Title: Glia: a new cellular target in the treatment of major depression.

Authors: Guiard B

Journal: Current drug targets

Year: 2013 Oct

Volume: 14

Issue: 11

Pages: 1219

Special Issue for CURRENT DRUG TARGETS

Guest Editor(s): Bruno Guiard

GLIA: A NEW CELLULAR TARGET TO TREAT MAJOR DEPRESSION

Title: Lentiviral vectors: a powerful tool to target astrocytes *in vivo*

Running title: Lentiviral vector and *in vivo* astrocytic targeting

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ABSTRACT

The morphological and functional diversity of astrocytes, and their essential contribution in physiological and pathological conditions, are starting to emerge. However, experimental systems to investigate neuron-glia interactions and develop innovative approaches for the treatment of CNS disorders are still very limited. Fluorescent reporter genes have been used to visualize populations of astrocytes and produce an atlas of gene expression in the brain. Knock-down or knock-out of astrocytic proteins using transgenesis have also been developed, but these techniques remain complex and time-consuming. Viral vectors have been developed to overexpress or silence genes of interest as they can be used for both *in vitro* and *in vivo* studies in adult mammalian species. In most cases, high transduction efficiency and long-term transgene expression can be observed in neurons but there is limited expression in astrocytes. Several strategies have been developed to shift the tropism of lentiviral vectors (LV) and allow local and controlled gene expression in glial cells. In this review, we describe how modifications of the interactions between the LV envelope glycoprotein and the surface receptor molecules on target cells, or the integration of cell-specific promoters and miRNA post-transcriptional regulatory elements have been used to selectively express transgenes in astrocytes.

Keywords

Astrocytes, gene transfer, lentiviral vector, targeting, detargeting, miRNA

Experimental approaches to target astrocytes

Experimental systems aiming to decipher the role of astrocytes in physiological and pathophysiological conditions are still in their infancy due to limited understanding of astrocytic biology. There are also limited resources devoted to the characterization of cell-type-specific expression patterns and the identification of proximal and distal regulatory elements leading to astrocyte-specific expression at the genome level. The first strategy to target astrocytes was based on transgenic mouse expressing reporter genes [1-2]. Transgenic mice expressing the LacZ reporter gene under the control of the glial fibrillary acidic protein (GFAP) promoter were used to study the kinetics of astroglial activation after brain trauma [3]. This revealed prominent LacZ expression in hippocampal formation and selected white matter tracts. Heterogeneous GFAP activity was reported in various brain areas, potentially reflecting functional differences. As a complementary approach, knockout mice such as vimentin -/- mice have been used [4]. These mice revealed the contribution of vimentin in the organization of the GFAP network after injury. Reactive astrocytes, which normally express both GFAP and vimentin cytoskeletal proteins, failed to do so in vimentin-null mice. Following these initial studies, numerous transgenic mice expressing selected transgenes have been produced to study signaling pathways during development, disease states or to identify therapeutic genes [5-9]. A cell-type specific conditional gene expression method has been developed by fusing the Cre protein with the mutant ligand-binding domain of the human estrogen receptor (CreER). This enables spatial regulation of gene expression via the CreER/loxP system [10-11] and, temporally, by using a mutated version of Cre recombinase activated by tamoxifen (Cre-ER^{T2}) [12-13].

Synthetic vectors or viral gene transfer technologies have been proposed as complementary approaches to study the biology of astrocytes or to help identify therapeutic candidates for CNS pathologies. Somatic gene transfer approaches are more flexible and less time consuming than transgenesis. Non-viral delivery systems (RNA and DNA oligonucleotides/plasmids) are safe, easy to manufacture and are suitable for large DNA sequences. Limitations to *in vivo* applications in the CNS include low transfection rate, short timeframe for transgene expression and restricted diffusion [14-15]. In contrast, viral vectors including lentiviral vectors (LVs) and adeno-associated viral vectors (AAVs) have high transduction efficiencies in the CNS and lead to long-term transgene expression [16-20]. The choice of the vector will depend on each specific application and parameters such as packaging capacity, host range, tissue-specific targeting, genome integration, duration of transgene expression and diffusion in the brain [21]. Initial studies in the CNS demonstrate a strong neuronal tropism for VSV-G pseudotyped LVs [22] and AAV2 serotypes [23-25] when the transgenes were under the control of ubiquitous promoters (cytomegalovirus (CMV) and phosphoglycerate kinase 1 (PGK) promoters). These viral vectors confer high level of transduction without affecting cell viability or inducing an immune response [26-27].

This tropism in the CNS can be influenced by i) the envelopes/serotypes of viral vectors, ii) the transcriptional and post-transcriptional elements used to control transgene expression, iii) the developmental stage, iv) the species considered, v) the brain areas targeted ,vi) the purity of the vectors and vii) the intracerebral delivery protocol [28-31]. A VSV-G-pseudotyped LV-CMV-GFP, microdelivered into the rat dorsal spinal cord, leads to a preferential expression of the fluorescent reporter gene in astrocytes and microglial cells [32]; whereas in striatum, it is associated with neuronal expression [22]. Interestingly, AAV9 vectors have a unique ability to cross the blood-brain barrier (BBB) but the tropisms following peripheral injection are contrasted. Tail vein injection of self-complementary (sc) AAV9-CBA-GFP (chicken-β actin promoter) in adult mice led to a robust transduction of astrocytes throughout the entire CNS whereas neuronal transduction was observed in neonatal

mice [33]. In contrast, Barkats and coworkers showed that AAV9sc-CMV-GFP (cytomegalovirus promoter) can efficiently transduce motoneurons in both newborn and adult animals [34]. Hence, additional studies are warranted to further define the relevance of use AAV9 for targeting. AAVs represent a powerful system for CNS applications but have one main limitation: the size of the expression cassette (4.5kb) precludes the integration of complex transcriptional and post-transcriptional regulatory elements. Similarly to AAVs, LVs are extensively used in laboratories [35] and are particularly useful for CNS studies due to their high transduction efficiency, sustained transgene expression and relatively large cloning capacity [22, 36]. Both AAVs and LVs are used in clinical practice and ongoing studies use these methods to explore new treatments of CNS pathologies [37-40].
In this review, we focus on LV gene transfer in the CNS. We discuss three strategies, which take advantage of the entry mechanism, transcription and post-transcriptional regulatory elements, to specifically target astrocytes.

Lentiviral gene transfer

Lentiviruses are part of the retroviridae family. Five groups of lentiviruses are indexed, reflecting the vertebrate hosts with which they are associated: human or siman immunodeficiency virus (HIV-1 and 2, simian foamy virus), equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV) and Visna-Maedi virus (ovine lentivirus). Viral vectors have been developed from most of these viruses [22, 41-46]. Vectors are designed to eliminate the natural pathogenicity of the virus and delete all information necessary for its replication. Third generation, multiply-attenuated and replication-deficient LVs are produced in biosafety level 2-3 (BSL2-3) laboratories depending on the transgene and specific national regulations [47]. These vectors can overexpress or block target gene expression to dissect neurobiological functions and are used to develop pertinent animal models of human pathologies [48].
Lentiviral vectors have been shown to efficiently deliver genes to post-mitotic cells and, in particular, to neuronal cells [22]. The heterologous envelope (Env) of the vesicular stomatitis virus glycoprotein (VSV-G protein, is often used to replace the natural virus envelope (a strategy called pseudotyping), due to its high stability and broad tropism [22, 49-50]. The stereotaxic injection of LVs in the CNS leads to a local, efficient and sustained transgene expression [48]. However, additional methods have been developed to further increase the diffusion of molecules in the CNS and reach the global gene delivery needed to treat pathologies affecting large brain areas. Strategies considered include those that take advantage of neuronal circuitry to facilitate the transfer of LVs or proteins (retrograde or anterograde transport) and the expression of secreted molecules [19, 49, 51].
Three regulatory steps of viral vector biology have been targeted to modify the neuronal tropism of LV: the entry of the vector in target cells, the use of cell-type specific promoters, and the integration of microRNA regulatory elements [52]. Combining one or all these elements shifts the tropism of LVs toward astrocytic fates whilst maintaining high transduction efficiency and a transgene expression level compatible with *in vivo* studies (Figure 1).

Modification of viral entry mechanisms to target astrocytes

LV tropism is first determined by the interaction of viral-surface proteins with receptor molecules expressed on target cells. Receptors of several retroviruses have been identified and their role in mediating virus entry has been demonstrated by functional studies. CD4 was the first component identified as an essential component of the HIV cell surface receptor [53]. Receptors of human and simian immunodeficiency retroviruses (HIV and

SIV) are unique in that the receptor has multiple components with a principal binding domain and fusion partners (CCR5 or CXCR4 co-receptors) [54].

Targeting specific cellular populations can potentially be achieved through natural viral tropism or by replacing the original Env protein with other viral glycoproteins (a mechanism/strategy known as pseudotyping). The formation of mixed phenotypic particles is a process that naturally occurs during viral particle assembly in cells infected by different viruses [55]. Page and collaborators were the first to design and test HIV-1-based vector particles harboring heterologous glycoproteins [56]. Pseudotyped particles may also acquire interesting properties. For example, LVs pseudotyped with a rabbies envelope are retrogradely transported [49]. HIV-1 can be pseudotyped by multiples envelopes but this is not the case for all retroviruses. The unusual particle morphogenesis of the foamy virus (FV), requiring capsid and glycoprotein for viral budding [57-58], has prevented any pseudotyping until recently [59].

The most commonly used envelope for LVs is the vesicular stomatitis virus G protein (VSV-G). Naldini and coworkers shown high transduction in the CNS without detectable pathological consequences due to the vector [22]. The VSV-G envelope confers several novel features to LV particles: i) it dramatically broadens vector tropism by facilitating transduction of various cell types in different species *in vitro* and *in vivo*, ii) it stabilizes the vector particles from shear forces during centrifugation, retaining vector concentration and iii) it directs the lentiviral vector entry via an endocytic pathway which reduces the need for viral accessory proteins for target cell transduction [60]. Extensive neuronal tropism can be seen *in vivo* when LVs are pseudotyped using VSV-G and carrying ubiquitous promoters (Figure 2) [19, 50, 61-63]. However, it is important to note that specific targeting of a viral vector *in vitro* does not necessarily imply that the same tropism will be observed in adult and neonatal brains [64]. There is currently no method to predict the tropism of viral vectors *in vivo* and all available data are based on empirical assessments. Bloor and collaborators reported that cells without the chaperone gp96 (localized in the endoplasmic reticulum), or with catalytically inactive gp96, do not bind VSV-G and are resistant to VSV-G-pseudotyped LV infection [65]. The ubiquitous expression of gp96 might therefore explain the broad tropism of VSV-G. Additional parameters such as the targeted brain structure, the animal model used (physiological or disease state), the viral load, the LV mode of production and administration or cell-type specific signaling pathways could influence the level of transgene expression. For example, recent studies suggest that JNK is required for lentivirus entry into target cells [66]. It was thought that phosphatidylserine (PS) was the cell surface receptor for the VSV-G, but recent studies suggest that there is no correlation between cell surface PS levels and VSV-G infection or binding [67]. PS may be involved in a post-binding step of the VSV-G-LV [68].

Numerous studies have evaluated LV tropism in the CNS. LVs pseudotyped with the Mokola envelope (MOK-G, *Rhabdoviridae* family) preferentially target astrocytes over other cell populations (Figure 2) [50, 69]. Using such a vector, it has been shown that glial cell line-derived neurotrophic factor (GDNF) delivery in astrocytes improves cognitive performances in aged rats [70]. However, there are inconsistent reports of astrocytic targeting with MOK-G-pseudotyped LV: a glial tropism has been observed in mice and rats injected with a MOK-G LV-CMV-GFP vector but preferential neuronal tropism was observed in other studies (in rats and in human cells) [71-73]. LV pseudotyping using lymphocytic choriomeningitis virus glycoprotein (LCMV) is associated with astrocytic transgene expression, but with limited transduction efficiency (few infected cells) [71]. The parent LCM virus, from which the envelope glycoprotein is derived, enters cells by binding to a gylcosylated and O-mannosylated form of α-dystroglycan [74-75]. No correlation were observed between the pattern of receptor expression and the tropism of the viral vector [71]. The LCMV and Mokola pseudotypes offer

an advantage over VSV-G as they are significantly less cytotoxic when expressed in packaging cells, and may therefore be useful to generate stable packaging cell lines [76]. Although these different envelopes partially shift LV tropism, residual expression in neurons is still observed in most cases.

Transcriptional regulation to restrict transgene expression in astrocytes

Specific transcriptional elements may be sued to drive transgene expression and overcome the lack of LV specificity. However, tissue-specific and cell type-specific promoters are poorly characterized and the packaging size of lentiviral vector restrains the size of promoters that can be inserted. Current regulatory element databases are supported by limited experimental data or information about putative binding sites. Recent results from chromatin immunoprecipitation and sequencing (ChIP-seq) studies enables genome-wide localization of transcription factors (TFs) and should greatly contribute to decoding cell-type-specific gene expression programs. The ENCODE project aims to establish a catalog of all regulatory elements in the human genome [77-79]. Preliminary data indicate that, if cell-type-specific DNA binding profile and TF complexes control cell-type-specific expression, histone modifications, DNA methylation and accessibility also contribute to cell-type-specific TF binding [80]. There are approximately 1,400 characterized and sequence-specific TFs, which bind to proximal regulatory elements close to the transcription start site (TSS) but also to enhancers, silencers, insulators and locus control regions which are sometimes located far away from the TSSs [80-83]. New methodologies are currently being developed to better predict cell-type-specific gene expression based on *cis*-regulatory sequences and to investigate the relationship between TF binding and gene expression in a systematic and quantitative manner [84-85]. In parallel, transcriptomic databases and information regarding miRNA distribution have been established for astrocytes, neurons and oligodendrocytes [86-88]. These high-throughput methods, and use of BAC-EGFP reporter and BAC-Cre recombinase driver mouse lines (GENSAT, the Gene Expression Nervous System Atlas project) [89-91], will greatly facilitate the development of controlled temporal and tissue-specific cassettes for CNS applications. They are also of use in experimental gene function manipulations with cellular resolution, particularly in astrocytes [86, 92]. One consortium is already using such data to develop mini-promoters that are suitable for viral-mediated gene expression (Pleiades promoter project) [93-95].

Most of the published data are based on a very limited set of astrocyte-specific promoters. The GFAP promoter is the most documented astrocytic promoter [1, 96] and fragments of the GFAP promoter responsible for the cell-type expression were identified in the early 1990s [97]. Dr Brenner and coworkers identified the minimal region sufficient for an astrocyte-specific expression (GFA-ABC1D promoter, 681 bp) [98]. Cloning of a gfa2 fragment (2.2 kb) into adenovirus 5 (Ad5) and AAVrh43 vectors restricts transgene expression in rat astrocytes [99-102]. Combining AAV5, AAV8, AAVrh43 and the human gfa2 promoter resulted in an efficient astrocytic transgene expression in the striatum of adult rats [1, 29, 101].

An eight fold increase in transgene expression in the striatum of adult rats can result from upregulated GFAP expression in reactive astrocytes after a brain lesion [103]. Therefore, this promoter is wells suited to provide strong transgene expression in conditions of astrocyte reactivity. Similarly, OASIS, (originally identified as a gene that is specifically induced in cultured astrocytes) is also upregulated in reactive astrocytes after kainic acid treatment and the number of glial fibrillary acidic protein (GFAP)-positive astrocytes is low in OASIS knockout mice. OASIS transcription is induced during endoplasmic reticulum stress in astrocytes, but not in other cell types. This basic leucine zipper transcription factor of the CREB/ATF family represents a potential candidate for controlled transgene regulation in normal and pathophysiological conditions [104-105].

Additional astrocytic promoters are emerging and appear to be promising candidates for cell-type specific expression. These include the aldehyde dehydrogenase 1 family member L1 (Aldh1L1), apolipoprotein E,

aquaporin-4, glutamine synthetase, excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2) and connexin 30 promoters but some of them have weak transcriptional activities [86, 106-112]. Mechanisms controlling the transcription of astrocyte markers are emerging and recent data demonstrate that nuclear factor-I (NFI) is involved in cell-type specific transcription of GFAP and α1-antichymotrypsin. The binding of stimulating proteins (SPs) 1 and 3 to a GC-box near the TSS in the excitatory amino acid transporter 1 (EAAT1 or GLAST) contributes to transcription regulation in astrocytes, however in that case, NFI is not implicated [108, 113]. First generation promoters, including GFAP and Aldh1L1, are based on widely expressed homogeneous astrocyte-specific markers. New promoters are needed to further characterize subsets of astrocytes in different brain regions and take into account the substantial differences in gene expression between cortical cells, cerebellar astrocytes, Bergman glia, Müller cells, radial glial cells, resting or reactive astrocytes. Their heterogeneous response to injury and disease also reflect considerable functional diversity [114-115]. Meanwhile, post-transcriptional regulation represents an interesting alternative strategy to restrict transgene expression in astrocytes.

Post-transcriptional regulation with miRNA

Post-transcriptional regulation or detargeting strategy takes advantage of microRNA (miRNA) biology to prevent transgene expression in non-desired cell types. MicroRNAs (miRNAs) are non-coding RNAs (~22 nt long), which bind to the 3'-untranslated region (3'-UTR) of a large number of target mRNAs. They repress their expression by various mechanisms including translational inhibition and mRNA degradation [116-118]. Recent data suggest that miRNA regulation might be far more complex than initially reported and has a role in the activatation of gene expression as well as repression [119]. A large proportion of all identified miRNAs (approximately 1,000 human miRNAs) are expressed in the mammalian brain. A group of miRNAs are particularly enriched in subcellular compartments such as synapses or dendrites, suggesting a role in local protein expression [117, 120]. Numerous functional pathways are targeted by miRNAs and some of these are linked to human diseases [121-126]. The negative regulation of gene expression is mediated through base-pairing with complementary regions within the 3'-UTR of their target mRNAs; this interaction is mainly mediated by the seed region (2^{nd} to $7^{th}/8^{th}$ nucleotides) [118, 127-130]. Simultaneous ribosome profiling and mRNA measurement suggest that miRNA predominantly act by lowering mRNA levels [131]. The "detargeting strategy" was suggested [52, 132-133] to suppress transgene expression in hematopoietic cells and therefore prevent the appearance of an immune response following systemic LV administration. The hematopoietic-specific miR223 was shown to recognize its target sequence within the transgene and prevent its expression in antigen-presenting cells [134]. Naldini and collaborators demonstrated a synergistic effect by concatemerizing miR-targets (miRT), with higher repression when several miRT sequences (up to four) are juxtaposed [52, 132, 135]. In subsequent studies, they then showed that tissue-specific miRNAs from a broad array of cell types successfully restrict transgene expression. There is a relationship between miRNA abundance within a cell and the extent to which that miRNA suppress gene expression [52]. When a miRT sequence with partial complementarity to its miRNA (a bulged miRT) is present in the 3'-UTR of a gene of interest, both post-transcriptional repression (mRNA degradation) and translational repression occurs [132, 135-136]. In contrast, mRNA degradation is the main mechanism of action for synthetic miRT, which is fully complementary to its miRNA [52, 132, 137-138]. Synthetic miRT with multiple copies of a perfectly complementary miRNA target sequence optimizes transgene repression [52, 134, 137]. However, the multimeric complex RISC (RNA-induced silencing complex), containing argonaute (Ago) subunits that bind to miRNA, significantly differs in its capacity

to direct translational repression or mRNA degradation. The abundance of catalytic (Ago2) and noncatalytic proteins (Ago1, Ago 3 and Ago 4) is cell-type specific [137]. Ago2 endonuclease acts with fully homologous miRT and cleaves mRNA molecules whereas Ago1, Ago3 and Ago4 can act with partially homologous miRT sequences. These parameters will have important implications for the efficacy and specificity of detargeting strategies. The ability to blocking expression in unwanted cells can be dependent on the species, the brain structure and the subpopulation of cells considered. Further studies are warranted to determine the expression profile of Ago proteins in various CNS cells [137].

Neuronal miRNAs to targets astrocytes

The detargeting strategy has been used to restrict transgene expression in astrocytes in the context of LV gene transfer. The first step is to select a miRNA that is expressed in cells, which are not of interest but is not expressed in astrocytes. A natural target sequence (with partial homology) or a sequence fully complementary to the mature miRNA (miRT) is cloned in the 3' untranslated region (3' UTR) of the LV transfer vector (Figure 3). When LV-Transgene-miRT enters a non-targeted cell, the endogenous miRNA recognizes the miRT present on the mRNA encoding the transgene and blocks its expression [139-140]. However, when LVs enter astrocytes, which do not express the miRNA, the transgene is expressed (Figure 3).

The expression profiles of miRNAs in the adult mouse CNS and throughout development are available [141-143] but have very limited information concerning cell-type-specific expression. Expression profiling in mouse embryonic stem cells show that several miRNA are expressed during neuronal differentiation, particularly the miR124 and miR9 molecules, which control neural lineage [144]. MiR124 plays an important role in neuronal development and differentiation through its interaction with mRNAs that code for proteins implicated in neurogenesis such as neuroD1, Notch, PTBP1, PTBP2 (polypyrimidine tract-binding protein) and SCP1 (small C-terminal domain phosphatase 1) [145-150]. It has been found that miR124 is expressed in neurons but not in astrocytes whereas miR9 is expressed in neural progenitors and some neurons [124, 151]. Based on these data, we identified a natural miR124 target sequence (mir124T) in the mouse integrin-β1 gene (partial homology) and integrated four copies in the 3'-UTR of a LV [152] (Figure 4). The rat and mouse integrin-β1 sequences differ by one nucleotide, which may explain the difference in detargeting between mice and rats (Figure 4C). In contrast, the synthetic sequence has similar effects in both species. Experiments in primary striatal neurons and astrocyte co-cultures indicated that the presence of a miR124T in LV reduced transgene expression exclusively in NeuN-positive neurons. RT-PCR analysis revealed that the silencing of the transgene was mainly due to mRNA cleavage and not due to inhibition of translation (in agreement with Guo et al., 2010). Expression of integrin-β1 mRNA was unaltered, indicating that the miRNA regulatory pathway was not saturated or dysfunctional, which could be deleterious to astrocytes. To eliminate the limited residual expression in neurons and further improve cell-type specificity, we could consider including additional miRT sequences as previously proposed for targeting liver endothelial cells [52]. Based on the limited information available miR9T [153-154], miR10T [125] or miR128T [124] may be integrated to LV genome to restrict transgene expression to astrocytes. It would be important to first establish the cell-type specific expression profile of these miRNAs both *in vitro* [87] and *in vivo*. However, studies of the striatum and hippocampus of adult mice demonstrated that combining LV pseudotyping with Mokola envelope and a miR124T detargeting strategy can efficiently restrict transgene expression to astrocytes [152].

Using this astrocyte-specific LV, Quesseveur and collaborators selectively overexpress brain-derived neurotrophic factor (BDNF) in the hippocampus to investigate the contribution of astrocytic BDNF in the activity of the antidepressant drug, fluoxetine [155]. The results demonstrate that BDNF can act through neurogenesis-dependent and independent mechanisms to regulate different aspects of anxiolytic-/antidepressant-like responses. BDNF expression elicited anxiolytic-/antidepressant-like effects in the novelty suppressed feeding test, an effect, which was neurogenesis-dependent. Furthermore, BDNF potentiated the anxiolytic-like activity of fluoxetine in the elevated plus maze test, a phenomenon, which was independent of neurogenesis. Remarkably, at presynaptic level, BDNF decreased 5-HT neurotransmission through a functional desensitization of the 5-HT_{1A} autoreceptor.

In a second study with this astrocyte-specific LV, mutant huntingtin fragments were overexpressed in striatal astrocytes to assess the contribution of these cells to Huntington's disease pathogenesis [156]. A progressive increase of GFAP immunostaining, morphological changes, associated with a decreased expression of glutamate transporters, and glutamate uptake was observed in infected astrocytes. This astrocytic phenotype was associated with neuronal dysfunction further supporting the importance of neuron-astrocyte cross-talk in neurodegenerative disorders.

Perspectives

Although a first generation LV and AAV serotypes are available to target astrocytes *in vivo* [1, 26, 29, 98, 101, 157], new viral engineering developments are still required to take into account the heterogeneity of astrocytes and better understand the functionality of these cells in pathophysiological conditions. It is necessary to combine strategies to achieve high expression levels (cis-regulatory elements for spatial, temporal and cell-type specific transgene expression, specific silencer and enhancer sequences) and bioinformatic tools (to generate synthetic regulatory elements and mini-promoters adapted to viral vector backbones) to advance our understanding and ability to treat CNS pathologies [94, 158]. These new vectors will be essential to allow the investigation of the functional diversity of astrocyte subsets and go beyond the 'pan-astrocyte' markers currently used. Increasing evidence demonstrates that astrocytes may play a prominent role in neurodevelopment, neurodegenerative and neuropsychiatric diseases [159-160]. Functional alteration and protein aggregation have been reported in Alzheimer's, Huntington's and Alexander's diseases. Aβ aggregation and internalization in astrocytes profoundly alter their metabolic phenotype with deleterious consequences for neuronal viability *in vitro* [161]. A reduction of astrocytic arborization and shrinkage of the astroglial domain occurred at early stages in a mouse model of Alzheimer's disease [162] and astrocyte networks is perturbed and might contribute to cortical dysfunction [163].

In addition, signaling cascades are being investigated to develop new astrocyte-specific therapies, and astrocytes are being considered as potential host for the delivery of therapeutic candidates promoting neuron survival and recovery [29]. Targeting astrocytes could be a potent strategy for many brain diseases in which neurons would benefit from the supportive functions of astrocytes [164]. For example, interfering with the NFAT-calcineurin pathway in astrocytes using gene transfer reduces glial activation and lead to an improvement of cognitive and synaptic functions, and lower amyloid levels in APP/PS1 mice [157, 165]. Similarly, GDNF delivery to astrocytes of mice with Parkinson's disease induces neurogenesis, enhances neuronal function, protects nigral dopaminergic neurons and improves cognitive functions [29, 70, 166]. Rett's syndrome is an X-chromosome-linked autism; the restoration of MeCP2 (methyl CpG-binding protein 2) in astrocytes of deficient mice has been shown to significantly improve locomotion and anxiety levels, restore respiratory abnormalities and prolong

lifespan [167]. Finally, the overexpression of a glutamate transporter (GLT-1) partly rescues the astrocyte phenotype in cases of Huntington's disease [156].

New generation viral vectors could contribute to a better understanding of the mechanisms underlying astrocyte signaling and reactive astrogliosis. Related studies should provide new opportunities to identify and administer candidates that might delay or block injury or disease outcomes in various CNS pathologies.

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Figures

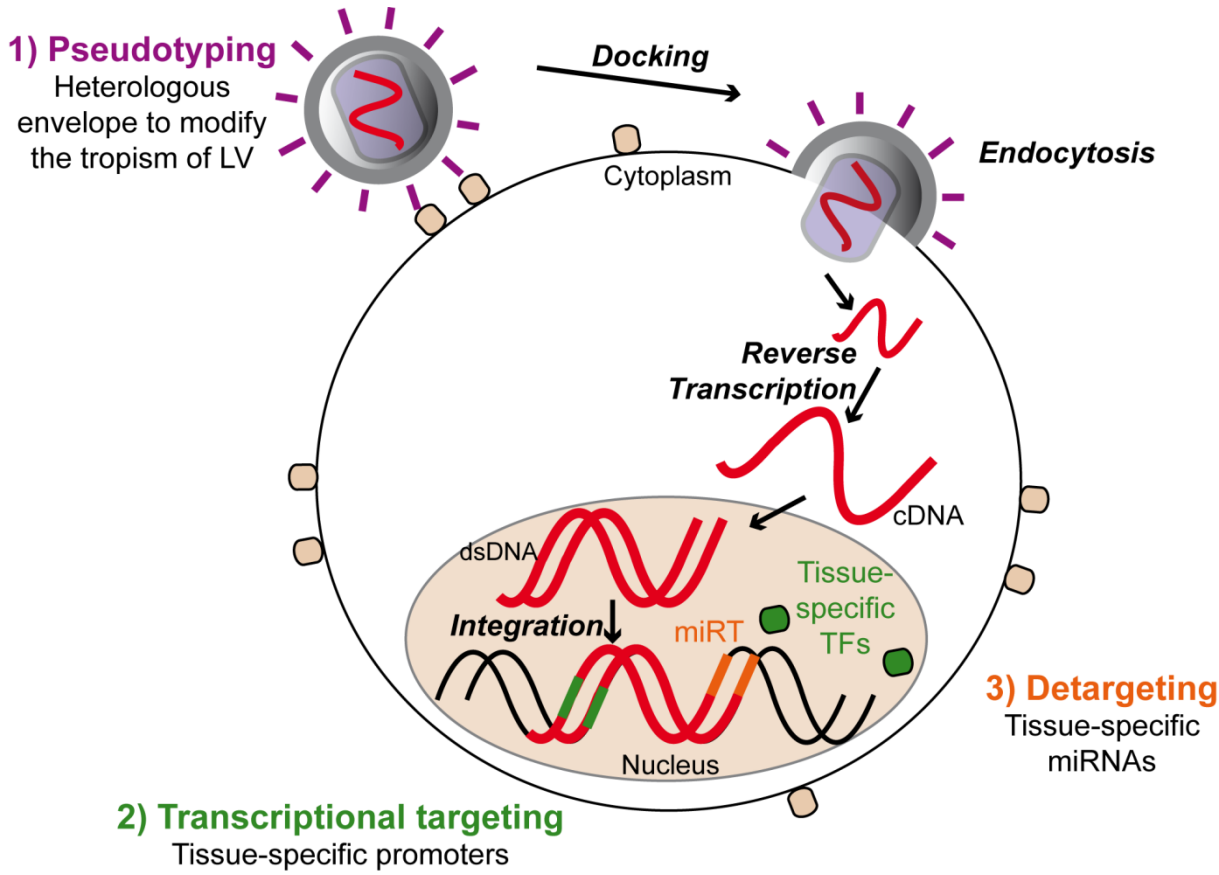

Figure 1: Strategies to modify the tropism of LVs. 1) use of heterologous envelope to pseudotype LVs and modify the entry of the vector into cells, 2) integrate tissue-specific promoter to restrict transgene expression and 3) integrate post-transcriptional regulatory elements (tissue-specific microRNA target sequences) in the 3' untranslated region (3'-UTR) of the transgene to block transgene expression in unwanted cells.

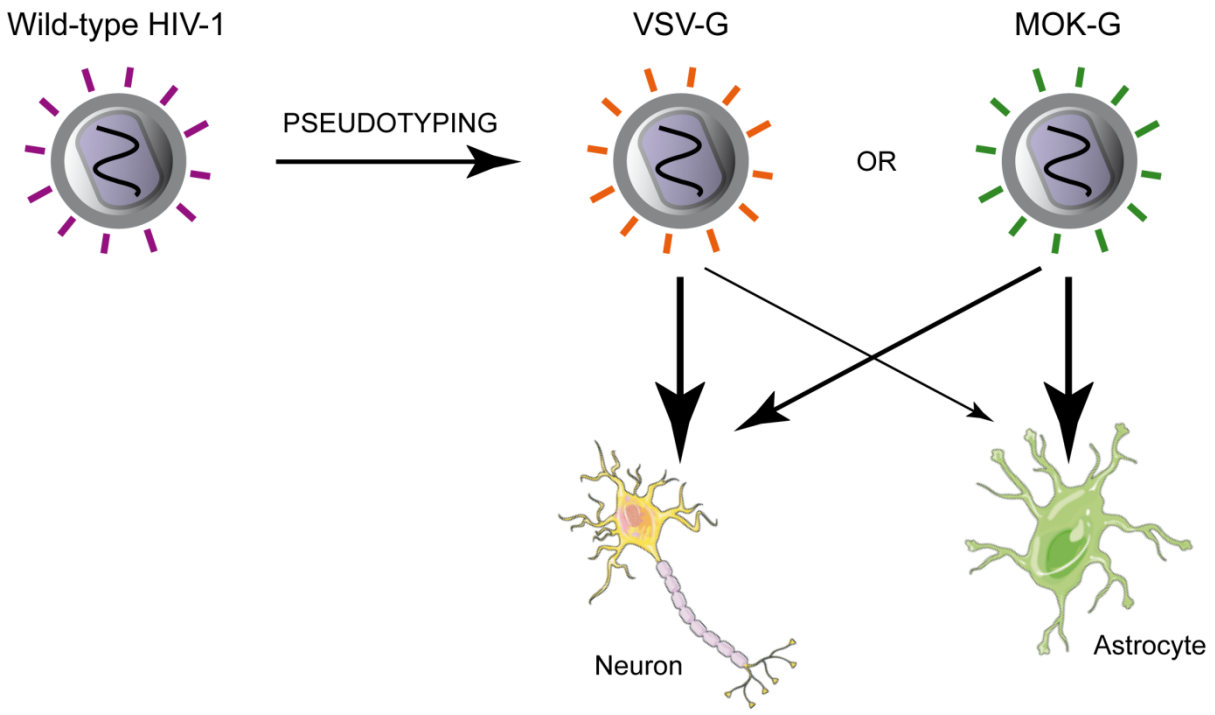

Figure 2: LV pseudotyping and tropism in the CNS. VSV-G pseudotyped LVs mainly transduce neurons whereas MOK-G pseudotyped LVs have an astrocytic tropism.

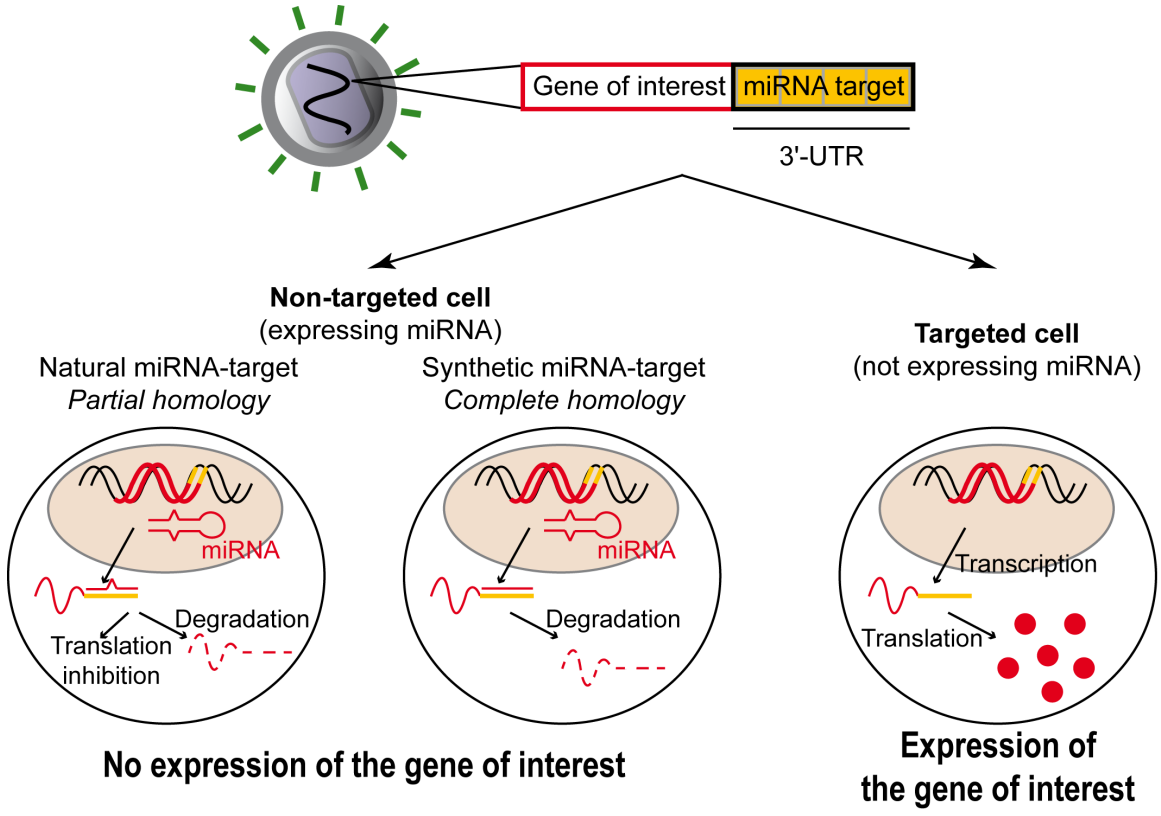

Figure 3: The microRNA target sequences (miRT, usually 4 copies to maximize the detargeting effect) integrated in the 3'-untranslated region (3'UTR) of LV are recognized by a tissue-specific miRNA and prevent transgene expression in unwanted cells.
The expression of the miRNA in non-targeted cells (left panel) and recognition of miRT sequence in the 3'-UTR of the transgene leads either to an inhibition of translation or degradation of mRNA (depending whether a natural

miRT sequence with a partial homology or a synthetic miRT with a full homology with the miRNA has been cloned in the LV).
A contrario, in targeted cells, which do not express the miRNA (right panel), the transgene is expressed.

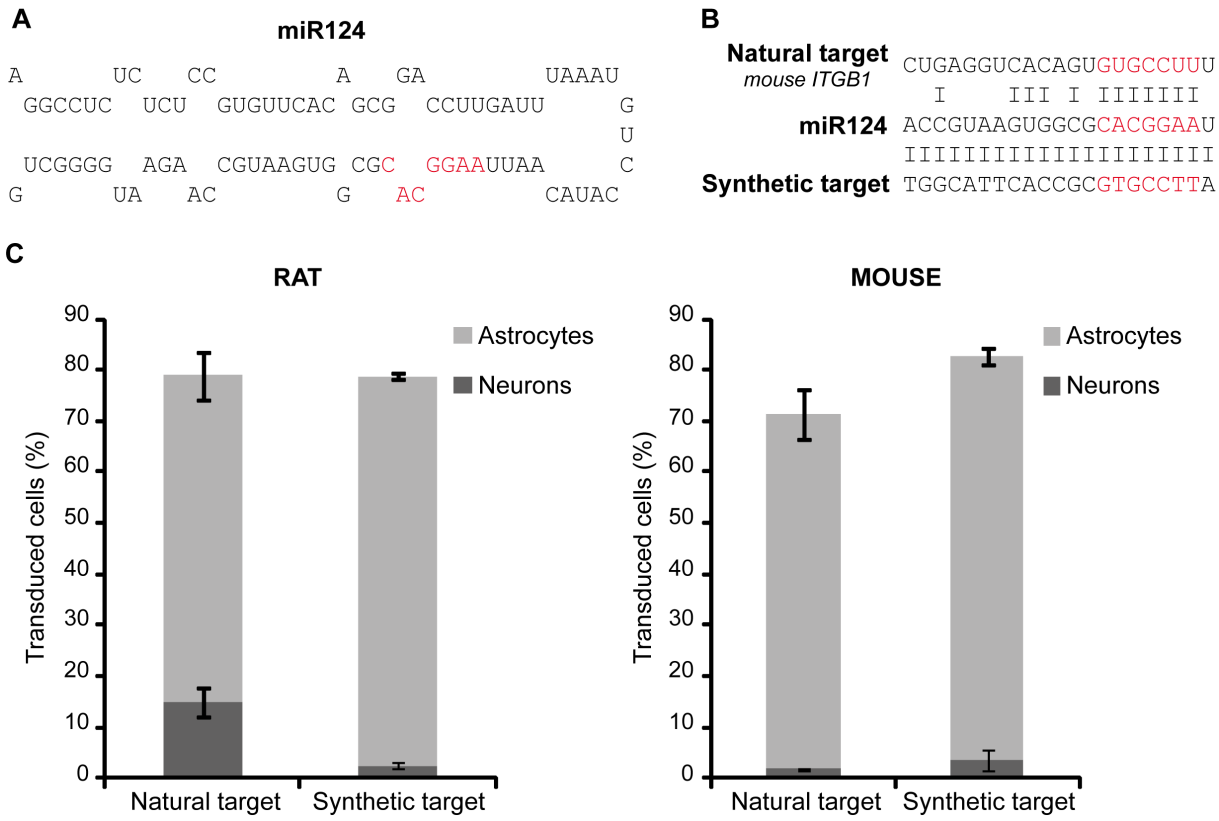

Figure 4: Potency of the neuron-specific miR124 for LV detargeting in the CNS. A) Sequence of the human miR124 with the seed region represented in red. B) To develop an astrocyte-specific LV, two types of miR124 target sequences were integrated in the LVs. As a first strategy, a natural miR124 target (miR124T), here from the integrin β1 (ITGB1) gene, which is partially complementary to miR124 was used. As a second strategy, a synthetic target fully homologous with miR124 was used. C) I*n vivo* data in the striatum of adult mice and rats injected with an MOK-LV-PGK-GFP-miR124T showing the astrocytic tropism. The difference in detargeting between mice and rats is probably due to the presence of one nucleotide mismatch between the rat/mouse ITGB1 sequence (natural miR124T). In contrast, the synthetic miR124T is highly efficient in both species.