

DOES INCREASING CELL SURVIVAL ENHANCE POST-STROKE LEARNING AND MEMORY?



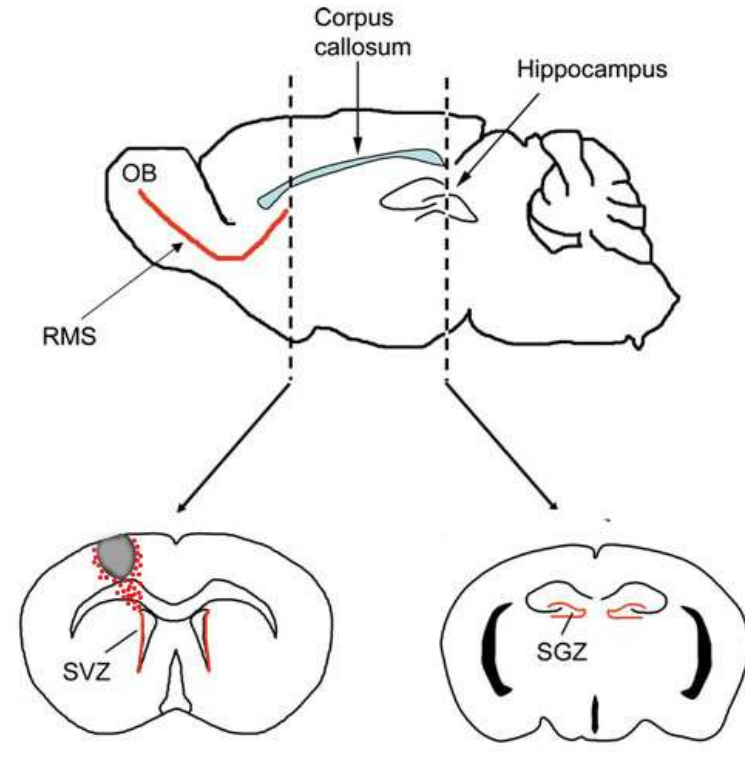
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INTRODUCTION

The 50,000 Canadians who suffer from stroke every year have a long and slow recovery process that aims to improve motor and cognitive function [1]. Remarkably, post-stroke patients often have an innate capacity to recover, which is hypothesized to be due to many forms of plasticity, including adult neurogenesis: the generation of new neurons in the adult brain [2].

Dividing progenitor cells (PCs) reside in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus [3-5]. Following a stroke, there is a 5-12 fold increase in the proliferation of PCs, which can migrate to the site of stroke damage [3]. However many of the PCs die by apoptosis, highlighting the need to increase survival of PCs as a potential therapeutic goal for recovery.

Enhancing the survival of PCs may also improve learning and memory post stroke since the adult-generated neurons in the hippocampus are hypothesized to regulate cognitive function.



AIM & HYPOTHESIS

Aim: To determine if increasing PC survival enhances spatial learning and memory post-stroke.

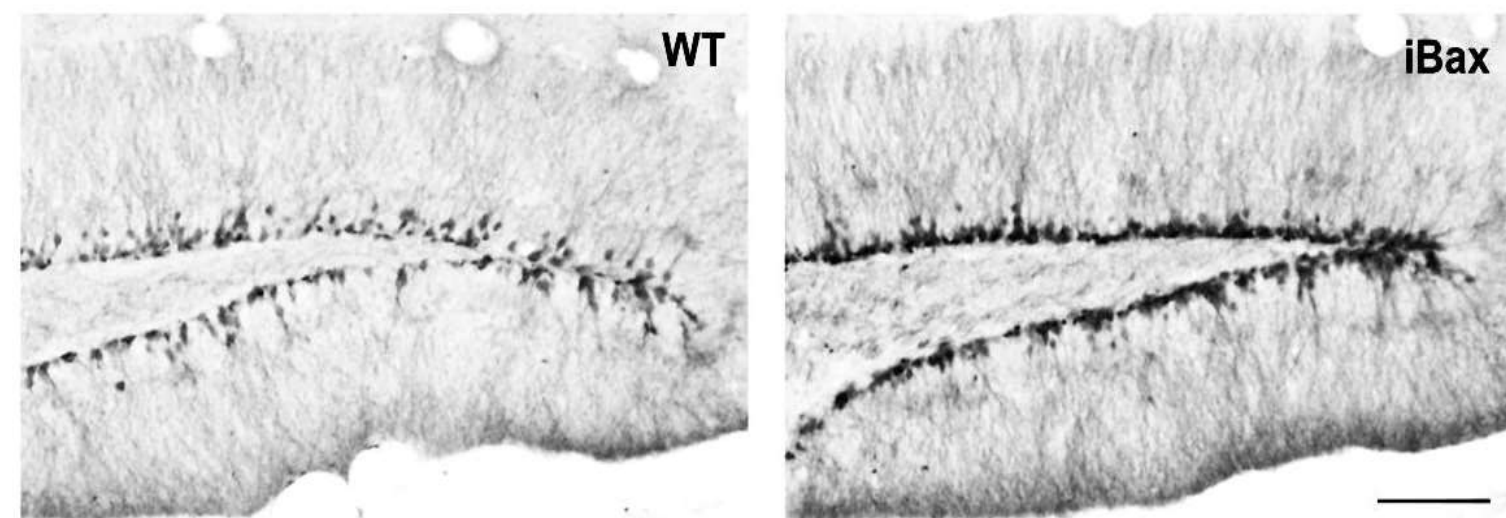
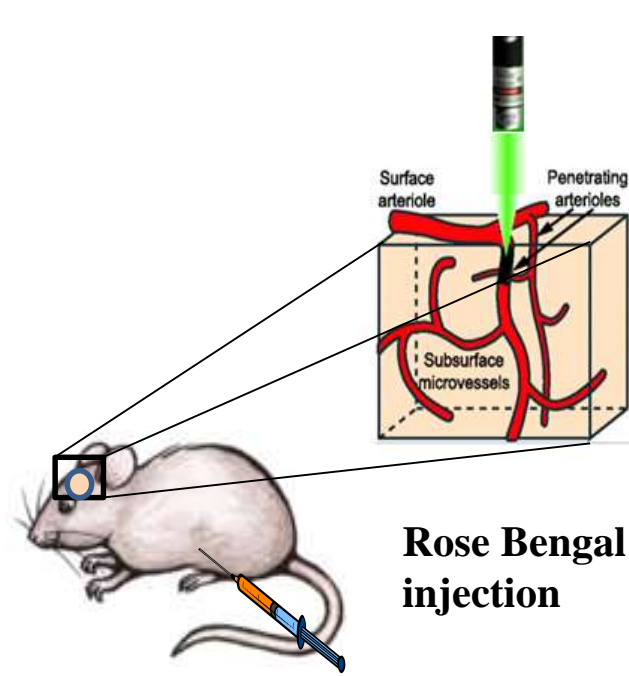
Hypothesis: Increasing survival of PCs will enhance learning and memory post-stroke.

METHODS

Phot thrombosis Stroke Surgery:

Mice were injected with 10 mg/mL Rose Bengal (10 µl/g mouse) intraperitoneally (IP) 5 minutes prior to activation of the 532nm laser at the sensorimotor cortex for 10 minutes to induce the formation of a clot.

iBax Mouse: This mouse (nestinCreERT²-floxed BAX) has an increase in PC survival by inducible knock out of the pro-apoptotic protein Bax. To induce the removal of BAX and expression of YFP from the PCs, tamoxifen (TAM) was administered 160 mg/kg/day IP for 5 days to adult mice.



iBax mice have an increased number of PCs in the SGZ following TAM treatment

Barnes Maze: A spatial memory test for rodents that was used to test learning, memory and re-learning.

Histology: Mice were transcardially perfused with 4% paraformaldehyde and cryoprotected in 30% sucrose in 0.01% sodium azide. Brains were sectioned on a freezing microtome at 40 µm and slices were stained using cresyl violet to identify the stroke region.

Analysis: Cresyl violet stained sections were imaged on a stereoinvestigator microscope, and the volume of the infarct was measured using ImageJ. Data analyzed using GraphPad Prism 6.

Stats: Results are expressed as Mean±SEM.

RESULTS

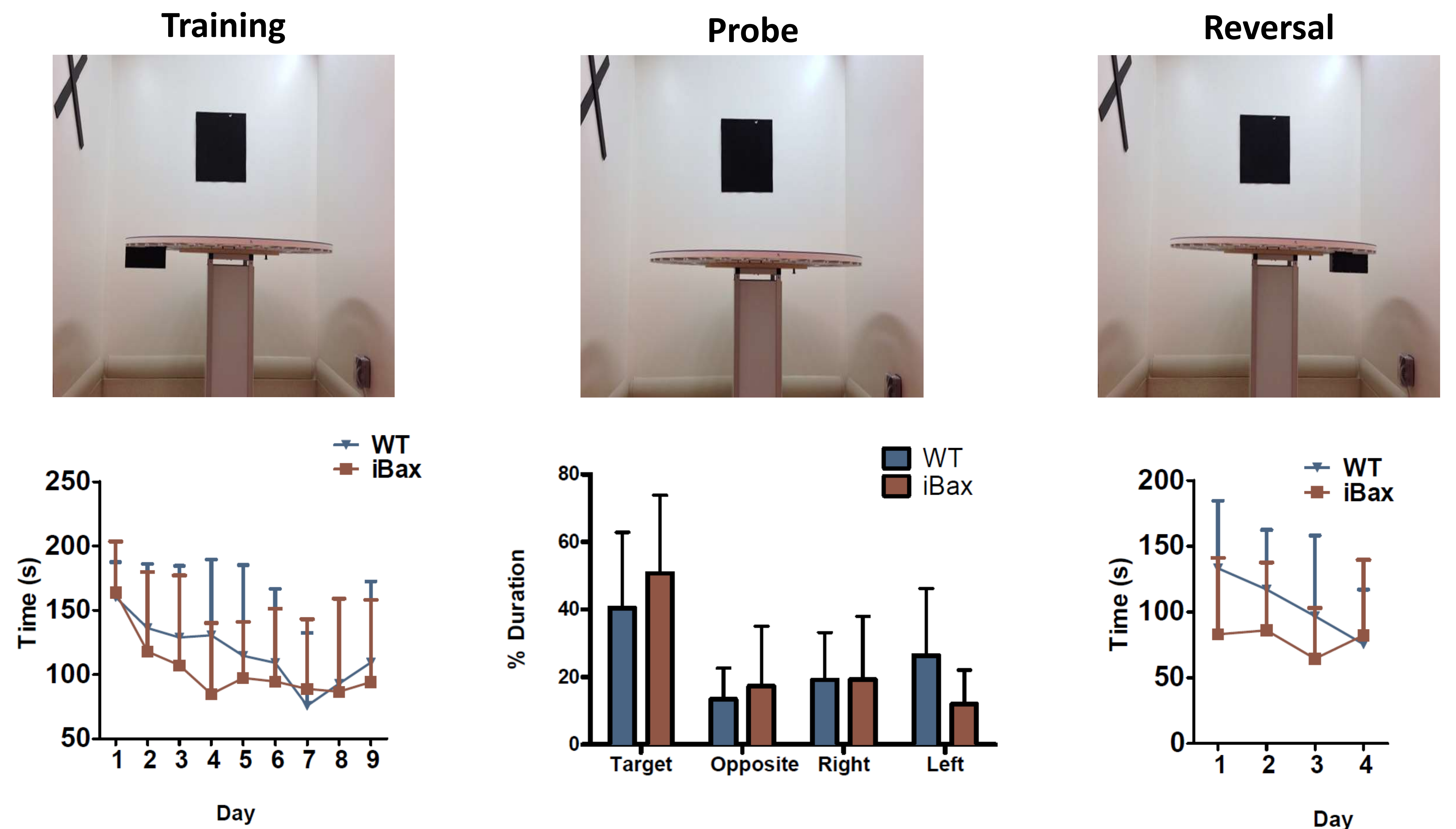
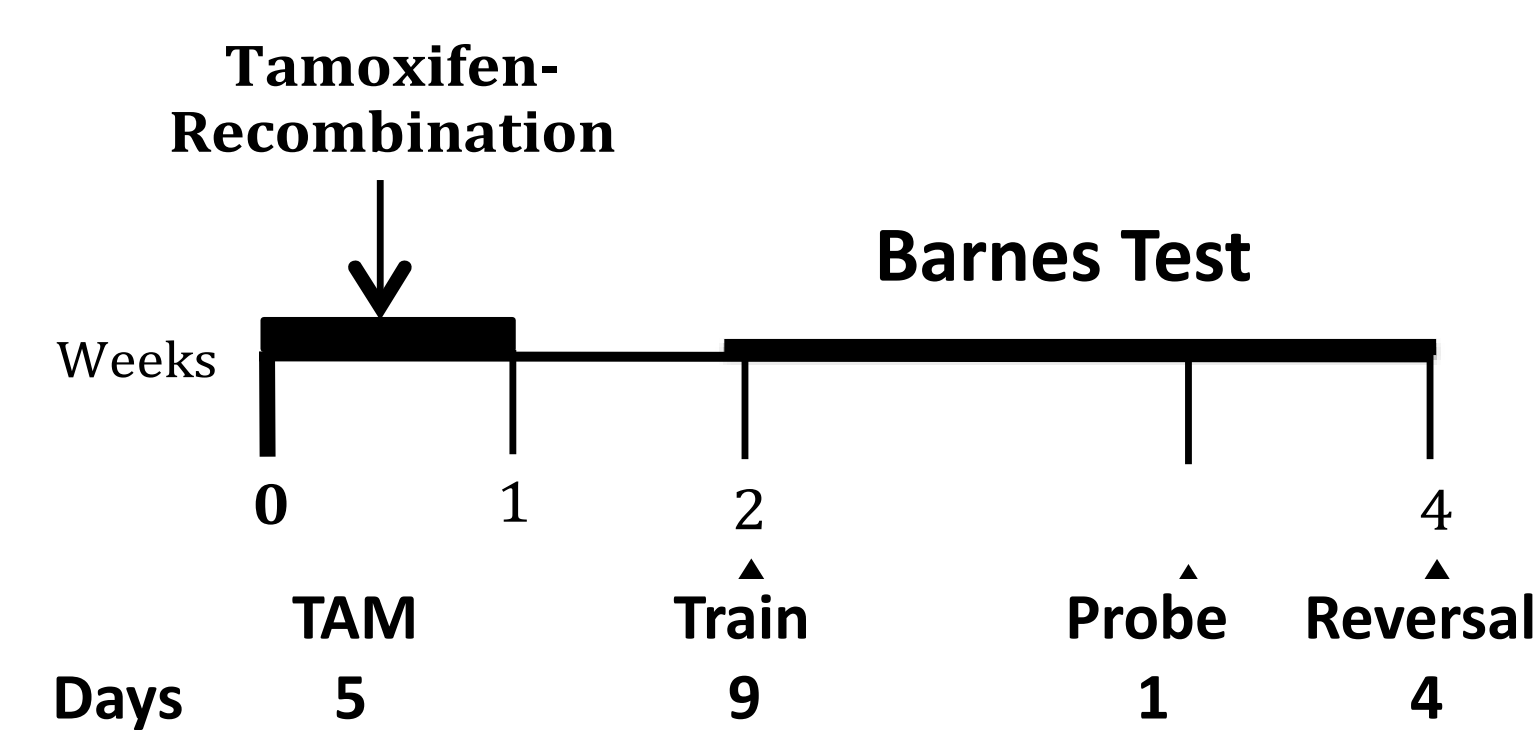
Increasing PC survival is not sufficient to enhance spatial learning and memory in naïve conditions

Experimental Groups:

WT (n=13)

iBax (n=7)

Excluded: n=1, did not learn task

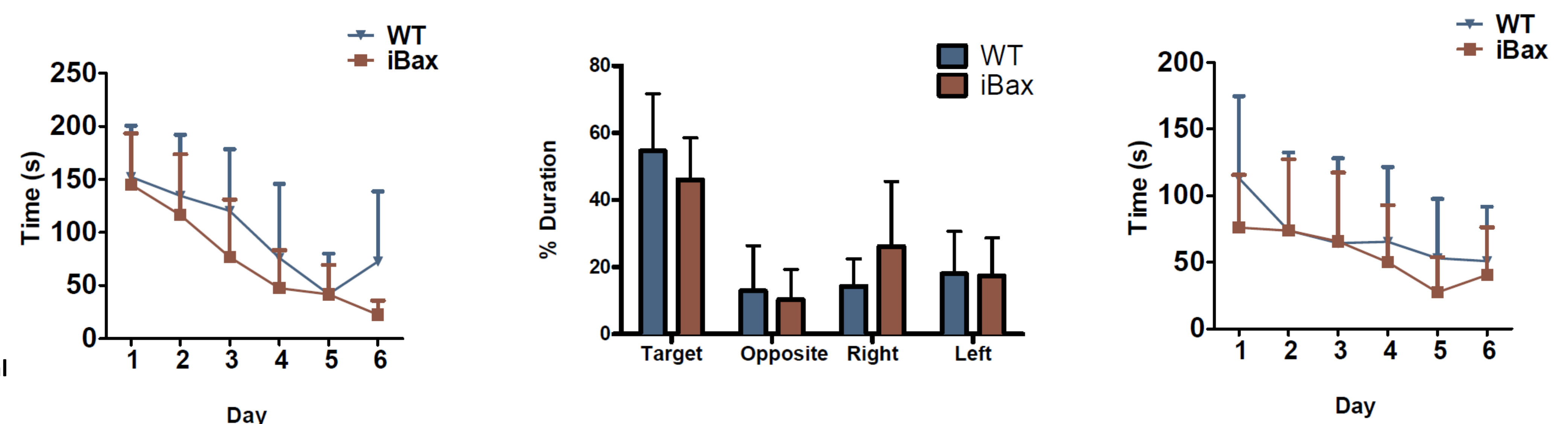
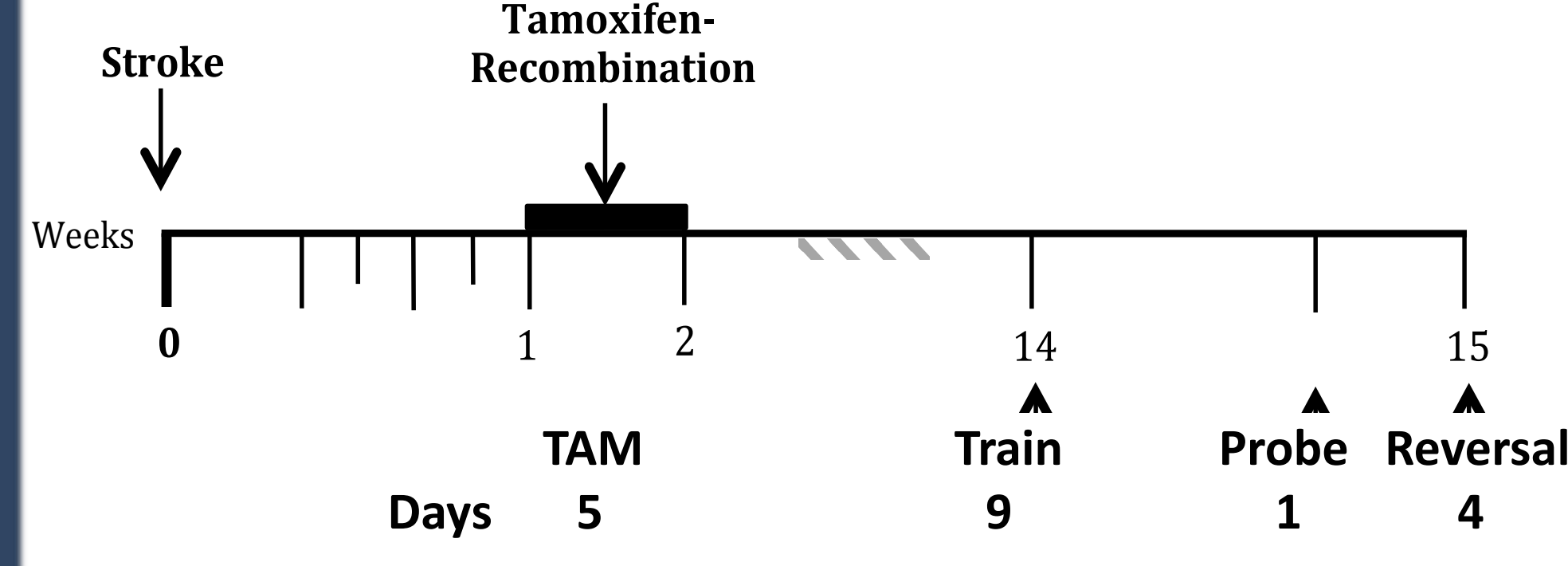


Increasing PC survival is not sufficient to enhance spatial learning and memory post-stroke

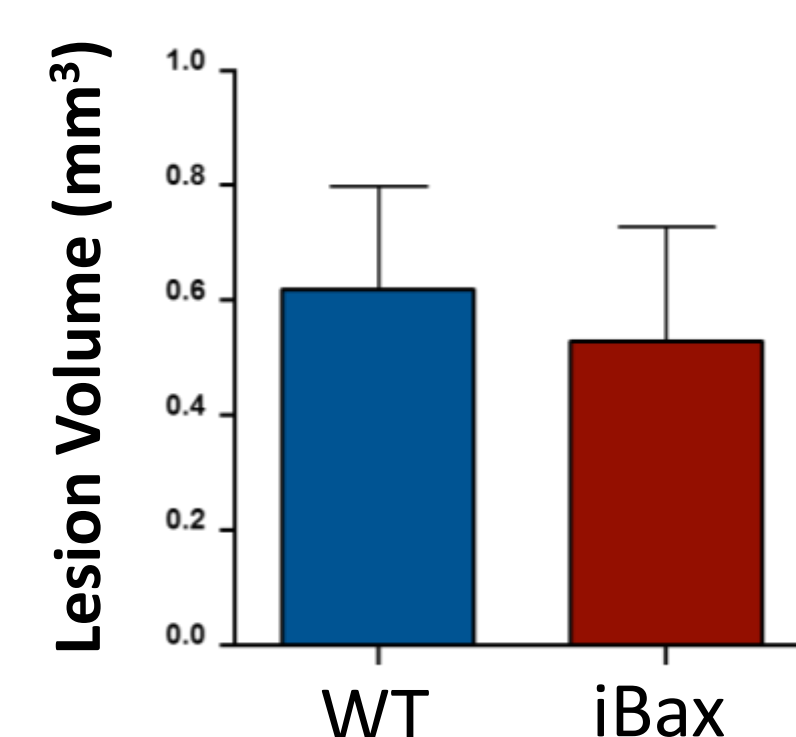
Experimental Groups

WT (n=8)

iBax (n=11)



iBax and WT mice have similar lesion volumes



DISCUSSION & FUTURE WORK

iBax and WT mice have similar stroke volumes which suggests that performance between the two genotypes is not being masked by differences in infarct sizes and that increasing survival of the PCs does not alter infarct size.

- Future work involves histological analysis to confirm the increase in PC survival in the iBax mice compared to their WT counterparts. In addition, immunohistochemical staining will be performed to phenotype and quantify the PCs, which will provide insight as to what proportion of them are becoming neurons.

iBax and WT mice perform similarly on the Barnes Maze in both naïve and stroke conditions. This suggests that an increase in PC survival is not sufficient to enhance spatial learning and memory, and thus may not be useful as a therapeutic strategy post-stroke.

- Sahay et al. (2011, [6]) show that naïve iBax mice perform better than their WT counterparts on pattern separation tasks, which is more demanding. Therefore, future work will determine if an increase in PC survival post-stroke plays a role in pattern separation.

REFERENCES

- Foundation., C.H.a.S. Statistics – Heart and Stroke Foundation Canada. 2011.
- Cramer, S.C. Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. *Ann. Neurol.* 63, 272-287 (2008).
- Thored, P., et al. Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. *Stroke.* 38(11): 3032-9 (2007).
- Li, L., Harms, K.M., Ventura, P.B., Lagace, D.C., Eisch, A.J. & Cunningham, L.A., Focal Cerebral Ischemia Induces a Multi lineage Cytogenic Response from Adult Subventricular Zone that is Predominantly Gliogenic. *Glia.* 58: 1610- 1619 (2010).
- Liu, J., et al. Increased Neurogenesis in the Dentate Gyrus After Transient Global Ischemia in Gerbils. *J Neuro.* 18(19): 7768-7778 (1998).
- Sahay, A., et al. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature.* 472, 466-470 (2011).

ACKNOWLEDGMENTS

Funded by Undergraduate Research Opportunity Program (MAV), Canadian Institute of Health Research (DCL) and Canadian Partnership for Stroke Recovery (DCL/MC). Thanks to Anthony Carter and the Animal Behaviour Core facility for assistance with stroke surgery, behaviour testing and data processing. Thanks to all members of the Lagace lab for their continued support and mentorship.



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