

Genetic engineering within the adult brain: Implications for molecular approaches to behavioral neuroscience

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Abstract

Currently, the most popular technology used to modify the molecular makeup of the nervous system is through germline modifications of early embryos. This allows to construct gene 'knock-ins' (gene overexpression) or 'knock-outs' (gene deletions). This technology leads to gene additions or deletions from the earliest developmental stages. This can potentially lead to compensatory genetic changes. The technology to achieve inducible and cell-type-specific changes in gene expression in transgenic animals has been established. However, it is not yet possible, to reliably turn a particular gene 'on' or 'off' exclusively in adult animals. Alternatively, the use of gene transfer technology in fully mature animals could overcome many of these shortcomings. Gene therapy is the use of nucleic acids as drugs, and uses gene transfer technology to genetically engineer adult animals. Viral and nonviral vectors have been modified to serve as vectors for nucleic acid sequences of interest. Thus, over the last two decades, methods have been developed to deliver particular nucleic acids directly to target tissues. Further technological advances allow delivery of transgenes or antisense mRNAs directly to predetermined cell types, as well as their delivery under the control of inducible promoter elements. Combined transgenic (i.e., germline modifications) and viral vector technology will also be very powerful in allowing the genetic modification of selected neuronal populations in adult animals. In this review, we discuss the potential of gene delivery to the brain to analyze the effect of genetic engineering of particular neuronal groups on behavior, as well as recent developments and applications of newly engineered vector systems to allow transgenesis within nervous structures of adult animals. © 2001 Elsevier Science Inc. All rights reserved.

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When you have eliminated the impossible, whatever remains, however improbable, must be the truth.
(Sir Arthur Conan Doyle)

1. Introduction

Human gene therapy is entering middle age, as illustrated by recent 'trials and tribulations', and a book that traces its history [1,2]. Importantly, the rise of clinical gene therapy

has sponsored a veritable growth in techniques that allow the genetic engineering of adult organs, including the brain, without the need of germline modifications [2–5]. Modern gene transfer techniques using viral or nonviral vectors allow to modify the genetic makeup of individual brain cells to assess its effects on neuronal physiology, and eventually on whole animal behavior without having to engineer the development of the experimental subjects. In spite of many recent advances in the development of safer, stable, and more efficient vectors for gene transfer into the brain, their use to modify adult neurons to dissect their role in behavior is still underrepresented in the scientific literature [2–11].

The main reasons holding back more widespread and common use of gene transfer into the brain must be found in the technical challenges of current transgene delivery systems, i.e., the gene transfer vectors [2–6,11,12], and the lack of easily available practical gene transfer technology

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expertise available in many neuroscience and behavioral laboratories. The main perceived limitations of gene transfer remain the short-term expression of transgenes, and vector instability in the presence of inflammatory and immune responses directed against vectors and/or transgenes [13–18]. However, most of these shortcomings are being overcome, and major progress has been achieved during the last 2–3 years [17–19].

Recent data using helper-dependent high-capacity, or ‘gutless’ adenoviruses, adenovirus-associated virus (AAV), and lentiviral vectors indicate that long-term expression can be achieved both in the presence or absence of vector genome integration [17–24]. Long-term expression of transgenes in the brain has been achieved for up to 12 months and longer for each of these types of viral vector. Whether transgene expression will be shown to be 100% stable over such long periods of time, or whether it will slowly taper off, remains to be assessed in quantitative detail. Importantly, decrease in transgene expression over time is not specific to the brain, but occurs in all organs in which long-term expression has been examined.

Mechanisms underlying the instability of long-term transgene expression are likely to be vector- and transgene-specific and remain to be determined for particular vector/transgene combinations. Furthermore, although in many experimental paradigms, inflammatory and immune processes will down-regulate transgene expression [13–18], inflammatory and immune cells have been shown to be beneficial for neuronal survival to injury [24,25]. Therefore, the long-term implications of inflammatory and immune responses for transgene longevity in the central and peripheral nervous system need to be thoroughly assessed [13–18,24,25], for each vector system [19–22]).

Although vector genome integration into target cells’ DNA is thought to support long-term transgene expression, transgene expression in the CNS may not depend on this. In fact, herpes simplex virus type 1 (HSV-1) normally infects dorsal root ganglia neurons and during latency remains episomally in such neuronal nuclei, stably expressing a few species of mRNAs during the life of the infected individual [11,20–21,26]. Thus, strong evidence indicates that viral genome integration is not absolutely necessary to achieve long-term stable persistence of viral genomes in neuronal cells. Further evidence from AAV vectors also suggests that expression may occur from both integrated and nonintegrated copies of the vectors’ genomes [27].

2. Recent vector developments: implications for the molecular analysis of behavior

Two changes need to be introduced into a virus to make it into a gene transfer vector: its replication needs to be inhibited to reduce or eliminate its cytopathogenic potential, and a transgenic expression cassette has to be inserted into the vector’s genome [2–6]. Several viruses have been

developed for gene transfer applications: the most relevant for gene transfer into the brain are those vectors derived from murine retroviruses, human and animal lentiviruses, adenoviruses, HSV-1, AAV, Semliki–Forest virus, and Sindbis virus (reviewed in Refs. [2–6]).

Murine retroviruses remain the classic gene transfer vector, and have spearheaded much of viral vector development. Murine retroviruses have been mainly used in applications of gene transfer into cells that are then transplanted directly into the brain. In this capacity, murine retroviruses are powerful vectors, even if they are still limited by the eventual turning off of promoter activity after long-term implantation of such genetically engineered cells into the brain. Their main limitation remains their incapacity to transduce nondividing brain cells, and very low levels of general transduction after their direct injection into the brain ‘in vivo’.

Lentiviral vectors have been initially derived from the human immunodeficiency virus type 1, but other animal lentiviruses have since been engineered to serve as vectors, e.g., simian immunodeficiency virus, feline immunodeficiency virus, and equine infectious anemia virus [2,3,28]. The main advantage of lentiviral vectors over previously clinically employed murine retroviral vectors is their capacity to express transgenes in nondividing cells. This is due to lentiviruses’ capacity to cross the nuclear membrane, and thereby insert their genomes into the target cells’ DNA in the absence of nuclear membrane breakdown during mitosis [2,3] (although this has been contested [29]). The development of lentiviruses for in vivo gene transfer has generated much excitement, especially for gene transfer into the postmitotic cells constituting the adult brain [19,28,30–32]; the longevity of transgene expression, furthermore, remains to be thoroughly characterized. Nevertheless, the packaging capacity of lentiviruses (i.e., the amount of transgenic sequence carried by lentiviral vectors) remains, as for retroviral vectors, below 10 kbp [28], and the random integration into host chromosomes will remain a potential drawback.

AAV have also been shown to integrate their genomes into nondividing cells. While in certain cell types, the AAV genome has been shown to integrate into host chromosomes, this remains to be demonstrated in neurons. However, it is even unclear whether such integration is necessary for long-term transgene expression within nerve cells. Importantly, recent advances in AAV vector technology have shown long-term transgene expression in the CNS. Also, AAV vectors have been engineered to achieve targeted delivery of transgenes to particular types of brain cells, vectors allowing the encoding of larger constructs have been produced, and it was recently discovered that different AAV serotypes are able to transduce different cell types within the CNS of adult animals [23,30,33,34].

HSV-1 is a neurotropic virus that remains latent for decades in the cell bodies of dorsal root ganglion neurons, while continuing to express just a few mRNA species from

its LAT region. HSV-1 is being made into powerful vectors by reducing or eliminating its cytotoxicity, and impairing its capacity for viral reactivation. However, HSV-1-derived vectors able to express transgenes for long term in neurons, without major associated neurotoxicity in the forebrain, have constituted an important technical challenge. More recently, HSV-1 vectors expressing stably for up to 6 months in neurons from the dorsal root ganglia and brainstem have been produced, and various alternative promoter/vector systems for long-term transgene expression are being developed. Much of the toxicity of HSV-1 vectors is due to remaining expression of the immediate early genes (e.g., ICP0) [4,22,27,47]. Thus, several groups have aimed to reduce or eliminate expression of immediate early genes. Long-term expression is then attempted through manipulations of the promoter/enhancers within the LAT region of HSV-1 and interesting results on long-term expression have been reported recently [7–11,20–22,26,36]. Whether vectors active in dorsal root ganglia and brainstem could be used also for long-term transgene expression in forebrain neurons of the striatum and/or neocortex remains to be determined. Importantly, helper-dependent HSV-1 ‘amplicon’ vectors have now been generated in the absence of any contaminating helper virus. These allow the insertion of large transgenic sequences. In addition, they do not express any viral proteins, apart from those carried by HSV-1 in its capsid and tegument. Such vectors completely deleted of any wildtype virus genes are very promising to achieve long-term, safe, and stable gene delivery to the brain [7–10,26,37].

A separate use of particular interest to neurobiologists has been made of certain herpes viruses. Within the herpes viruses, alpha herpes viruses (especially pseudorabies virus) have been exploited as neuroanatomical tracers. Because of their capacity to cross synapses (and different virus strains can do so in an anterograde or retrograde fashion) they have been extensively used as transneuronal tracers in the mapping of neuronal circuits [38] (for details, see Refs. [4,5]).

Helper-dependent, high-capacity (‘gutless’) adenoviruses represent a significant improvement in the engineering of adenoviruses as viral vectors [39]. These vectors only retain viral sequences necessary for genome packaging (ψ) and replication (viral inverted terminal repeats), and the viral protein capsid itself; ‘gutless’ vectors are grown with helper viruses that provide in trans all sequences necessary for viral replication, packaging, and encapsidation into viral capsids. The advantages of ‘gutless’ adenoviruses are that they do contain a very limited amount of adenoviral genomic material, none of which encodes for proteins. Initial reports highlighted that these artificial genomes turned out to be very unstable in cells, resulting only in transitory transgenic expression. Newer constructs however, containing large genomic sequences from the human hypoxanthine guanine phosphoribosyl transferase (HPRT) locus, have shown to be stable over several months, allowing sustained transgene expression in several organs, including

the rodent brain [17,18,39]. These vectors turned out to be very stable in naïve animals, when compared to first-generation vectors, especially in the long term. In addition, these vectors were completely stable in the brain, following an immunization of experimental animals against first-generation viral vectors.

Further, more recent experiments have shown that in preimmunized animals, while expression from first-generation adenoviruses is eliminated within the first month, expression from gutless vectors, although reduced, remains stable. The reason for their stability remains to be elucidated. Most likely, this depends on the complete absence of synthesis of any viral proteins encoded by the vector genome of first-generation vectors. Various experiments have now demonstrated that the genomes of first-generation vectors do express viral proteins, even in the brain, and even in the absence of any E1 gene products. Surprisingly, the genomes of first-generation adenoviruses may also turn out to be more stable than what was initially thought, and remain within infected cells for long periods of time. However, the immune response, apparently against viral proteins, eventually completely eliminates transgene expression. In the brain, phenotype reversion in a model of diabetes insipidus was achieved over 6 months using a first-generation adenovirus [40]. It is likely that longevity of transgene expression will also depend on the particular promoters being utilized.

Semliki-Forest virus and Sindbis virus are RNA virus-derived vectors that have also been exploited for gene transfer into neurons [41] (for details, see Ref. [4]). RNA viruses provide high levels of transgene expression. The particular biology of RNA viruses, however, determines that expression from these vectors has not been regulated, and the very high level of unregulated transgene expression are likely to be cytotoxic to transduced cells in the medium to long term. Although these vectors appear to infect neurons with high efficiency, applications of RNA virus-derived vectors need to remain limited to experiments that require short-term expression.

3. Perceived limitations of in vivo gene transfer?

In November 1999, we published a paper that described some of the long-term consequences encountered in a syngeneic model of brain glioblastoma in rats after successful inhibition of brain tumor growth [14]. We described the existence of a chronic inflammatory infiltration, evidence of neurodegeneration, and the presence of demyelinated lesions (of cytotoxic, though not autoimmune origin) ipsilateral to the administration of the gene therapy. We also demonstrated that chronic brain inflammation was due to the virus itself and the expression of the conditional cytotoxic gene, herpes simplex type 1 thymidine kinase in the presence of the prodrug ganciclovir. This report was published 1 month after the death of the first gene therapy

patient, Jesse Gelsinger, was announced publicly. This tragedy occurred during a clinical trial for the treatment of ornithine transcarbamylase deficiency. Mr. J. Gelsinger received the highest dose of an E1/E4-deleted adenovirus vector expressing the missing enzyme during a clinical trial, and died 2 days after the injection of the recombinant virus [2]. Detailed reports on the analysis of this sad and serious adverse event during a clinical gene therapy trial are now being made public shortly.

Since then, others, and we, have made major improvements to the safety of adenoviral vectors [16,17,39,42–45]. Improvements needed to abide by the stringent criteria applied when planning human clinical trials, certainly benefit the development of vectors for their application in basic neuroscience research.

A particular issue that remained poorly understood is the generally perceived low efficiency of vectors needed to transduce enough cells in order for transgene expression to be detected. This meant that, for all vectors in current use, very high doses were being injected into the brains of experimental animals. The use of high doses, in turn, meant that target cells were being infected by multiple viral particles. Whether this was due to poor infection of brain cells, low levels of expression from viral promoters in neuronal cells, or vector cytotoxicity remained unknown. The high doses of vectors used, however, were usually accompanied by important inflammatory reactions. Using an extremely powerful promoter, namely the major immediate early murine cytomegalovirus promoter, we have now shown that one single infectious adenoviral particle is all that is needed to transduce a particular brain cell, in our case, astrocytes [42]. This indicates that much more work needs to be done on developing adequate high-level expression promoters. Importantly, however, it indicates that, if these are used, very low doses of vectors can be shown to be very effective. Infection of brains with low doses of viral vectors also allows reducing the vector-related cytotoxicity and inflammation.

Strong promoters in combination with the use of safer vectors, such as the gutless adenoviruses, will undoubtedly allow the safe, stable, noncytotoxic, and noninflammatory transfer of genes into the adult CNS. This should allow the use of lower doses of vectors to transduce a predictable number of cells in the brain, to assess the behavioral role of low numbers of genetically engineered neurons, or restrict modifications of neuronal physiology and/or architecture.

4. In vivo gene transfer into the CNS: what has been achieved so far?

A wide variety of improvements to existing viral vectors have been developed over the last few years. Powerful vectors, which are devoid of all viral genes, derived from lentivirus [19,30–32,34], HSV-1 ‘amplicons’ [7–10,37,46], HSV-1 recombinant vectors [11,20–22], and adenovirus

(‘high-capacity, helper-dependent gutless’) [17,18,39] can now be constructed. Although viral vectors will always need to retain the viral capsids, they are devoid of any viral genomic sequences able to synthesize any immunogenic proteins that can be targeted by the adaptive immune system. Regulatory transcriptional systems have also been developed that allow transgenes to be turned ‘on’ or ‘off’ at will and that will allow the level of the transgene to be manipulated experimentally in whole animals and human patients and have been engineered into various types of viral vectors. Further, various systems have been recently developed that allow transgene expression to be induced in individual predetermined cell types. A number of groups have now combined the astrocyte specific (GFAP) promoter with the tetracycline-inducible transcriptional system to achieve astrocyte-specific and inducible transgene expression from adenoviral vectors. Further, neuronal-specific and inducible transgene expression has been achieved through the use of the synapsin or NSE promoters [43,44]. In addition to this transcriptional targeting of transgene expression, there is much ongoing work on the direct targeting of viral capsids [47,48].

New generations of lentivirus-, adenovirus-, AAV-, Semliki-Forest virus-, Sindbis virus-, or HSV-1-derived vectors described above are currently available, which allow gene transfer to large areas of the brain, with some vectors being able to do so in the absence of acute inflammation [19,42]. Further systems have been developed that can retarget transgene expression to infect specific cell types (through genetic and molecular modification of viral capsids) [47–49] or allow expression in particular predetermined cells, when transgene expression is placed under the control of cell type and inducible promoter elements [43,44], and stably, for several months. We have recently shown that the use of high-capacity helper-dependent new-generation adenoviral vectors bypasses the immune-mediated elimination of transgene expression in the brain of transduced animals, which were immunized against adenovirus [17,18].

Unpublished evidence from a number of laboratories, including our own, also indicates that transgene expression from these vectors can be achieved in preimmunized animals [18]. Further data from our laboratory have shown that the use of strong promoters can reduce the viral doses needed by up to three logs [42]. This impressive reduction in the dose of vector needed to transduce relatively large brain areas allows doing so in the complete absence of any acute inflammatory responses against adenoviral vectors. This, together with the longevity of transgene expression (up to 12 months), has allowed us to demonstrate that long-term, stable, noninflammatory, and nonimmune transgene expression can be achieved in the brain for long periods, even in immunized animals (Fig. 1). Also, vectors have been used to image the expression of transgenes in vivo utilizing novel imaging techniques [50].

Although some viral-based methods to selectively inhibit the electrophysiological function of individual neurons have

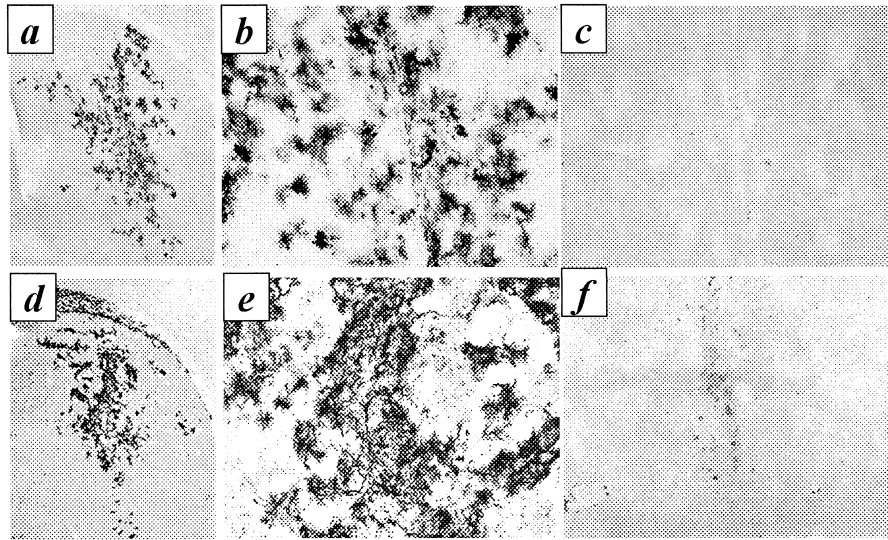


Fig. 1. This illustrates that transgene expression from the novel high-capacity adenoviral vectors (injected at 10^7 infectious units) persists, even after the peripheral immunization with adenovirus [(a) low-power, β -galactosidase immunocytochemistry; (b) high-power, β -galactosidase immunocytochemistry] and in the complete absence of inflammation [(c) infiltrating CD8 cells]. The use of a very powerful promoter illustrates comparable transgene expression at 10^5 infectious units [(d) low-power, β -galactosidase immunocytochemistry; (e) high-power, β -galactosidase immunocytochemistry], also in the complete absence of inflammation [(f) infiltrating CD8 cells]. Images are modified from Refs. [17,42].

been described [51], it is likely that more general vectors of this type will be developed in the near future. Given that highly efficient vector systems can now be engineered and that, in addition, allow long-term, safe, noncytotoxic, and nonimmunogenic transgene expression that furthermore can be imaged in brain slices, and eventually in whole animals, it can be predicted that highly efficient tools for the selective turning on or off, and/or for the specific genetic engineering of predetermined neuronal groups will be developed in the near future.

5. In vivo gene transfer: potential use for transient and reversible behavioral modifications of adult animals

All types of vectors described herein can be potentially used to transfer genes into the brains of adult animals to affect behavioral changes. Viral vectors have been most recently used in the following applications of transient transgenesis of adult animals: marking experiments, regulated transgene expression, imaging of transgene expression, growth factor delivery in models of neurodegeneration, physiological modifications of neuronal function, etc.

Of great interest are the recent behavioral modifications induced by overexpression of NGF in adult animals [7,8]. In these experiments, animals were engineered to contain an NGF transgene under the control of a promiscuous promoter. However, NGF could not be expressed, because the transgene had been engineered in the direction opposite of the promoter, and was flanked by loxP sites. Thus, expression of *cre* recombinase in target cells would allow expression of NGF. Infection of the hippocampus with HSV-1

vectors expressing the *cre* recombinase led to restricted NGF overexpression. The authors then analyzed the behavioral consequences of localized unilateral hippocampal overexpression of NGF in adult animals. Overexpression of NGF allowed compensating faster and at higher levels the effects of a lesion to the septohippocampal system.

Various papers have now examined the role of particular signal transduction pathways in neuronal apoptosis [52], membrane receptor dynamics [10,53], or the molecular basis of surface expression of neurotransmitter receptors [54]. Although most of this work has been done using adenoviral or HSV-1 vectors, this is possibly related to the ease of production, and larger size of transgenic sequences that can be inserted into such vectors. Nevertheless, even if most vectors could have been adapted for use in these experiments, in planning particular experimental designs it is important to carefully balance pros and cons of each vector system.

Of particular interest to gene therapy and neuroscience scientists is the long-term modification of the brain, especially concerning the expression of growth and survival factors. Adenoviral vectors have been used to reverse hypothalamic diabetes insipidus in Brattleboro rats [40]. Further, AAV, adenoviral vectors, lentiviral vectors, and HSV-1-derived vectors have been utilized to transduce the adult brain with specific neuronal growth factors and other therapeutic transgenes in order to avoid neurodegeneration. One of the best studied has been GDNF, in animal models of aging and Parkinson's disease, and lysosomal enzymes in models of mucopolysaccharidoses. Various types of viral vectors expressing GDNF and β -glucuronidase respectively, have been shown to promote the survival and/or regeneration of

lesioned nigrostriatal dopaminergic neurons or striatal and cortical neurons [19,23,32, 34,35,55,56]. Of special interest has been the recent report of lentiviral vectors expressing GDNF, which have been shown to counteract both the effects of normal aging, as well as those of MPTP neurotoxin, on the number of nigrostriatal neurons in nonhuman primates [19]. In addition, other experimental models have been used to explore the effects of replacing missing genes, in animal models of inherited neurological disorders. It is important to take into account that in both experimental Parkinson's disease models and in animal models such as MPSVII, in which the missing gene product is a factor active in the extracellular space, or is a secreted enzyme that can be taken up by other cells, widespread delivery of growth factor or correction of enzyme deficiency throughout the brain has been achieved [19,23,32,34,35,55,56,57]. The challenge remains to achieve similar high levels of therapeutic activity with transgene products active intracellularly.

6. Conclusions

In summary, in spite of relatively few groups having exploited current gene transfer technology to dissect molecular pathways underlying adult brain function, it can be predicted that these studies will increase in popularity over the next decade. Even if the construction of each viral vector still remains a complex procedure requiring the collaboration of specialized and dedicated laboratories, more and more commercial laboratories are offering these services. Recent important advances in producing safe, noninflammatory, noncytotoxic, cell-type-specific, and inducible viral vectors ought to allow their exploitation by the behavioral neuroscience community. Their more generalized use will open up the examination of the role of individual genes encoding for neurotransmitter receptor subtypes, signal transduction molecules, neuronal growth factors, or extracellular matrix proteins in the function of not only individual neurons, but also of entire neuronal networks underlying specific behaviors, in otherwise genetically intact animals.

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References

- [1] Friedmann T, editor. The development of human gene therapy. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1999.
- [2] Somia N, Verma IM. Gene therapy: trials and tribulations. *Nat Rev Genet* 2000;1:91–9.
- [3] Glorioso JC, Naldini L, Kay MA. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat Med* 2001;7:33–40.
- [4] Lowenstein PR, Enquist LW. Protocols for gene transfer in neuroscience: towards gene therapy of neurological disorders. Chichester: Wiley, 1996.
- [5] Kaplitt MG, Loewy AD. Viral vectors: gene therapy and neuroscience applications. San Diego: Academic Press, 1995.
- [6] Verma IM, Somia N. Gene therapy: promises, problems and prospects. *Nature* 1997;389:239–42.
- [7] Brooks AI, Cory-Slechta DA, Bowers WJ, Murg SL, Federoff HJ. Enhanced learning in mice parallels vector-mediated nerve growth factor expression in hippocampus. *Hum Gene Ther* 2000;11:2341–52.
- [8] Brooks AI, Cory-Slechta DA, Federoff HJ. Gene–experience interaction alters the cholinergic septohippocampal pathway of mice. *Proc Natl Acad Sci USA* 2000;97:13378–83.
- [9] Neve RL, Geller AI. Genetic analysis of neuronal physiology with defective herpes simplex virus vectors. *Adv Neurol* 1999;79:1027–32.
- [10] Telfeian AE, Federoff HJ, Leone P, During MJ, Williamson A. Overexpression of GluR6 in rat hippocampus produces seizures and spontaneous nonsynaptic bursting in vitro. *Neurobiol Dis* 2000;7:362–74.
- [11] Simonato M, Manservigi R, Marconi P, Glorioso J. Gene transfer into neurons for the molecular analysis of behaviour: focus on herpes simplex vectors. *TINS* 2000;23:183–90.
- [12] Castro MG, Southgate T, Lowenstein PR. Molecular therapy in a model neuroendocrine disease: developing clinical gene therapy for pituitary tumours. *Trends Endocrinol Metab* 2001;12:58–64.
- [13] Wood MJ, Charlton HM, Wood KJ, Kajiwara K, Byrnes A. Immune responses to adenovirus vectors in the nervous system. *TINS* 1996;19:497–501.
- [14] Dewey RA, Morrissey G, Cowsill CM, Stone D, Bolognani F, Dodd NJ, Southgate TD, Klatzmann D, Lassmann H, Castro MG, Lowenstein PR. Chronic brain inflammation and persistent HSV1-TK expression in survivors of syngeneic glioma treated by adenovirus-mediated gene therapy: implications for clinical trials. *Nat Med* 1999;5:1256–63.
- [15] Kafri T, Morgan D, Krahl T, Sarvetnick N, Sherman L, Verma IM. Cellular immune response to adenoviral vector infected cells does not require de novo viral gene expression: implications for gene therapy. *Proc Natl Acad Sci USA* 1998;95:11377–82.
- [16] Thomas CE, Birkett D, Anozie I, Castro MG, Lowenstein PR. Acute direct adenoviral vector cytotoxicity and chronic, but not acute, inflammatory responses correlate with decreased vector-mediated transgene expression in the brain. *Mol Ther* 2001;3:36–46.
- [17] Thomas CE, Schiedner G, Kochanek S, Castro MG, Lowenstein PR. Peripheral infection with adenovirus causes unexpected long term brain inflammation in animals injected intracranially with first-generation, but not with high-capacity, adenovirus vectors: toward realistic long-term neurological gene therapy for chronic diseases. *Proc Natl Acad Sci USA* 2000;97:7482–7.
- [18] Thomas CE, Schiedner G, Kochanek S, Castro MG, Lowenstein PR. Pre-existing anti-adenoviral immunity is not a barrier to efficient and stable transduction of the brain, mediated by novel high-capacity adenovirus vectors. *Hum Gene Ther* 2001;12:839–46.
- [19] Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L,

- Sands MS, Chen EY, Palfi S, Roitberg BZ, Bown WD, Holden JE, Pyzalski R, Taylor MD, Carvey P, Ling Z, Trono D, Hantraye P, Deglon N, Aebischer P. Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 2000;290:767–73.
- [20] 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;5:1593–603.
- [21] Smith C, Lachmann RH, Efstathiou S. Expression from the herpes simplex virus type 1 latency-associated promoter in the murine central nervous system. *J Gen Virol* 2000;81:649–62.
- [22] Palmer JA, Branston RH, Lilley CE, Robinson MJ, Groutsi F, Smith J, Latchman DS, Coffin R. Development and optimization of herpes simplex virus vectors for multiple long term gene delivery to the peripheral nervous system. *J Virol* 2000;74:5604–18.
- [23] Skorupa AF, Fischer KJ, Wilson JM, Parente MK, Wolfe JH. Sustained production of β -glucuronidase from localized sites after AAV vector gene transfer results in widespread distribution of enzyme and reversal of lysosomal storage lesions in a large volume of brain in mucopolysaccharidosis VII mice. *Exp Neurol* 1999;160:17–27.
- [24] Schwartz M, Cohen IR. Autoimmunity can benefit self-maintenance. *Immunol Today* 2000;21:265–8.
- [25] Hohfeld R, Kerschenstein M, Stadelmann C, Lassmann H, Wekerle H. The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis. *J Neuroimmunol* 2000;107:161–6.
- [26] Jacobs A, Breakefield XO, Fraefel C. HSV-1-based vectors for gene therapy of neurological diseases and brain tumors: Part I. HSV-1 structure, replication and pathogenesis. *Neoplasia* 1999;1:387–401.
- [27] Malik P, McQuiston SA, Yu XJ, Pepper KA, Krall WJ, Podsakoff GM, Kurtzman GJ, Kohn DB. Recombinant adeno-associated virus mediates a high level of gene transfer but less efficient integration in the K562 human hematopoietic cell line. *J Virol* 1997;71:1776–83.
- [28] Trono D. Lentiviral vectors: turning a deadly foe into a therapeutic agent. *Gene Ther* 2000;7:20–3.
- [29] Park F, Ohashi K, Chiu W, Naldini L, Kay MA. Efficient lentiviral transduction of liver requires cell cycling in vivo. *Nat Genet* 2000;24:49–52.
- [30] Alisky JM, Hughes SM, Sauter SL, Jolly D, Dubensky TW, Chiorini JA, Staber JA, Davidson BL. Transduction of murine cerebellar neurons with recombinant FIV and AAV5 vectors. *NeuroReport* 2000;11:2669–73.
- [31] Takahashi M, Miyoshi H, Verma IM, Gage FH. Rescue from photo-receptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. *J Virol* 1999;73:7812–6.
- [32] Bosch A, Perret E, Desmaris N, Trono D, Heard JM. Reversal of pathology in the entire brain of mucopolysaccharidosis type VII mice after lentivirus-mediated gene transfer. *Hum Gene Ther* 2000;11:1139–50.
- [33] Monahan PE, Samulski RJ. Adeno-associated virus vectors for gene therapy: more pros than cons? *Mol Med Today* 2000;6:433–40.
- [34] Bjorklund A, Kirik D, Rosenblad C, Georgievska B, Lundberg C, Mandel RJ. Towards a neuroprotective gene therapy of Parkinson's disease: use of adenovirus, AAV, and lentivirus vectors for gene transfer of GDNF to the nigrostriatal system in the rat Parkinson model. *Brain Res* 2000;15:82–98.
- [35] Bosch A, Perret E, Desmaris N, Heard JM. Long-term and significant correction of brain lesions in adult mucopolysaccharidosis type VII mice using recombinant AAV vectors. *Mol Ther* 2000;1:63–70.
- [36] Latchman DS, Coffin RS. Viral vectors in the treatment of Parkinson's disease. *Mov Disord* 2000;15:9–17.
- [37] Lognivoff C, Epstein AL. Genetic engineering of herpes simplex virus and vector genomes carrying loxP sites in cells expressing Cre recombinase. *Virology* 2000;267:102–10.
- [38] Enquist LW, Husak PJ, Banfield BW, Smith GA. Infection and spread of alpha herpesviruses in the nervous system. *Adv Virus Res* 1998;51:237–347.
- [39] Kochanek S. High-capacity adenoviral vectors for gene transfer and somatic gene therapy. *Hum Gene Ther* 1999;10:2451–9.
- [40] Geddes BJ, Harding TC, Lightman SL, Uney JB. Long-term gene therapy in the CNS: reversal of hypothalamic diabetes insipidus in the Brattleboro rat by using an adenovirus expressing arginine vasopressin. *Nat Med* 1997;3:1402–4.
- [41] Ehrenguber MU, Lundstrom K, Schweitzer C, Heuss C, Schlesinger S, Gahwiler BH. Recombinant Semliki Forest virus and Sindbis virus efficiently infect neurons in hippocampal slice cultures. *Proc Natl Acad Sci USA* 1999;96:7041–6.
- [42] Gerdes CA, Castro MG, Lowenstein PR. Strong promoters are the key to highly efficient, noninflammatory and noncytotoxic adenoviral-mediated transgene delivery into the brain in vivo. *Mol Ther* 2000;2:330–8.
- [43] Ralph GS, Bienemann A, Harding TC, Hopton M, Henley J, Uney JB. Targeting of tetracycline-regulatable transgene expression specifically to neuronal and glial cell populations using adenoviral vectors. *NeuroReport* 2000;11:2051–5.
- [44] Smith-Arica JR, Morelli AE, Larregina AT, Smith J, Lowenstein PR, Castro MG. Cell-type specific and regulatable transgenesis in the adult brain: adenovirus-encoded combined transcriptional targeting and inducible transgene expression. *Mol Ther* 2000;2:579–87.
- [45] Umana P, Gerdes CA, Davis JRE, Castro MG, Lowenstein PR. An efficient, in vitro-evolved FLPe enables scalable production of helper-dependent adenoviral vectors with negligible helper contamination. *Nat Biotechnol* 2001;19:582–5.
- [46] Jacobs A, Breakefield XO, Fraefel C. HSV-1-based vectors for gene therapy of neurological diseases and brain tumors: Part II. Vector systems and applications. *Neoplasia* 1999;1:402–16.
- [47] Curiel DT. Strategies to adapt adenoviral vectors for targeted delivery. *Ann NY Acad Sci* 1999;886:158–71.
- [48] Wickham TJ. Targeting adenovirus. *Gene Ther* 2000;7:110–4.
- [49] Kafri T, van Praag H, Gage FH, Verma IM. Lentiviral vectors: regulated gene expression. *Mol Ther* 2000;1:516–21.
- [50] Chen BE, Lendvai B, Nimchinsky EA, Burbach B, Fox K, Svoboda K. Imaging high-resolution structure of GFP-expressing neurons in neocortex in vivo. *Learn Mem* 2000;7:433–41.
- [51] Johns DC, Marx R, Mains RE, O'Rourke B, Marban E. Inducible genetic suppression of neuronal excitability. *J Neurosci* 1999;19:1691–7.
- [52] Harding TC, Xue L, Bienemann A, Haywood D, Dickens M, Tolkovsky AM, Uney JB. Inhibition of c-Jun N-terminal kinase by over-expression of the JNK binding domain of JIP-1 prevents apoptosis in sympathetic neurons. *J Biol Chem* 2001;276:4531–4.
- [53] Hislop JN, Madziva MT, Everest HM, Harding T, Uney JB, Willars GB, Millar RP, Troskie B, Davidson JS, McArdle CA. Desensitization and internalization of human and xenopus gonadotropin-releasing hormone receptors expressed in alphaT4 pituitary cells using recombinant adenovirus. *Endocrinology* 2000;141:4564–75.
- [54] Noel JS, Ralph GS, Pickard L, Williams J, Molnar E, Uney JB, Collingridge GL, Henley JM. Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism. *Neuron* 1999;23:365–76.
- [55] Choi-Lundberg DL, Lin Q, Chang YN, Chiang YL, Hay CM, Mohajeri H, Davidson BL, Bohn MC. Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* 1997;275:838–41.
- [56] Ghodsi A, Stein C, Derksen T, Yang G, Anderson RD, Davidson BL. Extensive beta-glucuronidase activity in murine central nervous system after adenovirus-mediated gene transfer to brain. *Hum Gene Ther* 1998;9:2331–40.
- [57] Daly TM, Vogler C, Levy B, Haskins ME, Sands MS. Neonatal gene transfer leads to widespread correction of pathology in a murine model of lysosomal storage disease. *Proc Natl Acad Sci USA* 1999;96:2296–300.