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Transfer of host-derived alpha synuclein to grafted dopaminergic neurons in rat

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ABSTRACT

Multiple laboratories have recently demonstrated that long-term dopaminergic transplants form Lewy bodies in patients with Parkinson's disease. Debate has arisen as to whether these Lewy bodies form from the transfer of alpha synuclein from the host to the graft or whether they form from intrinsic responses of the graft from being placed into what was, or became, an inflammatory focus. To test whether the former hypothesis was possible, we grafted fetal rat ventral mesencephalon into the dopamine depleted striatum of rats that had previously received 6-hydroxydopamine lesions. One month after the transplant, rats received viral over expression of human alpha synuclein (AAV2/6-alpha synuclein) or green fluorescent protein (AAV2/6-GFP) into the striatum rostral to the grafts. Care was taken to make sure that the AAV injections were sufficiently distal to the graft so no cells would be directly transfected. All rats were sacrificed five weeks after the virus injections. Double label immunohistochemistry combined with confocal microscopy revealed that a small number of grafted tyrosine hydroxylase (TH) neurons (5.7% ± 1.5% (mean ± SEM) of grafted dopamine cells) expressed host derived alpha synuclein but none of the grafted cells expressed host-derived GFP. The alpha synuclein in a few of these cells was misfolded and failed to be digested with proteinase K. These data indicate that it is possible for host derived alpha synuclein to transfer to grafted neurons supporting the concept that this is one possible mechanism by which grafted dopamine neurons form Lewy bodies in Parkinson's disease patients.

Introduction

Excess alpha synuclein, either due to genetic aberrations or experimental manipulations induces degeneration of substantia nigra dopamine neurons in both experimental and human Parkinson's disease. In addition to neurons within the substantia nigra degenerating, we (Chu and Kordower, 2010; Kordower et al., 2008a, 2008b) and others (Li et al., 2008, 2010) have recently demonstrated that human nigral neurons grafted into the putamen of patients with Parkinson's disease (PD) for greater than 10 years, display Lewy bodies that are indistinguishable from the Lewy bodies seen in host brain regions including the substantia nigra. In this regard, they display the morphological features of Lewy bodies as seen in hemotoxylin and eosin stained sections (Li et al., 2008, 2010) which express alpha synuclein (Chu and Kordower, 2010; Kordower et al., 2008a, 2008b; Li et al., 2008, 2010) and ubiquitin (Chu and Kordower, 2010; Kordower et al., 2008a, 2008b; Li et al., 2010) and are thioflavin S positive (Kordower et al., 2008b; Li et al., 2010), the most definitive light microscopic marker for Lewy bodies.

While the presence of Lewy bodies in grafted neurons is incontrovertible, the means by which they occur have been subject to debate. Some have argued that the presence of Lewy bodies is a response to grafts being placed into an environment altered by aging, or a result of grafting techniques that are conducive to producing immune or inflammatory responses (Cooper et al., 2009). Others have argued that alpha synuclein can transfer from the host to the graft in a prion-like process (Angot et al., 2010; Brundin et al., 2008) analogous to the proposed spread of PD pathology in the brain from the olfactory bulb and caudal brainstem, which then spreads caudally and rostrally from these two regions respectfully. This model has been termed the Braak hypothesis (Braak et al., 2006).

The present study was performed to test a single hypothesis: can grafted neurons take up host-derived alpha synuclein and express this protein within their cell soma in a manner seen in patients with long-term fetal nigral grafts? Towards this end, fetal nigral allografts were placed within the striatum of dopamine denervated rats. Subsequent to the grafting, alpha synuclein or a reporter gene were virally over-expressed within the striatum in regions distal to the graft using viral vectors. Only alpha synuclein was seen within grafted nigral perikarya with some appearing to be aggregated. These data demonstrate that grafted nigral neurons can uptake and transfer alpha synuclein from the host and this may be a mechanism by which Lewy bodies form in grafted dopamine neurons.

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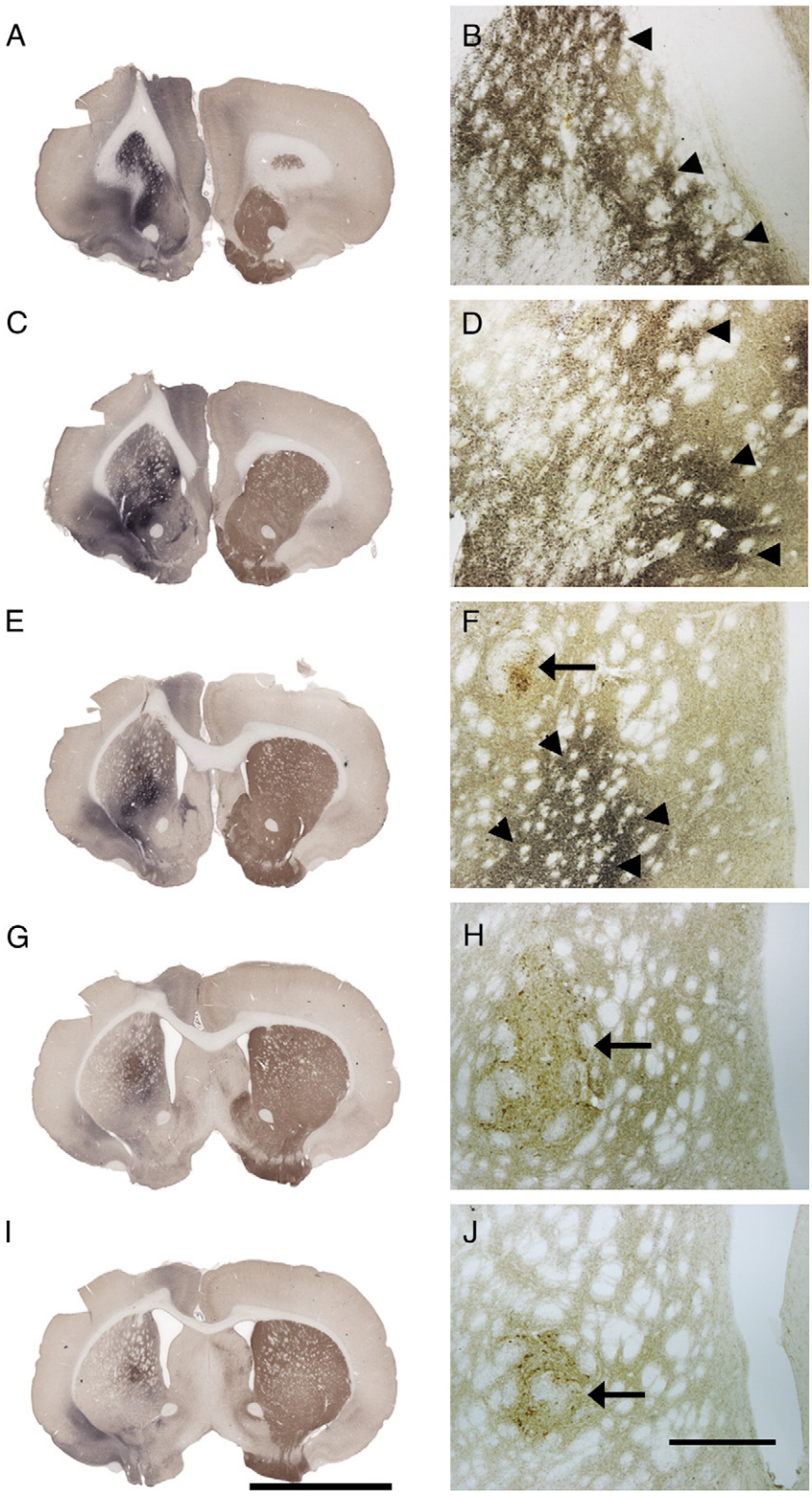

Fig. 1. Photomicrograph displaying human alpha synuclein (blue-black) and TH (brown) double peroxidase immunostained sections. A, C, E, G, and I represent low power photomicrographs, while B, D, F, H and J represent high power photomicrographs showing TH-ir grafted cells and virally expressed human alpha synuclein protein. Arrowheads represent the human alpha synuclein vector injection and arrows represent the grafted cells injection site. Note the presence of grafted cells in the absence of human alpha synuclein immunoreactivity directly from the injection indicating that the grafted cells were not directly transfected by the vector. Scale bar in I represents 5 mm and applies to (A, C, E, G, and I). Scale bar in J represents 500 μm and applies to (B, D, F, H, and J) respectively.