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Research report

Donor age dependent graft development and recovery in a rat model of Huntington's disease: Histological and behavioral analysis

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HIGHLIGHTS

- Ganglionic eminence graft donor age impacts on functional recovery and cell composition.
- E13 WGE (cf. E14 and E15) grafts showed the best anatomical and functional integration.
- The earliest grafts provided the best long-term behavioral recovery.
- Better understanding striatal development is necessary for tissue selection in clinical trials.

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ABSTRACT

Neural cell replacement therapy using fetal striatal cells has provided evidence of disease modification in clinical trials in Huntington's disease (HD) patients, although the results have been inconsistent. One of the contributing factors to the variable outcome could be the different capacity of transplanted cells derived from the primordial striatum to proliferate and maturate into striatal projection neurons. Based on the rodent lesion model of HD, the current study investigated how intrastriatal-striatal grafts from variable aged donors develop in vivo and how they influence functional recovery.

Young adult female Sprague-Dawley rats were lesioned unilaterally in the dorso-striatum with quinolinic acid (0.12 M) and transplanted 14 days later with single cell suspension grafts equivalent of one whole ganglionic eminence (WGE) from donors of embryonic developmental age E13, E14, or E15; animals with or without striatal lesion served as controls. All animals were tested on the Cylinder and the Corridor tests, as well as on apomorphine-induced rotation at baseline, post-lesion/pre-grafting, and at 6 and 10 weeks post-grafting. A week prior to perfusion, a sub-group in each grafted group received fluorogold injections into the ipsilateral globus pallidus to study graft efferent projections. In summary, the data demonstrates that the age of the embryonic donor tissue has an impact on both the graft mediated functional recovery, and on the in vivo cellular composition of the striatal transplant. E13 tissue grafts gave the best overall outcome indicating that WGE from different donor ages have different potential to promote functional recovery. Understanding the stages and process in rodent striatal development could improve tissue selection in clinical trials of cell therapy in HD.

1. Introduction

Huntington's disease (HD) is an autosomal dominant hereditary disease caused by the pathological expansion of the CAG trinucleotide repeat in the gene coding for huntingtin with disease manifestation occurring mainly after the age of 35 and the disease duration typically being 15–30 years, ending in complete care dependence and finally death [1,2]. The mutation leads to progressive neurodegeneration affecting the projection neurones from the striatum, but imaging studies have confirmed that degeneration occurs in other regions as well, including the cortex and the hypothalamus [3,4]. The order of the symptoms vary from person to person, but by the end stage patients develop motor, cognitive and psychiatric disturbances, and death is commonly caused by secondary reasons like pneumonia and depression, or suicide.

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Currently medical science offers no cures for HD, only symptomatic pharmacological treatments are available that can temporarily moderate some of the choreic movements so prominent amongst the patients [5,6]. Recent experimental evidence suggests that during late disease stages, pathology in non-neural tissues appear (e.g. muscle tissue) which can contribute to the impairment [7].

Cell replacement therapy has been tested in HD patients in several waves of clinical trials using primary neurons with the outcomes suggesting that – in some case and under certain conditions – this strategy can modify the progression of the disease and influence positively the patient's quality of life [8,9]. In experimental studies, transplantation of cells prepared from embryonic ganglionic eminence tissue – the structure that becomes the striatum in the adult – into animal models of HD have the potential to replace damaged striatal neurons, to provide the substrate for the reconstruction of dysfunctional neuronal circuits, and to provide partial to complete behavioral recovery in a task specific manner [10,11]. Selective dissection of the ganglionic eminence, the surgical protocol used to transplant the cells, as well as the post-grafting training experience, have been shown to influence both the graft-mediated functional recovery as well as the cellular composition of the striatal grafts [12–15]. However, more needs to be known about the key factors and the fundamental mechanisms that impact on the anatomical and functional outcome in order to enhance the translational value of cell replacement therapy.

The ganglionic eminence, the tissue source for striatal grafts, appears at embryonic day E10.5 in the rat and goes through several developmental waves generating different sub-sets of striatal neurons between the age of E12.5 and P2. Striatal grafts obtained from E14 ganglionic eminence are most commonly used as this time point corresponds to an early wave of striatal neurogenesis (E12.5–E14.5) and it precedes the formation of afferent or efferent connections [16]. Although there is a second wave of neurogenesis, between E17.5 and P2, older striatal grafts have a significantly lower capacity to survive and to promote recovery [17,18]. The current study aimed to investigate in more detail the in vivo properties of the tissue corresponding to the first striatal neurogenesis, i.e. E13, E14, and E15, and specifically comparing development, cellular content, integration with the host, and influence on behavioral recovery across the ages. The data clearly underlies the different potential of the donor ages, pointing towards the younger tissue sources, closest to the onset of neurogenesis, to have the most potential to promote functional recovery.

2. Methods and materials

2.1. Subjects

Rats were housed in standard cages in groups of up to five animals in a temperature-controlled room (temperature of 21 ± 1 °C, 50–60% relative humidity) on a 12 h light/12 h dark schedule. During the periods when behavioral testing took place, the animals had restricted access to food and were kept at 90% of their normal body weight during the testing period. Outside of testing periods – when food was restricted to 12 g/rat – animals had ad libitum access to laboratory food and to water, and were weighed once per week. All animals were treated according to the ethical guidelines set by the local ethical board of the University of Freiburg and the Regierungspräsidium Freiburg, Germany.

2.2. Experimental design

The experimental design is summarized in Table 1. Adult female Sprague-Dawley rats (Charles River, Germany) weighing between 225 and 250 g at the beginning of the study were used. All animals ($n = 48$) received Baseline behavioral testing prior to receiving unilateral dorsostriatal quinolinic acid lesions followed by a new round of behavioral tests. Based on their performance the lesioned animals were matched, and 10–14 days after the lesion, a sub-group of them received stereotactic implantation of embryonic whole ganglionic eminence cell grafts. At 6 weeks and at 10 weeks post-transplantation, all animals were subjected to behavioral testing. Seven days prior to perfusion, a randomly selected sub-group of animals received unilateral fluorogold injection into the globus pallidus. All the animals were perfused at 12 weeks post-graft survival. The final experimental groups were: Control ($n = 8$), Lesion ($n = 10$), and E13 ($n = 10$), E14 ($n = 10$), E15 ($n = 10$) grafting groups.

2.3. Excitotoxic lesion

Surgery was carried out under gas anesthesia with isoflurane (Abbott, Germany) using O_2 as carrier gas (induction: 5 vol% of isoflurane with 1.5 L/O_2/min; maintenance: 1.5–2.0 vol% isoflurane in 1.5 L/O_2/min). Using a stereotactic frame (Stoelting, USA), animals received unilateral striatal lesions by injecting 4 × 0.25 μl of 0.12 M quinolinic acid (QA, Sigma, USA) dissolved in 0.1 M phosphate-buffered saline (1 × PBS solution), pH = 7.4, at two depths in each of the two tracks in the left neostriatum. Each of the four injections was made over 90 s via a 26-gauge stainless steel cannula connected to a 10 μl Hamilton syringe (Hamilton Europe, Switzerland) which was placed on a micro pump (World Precision Instruments Inc., UK). In total, the animals received 1.0 μl of 0.12 M quinolinic acid solution (=120 nmol of QA) with an injection rate of 0.13 μl/min. Injection coordinates with measurements in mm anterior (*A*) in front of bregma, lateral (*L*) to the midline, vertical (*V*) below dura were: *A* = −0.4, *L* = +3.7, *V* = 5.2/4.2; *A* = +1.2, *L* = +2.9, *V* = 5.2/4.2. The inter-aural line was set at −2.3 mm. The cannula was held in place for 2 min before retraction to reduce leakage. During surgery the eyes were protected with an ointment (Dexpanthenol and Vaselin (Bepanthen®)), and the animals received s.c. Temgesic (0.05 mg Buprenorphinhydrochlorid/kg bodyweight; Essex Pharma, Germany) in the neck as post-surgical analgesic. Additional post-surgery care is described in detail elsewhere [19].

2.4. Time mating of animals to obtain donor tissue of specific developmental age

Female rats aged between 10 weeks and 6 month and male rats aged between 3 and 10 months were used. Male rats were placed into individual cages with free access to food and water between the hours of 0900 and 2000, after which the food was removed and the female rats were paired up with the males in a 2:1 ratio. Next day, exactly 12 h later the rats were separated, and the morning was designated as embryonic day 0 (E0). At day 13 the female rats were palpated under CO_2 narcosis and in case of pregnancy two were removed to be used as embryonic donors. The same procedure was repeated at day 14 (E14) and 15 (E15).

2.5. Tissue dissection and cell suspension preparation

Time-pregnant dams (E13, E14, E15) were terminally anesthetized with Xylazine hydrochloride (15 mg/kg) and *Ketamine* Hydrochloride (150 mg/kg, Ketamine Essex, Germany) by an intraperitoneal injection. All instruments were disinfected in 70% ethanol before using. The donor's abdomen was disinfected with 70% ethanol and the stomach was opened by a C-section. The bicornal uterus was removed and washed in sterile PBS. The dam was killed by cardiotomy. Afterwards the embryos were removed from uterus under semi-sterile conditions and transferred to a sterile petri-dish containing sterile DMEM. The crown-rump-length (CRL) of the embryos was measured to ascertain the developmental age, and the whole ganglionic eminence (WGE including both lateral and medial regions) was dissected and collected in DMEM containing 0.05% DNAse to avoid agglutination of the tissue pieces due to free DNA.

The tissue was incubated in 0.1% Trypsin/0.05% DNAse/DMEM at 37 °C for 25 min and then rinsed carefully 4 times in 0.05% DNAse using a pipette. Afterwards, the pieces were mechanically dissociated with fire-polished tips until a homogenous suspension was created. The suspension was centrifuged at 600 rpm for 5 min at 24 °C, the supernatant removed, and the single cells were resuspended in 0.05% DNAse/DMEM.

2.6. Micro-transplantation of striatal cells from the whole ganglionic eminence of E13, E14, E15 rat embryos

Cell viability was determined by a Trypan blue exclusion assay and varied between 98% and 99% at all developmental ages. The concentration of the final cell suspension was calculated so that all grafted animals received the equivalent of 1 WGE prepared in 2 μl, whether it be for the E13, E14 or E15 grafts; this way the final number of transplanted cells were 3.8×10^5, 4.8×10^5, and 5.8×10^5 cells/graft for the E13, E14 and E15 grafts, respectively. The animals were anesthetized using isoflurane and fixed in the stereotactic frame as described for the lesioning procedure. For the injection of the single cell suspension a glass capillary with a diameter of 50 μm connected to a 2 μl Hamilton syringe was used. Using a one track/two 1 μl deposit grafting protocol, the cells were injected at the following stereotactic coordinates with measurements in mm anterior (A) in front of bregma, lateral (L) to the midline, vertical (V) below dura were: *A* = +0.4, *L* = +3.3, *V* = −5.0/−4.4. The inter-aural setting was −2.3 mm. Each 1 μl deposit was injected slowly and progressively over a 60-s period, and a 30-s pause was introduced between the first and the second deposit. Following the second deposit the capillary was kept in place for 3 min before slow retraction and rinsing/cleaning in 0.05% DNAse/DMEM medium. The wound was sutured with clips and the animals were given a subcutaneous injection of Buprenorphin (Temgesic®) according to their bodyweight (0.05 μg/g bodyweight).