Adeno-Associated Viral Vectors for Mapping, Monitoring, and Manipulating Neural Circuits

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Abstract

Understanding the structure and function of neural circuits is central is neuroscience research. To address the associated questions, new genetically encoded tools have been developed for mapping, monitoring, and manipulating neurons. Essential to implementation of these tools is their selective delivery to defined neuronal populations in the brain. This has been facilitated by recent improvements in cell type–specific transgene expression using recombinant adeno-associated viral vectors. Here, we highlight these developments and discuss areas for improvement that could further expand capabilities for neural circuit analysis.

Introduction

THE CENTRAL NERVOUS SYSTEM orchestrates exceptionally diverse and complex biological processes, including learning, emotion, and decision-making. These and other brain functions are mediated by collections of neurons that are intricately wired into circuits through synaptic connections (Shepherd, 2004). Understanding the structure and function of these neural circuits is a key goal of neuroscience research. The combination of recently developed genetically encoded molecular tools for visualizing and perturbing circuits (Luo et al., 2008) and precisely targeted cell type–selective gene delivery approaches is dramatically expanding capabilities for neural circuit analysis.

Recombinant adeno-associated virus (rAAV) (Samulski et al., 1989) is widely used for gene delivery to the brain because it permits nontoxic transduction of postmitotic cells and long-term gene expression in neurons (Klein et al., 1998; McCown et al., 1996). These basic properties, which make rAAV promising for human gene therapy, also facilitate research into the structure and function of neural circuits (McCown 2005; Buning et al., 2008). Key improvements in rAAV vector design have advanced selective gene delivery to molecularly-defined neuron populations. Here, we review these recent developments with a focus on the application of rAAV vectors to (1) achieve cell type–specific transgene expression and (2) to deliver molecular tools for mapping, monitoring, and manipulating neural circuits.

Cell-Type Specificity and Neural Transduction

Within neural circuits, neurons with similar connectivity and functional roles are treated as circuit nodes (Fig. 1A). A major challenge has been identifying and manipulating these nodes. Neuronal subtypes are categorized based on morphological, physiological, and molecular characteristics (Gupta et al., 2000). However, investigation of individual cell types within circuits is often complicated by their intermingled distribution within the same anatomical domain (Klausberger and Somogyi 2008), making them neither readily distinguishable nor accessible for selective manipulation.

The divergent molecular characteristics of neurons provide a solution to this problem in which unique gene expression patterns define distinct neuronal cell types (Fig. 1B). These expressed marker genes can serve as entry points for delivery of genetically encoded molecular tools by using the corresponding gene promoter region to drive transgene expression (Tsien et al., 1996). Because of the diversity of possible genetic modifications, including expression of transgenes that permit neuron visualization and perturbation, gene expression patterns are being increasingly used to specify neuronal populations for neural circuit analysis (Arenkiel and Ehlers, 2009; Zagoraiou et al., 2009).

One limitation of most molecular marker genes is that often they are not restricted in expression to a unique neuronal population throughout the brain. Nevertheless, many markers are specific for neuronal subtypes within circumscribed brain regions (Lein et al., 2007) (Fig. 1B). Therefore, one challenge in applying this genetic approach is to restrict transgene expression regionally. rAAV vectors enable regional gene expression by local injection of the virus. Despite this, the application of rAAV for cell type–specific gene expression has been limited by technical challenges associated with insufficient transgene expression or lack of specificity.
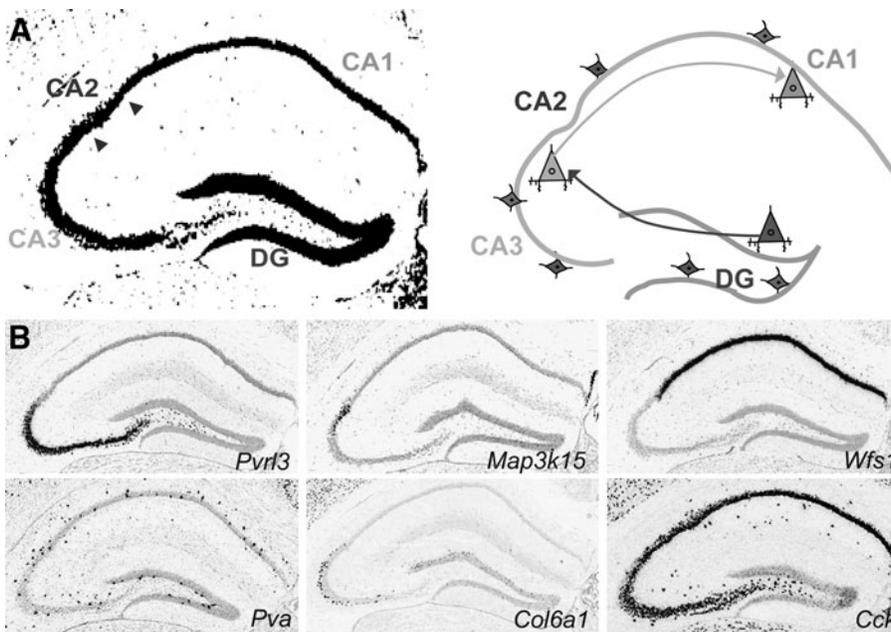
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FIG. 1. Marker genes specify circuit nodes in hippocampal circuitry. **(A)** Connections between anatomical regions of the hippocampus. In hippocampal circuits, granule cells located in the dentate gyrus (DG) send axonal projections to the CA3 region. The pyramidal cells in CA3 send axonal projections to CA1 neurons. Interspersed throughout the hippocampus are multiple classes of local inhibitory interneurons (diamond shaped in scheme). **(B)** Gene expression patterns label neurons selectively in the three main anatomical domains of the hippocampus. Pvrl3 marks CA3; Col6a1 marks a subset of CA3 neurons; Map3k15 marks CA2; Wfs1 marks CA1. Two inhibitory interneuron populations interspersed throughout the hippocampus can be distinguished by the expression of Pva or Cck. Within the hippocampal region, these genes mark neurons in distinct anatomical domains, however the expressed genes are not exclusive to these hippocampal regions, as they are also expressed in other anatomical domains of the brain. Images taken from: Allen Mouse Brain Atlas. Seattle (WA): Allen Institute for Brain Science. Copyright, 2009. (Lein *et al.*, 2007) Available from: http://mouse.brain-map.org.

Recent advances employing cell type–specific promoters, selective viral infectivity, and intersections of viral techniques with genetically modified organisms have largely overcome these limitations, opening new possibilities for widespread use of rAAV vectors in neural circuit research.

Cell type specificity for AAV vector expression: promoters

Promoters are gene regulatory elements that determine the strength and the cell-type specificity of expression. Initial studies using rAAV vectors in neurons focused on stable expression of a transgene. While expression driven by the cytomegalovirus (CMV) promoter decreased with time and led to transgene expression in neurons as well as some astrocytes, rAAV vectors with the neuron-specific enolase (NSE) promoter were shown to be suitable for long-term, neuron-selective expression of brain-derived neurotrophic factor (Fig. 2A) (Klein *et al.*, 1998).

Because promoter regions in mammals are often large (tens to hundreds of kilobases), promoter size is a key challenge for using rAAV due to its small packaging capacity (up to 4.7–5.0 kb) (Dong *et al.*, 1996). A truncated human *synapsin-1* promoter has proven effective for neuron-selective gene expression (Glover *et al.*, 2002; Kugler *et al.*, 2003) and only occupies 10% of the rAAV genome. Development of rAAV-compatible promoters that are selective for well-defined neuronal subsets was first demonstrated in neurons expressing the gene for melanin-concentrating hormone (MCH) (van den Pol *et al.*, 2004). rAAV containing a short MCH promoter fragment selectively drove expression of green fluorescent protein (GFP) in this subpopulation of lateral hypothalamic neurons and allowed investigation of their physiological properties (Fig. 2A).

This approach sparked efforts to identify other promoter fragments that drive expression in defined neural subtypes. However, determination of short cell type–specific promoters has proven challenging. A systematic attempt to identify such regulatory elements for mouse cortical inhibitory interneurons highlights the difficulties (Nathanson *et al.*, 2009a, 2009b). In an elegant study, conserved promoter elements from inhibitory neuron marker genes were derived from the pufferfish genome, which has a compact gene regulatory structure that is compatible with size limitations of rAAV. Unfortunately, these promoters were not sufficient to control transgene expression in previously defined subtypes of mammalian cortical inhibitory neurons (Nathanson *et al.*, 2009a). This is likely due to the importance of multiple regulatory elements for recapitulating gene expression patterns in the mammalian brain (Gong *et al.*, 2003), which often are not known and, further, would not be compatible with the limited packaging size of rAAV. More research into identification of the DNA regulatory sequences that underlie cell type–specific gene expression is clearly required to facilitate design of small synthetic promoter fragments. The primary limitation to the faithful reproduction of an endogenous gene expression pattern by a rAAV-expressed transgene continues to be viral genome packaging constraints.

Cell-type specificity for AAV vector expression: tropism

The tropism (cell type–selective infectivity) of AAV serotypes has also been investigated for targeting transgenes to specific cell populations. AAV serotypes differ in capsid protein sequence (Rutledge *et al.*, 1998). These sequence differences affect receptor-mediated endocytosis of AAV particles, which are based on interactions of capsid proteins with receptors on the neuronal surface (Fig. 2B). Multiple recom-

binant serotypes have been identified that have improved the potency and broadened the tropism for rAAVs (Cearley and Wolfe 2006; Gao *et al.*, 2005; Taymans *et al.*, 2007). Some of these molecular interactions have been characterized for AAV serotypes (Buning *et al.*, 2008), and a comparative analysis of neuronal infectivity for different serotypes showed different patterns of transduction that varied by brain region (Taymans *et al.*, 2007); however, cell-type selectivity was not investigated.

Recently, promising strategies have been developed to generate and select for novel capsid sequences using ligand-mediated receptor targeting, mutagenesis, and DNA shuffling coupled with selection techniques (for a review see [Michelfelder and Trepel, 2009]). Ultimately, such techniques may be used to generate cell type–specific rAAV vectors for use in neural circuits. In one case, the ultimate goal of generating cell type–specific rAAV tropism was achieved by screening a series of mutant AAV6 capsids for astrocyte selectivity. One of these mutant capsids efficiently transduced

Muller glial cells in the retina with high selectivity over other cell types (Klimczak *et al.*, 2009). Additional work will show if this can be generalized to engineer capsids with selectivity for specific neuronal cell types.

Cell-type specificity for AAV vector expression: intersectional genetic switches

Currently, the most effective approaches for cell type–specific transgene expression are based on intersectional genetic manipulations (Tsien *et al.*, 1996; Dymecki *et al.*, 2010). These approaches have been adapted recently for use with rAAV vectors containing a strong, ubiquitously expressed promoter that is only active in the presence of a second, selectively expressed genetic component such as Cre-recombinase (Cre) (Atasoy *et al.*, 2008; Kuhlman and Huang, 2008). Selective expression of Cre is achieved by genetic modification of the mouse genome using large upstream promoter regulatory regions that cannot be accommodated in the AAV genome (Gong *et al.*, 2007). To render rAAV expression vectors Cre-dependent requires a compact genetic element for achieving conditional expression. One method uses a Cre-dependent transcriptional stop cassette placed between the promoter and the transgene (Fig. 2Ci). However, stop cassettes are large and typically occupy a significant portion of the AAV genome, which restricts the size of the promoter and the transgene (Kuhlman and Huang, 2008).
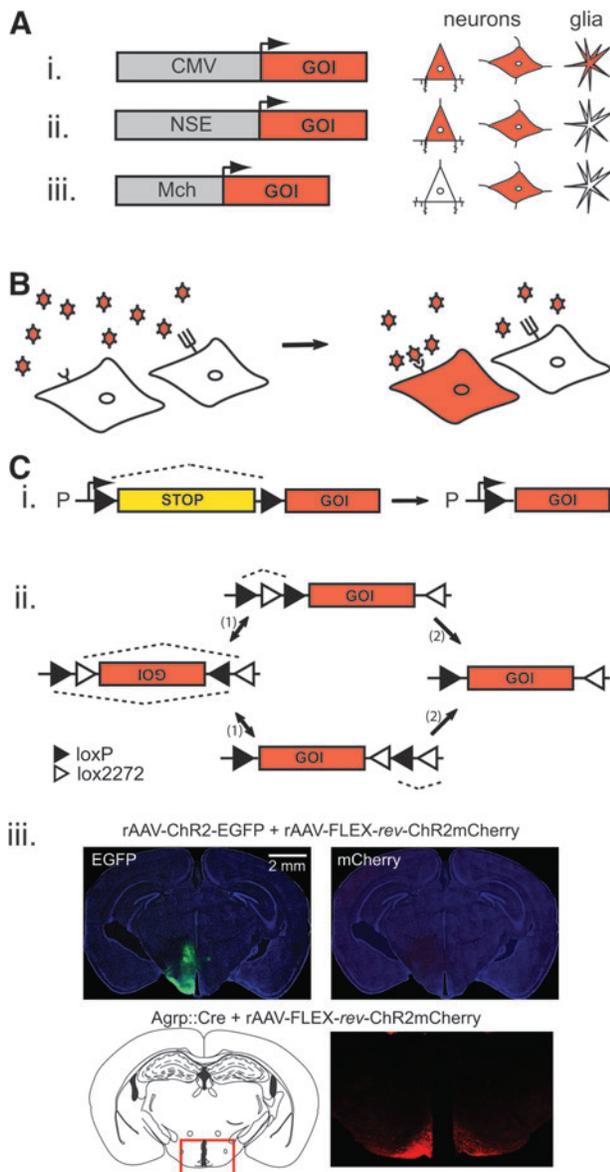

FIG. 2. Strategies for cell type–selective transgene expression with recombinant adeno-associated virus (rAAV). **(A)** Promoter specificity. Schematic showing cell-type specificity of short promoter fragments in rAAV. The cytomegalovirus (CMV) promoter drives expression nonselectively in neurons and glia, while the neuron-specific enolase (NSE) promoter is selective for neurons, and the melanin-concentrating hormone (MCH) promoter targets expression to a subpopulation of neurons in the lateral hypothalamus. **(B)** Tropism specificity. AAV capsid proteins lead to differential infectivity between cell populations based on recognition between capsids and cell surface receptors. **(C)** Cre-dependent intersectional genetic switches. (i) Insertion of a 1–2 kb stop cassette after the promoter (P) and upstream of a gene of interest (GOI). The stop cassette is flanked by two loxP sites which are the recognition sequences that recombine in the presence of Cre. After recombination, the stop cassette is excised (one loxP site remains) and transcription of the GOI proceeds. (ii) Use of a flip-excision (FLEX) switch to activate gene expression specifically in the presence of Cre-recombinase. The GOI is inverted between two pairs of heterotypic, antiparallel lox-type recombination sites, which first undergo an inversion of the coding sequence, followed by excision of two sites, leading to one of each orthogonal recombination site oppositely oriented and incapable of further recombination. (iii) Demonstration of Cre-dependent transgene expression using a FLEX switch. The top panels show tissue from a wild-type mouse brain co-injected with both a rAAV2/1-ChR2-EGFP virus and a rAAV2/1-FLEX-rev-ChR2-mCherry virus. Expression of EGFP is broadly observed while mCherry is not expressed in the absence of Cre. Injection of rAAV2/1-FLEX-rev-ChR2-mCherry into an Agrp::Cre mouse (bottom panel) shows expression of the mCherry signal only in the arcuate nucleus, which is the sole location of AGRP neurons in the brain. Adapted from *Journal of Neuroscience* (Atasoy *et al.*, 2008), copyright 2008.

An alternative strategy is a flip-excision (FLEX) switch, which involves stable Cre-dependent transgene inversion (Fig. 2Cii) (Atasoy *et al.*, 2008). This approach is especially well-suited for use in rAAV due to the high efficiency and compactness (only ~5% of AAV packaging size) of the FLEX switch. Furthermore, in the absence of Cre, the transgene is inverted with respect to the promoter, and thus expression is very tightly regulated (Fig. 2Ciii), unlike with the transcrip-

tional stop cassette, which experiences noticeable levels of "read-through" of the transgene (Kuhlman and Huang, 2008). Most importantly, intersectional approaches transfer the challenging problem of using cell type–specific promoters to the realm of mouse genetics, where manipulation of large DNA fragments is not a limitation. With the appropriate Cre-expressing mouse line, Cre-dependent rAAV allows facile, cell type–specific targeting of any transgene
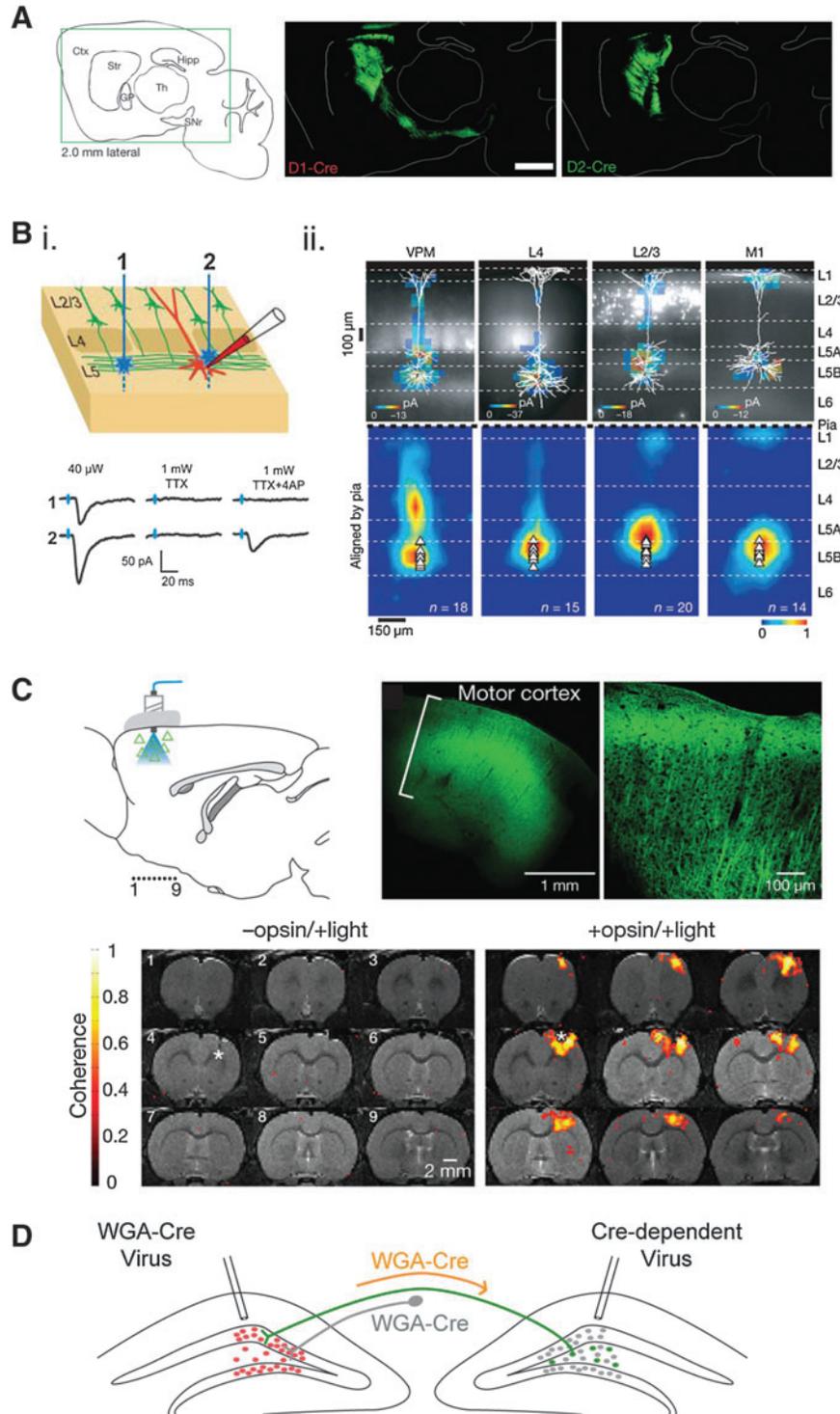
(within the size constraints of the rAAV genome). This capability has expanded the application of rAAV vectors for neural circuit analysis.

Use of rAAV With Genetically Encoded Tools for Mapping, Monitoring, and Manipulating Neural Circuits

rAAV for circuit mapping

Circuit mapping aims to identify and characterize synaptic connections between neuronal cell types. Anatomical and functional circuit mapping strategies are being used in conjunction with cell type–specific rAAV vectors to dissect neural circuits with unprecedented precision. Targeted injection of rAAV vectors expressing fluorescent proteins drives high expression levels in localized brain regions, which allows visualization of long-range axon projections (Chamberlin *et al.*, 1998). Until recently, limited progress was made by using rAAV for cell type–specific circuit mapping, because specific promoters typically did not deliver the high levels of fluorescent protein expression necessary to label axons. However, the development of Cre-dependent viral vectors with strong and ubiquitous promoters has greatly enhanced the ability to label axons that project throughout the brain. In one example, this approach was used to distinguish the projection patterns of two intermingled striatal projection neuron populations defined by expression of either dopamine receptor type 1 or type 2 (D1R or D2R) (Kravitz *et al.*, 2010). Using a Cre-dependent viral vector with a strong promoter (EF-1α) driving a GFP transgene in combination with genetically modified mice expressing Cre under the D1R and D2R promoter revealed the strikingly distinct striato-nigral and striato-pallidal projection patterns for the intermingled D1R and D2R cell populations, respec-

tively (Fig. 3A). While these cell type–specific anatomical techniques show the regional targets of neuron projections, they often do not give definitive confirmation of synaptic connectivity to a particular cell type. Because anatomical techniques also provide limited insight into the properties of a synaptic connection, functional circuit mapping techniques in combination with rAAV transgene targeting are proving especially useful for establishing connections and simultaneously characterizing their strength, sign, and other properties.

A powerful new functional circuit mapping technique combines electrophysiological recordings of synaptic responses with neuronal expression of the algal light-activated ion channel, channelrhodopsin-2 (ChR2). ChR2 depolarizes neurons after a light flash and can selectively induce action potential firing in a molecularly defined cell type with millisecond precision (Boyden *et al.*, 2005; Li *et al.*, 2005). This genetically encoded tool is the basis for ChR2-assisted circuit mapping (CRACM) (Petreanu *et al.*, 2007; Wang *et al.*, 2007). ChR2 distributes throughout a neuron, including the axonal arbor, and allows selective activation of axons from a defined neuron population, in a background of axons originating from other cell types. Electrophysiology in brain slices containing these ChR2-expressing axons permits synaptic connections to be identified by light activation of the presynaptic axon while recording the evoked synaptic current in the postsynaptic neuron. rAAV-mediated expression of ChR2 has been critical for generalizing the use of this tool because ChR2 has a very low ionic conductance and, thus, large quantities of the channel must be expressed to render neurons and their axons reliably photoexcitable. In combination with Cre-dependent rAAV vectors, CRACM has been used to dissect intermingled circuits in the hypothalamus that are involved in regulating feeding behavior (Atasoy *et al.*,

FIG. 3. Applications of rAAV for neural circuit analysis. **(A)** Selective, Cre-dependent viral-mediated expression of YFP in two distinct populations of striatal neurons. D1R-expressing neurons project to the substantia nigra pars reticulata (SNr), D2R-expressing neurons project to the globus pallidus (GP). These experiments highlight discrete projection patterns for molecularly defined neurons occupying the same anatomical, indicating distinct neuronal functions. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Kravitz *et al.*, 2010), copyright 2010. www.nature.com. **(B)** Mapping the subcellular distribution of inputs onto pyramidal neurons in the cortex using sCRACM. (i) The top panel shows two photostimulation configurations, where the postsynaptic neuron (red) is being recorded from and the blue stars indicate spots where the ChR2 expressing axons (green) are stimulated. The bottom panel provides representative traces of excitatory postsynaptic currents evoked by photostimulation corresponding to the locations indicated in the sketch. In the absence of the voltage-gated sodium channel blocker tetrodotoxin (TTX), both stimulation sites evoke synaptic responses, but these are both blocked with TTX, which prevents action potentials. Addition of the potassium channel blocker 4-aminopyridine (4-AP) renders ChR2 activation at a synaptic connection sufficient to drive release and evoke a synaptic response. Blue tickmarks indicate the laser pulse. Laser power is also shown for these conditions. (ii) Shown in the top panel are examples of sCRACM maps overlaid on somato-dendritic reconstructions. The fluorescent neurons are ChR2-positive neurons that are being mapped onto a pyramidal neuron. The bottom panel shows group averages of the inputs onto the post-synaptic neuron. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Petreanu *et al.*, 2009), copyright 2009. www.nature.com. **(C)** Functional magnetic resonance imaging (fMRI) with blood oxygenation level detection (BOLD) signal detects activation of neurons resulting from cortical neuron stimulation. Diagram shows cortical neurons transduced by a virus expressing the ChR2-EYFP transgene as triangles and the path of the photostimulation in blue. Adjacent images are of ChR2-EYFP expressing neurons in the motor cortex. In brain slices that lack the ChR2 opsin, negligible levels of BOLD activation are observed; however, when AAV5-ChR2 is expressed in the cortex (+opsin), BOLD activation is observed. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Lee *et al.*, 2010), copyright 2010. www.nature.com **(D)** Target region specificity. Injection of a Cre-dependent virus to infect neurons in one region of the central nervous system can be combined with an injection of a virus expressing wheat germ agglutinin (WGA)-Cre in a known anatomical target region of a subset of neurons in the original infection region. The fusion of WGA to the Cre recombinase promotes retrograde transport from the axon terminals of all neurons contributing to that projection field back to the cell body. Once at the nucleus, the Cre-recombinase unmasks the expression of the gene of interest (green fluorescent protein [GFP] in this case) of only the neurons in the original infection field that project to the appropriate target region. Reprinted by permission from Elsevier: *Cell* (Gradinaru *et al.*, 2010), copyright 2010. www.sciencedirect.com/science/journal/00928674

2008). The simplicity of this approach, requiring standard electrophysiological equipment and a relatively inexpensive fiber-coupled laser, makes it a rapid technique for definitively establishing and characterizing synaptic connections with cell type–specificity.

Cell type–specific neuron activation has enabled two further refinements, opposite in scale, for neural circuit mapping: (1) the subcellular distribution of synaptic inputs and (2) the influence of a localized neuron population on activity throughout the whole brain. Common to both approaches is the use of rAAV vectors to achieve high levels of ChR2 expression.

CRACM has been extended to identify the subcellular domains of synaptic contacts (sCRACM). In CRACM, an action potential propagates to all of the synapses made by a given axon, which makes it difficult to identify the origin of a synaptic input (Fig. 3Bi). However, under conditions in which action potentials are blocked by the voltage-gated sodium channel blocker tetrodotoxin and potassium channels are inhibited by 4-aminopyridine, ChR2 activation with a focused beam of laser light directly drives synaptic vesicle release but only in the vicinity of the laser spot (Fig. 3Bi). Thus, the position at which photostimulation evokes a synaptic response correlates with the region of synaptic contact. The location of the photostimulus and amplitude of the response are used to generate a map that reflects the subcellular distribution of synaptic connection strengths from inputs that originate from a defined ChR2-expressing presynaptic cell population, which can be overlaid with the morphological reconstruction of the postsynaptic neuron. This technique has been applied to map the spatial segregation of intracortical and cortico-thalamic inputs to cortical neuron dendritic arbors (Fig. 3Bii) (Petreanu et al., 2009). This information contributes to understanding neural computations in the postsynaptic neuron.

Functional connectivity has also been examined on a whole-brain scale by activating a subset of neurons while monitoring the resultant activity throughout the brain. In this approach, ChR2 is used to selectively drive action potentials in a cell population while using functional magnetic resonance imaging (fMRI) with blood oxygenation level detection (BOLD), which is thought to correlate with electrical activity. Using a CaMKIIa promoter in AAV2/5, ChR2 was expressed selectively in excitatory neurons in rat motor cortex. Optical activation of these neurons in anesthetized animals induced a BOLD signal in discrete regions throughout the brain (Lee et al., 2010) (Fig. 3C) (for a related approach in awake animals, see Desai et al., 2010). Specific ChR2 expression in motor cortex coupled with optical stimulation allowed unambiguous determination of the activated neuron population that served as the origin of the stimulus, in contrast to traditional electrical stimulation techniques, in which neurons as well as axon fibers of passage are both excited. Nevertheless, this technique involves exogenously stimulating populations of neurons with trains of action potentials and aims to measure the consequences of activating a neuronal network instead of identifying monosynaptic connections.

AAV for monitoring neuron activity

The relationship of neuron electrical activity to sensory inputs or ongoing behavior is widely investigated for ex-

ploring the function of neurons, and more recently, neuronal networks. Monitoring neuron activity in a network is a challenge that is increasingly being approached by large-scale, multi-neuronal imaging of calcium levels, which has been shown to reflect electrical activity (Tian et al., 2009). rAAV vectors can deliver genetically encoded calcium indicators for imaging calcium dynamics in vivo with sufficient expression levels for efficient detection. rAAV expressing the camgaroo-2 indicator under the control of a tetracycline transactivator (tTA) responsive promoter element was injected into the olfactory bulb of CaMKIIa-tTA transgenic mice (Hasan et al., 2004). This permitted expression in olfactory sensory axons and granule cells, and calcium dynamics were monitored in response to different odorants. More recently, another calcium indicator, GCaMP3, was targeted to the mouse motor cortex using rAAV2/5, which allowed dynamic activity in multiple neurons to be simultaneously monitored in the running mouse (Tian et al., 2009). These tools could be further extended to molecularly defined subpopulations with the cell type–specific expression strategies described above.

Manipulation of neural circuitry

Another recent advance has been the development of genetically encoded tools for reversibly manipulating neuron activity, which permits the causal relationship between neural activity and behavior to be explored. These tools are rapidly (millisecond to minute timescales) switchable in response to ligand binding or a light flash. Thus, they can be expressed for long periods without perturbing cellular function until they are engaged under the conditions chosen for a particular experiment. Because rAAV provides strong, cell type–specific transgene expression, it is increasingly being used for studies that involve activating or silencing molecularly defined neural populations.

Genetically encoded neuronal silencers are used to test the necessity of a neuron population for a behavioral function. For example, somatostatin (SST)-expressing cells in the pre-Botzinger complex, a hindbrain structure that regulates breathing, were selectively silenced to test their necessity in respiration. This study used the G-protein–coupled allostatin receptor (AlstR) from Drosophila that can silence mammalian neurons when complexed to its ligand, allatostatin, by activating a potassium conductance (Tan et al., 2006). Separately, the ligand and its receptor are thought not to perturb mammalian neurons. Using rAAV2/2, AlstR was specifically expressed in the subpopulation of SST cells in the medulla under the control of a somatostatin promoter fragment (Tan et al., 2008). SST cells could be reversibly silenced by application of allatostatin, which eliminated the respiratory rhythm until allatostatin washout. These experiments demonstrated the necessity of the SST neuron subpopulation in the pre-Botzinger complex for respiration. Recently, other reversible neuronal silencers using either pharmacological (Armbruster et al., 2007; Karpova et al., 2005; Lerchner et al., 2007) or optical (Zhang et al., 2007) triggers have been developed and could be employed in a similar manner.

For selective neuron activation, ChR2 has emerged as an effective tool for investigating the sufficiency of specific neuronal cell types to contribute to behavior or physiology. Stereotaxic injection of a Cre-dependent rAAV2/1 vector

using the EFα-1 promoter was used to target ChR2 to a midbrain-localized subset of dopamine-expressing neurons in transgenic mice expressing Cre under the control of the tyrosine hydroxylase promoter (Tsai *et al.*, 2009). Stimulation of midbrain dopamine neurons induced a preference for the environment in which stimulation was delivered. Cell type–specific photostimulation of these neurons was used to probe preferred firing patterns for forming a place preference. These experiments showed that a burst of dopamine neuron firing was more effective for inducing a preference than a tonic stimulation pattern (Tsai *et al.*, 2009). Similar cell type–specific viral tools have also been used for investigation of the causal relationship between activity patterns and behaviors such as sleep (Adamantidis *et al.*, 2007), locomotion (Kravitz *et al.*, 2010), addiction (Lobo *et al.*, 2010), and feeding (Aponte *et al.*, 2011).

Future directions for rAAV in neural circuit research

As reviewed here, improvements in cell type–specific gene expression strategies have increased the use of rAAV for neural circuit research. However, these techniques are best developed for use in genetically modified organisms. Future advances for rAAV vectors in neural circuit research are likely to come from new approaches to access functionally useful neuron populations in genetically unmodified organisms.

One attractive approach would be to further develop methods to efficiently engineer viral tropism for selective infection of a desired cell population. For this, a promising research direction is the development of pseudotyped rAAV vectors. Lentiviral vectors have been pseudotyped with the envelope glycoproteins of other viruses to alter or enhance the tropism (Stitz *et al.*, 2000). The envelope glycoproteins, which bind cell surface receptors, are necessary for entry into the target cell, and changing the recognition properties of these ligands may enhance cell type selectivity (reviewed in Bischof and Cornetta, 2010). Pseudotyped AAV capsids with single chain antibodies (Yang *et al.*, 1998) or ligands for cell surface receptors (Wu *et al.*, 2000) have been investigated; however, the endogenous tropism of the virus can be a complication to engineering high specificity.

Another consideration for neural circuit dissection is to distinguish cell types, in part, by their projection patterns. Neurons in the same brain region show divergent axon projections, indicating distinct functions. Thus, an additional area for further development is the capacity for rAAV to selectively infect axon terminals. Several viral vectors have this property including pseudorabies virus (Braz *et al.*, 2009), rabies virus (Wickersham *et al.*, 2007), herpes virus (Lima *et al.*, 2009), and canine adenovirus (Darvas and Palmiter 2009); however, the efficiency of axon transduction is variable and some of these vectors can be toxic to neurons. Recent reports suggested that self-complementary AAV vectors, specifically AAV1, exhibit efficient retrograde transduction properties from the neuromuscular junction back to motor neurons (Hollis *et al.*, 2008). Also, other pseudotypes of AAV, including AAV6, have been shown to have retrograde transduction properties (Towne *et al.*, 2010); however, the efficiency of these vectors supplied at central targets remains to be determined. Improvements may come from the design and/or guided evolution of novel rAAV serotypes with efficient retrograde transduction properties.

A complementary strategy that may prove useful for targeting neurons based on axonal projections involves the intersection of two viral vectors. rAAV expressing Cre fused to the plant lectin wheat germ agglutinin (WGA-Cre) (Gradinaru *et al.*, 2010) is targeted to a brain region receiving axon projections from a neuron population of interest. Expression of WGA-Cre at the injection site leads to its being taken up by axon terminals in this region and transported back to cell bodies, for example on the contralateral side of the brain. If a second rAAV with a Cre-dependent transgene is injected into this contralateral side, then only the subset of neurons with those projections will have the two components required for selective activation of the Cre-dependent transgene (Fig. 3D). Such an approach also has the advantage of not requiring a genetically modified organism, potentially increasing general applicability.

With advances in new molecular and genetic properties, rAAV vectors will continue to be a prominent tool for illuminating the circuit-based underpinnings of brain function. Additional progress could be maximized by close interaction of virologists that are discovering and designing new rAAV properties and neuroscience researchers with specific neural circuit applications. In light of the critical role of viral tools for dissecting neural circuits and the challenges for testing and optimizing new tools, such cross-disciplinary approaches will likely be important for advances in both fields.

Author Disclosure Statement

No competing financial interests exist.

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