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Brief Communication

Neuronal progenitor transplantation and respiratory outcomes following upper cervical spinal cord injury in adult rats

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ABSTRACT

Despite extensive gray matter loss following spinal cord injury (SCI), little attention has been given to neuronal replacement strategies and their effects on specific functional circuits in the injured spinal cord. In the present study, we assessed breathing behavior and phrenic nerve electrophysiological activity following transplantation of microdissected dorsal or ventral pieces of rat fetal spinal cord tissue (FSC_D or FSC_V, respectively) into acute, cervical (C2) spinal hemisections. Transneuronal tracing demonstrated connectivity between donor neurons from both sources and the host phrenic circuitry. Phrenic nerve recordings revealed differential effects of dorsally vs. ventrally derived neural progenitors on ipsilateral phrenic nerve recovery and activity. These initial results suggest that local gray matter repair can influence motoneuron function in targeted circuits following spinal cord injury and that outcomes will be dependent on the properties and phenotypic fates of the donor cells employed.

Introduction

Various cellular therapies are considered to have significant potential for promoting functional repair of the injured spinal cord (Eftekharpour et al., 2008; Reier, 2004). This is especially reflected by interest in transplantation of myelin-competent cells for remyelination of spared axons (Cao et al., 2010; Karimi-Abdolrezaee et al., 2010; Kocsis et al., 2004; Sasaki et al., 2007; Sharp et al., 2010). Less frequently considered, however, is the fact that focal neuronal loss after trauma or ischemic insult can also have functional consequences even when gray matter damage does not involve the spinal enlargements (Vierck et al., 2000; Yezierski et al., 1998) or when motoneuron pools are spared (Cizkova et al., 2007; Hadi et al., 2000; Magnuson et al., 2005).

Several laboratories have reported post-SCI functional improvements associated with intraspinal grafts containing neuronal progenitors either alone or in combination with other cells or interventions (Bonner et al., 2010; Hooshmand et al., 2009; Kim et al., 2006; Mitsui et al., 2005; Nikulina et al., 2004). However, little attention has been given to demonstrations of graft integration with functionally and neuroanatomically defined spinal circuits and whether distinct donor neuronal phenotypes differentially affect recovery (Goldman and Windrem, 2006).

In the present study, neural tissue grafts, which were predominantly comprised of interneuronal precursors, were introduced into an acute, cervical (C2) spinal hemisection (Hx) model of respiratory compromise (Fuller et al., 2008; Goshgarian, 2003; Lane et al., 2008a). Recent pharmacological and neuroanatomical studies have led to the suggestion that interneurons in dorsal versus intermediate or ventral gray matter at the level of the phrenic nucleus may play contrasting modulatory roles in spontaneous recovery of ipsilateral phrenic motoneuron (PhMN) function following C2Hx injuries (Lane et al., 2009; Zimmer and Goshgarian, 2007). Therefore, we investigated whether grafts of dorsal or ventral regions of rat fetal spinal cord (FSC) tissue would differentially influence respiratory recovery post-C2Hx and whether connectivity between donor neurons and the host phrenic circuit could be demonstrated.

Methods

All surgical and animal care procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida. Adult, female Sprague–Dawley, (205–265 g) rats were deeply anesthetized with xylazine (3 mg/kg; Phoenix Pharmaceutical, Inc., St. Joseph, MO) and ketamine (90 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). After laminectomy, a dural incision was made and hemisection cavity created at the C2 level (Jakeman and Reier, 1991). Post-hoc histological analysis confirmed the extent of hemisection and animals with spared ipsilateral tissue were excluded from the study (Fuller et al., 2009).

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Pregnant Sprague–Dawley rats were anesthetized with xylazine and ketamine at 14 days of gestation (E13.5–E14). Embryos were removed and meninges/dorsal root ganglion-free spinal cords were isolated in Hank's balanced salt solution (Gibco) (Jakeman and Reier, 1991). Dorsal and ventral strips of solid FSC tissue were then microdissected from the extreme alar and basal plate regions, respectively, as shown in Fig. 1A. Multiple FSC strips of dorsal (FSC_D; dorsal (alar plate) grafts, $n = 17$) or ventral (FSC_V; ventral