Neuropharmacology xxx (2017) 1–10

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Invited review

Gene therapy and editing: Novel potential treatments for neuronal channelopathies

R.C. Wykes*, G. Lignani*

Department of Clinical and Experimental Epilepsy, Institute of Neurology, UCL, London, UK

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
25 May 2017
Accepted 26 May 2017
Available online xxx

Keywords:
Channelopathy
Gene therapy
Gene editing
CRISPR/Cas9
Viral vectors

ABSTRACT

Pharmaceutical treatment can be inadequate, non-effective, or intolerable for many people suffering from a neuronal channelopathy. Development of novel treatment options, particularly those with the potential to be curative is warranted. Gene therapy approaches can permit cell-specific modification of neuronal and circuit excitability and have been investigated experimentally as a therapy for numerous neurological disorders, with clinical trials for several neurodegenerative diseases ongoing. Channelopathies can arise from a wide array of gene mutations; however they usually result in periods of aberrant network excitability. Therefore gene therapy strategies based on up or downregulation of genes that modulate neuronal excitability may be effective therapy for a wide range of neuronal channelopathies. As many channelopathies are paroxysmal in nature, optogenetic or chemogenetic approaches may be well suited to treat the symptoms of these diseases. Recent advances in gene-editing technologies such as the CRISPR-Cas9 system could in the future result in entirely novel treatment for a channelopathy by repairing disease-causing channel mutations at the germline level. As the brain may develop and wire abnormally as a consequence of an inherited or de novo channelopathy, the choice of optimal gene therapy or gene editing strategy will depend on the time of intervention (germline, neonatal or adult).

Contents

* Corresponding authors.
E-mail addresses: r.wykes@ucl.ac.uk (R.C. Wykes), g.lignani@ucl.ac.uk (G. Lignani).

http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

1. Introduction

Genetic channelopathies result from loss or gain of function mutations in genes coding ion channels or their accessory subunits. For the majority of channelopathies, therapy is palliative, treating either the symptoms of the disease, or using drugs which target the mutated ion channel. In either case, for a significant number of patients, therapy is often limited due to a lack of efficacy and tolerability. As many drugs that target specific families of ion channels are unable to discriminate between ion channel isoforms, they usually result in unwanted or unacceptable side-effects. The number of recognised genetic channelopathies, either inherited or de novo, is increasing as testing becomes more routine. Many channelopathies are extremely rare, and although pharmaceutical companies run rare/ultra-rare disorders programs, the development of alternative therapies is warranted. Therefore for the treatment of channelopathies, it may be prudent to move away from traditional drug-discovery programs and focus on the development of gene therapy and gene editing tools that can be tailored to the needs of each individual person. Gene therapy approaches can permit cell-specific modification of neuronal and circuit excitability and have been investigated experimentally as a therapy for numerous neurological disorders (Simonato et al., 2013). Recent advances in gene-editing technologies such as the CRISPR-Cas9 system could in the future result in entirely novel treatment for a channelopathy by repairing disease-causing channel mutations at a genomic level (McMahon and Cleveland, 2017).

2. Classical gene therapy approaches

Proof-of-concept studies in cell cultures and animal models have demonstrated the effectiveness of gene therapy in treating many neurological disorders, usually by either overexpression of a therapeutic gene, by either boosting expression of the non-mutated wild-type gene or by expressing a gene unrelated to the channelopathy that permits improvement of pathology. Alternatively one could inhibit the expression of the disease causing gene if the mutation is gain of function, such as for SCN8A mutations related with early-infantile epileptic encephalopathies (Wagnon and Meisler, 2015). Recently there has been significant progress in the advancement of the molecular tools required to translate these genetic strategies in experimental models to treat patients with a wide-range of neurological syndromes including, Parkinson's disease, Alzheimer's disease, Huntington's disease and epilepsy (Simonato et al., 2013; Choong et al., 2016). Gene therapy clinical trials for many of these diseases have already commenced. Few of these phase I/II clinical trials have so far reported significant clinical benefit (O'Connor and Boulis, 2015; Blits and Petry, 2016), although some are encouraging (LeWitt et al., 2011). With further basic science improvements in transgene vector design and delivery, and the identification of new therapeutic targets, there is optimism that results from ongoing and future trials will provide more favourable clinical outcomes.

2.1. Modulating neuronal excitability

Neuronal channelopathies often result in alterations in action potential properties and/or synaptic transmission resulting in an aberrant cellular and network excitability. The resulting pathological phenotype will depend on the expression pattern of the ion channel or accessory subunit that is mutated and the functional consequence of this mutation. A key feature of these diseases is that they are often paroxysmal in nature (Kullmann, 2010). Epilepsy is a disease characterised by discreet episodes of network hyper-excitability and although there are many distinct mechanisms that can cause epilepsy some result from monogenic mutations in different classes of neuronal ion channels (Lerche et al., 2013; Snowball and Schorge, 2015; Spillane et al., 2016). Therefore gene therapy strategies based on modulating either cellular or circuit excitability proven to be effective in experimental models of epilepsy may also be promising approaches to treat a broad range of channelopathies. Over the last decade there have been many experimental studies investigating gene therapy approaches to prevent epileptogenesis or treat established epilepsy (Simonato, 2014). The majority of these studies have not used animal models of epilepsy that arise from inherited mutations of ion channels with widespread brain expression. Instead, for reasons mainly associated to limited transduction volume of the viral vectors used to deliver the transgenes, they have focused on acquired epilepsies with a discreet epileptogenic zone (Wykes et al., 2012; Kullmann et al., 2014). Gene therapy approaches to treat epilepsy include targeting inflammatory pathways (Mazzuferi et al., 2013), preventing neuronal damage (Paradiso et al., 2009, 2011; Bovolenta et al., 2010) or manipulating expression of whole cohorts of genes that change during epileptogenesis by modulating transcription factors or microRNAs (McClelland et al., 2011; Jimenez-Mateos et al., 2012). However three main strategies investigated, based on manipulating neuronal excitability, can potentially be applied to treat abnormal network excitability resulting from a given neuronal channelopathy. The first is to increase inhibitory tone via increased expression of inhibitory neuropeptides or adenosine. The second involves manipulating intrinsic cell excitability by increasing or decreasing expression of ion channel genes. The third relies on expression of an exogenous ion channel or receptor, which when activated by a specific ligand, can result in neuronal firing or silencing.

2.2. Increasing inhibitory tone

Increasing expression of a number of inhibitory neuropeptides including galanin and neuropeptide Y (NPY), either in isolation or together with inhibitory Y receptor type 2, has been shown to exhibit anticonvulsant properties (Haberman et al., 2003; Richichi et al., 2004; Lin et al., 2006; McCown, 2006; Woldbye et al., 2010). A conceptual advantage to this approach is that the expression of the neuropeptides does not need to be restricted to specific cell types. Secretion of an inhibitory neuropeptide could affect targets remote from their site of release. Increasing NPY levels in the hippocampus was effective in reducing spontaneous seizure frequency in models of temporal lobe epilepsy (Noe et al., 2007; Ledri et al., 2016). Adenosine is an endogenous brain anticonvulsant and increasing levels of this protein by siRNA mediated knockdown of adenosine kinase has been reported to reduce spontaneous seizures (Theofilas et al., 2011). Although dosage control is more difficult with gene therapy than pharmaceutic compounds, there are numerous advantages to gene therapy including cell specificity and either constitutive or long-lasting effects.

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

2.3. Manipulating expression levels of ion channels

At a cellular level, overexpression of an inhibitory ion channel, such as the α1 subunit of the γ-aminobutyric acid ($GABA_A$) receptor (Raol et al., 2006), has been reported to decrease the number of seizures observed following a period of status epilepticus. Alternatively, decreasing excitability of pyramidal neurons by overexpression of the potassium channel KV1.1 was effective in suppressing established epileptiform activity (Wykes et al., 2012). An alternative approach would be to reduce pyramidal neuron excitability by reducing the expression of sodium channels using siRNA techniques (Boison, 2010). For these strategies to be effective the transgenes must be expressed only in specific populations of neurons, principle (excitatory) or interneuron (inhibitory), otherwise the opposite of the desired effect may occur (Haberman et al., 2002).

With these approaches, changes in inhibitory tone or ion channel expression, it will be difficult to quantify how much overexpression or siRNA-mediated reduction in protein will be required for the treatment of the pathology. Additionally, it is not known whether a sustained increase or decrease in expression levels of a certain protein will result in homeostatic compensation, or alterations in normal physiology and behaviour. The regulation of therapeutic function is not easily controllable as the expression of the exogenous gene is dependent on the promoter used which may have a different activity to the endogenous promoter of that gene.

2.4. Optogenetics and chemogenetics

Many patients with a channelopathy often appear normal between episodes of attack, presumably due to homeostatic mechanisms that are able to regulate membrane excitability within acceptable limits (Ryan and Ptacek, 2010). However when stressed in some way these homeostatic mechanisms are no longer able to keep the network within normal excitability parameters. Examples of a precipitating factor include consumption of certain foods that raise or lower serum potassium in channelopathies that result in periodic paralyses or warm temperatures in channelopathies that result in erythermelalgia (Ryan and Ptacek, 2010; Swann and Rho, 2014).

In this respect, instead of permanently up or down regulating proteins as suggested above we could focus on developing new therapies that can 'on-demand' reset network excitability only when required to do so.

Optogenetics is the combination of optical and genetic methods to control the activity of specific populations of excitable cells by light with high temporal and spatial resolution (Deisseroth, 2010). Derived from microbial organisms 'opsin' genes encoding light-activated ion channels and pumps can be genetically targeted to defined neuronal populations in mammalian brains using viral vectors. When exposed to light of an appropriate wavelength, activation or inhibition of neuronal excitability can be optically induced on a millisecond timescale (Fenno et al., 2011). In vivo epilepsy-related optogenetic research has focused on expressing inhibitory opsins (halorhodopsin) in excitatory principal neurons (Wykes et al., 2012; Krook-Magnuson et al., 2013; Paz et al., 2013; Sukhotinsky et al., 2013; Berglind et al., 2014; Sorokin et al., 2017) or excitatory opsins (Channelrhodopsin) in inhibitory interneurons within the epileptic focus (Krook-Magnuson et al., 2013; Chiang et al., 2014; Ladas et al., 2015; Assaf and Schiller, 2016). Although other approaches include optogenetically targeting structures that project to areas involved in seizure initiation (Krook-Magnuson et al., 2014, 2015; Kros et al., 2015; Soper et al., 2016; Xu et al., 2016). Optogenetic approaches have successfully suppressed seizure activity *in vivo* although there is an as yet unresolved debate as to which are the best subpopulations of interneurons to activate and when during periods of ictal and inter-ictal activity it is best to activate them (Wykes et al., 2016). There are translational concerns specific to a gene therapy approach based on optogenetics, including safety issues, concerning an immunological response, in regard to long-term expression of non-mammalian proteins and the need for a light source to be implanted into the brain. As brain penetrance by light is poor this approach is only likely to be feasible where optical stimulation of neurons in a focal area of the brain is required (Wykes et al., 2016). However the ability to use optogenetic technology to turn light-gated ion channels or pumps on and off only in response to changes in pathological brain network excitability is an attractive proposal for patients with an episodic neuronal channelopathy. This will allow brain circuits to operate normally at other times avoiding the side-effects of drugs or any potential adverse behaviour due to chronic up or downregulation of endogenous proteins.

Chemogenetics is a term used to describe the mutation of a ligand binding site in an ion channel or G protein-coupled receptor (GPCR) rendering the protein unable to respond to the endogenous ligand, but enabling it to be activated by an exogenous ligand. These mutated proteins are referred to as designer receptors exclusively activated by a designer drug (DREADD) (Roth, 2016). Specificity of DREADDs can be achieved by regional and cell-type specific expression using viral vectors. Neurons transduced to express a chemogenetic protein are not permanently altered in their intrinsic properties, but can respond with changes in neuronal excitability when a specific exogenous ligand is delivered (Pei et al., 2010). In contract to optogenetic techniques where invasive light delivery is required to activate the channels or pumps, the exogenous ligands used to activate DREADDs can usually be delivered systemically. Additionally this approach allows for larger and deeper areas of the brain to be targeted for modulation compared to optogenetics. A prototypical example of a DREADD is the engineered inhibitory Gi-coupled human muscarinic receptor hM4Di which has been rendered insensitive to the endogenous ligand acetylcholine and sensitive to a metabolically inert derivative of clozapine, clozapine-N-oxide (CNO) (Armbruster et al., 2007; Ferguson and Neumaier, 2012). hM4Di activation results in opening of G-protein gated inwardly rectifying potassium channels inducing neuronal inhibition via membrane hyperpolarization (Armbruster et al., 2007). CNO-mediated activation of hM4Di expressed in principle neurones within the epileptic focus was shown to inhibit acute and spontaneous seizures in rodent models of epilepsy (Katzel et al., 2014). Whereas optogenetic control of neurons can be achieved on the millisecond timescale, modulation of neuronal excitability via chemogenetic methods is orders of magnitude slower (minutes to hours). Chemogenetic approaches could be highly suitable in the treatment of a paroxysmal channelopathy such as migraine when a known precipitating factor such as hormonal fluctuation has been identified as a trigger. For many women, migraines are connected to their menstrual cycle, occurring for a few days when estrogen levels drop (Martin and Lipton, 2008). In fact as many as 1 in 4 people with migraine experience a prodromal phase, which can occur as early as 24 h before the migraine starts. For these patients brain areas can be transduced with a DREAAD and the orally bioavailable ligand taken to coincide with the start of menstruation or at the onset of prodromal symptoms.

A major advantage of both of these techniques is that they are potentially amenable to a closed-loop design by coupling abnormal network excitability to delivery of the ligand (light in the case of optogenetics and agonist in the case of chemogenetics). Advances in closed-loop, on-demand systems that recognize diverse human epileptic EEG signatures will be required, as will the development

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

of small, powerful, implantable light/drug delivery devices with a long-life battery. Implantable devices capable of real-time seizure detection and light emission in rodents are now being developed (Armstrong et al., 2013 and Kullmann DM & Wykes RC, unpublished data) and such devices, if successfully translated, offer the prospect of a device similar to an automatic implantable cardiac defibrillator to stop seizures without the permanent alteration of neuronal properties.

3. The gene editing challenge

An ideal treatment for a genetic channelopathy would be the ability to edit the faulty gene and correct the mutations(s). Gene editing is an exciting and promising gene therapy approach as this strategy offers the prospect of not only treating a channelopathy but curing the disease permanently. However, there are several limitations, in particular for neurological application of these techniques, which will have to be overcome before a translational breakthrough (Heidenreich and Zhang, 2016; Lee et al., 2016).

Several molecular mechanisms exist that can potentially correct the protein product of a faulty gene. These include RNA repair mechanisms which work at the level between the gene and the protein such as Spliceosome-Mediated RNA Trans-Splicing (Yang and Walsh, 2005), and gene editing tools such as ZFN, TALEN, and CRISPR/Cas (Kim and Kim, 2014). Of these CRISPR/Cas may be best suited to allow translational therapy for pathologies caused by genetic modifications (Sander and Joung, 2014) and will be discussed further. CRISPR is an innate protective mechanism part of the bacteria and archaea immune system, and it functions to protect them from viral and plasmid attacks (Horvath and Barrangou, 2010; Wiedenheft et al., 2012; Wright et al., 2016). Engineering of this system a few years ago permitted gene editing in mammalian cells (Cong et al., 2013; Mali et al., 2013). The CRISPR system is composed of two distinct parts: a nuclease (e.g. Cas9) able to cut the double DNA strand, and a small guide RNA (gRNA) which drives the nuclease in a precise genome location (Sander and Joung, 2014; Wright et al., 2016). This permits a precise double strand break of the DNA in a defined genome region.

Thereafter, at least two innate cell mechanisms of DNA repairing can be activated, the homology-directed repair (HDR) and the non-homologous end joining (NHEJ) (Sander and Joung, 2014). HDR requires the presence of an appropriately designed DNA repair template and corrects the break without introducing errors, essentially replacing the short stretch of faulty DNA with a corrected version. NHEJ is an error-prone mechanism of repair, frequently introducing insertion/deletion mutations, but does not require a DNA template (Sander and Joung, 2014; Chu et al., 2015; Maruyama et al., 2015). In neurons, and in general in all post-mitotic cells, HDR is less active than NHEJ (Cox et al., 2015). For this reason, at the moment, using HDR as a gene editing tool to treat neurons *in vivo* is still a big challenge (Heidenreich and Zhang, 2016; Lee et al., 2016).

In addition to potentially treating neurological diseases, genome-editing technology can be used to provide a greater understanding of diseases themselves (Heidenreich and Zhang, 2016; Lee et al., 2016). HDR has already been used to generate animal models of neurological diseases (Niu et al., 2014; Heidenreich and Zhang, 2016) as well as to insert or correct single mutations in cell lines and in induced pluripotent stem cells (iPSC) facilitating the investigation of channelopathies such as Dravet Syndrome (Liu et al., 2016). Although iPSC-derived neurons are a fundamental model to study neurological diseases and CRISPR considerably improved their potentiality (Heidenreich and Zhang, 2016; Rubio et al., 2016), an *in vivo* approach to understand the pathological effects of neuronal channelopathies on complex connected networks is required. As homeostatic and developmental changes are likely to occur as a result of a channelopathy, gene editing *in vivo* in animal models will be crucial to determine at which stage in an animals life, (embryonic, neonatal, adult), correction of the faulty gene will result in a full reversal of pathology.

CRISPR has been already successfully applied *in vivo* and *in vitro* in post-mitotic neurons mostly to disrupt gene function, such as those that code for iontropic neurotransmitter receptors (NMDA and AMPA), or proteins implicated in neurological disease such as the silencing factor MECP2 (Incontro et al., 2014; Straub et al., 2014; Swiech et al., 2015). Moreover, modified CRISPRs, not able to cut the DNA but still able to target a defective Cas9 (dCas9) to a precise genome location, have been used to increase or decrease gene expression by fusing cas9 with activator or repressor elements, as well as epigenetically controlling gene transcription by fusing cas9 with chromatin regulator factors (Qi et al., 2013; Hilton et al., 2015). Although the use of CRISPR/Cas9 in post-mitotic cells is limited by HDR low activity, recently two similar approaches, based on NHEJ, opened new possibilities for gene editing *in vivo* (Nakade et al., 2014; Suzuki et al., 2016). Suzuki et al. demonstrated for the first time that using homology-independent targeted integration (HITI), based on NHEJ, it is possible to precisely insert a DNA sequence in a genome location in neurons *in vivo* using CRISPR/Cas9 (Suzuki et al., 2016). HITI successfully improved vision in a rat model of the retinal degeneration condition *retinitis pigmentosa* providing proof of principle that a gene therapy approach based on CRISPR/Cas9 can treat a neurological genetic disease. In this study a copy of a non-mutated exon 2 was inserted into the first intron of the faulty gene. Importantly this was achieved in mature neurons (Suzuki et al., 2016). The big challenge will be to precisely correct single mutations in ion channels in neurons. This approach could rescue severe channelopathies due to mutations in channels such as SCN1A, SCN2A, KCNQ2/3, CACNA1, KCN1A or GABRA1 which at present have few or no effective treatment options (Kullmann and Waxman, 2010; Spillane et al., 2016). The direct modification of the single mutation in an ion channel gene, the disruption of a faulty gene as well as the regulation of endogenous gene expression have advantages compared to the current strategies such as drug administration, optogenetics, chemogenetics, channel over-expression or RNA interference. However several challenges exist which must be overcome before gene editing can be used to rescue both acquired and genetic channelopathies (Bernard et al., 2004; Heidenreich and Zhang, 2016; Spillane et al., 2016). Some limitations are shared with “classical” gene therapy approaches (e.g. viral transduction efficiency, see below) others are more specific such as the size of the Cas9 or the possibility of off-target recognition by sequences of the gRNAs (Heidenreich and Zhang, 2016; Lee et al., 2016; Suzuki et al., 2016). Continued development of viral vectors and new CRISPR systems (saCAS9, SpCas9-HF1) are helping to overcome these limitations (Heidenreich and Zhang, 2016; Kleinstiver et al., 2016; Wright et al., 2016). Regarding the HITI and in general the gene editing mediated by NHEJ, it will be important to determine whether the inclusion of a few base pairs before and after the reintroduced nucleotide sequence will alter post-translational modifications to the channel and/or affect correct protein trafficking (Suzuki et al., 2016). Although there may be the possibility of gene editing at an embryonic stage (Callaway, 2016b) for genetic channelopathies, it is most likely, and only possible for acquired channelopathies, to use a viral approach to target CRISPR-Cas nucleases to multiple neurons in the mature brain. CRISPR development could permit a new era of treatments for neurological channelopathies. The ideal system would be to revert back to wild-type amino acid sequences. However with current methodology, additional amino nucleotides may be introduced. In this context it is important to insure that the coding

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

sequence will not be affected, especially for highly structured proteins such as ion channels.

4. Viral vector mediated delivery of gene therapies into neurons

The efficacy of a neuronal gene therapy strategy will depend on targeting disease-modifying agents to where they need to be, not only transduction of the correct cells and circuits but also to the appropriate intracellular localization. A viral strategy for the delivery of a therapeutic gene or gene-editing tools into a neuron is most likely, although alternatives such as liposomes or nanoparticle mediated delivery can also be considered (Naldini, 2015). The key to development of an effective viral vector is to harness the virus biology for transgene expression and to modify or remove the remaining viral genome in a manner that prevents pathogenic properties such as viral replication after host transduction. A number of genetically modified viruses are being developed that can be used to introduce heterologous genes or sequences of DNA into neurons in a safe manner. At present two main classes are most prominent: the lentiviruses and the adeno-associated viruses (AAVs). Other viruses transduce neurons, but can produce unacceptable toxicity, immune responses or transient transgene expression (HSV, Rabies, semliki forest virus) (Manfredsson and Mandel, 2010). In this article we will discuss the suitability of lentiviruses and AAVs to treat CNS channelopathies. However it should be noted that there are channelopathies which affect the PNS where a different viral approach may be preferential. For example non-replicating Herpes Simplex viruses are promising vehicles for delivery of therapeutic transgenes to the PNS (Glorioso and Fink, 2004).

4.1. Lentivirus

Lentiviral vectors are a popular vector for CNS gene therapy as they result in a long-lasting gene expression within neurons without inducing a significant host immune response (Abordo-Adesida et al., 2005). They permit a transgene capacity of ~9 kb, which is 2–3 times larger than AAVs. As lentivirus can integrate into the genome there is a hypothetical risk of insertional mutagenesis. To address potential concerns regarding chromosomal mutagenesis owing to the insertion of viral genes non-integrating constructs have been developed (Rahim et al., 2009; Wanisch and Yanez-Munoz, 2009). Lentiviruses cannot be delivered systemically and are required to be injected directly into the brain. Due in part to the large size of lentivirus (compared to AAV's) their diffusion through the extracellular space is constrained and therefore the spread of virus is highly restricted. In non-human primate brain, injection of 1 μl into the visual cortex of a lentivirus resulted in protein expression in the majority of neurons within 750 μm radius of the injection site (Lerchner et al., 2014). To achieve appropriate cortical coverage injections should be delivered within a distance of 1.5 mm from each other. Therefore lentiviruses are a more favourable choice for a channelopathy with a focal rather than global pathology.

4.2. AAVs

AAVs are currently the preferred gene delivery vector for treatment of CNS disorders (Blessing and Deglon, 2016; Choudhury et al., 2016a). They provide long-term transgene expression with minimal pathogenicity. There are numerous AAV serotypes and these can have different tropisms and distributions (Wu et al., 2006). AAV–delivered DNA usually exists in extrachromosomal episomes, although under rare circumstances it can integrate into the genome (High and Aubourg, 2011). As with lentiviruses there therefore exists a potential risk for insertional mutagenesis. Some AAV serotypes have a relatively high natural immunity (Zaiss and Muruve, 2005). Neutralising antibodies can prevent brain transduction (Boutin et al., 2010), therefore AAV serotypes for which the prevalence in the human population is low or non-existent will be required. Additionally acquired immunity could potentially complicate repeated treatment. However these concerns have not stopped clinical trials using these viruses for CNS disorders (Marks et al., 2010; LeWitt et al., 2011). The most significant shortcoming of AAVs may be their small payload capacity (~4.5 kb of DNA), which will preclude them from applications where delivery of large genes or regulatory sequences is required. Importantly more efficient self-complementary design AAV with great clinical efficacy are even more restrictive to the payload (2.2 kb) (Armbruster et al., 2016).

4.3. Promoters

Many experimental gene therapy studies utilise strong promotors in their viral vectors such as *cytomegalovirus* (CMV) to drive high transgene expression. In order to fit in the viral vectors functional fragments of promoters are often used instead the full-length promoters. Dependent on the type or serotype of virus used relatively specific expression in subpopulations of neurons can be achieved. However the viral tropism for subpopulations of neurons may not be consistent across species and raises concerns for translation. We have previously reported that lentiviral delivery of a transgene under the CMV promotor to rat motor cortex results in preferential transduction of pyramidal (excitatory) neurones (Wykes et al., 2012). However a different study, also using a lentivirus with a CMV promotor reported preferred transduction of glial cells when the virus was injected into non-human primate cortex (Lerchner et al., 2014). Depending on your gene therapy strategy it may be crucial to restrict expression of your transgene to a particular type of neuron. This could be relatively broad, for example expression only in either excitatory or inhibitory neurones. Or it may be necessary to be more specific and to discriminate between different subtypes of neurones for example parvalbumin or somatostatin positive inhibitory neurons. To achieve specific expression in excitatory glutamatergic neurons the CaMKIIa promotor is usually employed. To target GABAergic interneurons glutamate decarboxylase isoforms 65 or 67 promotors can be used, however due to the large size of mammalian interneuron specific promotor's popular viral vectors with limited payload capacity such as AAVs are no longer an option. There are also concerns regarding the specificity of these promoters *in vivo* (Mantoan Ritter et al., 2016). To allow use of smaller viral vectors in combination with interneuron specific promotors efforts have been made to find alternatives. These include expressing interneuron gene regulatory sequences from the fugu fish to drive expression of transgenes in mammalian brains (Nathanson et al., 2009) or inserting mDlx enhancers from DLX5/6 genes to drive transgene expression restricted to GABAergic interneurons in any vertebrate species (Dimidschstein et al., 2016). If available the use of endogenous promoter elements instead of exogenous promoters will be optimal for the regulation of expression of an endogenous channel. For the overexpression of exogenous therapeutic genes, such as a DREADD or opsins, neuronal subtype specific promoters are more appropriate.

4.4. Focal or global viral-mediated delivery of transgenes or CRISPR-Cas

Although many viral-mediated approaches have worked very well in experimental rodent studies there are additional factors to

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

consider in terms of translation to human brains, chief among these is the large difference in brain size. Some channel genes undergo differential splicing which varies among brain regions and neuronal populations restricting the protein product of these disease-associated mutations to discreet areas of the brain. In these cases viral vectors can be injected directly into the area of the brain required to reverse pathology. Many genetic channelopathies however have a global pathology. For an effective treatment widespread distribution of the vector will be required. Widespread transgene expression could be achieved via multiple distributed injections of virus (Simonato et al., 2013). Alternatively, injection into densely packed cell bodies that project their axons across large areas of the brain could result in extensive modulation of excitability across the brain. For example, the majority of cholinergic afferents to neocortex are from neurons in nucleus basalis. Targeting cell bodies within the small area of the nucleus basalis with a gene therapy could result in wide spread changes in acetylcholine modulation throughout the cortex (Kalmbach et al., 2012). Vectors could be injected into cerebrospinal fluid to allow distribution within the brain via the circulation. Alternatively some serotypes of AAV have the ability to penetrate the blood brain barrier (BBB), raising the possibility of a systemic administration of a virus that targets neurons without the need for surgery (Gray et al., 2010). AAV9 has been shown to cross the BBB and transduce large numbers of neurons and glia in rodents (Duque et al., 2009; Foust et al., 2009). Peripheral administration of viral vectors presents several challenges to overcome before translation to humans can occur. Systemic delivery will lead to transduction of cells outside the CNS, such as those in the liver, heart and skeletal muscle. Depending on the transgene selected for expression this could have serious and significant consequences for safety. To address these concerns in addition to using neuronal specific promotors to drive protein expression, micro-RNA motifs can be added to the viral DNA to minimise non-neuronal transduction. Incorporation of a liver specific micro-RNA (miR-122) binding site into the backbone of the AAV vector resulted in a dramatic reduction in the transduction of liver cells without affecting transduction efficiency in other cell types (Qiao et al., 2011). A similar strategy where several microRNA binding sites designed to repress AAV expression outside of the CNS were incorporated into the AAV9 vector resulted in high transduction efficiency in the CNS and low transduction efficiency in peripheral organs following intravascular delivery (Xie et al., 2011). Intense screening of naturally occurring, chimeric, or engineered AAV capsids with these desired characteristics, widespread CNS transduction with minimal transgene expression in off-target organs following intravenous injection, has resulted in AAV variants such as AAV2g9 (Murlidharan et al., 2016), AAV-PHP.B (Deverman et al., 2016; Jackson et al., 2016) or AAV-B1 (Choudhury et al., 2016b). These new generation of AAVs are capable of efficient widespread transfer of genes throughout the CNS following systemic injection.

4.5. Control of transgene expression

With the viral vector approaches previously discussed there is no control for transgene expression once a neuron has been transduced. As some ion channels undergo age-dependent changes in expression the ability to switch on or off the transgene of interest may be useful in the treatment of channelopathies where the pathology is only apparent at certain ages. In fact it may be prudent to discontinue expression of the therapeutic transgene once transcription of the faulty gene ceases. For these cases the use of promotors that can be induced in response to oral drugs can be used (Naidoo and Young, 2012). Currently these inducible promoters rely on antibiotic drugs and long-term use of these is undesirable, although prolonged antibiotic administration is used to treat other diseases such as acne (Bienenfeld et al., 2017). Therefore the development of non-antibiotic new molecules to induce promoter activity will be preferable.

5. When should gene therapy be delivered?

Of critical relevance to treat a channelopathy will be when to deliver the gene therapy. For gene editing therapies aimed at reversing the mutation underlying a severe channelopathy such as Dravet Syndrome, treatment may have to occur early in development. Dravet syndrome is mostly due to heterozygous single nucleotide mutations of the SCN1A gene (Brunklaus and Zuberi, 2014). Children with Dravet syndrome suffer from drug-resistant seizures, intellectual disability, behaviour and sleep problems (Wirrell, 2016). Once the formation of abnormal neuronal circuits during critical periods of development occurs, as a consequence, of the mutated ion channel, it is unlikely that a gene editing therapy aimed at reversing this mutation alone will be effective at treating all the symptoms of the disease if administered at a later stage in development (Moody and Bosma, 2005; Lai and Jan 2006). In contrast, in an adult brain, treatment of a channelopathy with a milder phenotype, where brain behaviour is relatively normal in between episodic attacks, is more likely to be amenable to a wider range of gene therapy strategies. Gene therapy approaches can be administered at both the germline and somatic genome level.

5.1. Germline genetic correction

Gene editing at the germline level could be the perfect treatment to eradicate inherited neurological channelopathies as well as other monogenic diseases (Baltimore et al., 2015; Vassena et al., 2016). This approach is permanent and will be passed on to further generations. Three different germline gene editing approaches are possible. 1. Gene editing in embryos before implantation. 2. Male and/or female germ cell modification. 3. iPS cells editing and differentiation (Vassena et al., 2016). CRISPR/Cas9 mediated gene editing for all these processes is relatively simple and efficient. The use of CRISPR/Cas9 to correct genes in embryos has already been published (Liang et al., 2015; Kang et al., 2016) and new studies are ongoing (Callaway, 2016a, b). This presents however several ethical concerns and technical limitations. The ability to change DNA at germline level permits modification of an organism before its birth. Ethical debate surrounding these issues both in the scientific community and wider public are ongoing (Evitt et al., 2015; Flotte, 2015; Lanphier et al., 2015). Technically the biggest hurdle to overcome for gene editing with CRISPR/Cas9 at the germline level will be to prevent off-targets effects. Unwanted changes in other genes due to similarity with the target sequence must be avoided (Kleinstiver et al., 2016).

Germline gene correction of mutations could potentially eradicate early-childhood devastating diseases such as Dravet Syndrome by preventing the development of the widespread network circuits abnormalities that lead to severe epilepsy, cognitive deficits and autistic behaviours (Brunklaus and Zuberi, 2014). This approach can also be used to treat channelopathies where expression of the protein is not only restricted to the brain. Timothy syndrome is a multi-organ system channelopathy due to gain of function mutations in the CACNA1C gene coding for the calcium channel Cav1.2. This disorder comprises long QT syndrome, autism, epilepsy, development deficits and immunodeficiency. Children mostly die prematurely for cardiac ventricular fibrillation (Heyes et al., 2015; Imbrici et al., 2016). Due to enhanced safety and ethical concern this approach is still far from clinical translation but could potentially be a game changer in the future.

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

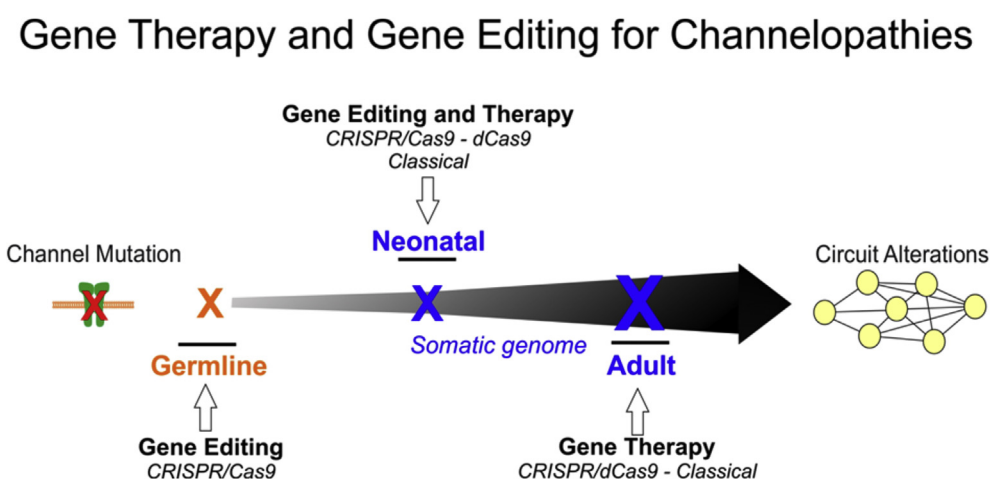

Fig. 1. Graphical representation of a developmental time course, indicating when gene therapy and gene editing interventions to treat a neuronal channelopathy are most likely to be effective. An ion channel mutation may result in abnormal developmental of the brain or circuit alterations. The optimal gene editing or gene therapy strategy will depend when during development they are delivered. Orange: germline intervention; blue: somatic genome intervention.

5.2. Somatic genome

Gene therapies on somatic cells imply that the treatments will be only for that patient and will not pass to offspring. These approaches can be applied at late-embryonic, neonatal or adult stage. The earlier the developmental stage, the easier it will be to target large brain regions. At an early developmental stage gene editing with CRISPR/Cas9 can be used to achieve gene correction (Suzuki et al., 2016). Once neuronal circuits are already established, classical gene therapy approaches to manipulate network excitability, either constitutively by up or downregulating endogenous proteins (overexpression of genes, siRNA, or CRISPR/dCas9); or on demand using optogenetics or chemogenetics are more appropriate as they have the ability to modulate network activity.

6. Conclusions

Gene therapies for non-neurological diseases are now achieving regulatory approval (Bryant et al., 2013; Gaudet et al., 2013). There has been remarkable progress in the last decade advancing the translational suitability of viral vectors and gene therapy for application to neurological disorders. The AAVs and lentiviruses that are currently used to deliver transgenes are increasingly reliable in terms of expressing the transgene, and data on long-term safety are accumulating from several neurological diseases (Simonato et al., 2013). Several clinical trials for degenerative neurological disorders have been reported (O'Connor and Boulis, 2015) and although the clinical outcome of these initial trials have usually failed to show efficacy they have demonstrated that delivery of gene therapies to the CNS is safe and well-tolerated (Bartus et al., 2014; Palfi et al., 2014).

Channelopathies usually manifest with abrupt periods of abnormal brain excitability. Decades of rodent experimental research in the epilepsy field has shown that gene therapy approaches can treat diseases of the brain that affect network excitability and some of these strategies may start clinical trials in the near future (Kullmann et al., 2014). Therefore adapting some of the gene therapy strategies used to treat epilepsy may be useful in the treatment of neuronal channelopathies.

Guide RNA-mediated CRISPR–Cas nucleases are powerful technologies for the engineering of mammalian genomes. Distinct approaches can be used to treat a channelopathy using a CRISPR-Cas strategy: disruption of gene expression, thereby reducing mutant protein levels; regulation of endogenous gene expression and epigenetic modifications; and finally, the repair of point mutations in a faulty gene, restoring normal function to the translated protein. Further developments in CRISPR technology will permit not only a novel treatment for neurological channelopathies but a potential cure for these diseases.

An important consideration in terms of which gene therapy or gene editing approach to take will be the time of intervention and whether the mutation results in widespread alterations in brain development and behaviours (Fig. 1). If it is possible to detect the channelopathy before permeant abnormal changes in brain function develop then treatment using CRISPR/Cas9 will potentially be the best therapy. However if the brain has developed and wired differently as a consequence of the channelopathy then 'fixing' the mutated gene underlying the disease in adult neurons may not be enough to fully reverse the pathology. In these cases classic gene therapy approaches to suppress or augment neuronal excitability are more likely to be therapeutically beneficial. Gene therapy approaches that optogenetically or chemogenetically supress or excites neurons on-demand are particularly attractive options to treat paroxysmal disorders.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgments

RCW is funded by an Epilepsy Research UK (F1401) fellowship and GL is funded by a Marie Skłodowska-Curie (658418) individual fellowship.

References

Abordo-Adesida, E., Follenzi, A., Barcia, C., Sciascia, S., Castro, M.G., Naldini, L., Lowenstein, P.R., 2005. Stability of lentiviral vector-mediated transgene expression in the brain in the presence of systemic antivector immune responses. Hum. Gene Ther. 16, 741–751.

Armbruster, B.N., Li, X., Pausch, M.H., Herlitze, S., Roth, B.L., 2007. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc. Natl. Acad. Sci. U. S. A. 104, 5163–5168.

Armbruster, N., Lattanzi, A., Jeavons, M., Van Wittenberghe, L., Gjata, B., Marais, T., Martin, S., Vignaud, A., Voit, T., Mavilio, F., Barkats, M., Buj-Bello, A., 2016.

Efficacy and biodistribution analysis of intracerebroventricular administration of an optimized scAAV9-SMN1 vector in a mouse model of spinal muscular atrophy. Mol. Ther. Methods Clin. Dev. 3, 16060.
Armstrong, C., Krook-Magnuson, E., Oijala, M., Soltesz, I., 2013. Closed-loop optogenetic intervention in mice. Nat. Protoc. 8, 1475–1493.
Assaf, F., Schiller, Y., 2016. The antiepileptic and ictogenic effects of optogenetic neurostimulation of PV-expressing interneurons. J. Neurophysiol. 116, 1694–1704.
Baltimore, D., Berg, P., Botchan, M., Carroll, D., Charo, R.A., Church, G., Corn, J.E., Daley, G.Q., Doudna, J.A., Fenner, M., Greely, H.T., Jinek, M., Martin, G.S., Penhoet, E., Puck, J., Sternberg, S.H., Weissman, J.S., Yamamoto, K.R., 2015. Biotechnology. A prudent path forward for genomic engineering and germline gene modification. Science 348, 36–38.
Bartus, R.T., Weinberg, M.S., Samulski, R.J., 2014. Parkinson's disease gene therapy: success by design meets failure by efficacy. Mol. Ther. 22, 487–497.
Berglind, F., Ledri, M., Sorensen, A.T., Nikitidou, L., Melis, M., Bielefeld, P., Kirik, D., Deisseroth, K., Andersson, M., Kokaia, M., 2014. Optogenetic inhibition of chemically induced hypersynchronized bursting in mice. Neurobiol. Dis. 65, 133–141.
Bernard, C., Anderson, A., Becker, A., Poolos, N.P., Beck, H., Johnston, D., 2004. Acquired dendritic channelopathy in temporal lobe epilepsy. Science 305, 532–535.
Bienenfeld, A., Nagler, A.R., Orlow, S.J., 2017. Oral antibacterial therapy for acne vulgaris: an evidence-based review. Am. J. Clin. Dermatol 1–22. http://dx.doi.org/10.1007/s40257-017-0267-z.
Blessing, D., Deglon, N., 2016. Adeno-associated virus and lentivirus vectors: a refined toolkit for the central nervous system. Curr. Opin. Virol. 21, 61–66.
Blits, B., Petry, H., 2016. Perspective on the road toward gene therapy for Parkinson's disease. Front. Neuroanat. 10, 128.
Boison, D., 2010. Inhibitory RNA in epilepsy: research tools and therapeutic perspectives. Epilepsia 51, 1659–1668.
Boutin, S., Monteilhet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M.F., Masurier, C., 2010. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum. Gene Ther. 21, 704–712.
Bovolenta, R., Zucchini, S., Paradiso, B., Rodi, D., Merigo, F., Navarro Mora, G., Osculati, F., Berto, E., Marconi, P., Marzola, A., Fabene, P.F., Simonato, M., 2010. Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. J. Neuroinflammation 7, 81.
Brunklaus, A., Zuberi, S.M., 2014. Dravet syndrome–from epileptic encephalopathy to channelopathy. Epilepsia 55, 979–984.
Bryant, L.M., Christopher, D.M., Giles, A.R., Hinderer, C., Rodriguez, J.L., Smith, J.B., Traxler, E.A., Tycko, J., Wojno, A.P., Wilson, J.M., 2013. Lessons learned from the clinical development and market authorization of Glybera. Hum. Gene Ther. Clin. Dev. 24, 55–64.
Callaway, E., 2016a. UK scientists gain licence to edit genes in human embryos. Nature 530, 18.
Callaway, E., 2016b. Gene-editing research in human embryos gains momentum. Nature 532, 289–290.
Chiang, C.C., Ladas, T.P., Gonzalez-Reyes, L.E., Durand, D.M., 2014. Seizure suppression by high frequency optogenetic stimulation using in vitro and in vivo animal models of epilepsy. Brain Stimul. 7, 890–899.
Choong, C.J., Baba, K., Mochizuki, H., 2016. Gene therapy for neurological disorders. Expert Opin. Biol. Ther. 16, 143–159.
Choudhury, S.R., Hudry, E., Maguire, C.A., Sena-Esteves, M., Breakefield, X.O., Grandi, P., 2016. Viral vectors for therapy of neurologic diseases. Neuropharmacology 1–18 pii: S0028-3908(16)30048-X. http://dx.doi/10.1016/j.neuropharm.2016.02.013 [Epub ahead of print].
Choudhury, S.R., Fitzpatrick, Z., Harris, A.F., Maitland, S.A., Ferreira, J.S., Zhang, Y., Ma, S., Sharma, R.B., Gray-Edwards, H.L., Johnson, J.A., Johnson, A.K., Alonso, L.C., Punzo, C., Wagner, K.R., Maguire, C.A., Kotin, R.M., Martin, D.R., Sena-Esteves, M., 2016b. Vivo selection yields AAV-B1 capsid for central nervous system and muscle gene therapy. Mol. Ther. 24, 1247–1257.
Chu, V.T., Weber, T., Wefers, B., Wurst, W., Sander, S., Rajewsky, K., Kuhn, R., 2015. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. Nat. Biotechnol. 33, 543–548.
Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., Zhang, F., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819–823.
Cox, D.B., Platt, R.J., Zhang, F., 2015. Therapeutic genome editing: prospects and challenges. Nat. Med. 21, 121–131.
Deisseroth, K., 2010. Controlling the brain with light. Sci. Am. 303, 48–55.
Deverman, B.E., Pravdo, P.L., Simpson, B.P., Kumar, S.R., Chan, K.Y., Banerjee, A., Wu, W.L., Yang, B., Huber, N., Pasca, S.P., Gradinaru, V., 2016. Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat. Biotechnol. 34, 204–209.
Dimidschstein, J., et al., 2016. A viral strategy for targeting and manipulating interneurons across vertebrate species. Nat. Neurosci. 19, 1743–1749.
Duque, S., Joussemet, B., Riviere, C., Marais, T., Dubreil, L., Douar, A.M., Fyfe, J., Moullier, P., Colle, M.A., Barkats, M., 2009. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. Mol. Ther. 17, 1187–1196.
Evitt, N.H., Mascharak, S., Altman, R.B., 2015. Human germline CRISPR-cas modification: toward a regulatory framework. Am. J. Bioeth. 15, 25–29.
Fenno, L., Yizhar, O., Deisseroth, K., 2011. The development and application of optogenetics. Annu. Rev. Neurosci. 34, 389–412.
Ferguson, S.M., Neumaier, J.F., 2012. Grateful DREADDs: engineered receptors reveal how neural circuits regulate behavior. Neuropsychopharmacology 37, 296–297.
Flotte, T.R., 2015. Therapeutic germ line alteration: has CRISPR/cas9 technology forced the question? Hum. Gene Ther. 26, 245–246.
Foust, K.D., Nurre, E., Montgomery, C.L., Hernandez, A., Chan, C.M., Kaspar, B.K., 2009. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat. Biotechnol. 27, 59–65.
Gaudet, D., Methot, J., Dery, S., Brisson, D., Essiembre, C., Tremblay, G., Tremblay, K., de Wal, J., Twisk, J., van den Bulk, N., Sier-Ferreira, V., van Deventer, S., 2013. Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for lipoprotein lipase deficiency: an open-label trial. Gene Ther. 20, 361–369.
Glorioso, J.C., Fink, D.J., 2004. Herpes vector-mediated gene transfer in treatment of diseases of the nervous system. Annu. Rev. Microbiol. 58, 253–271.
Gray, S.J., Blake, B.L., Criswell, H.E., Nicolson, S.C., Samulski, R.J., McCown, T.J., Li, W., 2010. Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood-brain barrier (BBB). Mol. Ther. 18, 570–578.
Haberman, R., Criswell, H., Snowdy, S., Ming, Z., Breese, G., Samulski, R., McCown, T., 2002. Therapeutic liabilities of in vivo viral vector tropism: adeno-associated virus vectors, NMDAR1 antisense, and focal seizure sensitivity. Mol. Ther. 6, 495–500.
Haberman, R.P., Samulski, R.J., McCown, T.J., 2003. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. Nat. Med. 9, 1076–1080.
Heidenreich, M., Zhang, F., 2016. Applications of CRISPR-Cas systems in neuroscience. Nat. Rev. Neurosci. 17, 36–44.
Heyes, S., Pratt, W.S., Rees, E., Dahimene, S., Ferron, L., Owen, M.J., Dolphin, A.C., 2015. Genetic disruption of voltage-gated calcium channels in psychiatric and neurological disorders. Prog. Neurobiol. 134, 36–54.
High, K.A., Aubourg, P., 2011. rAAV human trial experience. Methods Mol. Biol. 807, 429–457.
Hilton, I.B., D'Ippolito, A.M., Vockley, C.M., Thakore, P.I., Crawford, G.E., Reddy, T.E., Gersbach, C.A., 2015. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat. Biotechnol. 33, 510–517.
Horvath, P., Barrangou, R., 2010. CRISPR/Cas, the immune system of bacteria and archaea. Science 327, 167–170.
Imbrici, P., Liantonio, A., Camerino, G.M., De Bellis, M., Camerino, C., Mele, A., Giustino, A., Pierno, S., De Luca, A., Tricarico, D., Desaphy, J.F., Conte, D., 2016. Therapeutic approaches to genetic ion channelopathies and perspectives in drug discovery. Front. Pharmacol. 7, 121.
Incontro, S., Asensio, C.S., Edwards, R.H., Nicoll, R.A., 2014. Efficient, complete deletion of synaptic proteins using CRISPR. Neuron 83, 1051–1057.
Jackson, K.L., Dayton, R.D., Klein, R.L., 2016. Gene vector 'magic bullet': targeted expression in the central nervous system after peripheral delivery using the synapsin promoter. Expert Opin. Ther. Targets 20, 1153–1154.
Jimenez-Mateos, E.M., Engel, T., Merino-Serrais, P., McKiernan, R.C., Tanaka, K., Mouri, G., Sano, T., O'Tuathaigh, C., Waddington, J.L., Prenter, S., Delanty, N., Farrell, M.A., O'Brien, D.F., Conroy, R.M., Stallings, R.L., DeFelipe, J., Henshall, D.C., 2012. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. Nat. Med. 18, 1087–1094.
Kalmbach, A., Hedrick, T., Waters, J., 2012. Selective optogenetic stimulation of cholinergic axons in neocortex. J. Neurophysiol. 107, 2008–2019.
Kang, X., He, W., Huang, Y., Yu, Q., Chen, Y., Gao, X., Sun, X., Fan, Y., 2016. Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. J. Assist. Reprod. Genet. 33, 581–588.
Katzel, D., Nicholson, E., Schorge, S., Walker, M.C., Kullmann, D.M., 2014. Chemical-genetic attenuation of focal neocortical seizures. Nat. Commun. 5, 3847.
Kim, H., Kim, J.S., 2014. A guide to genome engineering with programmable nucleases. Nat. Rev. Genet. 15, 321–334.
Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z., Joung, J.K., 2016. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529, 490–495.
Krook-Magnuson, E., Armstrong, C., Oijala, M., Soltesz, I., 2013. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. Nat. Commun. 4, 1376.
Krook-Magnuson, E., Gelinas, J.N., Soltesz, I., Buzsaki, G., 2015. Neuroelectronics and biooptics: closed-loop technologies in neurological disorders. JAMA Neurol. 72, 823–829.
Krook-Magnuson, E., Szabo, G.G., Armstrong, C., Oijala, M., Soltesz, I., 2014. Cerebellar directed optogenetic intervention inhibits spontaneous hippocampal seizures in a mouse model of temporal lobe epilepsy. eNeuro 1.
Kros, L., Rooda, O.H., De Zeeuw, C.I., Hoebeek, F.E., 2015. Controlling cerebellar output to treat refractory epilepsy. Trends Neurosci. 38, 787–799.
Kullmann, D.M., 2010. Neurological channelopathies. Annu. Rev. Neurosci. 33, 151–172.
Kullmann, D.M., Waxman, S.G., 2010. Neurological channelopathies: new insights into disease mechanisms and ion channel function. J. Physiol. 588, 1823–1827.
Kullmann, D.M., Schorge, S., Walker, M.C., Wykes, R.C., 2014. Gene therapy in epilepsy-is it time for clinical trials? Nat. Rev. Neurol. 10, 300–304.
Ladas, T.P., Chiang, C.C., Gonzalez-Reyes, L.E., Nowak, T., Durand, D.M., 2015. Seizure reduction through interneuron-mediated entrainment using low frequency

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

optical stimulation. Exp. Neurol. 269, 120–132.
Lai, H.C., Jan, L.Y., 2006. The distribution and targeting of neuronal voltage-gated ion channels. Nat. Rev. Neurosci. 7, 548–562.
Lanphier, E., Urnov, F., Haecker, S.E., Werner, M., Smolenski, J., 2015. Don't edit the human germ line. Nature 519, 410–411.
Ledri, L.N., Melin, E., Christiansen, S.H., Gotzsche, C.R., Cifra, A., Woldbye, D.P., Kokaia, M., 2016. Translational approach for gene therapy in epilepsy: model system and unilateral overexpression of neuropeptide Y and Y2 receptors. Neurobiol. Dis. 86, 52–61.
Lee, H.B., Sundberg, B.N., Sigafoos, A.N., Clark, K.J., 2016. Genome engineering with TALE and CRISPR systems in neuroscience. Front. Genet. 7, 47.
Lerche, H., Shah, M., Beck, H., Noebels, J., Johnston, D., Vincent, A., 2013. Ion channels in genetic and acquired forms of epilepsy. J. Physiol. 591, 753–764.
Lerchner, W., Corgiat, B., Der Minassian, V., Saunders, R.C., Richmond, B.J., 2014. Injection parameters and virus dependent choice of promoters to improve neuron targeting in the nonhuman primate brain. Gene Ther. 21, 233–241.
LeWitt, P.A., et al., 2011. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. Lancet Neurol. 10, 309–319.
Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., Lv, J., Xie, X., Chen, Y., Li, Y., Sun, Y., Bai, Y., Songyang, Z., Ma, W., Zhou, C., Huang, J., 2015. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell 6, 363–372.
Lin, E.J., Young, D., Baer, K., Herzog, H., During, M.J., 2006. Differential actions of NPY on seizure modulation via Y1 and Y2 receptors: evidence from receptor knockout mice. Epilepsia 47, 773–780.
Liu, J., Gao, C., Chen, W., Ma, W., Li, X., Shi, Y., Zhang, H., Zhang, L., Long, Y., Xu, H., Guo, X., Deng, S., Yan, X., Yu, D., Pan, G., Chen, Y., Lai, L., Liao, W., Li, Z., 2016. CRISPR/Cas9 facilitates investigation of neural circuit disease using human iPSCs: mechanism of epilepsy caused by an SCN1A loss-of-function mutation. Transl. Psychiatry 6, e703.
Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., Church, G.M., 2013. RNA-guided human genome engineering via Cas9. Science 339, 823–826.
Manfredsson, F.P., Mandel, R.J., 2010. Development of gene therapy for neurological disorders. Discov. Med. 9, 204–211.
Mantoan Ritter, L., Macdonald, D.C., Ritter, G., Escors, D., Chiara, F., Cariboni, A., Schorge, S., Kullmann, D.M., Collins, M., 2016. Lentiviral expression of GAD67 and CCK promoter-driven opsins to target interneurons in vitro and in vivo. J. Gene Med. 18, 27–37.
Marks Jr., W.J., et al., 2010. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. Lancet Neurol. 9, 1164–1172.
Martin, V.T., Lipton, R.B., 2008. Epidemiology and biology of menstrual migraine. Headache 48 (Suppl. 3), S124–S130.
Maruyama, T., Dougan, S.K., Truttmann, M.C., Bilate, A.M., Ingram, J.R., Ploegh, H.L., 2015. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. Nat. Biotechnol. 33, 538–542.
Mazzuferi, M., Kumar, G., van Eyll, J., Danis, B., Foerch, P., Kaminski, R.M., 2013. Nrf2 defense pathway: experimental evidence for its protective role in epilepsy. Ann. Neurol. 74, 560–568.
McClelland, S., Flynn, C., Dube, C., Richichi, C., Zha, Q., Ghestem, A., Esclapez, M., Bernard, C., Baram, T.Z., 2011. Neuron-restrictive silencer factor-mediated hyperpolarization-activated cyclic nucleotide gated channelopathy in experimental temporal lobe epilepsy. Ann. Neurol. 70, 454–464.
McCown, T.J., 2006. Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. Mol. Ther. 14, 63–68.
McMahon, M.A., Cleveland, D.W., 2017. Gene therapy: gene-editing therapy for neurological disease. Nat. Rev. Neurol. 13, 7–9.
Moody, W.J., Bosma, M.M., 2005. Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. Physiol. Rev. 85, 883–941.
Murlidharan, G., Sakamoto, K., Rao, L., Corriher, T., Wang, D., Gao, G., Sullivan, P., Asokan, A., 2016. CNS-restricted transduction and CRISPR/cas9-mediated gene deletion with an engineered AAV vector. Mol. Ther. Nucleic Acids 5, e338.
Naidoo, J., Young, D., 2012. Gene regulation systems for gene therapy applications in the central nervous system. Neurol. Res. Int. 2012, 595410.
Nakade, S., Tsubota, T., Sakane, Y., Kume, S., Sakamoto, N., Obara, M., Daimon, T., Sezutsu, H., Yamamoto, T., Sakuma, T., Suzuki, K.T., 2014. Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9. Nat. Commun. 5, 5560.
Naldini, L., 2015. Gene therapy returns to centre stage. Nature 526, 351–360.
Nathanson, J.L., Jappelli, R., Scheeff, E.D., Manning, G., Obata, K., Brenner, S., Callaway, E.M., 2009. Short promoters in viral vectors drive selective expression in mammalian inhibitory neurons, but do not restrict activity to specific inhibitory cell-types. Front. Neural Circuits 3, 19.
Niu, Y., et al., 2014. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell 156, 836–843.
Noe, F., Nissinen, J., Pitkanen, A., Gobbi, M., Sperk, G., During, M., Vezzani, A., 2007. Gene therapy in epilepsy: the focus on NPY. Peptides 28, 377–383.
O'Connor, D.M., Boulis, N.M., 2015. Gene therapy for neurodegenerative diseases. Trends Mol. Med. 21, 504–512.
Palfi, S., et al., 2014. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. Lancet 383, 1138–1146.
Paradiso, B., Zucchini, S., Su, T., Bovolenta, R., Berto, E., Marconi, P., Marzola, A., Navarro Mora, G., Fabene, P.F., Simonato, M., 2011. Localized overexpression of FGF-2 and BDNF in hippocampus reduces mossy fiber sprouting and spontaneous seizures up to 4 weeks after pilocarpine-induced status epilepticus. Epilepsia 52, 572–578.
Paradiso, B., Marconi, P., Zucchini, S., Berto, E., Binaschi, A., Bozac, A., Buzzi, A., Mazzuferi, M., Magri, E., Navarro Mora, G., Rodi, D., Su, T., Volpi, I., Zanetti, L., Marzola, A., Manservigi, R., Fabene, P.F., Simonato, M., 2009. Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. Proc. Natl. Acad. Sci. U. S. A. 106, 7191–7196.
Paz, J.T., Davidson, T.J., Frechette, E.S., Delord, B., Parada, I., Peng, K., Deisseroth, K., Huguenard, J.R., 2013. Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. Nat. Neurosci. 16, 64–70.
Pei, Y., Dong, S., Roth, B.L., 2010. Generation of designer receptors exclusively activated by designer drugs (DREADDs) using directed molecular evolution. Curr. Protoc. Neurosci. 33. Chapter 4:Unit 4.
Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., Lim, W.A., 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152, 1173–1183.
Qiao, C., Yuan, Z., Li, J., He, B., Zheng, H., Mayer, C., Li, J., Xiao, X., 2011. Liver-specific microRNA-122 target sequences incorporated in AAV vectors efficiently inhibits transgene expression in the liver. Gene Ther. 18, 403–410.
Rahim, A.A., Wong, A.M., Howe, S.J., Buckley, S.M., Acosta-Saltos, A.D., Elston, K.E., Ward, N.J., Philpott, N.J., Cooper, J.D., Anderson, P.N., Waddington, S.N., Thrasher, A.J., Raivich, G., 2009. Efficient gene delivery to the adult and fetal CNS using pseudotyped non-integrating lentiviral vectors. Gene Ther. 16, 509–520.
Raol, Y.H., Lund, I.V., Bandyopadhyay, S., Zhang, G., Roberts, D.S., Wolfe, J.H., Russek, S.J., Brooks-Kayal, A.R., 2006. 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. 26, 11342–11346.
Richichi, C., Lin, E.J., Stefanin, D., Colella, D., Ravizza, T., Grignaschi, G., Veglianese, P., Sperk, G., During, M.J., Vezzani, A., 2004. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. J. Neurosci. 24, 3051–3059.
Roth, B.L., 2016. DREADDs for neuroscientists. Neuron 89, 683–694.
Rubio, A., Luoni, M., Giannelli, S.G., Radice, I., Iannielli, A., Cancellieri, C., Di Berardino, C., Regalia, G., Lazzari, G., Menegon, A., Taverna, S., Broccoli, V., 2016. Rapid and efficient CRISPR/Cas9 gene inactivation in human neurons during human pluripotent stem cell differentiation and direct reprogramming. Sci. Rep. 6, 37540.
Ryan, D.P., Ptacek, L.J., 2010. Episodic neurological channelopathies. Neuron 68, 282–292.
Sander, J.D., Joung, J.K., 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol. 32, 347–355.
Simonato, M., 2014. Gene therapy for epilepsy. Epilepsy Behav. 38, 125–130.
Simonato, M., Bennett, J., Boulis, N.M., Castro, M.G., Fink, D.J., Goins, W.F., Gray, S.J., Lowenstein, P.R., Vandenberghe, L.H., Wilson, T.J., Wolfe, J.H., Glorioso, J.C., 2013. Progress in gene therapy for neurological disorders. Nat. Rev. Neurol. 9, 277–291.
Snowball, A., Schorge, S., 2015. Changing channels in pain and epilepsy: exploiting ion channel gene therapy for disorders of neuronal hyperexcitability. FEBS Lett. 589, 1620–1634.
Soper, C., Wicker, E., Kulick, C.V., N'Gouemo, P., Forcelli, P.A., 2016. Optogenetic activation of superior colliculus neurons suppresses seizures originating in diverse brain networks. Neurobiol. Dis. 87, 102–115.
Sorokin, J.M., Davidson, T.J., Frechette, E., Abramian, A.M., Deisseroth, K., Huguenard, J.R., Paz, J.T., 2017. Bidirectional control of generalized epilepsy networks via rapid real-time switching of firing mode. Neuron 93, 194–210.
Spillane, J., Kullmann, D.M., Hanna, M.G., 2016. Genetic neurological channelopathies: molecular genetics and clinical phenotypes. J. Neurol. Neurosurg. Psychiatry 87, 37–48.
Straub, C., Granger, A.J., Saulnier, J.L., Sabatini, B.L., 2014. CRISPR/Cas9-mediated gene knock-down in post-mitotic neurons. PLoS One 9, e105584.
Sukhotinsky, I., Chan, A.M., Ahmed, O.J., Rao, V.R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K., Majewska, A.K., Cash, S.S., 2013. Optogenetic delay of status epilepticus onset in an in vivo rodent epilepsy model. PLoS One 8, e62013.
Suzuki, K., et al., 2016. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature 540, 144–149.
Swann, J.W., Rho, J.M., 2014. How is homeostatic plasticity important in epilepsy? Adv. Exp. Med. Biol. 813, 123–131.
Swiech, L., Heidenreich, M., Banerjee, A., Habib, N., Li, Y., Trombetta, J., Sur, M., Zhang, F., 2015. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat. Biotechnol. 33, 102–106.
Theofilas, P., Brar, S., Stewart, K.A., Shen, H.Y., Sandau, U.S., Poulsen, D., Boison, D., 2011. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 52, 589–601.
Vassena, R., Heindryckx, B., Peco, R., Pennings, G., Raya, A., Sermon, K., Veiga, A., 2016. Genome engineering through CRISPR/Cas9 technology in the human germline and pluripotent stem cells. Hum. Reprod. Update 22, 411–419.
Wagnon, J.L., Meisler, M.H., 2015. Recurrent and non-recurrent mutations of SCN8A in epileptic encephalopathy. Front. Neurol. 6, 104.
Wanisch, K., Yanez-Munoz, R.J., 2009. Integration-deficient lentiviral vectors: a slow coming of age. Mol. Ther. 17, 1316–1332.
Wiedenheft, B., Sternberg, S.H., Doudna, J.A., 2012. RNA-guided genetic silencing systems in bacteria and archaea. Nature 482, 331–338.

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029

Wirrell, E.C., 2016. Treatment of Dravet syndrome. Can. J. Neurol. Sci. 43 (Suppl. 3), S13–S18.
Woldbye, D.P., Angehagen, M., Gotzsche, C.R., Elbrond-Bek, H., Sorensen, A.T., Christiansen, S.H., Olesen, M.V., Nikitidou, L., Hansen, T.V., Kanter-Schlifke, I., Kokaia, M., 2010. Adeno-associated viral vector-induced overexpression of neuropeptide Y Y2 receptors in the hippocampus suppresses seizures. Brain 133, 2778–2788.
Wright, A.V., Nunez, J.K., Doudna, J.A., 2016. Biology and applications of CRISPR systems: harnessing Nature's toolbox for genome engineering. Cell 164, 29–44.
Wu, Z., Asokan, A., Samulski, R.J., 2006. Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol. Ther. 14, 316–327.
Wykes, R.C., Kullmann, D.M., Pavlov, I., Magloire, V., 2016. Optogenetic approaches to treat epilepsy. J. Neurosci. Methods 260, 215–220.
Wykes, R.C., Heeroma, J.H., Mantoan, L., Zheng, K., MacDonald, D.C., Deisseroth, K., Hashemi, K.S., Walker, M.C., Schorge, S., Kullmann, D.M., 2012. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci. Transl. Med. 4, 161ra152.
Xie, J., Xie, Q., Zhang, H., Ameres, S.L., Hung, J.H., Su, Q., He, R., Mu, X., Seher Ahmed, S., Park, S., Kato, H., Li, C., Mueller, C., Mello, C.C., Weng, Z., Flotte, T.R., Zamore, P.D., Gao, G., 2011. MicroRNA-regulated, systemically delivered rAAV9: a step closer to CNS-restricted transgene expression. Mol. Ther. 19, 526–535.
Xu, Z., Wang, Y., Chen, B., Xu, C., Wu, X., Wang, Y., Zhang, S., Hu, W., Wang, S., Guo, Y., Zhang, X., Luo, J., Duan, S., Chen, Z., 2016. Entorhinal principal neurons mediate brain-stimulation treatments for epilepsy. EBioMedicine 14, 148–160.
Yang, Y., Walsh, C.E., 2005. Spliceosome-mediated RNA trans-splicing. Mol. Ther. 12, 1006–1012.
Zaiss, A.K., Muruve, D.A., 2005. Immune responses to adeno-associated virus vectors. Curr. Gene Ther. 5, 323–331.

Please cite this article in press as: Wykes, R.C., Lignani, G., Gene therapy and editing: Novel potential treatments for neuronal channelopathies, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.05.029