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Emerging role of viral vectors for circuit-specific gene interrogation and manipulation in rodent brain

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Abstract

Over the past half century, novel tools have allowed the characterization of myriad molecular underpinnings of neural phenomena including synaptic function, neurogenesis and neurodegeneration, membrane excitability, and neurogenetics/epigenetics. More recently, transgenic mice have made possible cell type-specific explorations of these phenomena and have provided critical models of many neurological and psychiatric diseases. However, it has become clear that many critical areas of study require tools allowing the study and manipulation of individual neural circuits within the brain, and viral vectors have come to the forefront in driving these circuit-specific studies. Here, we present a surface-level review of the general classes of viral vectors used for study of the brain, along with their suitability for circuit-specific studies. We then cover in detail a new long-lasting, retrograde expressing form of herpes simplex virus termed LT-HSV that has become highly useful in circuit-based studies. We detail some of its current uses and propose a variety of future uses for this critical new tool, including circuit-based transgene overexpression, gene editing, and gene expression profiling.

Keywords

Viral vectors; Circuits; Retrograde tracing; CRISPR; HSV; Gene expression

1. General introduction to viral vectors

Engineered viral vectors provide transport for a genetic payload and their utility derives from the great diversity of potential cargo and cellular targets. Because viral vectors enable or enhance the function of many other technologies, they have become indispensable in modern neuroscience research. In combination with Cre-recombinase driver lines, they allow cell-type specific manipulation of gene expression, while viral delivery of genetic calcium indicators or optogenetic and pharmacogenetics tools makes possible circuit-specific observation and manipulation of neuronal activity in vivo. Many different viral vectors have been widely used for the manipulation of gene expression in the brain, and each vector has its own set of advantages and disadvantages. The most efficient choice of virus (i.e., adeno-associated virus (AAV) vs herpes simplex virus (HSV)), serotype (i.e., AAV2 vs AAV8), and mode of administration (i.e., systemic vs stereotaxic injection) will depend upon many factors that stem from the experimental hypothesis, the animal model, the transgene, and the

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cells and region in question. The nature of the target cells (i.e., dividing cells like glia or non-dividing mature neurons) is critical: some viruses exclusively infect cells at a specific point in the cell cycle. Other important factors include the size and nature of the transgene insert. For example, some viruses are more suitable for expression of transgenes encoding small proteins or short RNAs (like siRNA (Pfeifferberger et al., 2016)) rather than larger transgenes needed for high molecular weight receptors, or larger tagged proteins. If the viral vector integrates the transgene into the host genome, then oncogenes and genes with high potential to mutate into oncogenes are inappropriate in the transgene insert. Immunogenicity is another important factor as some vectors can produce mild to strong innate and adaptive immune responses in the host. Cell specific tropism and duration of transgene expression will also influence the vector chosen, and some viral vectors even have the potential to become replication competent and require BSL-2 laboratory facilities. Another key factor is the direction and mechanism of viral infection (i.e. anterograde, retrograde, anterograde trans-synaptic and retrograde transsynaptic), which varies by viral capsid type and by promoter, and can be leveraged for circuit-specific studies, as will be described in later sections. In this section, we will summarize the nature of various viral vectors and the uses for which each is best suited (see Table 1 for brief summary). It is also critical to note that some of the properties of viral transduction can be purposely modified by the choice of promoter used to drive transgene expression. For instance, the cytomegalovirus (CMV) promoter is frequently chosen for high-copy expression of things like fluorophores (Grueter et al., 2013), while the human synapsin (hSyn) promoter can drive less-robust, but neuron specific, expression of receptors or enzymes to alter cell signaling (see below for more detail).

1.1. Lentivirus

Lentiviruses (Sakuma et al., 2012) are a subclass of retroviruses, so named not because of retrograde neuronal transduction, but rather because they carry a homodimer of linear, positive-sense, single-stranded RNA genomes that are retro-transcribed into linear double-stranded DNA and integrated into the host chromosome. Unlike most retroviruses which only infect dividing cells, lentiviruses can infect both dividing and non-dividing cells in a wide range of hosts including neurons. In addition, these modified Human Immunodeficiency Virus (HIV)-1 vectors have few cytotoxic effects in many cell types, and thus it has been suggested that lentiviruses may be extremely useful for gene therapy and therapeutic applications (Kobayashi et al., 2017). Because their genetic complement is RNA, and because they can carry a payload of no > 8 kb of exogenous DNA expressed in place of viral genes, they are often a vector of choice for siRNA delivery but not for expression of large transgenes or complex promoters.

Cell types targeted by a given lentivirus are determined by the envelope glycoproteins and their interactions with receptors on target cells, and these glycoproteins can be further post-translationally modified by the packaging cell line, allowing for a wide variety of cell targets (Delzor et al., 2013). This can be further modified by a process called pseudotyping, in which the lentiviral envelope proteins can be replaced by envelope proteins from other viruses, significantly expanding the range of potential target cell types. Some retroviral vectors require nuclear membrane dissolution in order for integration of payload genetic

material to occur, making them unsuitable for transduction of non-dividing cells like neurons (Park and Kay, 2001). Nevertheless, preserving the original cell-cycle specificity can actually make lentiviral vectors ideal for studying immature neurons and neurogenesis. For example, in the hippocampus, this type of lentivirus mediated trans-gene expression has been shown to be restricted to a subpopulation of neural progenitor cells and immature neurons present in the inner granule cell layer, while mature neurons in the outer layers are resistant to this transduction (van Hooijdonk et al., 2009).

In general, because they integrate their genetic payload into the host genome, lentivirus-delivered transgenes continue to express for the life of the cell, and thus the long period of expression of lentivirus allows delineation of the long-term impact of gene expression. For example, long-term lentiviral-mediated overexpression of melanocyte stimulating hormone in the arcuate nucleus of the hypothalamus reduced weight gain and improved glucose tolerance in obese mice over the course of months (Eerola et al., 2013). On the other hand, the recombination capacity needed for integration into the host genome allows for rare series recombinations to restore replication competence to the vector. However, the frequency of these events in more recent generations of lentiviral vectors is astronomically low, as the packaging systems have incorporated more and more separate but necessary gene compliments, making the number of ordered recombinations required to restore competence higher and higher (Pfeifferberger et al., 2016; Sakuma et al., 2012). Another major disadvantage of lentiviruses (and other retroviruses) is the random integration of the transgene into host genomic DNA, which can occur anywhere within the genome and may interrupt important gene sequences causing loss of function mutations, or produce oncogenes, although the rate of these effects is lower for lentiviral vectors than for other retroviruses (Modlich et al., 2009; Montini et al., 2006). However, more recent advances, including the advent of integration-deficient lentiviral vectors (Saenz et al., 2004), has partially mollified these concerns (Sakuma et al., 2012).

1.2. Adenovirus and adenovirus-associated vectors

Unlike lentiviruses, the adenoviral vector (AVV) genome is packaged as double stranded DNA and does not integrate into the host genome. Adenoviruses are the largest non-enveloped virus and are transported through endosomes, but first generation AVVs were generally very immunogenic. The advent of helper-dependent AVVs has reduced immunogenic concerns in the central nervous system (Zou et al., 2002), and replication incompetent AVVs have been used successfully to express transgenes in neurons. However, when compared to lentivirus, AVVs are less neuron-specific, and generally more successful in transfecting glia (Duale et al., 2005). It is generally accepted that AVVs can be amplified very rapidly with high yields and have a high efficiency of transduction and level of gene expression (Duale et al., 2005; Teschemacher et al., 2005), but this expression is transient, and typically disappears within a few weeks.

AVVs have no envelope that can be manipulated for recognition by specific cells and receptors allowing endosomal uptake of AVV are expressed ubiquitously, reducing their utility in cell type-specific investigations. These characteristics make AVVs quite useful for experiments involving cultured cells, and they transduce many cell types in culture

(Teschemacher et al., 2005). However, due to concerns over immunogenicity and the limited cell type-specificity, the last decade has seen a shift away from human-derived adenoviral vectors and toward canine adenovirus type-2 (CAV-2) and adeno-associated viral vectors (AAVs).

CAV-2 has little immunogenicity and preferentially transduces neurons through a variety of exposure methods, including intranasal, intramuscular and intracerebral injections (Soudais et al., 2001). CAV-2 is taken up by neurons through interactions with receptors localized to the presynaptic compartment, and are then distributed widely by axonal transport (Junyent and Kremer, 2015). This retrograde transport makes CAV-2 an important tool for circuit-tracing experiments, and it has been also used to determine the molecular profiling of neurons on the basis of their connectivity (Ekstrand et al., 2014). Importantly, through the use of helper plasmids during viral packaging, the capacity of CAV-2 vectors is up to 30 kb, making them versatile tools for expression of multiple target genes (Junyent and Kremer, 2015).

AAVs were discovered as a contaminant in adenovirus preparations from infected humans (Atchison et al., 1965), and have a variety of features making them useful for the expression of transgenes in brain. Like adenovirus, AAV is a small, nonenveloped virus that packages a single-stranded linear DNA genome, approximately 5 kb long, and infects non-dividing and dividing cells. The viral genome generally does not integrate into the host genome, but exists as circular episomes containing multiple copies of the transgene cassette (Samulski and Muzyczka, 2014). Occasional integration is seen, most commonly in chromosome 19 in human tissue (Kotin et al., 1990), and particularly in hepatocytes, which show an integration rate of about 1% in infected cells (Nakai et al., 2001), but is more rare in heart, muscle and brain tissue (Samulski and Muzyczka, 2014). Critically, AAV is unable to propagate by itself, and requires a helper virus like adenovirus to replicate (Samulski and Muzyczka, 2014), meaning infections do not spread. Moreover, AAVs produce very mild to no innate or adaptive immune response in the host and are not associated with any animal or human diseases. Like adenovirus, the expression of AAV also tends to be persistent, lasting upwards of 6 months in vivo (McCown et al., 1996).

For these reasons, AAVs have become an important neuroscience tool for the study synaptic plasticity and connectivity. Perhaps their most important application has been in the development of optogenetics, where they have been used to express light-activated channels allowing the control of neuronal activity in vivo (Fenno et al., 2014; Mattis et al., 2011). AAVs have also been used to study a variety of diseases and behaviors, from overexpression of alpha synuclein in the rat substantia nigra to produce a rodent Parkinson's model (Ulusoy et al., 2010), to expression or inhibition of the transcription factor FosB to study addiction (Kelz et al., 1999), depression (Robison et al., 2014; Vialou et al., 2010), or learning (Eagle et al., 2015).

To increase the specificity and tropism of AAVs, many different serotypes have been created via capsid modifications that allow the virus to interact with proteoglycans expressed by specific cell types. Tissue-specific and inducible promoters have also been developed to increase tissue specificity and control of gene expression respectively (Salganik et al., 2015).

In addition, recombinant AAVs have been engineered to permit retrograde transport of transgenes, and their high level of transgene expression and low immunogenicity make them very attractive. The rAAV2-retro gene delivery system infects neuron cell bodies as well as axons to achieve long-term, high-level transgene expression in a retrograde fashion (Tervo et al., 2016). AAVs have also been used in the transgenic multicolored labeling strategy known as “Brainbow” (Richier and Salecker, 2015). In this system, transgenic Cre expressing mice are infected with AAVs to provide spatial and temporal control over expression of fluorescent proteins, allowing improved visualization of specific neurons in the Brainbow transgenic mice (Cai et al., 2013).

Another newly emerging property of AAV vectors is anterograde trans-synaptic transmission. Previously, retrograde trans-synaptic tracers such as rabies virus and pseudorabies virus have been used for mapping neural circuits, but they have a number of limitations, including toxicity (see below). Recently, Zingg et al. produced robust labeling of neurons downstream of the injection location using an AAV1 serotype vector in combination with a Cre-dependent fluorescent reporter (Zingg et al., 2017). The labeling was consistent with a trans-synaptic mechanism of spread, with no labeling of surrounding neurons or glia, though it may exhibit retrograde transport under some conditions. These new tools, when combined with intersectional strategies described below, can be used to categorize neurons by their inputs and molecular identity, and allow forward screening of distinct functional neural pathways embedded in complex brain circuits (Zingg et al., 2017).

This trans-synaptic AAV has recently been adapted to target the peripheral nervous system (Chan et al., 2017). The AAV capsid variant AAV-PHP.S can transduce neurons in the enteric nervous system, cardiac ganglia, DRG, and other peripheral ganglia, and when used with Cre transgenic lines or cell-type-specific promoters, can provide system wide delivery to these populations. The implications of retroviral transduction in the peripheral nervous system include advancing the NIH initiative for Stimulating Peripheral Activity to Relieve Conditions (SPARC) and developing new adaptations of genetic tools such as optogenetics and chemogenetics. Several enhancers and promoters have been shown to enhance the expression of AAV-PHP.eB, including *Dlx5/6*, which enhances viral expression in GABAergic neurons when delivered using intravenous AAV.eB. In addition, the same study details sparse multicolor labeling enabled by these novel capsids, which they term vector-assisted spectral tracing (VAST) (Chan et al., 2017). This method allows segmentation of several local dendritic arbors after tissue clearing, a potentially important tool for the study of plasticity of neuronal architecture in specific central and peripheral circuits.

1.3. Rabies and pseudorabies virus

Early studies of the rabies virus in rats demonstrated the virus’s extreme toxicity upon entering the CNS (Baer et al., 1965). However, recombinant rabies viruses incorporating glycoprotein deletions (such as SAD-B19) have now become powerful tools for retrograde trans-synaptic tracing. This glycoprotein deletion prevents viral budding and transfer, allowing selective monosynaptic mapping of first-order presynaptic neurons. While these viruses have been engineered to have less neurotoxicity, they are still neurotoxic in larger concentrations and have weak efficiency of transgene expression. More recent

improvements, including the CVS-N2cdeltaG strain, have lower levels of neurotoxicity and enhanced retrograde synaptic transfer (Reardon et al., 2016). Retrograde monosynaptic tracing using rabies virus was made possible by novel pseudotyping, enabling high-level expression of genes of interest in synaptically coupled neurons and robust labelling of connections made to single cells (Wickersham et al., 2007a; Wickersham et al., 2007b). More recently, the same technology has been further adapted, combining multiple monosynaptic retrograde and retrograde transsynaptic viruses to allow the tracing of multi-neuron circuits (Watabe-Uchida et al., 2012), including three-node tracing strategies. The rabies virus is pseudotyped with an avian virus envelope protein (EnvA) and therefore cannot infect mammalian cells, thus restricting mouse brain infection to neurons engineered to express a cognate avian receptor. Further, the envelope glycoprotein (RG) that allows transsynaptic spread is removed, such that transsynaptic transfer occurs only from neurons that exogenously express RG. By expressing RG and the avian receptor under cre-lox control, the authors were able to demonstrate that dopamine neurons of substantia nigra and VTA receive inputs from quite different brain regions. In another use of this technology (Schwarz et al., 2015), rabies virus-based input mapping indicated that norepinephrine neurons in the locus coeruleus receive convergent synaptic input from many previously identified brain regions as well as newly identified synaptic partners, including cerebellar Purkinje fibers. Used in conjunction with similar projection-based viral labeling, many diverse inputs and outputs of the locus coeruleus were visualized, indicating that these strategies can be used to confirm the existence of known circuits and to uncover novel or understudied cell type-specific pathways.

Pseudorabies virus (PRV) is a form of swine alpha herpesvirus that has a very wide host-range, making it useful for studying a variety of cell types. PRV carries a viral load of double-stranded DNA within a protein capsid and an envelope acquired from the host cell. In its natural state, PRV is quite cytotoxic, killing cells within hours or days of infection and likely altering neuronal activity and connectivity (McCarthy et al., 2009). However, there are many well-characterized attenuated strains with greatly reduced toxicity that also possess the critical innate property of trans-synaptic spread. Critically, PRVs have also been engineered to express multiple distinct fluorescent proteins in a time shifted manner, allowing the experimenter to determine time of expression and differentiate the synaptic order of infected neurons in a circuit (Boldogkoi et al., 2009). These same tools have also proved useful in monitoring circuit-specific activity through overexpression of a ratiometric Ca^{2+} indicator (Boldogkoi et al., 2009). Alternatively, strains like PrV-Kaplan provide a tool useful for anterograde tracing, particularly in sensory neuronal systems (Rothermel et al., 2007).

1.4. Herpes simplex virus

In the same herpesviridae family as PRV are herpes simplex viruses (HSVs). These large, icosahedral enveloped viruses contain double-stranded DNA. The HSV genome is quite large in comparison to the other viral vectors, stretching to about 150 Kb, and it can be readily modified to include large transgene inserts. However, even slight mutations can impair the virus's ability to replicate, which is of course not a concern when using replication-deficient amplicons. Native HSV is toxic to cells and may alter neuronal activity, but modern HSV-1 amplicons are replication-incompetent, nontoxic, and can express

multiple genes at high levels (Jerusalinsky et al., 2012; Jerusalinsky and Epstein, 2006). After infection, expression is robust beginning around 10 h, peaking around three to five days and disappearing after eight to ten days (Neve, 2016). This relatively short expression window can be an advantage, and HSV has been used to study overexpression or inhibition of various proteins in a time-sensitive manner. For instance, HSVs have been used to examine changes in protein stability in the brain due to specific phosphorylation events (Cates et al., 2014; Ulery-Reynolds et al., 2009) or roles for transcription factors at specific stages of behavioral acquisition and expression (Eagle et al., 2015; Robison et al., 2013).

Recently, a novel form of HSV has emerged, designed to achieve longer expression patterns and so termed long-term HSV or LT-HSV (Fenno et al., 2014). In this pseudotype, the polyadenylation (pA) signal was altered from SV40 late pA to SV40 early pA, and the 692-bp Woodchuck Hepatitis Virus posttranscriptional regulatory element (Zufferey et al., 1999) was added. These modifications increase expression time, with peak expression occurring around two weeks and lasting indefinitely (Fenno et al., 2014; Gremel et al., 2016; Tovote et al., 2016). In addition, LT-HSV was found to possess a retrograde transfer property unique among HSVs (Fig. 1). The retrograde expression appears to be monosynaptic, and occurs across multiple synapse types (Fenno et al., 2014; Gremel et al., 2016; Tovote et al., 2016). LT-HSVs, originally available from the Massachusetts Institute of Technology Viral Vector Core and now produced at Massachusetts General Hospital Viral Vector Core, allow for robust retrograde expression of large genetic loads, such that complex multipart expression tools can now be used in circuit-specific manners. For instance, the large packaging capacity of these LT-HSVs allows retrograde delivery of multiple Cre-dependent transgenes, using floxed stop cassettes (lox-STOP-lox, LS1L; (Fenno et al., 2014)) in combination with internal ribosomal entry site (IRES) sequences that produce multiple protein products from a single Cre-dependent message, something that cannot be easily done with other vectors that have limited payload size, such as AAV and lentivirus.

2. Cell type-specific viral tools

As understanding of the specific contributions of various neuronal and non-neuronal cell types to brain function has increased, the need has arisen for tools that allow modification of gene expression and function of specific cell types in the brain. The most prominent strategy for achieving this end has been the advent of transgenic mouse lines expressing recombinases under the control of promoters derived from genes that are enriched in specific cell types. For instance, tyrosine hydroxylase and dopamine transporter promoters have been utilized to drive expression of Cre recombinase primarily in dopaminergic cells, though patterns of expression differ and the exclusive nature of this expression is dependent on the specific mouse lines (Lammel et al., 2015; Stuber et al., 2015). Such mouse lines can be combined with viral vectors engineered to express their payload in a recombinase-dependent manner. Thus, although many different cell types in a region of interest may be transduced by the viral vector, the transgene will only be expressed in the cells of that region also expressing the recombinase. Examples of the utility of this approach include specific expression of transgenes in D1- vs D2-type medium spiny neurons (MSNs) of the nucleus accumbens (Grueter et al., 2013) and manipulation of activity of those same cells (Francis et al., 2015). Such manipulation can be achieved by cell type-specific expression of

optogenetic channels, as in these MSNs (Francis et al., 2015) or in dopaminergic neurons of the ventral tegmental area (Chaudhury et al., 2013), or using designer receptors specifically activated by designer drugs (DREADDs), which have also been used to manipulate the activity of many specific cell types (Bradley et al., 2017; Locke et al., 2018; Vazey and Aston-Jones, 2014). This approach is amenable to many viral vectors, including HSVs (Grueter et al., 2013) and AAVs (Woodworth et al., 2017), but has the disadvantage of requiring the use of transgenic mice.

Cell type specificity has also been achieved using viral vectors that express their transgene of interest using a cell type-selective promoter directly, eliminating the need for transgenic mouse lines expressing recombinases. Many viral vectors use specific promoters to restrict expression to neurons rather than glia (i.e., using a synapsin promoter (Lopez et al., 2016)), or vice versa, using a glia-specific promoter (Shigetomi et al., 2013). However, the same approach has more recently been applied to targeting of specific subtypes of neurons. For instance, to directly study the roles of striatal MSNs, the Neumaier group created HSVs expressing DREADDs under the control of the dynorphin or enkephalin promoters, restricting their expression to D1 or D2 MSNs, respectively (Ferguson et al., 2011). The success of such cell type-specific approaches is dependent on both the promoter and type of viral vector, and although targeted design of such promoters is possible, serendipitous discovery of inherent cell type-specificity may sometimes provide the best tools. For instance, various serotypes of AAV selectively transduce differential subpopulation of hippocampal cells and cultured cells, and this can be leveraged for cell type-specific targeting of transgene expression (Burger et al., 2004; Royo et al., 2008). Thus, in practice, the choice of viral tools is often driven empirically, using trial and error to find a promoter or serotype that offers the appropriate level and specificity for the experimental needs. It will be critical in future studies to design and/or uncover novel viral tools capable of more specific and stronger transgene expression in targeted cell populations.

3. Current and potential circuit-specific viral manipulations

To assess the role of individual circuits in animal behavior or the circuit-specific contribution of individual genes to cell function or behavior, novel retrograde viral vector approaches will be critical. For instance, a combinatorial strategy using LT-HSV technology has made possible the manipulation of not only a specific projection, but specific cell types within that projection. Targeting dopaminergic cells of the ventral tegmental area (VTA) projecting to nucleus accumbens (NAc) requires an intersectional strategy (Fenno et al., 2014), as not all VTA neurons projecting to NAc are dopaminergic (some are GABAergic), and not all dopaminergic VTA neurons project to NAc (some project to cortex). Methodology for this intersectional strategy has been demonstrated (Fenno et al., 2014), wherein LT-HSV-LS1L virus driving expression of flipase was injected into the NAc of mice that express Cre in only dopaminergic cells (*TH-Cre*). Subsequent injection of double conditional (fDIO – requiring both Cre recombinase and flipase) AAV expressing channelrhodopsin (ChR2) into VTA allowed expression of the ChR2 only in VTA dopamine cells projecting to NAc. More recently, such an intersectional LT-HSV approach was used functionally to selectively stimulate glutamatergic ventrolateral periaqueductal grey (vlPAG) neurons projecting specifically to the magnocellular nucleus (Mc). LT-HSV expressing flipase in a Cre-

dependent manner was injected into the Mc of mice expressing Cre only in glutamatergic neurons (*Vglut2-ires-Cre*). Double-conditional (fDIO) AAV expressing ChR2 was then injected into vIPAG, allowing selective expression of ChR2 into vIPAG glutamatergic neurons projecting to Mc, allowing the authors to separate the circuit-specific analgesic and anxiogenic outputs of vIPAG (Tovote et al., 2016).

This sort of intersectional strategy could also be used for circuit-specific interrogation of the role of a gene of interest in a particular behavioral output. For instance, by combining a retrograde virus expressing Cre injected into a target region with a locally expressing virus overexpressing a gene of interest in a Cre-dependent manner injected into a region sending projections to that target, one could measure the behavioral effects of the overexpression on the function of neurons specifically projecting from one region to another, as well as subsequent behavior of the animal (Fig. 2A). Such an experiment allows determination of the sufficiency of a particular gene product within a specific circuit for a given cellular or behavioral phenotype. This method could also be used to inhibit expression of a specific gene product by combining retrograde viral Cre expression with local viral Cre-dependent expression of a short hairpin RNA or small interfering RNA complementary the mRNA of the target gene. This would result in circuit-specific knockdown of the targeted gene product, allowing determination of the necessity of a particular gene product within a specific circuit for a given cellular or behavioral phenotype. A similar result could be obtained using Cre-dependent, circuit-specific overexpression of an inhibitor peptide or dominant negative version of the gene of interest.

These overexpression or RNA knockdown strategies are relatively simple, but may be considered artificial as they rely on exogenous overexpression or RNA manipulation rather than direct manipulation of the target gene itself, as in true knockout animals. To address this, our lab and others have begun to pilot a CRISPR/Cas9 approach that allows circuit-specific editing of the chromosomal DNA encoding a target gene. The CRISPR/Cas9 system consists of the Cas9 nuclease and a single guide RNA (gRNA), which is a fusion of a CRISPR RNA (crRNA) and a trans-activating crRNA that binds Cas9 nuclease and directs it to a target sequence based on a complementary base-pairing rule (Chira et al., 2017). By combining retrograde viral expression of the Cas9 enzyme with a local viral expression of guide RNA, CRISPR-mediated editing of a target gene could be used to determine its circuit-specific role (Fig. 2B). A critical issue in the use of CRISPR technology is the potential for off-target effects and general toxicity of Cas9 overexpression (Jiang and Doudna, 2017). For this reason, our lab and others piloting this technique have combined the local expression of gRNA with local expression of Cre, and made the retrograde Cas9 expression Cre-dependent using the LS1L lox-stop-method (Eagle et al., 2016), thus preventing any effects of general Cas9 overexpression in non-targeted projection circuits. This method has the added advantage that the retrograde Cre-dependent expression of Cas9 can be combined with retrograde Cre-dependent overexpression of the very target gene the gRNA/Cas9 complex is meant to silence, thus achieving a circuit-specific rescue of a circuit-specific gene knockout. Such complex conditional multi-gene expression will require retrograde viral tools with large expression capacity, and the novel LT-HSVs described above are ideally suited for such experiments. Of course, in the absence of these Cre-dependent viral methods, a similar circuit-specific CRISPR approach could be combined

with transgenic Cre driver lines to allow cell-type and circuit-specific manipulation of genes of interest, critical steps for our understanding of their role in the pathophysiology of psychiatric disease.

The number of genes identified as critical for neuronal function and animal behavior has ballooned in the past few decades, and analyzing the individual roles of every gene in every circuit certainly seems beyond the capacity of current technology. Thus, it will be critical to develop unbiased methods for identifying genes regulated under specific conditions in an individual circuit, thus providing potential candidates for circuit-specific gene manipulation. One potential method for identifying genes regulated within a specific brain circuit is translating ribosome affinity purification (TRAP), which uses immunoprecipitation of epitope-tagged ribosomes expressed only in cells of interest to enrich for mRNAs being translated within that specific cell population (Doyle et al., 2008; Heiman et al., 2014; Heiman et al., 2008). Transgenic mice have made this technique extremely versatile, allowing Cre-dependent expression of GFP-tagged ribosomal subunits like L10a (Heiman et al., 2014). Such mice could be injected in a target brain region with retrograde virus expressing Cre, thus causing tagged ribosome expression in all neurons projecting to that brain region. Excision of a specific brain region that projects to the target region and subsequent TRAP (Fig. 2C) would allow purification of actively translating mRNAs from an individual brain circuit, which can then be sequenced. Such an approach was recently used to uncover genes enriched in hypothalamic and midbrain neurons projecting to the mesolimbic dopamine circuit, making use of pseudorabies virus tools to express GFP-tagged ribosomes in this circuit (Pomeranz et al., 2017). In the future, comparing sequencing results from animals treated with a drug or undergoing a specific behavioral paradigm to relevant controls could reveal genes regulated by a specific stimulus in a specific circuit. Moreover, a similar approach could be taken in floxed mouse strains to allow interrogation of the effects of circuit-specific knockout of a given gene on the expression of other genes in the same circuit. This approach could be particularly useful in interrogating the role of individual transcription factors in a specific circuit.

4. Conclusions

A variety of tools and techniques have emerged in recent years to revolutionize the study of the brain, from light-sensitive ion channels to genetically encoded calcium indicators to transgenic animal models. Of the many findings stemming from this ongoing technological wave, the prominence of circuit-specific drives on brain function and animal behavior has been universal. Although lesions and pharmacology have allowed a blunt interrogation of circuit function for many decades, there is a clear demand for more subtle circuit-based tools to allow the manipulation of neuronal function or individual genes within specific circuits, and viral vectors have risen to the forefront as the tools of choice for these critical investigations. Here, we summarized the general classes of viral vectors and detailed a novel long-term expressing retrograde HSV that has contributed to multiple recent innovative studies. It is clear that many new experiments will be performed in the coming years with LT-HSVs and other vectors, and we have attempted to cover some of the potential designs for such experiments and tools best suited to execute them. It is now up to our group and the

rest of the field to use these tools to continue to explore brain circuitry and its role in animal behavior and disease.

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References

- Atchison RW, Casto BC, Hammon WM, 1965 Adenovirus-associated defective virus particles. *Science* 149 (3685), 754–756. [PubMed: 14325163]
- Baer GM, Shanthaveerappa TR, Bourne GH, 1965 Studies on the pathogenesis of fixed rabies virus in rats. *Bull. World Health Organ.* 33 (6), 783–794. [PubMed: 5295402]
- Boldogkoi Z, Balint K, Awatramani GB, Balya D, Busskamp V, Viney TJ, Lagali PS, Duebel J, Pasti E, Tombacz D, Toth JS, Takacs IF, Scherf BG, Roska B, 2009 Genetically timed, activity-sensor and rainbow transsynaptic viral tools. *Nat. Methods* 6 (2), 127–130. [PubMed: 19122667]
- Bradley SJ, Tobin AB, Prihandoko R, 2017 The use of chemogenetic approaches to study the physiological roles of muscarinic acetylcholine receptors in the central nervous system. *Neuropharmacology*. 10.1016/j.neuropharm.2017.11.043.
- Burger C, Gorbatyuk OS, Velardo MJ, Peden CS, Williams P, Zolotukhin S, Reier PJ, Mandel RJ, Muzyczka N, 2004 Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. *Mol. Ther* 10 (2), 302–317. [PubMed: 15294177]
- Cai D, Cohen KB, Luo T, Lichtman JW, Sanes JR, 2013 Improved tools for the Brainbow toolbox. *Nat. Methods* 10 (6), 540–547.
- Cates HM, Thibault M, Pfau M, Heller E, Eagle A, Gajewski P, Bagot R, Colangelo C, Abbott T, Rudenko G, Neve R, Nestler EJ, Robison AJ, 2014 Threonine 149 phosphorylation enhances DeltaFosB transcriptional activity to control psychomotor responses to cocaine. *J. Neurosci* 34 (34), 11461–11469. [PubMed: 25143625]
- Chan KY, Jang MJ, Yoo BB, Greenbaum A, Ravi N, Wu WL, Sanchez-Guardado L, Lois C, Mazmanian SK, Deverman BE, Gradinaru V, 2017 Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nat. Neurosci* 20 (8), 1172–1179. [PubMed: 28671695]
- Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, Ferguson D, Tsai HC, Pomeranz L, Christoffel DJ, Nectow AR, Ekstrand M, Domingos A, Mazei-Robison MS, Mouzon E, Lobo MK, Neve RL, Friedman JM, Russo SJ, Deisseroth K, Nestler EJ, Han MH, 2013 Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* 493 (7433), 532–536. [PubMed: 23235832]
- Chira S, Gulei D, Hajitou A, Zimta AA, Cordelier P, Berindan-Neagoe I, 2017 CRISPR/Cas9: transcending the reality of genome editing. *Mol. Ther. Nucleic Acids* 7, 211–222. [PubMed: 28624197]
- Delzor A, Escartin C, Deglon N, 2013 Lentiviral vectors: a powerful tool to target astrocytes in vivo. *Curr. Drug Targets* 14 (11), 1336–1346. [PubMed: 24020977]
- Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, Gong S, Greengard P, Heintz N, 2008 Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell* 135 (4), 749–762. [PubMed: 19013282]
- Duale H, Kasparov S, Paton JF, Teschemacher AG, 2005 Differences in transductional tropism of adenoviral and lentiviral vectors in the rat brainstem. *Exp. Physiol* 90 (1), 71–78. [PubMed: 15542614]
- Eagle AL, Gajewski PA, Yang M, Kechner ME, Al Masraf BS, Kennedy PJ, Wang H, Mazei-Robison MS, Robison AJ, 2015 Experience-dependent induction of hippocampal DeltaFosB controls learning. *J. Neurosci* 35 (40), 13773–13783. [PubMed: 26446228]

- Eagle AL, M. C, Gajewski PA, Achargui R, Gron LE, Boyce FM, Neve RL, Robison AJ, 2016 Activity-Dependent Transcriptional Regulation of Hippocampal Projections in Fear/Anxiety and Cocaine Reward. Society for Neuroscience Annual Meeting, San Diego, CA.
- Eerola K, Nordlund W, Virtanen S, Dickens AM, Mattila M, Ruohonen ST, Chua SC, Jr., Wardlaw SL, Savontaus M, Savontaus E, 2013 Lentivirus-mediated alpha-melanocyte-stimulating hormone overexpression in the hypothalamus decreases diet induced obesity in mice. *J. Neuroendocrinol* 25 (12), 1298–1307. [PubMed: 24118213]
- Ekstrand MI, Nectow AR, Knight ZA, Latcha KN, Pomeranz LE, Friedman JM, 2014 Molecular profiling of neurons based on connectivity. *Cell* 157 (5), 1230–1242. [PubMed: 24855954]
- Fenno LE, Mattis J, Ramakrishnan C, Hyun M, Lee SY, He M, Tucciarone J, Selimbeyoglu A, Berndt A, Grosenick L, Zalocusky KA, Bernstein H, Swanson H, Perry C, Diester I, Boyce FM, Bass CE, Neve R, Huang ZJ, Deisseroth K, 2014 Targeting cells with single vectors using multiple-feature Boolean logic. *Nat. Methods* 11 (7), 763–772. [PubMed: 24908100]
- Ferguson SM, Eskenazi D, Ishikawa M, Wanat MJ, Phillips PE, Dong Y, Roth BL, Neumaier JF, 2011 Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat. Neurosci* 14 (1), 22–24. [PubMed: 21131952]
- Francis TC, Chandra R, Friend DM, Finkel E, Dayrit G, Miranda J, Brooks JM, Iniguez SD, O'Donnell P, Kravitz A, Lobo MK, 2015 Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biol. Psychiatry* 77 (3), 212–222. [PubMed: 25173629]
- Gremel CM, Chancey JH, Atwood BK, Luo G, Neve R, Ramakrishnan C, Deisseroth K, Lovinger DM, Costa RM, 2016 Endocannabinoid modulation of orbitostriatal circuits gates habit formation. *Neuron* 90 (6), 1312–1324. [PubMed: 27238866]
- Grueter BA, Robison AJ, Neve RL, Nestler EJ, Malenka RC, 2013 FosB differentially modulates nucleus accumbens direct and indirect pathway function. *Proc. Natl. Acad. Sci. U. S. A* 110 (5), 1923–1928. [PubMed: 23319622]
- Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, Suarez-Farinas M, Schwarz C, Stephan DA, Surmeier DJ, Greengard P, Heintz N, 2008 A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135 (4), 738–748. [PubMed: 19013281]
- Heiman M, Kulicke R, Fenster RJ, Greengard P, Heintz N, 2014 Cell type-specific mRNA purification by translating ribosome affinity purification (TRAP). *Nat. Protoc* 9 (6), 1282–1291. [PubMed: 24810037]
- van Hooijdonk LW, Ichwan M, Dijkmans TF, Schouten TG, de Backer MW, Adan RA, Verbeek FJ, Vreugdenhil E, Fitzsimons CP, 2009 Lentivirus-mediated transgene delivery to the hippocampus reveals sub-field specific differences in expression. *BMC Neurosci.* 10, 2. [PubMed: 19144149]
- Jerusalinsky D, Epstein AL, 2006 Amplicon vectors as outstanding tools to study and modify cognitive functions. *Curr. Gene Ther* 6 (3), 351–360. [PubMed: 16787186]
- Jerusalinsky D, Baez MV, Epstein AL, 2012 Herpes simplex virus type 1-based amplicon vectors for fundamental research in neurosciences and gene therapy of neurological diseases. *J. Physiol. Paris* 106 (1–2), 2–11. [PubMed: 22108428]
- Jiang F, Doudna JA, 2017 CRISPR-Cas9 structures and mechanisms. *Annu. Rev. Biophys* 46, 505–529. [PubMed: 28375731]
- Junyent F, Kremer EJ, 2015 CAV-2—why a canine virus is a neurobiologist's best friend. *Curr. Opin. Pharmacol* 24, 86–93. [PubMed: 26298516]
- Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranaukas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ, 1999 Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 401 (6750), 272–276. [PubMed: 10499584]
- Kobayashi K, Inoue KI, Tanabe S, Kato S, Takada M, Kobayashi K, 2017 Pseudotyped lentiviral vectors for retrograde gene delivery into target brain regions. *Front. Neuroanat* 11, 65. [PubMed: 28824385]
- Kotin RM, Siniscalco M, Samulski RJ, Zhu XD, Hunter L, Laughlin CA, McLaughlin S, Muzyczka N, Rocchi M, Berns KI, 1990 Site-specific integration by adeno-associated virus. *Proc. Natl. Acad. Sci. U. S. A* 87 (6), 2211–2215. [PubMed: 2156265]

- Lammel S, Steinberg EE, Foldy C, Wall NR, Beier K, Luo L, Malenka RC, 2015 Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. *Neuron* 85 (2), 429–438. [PubMed: 25611513]
- Locke TM, Soden ME, Miller SM, Hunker A, Knakal C, Licholai JA, Dhillon KS, Keene CD, Zweifel LS, Carlson ES, 2018 Dopamine D1 receptor-positive neurons in the lateral nucleus of the cerebellum contribute to cognitive behavior. *Biol. Psychiatry* 10.1016/j.biopsych.2018.01.019.
- Lopez AJ, Kramar E, Matheos DP, White AO, Kwapis J, Vogel-Ciernia A, Sakata K, Espinoza M, Wood MA, 2016 Promoter-specific effects of DREADD modulation on hippocampal synaptic plasticity and memory formation. *J. Neurosci* 36 (12), 3588–3599. [PubMed: 27013687]
- Mattis J, Tye KM, Ferenczi EA, Ramakrishnan C, O'Shea DJ, Prakash R, Gunaydin LA, Hyun M, Fenno LE, Gradinaru V, Yizhar O, Deisseroth K, 2011 Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat. Methods* 9 (2), 159–172. [PubMed: 22179551]
- McCarthy KM, Tank DW, Enquist LW, 2009 Pseudorabies virus infection alters neuronal activity and connectivity in vitro. *PLoS Pathog.* 5 (10), e1000640. [PubMed: 19876391]
- McCown TJ, Xiao X, Li J, Breese GR, Samulski RJ, 1996 Differential and persistent expression patterns of CNS gene transfer by an adeno-associated virus (AAV) vector. *Brain Res.* 713 (1–2), 99–107. [PubMed: 8724980]
- Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH, Schambach A, Charrier S, Galy A, Thrasher AJ, Bueren J, Baum C, 2009 Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. *Mol. Ther* 17 (11), 1919–1928. [PubMed: 19672245]
- Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, Sergi L, Benedicenti F, Ambrosi A, Di Serio C, Doglioni C, von Kalle C, Naldini L, 2006 Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. *Nat. Biotechnol* 24 (6), 687–696. [PubMed: 16732270]
- Nakai H, Yant SR, Storm TA, Fuess S, Meuse L, Kay MA, 2001 Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo. *J. Virol* 75 (15), 6969–6976. [PubMed: 11435577]
- Neve RL, 2016 The HSV Manual (v1.10). McGovern Institute for Brain Research at MIT.
- Park F, Kay MA, 2001 Modified HIV-1 based lentiviral vectors have an effect on viral transduction efficiency and gene expression in vitro and in vivo. *Mol. Ther* 4 (3), 164–173. [PubMed: 11545606]
- Pfeifferberger E, Sigl R, Geley S, 2016 Conditional RNAi using the lentiviral GLTR system. *Methods Mol. Biol* 1448, 121–138. [PubMed: 27317178]
- Pomeranz LE, Ekstrand MI, Latcha KN, Smith GA, Enquist LW, Friedman JM, 2017 Gene expression profiling with cre-conditional pseudorabies virus reveals a subset of midbrain neurons that participate in reward circuitry. *J. Neurosci* 37 (15), 4128–4144. [PubMed: 28283558]
- Reardon TR, Murray AJ, Turi GF, Wirblich C, Croce KR, Schnell MJ, Jessell TM, Losonczy A, 2016 Rabies virus CVS-N2c(DeltaG) strain enhances retrograde synaptic transfer and neuronal viability. *Neuron* 89 (4), 711–724. [PubMed: 26804990]
- Richier B, Salecker I, 2015 Versatile genetic paintbrushes: Brainbow technologies. *Wiley Interdiscip. Rev. Dev. Biol* 4 (2), 161–180. [PubMed: 25491327]
- Robison AJ, Vialou V, Mazei-Robison M, Feng J, Kourrich S, Collins M, Wee S, Koob G, Turecki G, Neve R, Thomas M, Nestler EJ, 2013 Behavioral and structural responses to chronic cocaine require a feedforward loop involving DeltaFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *J. Neurosci* 33 (10), 4295–4307. [PubMed: 23467346]
- Robison AJ, Vialou V, Sun HS, Labonte B, Golden SA, Dias C, Turecki G, Tamminga C, Russo S, Mazei-Robison M, Nestler EJ, 2014 Fluoxetine epigenetically alters the CaMKIIalpha promoter in nucleus accumbens to regulate DeltaFosB binding and antidepressant effects. *Neuropsychopharmacology* 39 (5), 1178–1186. [PubMed: 24240473]
- Rothermel M, Schobel N, Damann N, Klupp BG, Mettenleiter TC, Hatt H, Wetzel CH, 2007 Anterograde transsynaptic tracing in the murine somatosensory system using pseudorabies virus

(PrV): a “live-cell”-tracing tool for analysis of identified neurons in vitro. *J. Neuro-Oncol.* 13 (6), 579–585.

- Royo NC, Vandenbergh LH, Ma JY, Hauspurg A, Yu L, Maronski M, Johnston J, Dichter MA, Wilson JM, Watson DJ, 2008 Specific AAV serotypes stably transduce primary hippocampal and cortical cultures with high efficiency and low toxicity. *Brain Res.* 1190, 15–22. [PubMed: 18054899]
- Saenz DT, Loewen N, Peretz M, Whitwam T, Barraza R, Howell KG, Holmes JM, Good M, Poeschla EM, 2004 Unintegrated lentivirus DNA persistence and accessibility to expression in nondividing cells: analysis with class I integrase mutants. *J. Virol* 78 (6), 2906–2920. [PubMed: 14990709]
- Sakuma T, Barry MA, Ikeda Y, 2012 Lentiviral vectors: basic to translational. *Biochem. J* 443 (3), 603–618. [PubMed: 22507128]
- Salganik M, Hirsch ML, Samulski RJ, 2015 Adeno-associated virus as a mammalian DNA vector. *Microbiol. Spectr* 3 (4).
- Samulski RJ, Muzyczka N, 2014 AAV-mediated gene therapy for research and therapeutic purposes. *Ann. Rev. Virol* 1 (1), 427–451. [PubMed: 26958729]
- Schwarz LA, Miyamichi K, Gao XJ, Beier KT, Weissbourd B, DeLoach KE, Ren J, Ibanes S, Malenka RC, Kremer EJ, Luo L, 2015 Viral-genetic tracing of the input-output organization of a central noradrenaline circuit. *Nature* 524 (7563), 88–92. [PubMed: 26131933]
- Shigetomi E, Bushong EA, Hausteiner MD, Tong X, Jackson-Weaver O, Kracun S, Xu J, Sofroniew MV, Ellisman MH, Khakh BS, 2013 Imaging calcium micro-domains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. *J. Gen. Physiol* 141 (5), 633–647. [PubMed: 23589582]
- Soudais C, Laplace-Builhe C, Kissa K, Kremer EJ, 2001 Preferential transduction of neurons by canine adenovirus vectors and their efficient retrograde transport in vivo. *FASEB J.* 15 (12), 2283–2285. [PubMed: 11511531]
- Stuber GD, Stamatakis AM, Katak PA, 2015 Considerations when using cre-driver rodent lines for studying ventral tegmental area circuitry. *Neuron* 85 (2), 439–445. [PubMed: 25611514]
- Tervo DG, Hwang BY, Viswanathan S, Gaj T, Lavzin M, Ritola KD, Lindo S, Michael S, Kuleshova E, Ojala D, Huang CC, Gerfen CR, Schiller J, Dudman JT, Hantman AW, Looger LL, Schaffer DV, Karpova AY, 2016 A designer AAV variant permits efficient retrograde access to projection neurons. *Neuron* 92 (2), 372–382. [PubMed: 27720486]
- Teschemacher AG, Wang S, Lonergan T, Duale H, Waki H, Paton JF, Kasparov S, 2005 Targeting specific neuronal populations using adeno- and lentiviral vectors: applications for imaging and studies of cell function. *Exp. Physiol* 90 (1), 61–69. [PubMed: 15542618]
- Tovote P, Esposito MS, Botta P, Chaudun F, Fadok JP, Markovic M, Wolff SB, Ramakrishnan C, Fenno L, Deisseroth K, Herry C, Arber S, Luthi A, 2016 Midbrain circuits for defensive behaviour. *Nature* 534 (7606), 206–212. [PubMed: 27279213]
- Ulery-Reynolds PG, Castillo MA, Vialou V, Russo SJ, Nestler EJ, 2009 Phosphorylation of DeltaFosB mediates its stability in vivo. *Neuroscience* 158 (2), 369–372. [PubMed: 19041372]
- Ulusoy A, Decressac M, Kirik D, Bjorklund A, 2010 Viral vector-mediated overexpression of alpha-synuclein as a progressive model of Parkinson's disease. *Prog. Brain Res* 184, 89–111. [PubMed: 20887871]
- Vazey EM, Aston-Jones G, 2014 Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. *Proc. Natl. Acad. Sci. U. S. A* 111 (10), 3859–3864. [PubMed: 24567395]
- Vialou V, Robison AJ, Laplant QC, Covington HE, 3rd, Dietz DM, Ohnishi YN, Mouzon E, Rush AJ, 3rd, Watts EL, Wallace DL, Iniguez SD, Ohnishi YH, Steiner MA, Warren BL, Krishnan V, Bolanos CA, Neve RL, Ghose S, Berton O, Tamminga CA, Nestler EJ, 2010 DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat. Neurosci* 13 (6), 745–752. [PubMed: 20473292]
- Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N, 2012 Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* 74 (5), 858–873. [PubMed: 22681690]
- Wickersham IR, Finke S, Conzelmann KK, Callaway EM, 2007a Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat. Methods* 4 (1), 47–49. [PubMed: 17179932]

- Wickersham IR, Lyon DC, Barnard RJ, Mori T, Finke S, Conzelmann KK, Young JA, Callaway EM, 2007b Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* 53 (5), 639–647. [PubMed: 17329205]
- Woodworth HL, Beekly BG, Batchelor HM, Bugescu R, Perez-Bonilla P, Schroeder LE, Leininger GM, 2017 Lateral hypothalamic neurotensin neurons orchestrate dual weight loss behaviors via distinct mechanisms. *Cell Rep.* 21 (11), 3116–3128. [PubMed: 29241540]
- Zingg B, Chou XL, Zhang ZG, Mesik L, Liang F, Tao HW, Zhang LI, 2017 AAV-mediated anterograde transsynaptic tagging: mapping corticocollicular input-defined neural pathways for defense behaviors. *Neuron* 93 (1), 33–47. [PubMed: 27989459]
- Zou L, Yotnda P, Zhao T, Yuan X, Long Y, Zhou H, Yang K, 2002 Reduced inflammatory reactions to the inoculation of helper-dependent adenoviral vectors in traumatically injured rat brain. *J. Cereb. Blood Flow Metab* 22 (8), 959–970. [PubMed: 12172381]
- Zufferey R, Donello JE, Trono D, Hope TJ, 1999 Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol* 73 (4), 2886–2892. [PubMed: 10074136]

Fig. 1.

Retrograde transducing LT-HSVs label multiple circuits. A) Schematic depicting injection of retrograde LT-HSV expressing Cre recombinase into NAc (purple) of L10-GFP reporter mice. Brain regions projecting to NAc, such as ventral hippocampus (blue), amygdala (red), and ventral tegmental area (VTA, yellow) will express GFP in the specific projection neurons. B) Confocal fluorescent microscopy reveals neurons specifically projecting to NAc (green), from posterior amygdala (PA), from the CA1 region of ventral hippocampus, and from the VTA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) (Adapted from (Eagle et al., 2016)).

Fig. 2.

Current and potential methods for circuit-specific interrogation of gene function and expression. A) Schematic depicting the combination of a retrograde Cre virus (green) injected into a target region (purple) with a local virus expressing a transgene of interest in a Cre-dependent manner (orange) injected into a projecting region (pink). Such a strategy would result in overexpression of the IEG only in projection region neurons specifically terminating in the target region. B) Schematic of a converse strategy: the combination of a retrograde virus expressing Cas9 (red) in a target region (blue) with a local virus expressing a guide RNA targeting a gene of interest (green) in a projecting region (yellow) could be used to silence a single gene only in a specific circuit. C) Schematic of a strategy for uncovering circuit-specific gene expression. Using a retrograde Cre virus (green) injected into a target region (purple) of a L10-GFP reporter mouse to induce expression of GFP-tagged ribosomes in the projection region (pink) allows the use of TRAP to determine gene expression changes in the circuit. By combining this approach with mice floxed for a specific gene of interest, circuit-specific transcriptional effects of gene silencing could also be revealed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

General Viral Vector Information.

Viral vector	Lentivirus	Adenovirus	Canine adenovirus type-2	Adeno-associated virus	Rabies virus	Herpes simplex virus
Genome	RNA 3–9 kb	dsDNA>8 kb	dsDNA>30 kb	ssDNA 5 kb	RNA<3.7 kb	dsDNA 120–200 kb
Capacity	<8.5 kb	>8 kb	>30 kb	<4.5 kb	<3.7 kb	>30 kb
Tropism	Can be pseudotyped to enhance specificity across a range of hosts	Specificity is a challenge	Broadly neurotropic, exclusively retrograde	Broad tropism, enhanced by pseudotyping, can be retrograde	Broadly neurotropic, exclusively retrograde	Broadly neurotropic, can be short- or long-lasting with retrograde option