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Monosynaptic Tracing using Modified Rabies Virus Reveals Early and Extensive Circuit Integration of Human Embryonic Stem Cell-Derived Neurons

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SUMMARY

Human embryonic stem cell (hESC)-derived dopamine neurons are currently moving toward clinical use for Parkinson's disease (PD). However, the timing and extent at which stem cell-derived neurons functionally integrate into existing host neural circuitry after transplantation remain largely unknown. In this study, we use modified rabies virus to trace afferent and efferent connectivity of transplanted hESC-derived neurons in a rat model of PD and report that grafted human neurons integrate into the host neural circuitry in an unexpectedly rapid and extensive manner. The pattern of connectivity resembled that of local endogenous neurons, while ectopic connections were not detected. Revealing circuit integration of human dopamine neurons substantiates their potential use in clinical trials. Additionally, our data present rabies-based tracing as a valuable and widely applicable tool for analyzing graft connectivity that can easily be adapted to analyze connectivity of a variety of different neuronal sources and subtypes in different disease models.

INTRODUCTION

Dopamine (DA) cell replacement for Parkinson's disease (PD), using transplantation of human fetal ventral mesencephalic tissue to the striata of PD patients, has provided proof of principle that DA-neuron-rich grafts can survive long term and restore striatal DAergic function (Barker et al., 2013). The lack of available fetal tissue, as well as the logistic and ethical issues associated with the use of obtaining such tissue, has spurred the development of protocols for the generation of authentic and functional midbrain DA neurons from human embryonic stem cells (hESCs) (Kirkeby et al., 2012; Kriks et al., 2011). Pre-clinical validation of such hESC-derived neurons in a rat model of PD shows that they function with equal potency and efficacy to fetal DA neurons after transplantation (Grealish et al., 2014). With the overall goal of mapping how transplants of stem cell-derived human neurons integrate in the host brain in a preclinical rat model of PD, we used a method based on modified rabies virus (ΔG-rabies) that allows for the tracing of afferent and efferent connections of transplanted human neurons. ΔG-rabies is a deletion mutant virus in which the gene coding for glycoprotein (GP; necessary for transsynaptic spread) is replaced by the gene coding for a fluorescent protein, and the envelope is pseudotyped to only infect cells expressing the TVA receptor (Wickersham et al., 2007). Therefore, the initial infection can be targeted to any cell engineered to express the TVA receptor. If that cell is modified to also express GP, the ΔG-rabies can assemble into infectious particles in this cell and spread retrograde across one synapse. This system has previously been used to trace connectivity of endogenous neural circuitry (Miyamichi et al., 2011; Watabe-Uchida et al., 2012), as well as newly born neurons in the olfactory bulb and hippocampus (Deshpande et al., 2013; Vivar et al., 2012). Here, we use the system to trace connectivity of transplanted hESC-derived neurons in a rat model of PD.

RESULTS

We used a polycistronic lentiviral tracing vector (Miyamichi et al., 2011) expressing a histone-tagged GFP; the TVA receptor, which allows for selective infection of ΔG-rabies; and rabies GP to allow for transsynaptic spread (Figure 1A). To control for unspecific labeling, we used a non-synaptic spreading of ΔG-rabies, a control vector containing GFP and TVA, but lacking GP (Figure 1A) in parallel.

The targeted cells, here termed *starter neurons*, can be visualized by nuclear GFP expression (Figure 1B). Upon infection with the ΔG-rabies vector encoding mCherry, the targeted starter neurons expressing the TVA receptor can be infected by ΔG-rabies and easily identified by expression of GFP and mCherry (Figure 1B). These neurons are engineered to contain GP, and hence, ΔG-rabies can assemble into infectious particles in the starter cells. Any neuron that forms presynaptic contacts with the starter

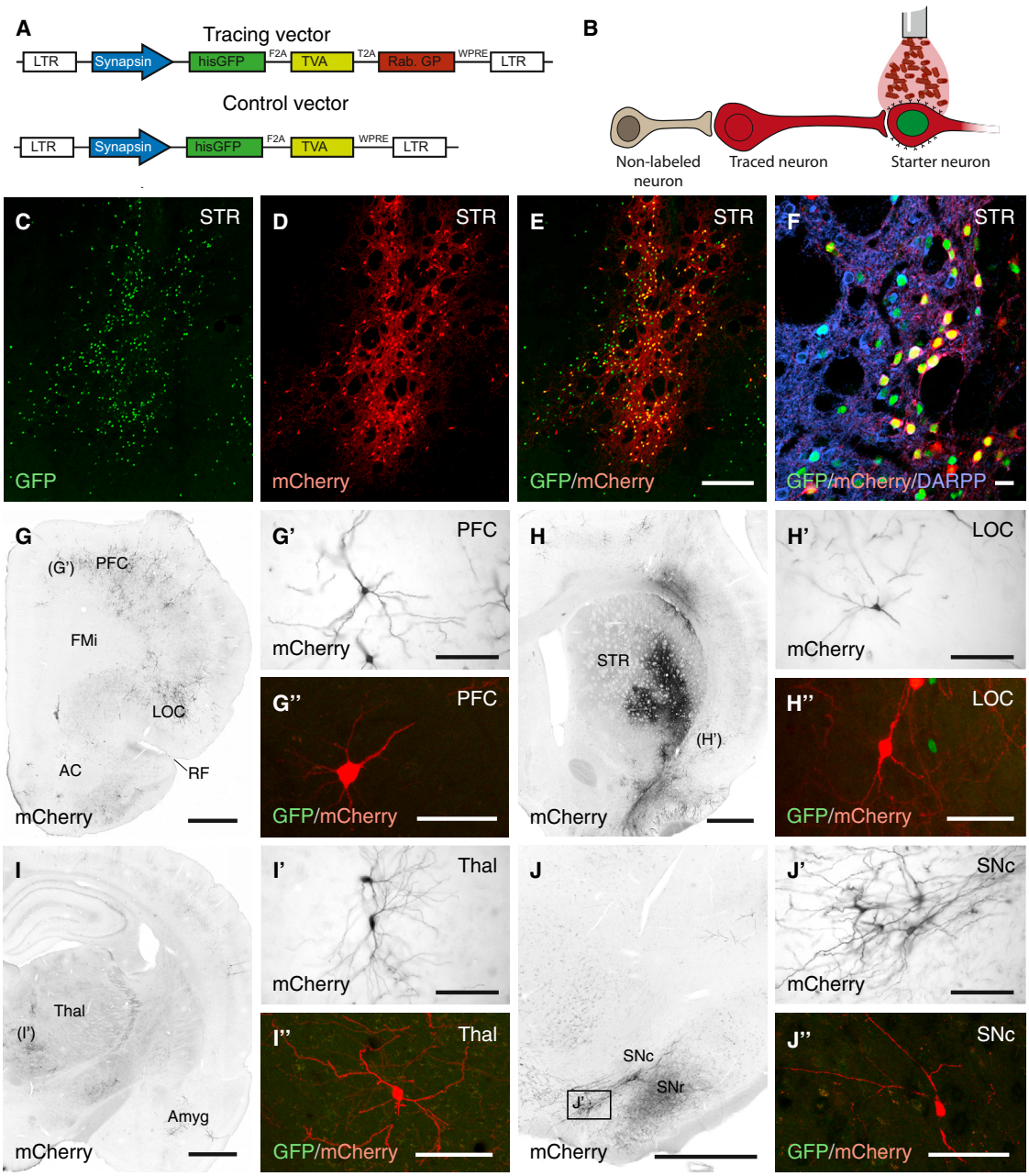

Figure 1. Overview of Monosynaptic Tracing Methodology

(A) Schematic representation of the lentivector constructs used in the study. The tracing vector labels cells with a histone-tagged GFP, the TVA receptor necessary for ΔG-rabies infection as well as the rabies GP that allows ΔG-rabies to transmit across synapses. The control vector lacks the GP and thus cannot transmit.

(B) Neurons that have been infected with the tracing construct are termed *starter neurons*. After ΔG-rabies infection, these cells turn mCherry$^+$, and due to the presence of GP in the starter neuron, ΔG-rabies is transmitted retrogradely to label the *traced neuron* and cannot transmit further due to the lack of GP.

(C) Upon injection of the tracing lentivector to the rat striatum (n = 6), strong nuclear GFP$^+$ expression is observed after 4 weeks.

(D and E) At 7 days after ΔG-rabies injection, a clear mCherry$^+$ signal is observed co-localized with GFP$^+$ nuclei.

(F) The main starter neuron population in this paradigm was DARPP32-expressing MSNs of the striatum.

(G–H″) Traced mCherry^{+}/GFP$^-$ neurons could also be observed in rostral structures, such as the prefrontal and lateral orbital cortex and close to the injection site within the striatum.

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