Effects of Developmental Restriction on Neural Progenitor Cell Differentiation *In Vitro*

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Background

- Neural progenitor cell (NPC) transplantation is a promising therapeutic strategy following spinal cord injury (SCI).
- Previous studies have shown that NPCs grafted into sites of SCI differentiate into mature neurons that integrate into host spinal cord circuitry, underscoring their ability to support recovery of function [1-3].
- There is still a limited understanding of how distinct subtypes of graft neurons can integrate into specific neural circuits. Further understanding of graft-derived cell phenotypes is necessary for graft optimization and successful clinical translation.
- In experimental models of SCI, most NPCs are obtained from E12.5 embryonic mouse spinal cord tissue. These cells mature and populate into a variety of neuronal and glial subtypes.
- Spinal cord neurogenesis normally occurs over E9.5-E13.5, with distinct neural progenitor classes exhibiting different birthdates (Fig. 1), suggesting that grafts of different embryonic "age" may significantly differ in their cellular composition upon maturation.
- We hypothesized that the developmental stage of NPC isolation is a critical factor determining cell fate in culture or following transplantation into sites of SCI. To address this, in the current study we investigated the effects of developmental restriction on NPC differentiation *in vitro*.

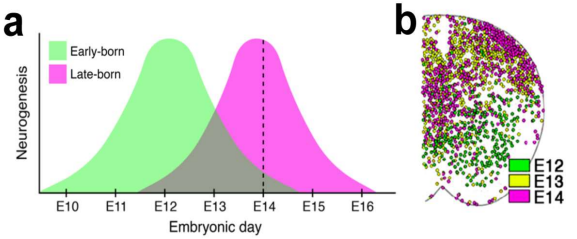


Figure 1. Neurogenesis in the rodent spinal cord. (**a**) Graph depicting the relative proportions of neurogenesis in early-born versus late-born spinal cord neuronal populations during embryonic development. (**b**) Cartoon showing the distribution of BrdU+ cells in the neonatal rat spinal cord after pulsing with BrdU at E12, E13, or E14; image adapted from Petracca *et al.*, 2016 [4].

Experimental Approach

- Neural progenitor cells were isolated from mouse embryonic spinal cords on days E11.5, E12.5, and E13.5 (Fig. 2).
- A total of 1 million cells per well (n=2) were plated for each timepoint and cultured for 7 days.
- Cultures were fixed and immunolabeled with DAPI and the following antibodies and Alexa Fluor secondaries:
  Goat Sox9 – 488; Guinea Pig NeuN – 555; Rabbit Olig2 – 647
- Cultures were imaged at 10X, with a total of 9 fields per well (3X3).
- Cell types were quantified using ImageJ software.

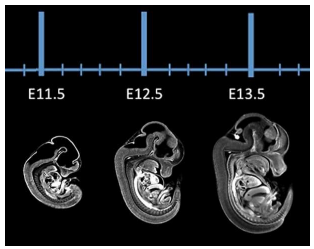
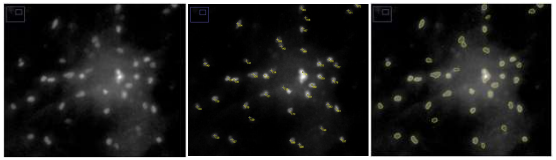
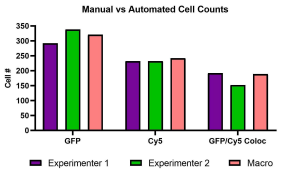


Figure 2. As mouse embryos age, the spinal cord develops quickly, and NPCs mature into motor and sensory neurons as time progresses. Timeline depicting the development and growth of mouse embryos from E11.5 to E14 ; image adapted from Wong *et al.*, 2015.

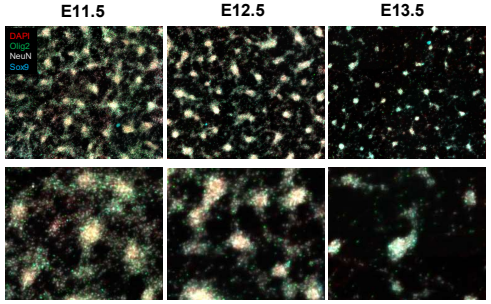
Custom Image Quantification Macro is Reliable

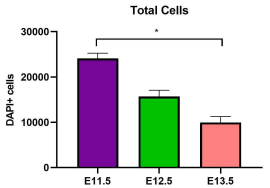




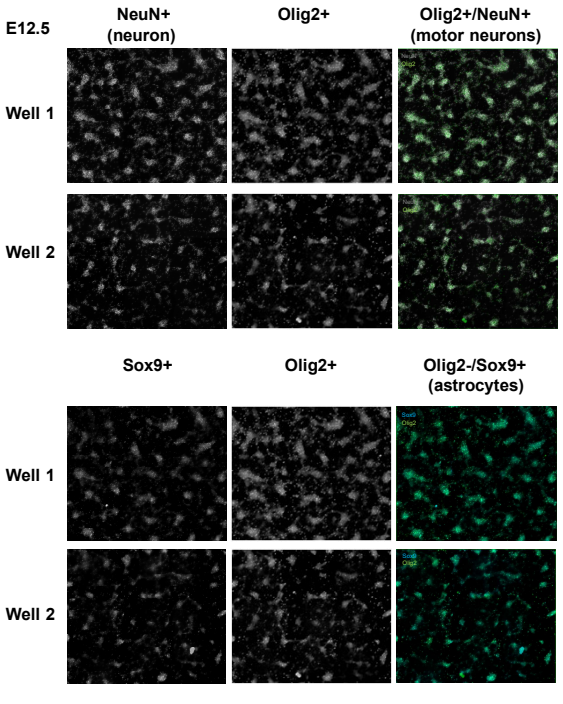
A comparison between the original image, the hand count, and the macro count, reveal that the macro was relatively accurate and identified most if not all of the cells in the image.

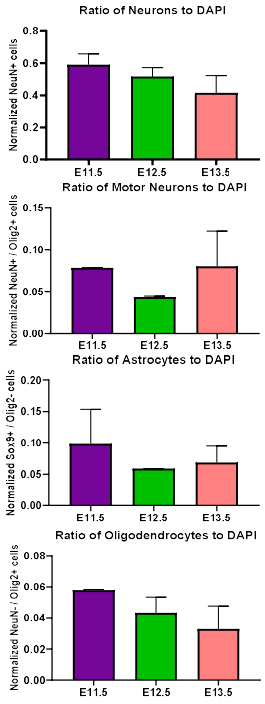
Earlier-stage NPCs Exhibit Greater Proliferative Capacity





Varying Trends seen in Neurons, Oligodendrocytes, and Astrocytes





Results

- Customized ImageJ macro generates reliable and precise results for cell type quantification
- Early-stage NPCs exhibit greater proliferation due to a higher proliferative capacity, decreasing over time
- Cell populations normalized to total cells present in each culture
- Proportion of neurons and oligodendrocytes decreased steadily from E11.5 to E13.5
- Astrocyte proliferation greatest in E11.5 and similar in E12.5 and E13.5
- Surprisingly, E12.5 cultures produced the lowest proportion of motor neurons; indicates that the developmental stage plays a significant role in NPC cell fate

Future Directions

- Our data suggests that the NPC developmental stage at the time of isolation plays a critical role in determining cell fate
- These findings have important implications in rodent transplantation studies, where the golden standard has long been E12 mouse NPCs
- However, E12 may not be optimum, and we are currently testing this in ongoing studies in vivo, grafting developmentally-restricted NPCs into sites of SCI (Fig 3), allowing us to:

1. Characterize the affect of developmental restriction on graft composition in vivo
2. Assess graft/host integration of specific graft-derived neuron subtypes
3. Evaluate if "phenotypically-appropriate" grafts improve behavioral outcomes following experimental SCI

- Altogether, the aim of this work is to establish important new guidelines for engineering new and effective cell sources for SCI clinical trials.

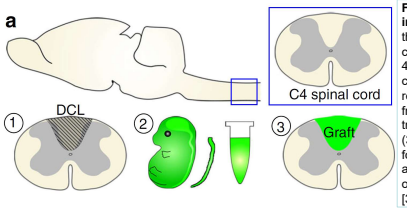


Figure 3. NPC transplantation in a mouse model of SCI. In this type of injury, the dorsal columns are axotomized at the 4th cervical segment of the spinal cord (1). Developmentally-restricted NPCs are isolated from GFP+ embryos (2) and transplanted into the lesion site (3). Grafts are allowed to mature for 4 weeks post-transplantation and assessed using IHC. Image obtained from Dulin *et al.*, 2018 [3].

References

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Acknowledgments

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