparing of neuronal function postseizure with gene therapy. Proc Natl Acad Sci USA 2000;97:12804–12809. [PubMed: 11058147]

McMenamin MM, Byrnes AP, Charlton HM, Coffin RS, Latchman DS, Wood MJA. A gamma34.5 mutant of herpes simplex 1 causes severe inflammation in the brain. Neuroscience 1998;83:1225–1237. [PubMed: 9502260]

McPhee SW, Janson CG, Li C, Samulski RJ, Camp AS, Francis J, Shera D, Lioutermann L, Feely M, Freese A, et al. Immune responses to AAV in a phase I study for Canavan disease. J Gene Med 2006;8:577–588. [PubMed: 16532510]

Morris MJ, Gannan E, Stroud LM, Beck-Sickinger AG, O'Brien TJ. Neuropeptide Y suppresses absence seizures in a genetic rat model primarily through effects on Y receptors. Eur J Neurosci 2007;25:1136–1143. [PubMed: 17331209]

Muzyczka N, Warrington KH Jr. Custom adeno-associated virus capsids: the next generation of recombinant vectors with novel tropism. Hum Gene Ther 2005;16:408–416. [PubMed: 15871672]

Noe F, Nissinen J, Pitkanen A, Gobbi M, Sperk G, During M, Vezzani A. Gene therapy in epilepsy: the focus on NPY. Peptides 2006;28:377–383. [PubMed: 17196301]

Noè F, Pool AH, Nissinen J, Gobbi M, Bland R, Rizzi M, Balducci C, Ferraguti F, Sperk G, During MJ, Pitkänen A, Vezzani A. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. Brain 2008;131:1506–1515. [PubMed: 18477594]

Noebels JL. Targeting epilepsy genes. Neuron 1996;16:241–244. [PubMed: 8789939]

O'Connor WM, Davidson BL, Kaplitt MG, Abbey MV, During MJ, Leone P, Langer D, O'Conner MJ, Freese A. Adenovirus vector-mediated gene transfer into human epileptogenic brain slices: prospects for gene therapy in epilepsy. Exp Neurol 1997;148:167–178. [PubMed: 9398459]

Patrylo PR, Van Den Pol AN, Spencer DD, Williamson A. NPY inhibits glutamatergic excitation in the epileptic human dentate gyrus. J Neurophysiol 1999;82:478–483. [PubMed: 10400974]

Peden CS, Burger C, Muzyczka N, Mandel RJ. Circulating anti-wild-type adeno-associated virus type 2 (AAV2) antibodies inhibit recombinant AAV2 (rAAV2)-mediated, but not rAAV5-mediated, gene transfer in the brain. J virol 2004;78:6344–6359. [PubMed: 15163728]

Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC, Wong LF, Bilsland LG, Greensmith L, Kingsman SM, Mitrophanous KA, Mazarakis ND, Azzouz M. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat Med 2005;11:429–433. [PubMed: 15768029]

Rao MS, Hattiangady B, Rai KS, Shetty AK. Strategies for promoting anti-seizure effects of hippocampal fetal cells grafted into the hippocampus of rats exhibiting chronic temporal lobe epilepsy. Neurobiol Dis 2007;27:117–132. [PubMed: 17618126]

Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. J Neurosci 2006;26:11342–11346. [PubMed: 17079662]

Rathjen J, Rathjen PD. Mouse ES cells: experimental exploitation of pluripotent differentiation potential. Curr Opin Genet Dev 2001;11:587–594. [PubMed: 11532403]

Rebola N, Porciuncula LO, Lopes LV, Oliveira CR, Soares-da-Silva P, Cunha RA. Long-term effect of convulsive behavior on the density of adenosine A1 and A 2A receptors in the rat cerebral cortex. Epilepsia 2005;46 Suppl 5:159–165. [PubMed: 15987272]

Reibel S, Larmet Y, Carnahan J, Marescaux C, Depaulis A. Endogenous control of hippocampal epileptogenesis: a molecular cascade involving brain-derived neurotrophic factor and neuropeptide Y. Epilepsia 2000;41 Suppl 6:S127–S133. [PubMed: 10999534]

Rettig GR, Rice KG. Non-viral gene delivery: from the needle to the nucleus. Expert Opin Biol Ther 2007;7:799–808. [PubMed: 17555366]

Richichi C, Lin EJ, Stefanin D, Colella D, Ravizza T, Grignaschi G, Veglianese P, Sperk G, During MJ, Vezzani A. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. J Neurosci 2004;24:3051–3059. [PubMed: 15044544]

Riess P, Molcanyi M, Bentz K, Maegele M, Simanski C, Carlitscheck C, Schneider A, Hescheler J, Bouillon B, Schäfer U, Neugebauer E. Embryonic stem cell transplantation after experimental traumatic brain injury dramatically improves neurological outcome, but may cause tumors. J Neurotrauma 2007;24:216–225. [PubMed: 17263685]

Robert JJ, Bouilleret V, Ridoux V, Valin A, Geoffroy MC, Mallet J, Le Gal La Salle G. Adenovirus-mediated transfer of a functional GAD gene into nerve cells: potential for the treatment of neurological diseases. Gene Ther 1997;4:1237–1245. [PubMed: 9425448]

Ruitenberg MJ, Eggers R, Boer GJ, Verhaagen J. Adeno-associated viral vectors as agents for gene delivery: application in disorders and trauma of the central nervous system. Methods 2002;28:182–194. [PubMed: 12413416]

Ruppert C, Sandrasagra A, Anton B, Evans C, Schweitzer ES, Tobin AJ. Rat-1 fibroblasts engineered with GAD65 and GAD67 cDNAs in retroviral vectors produce and release GABA. J Neurochem 1993;61:768–771. [PubMed: 8336154]

Saar K, Mazarati AM, Mahlapuu R, Hallnemo G, Soomets U, Kilk K, Hellberg S, Pooga M, Tolf BR, Shi TS, Hokfelt T, Wasterlain C, Bartfai T, Langel U. Anticonvulsant activity of a nonpeptide galanin receptor agonist. Proc Natl Acad Sci U S A 2002;99:7136–7141. [PubMed: 12011470]

Sacchettoni SA, Benchaibi M, Sindou M, Belin MF, Jacquemont B. Glutamate-modulated production of GABA in immortalized astrocytes transduced by a glutamic acid decarboxylase-expressing retrovirus. Glia 1998;22:86–93. [PubMed: 9436790]

Shetty AK, Turner DA. Fetal hippocampal grafts containing CA3 cells restore host hippocampal glutamate decarboxylase-positive interneuron numbers in a rat model of temporal lobe epilepsy. J Neurosci 2000;20:8788–8801. [PubMed: 11102487]

Simonato M, Tongiorgi E, Kokaia M. Angels and demons: neurotrophic factors and epilepsy. Trends Pharmacol Sci 2006;27:631–638. [PubMed: 17055067]

Sperk G, Herzog H. Anticonvulsant action of neuropeptide Y. Neuropeptide Y may act as an endogenous anticonvulsant through Y5 receptors suggesting a new target for antiepileptic drugs. Nat Med 1997;3:728–729. [PubMed: 9212097]

Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 1995;11:201–203. [PubMed: 7550350]

Stief F, Zuschratter W, Hartmann K, Schmitz D, Draguhn A. Enhanced synaptic excitation-inhibition ratio in hippocampal interneurons of rats with temporal lobe epilepsy. Eur J Neurosci 2007;25:519–528. [PubMed: 17284194]

Taymans JM, Vandenberghe LH, Haute CV, Thiry I, Deroose CM, Mortelmans L, Wilson JM, Debyser Z, Baekelandt V. Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain. Hum Gene Ther 2007;18:195–206. [PubMed: 17343566]

Thompson K, Anantharam V, Behrstock S, Bongarzone E, Campagnoni A, Tobin AJ. Conditionally immortalized cell lines, engineered to produce and release GABA, modulate the development of behavioral seizures. Exp Neurol 2000;161:481–489. [PubMed: 10686070]

Tuszynski MH, Thal L, Pay M, Salmon DP, U HS, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat Med 2005;11:551–555. [PubMed: 15852017]

Van Den Pol AN, Acuna-Goycolea C, Clark KR, Ghosh PK. Physiological properties of hypothalamic MCH neurons identified with selective expression of reporter gene after recombinant virus infection. Neuron 2004;42:635–652. [PubMed: 15157424]

Vezzani A, Sperk G, Colmers WF. Neuropeptide Y: emerging evidence for a functional role in seizure modulation. Trends Neurosci 1999;22:25–30. [PubMed: 10088996]

Vezzani A. Gene therapy in epilepsy. Epilepsy Curr 2004;4:87–90. [PubMed: 16059458]

Vezzani A, Sperk G. Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides 2004;38:245–252. [PubMed: 15337376]

Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, Dyke JP, Ballon D, Heier L, Greenwald BM, Christos P, Mazumdar M, Souweidane MM, Kaplitt MG, Crystal RG. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. Hum Gene Ther 2008;19:463–474. [PubMed: 18473686]

Xiao X, McCown TJ, Li J, Breese GR, Morrow AL, Samulski RJ. Adeno-associated virus (AAV) vector antisense gene transfer in vivo decreases GABA(A) alpha1 containing receptors and increases inferior collicular seizure sensitivity. Brain Res 1997;756:76–83. [PubMed: 9187316]

Yenari MA, Fink SL, Sun GH, Chang LK, Patel MK, Kunis DM, Onley D, Ho DY, Sapolsky RM, Steinberg GK. Gene therapy with HSP72 is neuroprotective in rat models of stroke and epilepsy. Ann Neurol 1998;44:584–591. [PubMed: 9778256]

Yoo YM, Lee CJ, Lee U, Kim YJ. Neuroprotection of adenoviral-vector-mediated GDNF expression against kainic-acid-induced excitotoxicity in the rat hippocampus. Exp Neurol 2006;200:407–417. [PubMed: 16690057]

Young D, Dragunow M. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. Neuroscience 1994;58:245–261. [PubMed: 8152537]

Zhang LX, Li XL, Smith MA, Post RM, Han JS. Lipofectin-facilitated transfer of cholecystokinin gene corrects behavioral abnormalities of rats with audiogenic seizures. Neuroscience 1997;77:15–22. [PubMed: 9044370]

Zhao J, Lever AM. Lentivirus-mediated gene expression. Methods Mol Biol 2007;366:343–355. [PubMed: 17568135]

Zufferey R, Nagy D, Mandel RJ, Naldini L, Trono D. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat Biotechnol 1997;15:871–875. [PubMed: 9306402]

Table 1

Summary of studies for gene therapy of epilepsy

Gene	Vector	Model	Authors
Adenosine	Cells expressing adenosine	Kindling	Huber et al., 2001
	Myoblasts delivering adenosine	Kindling	Guttinger et al., 2005
CCK	Lipofectin	Audiogenic rats	Zhang et al., 1997
ICP10PK	HSV-2	Kainate ip	Laing et al., 2006
GAD	Cells expressing GAD65	Kindling	Gernert et al., 2002
	Fetal cells	Kainate icv	Shetty & Turner, 2000
	Immortalized astrocytes expressing GAD67	In vitro	Sacchettoni et al., 1998
	Immortalized GABAergic cells	Kainate ip	Castillo et al., 2006
	AAV-GAD67	In vitro	Robert et al., 1997
	Fibroblasts, GAD65, GAD67	In vitro	Ruppert et al., 1993
	Cells expressing GAD65	Kindling	Thompson et al., 2000
	AAV-antisense GABA-A alpha1	Stim. of IC	Xiao et al., 1997
Galanin	AAV-preprogalanin	Kainate ih	Lin et al., 2003
	AAV-FIB-galanin	Kainate ip/stim. of IC	McCown, 2006
	AAV-FIB-galanin/AAV-galanin	Stim. of IC	Haberman et al., 2003
GDNF	Ad-GDNF	Kainate ip	Yoo et al., 2006
	AAV-GDNF	Kindling, SSLSE	Kanter-Schlifke et al., 2007
Glut1	HSV1	Kainate ih	McLaughlin et al., 2000
HSP72	HSV	Kainate ip	Yenari et al., 1998
Homer1	AAV	SSLSE	Klugmann et al., 2005
NPY	AAV-preproNPY	Kainate ip, kindling	Richichi et al., 2004
NPY	AAV-preproNPY	SSLSE	Noé et al., 2008
NR1	AAV – NR1 oral vaccine	Kainate ip	During et al., 2000
	AAV-NR1A/AAV tet off	Stim. of IC	Haberman et al., 2002

Ad, adenovirus; CCK, cholecystokinin; icv, intracerebroventricular; ih, intrahippocampal; ip, intraperitonneal; SSLSE, self-sustaining limbic status epilepticus; stim, stimulation.