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TRAINING SPECIFICITY, GRAFT DEVELOPMENT AND GRAFT-MEDIATED FUNCTIONAL RECOVERY IN A RODENT MODEL OF HUNTINGTON'S DISEASE

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Abstract—Neuronal function and morphology are affected by the environment and the behavioral experience. Here we report on the effects of differential training protocols on the development and the functional recovery mediated by intrastriatal striatal grafts. Rats were trained exclusively on the left or the right paw to perform on the skilled staircase task before being lesioned unilaterally in the dorsal striatum with quinolinic acid. E15 whole ganglionic eminence suspension grafts were implanted into the lesioned striatum. Subsequent testing probed unilateral performance of the affected contralateral paw, as well as bilateral performance. The grafted animals were initially as impaired as the lesioned, but partially recovered their performance with additional training. Grafted animals with appropriate previous experience initially performed better on the staircase test, but the advantage was transient. Furthermore, the grafted animals performed better with their affected paw under forced choice than under conditions when both paws were simultaneously probed. Improvements of the grafted animals were also observed on tests of forelimb akinesia and asymmetry. Morphological data suggest that the training conditions influenced the development specifically of striatal-like, but not of non-striatal like, neurones within the grafts. The grafts were smaller containing less striatal-like neurones in animals that were trained on the contralateral side prior to lesioning and grafting. The results support the hypothesis that unilateral training sensitizes the striatum that subserves the motor learning, leading to exacerbated excitotoxic lesions and to an environment less conducive for graft development.

Key words: transplantation, striatum, plasticity, training, Huntington's disease.

Neural transplantation is a potential therapy in Huntington's disease, a chronic neurodegenerative disorder affecting primarily the striatum (Peschanski et al., 2004). A single gene mutation, autosomal dominant, CAG trinucleotide repeat disorder, Huntington's disease typically results in motor, cognitive and psychiatric disturbances with a mean onset of 30–45 years of age (Leegwater-Kim and Cha, 2004). Clinical trials transplanting embryonic striatal tissue have demonstrated the safety and feasibility, as well as long-lasting motor, cognitive, and functional benefits of this therapy (Bachoud-Levi et al., 2000; Rosser et al., 2002). The grafted fetal striatal tissue has been shown to survive the transplantation process, develop and integrate anatomically with the host without being affected by the disease process itself (Freeman et al., 2000). Intrastriatal implantation of embryonic striatal tissue is, at the moment, the only therapy that has shown a long-term clinical benefit in patients with Huntington's disease. However, the benefits have been limited in terms of scale and restricted to a small number of patients (Bachoud-Levi et al., 2000, 2002). More needs to be known about the conditions that affect graft development and promote functional recovery.

Recent advances have improved understanding of cell death and survival (Brundin et al., 2000), differentiation (Perrier and Studer, 2003) and long-distance axonal growth (Armstrong et al., 2002) of grafted tissue within the host brain. Similarly, emerging evidence suggests that the host's experience and training, both prior to and following transplantation, are important components contributing to the plasticity of, and functional recovery provided by, neural grafts (Dobrossy and Dunnett, 2001, 2003, 2004). In many systems, for grafts to alleviate complex behavior deficits, they need to establish afferent and efferent connections with the host brain appropriate to the particular function that needs to be restored (Bregman, 1994; Dunnett and Bjorklund, 1994; Dunnett, 1995). Re-establishing neural pathways requires plasticity from both the intact host brain and the transplanted neurones, and the factors favoring optimal functional recovery are not known. However, it is becoming apparent that circuit reconstruction on its own may not be sufficient for functional recovery, but that appropriate patterns of behavior must be re-learnt through training (Brasted et al., 1999a; Coffey et al., 1989; Dobrossy and Dunnett, 2003). The training allows the transplant to provide the transduction of sensory experience, the interpretation of which is dependent upon conditioning, or to become the neural substrate for the conditioning itself (Dobrossy and Dunnett, 2001). Promoting intrinsic and graft-mediated recovery through the design of appropriate rehabilitation programs, combined with neurobiological and surgical strategies for repair, are likely to have a significant positive impact on cell therapies in the clinical treatment of neurodegenerative diseases and brain trauma (Polgar et al., 1997).

The present study explored the effects of differential training protocols on functional restoration and graft development in a rodent model of Huntington's disease. Animals

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Abbreviations: A, anterior of bregma; DARPP-32, dopamine adenosine 3′:5′-monophosphate regulated phospho-protein; L, lateral to the midline; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; PBS, phosphate-buffered saline; TH, tyrosine hydroxylase; V, vertical below dura; WGE, whole ganglionic eminence.

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Table 1. Experimental groups

Side of Pre-Training	Groups		*n*	Lesion*	Graft*	Pre-Training
Ipsilateral paw	Controls	C-PT I	7	—	—	✓
	Lesions	L-PT I	8	✓	—	✓
	Grafts	G-PT I	12	✓	✓	✓
Contralateral paw	Control	C-PT C	8	—	—	✓
	Lesions	L-PT C	8	✓	—	✓
	Grafts	G-PT C	12	✓	✓	✓

* Variation in these procedures defines the different groups.

were pre-trained exclusively either on the left or the right paw to perform on the skilled reaching task, the "staircase" test (Montoya et al., 1991), followed by unilateral striatal lesions and striatal grafts on the left side of the brain. Similar lesions and grafts have previously been shown to disrupt and partially restore, respectively, reaching performance with the contralateral paw (Fricker et al., 1997; Watts et al., 2000). Subsequent testing on the staircase test probed separately unilateral performance of the affected contralateral paw, as well as bilateral performance. We report that the graft and the training conditions exerted significant effects on the rate and the level of behavioral recovery. Furthermore, the training the animals received prior to transplantation was a factor in their graft development.

EXPERIMENTAL PROCEDURES

The experimental groups are summarized in Table 1 and the experimental design and sequence of tests in Table 2.

Subjects

Male Sprague–Dawley rats ($n=55$; Charles River, Kent, UK) weighing 250 g at the start of the experiment were used in the study. All animals were housed four rats per cage in a temperature (21 °C) and humidity (50%) controlled room with a 12-h light/dark cycle (lights on at 8:00 a.m.). The cages had sawdust-covered floors, a cardboard tunnel for basic enrichment, and were cleaned once a week. Animals were food-deprived and kept at 90% of their normal body weights throughout the training and testing period. Exceptions were made during the periods of testing drug-induced rotation, and a week before and after surgery. Graft tissues were collected from the embryos of pregnant rats of the same Sprague–Dawley strain. All animals in this study were treated in accordance with standards set out by the Animals (Scientific Procedures) Act 1986, and were used with approval from the UK Home Office. The number of animals used and their suffering was kept to a minimum.

Lesion

The animals were anesthetized by inhalation anesthetic (isoflurane; Abbott, UK; using O_2 and N_2O as carrier gas) and placed in a stereotaxic frame (Kopf Instruments). Unilateral dorsal striatal lesions were made by injecting 4×0.2 μl of 0.12 M quinolinic acid (Cambridge Research Biochemicals) dissolved in 0.1 M phosphate-buffered saline (PBS), pH=7.4, at two depths in each of two tracks in the right neostriatum. Each injection was infused over 1 min 40 s via a 30 gauge stainless steel cannula connected to a microdrive pump, with 2 min allowed for diffusion. Injection coordinates with measurements in mm anterior (A) in front of bregma, lateral (L) to the midline, vertical (V) below dura, and the nose bar set 2.3 mm below the interaural line were: A=−0.4; L=−3.7; V=5.2 and 4.2; and A=1.2; L=−2.9; V=5.2 and 4.2. Prior to regaining consciousness, animals received 5 ml glucose saline s.c. in the neck and 0.15 ml diazepam (i.m.; 5 mg/ml) in the hind leg. Dissolved paracetemol (1 g/l) was left in the animals' drinking water for 48 h after surgery.

Graft

Graft tissue was dissected from the whole ganglionic eminence (WGE) of E15 rat embryos (crown rump length=12 mm), incubated in trypsin, washed and prepared as dissociated cell suspensions as previously described (Fricker et al., 1996). Cell viability was assessed by Trypan Blue exclusion in a hemocytometer, yielding cell counts of 150,000 cells/μl and 97% viability. Host animals were again anesthetized using isoflurane and positioned in the stereotaxic frames. The cell suspension was injected via a 10 μl glass microsyringe with wide bore needle (SGE, Ringwood, Australia) targeted at A=0.4; L=3.3; V=5.0 and 4.4 mm. A volume of 1 μl was injected over 2 min at each of the two depths (approx. 300,000 cells/graft), followed by waiting 3 min for diffusion before retraction of the cannula, suturing the wound, and the same post-operative treatment as employed after the lesions.

Histology

Following completion of behavioral testing, the rats were anesthetized with a 2 ml lethal dose of Euthatal (pentobarbital sodium;

Table 2. Experimental design and sequence of tests

Weeks	Experimental treatments	Experimental tests
1–3		Paw reaching (test 1): Pre-Training on Ipsilateral or Contralateral paw
4	Lesions*	—
6	Graft*	—
12		Paw reaching (test 2): Only Contralateral paw
22		Paw reaching (test 3): both paws
25		Stepping test
26		Cylinder test
30	Perfusion	—

* These procedures differ between groups, as defined in Table 1.