A mouse model of small-vessel disease that produces brain-wide-identified microocclusions and regionally selective neuronal injury Gergely Silasi, Jennifer She, Jamie D Boyd, Songchao Xue and Timothy H Murphy
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Supplementary Material
Methods:

Microsphere Injection

Experiments were carried out on transgenic mice (n=15; Thy1-GFP-M) expressing fluorescent protein in a subset of neuronal populations [1], providing a Golgi-like label throughout the brain (Jackson Laboratory, Stock number: 007788). An additional group of $CX3CR1^{GFP/+}$ mice [2] (n=3) was used to visualize microglial activation at a 3-day survival time. To perform the injection, the CCA was isolated through a neck incision, and 2,000 microspheres (Polysciences, Catalog number: 19096-2) suspended in a 100µl of PBS are injected into the CCA over approximately 1 min with a 33G needle. During the injection, the external carotid artery and the pterygopalantine arteries are transiently blocked with a clip, however flow in the CCA is maintained, thus forcing all of the microsphere solution towards the circle of Willis. After removing the needle, bleeding is controlled by applying pressure with bioabsorbable Gelfoam (Pfizer, New York, USA) to the site until bleeding stops. The sham procedure consisted of all the same steps, except no beads were added to the PBS solution. This injection method was previously used for intra-arterial delivery of cell-therapies in a mouse stroke model, and was found to not produce any ischemic injury nor alterations in cerebral blood flow [3]. In preliminary studies we found that injecting 3,000 microspheres or more resulted in some mortality, usually within the first 24 h after surgery (data not shown), while injecting 2,000 did not result in any mortality. The procedure is quick and simple to perform (~20 min of anesthesia) and the number of injected microspheres has been titrated to produce no mortality at 10-day survival.

Histological assessment and imaging

This model of diffuse vascular injury was applied in Thy1-GFP-m mice, which express GFP in a subpopulation of neurons in the hippocampus, neocortex and cerebellum, as well as the thalamus and amygdala [1]. Additionally, white matter tracts such as the fornix, corpus callosum and anterior commissures as well as axonal fibers of passage in the thalamus and basal ganglia also express GFP. Mice were perfused 8-10 days after the microsphere injection, the brains sectioned at 100 or 150 µm on a vibratome (Leica, VT1000S) and examined with a 20x (0.85NA) objective on a confocal microscope (Zeiss, Meta-510). Microglial activation was assessed by ranking each occlusion site as either injured or normal. Injured sites contained activated microglia with amoeboid cell bodies, thickened processes and an increase in label intensity relative to background (see supplemental Figure 2).

Behavioural assessment

A blinded experimenter assessed a group of microsphere injected (n=5) and sham (n=4) mice on the neurological deficit scale (NDS) to monitor motor impairments. Scores from each subset of the test battery were combined to give an overall indication of impairment, with a maximum score of 11.

Results:

Unilateral microsphere injection produces transient impairments on gross motor tests

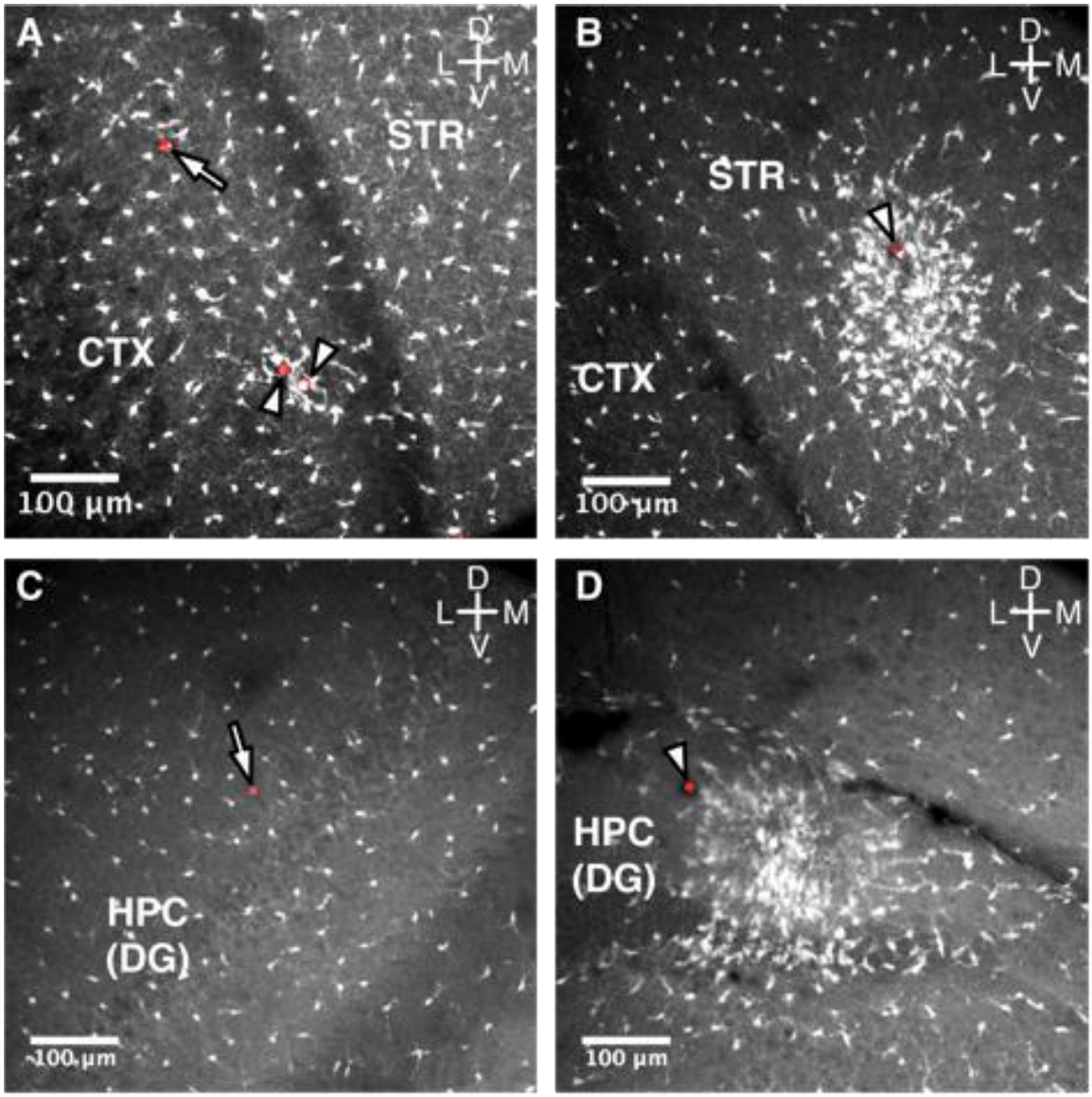
All 15 mice survived until the pre-planned euthanasia time of 9 or 10 days and required no special post-surgical care. There was a significant decrease in post-surgical body weight of microsphere injected mice (23.79 +/- 2.7) relative to sham animals (27.8 +/- 1.09; p=0.046). Microsphere injected mice also showed a slight, but significant impairment of gross motor abilities assessed by NDS on post-surgical day 1 (p=0.042) and 4 (p=0.045), but performance recovered to sham levels by day 7 (p=0.524; Supplemental Fig. 1B).

Supplemental References:

1. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M *et al.* Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* 2000; 28(1)**:** 41-51.

2. Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Molecular and cellular biology* 2000; 20(11)**:** 4106-4114.

3. Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X *et al.* Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2011; 31(5)**:** 1263-1271.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