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STEM CELLS

Transsynaptic tracing and its emerging use to assess graft-reconstructed neural circuits

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ABSTRACT

Fetal neural progenitor grafts have been evaluated in preclinical animal models of spinal cord injury and Parkinson's disease for decades, but the initial reliance on primary tissue as a cell source limited the scale of their clinical translatability. With the development of robust methods to differentiate human pluripotent stem cells to specific neural subtypes, cell replacement therapy holds renewed promise to treat a variety of neurodegenerative diseases and injuries at scale. As these cell sources are evaluated in preclinical models, new transsynaptic tracing methods are making it possible to study the connectivity between host and graft neurons with greater speed and detail than was previously possible. To date, these studies have revealed that widespread, long-lasting, and anatomically-appropriate synaptic contacts are established between host and graft neurons, as well as new aspects of host-graft connectivity which may be relevant to clinical cell replacement therapy. It is not yet clear, however, whether the synaptic connectivity between graft and host neurons is as cell-type specific as it is in the endogenous nervous system, or whether that connectivity is responsible for the functional efficacy of cell replacement therapy. Here, we review evidence suggesting that the new contacts established between host and graft neurons may indeed be cell-type specific, and how transsynaptic tracing can be used in the future to further elucidate the mechanisms of graft-mediated functional recovery in spinal cord injury and Parkinson's disease.

1 | INTRODUCTION

Human pluripotent stem cell (PSC)-based therapies to treat neurological diseases is at a very exciting time of development. Neural cells differentiated from stem cells have entered the clinical trial phase at some centers, while others have begun to recruit patients [1-3]. Compared to the use of primary human brain tissue as a cell source, PSC-based therapies have the advantages of better standardizability and greater availability - at scales that can enable their widespread use. These stem cell-based therapies aim to restore function lost to neuronal death as a consequence of injury or neurodegenerative disease. A guiding principle in the field has often been to attempt to supply the injured or degenerating tissue with functionally-appropriate subtypes of graft neurons, and to encourage the integration of those graft-derived neurons into functionally-appropriate host circuits. Using this principle, cell replacement therapies have demonstrated preclinical as well as potential clinical efficacy for the treatment of neurodegenerative diseases.

Conceptually, it is appealing to imagine that new graft neurons which are integrated into injured or degenerating circuits are modulated by the host in a similar fashion as the healthy endogenous neurons which they aim to replace. In the case of Parkinson's disease (PD), for example, appropriately-patterned and -integrated graft neurons are expected to release dopamine (DA) in a physiologically-regulated fashion, rather than as autonomous "dopamine pumps". A similar concept holds promise for the recovery of function following spinal cord injury (SCI), wherein graft-derived spinal neurons could provide new relay circuits to bridge injury sites for functional benefit - the "relay hypothesis" [4,5].

Indeed, since the inception of the neural cell replacement field, there has been a steady accumulation of evidence suggesting that many aspects of host-graft connectivity recapitulate functionally-relevant aspects of healthy endogenous connectivity after grafting new neurons to repair various damaged cerebral and spinal circuitries [6]. Additionally or alternatively, the functional impact of grafted neurons in certain contexts may also be attributed to a number of mechanisms other than cell replacement, including the normalization of disordered host neuronal activity or the trophic support of spared host neurons [7], without a prerequisite that the graft-derived connectivity recapitulates that of the intact system.

With improving access to specific phenotypic and genetic markers, combined with axonal tracers, the precision of host-graft and graft-host axonal outgrowth is being studied in greater detail. These experiments continue to reveal a remarkable resemblance between intact anatomy and the new connectivity established between host and graft in different lesion models. Generally, more is known about the patterns of axon extension to and from grafts than how grafted cells are synaptically integrated with host neurons. Until recently, it has been difficult to map host-to-graft connectivity in its entirety, since the methods available were largely limited to focused electrophysiological and ultrastructural analyses. Now, the increased use of transsynaptic tracing has enabled global mapping of inputs to and from graft-derived neurons, allowing researchers to assess host-graft connectivity more rapidly and with more precision than ever before. In this review, we highlight the similarities between intact and graft-reconstructed circuits in rodent models of SCI and PD, with a focus on the current and future utility of monosynaptic rabies tracing. In the future, the merger of transsynaptic tracing and opto- and chemogenetic circuit manipulation techniques can be combined to elucidate the extent to which circuit replacement contributes to graft-mediated functional recovery.

Significance Statement

Monosynaptic tracing has accelerated the pace and depth to which the connectivity between stem cell-derived grafts and the host nervous system can be studied. To date, these studies have revealed that transplant-derived neurons receive anatomically-appropriate inputs when transplanted into pre-clinical animal models, but do not yet address whether those inputs are critical to functional recovery. Here, we review how transsynaptic tracing has elucidated host-graft connectivity, and suggest how transsynaptic tracing can address what is unknown in the future. A better understanding of the mechanisms of graft-mediated neural repair can inform clinical efforts, towards improved patient outcomes.

2 | GRAFT-INITIATED MONOSYNAPTIC RABIES TRACING

The utility of transsynaptic viral tracing was first demonstrated with the use of herpes simplex viruses (HSV) and unmodified rabies viruses to map neural ensembles [8]. These polysynaptic methods rely on time-dependent viral propagation through neural circuits to determine which neurons are likely to be directly connected. More recently, monosynaptic tracing based on "modified" EnvA-pseutotyped glycoprotein-deleted (EnvA-ΔG) rabies virus has been used to map direct inputs to defined populations of postsynaptic neurons (reviewed in [9]). This technique can also be employed to study direct host-to-graft connectivity (Figure 1). In this system, graft neurons are engineered to express the rabies helper components - the TVA receptor needed for initial infection, and rabies G-protein needed for transsynaptic spread - either by pre-infection with a lentivirus [10], or by the isolation of transgenic [11] or genome-edited [12] donor cells prior to transplantation. Following graft maturation, EnvA-ΔG rabies is subsequently injected. This virus will only infect the cells expressing the TVA receptor (ie, the grafted cells) and as a consequence specifically initiate tracing from the transplant. ΔG rabies then spreads retrogradely from graft cells expressing the G-protein to presynaptic neurons. As those host neurons do not express the G-protein, the virus will not spread further, and as a result only monosynaptic host-to-graft inputs are mapped. A reverse approach can also be used to study graft-to-host synaptic inputs, wherein host neurons are first engineered to express the rabies helper components before grafting wild type cells [10].

3 | TRANSSYNAPTIC TRACING DEMONSTRATES THE CONNECTIVITY ESTABLISHED BETWEEN HOST AND GRAFT NEURONS

To date, transsynaptic tracing has indicated that transplants receive widespread and long-lasting synaptic inputs from appropriate types of host neurons. In the following sections, we summarize the current findings based on transsynaptic tracing in animal models of SCI and PD, as contextualized by long histories of observation using conventional tract tracing techniques in both cases.

3.1 | Transsynaptic tracing of transplant connectivity in the injured spinal cord

There is currently no clear consensus on the most efficient strategy to be used for cell replacement therapy in SCI. From a clinical perspective, the restoration of motor, autonomic, and sensory circuitry may each provide essential functional benefit. A complicating factor from an experimental perspective is that the injured spinal cord has the capacity to spontaneously recover lost functions by the sprouting and repurposing of spared axons to circumvent injury sites via newly-formed relays [5]. For future clinical applications, therefore, it is

important to understand the mechanism whereby grafts elicit functional recovery in each of these functional domains, and to what extent neural grafts can restore connectivity to serve as functional relays across injury sites.

Following SCI, grafts of fetal spinal tissue [13-15] survive and differentiate into a multitude of spinal interneuronal and glial subtypes (reviewed in [16]). In some cases, these grafts appear able to support functional recovery, either by the new formation of relays or the support of spared host relay circuits [17]. Conventional tract tracing and electron microscopy have revealed, for example: corticospinal [18], raphespinal, coerulospinal [19], reticulospinal [15], and sensory [20] host inputs to intraspinal grafts - as well as the extension of graft axons long distances into the host (reviewed in [21]).

There is evidence that newly-established host inputs may be specific for particular subtypes of graft neurons. For example, though corticospinal neurons extensively innervate grafts derived from fetal spinal neural progenitor cells (NPC) [18], neuron-rich pockets of graft tissue remain notably devoid of corticospinal axons [22]. Upon examination of these areas with antibodies specific for spinal interneuron markers, it was revealed that, akin to grafts of primary fetal tissue pieces [23], dissociated grafts had developed into multicellular laminated domains (Figure 2, bottom left, green and yellow cells) resembling the superficial laminae of the dorsal horn (Figure 2, top left, green and yellow cells), and that regenerating corticospinal neurons (green axons) respected those boundaries as they do in the intact spinal cord [22]. Similar observations were also made using a human stem cell source [24]. Nociceptive axons, on the other hand (Figure 2, cyan), do innervate the superficial layers of the intact dorsal horn and fetal spinal tissue pieces [25,26]. Accordingly, host nociceptive sensory fibers faithfully projected to these areas in dissociated NPC grafts, including increased c-Fos labeling upon subcutaneous capsaicin injection, indicating a degree of functional connectivity [22].

Using monosynaptic rabies tracing, synaptic inputs from the host have been observed from all major descending premotor nuclei, as well as from sensory neurons, using both rodent primary fetal spinal progenitors [11] and human embryonic stem cell (hESC)-derived cells [27] (Figure 2, bottom right). These studies provide a list of host nuclei that may participate in graft-derived relay circuits, including inputs from the corticospinal and nociceptive neurons that were observed to regenerate towards appropriate regions of grafts using conventional tract tracing [22]. These initial rabies tracing studies do not, however, address all components of graft relay circuits. The specific subtypes of synaptically-coupled host and graft neurons remain unknown, as do the subtypes of graft neurons which may contact motor or premotor neurons below the injury site. It is not yet clear, therefore, whether host-graft synaptogenesis is as cell type-specific as monosynaptic rabies tracing is able to reveal in the intact spinal cord (Figure 2, top right).

Polysynaptic tracing, using HSV or wild-type rabies viruses (see above), can also serve as a useful complement to monosynaptic tracing in the identification of circuit participants, particularly when there is some ambiguity regarding how functionally-relevant commands might flow to or from a graft. For instance, though corticospinal neurons are able to evoke motion, those motor commands coalesce at lower motor neurons via a variety of hindbrain and propriospinal relays, which are critical for the coordinated execution of movement in rodents [28-30]. Polysynaptic tracing therefore allows for a degree of agnosticism as to how functional host-graft circuits could be arranged anatomically, albeit with increased uncertainty as to which host and graft neurons are directly connected. For example, the H129 strain of HSV spreads polysynaptically, primarily in the anterograde direction [31], and has been used to identify graft neurons that are downstream of cortical neurons [24]. The Bartha strain of pseudorabies virus has similarly been used to identify graft neurons upstream of phrenic motor neurons [32]. Polysynaptic tracing can help to inform the design of monosynaptic tracing experiments aimed at the implication of specific subtypes of transplant neurons in a given modality of graft-mediated functional recovery, following SCI.

The current state of transsynaptic tracing in cell therapy to treat SCI is summarized in Figure 2. Conventional fiber tracing methods have revealed that injured and regenerating adult host axons project to appropriate subtypes of graft-derived neurons (bottom left), appearing similar in many ways to the anatomy of the intact spinal cord (top left). Monosynaptic rabies tracing studies have shown the high degree of specificity in the synaptic connectivity of the intact cord (top right, tracing sensory-motor synaptic selectivity for example), and that NPC grafts are capable of simultaneously receiving direct input from a variety of descending and ascending tracts (bottom right). What remains is to determine whether host-graft synaptic connectivity is similarly cell type-specific, and ultimately which among those connections are functionally-relevant.

3.2 | Transsynaptic tracing of dopaminergic transplants in models of PD

The widespread effort to treat PD with cell therapy is based on the relatively straightforward need to restore input from one subtype of neuron (DA neurons) in one area of the brain (the striatum, where DAergic fibers terminate). Decades of observations made through the grafting of primary fetal ventral midbrain progenitors in rodents and humans provide a template of attributes that PSC-derived dopaminergic progenitors should exhibit in order to predict their clinical success with high confidence. The “relay hypothesis” developed in the context of a transecting lesion of the spinal cord (see above) may be applied also in this case, meaning that successful dopaminergic grafts are able to 1) extend axons to functionally-relevant target areas, and 2) receive synaptic inputs from appropriate host neurons, which 3) regulate the release of dopamine in those target areas in a more physiological manner than is possible through the use of oral dopamine replacement therapy [33]. There are additional considerations however that are unique to the cell replacement strategy in PD. Midbrain-patterned cells are clinically grafted ectopically into their forebrain target structure, the striatum, rather than their endogenous location in the midbrain. In this section we review the established components of these host-graft circuits, and how the understanding of that connectivity in

a preclinical transplantation model has been accelerated with the use of transsynaptic tracing.

Fetal graft-derived dopaminergic projections were among the first to be visualized [34,35], and since that time, the ability of dopaminergic grafts to specifically innervate functionally-relevant dopamine targets has continued to be observed consistently [36-38]. For effective functional recovery, graft-derived axons must sufficiently reinnervate the dopamine-depleted dorsolateral striatum [39], which is associated with motor functions in the intact system [40]. Target-specific long-distance fiber outgrowth from dopaminergic grafts is most clearly visualized when grafts are placed in the substantia nigra [41-44]. In the case of human cells, target-directed axonal outgrowth proceeds gradually over a period of months [45], following the timecourse of functional recovery. There is also evidence that dopaminergic fiber outgrowth for grafts placed in the clinical location, the striatum, is both target-directed and subtype-specific [38,39,46].

Once graft-derived dopaminergic terminals have innervated the striatum they form synapses and release dopamine. Graft-derived dopaminergic neurons have been observed to synapse on striatal medium spiny and cholinergic interneurons using electron microscopy [47]. Optogenetic [48] and chemogenetic techniques [49] have revealed dopamine receptor-dependent modulation of host medium spiny neuron activity by graft neurons, and PET imaging has been used to demonstrate graft-mediated recovery of dopaminergic transmission [42,50]. Monosynaptic rabies tracing initiated from host cortical and striatal neurons has provided further evidence in support of direct synaptic input from graft neurons to host target areas [10].

Functional graft-to-host connectivity is therefore well-established in PD models. Less is known, however, about how host afferents modulate the function of dopaminergic grafts. Early conventional tract-tracing studies have demonstrated host-to-graft connectivity derived from striatum, cortex, and raphe nuclei [51,52]. Subsequent studies using field stimulation of cortical and striatal areas were found to induce both postsynaptic excitation and inhibition in graft neurons [53,54].

More recent studies using graft-initiated rabies tracing have shown that the host inputs to dopaminergic grafts are much more extensive than those detected with conventional tract-tracing methods, and that they, for the most part, are derived from regions that are known to modulate the activity of endogenous midbrain DA neurons, including the globus pallidus, cortex, and striatum [10,45,55]. Interestingly, dopaminergic grafts placed in the clinical striatal location are able to receive inputs from these regions despite their ectopic location. It seems possible that these inputs to graft-derived neurons arise, at least in part, via collateralization of host neurons which also innervate the host substantia nigra [55]. These data suggest that intrastriatal DA neuron grafts may be under regulatory control from anatomically-appropriate areas of the host brain. The studies performed so far, however, have not addressed to what extent afferent host control may contribute to the functionality of the graft.

It should be noted that the intrastriatal DA neuron grafts also receive inputs from host regions which are not known to connect to the midbrain. The possible impact of anomalous inputs to cells within the graft is unclear [55]. Beyond DA neurons, midbrain-patterned grafts also contain other cell types. Since the rabies tracing was initiated from all types of graft-derived neurons, it is not yet known whether these non-midbrain inputs are indeed ectopic. Furthermore, the anatomical origins of host input to grafts containing neurons of either midbrain or forebrain phenotypes were indistinguishable [55], indicating that the overall pattern of afferent inputs was determined by the location of the grafts rather than their neuronal content. These overlapping inputs suggest a degree of "promiscuity" of synaptic connectivity onto some of the grafted neurons, but they could also, at least to some degree, be due to a phenotypic overlap between neurons contained in the forebrain- and midbrain-patterned grafts.

In our recent study [55] we compared hESC-derived dopaminergic grafts transplanted ectopically to the striatum with similar grafts placed homotopically in the substantia nigra. The results are summarized in Figure 3 (bottom panel). Regardless of their location, dopaminergic grafts were found to receive inputs from the same three areas known to regulate midbrain DA neuron function in their normal location in the midbrain, *that is*, cortex, striatum, and globus pallidus. These neurons are known to simultaneously send collateral projections within the striatum [56-59]. This arrangement (Figure 3, bottom right) provides an anatomical substrate whereby intrastriatal grafts, despite their ectopic location, can receive input from functionally-appropriate subtypes of host excitatory (cortical) and inhibitory (striatal and pallidal) neurons. What remains to be determined is whether this host-graft synaptic connectivity is functionally relevant, and if so, whether those inputs are made directly onto graft dopamine neurons, or onto other subtypes of graft neurons, *for example*, interneurons that, in turn, provide regulatory control over the dopaminergic ones. Rabies tracing has provided a more comprehensive map of the components of these circuits, which can be combined with behavioral outcome measures in future studies.

4 | RABIES TRACING IS USEFUL BUT CARE SHOULD BE TAKEN TO AVOID OVER-INTERPRETATION

Monosynaptic rabies tracing has made it possible to obtain global maps of host-to-graft connectivity of stem cell-derived transplants in a way that has not been possible to achieve with alternative anatomical methods. When designing experiments to determine the functional consequence these inputs have on graft function, it becomes increasingly important to keep in mind technical considerations involved with the use of rabies tracing. Simply put, well-controlled rabies tracing provides strong evidence that neurons are synaptically connected, but reduced or absent labeling does not necessarily provide strong evidence for a lack of connectivity. As such, some experimental comparisons are more appropriate for qualitative rather than quantitative assessment. By extension, it should not be assumed that the host regions which are most heavily-labeled with rabies are necessarily the most likely to be important for graft function. Differences between

rodent and human anatomy may also be significant to consider in translational studies.

In Figure 4, we have summarized factors that can reduce the efficiency of transsynaptic tracing between neurons that are nevertheless synaptically connected. Transsynaptic tracing depends strongly on G-protein expression levels [60], which for example could vary as a consequence of a dependence on helper construct promoter activity related to neuronal phenotype or maturity. "First-generation" (SADΔG) rabies virus labels distant presynaptic partners less efficiently than those providing input locally [61]. Rabies transfer may also depend on neuronal activity at the time of tracing [62], as well as on the phenotypes of synapses traced [63] and terminals infected [64]. Further, highly interconnected graft neurons expressing G-protein can be expected to transfer rabies between themselves, which could lead to a selective amplification and bias in the starter neuron population.

5 | FUTURE DIRECTIONS

Monosynaptic tracing using modified rabies virus vectors has provided a powerful new tool to explore the connectivity of intracerebral and intraspinal neuronal grafts. The rabies tracing technique has given a detailed and comprehensive picture of the patterns of reciprocal host and graft connectivity. Monosynaptic tracing initiated from mixed populations of graft neurons has demonstrated stable inputs from host neurons which are generally anatomically-appropriate for the location and phenotype of the neurons contained in the grafts. Fundamental questions posed but not yet answered fully by these studies include: Are the specific patterns of axonal growth indicative of a similar degree of underlying synaptic specificity? Are host inputs necessary for graft-mediated functional recovery?

The apparent organotypic specificity of host inputs to NPC- or hESC-derived grafts provides an intuitive entry point into the design of future monosynaptic rabies tracing experiments to answer these questions. The initiation of monosynaptic tracing from specific therapeutically-relevant populations of neurons, relying on the use of transgenic Cre driver lines and/or intersectional tracing methods [65], is a clear next step. Good candidates for such an approach in PD and SCI models would be graft tissue prepared from dopaminergic and V2a interneuronal [66] Cre driver lines, respectively. Rabies tracing could also be initiated from host motor [67] or premotor neurons caudal to the injury site following SCI, in order to identify graft neurons that contribute to motor output, for example. Further, the advent of monosynaptically-restricted anterograde tracers, based on the use of herpes vectors (HSV) for example [68], may provide a complementary technique for the identification of direct graft inputs to host neurons.

The identification of graft neurons that are able to participate in relay circuits, and the specific host neurons which provide input to them, may be critical to successful clinical translation of stem cell-based therapies of neurological disease. For example, if a particular set of host inputs is demonstrated to influence graft function, it may be clinically important to consider if those inputs require an injury signal and/or a direct interface with the graft site, as has been demonstrated for the regeneration of the corticospinal tract [18]. If randomly-positioned graft components block the regeneration of host inputs, such as observed in the Dulin, et al. study [22], turn out to block functional recovery, it may be possible to arrange graft cells in a manner that better resembles intact anatomy. Such an effort could be aided by bioengineering approaches [69-72].

Graft neurons may also be extensively interconnected with one-another [51,73]. With the use of Cre driver lines to initiate rabies tracing from specific populations of graft neurons, important insights into graft-graft connectivity may also be revealed. This information could be particularly useful for next-generation translational efforts; if functionally-relevant graft neurons are found to be potently modulated by other neurons in transplants, graft preparations could be modified or mixed to better support beneficial forms of connectivity - a concept that has been previously explored with fetal grafts in the rat PD model [74]. The results of such experiments could also provide guidance as to whether grafts containing a single or multiple neuronal (and glial) subtypes would be ideal for translation to the clinic.

Monosynaptic tracing in its current form is limited to short-term experiments due to the inherent toxicity of the rabies virus vectors currently in use [75], generally precluding functional analyses in vivo. However, modifications to the original technique are beginning to make long-term assessments possible, using double-deletion (ΔGL) [76] or less-rapidly-replicating strains of ΔG rabies [61] to enable observation and manipulation of infected cells over months or years. The ΔGL approach relies on the reduction of viral transcript expression to trace levels, which are insufficient to elicit cytotoxicity but are sufficient to drive Cre- or Flp-mediated recombination. If the use of ΔGL rabies can be extended from retrograde projection mapping [76] to monosynaptically-restricted tracing, it may become an ideal tool to interrogate the functional consequence of graft-mediated relay circuits. In order to specifically implicate graft relay circuits as contributors to functional recovery, both graft activity and presynaptic host activity need to be orthogonally manipulated at scale, in order to control for the influence of host-host connectivity.

When (and if) the tools to perform behavioral experiments with defined synaptic partners become available, these systems could also be used in more therapeutically-relevant models. Graft-initiated tracing could be extended from corticospinal [11,77] and neurotoxic [10,45,55] lesion models to chronic contusion and alpha-synuclein overexpression [78] models, which may better represent the clinical situation in human patients with SCI and PD, respectively.

6 | CONCLUSION

For many decades, appropriate synaptic connectivity between host and graft neurons has been observed using traditional tract-tracing techniques, the scale of which has been better revealed using monosynaptic tracing. The ongoing development of next-generation monosynaptic tracing vectors is well-poised to assess the long-standing "relay hypothesis" of graft-mediated functional recovery. Studies on the ability of grafted neurons to integrate into host neural circuitry,

and the investigation of synaptic specificity among new circuits containing graft neurons, will help to guide the development and design of effective clinical cell replacement strategies. The demonstration and understanding of the extent to which neuronal connectivity is required for optimal functional benefit will also help to answer fundamental questions about the plasticity of the regenerating nervous system.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

M.P. declared patent holder of the U.S. patent application 15/093927 owned by Biolamina AB and EP17181588 owned by Miltenyi Biotec, consultancy role with Novo Nordisk and has stock ownership in Parmar Cells. The other authors declared no potential conflict of interest.

AUTHOR CONTRIBUTION

Andrew Adler, Anders Björklund, Malin Parmar: Wrote and approved the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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FIGURE 1 Graft-initiated monosynaptic rabies tracing of host-to-graft inputs

Monosynaptic host inputs to graft-derived neurons can be mapped across the entire host nervous system using modified rabies virus tracing. EnvA-pseudotyped and G-deleted (ΔG) rabies virus expressing mCherry is unable to infect neurons unless the TVA receptor is provided in trans, and ΔG rabies-infected neurons are unable to transmit virus to presynaptic partners unless the G-protein is also provided in trans, often in combination with a green fluorescent protein (GFP) to mark starter neurons. Graft neurons can be engineered to express the rabies helper components (TVA receptor and G-protein) using transgenic, CRISPR-Cas9, or viral approaches before grafting. Graft-initiated monosynaptic rabies tracing then proceeds in a retrograde fashion to presynaptic host neurons, and ceases without disynaptic transfer among host neurons, which do not express the G-protein. Righthand panels depict a histological example of rabies-traced host neurons (arrowheads, red) providing synaptic input to human neurons (red and green) derived from a transplant of dopaminergic progenitors placed in the dopamine-depleted rat striatum (scale bars = 50 μm).

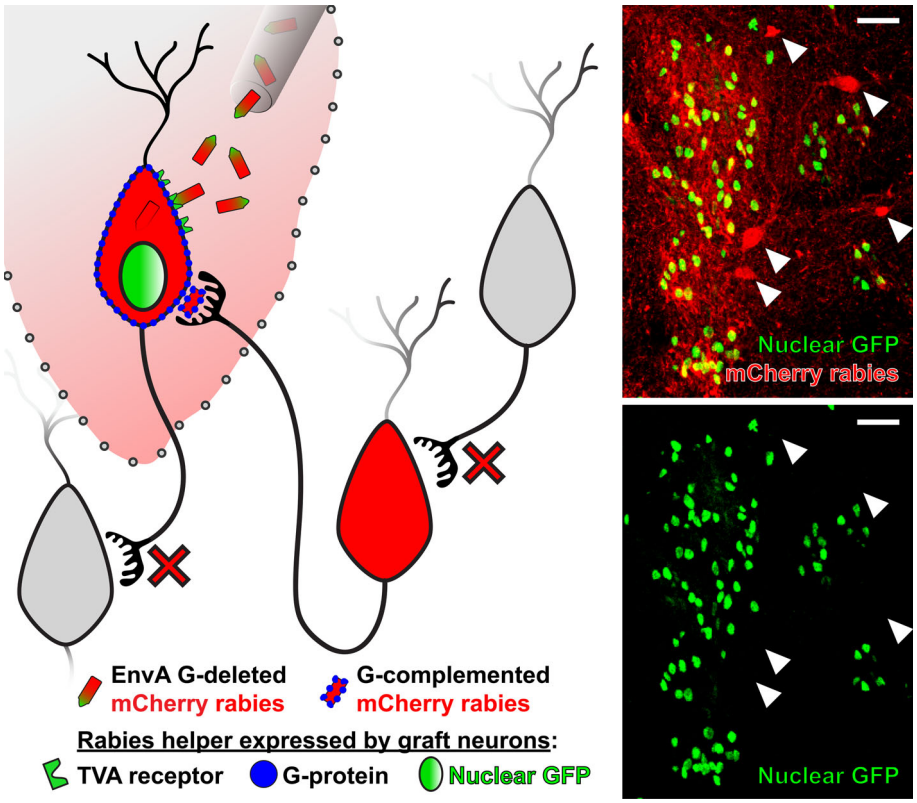


FIGURE 2 Neuroanatomical and monosynaptic tracing reveal homology between the intact and the graft-reconstituted spinal cord after lesion

(Top left) Neuroanatomical visualization of the intact spinal cord depicts an ordered projection pattern from sensory and cortical upper motor neurons to laminated groups of spinal interneurons in the dorsal horn.

(Top right) Monosynaptic rabies virus tracing initiated from cholinergic lower motor neurons in ChAT-Cre transgenic animals, combined with targeting of Cre-dependent rabies helper AAV to a single muscle group, highlights the strict requirement that neurons be synaptically coupled to enable transsynaptic rabies spread. Proprioceptive sensory neurons innervating targeted muscle groups are labeled transsynaptically, whereas those innervating antagonistic muscles are not, despite close juxtaposition of the targeted and antagonistic motor neuron dendrites [61].

(Bottom left) Neuroanatomical tracing reveals that injured and regenerating host sensory and motor axons strictly respect graft-derived spinal sensory interneuron domains, analogous to the organization of the intact spinal cord. Regenerating corticospinal axons appear unable to traverse graft-derived borders that they do not cross in the intact animal [22].

(Bottom right) Graft-initiated monosynaptic tracing from spinal cord progenitor cell grafts labels host neurons in functionally-appropriate nuclei [11]. Without restriction of rabies helper expression to specific subtypes of graft neurons it has not yet been possible to clarify whether host-graft synapse formation follows the same strict boundaries established by host axonal regeneration.

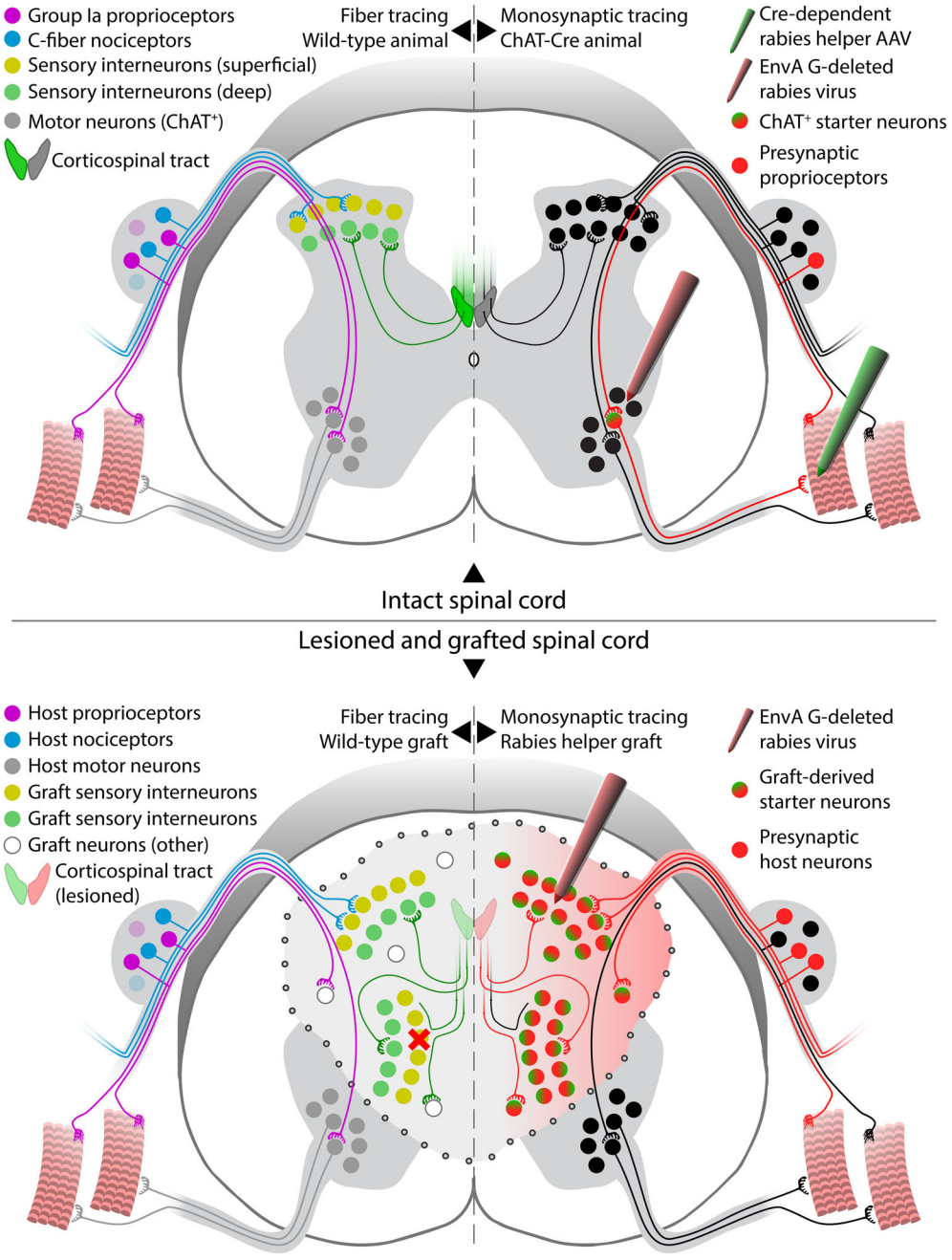


FIGURE 3 Neuroanatomical and monosynaptic tracing reveal homology between the intact and the graft-reconstituted basal ganglia after lesion

(Top left) Neuroanatomical visualization of intact basal ganglia circuitry depicts reciprocal projections between A9 dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and GABAergic medium spiny neurons in the dorsolateral striatum, as well as a "sparse" cortico-nigral projection [79]. A10 DA neurons in the ventral tegmental area (VTA) and nucleus accumbens (NAcc) are also reciprocally innervated.

(Top right) Monosynaptic tracing initiated specifically from SNpc DA neurons with DAT-Cre transgenic animals and anatomical targeting of a Cre-dependent rabies helper AAV confirms known connectivity between the SNpc and the dlSTR, but has, in addition, revealed widespread direct monosynaptic corticonigral inputs [59].

(Bottom left) Graft-derived DA neurons reinnervate the DA-depleted host basal ganglia [42], with an appropriate bias for A9 vs A10 target structures [46].

(Bottom right) Graft-initiated monosynaptic tracing from DAergic grafts labels host neurons in functionally-appropriate nuclei, regardless of whether they are placed in the substantia nigra or the striatum, but the phenotypes of synaptically coupled host and graft neurons are not yet fully known [10,45,55].

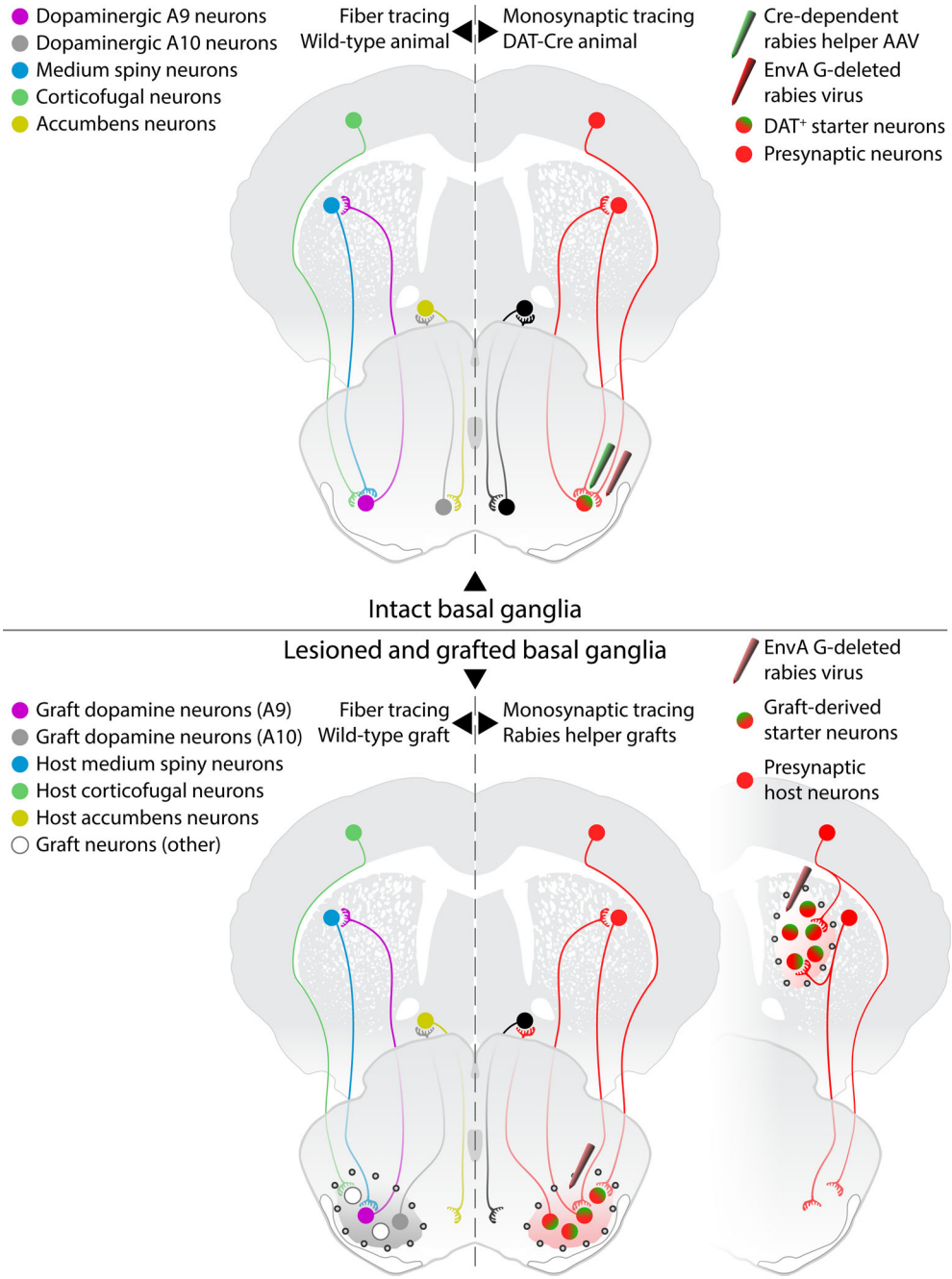
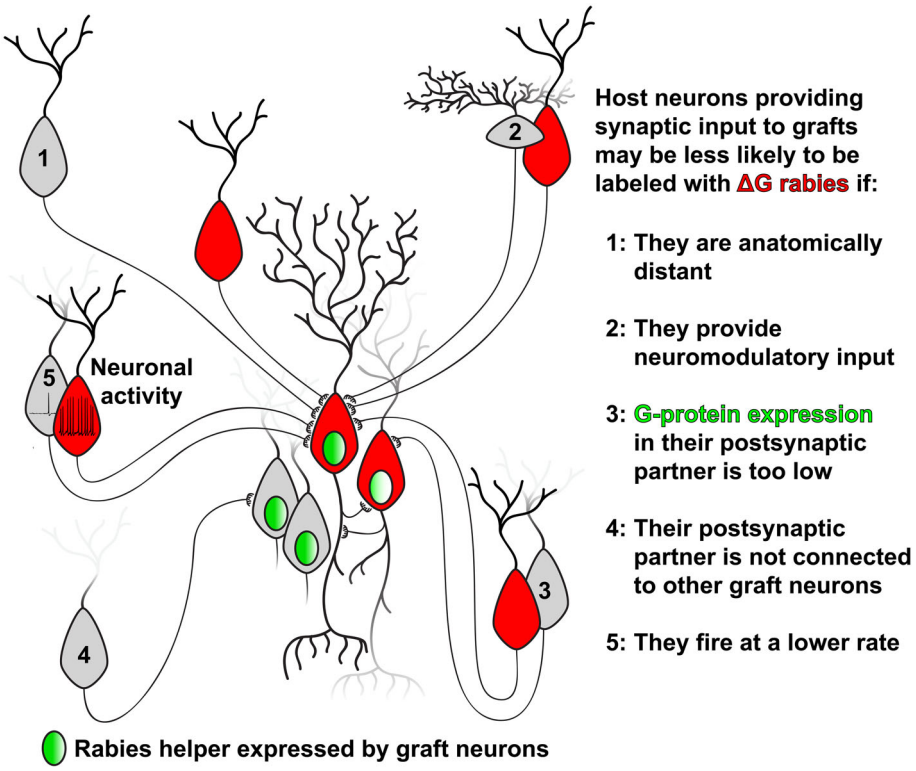


FIGURE 4 Rabies virus does not trace all synaptic connections with equal efficiency

Transsynaptic transfer of G-deleted rabies virus between synaptically-coupled neurons is a stochastic process, providing a low "false positive" rate, but also a high "false negative" rate. As such, a lack of transsynaptic rabies virus transfer generally does not provide strong evidence for a lack of synaptic connectivity. Clockwise from top left: (1) Rabies virus transfer efficiency decreases with a greater distance between pre- and postsynaptic neurons [61]. (2) Rabies virus transfer efficiency varies with synaptic phenotype, and appears to be lower for neuromodulatory contacts, in particular [63]. (3) Rabies virus transfer depends on G-protein expression levels in graft neurons, which could vary depending on graft maturity or starter neuron phenotype. (4) Rabies virus spreads polysynaptically among helper-expressing graft neurons, and may therefore bias the starter population towards more highly-interconnected neuronal subtypes. (5) For two identical presynaptic host neurons, one may be more likely to be labeled with rabies due to higher neuronal activity [62].



Graphical Abstract

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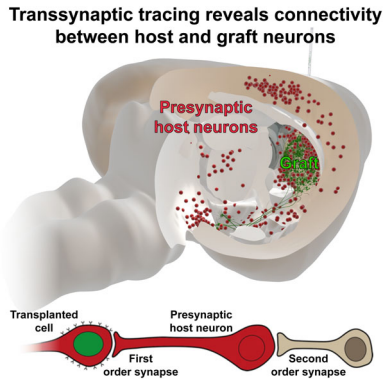

Monosynaptic rabies tracing can be used to map inputs to stem cell-derived neurons grafted to models of neurodegenerative disease and injury. These studies indicate that graft neurons receive anatomically-appropriate inputs, but it remains to be determined whether those inputs are necessary for functional recovery. Next-generation rabies tracing is poised to address this outstanding question, which may aid ongoing translational efforts.