Examining adult-generated cells that surround focal cortical strokes

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Introduction

Stroke occurs due to a obstruction of blood flow to the brain. Remarkably after a stroke there is an increase in the generation of dividing cells in the brain in a region called the subventricular zone (SVZ) through surrounds the ventricle**.** The dividing cells have been found to migrate to surround the injured tissue and express proteins such as Nestin[1].

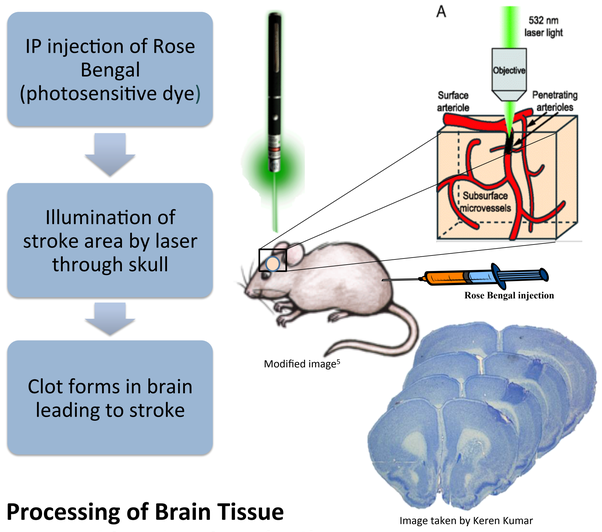
The fate of the cells that surround the stroke damaged tissue remains unknown. Studying the fate of the cells after stroke will inform us about how best to enhance regeneration and recovery post stroke. It has been hypothesized that the migrating cells are reactive astrocytes which is interesting because astrocytes have maintenance and restorative functions in the central nervous system, being especially active after brain injuries[2].

In order to determine if the cells that migrate to the site of stroke injury are astrocytes, this project measures the proportion of newly generated cells that express glial fibrillary acidic protein (GFAP), which is a marker of reactive astrocytes.

Method

Nestin GFP Reporter Mouse Model :
Adult Nestin-GFP transgenic mice[3] (n=6) were used for this study since in this reporter mouse the dividing adult-generated cells express nestin and therefore in this model express GFP.

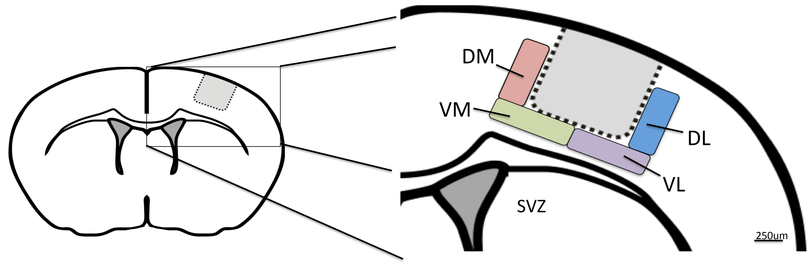
Stroke Model: Photothrombotic Ischemia Procedure:
The mice were given a left hemisphere cortical stroke using the photothrombosis stroke model[4].



Processing of Brain Tissue Immunohistochemistry (IHC):
Animals were euthanized and perfused at 7 days after stroke. The brains were cut on a microtome to produce 40um thick sections and the sections underwent immunohistochemistry to stain for GFP and GFAP. Pictures of the brains were taken with an inverted Zeiss AxioObserver.Z1 microscope at 40X and labeled cells were counted in defined areas around the stroke injury site.

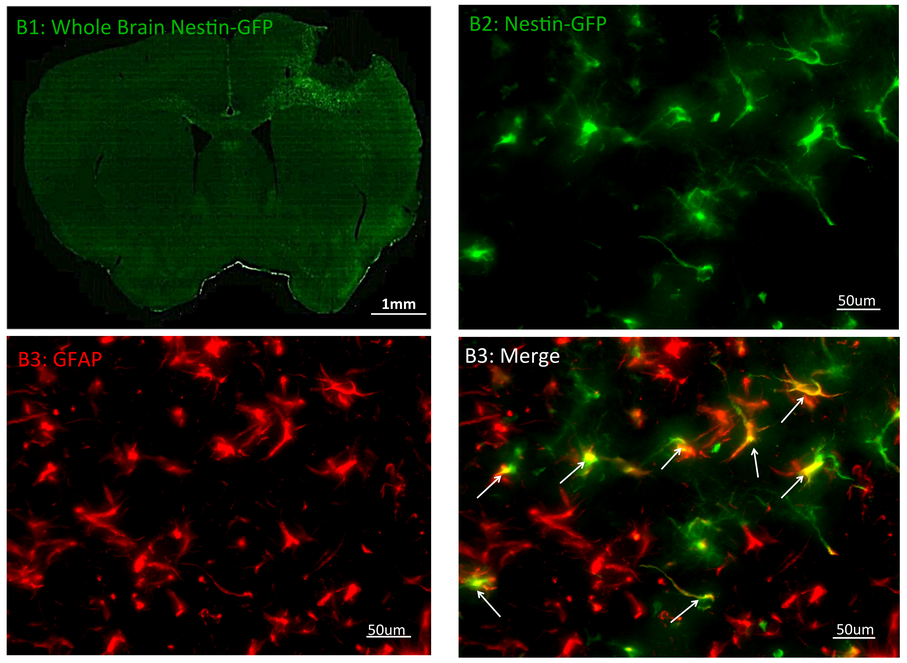
Results

Nestin-GFP cells in the peri-infarct area were analyzed in four areas:



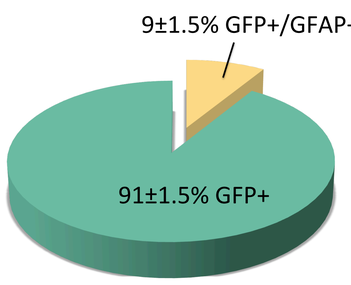
Stroke was induced in the motor and sensorimotor cortex. The area around the stroke was divided into four areas surrounding the infarct in grey. An area of 250um² on the most dorsal edge is excluded.

Representative images of a stroked brain and staining for Nestin-GFP expressing cells (GFP+) and GFAP-expressing astrocytes (GFAP+)

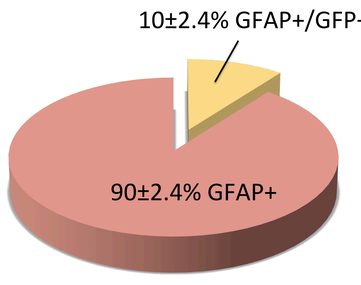


B1. Taken at 5X (image taken by Declan Lu). **B2.** Taken at 40X, Nestin-GFP labeled cells in green. **B3.** Taken at 40X, GFAP labeled cells in red. **B4.** Taken at 40X, merged image shown, arrows point to GFP and GFAP co-labeled cells.

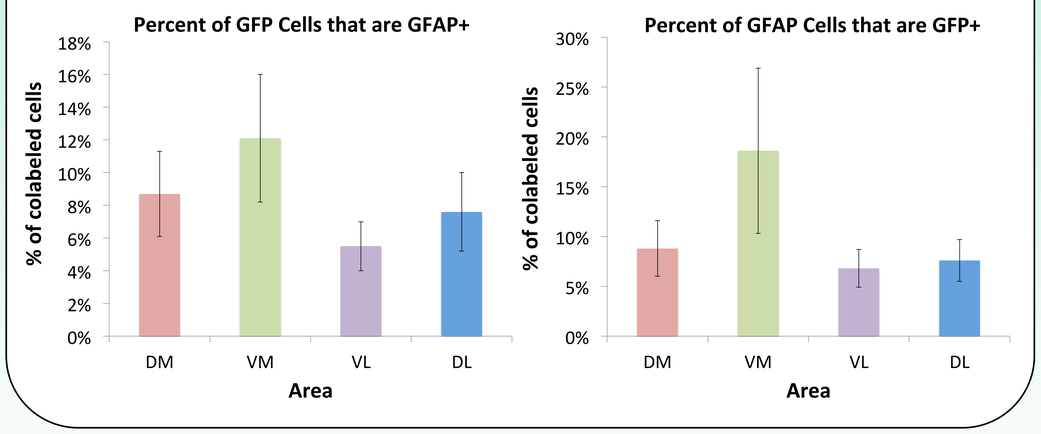
9% of GFP labeled cells express GFAP



10% of GFAP labeled cells express GFP



On average more dual labeled cells were present in the area (VM) that is closest to the SVZ



Discussion & Conclusions

- Surrounding the site of stroke injury, most of newly generated (GFP+ cells) are not reactive GFAP+ astrocytes. Similarly most of the GFAP+ astrocytes are not newly born cells that express Nestin-GFP.
- The ~10% of cells that are nestin-expressing adult generated cells and are reactive astrocytes could come from three possible origins: migration from the SVZ; migration from the cortex; or localized development.
- These results do not determine the origin of the cells but suggest they may migrate from the SVZ based on their location.

Future Studies

- Stroke sizes will be determined in order to determine if there is a relationship between size of the stroke and cell number or location.
- In order to apply our findings to recovery post storke, we will expand the time course for this study and determine whether the cell fate changes over time.
- Viral techniques and stereotaxic surgery will be used to specifically label the newborn SVZ cells. Labeling with a retrovirus at the time of stroke will allow tracking of the cells. If the virus labeled cells appear in the infarct area this would suggest that they migrated from the SVZ.

References

[1]Krogenberg *et al.* (2005) Nestin-expressing cells divide and adopt a complex electrophysiological phenotype after transient brain ischemia. *Journal of Cerebral Blood Flow & Metabolism.* 25:1613-1624.

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[3]Yamaguchi *et. al.* (2000) Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice. *Neuroreport.* 11(9):1991-1996.

[4]Watson *et. al.* (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol.* 17:497-504.

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