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Pseudotype-dependent lentiviral transduction of astrocytes or neurons in the rat substantia nigra

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ABSTRACT

Gene transfer to the central nervous system provides powerful methodology for the study of gene function and gene–environment interactions *in vivo*, in addition to a vehicle for the delivery of therapeutic transgenes for gene therapy. The aim of the present study was to determine patterns of tropism exhibited by pseudotyped lentiviral vectors in the rat substantia nigra, in order to evaluate their utility for gene transfer in experimental models of Parkinson's disease. Isogenic lentiviral vector particles encoding a GFP reporter were pseudotyped with envelope glycoproteins derived from vesicular stomatitis virus (VSV), Mokola virus (MV), lymphocytic choriomeningitis virus (LCMV), or Moloney murine leukemia virus (MuLV). Adult male Lewis rats received unilateral stereotactic infusions of vector into the substantia nigra; three weeks later, patterns of viral transduction were determined by immunohistological detection of GFP. Different pseudotypes gave rise to transgene expression in restricted and distinct cellular populations. VSV and MV pseudotypes transduced midbrain neurons, including a subset of nigral dopaminergic neurons. In contrast, LCMV- and MuLV-pseudotyped lentivirus produced transgene expression exclusively in astrocytes; the restricted transduction of astroglial cells was not explained by the cellular distribution of receptors previously shown to mediate entry of LCMV or MuLV. These data suggest that pseudotyped lentiviral vectors will be useful for experimental gene transfer to the rat substantia nigra. In particular, the availability of neuronal and astrocytic-targeting vectors will allow dissociation of cell autonomous and cell non-autonomous functions of key gene products *in vivo*.

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Introduction

Parkinson's disease (PD) is a common, sporadic, late-onset neurodegenerative disease, characterized pathologically by loss of discrete populations of cerebral neurons, including dopaminergic neurons of the substantia nigra (Forno, 1996). The resulting cardinal motor signs of bradykinesia, rigidity and tremor can be ameliorated by using drugs that augment striatal dopamine production or directly stimulate dopamine receptors. However, currently available treatments do not prevent or slow disease progression, and there is an urgent, and presently unmet, need for effective neuroprotective agents. There has been significant recent progress in determining the molecular basis of neurodegeneration in PD. Rare Parkinsonism phenocopies are caused by mutations in genes encoding α-synuclein, leucine-rich receptor kinase 2, Parkin, pten-induced kinase 1 (PINK1) and DJ-1 (Bonifati et al., 2003, Kitada et al., 1998, Paisan-Ruiz et al., 2004, Polymeropoulos et al., 1997, Valente et al., 2004, Zimprich et al., 2004). Although mutations in these genes account for a minority of cases of PD, it is possible that elucidation of the abnormal biochemical processes underlying these familial forms of Parkinsonism will identify pathogenic mechanisms that are shared with the common sporadic form of PD. There is consequently considerable interest in understanding the roles of these genes *in vivo*, particularly not only with regard to how they may inform on the mechanisms underlying selective vulnerability of dopaminergic neurons in PD, but also in relation to how the relevant gene products interact with environmental influences thought important in the pathogenesis of sporadic PD, for example pesticide exposure (Betarbet et al., 2000, Costello et al., 2009, Ritz et al., 2009). Methods for reliable experimental manipulation of gene expression in the substantia nigra may therefore yield valuable insights into the mechanisms underlying neurodegeneration in PD, in addition to providing a means for testing putative therapeutic approaches by effecting the ectopic expression of molecules such as growth factors or antioxidants.

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Direct gene transfer to the brain *in vivo* offers some advantages for these experimental applications. First, genetic manipulation is not restricted to organisms in which germline transgenic and gene inactivation technologies are applicable, broadening the range of animal models that can be subjected to these analyses. Second, gene transfer in adult animals circumvents the possibility of compensatory developmental changes altering observed phenotypes. Third, targeted alterations in gene expression allow elucidation of the neuroanatomical basis of behavioral phenotypes without confounding effects of widespread alterations in gene expression. Finally, gene expression changes that appear neuroprotective in models of sporadic PD could be developed as possible gene therapy approaches. The most effective gene delivery vehicles in the CNS *in vivo* are recombinant virus vectors, in which viral genes mediating pathogenic functions are replaced with transgene sequences of interest (Burton et al., 2005). A variety of viral vectors has been used to deliver transgenes to the substantia nigra *in vivo*. Of these, vectors based on adeno-associated virus (AAV) have received particular attention, because they show impressive tropism for nigral dopamine neurons, and the parent virus does not cause disease in humans. AAV vectors have been used recently in Parkinson's disease clinical gene therapy trials (Feigin et al., 2007, Kaplitt et al., 2007, Marks et al., 2008). However, the limited capacity for the insertion of heterologous genetic sequences into the AAV genome precludes the use of AAV-based vectors for applications demanding delivery of large transgene sequences. Lentiviral-based vectors can accommodate larger transgene sequences (up to ≈8 kb). The utility of the HIV-1 based lentivirus vectors commonly used experimentally has been greatly enhanced by pseudotyping, in which the endogenous viral envelope proteins are replaced by envelope proteins from other viruses, altering the tropic range of the vectors. For example, the native envelope glycoproteins of HIV-1 bind to cell surface receptors present on lymphocytes, and impart specific viral tropism for these cells in the pathogenesis of AIDS; replacement of these envelope proteins with the vesicular stomatitis virus G-protein allows the resulting vectors to transduce many different types of cells. A range of lentiviral pseudotypes has been described, including envelope proteins derived from natively neurotropic viruses. A systematic evaluation of the relative utility of these pseudotyped vectors for gene delivery to the substantia nigra has not yet been reported. Here we show that the type of cell, neuronal or astrocytic, transduced by lentiviral vectors in the substantia nigra *in vivo* can be altered by pseudotyping the particles with envelope proteins derived from different viruses.

Materials and methods

Unless otherwise noted, all chemical supplies were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Viral vectors

Lentiviral vectors expressing green fluorescent protein (GFP) were prepared by Penn Vector Core (University of Pennsylvania, Philadelphia, PA). The gene transfer plasmid pHR-EGFP contains HIV-1 LTRs/packaging signals and a cassette in which the immediate early promoter from cytomegalovirus drives GFP expression (Kobinger et al., 2001). Vector particles were prepared as described (Kobinger et al., 2001), by transient transfection of HEK293T cells with: (i) pHR-EGFP; (ii) packaging plasmid pCMVΔR8.2 encoding viral genes, except for envelope glycoproteins (Naldini et al., 1996); and (iii) a plasmid encoding envelope glycoproteins derived from vesicular stomatitis virus (VSV; pMD.G (Naldini et al., 1996)), Mokola virus (MV; pLTRMVG (Mochizuki et al., 1998)), lymphocytic choriomeningitis virus (LCMV; pHCMV-LCMV-GP(WE-HPI) (Beyer et al., 2002)) or Moloney murine leukemia virus (MuLV; pHIT456 (Kobinger et al., 2001)). Vector particles were harvested from culture supernatants, filtered and concentrated by ultracentrifugation, as described (Watson et al., 2002). For infectious titer determination, HEK293T cells were transduced with limiting dilutions of the concentrated vector suspension and the number of proviral DNA genomes determined by qPCR using a primer and probe set corresponding to the 5′ non-coding region downstream of the 5′ LTR. Titers were as follows: VSV, 1.1×10^{11} IU/mL; MV, 7.57×10^{9} IU/mL; LCMV, 1.03×10^{9} IU/mL; and MuLV, 2.50×10^{9} IU/mL.

Surgery

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and in accordance with National Institutes of Health guidelines. Adult male Lewis rats (4 per pseudotype; Hilltop Lab Animals, Inc., Scottdale, PA,

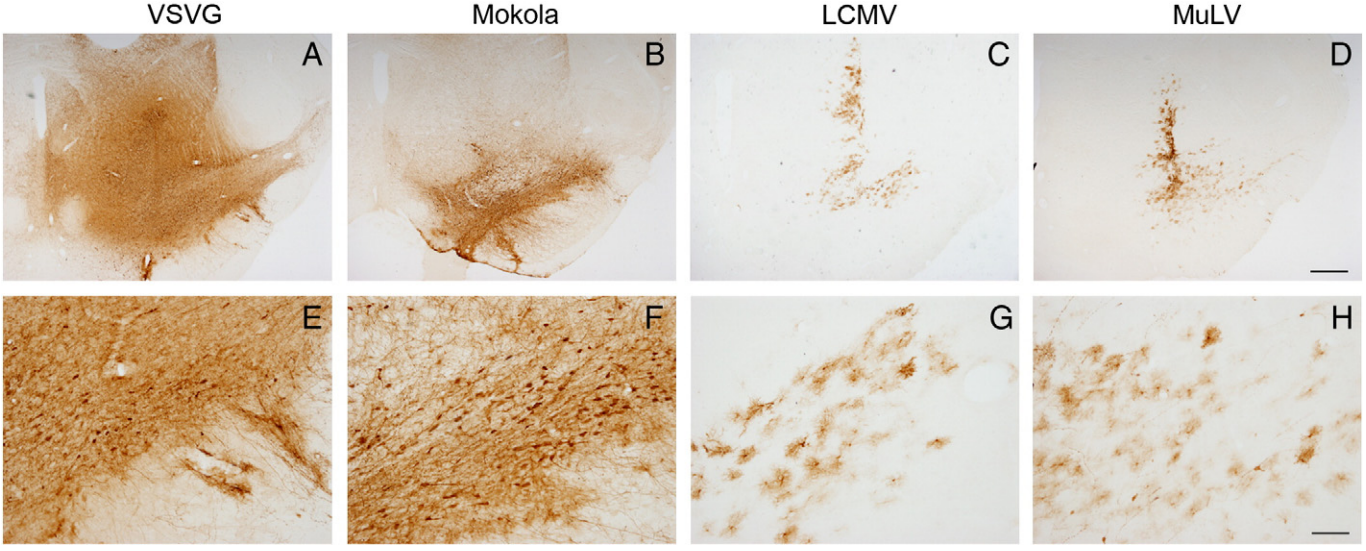

Fig. 1. GFP transgene expression in the midbrain following infusion of pseudotyped lentiviral vectors into the substantia nigra. Light micrographs of coronal midbrain sections are shown, 21 days after intranigral infusion of lentiviral vectors pseudotyped with envelope proteins derived from VSV (A, E), MV (B, F), LCMV (C, G), or MuLV (D, H). GFP expression was localized immunohistochemically, with a chromogenic substrate yielding a brown product. Low magnification images (A–D; bar = 500 μm) of sections are shown for each pseudotype, in order to illustrate the topographical distribution of transgene expression. High magnification images (E–H; bar = 100 μm) of the substantia nigra show the morphology of transduced cells.