Techniques for gene transfer into neurons

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To illuminate the function of the thousands of genes that make up the complexity of the nervous system, it is critical to be able to introduce and express DNA in neurons. Over the past two decades, many gene transfer methods have been developed, including viral vectors, liposomes and electroporation. Although the perfect gene transfer technique for every application has not yet been developed, recent technical advances have facilitated the ease of neuronal gene transfer and have increased the accessibility of these techniques to all laboratories. In order to select a transfection method for any particular experiment, the specific advantages and disadvantages of each technique must be considered.

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Current Opinion in Neurobiology 2002, 12:566–573

0959-4388/02/$ – see front matter

DOI 10.1016/S0959-4388(02)00365-3

Abbreviations

AAV	adeno-associated virus
AdV	adenovirus
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
EGFP	enhanced green fluorescent protein
HSV	herpes simplex virus
SFV	Semliki Forest virus
WPRE	woodchuck hepatitis virus posttranscriptional regulatory element

Introduction

A major challenge in current neuroscience research is to understand the functions of the thousands of brain-specific genes involved in neural development, plasticity, physiology, and function. To accomplish this goal, we must have access to techniques in which gene expression can be monitored and manipulated in healthy cells, slices, embryos, and adult animals. Historically, transfection of postmitotic neurons has been labor-intensive, inefficient, unreliable, and/or cytotoxic. This inability to express foreign proteins in postmitotic neurons has, until the past few years, hampered neuroscience research. Fortunately, a large number of diverse techniques for transferring genes into postmitotic neurons have recently been developed and optimized (reviewed in [1]).

It is now possible to express foreign genes in either a single neuron or a large population of neurons in dissociated cultures, cultured slices, or *in vivo*. For basic research purposes, the ideal transfection method should: first, be capable of transfecting postmitotic neurons with high efficiency; second, allow transfection of constructs of varying sizes, including cotransfection with multiple constructs; third, have limited cellular toxicity; and fourth, be easy and safe to perform. Despite major advances in this field in the past several years, the ideal gene delivery system for all applications has yet to be developed. Thus, the specific advantages and disadvantages of each echnique must be considered in selecting a transfection method for any particular experiment [2] (Table 1).

Because one ultimate goal of gene transfer lies in therapeutic remedies, much of the research into DNA delivery to the nervous system is geared towards gene therapy. However, reviewing the large and rapidly growing field of gene therapy is outside the scope of this review; for gene therapy issues, including information on the use of lentivirus in gene transfer, the reader is referred to several recent reviews and reports [3–9]. The objective of this review is, instead, to present an overview of neuronal transfection methods, to provide a few illustrative examples of applications of these techniques, and to compare the most common methods for their suitability for gene transfer into postmitotic neurons in the central nervous system (CNS; Table 1).

Recombinant virus-based technologies

Gene transfer into postmitotic neurons is a young field. One of the first major breakthroughs in transfecting postmitotic neurons came in 1988 with the demonstration of the first high-efficiency, virally mediated transfer of a foreign gene into neurons [10]. The increasing use of viral vectors for the transfer of DNA to neurons is undoubtedly due to extremely high infection efficiencies (up to 95% of neurons) compared with non-viral methods. This superiority of virus-based systems comes as little surprise, because one is benefiting from what viruses have evolved to do — insert their DNA or RNA into host cells and express it. This basic predisposition for infection makes viruses relatively easy to use in both young and adult tissue and on such diverse preparations as dissociated cells, slices and *in vivo*.

Because many recombinant viral vectors are replication-incompetent, most are also relatively safe to use. Recombinant viral vectors can be locally applied or focally injected into a group of neurons, either in culture or in tissue, to produce highly localized expression of a gene of interest. However, these advantages are counterbalanced by some serious limitations — potential toxicity to neurons, the effort and time to construct recombinant viral vectors, limitations on size of the DNA expression cassette, and potential safety hazards to laboratory personnel [1,2,11,12••,13]. There are a number of viral vectors currently being used to transfect postmitotic neurons. These viral vectors differ in terms of infection efficiency, expression levels, lag phase,

Table 1

Gene transfer methods for postmitotic neurons*.

	Recombinant viral vectors					Non-viral transfection methods				
	HSV	AdV	AAV	Vaccinia	Sindbis/SFV	Ca-Phos	Lipofection	Microinjection	Biolistics	Electropora-tion
Efficiency of neuronal transfection	High	Moderate for neurons; high for glia	High	High	Highest	Low	Low	Low	Low to medium	High
Level of expression	High	Low	High	High	High	Moderate	Moderate/ high	High	High	High
Onset of expression	Hours	Days	Weeks	Hours	Hours	Hours	Hours	Hours	Hours	Hours
Ratio of neuron/glia expression†	High	Low	High	Low	High	~1	~1	~1	~1	~1
Targeted expression	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Possible	Yes
Single neuron expression	No	No	No	No	No	No	No	Yes	No	Yes
Integration	No	No	Yes	No	No	No	No	No	No	No
Insert size	<30kb	<7.5kb	<5kb	<30kb	6.5kb	No limit	No limit	No limit	No limit	No limit
Application: dissociated neurons	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
slice cultures	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
in vivo	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes; embryos and oocytes	No	Yes
Toxicity/ cell damage	High (less with amplicon-based vectors)	High with high titers	Minimal	High in mammalian tissue; low in *Xenopus*	High after 24 hr (culture), 3–5 days (slices), 48–72 hrs (*in vivo*)	Minimal	Variable	Variable damage from cell injection	Minimal with optimization	Minimal

Information included in this table is based on current published reports. It is possible that results may vary depending on laboratory experience and, especially, the health of the neuronal preparation. *A comparison of the most commonly used methods for gene transfer for postmitotic neurons. Please find references for each point in text. †Ratio determined using general cytomegalovirus promoters; this could be changed by using neuron-specific promoters.

and toxicity for the host cell or animal [12••,14,15•] (Table 1). Thus, the choice of viral vector depends greatly on the experimental application.

Herpes simplex virus

The first virus to be used for gene transfer was herpes simplex virus (HSV) [10]. Neurons are a natural host for HSV and expression of HSV-transduced genes can last for months to years. However, because of its cellular toxicity, its difficulty to construct, and its high potential risk to humans, HSV is not commonly used [1]. Recent advances in amplicon-based HSV vectors [16], decreases in toxicity, and increasing ease of use may allow these viruses to live up to their early promise in the near future.

Adenovirus

Adenovirus (AdV) has historically been the most commonly used viral vector, with applications ranging from gene transfer *in vivo*, to *in vitro* slices and dissociated neurons [17–20]. The first reports of recombinant AdV as an effective gene delivery system for postmitotic neurons *in vivo* were published in 1993 [21–23]. Expression begins a few days following infection and lasts for weeks to months [11,12••]. Although this vector can transduce postmitotic neurons in culture well [17], the success of recombinant AdV in transducing postmitotic neurons in intact tissue can be variable [12••,17,18,20]. Furthermore, first-generation AdV is pathogenic at high titers, transduces glia better than neurons, is relatively difficult to construct, and can cause severe immune reactions *in vivo* [1,2]. The new, second-generation, helper-dependent, 'gutless' adenoviral vectors developed in the past few years may alleviate these disadvantages [15•] and recent adenoviral vectors designed with neuron-specific, inducible promoters are especially exciting [15•,24••].

Adeno-associated virus

One of the most promising viral vectors is adeno-associated virus (AAV) [25]. In 1994, Kaplitt *et al.* [26] discovered that AAV vectors can selectively transfect neurons. AAV is the least toxic of all viral vectors, leads to high levels of gene expression and has the potential for site-specific integration, leading to long-lasting gene expression. The limitations of AAV vectors are two-fold: the recombinant protein starts to be expressed after a delay of about two weeks post-infection and the maximal insert size is only about 5000 nucleotides [11,12••,13]. Recently, AAV vectors have been used to transduce postmitotic neurons *in vivo*, in dissociated primary cultures, and in cultured brain slices [12••,13,25,27,28].

Vaccinia virus

Vaccinia virus was one of the first viral vectors to be used successfully in transducing hippocampal slice cultures at extremely high efficiencies [29–31]. Recombinant protein starts to be expressed from 6–16 h post-infection [1]. In mammalian tissue, vaccinia quickly becomes highly toxic, causing 50% of transduced neurons to die within 18 h following infection (R Malinow, personal communication). However, this toxicity is not seen in non-mammalian organisms. In *Xenopus laevis*, vaccinia vectors have been used successfully to transduce tectal neurons in dissociated cultures (H Cline, personal communication) and *in vivo* (see [32,33•] for examples).

Sindbis and Semliki Forest viruses

Recently, the related RNA viruses, Sindbis and Semliki Forest virus (SFV) have received a lot of attention [12••,34]. These viruses are selective for neurons (depending on the strain) and can mediate recombinant protein expression rapidly, reliably, and to high levels [12••,34]. Relative to other viral vectors, they are less labor-intensive thanks to commercially available kits (Invitrogen). Sindbis and SFV have been used with great success *in vivo* and in dissociated neurons and cultured slices (see [35,36] for examples). In particular, Sindbis has been used to successfully transduce large numbers of hippocampal neurons in slices and *in vivo* [36–38,39•,40].

The potential major drawback to these viruses is that they shut off host protein synthesis within approximately 8 h of infection, leading to neuronal toxicity and death at variable times post-infection [1]. By carefully monitoring synaptic transmission, membrane potential, and input resistance, Malinow and colleagues have found that Sindbis infection leads to significant toxicity only after 48 h (and probably 72 h) post-infection in hippocampal slices (R Malinow, personal communication). Toxicity in dissociated neuronal cultures arises approximately 24–48 h after infection (J Sullivan, personal communication) and between 48 and 72 h *in vivo* (R Malinow, personal communication).

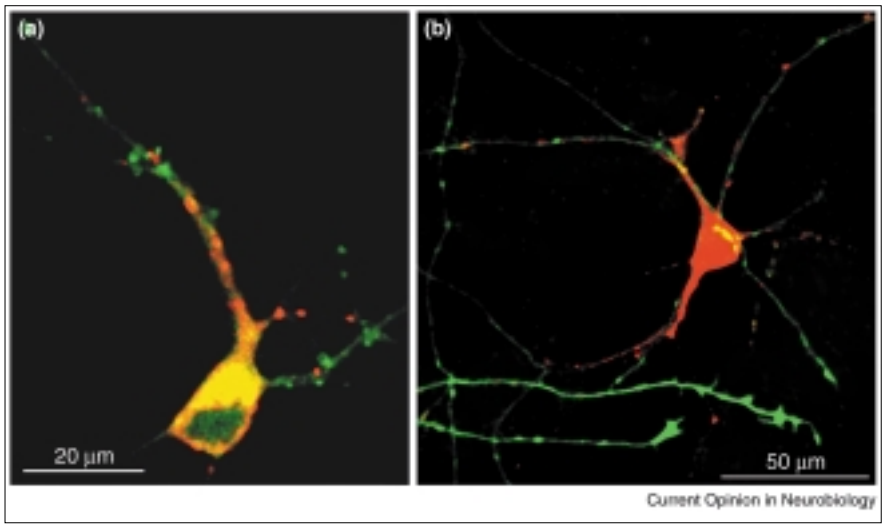
Non-viral transfection methods

Non-viral transfection methods comprise an eclectic mix of chemical, physical and electrical methods for gene transfer. Non-viral methods are advantageous for gene transfer into postmitotic neurons because they are generally easier to use, less toxic, and not constrained to delivering plasmids below a relatively small size (see Table 1 for comparison with viral techniques). However, transfection efficiencies resulting from non-viral transfection methods are generally considerably lower (except for electroporation) than efficiencies obtained with recombinant viral vectors [1] (Table 1).

Chemical transfection methods

The first subgroup of non-viral technologies, the chemical transfection methods, includes calcium phosphate coprecipitation, liposomes, non-liposomal lipids such as Effectene (Qiagen), and high molecular weight cationic polymers. Calcium phosphate-mediated transfection is one of the oldest methods for gene transfer and is, along with lipofection, one of the most commonly used gene transfer methods for basic neuroscience applications. The physical basis for this method is unclear, although it is believed that the DNA-calcium phosphate coprecipitate enters the neuron through endocytosis [1]. Although calcium phosphate coprecipitation has not been used to transfect neurons in intact tissue, it has been used extensively and successfully

Figure 1



Cis and *trans* cotransfection of fluorescently tagged proteins into young dissociated primary cortical cultures using lipofection [52•]. This figure demonstrates cotransfection of the same neuron with two constructs, or neighboring neurons with distinct constructs. Neurons were dissociated and cultured as described [52•] and then transfected using Lipofectamine 2000 (Gibco) at four days *in vitro*. (a) Neurons were *cis* cotransfected with a postsynaptic scaffolding protein (postsynaptic density protein 95kDa [PSD95]) linked to EGFP (PSD95–EGFP; in green) and an *N*-methyl-D-aspartate (NMDA) receptor subunit coupled to DsRed (NR1–DsRed; in red). Both fusion proteins are expressed in dendrites but show distinct subcellular distributions in young cortical pyramidal neurons. (b) Neurons were *trans* cotransfected with growth-associated protein 43 (GAP43) – an abundant protein in growth cones – coupled to EGFP (GAP43–EGFP; in green) and NR1–DsRed (in red). The NMDA receptor subunit is localized to dendrites, where it is highly expressed in the cell body and proximal apical dendrite, but expressed at levels comparable to endogenous levels in puncta in basal and distal apical dendrites [52•]. GAP43, transfected into a neighboring neuron out of the illustrated field, is expressed in the axons contacting the neuron transfected with NR1–DsRed.

to transfect dissociated neuronal cultures from the CNS and peripheral nervous system of many diverse species [41–44]. Cotransfection is also possible with calcium phosphate coprecipitation, leading to almost 100% cotransfection, although ratios of expression vary. The major drawback to this method is that transfection efficiencies are highly variable but consistently low, in the range of 1–3% [1,41,42].

Despite reduced transfection efficiency compared to viruses, gene transfer using liposomes (lipofection) has had a significant impact in many areas of neuroscience by virtue of its user-friendliness and versatility. Liposomes are positively charged lipid spheres with a diameter between 100 and 500 nm [45•]. The surface positive charges on liposomes attract the negative charges of both DNA and neuronal surfaces. In general, liposomes are believed to be endocytosed by cells, although the precise mechanisms of DNA entry into the cell and transport to the nucleus are unknown [46]. The charge ratio and size of the liposomal particles strongly influence the efficiency and cell specificity of endocytic uptake [45•,46]. Most recently, Invitrogen has developed a new mixture of lipids called Lipofectamine 2000, which significantly increases the efficiency of neuronal transfection (routinely 10–25%; PRMA Gomes and AK McAllister, unpublished data). Since the first description of lipofection in 1987 [47] and its first use *in vivo* in 1990 [48], lipofection has been used in several different applications *in vitro* [49–51,52•] (Figure 1) and *in vivo* [53•,54]. Recent attempts to improve on the transfection efficiency of lipofection have led to the discovery that anionic liposomes largely increase transfection efficiency of oligonucleotides in neurons [55•], but it remains to be seen whether this will be made commercially available.

Several additional methods related to lipofection can also be used to transfect postmitotic neurons. Effectene, a non-liposomal lipid produced by Qiagen, has been used to transfect dissociated neuronal cultures specifically to achieve low levels of protein expression [56•]. High molecular weight polycationic polymers have also been used successfully to transfect neurons [57]. Finally, immunoliposomes or antibody-directed liposomes can be generated by encapsulating liposomes with antibody-bound poly-ethylene glycol. These antibodies target the complexes to specific cells, even across the blood–brain barrier [58••], thus allowing brain-specific expression after intravenous administration. The importance of this method for gene therapy is striking and it should not be ignored by the basic neuroscience community, because immunoliposomes may constitute an inexpensive and less labor-intensive alternative to producing transgenic and knockout mice.

Physical transfection methods

The physical methods for transfection include microinjection and biolistics. Microinjection involves directly injecting plasmid DNA into the nucleus of a neuron [59], or injecting cRNA into the cytoplasm [60]. Whereas this method is standard for transfecting oocytes, *Xenopus* blastomeres (see [53•] for example), and invertebrate neurons, it requires considerable skill with mammalian CNS neurons and has not become a routine approach. Microinjection is quite labor-intensive and can be used on only a small number of neurons at a time. However, for applications in which only one identified neuron needs to be transfected, this method can be used effectively and elegantly [61•].

Biolistics, short for biological ballistics, involves bombarding neurons at high velocity with DNA-coated gold particles [62,63]. Neurons whose nuclei are penetrated by a gold particle have a high likelihood of becoming transfected. Transfection efficiencies are relatively low in dissociated cultures (1–5%), but higher in cultured slices (up to several hundred neurons per slice) [62]. Biolistics is straightforward and reliable but requires optimization to minimize physical damage to cells or tissue and investment in a gene gun (BioRad). Although biolistics has not, to date, been successful in transfecting neurons *in vivo*, it is particularly useful for transfecting neurons in a dispersed manner in slices and primary cultures [63,64].

Electrical transfection methods

Perhaps the most promising non-viral method for transfecting postmitotic neurons is electroporation. Although the physical basis for this method is unknown, it is believed that electric shock transiently opens pores in the cell membrane, allowing charged molecules to enter cells by electrophoresis [65•]. In the past, this method has been limited by the damage caused by these electrical pulses; however, recent advances have dramatically improved neuronal health. Unlike the other non-viral transfection methods, electroporation results in large numbers of healthy, highly expressing transfected neurons. Single cells to entire tissues can be transfected with single or multiple constructs by varying the size of the electrodes and modifying the pattern of stimulation. In fact, *in vivo* electroporation is now routinely used by both chick and mouse embryologists [66–68]. Electroporation has also been adapted to transfect dissociated neurons in culture [69]. Perhaps most exciting, Cline and colleagues have developed a new method to target gene transfer to single neurons *in vivo* using single-cell electroporation [33•,70••]. Electroporation is also the most versatile of the non-viral technologies; it can be used not only for gene transfer, but also potentially to target any charged macromolecule to neurons including dyes, drugs, antibodies, antisense oligonucleotides, double-stranded RNAs, and bacterial or yeast artificial chromosomes [65•].

Conclusions and future directions

Recent advances in technologies for gene transfer to postmitotic neurons present neuroscientists with an abundance of methods, each with their individual advantages and disadvantages (Table 1). Thus, researchers must choose a transfection technique which best serves their experimental goals. For transfecting dissociated cultures, both viral and non-viral approaches are options. Viruses, such as Sindbis and SFV, transduce large numbers of neurons with extremely high levels of expression, but take over the neuron's protein synthesis machinery after 8 h [1,2,12••]. Liposomes, calcium phosphate coprecipitation, and Effectene result in lower transfection efficiencies but can be used to express constructs at near endogenous levels for weeks, with the option of cotransfecting single neurons or synaptically coupled cells [41-43,52•,56•] (Figure 1). Transfecting neurons in slices is optimal using either viral vectors to transiently transduce large groups of neurons [12••,19,24••,31,37,38,40] or biolistics to achieve a large number of healthy, dispersed transfected neurons with long-lasting expression [63,71]. Viruses are particularly effective in transducing the large number of neurons necessary for biochemical analysis [38]. Finally, transfecting neurons *in vivo* has recently become much more successful using exciting new modifications to electroporation [65•,70••] and viruses [15•,32,33•,39•].

Technologies for transfecting postmitotic neurons have vastly improved in the last five years, providing basic researchers with many options and allowing experiments to be performed that were, until recently, technically impossible. The field of neuronal gene transfer for basic research applications is currently focused on two major issues — improving transfection efficiencies and targeting genes to specific neuronal types. The first goal — to improve transfection efficiencies — is steadily being achieved through rapid advances in both viral and non-viral transfection technologies. Recent reports suggest that combining viral and non-viral approaches may allow researchers the best of both worlds [72,73]. The second goal for the field is to develop ways in which near-endogenous expression levels and specific transfection of neuronal subtypes can be achieved. Currently, most transfected genes are driven by the ubiquitous and powerful cytomegalovirus promoter. However, neuronal specificity of transfection can be increased by using neuron-specific promoters [74], such as the platelet-derived growth factor β-chain promoter [12••] or the synapsin 1 promoter [24••,75••], and the timing of expression can be controlled by using neuron-specific, inducible promoters [24••]. Thus, recent advances in transfection technologies are making it possible to address the functions of proteins in neuronal development and adulthood in new and exciting ways.

Acknowledgements

We thank Holly Cline, Robert Malinow, Sam Young, and Jane Sullivan for critical information on recombinant viral methods and Leon Hall, Karl Murray, and Marty Usrey for informative discussions and critical reading of the manuscript. Our research is supported by the Alfred P Sloan and Pew Foundations (AK McAllister), the March of Dimes (AK McAllister) and National Institute of Health grant RO1 EY13584 (AK McAllister). P Washbourne is a Medical Investigation of Neurodevelopmental Disorders Institute Scholar.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Craig AM: Transfecting cultured neurons. In *Culturing Nerve Cells*, edn 2. Edited by Banker G, Goslin K. Cambridge MA; MIT Press; 1998:79-111.

2. Slack RS, Miller FD: Viral vectors for modulating gene expression in neurons. *Curr Opin Neurobiol* 1996, 6:576-583.

3. Kordower JH, Emborg ME, Bloch J, Ma SY, Chu YP, Leventhal L, McBride J, Chen EY, Palfi S, Roitberg BZ *et al.*: Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 2000, 290:767-773.

4. Trono D: Lentiviral vectors: turning a deadly foe into a therapeutic agent. *Gene Ther* 2000, 7:20-23.

5. Berry M, Barrett L, Seymour L, Baird A, Logan A: Gene therapy for central nervous system repair. *Curr Opin Mol Ther* 2001, 3:338-349.

6. Dumas TC, Sapolsky RM: Gene therapy against neurological insults: sparing neurons versus sparing function. *Trends Neurosci* 2001, 24:695-700.

7. Pfeifer A, Verma IM: Gene therapy: promises and problems. *Annu Rev Genomics Hum Genet* 2001, 2:177-211.

8. Li S, Ma Z: Nonviral gene therapy. *Curr Gene Ther* 2001, 1:201-226.

9. Nishikawa M, Huang L: Nonviral vectors in the new millennium: Delivery barriers in gene transfer. *Hum Gene Ther* 2001, 12:861-870.

10. Geller AI, Breakefield XO: A defective HSV1 vector expresses ***Escherichia coli*** beta-galactosidase in cultured neurons. *Science* 1988, 241:1667-1669.

11. Simonato M, Manservigi R, Marconi P, Glorioso J: Gene transfer into neurones for the molecular analysis of behaviour: focus on herpes simplex vectors. *Trends Neurosci* 2000, 23:183-190.

12. •• Ehrengruber MU, Hennou S, Bueler H, Naim HY, Deglon N, Lundstrom K: Gene transfer into neurons from hippocampal slices: comparison of recombinant Semliki Forest virus, adenovirus, adeno-associated virus, lentivirus, and measles virus. *Mol Cell Neurosci* 2001, 17:855-871.

This is one of the few careful, direct comparisons of several viral vectors in mediating gene transfer into postmitotic neurons in a single preparation. The authors compare several aspects of transfection properties for SFV, AdV, AAV, lentivirus and measles viral vectors. They conclude that SFV is most useful for short-term, and AAV and lentivirus for long-term gene transfer to postmitotic neurons in hippocampal slice cultures.

13. Janson CG, McPhee SWJ, Leone P, Freese A, During MJ: Viral-based gene transfer to the mammalian CNS for functional genomic studies. *Trends Neurosci* 2001, 24:706-712.

14. Lowenstein PR, Enquist LW: *Protocols For Gene Transfer In Neuroscience: Towards Gene Therapy Of Neurological Disorders*. Chichester: Wiley; 1996.

15. • Lowenstein PR, Castro MG: Genetic engineering within the adult brain: implications for molecular approaches to behavioral neuroscience. *Physiol Behav* 2001, 73:833-839.

This review focuses on recent developments and practical applications for newly developed viral vector systems. A critical examination of advantages and disadvantages of these new vectors for *in vivo* applications is provided.

16. Federoff HJ, Brooks A, Muhkerjee B, Corden T: Somatic gene transfer approaches to manipulate neural networks. *J Neurosci Methods* 1997, 71:133-142.

17. Slack RS, Belliveau DJ, Rosenberg M, Atwal J, Lochmuller H, Aloyz R, Haghighi A, Lach B, Seth P, Cooper E *et al.*: Adenovirus-mediated gene transfer of the tumor suppressor, P53, induces apoptosis in postmitotic neurons. *J Cell Biol* 1996, 135:1085-1096.

18. Moriyoshi K, Richards LJ, Akazawa C, O'Leary DDM, Nakanishi S: Labeling neural cells using adenoviral gene transfer of membrane-targeted GFP. *Neuron* 1996, 16:255-260.

19. Griesbeck O, Korte M, Gravel C, Bonhoeffer T, Thoenen H: Rapid gene transfer into cultured hippocampal neurons and acute hippocampal slices using adenoviral vectors. *Mol Brain Res* 1997, 44:171-177.

20. Vasquez EC, Beltz TG, Haskell RE, Johnson RF, Meyrelles SS, Davidson BL, Johnson AK: Adenovirus-mediated gene delivery to cells of the magnocellular hypothalamo-neurohypophyseal system. *Exp Neurol* 2001, 167:260-271.

21. La Salle GL, Robert JJ, Berrard S, Ridoux V, Stratford-Perricaudet LD, Perricaudet M, Mallet J: An adenovirus vector for gene transfer into neurons and glia in the brain. *Science* 1993, 259:988-990.

22. Akli S, Caillaud C, Vigne E, Stratford-Perricaudet LD, Poenaru L, Perricaudet M, Kahn A, Peschanski MR: Transfer of a foreign gene into the brain using adenovirus vectors. *Nat Genet* 1993, 3:224-228.

23. Davidson BL, Allen ED, Kozarsky KF, Wilson JM, Roessler BJ: A model system for ***in vivo*** gene transfer into the central nervous system using an adenoviral vector. *Nat Genet* 1993, 3:219-223.

24. •• 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, 11:2051-2055.

This study describes the development of a tetracycline-inducible adenoviral vector that makes use of neuron-specific and glial-specific promoters, synapsin-1 (see also [75••]) and glial fibrillary acidic protein promoters, to target foreign gene expression to specific cell types. Using these vectors, the authors demonstrate successful, inducible gene transfer to both dissociated hippocampal cultures and the adult hippocampus *in vivo*. This exciting technique allows inducible and cell-specific gene transfer to postmitotic neurons.

25. Peel AL, Klein RL: Adeno-associated virus vectors: activity and applications in the CNS. *J Neurosci Methods* 2000, 98:95-104.

26. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, During MJ: Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nat Genet* 1994, 8:148-154.

27. McCown TJ, Xiao X, Li J, Breese GR, Samulski RJ: Differential and persistent expression patterns of CNS gene transfer by an adeno-associated virus (AAV) vector. *Brain Res* 1996, 713:99-107.

28. Keir SD, House SB, Li J, Xiao X, Gainer H: Gene transfer into hypothalamic organotypic cultures using an adeno-associated virus vector. *Exp Neurol* 1999, 160:313-316.

29. Ozaki M, Matsumura K, Kaneko S, Satoh M, Watanabe Y, Aoyama T: A vaccinia virus vector for efficiently introducing into hippocampal slices. *Biochem Biophys Res Comm* 1993, 193:653-660.

30. Pettit DL, Perlman S, Malinow R: Potentiated transmission and prevention of further LTP by increased CAMKII activity in postsynaptic hippocampal slice neurons. *Science* 1994, 266:1881-1885.

31. Pettit OL, Koothan T, Liao DZ, Malinow R: Vaccinia virus transfection of hippocampal slice neurons. *Neuron* 1995, 14:685-688.

32. Wu GY, Zou DJ, Koothan T, Cline HT: Infection of frog neurons with vaccinia virus permits ***in vivo*** expression of foreign proteins. *Neuron* 1995, 14:681-684.

33. • Foa L, Rajan I, Haas K, Wu GY, Brakeman P, Worley P, Cline H: The scaffold protein, Homer1b/c, regulates axon pathfinding in the central nervous system ***in vivo.*** *Nat Neurosci* 2001, 4:499-506.

This study and [53•] illustrate the point that specific gene transfer techniques are best selected based on the experimental application and goal. The authors use a combination of three gene transfer techniques to postmitotic neurons – vaccinia viral infection, single-cell electroporation and targeted electroporation – in the optic tectum of *Xenopus* tadpoles to study the role of the Homer protein in axon guidance.

34. Malinow R, Hayashi Y, Maletic-Savatic M, Zaman SH, Poncer JC, Shi SH, Esteban JA: Introduction of green fluorescent protein into hippocampal neurons through viral infection. In *Imaging Neurons*. Edited by Yuste R, Lanni A, Konnerth A. Cold Spring Harbor Laboratory Press; 1999:58.51-58.58.

35. de Hoop MJ, Olkkonen VM, Ikonen E, Williamson E, von Poser C, Meyn L, Dotti CG: Semliki Forest virus as a tool for protein expression in cultured rat hippocampal neurons. *Gene Ther* 1994, 1:28-31.

36. Ehrengruber 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, 96:7041-7046.

37. Maletic-Savatic M, Malinow R, Svoboda K: Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 1999, 283:1923-1927.

38. Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R: Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 1999, 284:1811-1816.

39. • 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, 7:433-441.

These authors demonstrate that detailed neuronal morphology of postmitotic neurons in the neocortex of living mice can be visualized following *in vivo* infection with Sindbis viral vectors containing the gene for the enhanced green fluorescent protein (EGFP). This report shows that neurons in the barrel cortex of rats and mice can be successfully transfected throughout postnatal development. The complete fluorescent labeling of the dendritic

arbor of these neurons allows for *in vivo* imaging of dendritic morphology and dynamics.

40. D'Apuzzo M, Mandolesi G, Reis G, Schuman EM: Abundant GFP expression and LTP in hippocampal acute slices by ***in vivo*** injection of Sindbis virus. *J Neurophysiol* 2001, 86:1037-1042.

41. Xia ZG, Dudek H, Miranti CK, Greenberg ME: Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/Erk-dependent mechanism. *J Neurosci* 1996, 16:5425-5436.

42. Kohrmann M, Haubensak W, Hemraj I, Kaether C, Lessmann VJ, Kiebler MA: Fast, convenient, and effective method to transiently transfect primary hippocampal neurons. *J Neurosci Res* 1999, 58:831-835.

43. Bresler T, Ramati Y, Zamorano PL, Zhai R, Garner CC, Ziv NE: The dynamics of SAP90/PSD-95 recruitment to new synaptic junctions. *Mol Cell Neurosci* 2001, 18:149-167.

44. O'Brien R, Xu DS, Mi RF, Tang XP, Hopf C, Worley P: Synaptically targeted Narp plays an essential role in the aggregation of AMPA receptors at excitatory synapses in cultured spinal neurons. *J Neurosci* 2002, 22:4487-4498.

45. • de Lima MCP, Simoes S, Pires P, Faneca H, Duzgunes N: Cationic lipid–DNA complexes in gene delivery: from biophysics to biological applications. *Adv Drug Del Rev* 2001, 47:277-294.

This comprehensive review covers the biochemistry of different lipids used for transfecting neurons and the biophysics of formation of lipid–DNA complexes. Perhaps most importantly, the authors provide practical advice on how to optimize lipid composition for transfection of postmitotic neurons.

46. Ohki EC, Tilkins ML, Ciccarone VC, Price PJ: Improving the transfection efficiency of post-mitotic neurons. *J Neurosci Methods* 2001, 112:95-99.

47. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M: Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987, 84:7413-7417.

48. Holt CE, Garlick N, Cornel E: Lipofection of cDNAs in the embryonic vertebrate central nervous system. *Neuron* 1990, 4:203-214.

49. Craven SE, El-Husseini AE, Bredt DS: Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. *Neuron* 1999, 22:497-509.

50. Murray KD, McQuillin A, Stewart L, Etheridge CJ, Cooper RG, Miller AD, Gurling HMD: Cationic liposome-mediated DNA transfection in organotypic explant cultures of the ventral mesencephalon. *Gene Ther* 1999, 6:190-197.

51. Okamoto S, Li Z, Ju C, Scholzke MN, Mathews E, Cui JK, Salvesen GS, Bossy-Wetzel E, Lipton SA: Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. *Proc Natl Acad Sci USA* 2002, 99:3974-3979.

52. • Washbourne P, Bennet JE, McAllister AK: Rapid recruitment of NMDA receptor transport packets to nascent synapses. *Nat Neurosci* 2002, 5:751-759.

This paper illustrates that lipofection can be used to transfect dissociated neurons from rat visual cortex with genes encoding fluorescently tagged synaptic proteins. Lipofection was optimized to allow expression of the foreign protein at near-endogenous levels. The authors used lipofection to achieve two forms of cotransfection: *cis* cotransfection, in which single cortical neurons were transfected with multiple constructs, and *trans* cotransfection, in which neighboring, synaptically coupled neurons were separately transfected with two different constructs (Figure 1).

53. • Alsina B, Vu T, Cohen-Cory S: Visualizing synapse formation in arborizing optic axons ***in vivo***: dynamics and modulation by BDNF. *Nat Neurosci* 2001, 4:1093-1101.

This study and [33•] illustrate the point that specific gene transfer techniques should be selected based on the experimental application and goal. Here, both microinjection and lipofection were used to transfect neurons at different stages in embryonic *Xenopus* tadpoles to study the role of brain-derived neurotrophic factor (BDNF) in synapse formation *in vivo*.

54. Hutson LD, Bothwell M: Expression and function of ***Xenopus laevis*** p75(NTR) suggest evolution of developmental regulatory mechanisms. *J Neurobiol* 2001, 49:79-98.

55. • Lakkaraju A, Dubinsky JM, Low WC, Rahman YE: Neurons are protected from excitotoxic death by p53 antisense oligonucleotides delivered in anionic liposomes. *J Biol Chem* 2001, 276:32000-32007.

This study is one of the most compelling reports showing that anionic liposomes may be more effective than cationic liposomes for transfecting dissociated hippocampal neurons. Anionic liposomes were used to transfer both plasmids and oligonucleotides to neurons with efficiencies reported to be 100%. Although future studies using anionic liposomes are needed to characterize details of transfection, such as possible toxicity, this is a potentially promising development in non-viral transfection techniques.

56. • Fong DK, Rao A, Crump FT, Craig AM: Rapid synaptic remodeling by protein kinase C: Reciprocal translocation of NMDA receptors and calcium/calmodulin-dependent kinase II. *J Neurosci* 2002, 22:2153-2164.

This study illustrates the relatively uncommon, but effective, use of Effectene to transfect primary hippocampal cultures. The authors used Effectene to transfect relatively mature neuronal cultures (typically difficult to transfect) with a GFP fusion construct, to visualize the effects of protein kinase C on the distribution and dynamics of several postsynaptic proteins. The protocol using Effectene was optimized to obtain near-endogenous levels of expression of the recombinant, fluorescently tagged protein.

57. Godbey WT, Mikos AG: Recent progress in gene delivery using non-viral transfer complexes. *J Controlled Release* 2001, 72:115-125.

58. •• Pardridge WM: Drug and gene targeting to the brain with molecular Trojan horses. *Nat Rev Drug Discov* 2002, 1:131-139.

Although this review focuses on the field of drug delivery and gene therapy to the brain, the author reviews some of the most exciting new non-invasive methods for gene transfer to the mammalian CNS. In particular, the use of immunoliposomes and related techniques to direct transgene expression to the brain after intravenous administration are reviewed and discussed.

59. Martinou I, Fernandez PA, Missotten M, White E, Allet B, Sadoul R, Martinou JC: Viral proteins E1b19k and P35 protect sympathetic neurons from cell death induced by NGF deprivation. *J Cell Biol* 1995, 128:201-208.

60. Ikeda SR, Lovinger DM, McCool BA, Lewis DL: Heterologous expression of metabotropic glutamate receptors in adult rat sympathetic neurons - subtype-specific coupling to ion channels. *Neuron* 1995, 14:1029-1038.

61. • Kohara K, Kitamura A, Morishima M, Tsumoto T: Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science* 2001, 291:2419-2423.

These authors cleverly use microinjection to transfect a single neuron in culture to demonstrate activity-dependent secretion of BDNF specifically from presynaptic to postsynaptic neurons. Microinjection was selected specifically because of its ability to target gene transfer to a single neuron, to be absolutely certain of the origin of the expressed BDNF–EGFP.

62. Lo DC, McAllister AK, Katz LC: Neuronal transfection in brain slices using particle-mediated gene transfer. *Neuron* 1994, 13:1263-1268.

63. McAllister AK: Biolistic transfection of neurons. *Science's Signal Transduction Knowledge Environment* 2000. Published online at: http://stke.sciencemag.org/cgi/content/full/sigtrans;2000/51/pl1

64. McAllister AK, Katz LC, Lo DC: Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron* 1997, 18:767-778.

65. • Inoue T, Krumlauf R: An impulse to the brain — using ***in vivo*** electroporation. *Nat Neurosci* 2001, 4:1156-1158.

This review summarizes recent advances in the development of electroporation for gene transfer to postmitotic neurons. The authors discuss the advantages of this approach and its potential applications within basic neuroscience research.

66. Itasaki N, Bel-Vialar S, Krumlauf R: 'Shocking' developments in chick embryology: electroporation and ***in ovo*** gene expression. *Nat Cell Biol* 1999, 1:E203-E207.

67. Agarwala S, Sanders TA, Ragsdale CW: Sonic hedgehog control of size and shape in midbrain pattern formation. *Science* 2001, 291:2147-2150.

68. Fukuchi-Shimogori T, Grove EA: Neocortex patterning by the secreted signaling molecule FGF8. *Science* 2001, 294:1071-1074.

69. Teruel MN, Blanpied TA, Shen K, Augustine GJ, Meyer T: A versatile microporation technique for the transfection of cultured CNS neurons. *J Neurosci Methods* 1999, 93:37-48.

70. Haas K, Sin WC, Javaherian A, Li Z, Cline HT: Single-cell
•• electroporation for gene transfer ***in vivo.*** *Neuron* 2001, 29:583-591.

This Neurotechnique article describes recent modifications to electroporation that allow targeting of foreign genes to single neurons *in vivo*. The authors fully characterize and describe this promising transfection method, demonstrating long-lasting expression and the possibility of cotransfection in healthy postmitotic neurons in *Xenopus* tadpoles and rat hippocampal slices. This method is particularly exciting because it allows for both temporal and spatial control over gene transfer into postmitotic neurons *in vivo*.

71. Marrs GS, Green SH, Dailey ME: Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nat Neurosci* 2001, 4:1006-1013.

72. Paterna JC, Moccetti T, Mura A, Feldon J, Bueler H: Influence of promoter and WHV post-transcriptional regulatory element on AAV-mediated transgene expression in the rat brain. *Gene Ther* 2000, 7:1304-1311.

73. Tagawa T, Manvell M, Brown N, Keller M, Perouzel E, Murray KD, Harbottle RP, Tecle M, Booy F, Brahimi-Horn MC *et al.*: Characterization of LMD virus-like nanoparticles self-assembled from cationic liposomes, adenovirus core peptide mu and plasmid DNA. *Gene Ther* 2002, 9:564-576.

74. Kugler S, Meyn L, Holzmuller H, Gerhardt E, Isenmann S, Schulz JB, Bahr M: Neuron-specific expression of therapeutic proteins: evaluation of different cellular promoters in recombinant adenoviral vectors. *Mol Cell Neurosci* 2001, 17:78-96.

75. Glover CP, Bienemann AS, Heywood DJ, Cosgrave AS, Uney JB:
•• Adenoviral-mediated, high-level, cell-specific transgene expression: a SYN1-WPRE cassette mediates increased transgene expression with no loss of neuron specificity. *Mol Ther* 2002, 5:509-516.

In addition to demonstrating the use of the synapsin promoter to facilitate neuron-specific expression of foreign genes (see also [24••]), these authors show that gene expression can be significantly enhanced by inclusion of a portion of the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) in their expression cassette. Addition of the WPRE to the synapsin 1 promoter greatly increased EGFP expression levels with no loss of neuronal specificity in primary hippocampal cultures, slice cultures, and *in vivo*. Furthermore, this composite expression cassette allows successful *in vivo* transfection of postmitotic neurons using low titers of adenovirus, thereby reducing toxicity.