

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Long-term survival and development of fetal ventral spinal grafts into the motoneuron-depleted rat spinal cord: Role of donor age**Rosario Gulino^{a,*}, Laura Litrico^a, Giampiero Leanza^b^aDepartment of Physiological Sciences, University of Catania, Viale Andrea Doria 6, I95125 Catania, Italy^bB.R.A.I.N. Centre for Neuroscience, Department of Life Sciences, University of Trieste, Via Weiss 8, I34127 Trieste, Italy

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ABSTRACT

Fetal spinal cord (SC) tissue grafts can survive and develop into the lesioned SC, but no conclusive data are available concerning the long-term fate of transplanted material and the relation between the graft fate and the donor embryo age. Here, pre-labelled suspensions of ventral SC from E12 or E17 rat fetuses were grafted to the lumbar SC of adult rats with motoneuron depletion induced by perinatal injection of volkensin. E12 and E17 are presumably the stages when motoneuron development starts and terminates, respectively. Four or 10 months post-grafting, SCs were analyzed to check the graft survival rate and to follow the differentiation and spatial distributions of grafted cells. Neurotoxic lesion produced a 61% motoneuronal loss in the lumbar SC. In transplanted animals, all E12 fetal grafts survived until the observed time-points and developed various mature cell phenotypes. Many motoneuron-like labelled cells were found within the graft area or adjacent to it. Conversely, none of the E17 fetal grafts survived, since no graft-derived elements with neuronal morphology were found either in the site of graft placement or adjacent to it. The present findings indicate that spinal neuroblasts can survive for a long time and develop within the motoneuron-depleted SC, and that the donor embryo age is crucial for successful engraftment.

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1. Introduction

Neural transplantation represents a promising therapeutic strategy for brain repair as well as for repairing the damaged spinal cord (SC) (Christie and Mendez, 2001; Reier, 2004). Fetal spinal tissue grafts have been successfully used to replace lost populations of cells within the SC, such as motoneurons of the anterior horn (Sieradzan and Vrbová, 1989; Nográdi and Vrbová, 1994, 1996; Gulino et al., 2007), which can be depleted as a result of injury or neurodegenerative diseases (Martin

et al., 1999, 2000). For instance, we have previously shown that motoneuron-enriched fetal grafts could survive within the SC and produce functioning motoneurons (Gulino et al., 2007).

In order to achieve the best results in terms of graft survival, integration and functional recovery, the donor age should be appropriately defined. In several experiments of fetal tissue grafted to the adult mammalian brain, the donor age appeared inversely linked to the graft volume, graft survival rate, and affected also several aspects of anatomical and functional integration of the graft. In particular, the role of

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donor age has been proven to affect the outcome of cholinergic grafting to the rodent brain with septohippocampal damage (Cassel et al., 1991), as well as for the integration of striatal (Fricker et al., 1997; Watts et al., 2000) or dopamine (Brundin et al., 1988; Freeman et al., 1995; Annett et al., 1997) neuron suspensions grafted to the lesioned striatum. The effect of donor age on the fate of neocortical grafts have also been studied (Clinton and Ebner, 1988; Grabowski et al., 1994).

Little information is available about how survival and integration of motoneuron-enriched fetal spinal grafts are linked to the donor developmental stage. It is known that spinal motoneurons are generated at E11–E12 and that their maturation is complete at about E17 (Barber et al., 1991; Phelps et al., 1988), which is a critical time-point for motoneuron development (Harris, 1988; Sheard et al., 1984). Therefore, to test the possible difference of *in vivo* engraftment of donor cells obtained from these two markedly different developmental stages, we grafted motoneuron-enriched suspensions of fetal SC tissue obtained from either E12 or E17 rat embryos, to the SC of adult rats previously subjected to motoneuronal depletion by neurotoxic lesion at birth. Then, we analyzed the long-term survival and development of the graft within the host SC. We have found that the donor embryo age is crucial for graft survival within the adult lesioned rat SC. In particular,

E12 grafts were able to survive for at least 10 months and give rise to mature cells including motoneurons, whereas E17 grafts were unable to engraft into the host.

2. Results

All pups survived the effects of the lesion, gained weight normally and showed no episodes of autotomy of the treated hindlimb. All lesioned rats, included those subsequently grafted, showed ipsilateral hindlimb atrophy starting the day after lesion and during the entire survival period. The sham-lesioned rats did not differ at any stage of the study from the intact rats in any of the parameters analyzed. These two groups were therefore combined into a single Normal group.

At about 5 months post-lesion, bilateral injections of FG into the medial gastrocnemius muscle of both Normal and Lesioned animals resulted, 1 week later, in the retrograde labelling of numerous motoneurons in the ventral portions of lumbar (L3–L5) SC, corresponding to lamina IX (Figs. 1 and 2). In Normal animals, the estimated number of FG-labelled profiles was similar on both sides (paired *t*-test, $P=0.756$, Fig. 2), and averaged 128 ± 24 and 142 ± 36 , respectively ($n=4$),

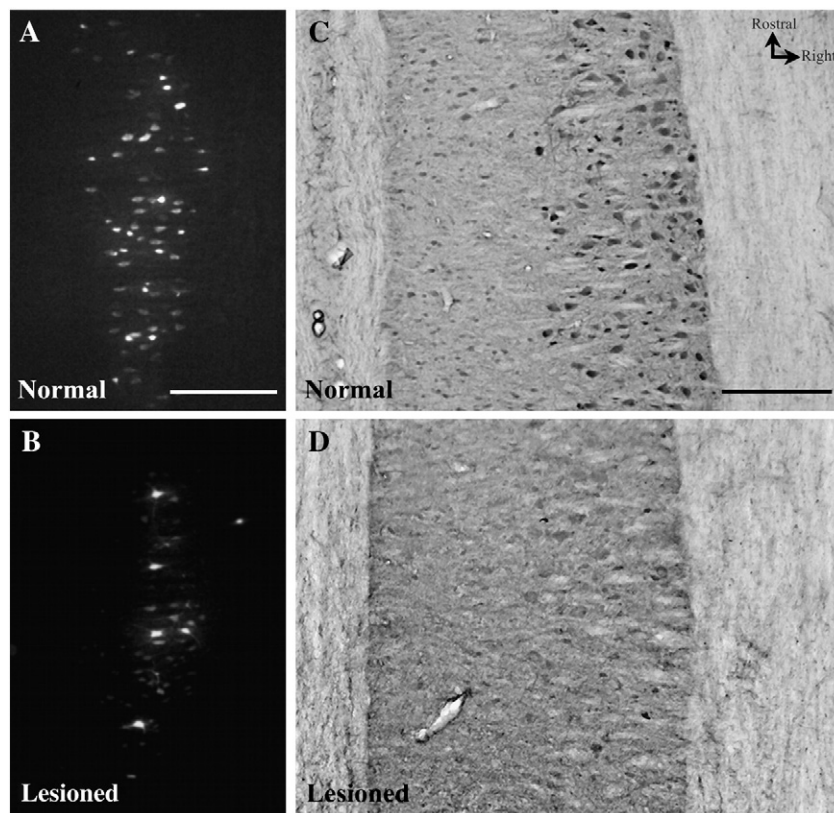


Fig. 1 – Effects of the lesion. Motoneuronal loss induced by perinatal injection of volkensin (2 ng) into the right medial gastrocnemius muscle. (A, B): Fluorescent micrographs of horizontal sections through the ventral portion (corresponding to lamina IX) of the lumbar (L3–L5) spinal cord showing the distribution of retrogradely labelled neurons 1–2 weeks following bilateral injections of the fluorescent tracer Fluoro-Gold (FG) into the medial gastrocnemius muscle. A marked depletion of FG-labelled motoneurons is evident in lesioned animals (B), relative to the normal SC (A). **(C, D):** Photomicrographs of ChAT-stained horizontal sections showing the distribution of lamina IX motoneurons in normal (C) or lesioned animals (D). Note the evident loss of ChAT-positive motoneurons in the right side, ipsilateral to the volkensin injection. Scale bars, 500 μ m.

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