Analysis of Arc expression in photothrombotic models of ischemic stroke

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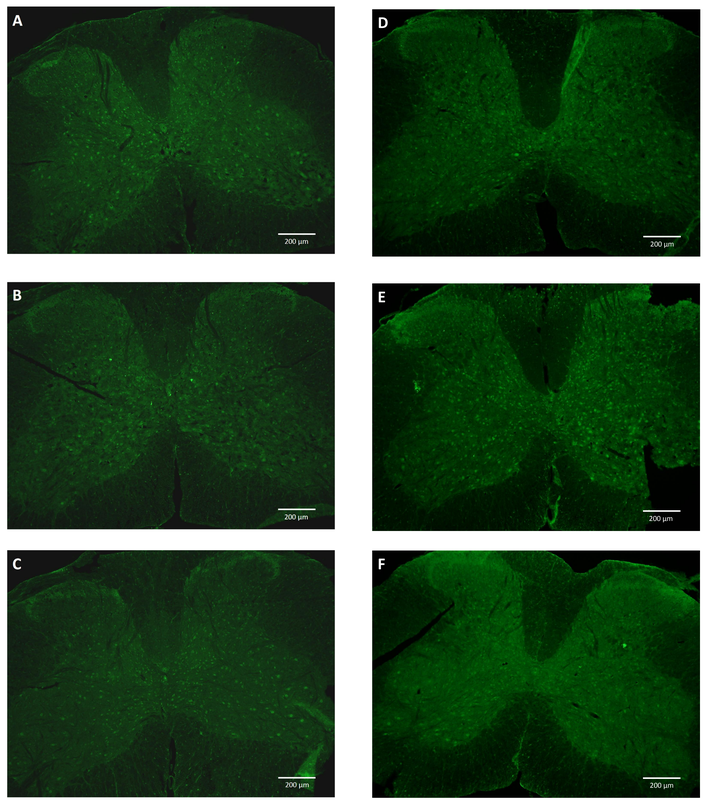
Introduction

Motor learning involves synaptic plasticity – the rewiring and formation of new neuronal connections in our brain. Rewiring of neural circuits, particularly those of the central nervous system, is necessary to accommodate for the development of novel forms of muscle coordination. This study aims to understand the role of the immediate early-gene (IEG) Arc (activity-regulated cytoskeleton-associated protein) which has been associated with synaptic plasticity and motor memory development [1].

As animals learn new motor skills, spinal neurons are hypothesized to upregulate their Arc expression. This aids the synaptic plasticity process that is required to form new neural circuits at the spinal cord level. Thereby, analysis of Arc expression following reacquisition of lost motor skills will allow us to examine which neurons of the spinal cord underwent synaptic plasticity.

Malfunctioning of synaptic plasticity can result in a wide range of neurodegenerative disorders [1]. Hence, our research aims to shed important insights on the neurobiological mechanisms that occur during the acquisition of new motor skills and thus deepen the knowledge of Arc's role in renewing neural circuits in neurodegenerative disease models.

Results



All images were taken at 10X. **A, B, C** Results from Arc-GFP experiment. **D, E, F** Results from Arc-Cre-YFP experiment. **A, D** Mice did not get adhesive test nor training; no stroke. **B, E** Mice were given adhesive test and training on right hand; no stroke. **C, F** Mice were given adhesive test and training on right hand; stroke was given on left brain.

Conclusion

Arc-GFP:

- Levels of Arc expression are similar in all conditions within Arc-GFP experiment.
- Results are inconclusive in terms of the difference in levels of Arc expression. GFP could have tagged all Arc protein expressed in non-stroke induced mice.

Arc-Cre-YFP:

- There is an increase in Arc expression in stroke induced mice.
- Levels of Arc expression are indifferent on either side of spinal cord.
- There is evidence of upregulation of Arc expression in stroke model; however, cannot conclude that there is upregulation on either side of spinal cord to compensate for loss of function due to induced stroke on left brain.

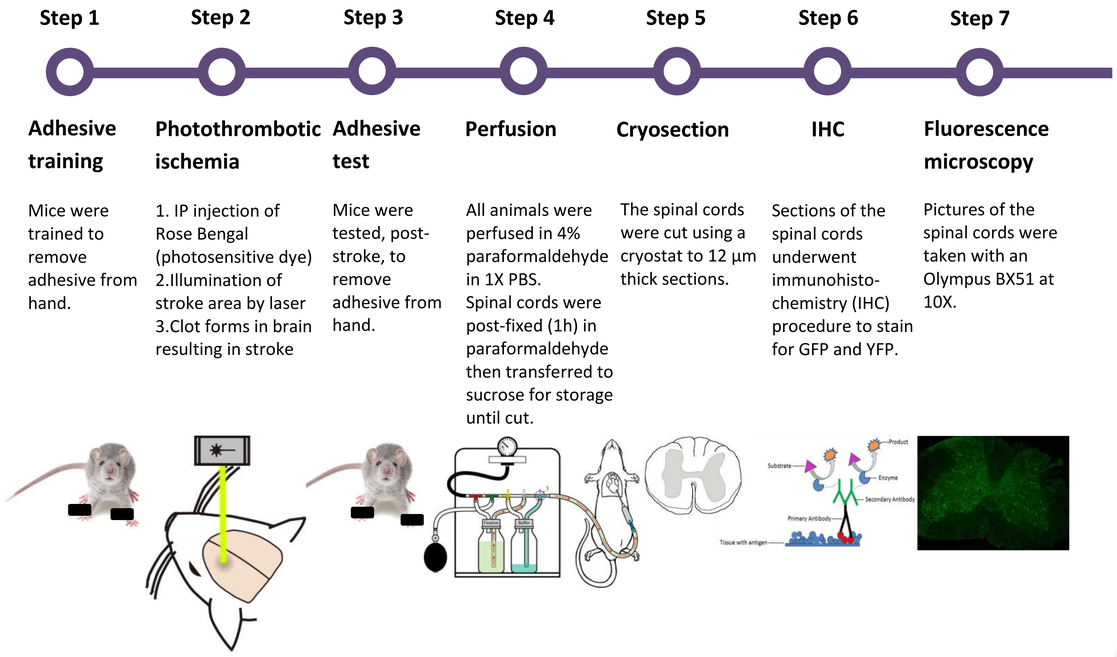
Future steps

- To quantify the number of cells that expressed Arc.
- To carry out an experiment where Arc-Cre is crossed with Arch-GFP. This is to drive the expression of GFP that is fused with a protein called Archeorhodopsin (Arch). Arch is a light-sensitive protein that allows us to inhibit neurons using light [4]. Arch-GFP fusion protein is expressed under the control of Cre and Cre is expressed whenever Arc is expressed. This experiment will be conducted in live mice where light is used to activate Arch temporarily.

Methods

Experiments:

1. **Arc-GFP**: destabilized form of GFP is expressed whenever Arc is expressed [2]. This is a way to mark neurons that recently expressed Arc. Perfusion took place 90 minutes after behaviour test.
2. **Arc-Cre-YFP**: YFP expression is controlled by Cre which is under the regulation of estradiol receptor (ER) [3]. Cre is only activated when an estradiol agonist (ex: Tamoxifen) is given to the mouse. YFP marks neurons that expressed Arc during training.



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