Developmental restriction alters the composition of neural progenitor grafts after spinal cord injury

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Background

- Neural progenitor cell (NPC) transplantation is a promising therapeutic strategy following spinal cord injury (SCI).
- NPCs transplanted into the injured spinal cord undergo specification through normal developmental processes, and give rise to diverse neuronal subtypes (Fig. 1A) & glia.
- Studies suggest that the graft cell identity may significantly affect circuit-appropriate integration and recovery of function, highlighting the importance of graft composition.
- Spinal cord neurogenesis occurs over multiple days with different progenitor classes exhibiting different temporal patterns (Fig.1B).
- In this study, we assessed the effects of developmental restriction on NPC graft composition.

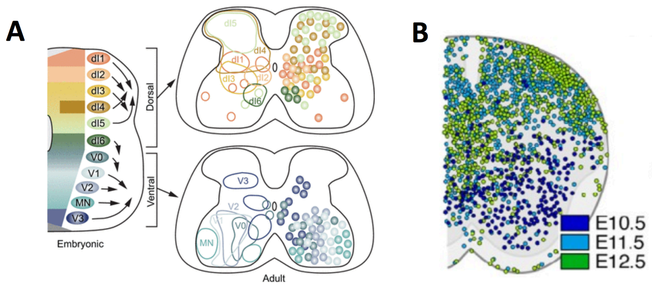

Figure 1. (**a**) Morphogen gradients in embryonic neural tube lead to development of distinct neuronal domains in the adult spinal cord; image adapted from Lai, *et al.*, 2016 [1]. (**b**) Distribution of BrdU+ cells in embryonic spinal cord after one pulse of BrdU. Image shows differing areas of proliferating cells at various embryonic time points; image adapted from Petracca, *et al.*, 2016 [2].

Experimental Approach

- Donor neural progenitor cells (NPCs) were isolated from GFP+ mouse embryonic spinal cords at time points E11.5, E12.5, and E13.5.
- NPCs were transplanted into wild type host mice following a C4 dorsal column lesion (1 million cells per subject).
- Four weeks post-transplantation, tissue was collected and sectioned at 20µm for immunohistochemical analysis.
- Graft composition was characterized using cell markers including NeuN, Sox9, Lbx1, Tlx3, Calbindin, and ChAT.
- Quantification was performed with ImageJ and an automated cell counting plugin created in our lab (Chen et al., *in prep*).

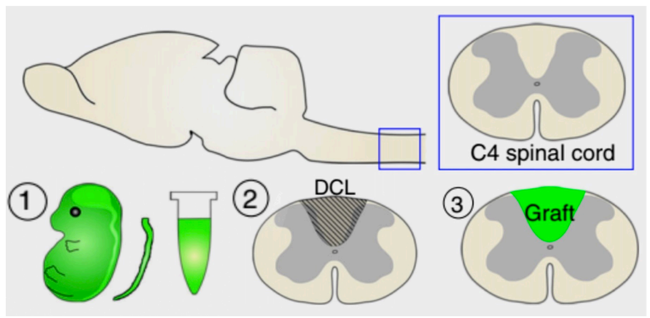

Figure 2. NPC transplantation into the lesion site in a mouse model of spinal cord injury. Image adapted from Dulin *et al.*, 2018 [2].

Effects on Graft Size

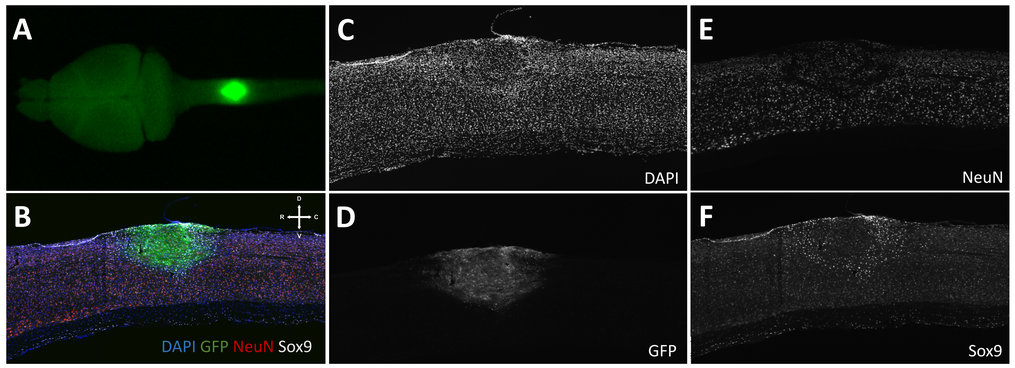

Figure 3. (**a**) Image of mouse CNS with GFP+ graft four-weeks post transplantation at 4X magnification. (**b**) Composite image of 20 µm sagittal spinal cord section taken at 10X magnification. (**c - f**) DAPI, GFP, NeuN, and Sox9 are shown in their respective channels. (**g**) Quantification of graft volume (mm^3) from NPCs harvested at embryonic dates E11.5, E12.5, and E13.5

Effects on Graft-derived Neurons

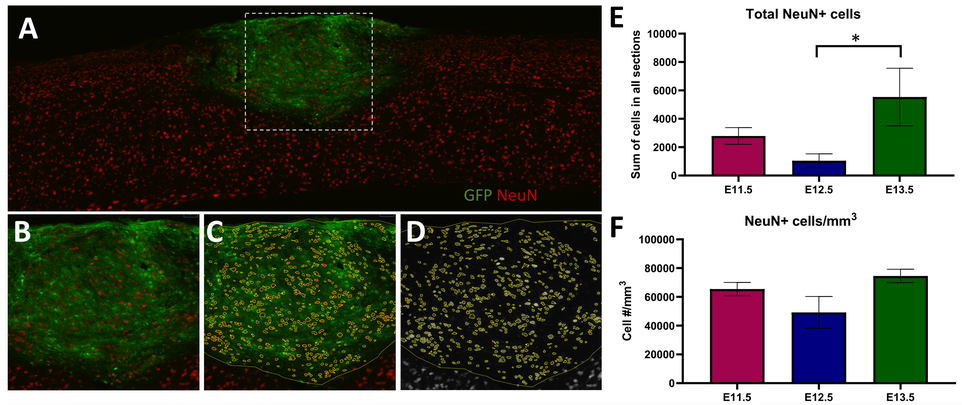

Figure 4. (**a**) Image of 20 µm sagittal section of the spinal cord showing GFP+ graft in green and NeuN in red. (**b**) Close up of area delineated by the square in A. (**c**) NeuN+ cells in the graft identified by our macro. (**d**) NeuN+ mask overlaid on the NeuN+ channel only. (**e**) Total NeuN+ cell counts in all sections. (**f**) NeuN+ cell density derived by dividing the total number of NeuN+ cells by the total graft volume.

Effects on Graft-derived Astrocytes

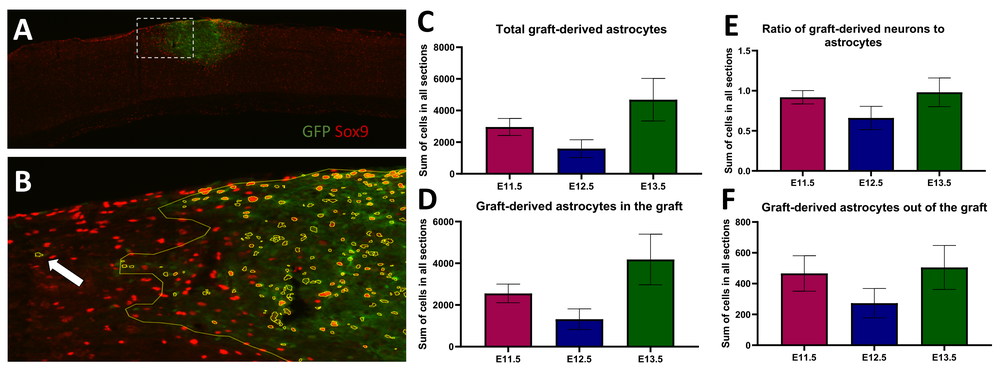

Figure 5. (**a**) Image of 20 µm sagittal section of the spinal cord showing GFP+ graft in green and Sox9 in red. (**b**) Graft-derived astrocytes identified by our macro, showing colocalization of GFP and Sox9. Arrow points to one of these cells present outside of the graft. (**c**) Quantification of total graft-derived astrocytes (**d**) Quantification of graft-derived astrocytes within the boundary of the graft. (**e**) Ratio of graft-derived neurons to astrocytes across embryonic time points. (**f**) Quantification of graft-derived astrocytes found outside of the graft and in the host tissue.

Phenotypic Characterization

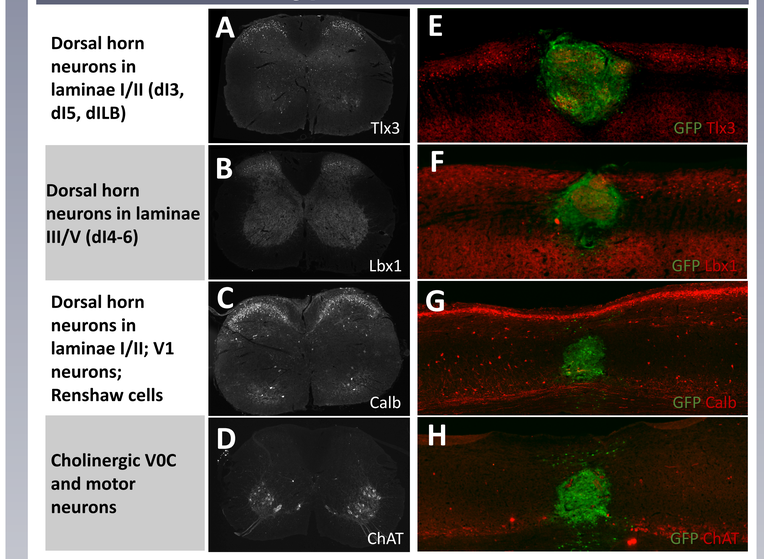

Figure 6. (a-d) Images show spinal expression of Tlx3, Lbx1, calbindin, and choline acetyltransferase in transverse sections at postnatal day 21 and (e-h) in sagittal sections at four weeks following NPC transplantation.

Discussion

- Our data suggests that the developmental stage during NPC harvesting may significantly affect graft composition.
- The E12.5 NPCs, considered the "golden standard" in cell transplantation studies, showed a tendency for smaller graft volume, and lower neuron and astrocyte numbers.
- We are currently characterizing the effects of developmental restriction on "phenotypic identity." Preliminary data suggests that earlier-stage grafts show a more ventral/motor profile, while later-stage grafts show a more dorsal/sensory profile.
- Follow-up experiments will be necessary to evaluate how different graft-derived neuron subtypes mediate graft/host integration and recovery of function.
- Importantly, these experiments could inform engineering of improved and optimized grafts for translation into the clinic.

References

1. Lai, Helen C., et al. "Making sense out of spinal cord somatosensory development." *Development* 143 (2016) 3434-3448.
2. Petracca, Yanina L., et al. "The late and dual origin of cerebrospinal fluid-contacting neurons in the mouse spinal cord." *Development* 143.5 (2016): 880-891.
3. Dulin, Jennifer N., et al. "Injured adult motor and sensory axons regenerate into appropriate organotypic domains of neural progenitor grafts." *Nature communications* 9.1 (2018): 1-13.

Acknowledgments

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