

Impact of dopamine to serotonin cell ratio in transplants on behavioral recovery and L-DOPA-induced dyskinesia

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ABSTRACT

Fetal dopamine (DA) cell transplantation has shown to be efficient in reversing behavioral impairments associated with Parkinson's disease. However, the beneficial effects on motor behavior and L-DOPA-induced dyskinesia have varied greatly in between clinical trials and patients within the same trial. Recently, the inclusion of serotonin (5-HT) neurons in the grafted tissue has been suggested to play an important negative role, in particular, on the effect of L-DOPA-induced dyskinesia. In the present study we have evaluated the influence of different ratios of DA neurons in relation to 5-HT neurons in the graft on spontaneous motor behavior and L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. We show that using the standard dissection method that gives rise to a DA:5-HT ratio in the graft of 2:1 to 1:2 there is significant and consistent improvement in spontaneous motor behavior and reversal of L-DOPA-induced dyskinesia. Increasing the ratio of 5-HT neurons in the graft, to a DA:5-HT ratio of in between 1:3 and 1:10, still induces significant reduction of L-DOPA-induced dyskinesia, suggesting that the detrimental effect of 5-HT neurons on L-DOPA-induced dyskinesia is prevented even by small numbers of DA neurons in the graft. Nonetheless, while the post-synaptic responses were normalized following peripheral L-DOPA delivery in animals with low DA:5-HT ratio, we observed a pharmacological indication of hyperactive pre-synaptic response in these animals. These data suggests that 5-HT cells within a graft are neither detrimental nor beneficial for functional effects of DA-rich transplants; however, in absence of sufficient numbers of DA neurons, the 5-HT neurons may induce negative effects following L-DOPA therapy. In summary, our data indicate that for future clinical trials the inclusion of 5-HT neurons in grafted tissue is not critical as long as there are sufficient numbers of DA cells in the graft.

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Introduction

Transplantation of dopamine (DA) neurons, derived from embryonic ventral mesencephalon (VM) has shown to be efficient in reversing behavioral deficits and to reduce the need for pharmacotherapy in patients with Parkinson's disease (PD) (Winkler et al., 2005). The results have, however, highly varied in between patients and clinical trials, and two double-blind trials have shown improvements in subgroups of patients but failed to reach an overall significant behavioral recovery (Freed et al., 2001; Ma et al., 2010; Olanow et al., 2003, 2009). Fetal cell transplantation has also shown various effects on the expression of L-DOPA-induced dyskinesia, one of the most common side effects of pharmacotherapy in PD. Some

patients have thus shown good benefit from cell therapy, while in others no change or even worsening in L-DOPA-induced abnormal involuntary movements (AIMs) has been reported (Hagell and Cenci, 2005).

Several parameters of the transplant protocols have been put forward to play a role for the functional outcome after fetal DA cell transplantation and to explain the discrepancies within patients and trials, including tissue preparation and graft composition (Brundin et al., 2010; Lane et al., 2010; Winkler et al., 2005). Recently, the inclusion of serotonin (5-HT) neurons in the grafts has emerged as a critical factor for the effect on treatment-induced dyskinesia. 5-HT neurons have been shown to possess the cellular machinery to take up peripheral L-DOPA, convert it to DA, store it in vesicles and release it in an activity dependent manner (Arai et al., 1994; Carta et al., 2010; Maeda et al., 2005; Navailles and De Deurwaerdère, 2011; Ng et al., 1970, 1971; Tanaka et al., 1999). However, in contrast to DA neurons these cells lack the auto-regulatory feedback by activation of D₂ receptors in order to control the release of its "false transmitter" DA. In fact, the serotonin system has shown to be a major player in induction and maintenance of L-DOPA-induced dyskinesias in animal models of

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PD (Carta et al., 2010). Thus, Carta et al. (2007) have shown that L-DOPA-induced dyskinesia can be blocked in the rat PD model by removing the intrinsic 5-HT system by toxic lesions or by pharmacologically blocking the 5-HT release. In addition, it has recently been shown that serotonin grafts can significantly worsen L-DOPA-induced dyskinesia in the rat PD model (Carlsson et al., 2007, 2009). This effect developed over time as the graft matured and the detrimental effect was present both as an increase in severity of dyskinesias as well as a prolonged dyskinetic response after a single dose of L-DOPA (Carlsson et al., 2007). In addition, dyskinesia in the 5-HT grafted animals was evident already after partial DA-denervating lesions and drastically worsened as the DA lesion extended suggesting a protective role of the DA system on the 5-HT-effect on dyskinesia (Carlsson et al., 2009). Since fetal grafts in PD patients may contain large numbers 5-HT neurons (Mendez et al., 2008; Politis et al., 2010), and since degeneration of the DA system will progress in these patients, these experimental data clearly indicate the importance of the 5-HT system in the outcome of fetal cell transplantation, and the need for better understanding the role of grafted 5-HT cells.

The effects of transplanted 5-HT neurons in animal PD models, however, have only been evaluated using “pure” 5-HT grafts and compared to “regular” grafts, containing DA and 5-HT neurons at a ratio of approximately 1:1. Using the rat model of PD, we investigated the effects of increasing the ratio of 5-HT neurons in relation to DA neurons in the graft from approximately 1:1 to 10:1 on functional changes in spontaneous motor behavior and L-DOPA-induced dyskinesia. We show that grafts containing sufficient numbers of DA neurons (more than 600 cells) inducing DA-reinnervation to at least 20% of normal in the caudolateral part of the striatum will always improve spontaneous motor function and reduce L-DOPA-induced dyskinesia by more than 50%, irrespective of the 5-HT-neuron number or the DA:5-HT ratio. Also in the group of animals with a DA:5-HT ratio between 1:3 and 1:10 there was significant reduction of L-DOPA-induced dyskinesia despite these animals not improving spontaneous motor function due to low DA neuron numbers, thus indicating that small numbers of grafted DA neurons are sufficient to block the effects of 5-HT neurons on the expression of dyskinesia. Nonetheless, an increased ratio of 5-HT neurons versus DA cells in the grafts may aggravate hyperactive pre-synaptic activity, after pharmacological manipulation, manifested as a strong tendency to

increase in L-DOPA-induced rotation, and an unchanged apomorphine-induced response.

Materials and methods

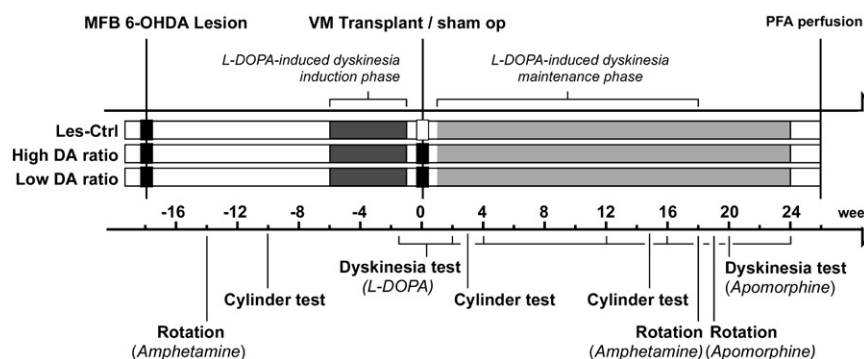
Subjects

Adult female Sprague–Dawley rats (Charles River, Sulzfeld, Germany) weighing 225 g at the beginning of the experiment were used in this study. Animals were housed under a 12 h light/dark cycle with water and food ad libitum. The experiments were carried out according to the ethical guidelines set by the ethical board of the Albert-Ludwigs University of Freiburg and the Regierungspräsidium Freiburg, Germany.

Experimental design

All animals received unilateral injections of 6-OHDA into the medial forebrain bundle (MFB) in order to obtain a complete DA depletion of the nigrostriatal pathway (see *experimental timeline* in Fig. 1A). The extent of the lesion was characterized by amphetamine-induced rotation and in the cylinder test at 4 and 5 weeks after the lesion, respectively. Animals exhibiting more than 5 full body turns/min towards the side of DA deficiency in amphetamine rotation and <30% contralateral paw use in the cylinder test were selected for the study, and subsequently treated chronically with 3,4-dihydroxyphenylalanine (L-DOPA) in combination with the peripheral decarboxylase inhibitor benserazide hydrochloride daily for 4 weeks. During this period (*L-DOPA-induced dyskinesia induction phase* in Fig. 1A), the animals were repeatedly tested for the expression of abnormal involuntary movements (AIMs) until dyskinesia scores had reached a plateau. Animals with moderate to severe dyskinesia were selected and allocated in 3 groups, before grafting, according to their dyskinesia scores and performance in amphetamine-induced rotation and cylinder test. Two of the groups received single cell suspension grafts containing a mixture of DA and 5-HT neuroblasts, obtained from the ventral mesencephalon (VM) and the raphe nucleus of 14 day old (E14) rat embryos. The first grafted group (*High DA ratio*) received cells of a regular VM dissection, where according to our previous studies part of the developing upper

A Experimental design



B Animal grouping according to rank order (TH: 5-HT cell numbers ratio)

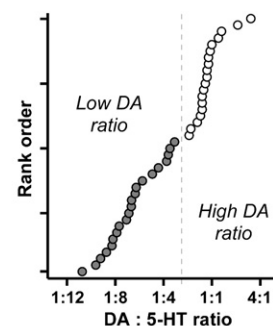


Fig. 1. Experimental design and individual animal rank order. (A) After the unilateral 6-hydroxydopamine (6-OHDA) lesion of the medial forebrain bundle (MFB) and selection of animals according to performance in amphetamine-induced rotation and cylinder test, animals received daily injections of L-DOPA to induce dyskinesia (*induction phase*, dark-gray area). Animals with moderate to severe dyskinesia were allocated and balanced in 3 groups prior to grafting according to their dyskinesia scores and performance in amphetamine-induced rotation and cylinder test. Animals received single cell suspension grafts containing different ratios of DA and 5-HT neuroblasts obtained from the ventral mesencephalon (VM) and the raphe nucleus of E14 rat embryos (*High DA ratio* and *Low DA ratio* groups), or sham operation (*Les-Ctrl* group). After grafting animals were kept on a maintenance regimen of twice-weekly L-DOPA (*maintenance phase*, light-gray area). Grafts effects on motor behaviors and dyskinesia were repeatedly assessed before perfusion with paraformaldehyde (PFA) for histological analysis. (B) Animals with VM grafts showed a TH:5-HT cell ratio between 3:1 and 1:3 (*High DA ratio* group), whereas animals with less VM cells but additional cells from the raphe nucleus showed a TH:5-HT ratio between 1:3 and 1:10 (postmortem analysis of numbers of grafted DA and 5-HT neurons showed that two animals in the *High DA ratio* group rather had a low TH:5-HT ratio between 1:3 and 1:10 and were therefore moved to the *Low DA ratio* group for further analysis).

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