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Intraspinal transplantation and modulation of donor neuron electrophysiological activity

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ABSTRACT

Rat fetal spinal cord (FSC) tissue, naturally enriched with interneuronal progenitors, was introduced into high cervical, hemi-resection (Hx) lesions. Electrophysiological analyses were conducted to determine if such grafts exhibit physiologically-patterned neuronal activity and if stimuli which increase respiratory motor output also alter donor neuron bursting. Three months following transplantation, the bursting activity of FSC neurons and the contralateral phrenic nerve were recorded in anesthetized rats during a normoxic baseline period and brief respiratory challenges. Spontaneous neuronal activity was detected in 80% of the FSC transplants, and autocorrelation of action potential spikes revealed distinct correlogram peaks in 87% of neurons. At baseline, the average discharge frequency of graft neurons was 13.0 ± 1.7 Hz, and discharge frequency increased during a hypoxic respiratory challenge ($p < 0.001$). Parallel studies in unanesthetized rats showed that FSC tissue recipients had larger inspiratory tidal volumes during brief hypoxic exposures ($p < 0.05$ vs. C2Hx rats). Anatomical connectivity was explored in additional graft recipients by injecting a transsynaptic retrograde viral tracer (pseudorabies virus, PRV) directly into matured transplants. Neuronal labeling occurred throughout graft tissues and also in the host spinal cord and brainstem nuclei, including those associated with respiratory control. These results underscore the neuroplastic potential of host–graft interactions and training approaches to enhance functional integration within targeted spinal circuitry.

Introduction

A longstanding focus of spinal cord repair has centered on cell-based approaches to promote axonal growth and restored neural communication across sites of injury (Reier, 2004). However, neuroanatomical and electrophysiological findings have indicated that intraspinal transplantation of neuronal progenitors also can promote functional changes (Mitsui et al., 2005) such as by serving as novel neuronal relays (Jakeman and Reier, 1991; Reier, 1985; Reier et al., 1986; White et al., 2010). Neuronal progenitor-enriched tissue repair strategies also have the potential to reconstruct disrupted spinal circuitries (i.e., "gray matter repair") (Bonner et al., 2010; Ketschek et al., 2012; Reier et al., 2002) either alone or in combination with other treatments (Hooshmand et al., 2009; Kim et al., 2006; Nikulina et al., 2004).

The efficacy of a neuronal progenitor-based therapeutic approach is dependent on many variables (Bonner et al., 2011; Reier et al., 2002). Whether specific neuronal precursors will be required or if donor neuronal activity can be entrained within a defined spinal circuit remains unclear. Being able to effectively entrain donor neuron activity and possibly evoke activity-dependent plasticity within donor cells could be advantageous for achieving optimal graft-mediated recoveries.

While there are many examples of activity induced plasticity within naïve or injured spinal circuitries (Baker-Herman et al., 2004; Fuller et al., 2001; Golder and Mitchell, 2005; Trumbower et al., 2012), to date there has been no attempt to determine if studies of activity-dependent plasticity would be feasible in neuronally-enriched intraspinal grafts. In the present study, transplants of FSC tissue were made into lesions immediately rostral to the phrenic motor nucleus (i.e., at C2). Since many cells in that circuit fire rhythmically in phase with the respiratory cycle (Lee and Fuller, 2011), we thus assessed whether matured, neuronally-enriched grafts acquired site-specific, physiologically-patterned bursting activity. To determine if studies of activity-dependent plasticity would be feasible in this model, the response of donor neurons to elevated CO_2 (i.e., hypercapnia) or reductions in O_2 (i.e., hypoxia) were evaluated. Both stimuli are known to

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increase respiratory-related output of the host phrenic motor system (Fuller et al., 2003; Lee et al., 2009; Vinit et al., 2009).

Materials and methods

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida. Adult (N = 27) Sprague–Dawley rats purchased from Harlan Inc. (Indianapolis, IN, USA) were used to investigate the bursting patterns of neurons in mature FSC grafts under baseline and respiratory challenge conditions, and also the impact of the FSC grafts on the pattern of breathing in unanesthetized rats. The experimental animals were divided into three groups: control, uninjured (N = 7), C2 hemisection (Hx) (N = 9), or C2Hx followed by immediate transplantation of embryonic day 14/15 FSC tissue (C2Hx + FSC, N = 11). An additional group of C2Hx + FSC rats was used exclusively for anatomical tracing studies (N = 6).

Spinal cord injury and FSC transplantation

Rats were injured at age 3 months as previously described (Lane et al., 2008; Lee et al., 2010). Briefly, the animals were anesthetized by injection of xylazine (10 mg/kg, s.c.) and ketamine (140 mg/kg, i.p., Fort Dodge Animal Health, IA, USA). A dorsal cervical incision was made from the base of the skull to the C3 segment followed by C2 laminectomy and left C2 dura section. A left C2Hx was performed by microscalpel incision and aspiration. In a subset of rats FSC was transplanted into the lesion cavity as previously described by our group (Jakeman and Reier, 1991; White et al., 2010). In brief, pregnant Sprague–Dawley rats at 14 days of gestation (E13.5–E14) were anesthetized by xylazine (10 mg/kg, s.c.) and ketamine (140 mg/kg, i.p.). Following a laparotomy, embryos were removed from the placenta and placed in Hank's balanced salt solution (Mediatech, Inc., Manassas, VA, USA). FSC tissue was then removed and cut into ~1–2 mm tissue blocks which were introduced into the lesion cavity until filling was achieved. The dura and overlying muscles were sutured with 9–0 (Ethicon, NJ, USA) and 4–0 (Webster Veterinary, MA, USA) polyglycolic acid sutures, respectively. The skin was closed with stainless steel wound clips (Stoelting, IL, USA), and animals received an injection of yohimbine (1.2 mg/kg, s.c., Lloyd, IA, USA) to reverse the effect of xylazine. Following surgery, animals were given an analgesic (buprenorphine, 0.03 mg/kg, s.c., Hospira, Inc., IL, USA) and sterile lactated Ringers solution (5 ml s.c.). The post-surgical care protocol included injection of lactated Ringers solution (5 ml/day, s.c.) and oral supply of Nutrical supplements (1–3 ml, Webster Veterinary, MA, USA), given until adequate volitional drinking and eating resumed.

Plethysmography

Tidal volume, minute ventilation and breathing frequency were quantified in unanesthetized animals at both 5 and 10 weeks post-injury using whole body plethysmography (Buxco Inc., Wilmington, NC, USA). The plethysmograph system was calibrated using standard procedures as previously described (Fuller et al., 2008). Animals were placed in Plexiglas chambers for a 60–90 min baseline period while the chamber was flushed with a normoxic gas mixture (21% O_2, balance N_2). The animals were then exposed to a 5 min hypoxic challenge during which the chamber was flushed with 10% O_2 (balance N_2).

Neurophysiology

Between 11 and 13 weeks post-injury, rats were anesthetized with urethane (1.6 g/kg, i.p.) and prepared for terminal neurophysiological recordings, as described previously (Lee and Fuller, 2010; Lee et al., 2009, 2010; Sandhu et al., 2010). Intubation was via an endotracheal tube below the larynx. Cannulae were inserted into the femoral artery and vein for blood pressure measurement and drug administration, respectively. The rectal temperature was monitored by an electrical thermometer and maintained at 37.5 ± 1 °C by a servo-controlled heating pad (model TC-1000, CWE Inc., Ardmore, PA, USA). The animal was then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) to stabilize the spinal cord, paralyzed with pancuronium bromide (2.5 mg/kg, i.v., Hospira, Inc., Lake Forest, IL, USA) and mechanically ventilated (Model 683; Harvard Apparatus, Inc., South Natick, MA, USA) with an oxygen/nitrogen mixture throughout the experimental procedure (50–60% O_2, balance N_2, volume = 7 ml/kg; frequency = 60–65/min). The partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) was analyzed with a Capnogard neonatal CO_2 monitor placed on the expiratory line of the ventilator circuit (Novametrix Medical Systems, Wallingford, CT, USA) and maintained at 45–50 mm Hg by adjusting inspired CO_2. This value was selected to ensure a robust inspiratory signal in the phrenic nerve recording.

The right phrenic nerve (i.e. contralateral to the C2Hx lesion) was exposed using a dorsal approach. The contralateral nerve was selected for recording since it provides a very robust host respiratory pattern after C2Hx (Doperalski and Fuller, 2006; Lee et al., 2010). The isolated nerve was sectioned distally and placed over monopolar hook silver electrodes. Recordings were amplified (1000×, Model 1700, A-M Systems, Carlsborg, WA, USA), band-pass filtered (0.3–10 kHz) and integrated (time constant of 100 ms; model MA-1000; CWE Inc., Ardmore, PA, USA). The raw and integrated neural signals were digitized by the CED Power 1401 data acquisition interface and recorded on a PC using Spike2 software (Cambridge Electronic Design Limited, Cambridge, England).

For FSC graft recordings, the C1–C3 vertebrate bone and dura were removed to re-expose the transplant site. Single (0.4–0.8 MΩ, carbostar-3, Kation Scientific, MN, USA) (N = 9) or array (20 kΩ, Tucker Davis Technologies, FL, USA) (N = 2) electrode with sixteen 50 μm tungsten electrodes with 250 × 500 μm column spacing was mounted on the microelectrode holder (David Kopf Instruments, Tujunga, CA, USA) and inserted into the graft. Neural signals from the single electrode were amplified (10,000×, ExAmp Extracellular amplifier, Kation Scientific), digitized by the CED Power 1401 and recorded on a PC as well as phrenic nerve signals. Neural signals recorded from multi-electrode array were amplified (5000×), band-pass filtered (0.5–6 kHz), and recorded with hardware (sampled at 24,414.06 Hz) and software from Tucker Davis Technologies (Alachua, FL, USA). After stable recording of graft neurons during the baseline condition (50–60% O_2, balance N_2; $P_{ET}CO_2$ = 45–50 mm Hg), animals were exposed to 3 min period of hypoxic (13–15% O_2, balance N_2) and/or hypercapnic ($P_{ET}CO_2$ = 70–80 mm Hg) gas.

Histological evaluation

At the end of experiments, each animal was intracardially perfused with paraformaldehyde [4% w/v in 0.1 M phosphate buffered saline (PBS), pH 7.4]. The spinal cord was then removed from the vertebral column and stored in paraformaldehyde (2% w/v in PBS) for histological processing. Cervical spinal cord tissue was processed for vibratome (40 μm) sectioning (described in detail in Lane et al., 2008). Serotonin immunohistochemistry was used to examine the distribution of serotonergic fibers. Longitudinal sections of cervical spinal cord (40 μm) were incubated overnight with primary antibody solution (anti-serotonin, 1:20,000), 0.75% triton X-100 and 1% normal goat serum. Sections were then incubated with a secondary antibody (goat-anti-rabbit, 1:100) and then reacted with DAB prior to mounting on glass slides and counterstained with cresyl violet.

To obtain an index of host–graft connectivity, a subset of animals (N = 6) received intratransplant injections of the transneuronal tracer pseudorabies virus (PRV152, titer of ~2.0×10^8). Once surgical plane of