

The Role of Neurovascular Coupling in Stroke Recovery

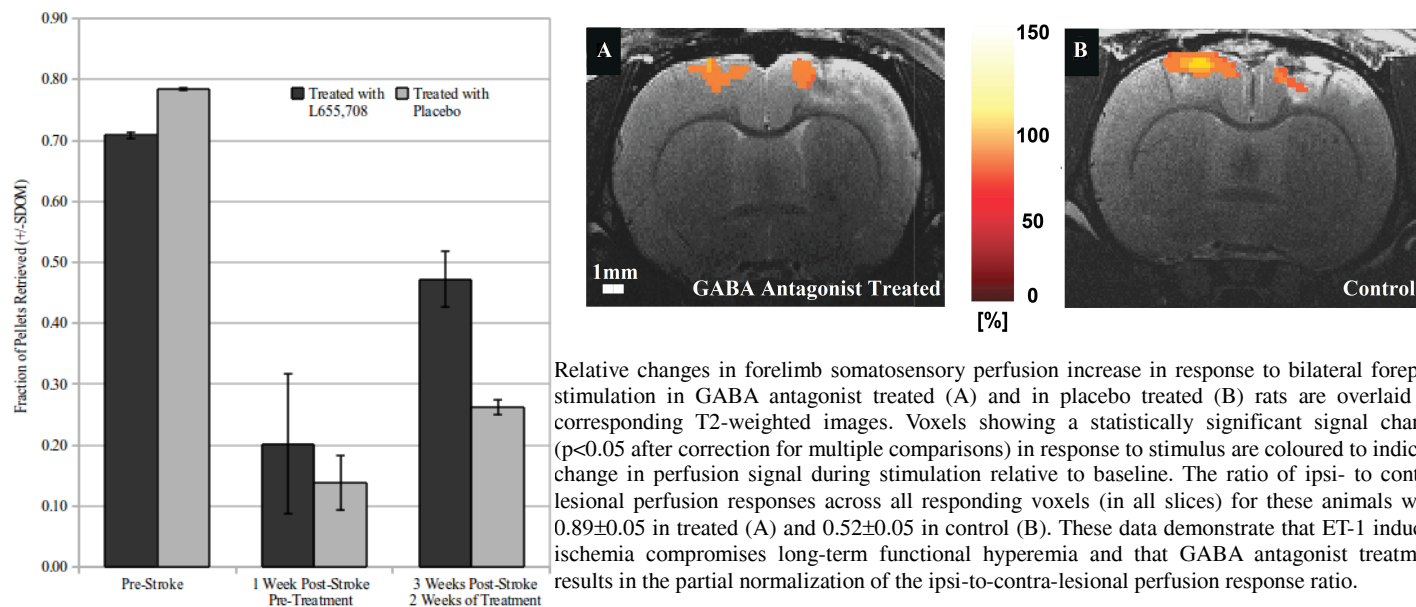
Evelyn Lake¹, Rafal Janik¹, Joydeep Chaudhuri¹, Greg Stanisz¹, and Bojana Stefanovic¹
¹Sunnybrook Research Institute, Toronto, Ontario, Canada

Target Audience: Those interested in stroke and preclinical MRI of disease models.

Purpose: Current treatment for ischemic stroke is limited to: administration of clot dissolving drugs that are effective only within 3-4.5 hours following the onset of symptoms (utilized in 2% of patients) and physiotherapy.¹ The majority of stroke survivors suffer significant disabilities and up to a third are institutionalized making stroke the leading cause of permanent neurological disability.² Effective rehabilitation is, to a large extent, hindered by uncertainty surrounding the underlying mechanisms of recovery. Whereas much is known about the molecular pathways underlying the acute stage (<24 hours following injury) response to stroke (e.g. neuronal cell death), the changes in the neurovascular unit in the peri-infarct zone during the days and weeks following ischemic insult are not well understood. The present work combines functional MRI with behavioural testing to assess the effect of well-timed, low dose gamma-aminobutyric acid (GABA) antagonism on recovery in the chronic stage (>1 week following injury) in a well established rat model of focal ischemia.³

Methods: Focal ischemic stroke was induced in 6 adult male Sprague Dawley rats using intracranial micro injections of endothelin-1 (ET-1), a potent vasoconstrictor.^{4,5} This model permits targeting of specific cortical domains (the right somatosensory cortex was damaged in this study) in a manner that is not achievable with most other stroke models and affords kinetics of perfusion decrease and restoration that more faithfully reproduce those seen in human ischemic stroke.^{4,5} Left forelimb sensorimotor deficits were evaluated at weekly intervals following stroke using the highly sensitive staircase skilled reaching task and compared to baseline reaching ability.⁶ MRI was performed prior to stroke and at weekly intervals following ischemia for a three week period on a 7T Bruker Biospec system, with a birdcage body coil for transmission and a quadrature receive-only coil for reception. Animals were anaesthetized with a continuous IV infusion of propofol (0.3mg/kg/min). At each imaging session, structural T2-weighted images (for lesion volume and inflammation) and functional continuous arterial spin labelling (CASL) images (for assessment of functional hyperemia) were collected. Our CASL protocol consisted of a 3s adiabatic pulse played out at the level of the carotid arteries, and a 0.2s post label delay. Single-average, single-shot echo-planar images were obtained from four 2-mm-thick coronal planes positioned over the sensorimotor cortex, with a 2.56 x 1.28cm² FOV, a 128x64 matrix (nominal in-plane resolution of 200 x 200 μ m²), and a TR/TE of 4000/17ms. Functional hyperemia was elicited bilaterally via electrical forepaw stimulation in ten 90s epochs, each consisting of a 30s on period (during which 0.3ms 5mA pulses were delivered at 3Hz), and a 60s off period. Beginning seven days following stroke, and continuing for a 14 day period, animals underwent daily subcutaneous pill implantations. Animals either received pills containing 58.5mg of HPMV matrix and 1.5mg of a novel GABA antagonist L-655,708 (treated group) or 60mg of HPV matrix (control group).⁷

Results: ET-1 microinjection into the right somatosensory cortex caused a pronounced deficit in left forelimb reaching ability (shown in the histogram below) one week following injury. After two weeks of daily pill implantations, GABA antagonist treated animals (N_T=3) exhibited significantly more recovery than that shown by the control group (N_C=3).



Relative changes in forelimb somatosensory perfusion increase in response to bilateral forepaw stimulation in GABA antagonist treated (A) and in placebo treated (B) rats are overlaid on corresponding T2-weighted images. Voxels showing a statistically significant signal change ($p < 0.05$ after correction for multiple comparisons) in response to stimulus are coloured to indicate change in perfusion signal during stimulation relative to baseline. The ratio of ipsi- to contra-lesional perfusion responses across all responding voxels (in all slices) for these animals were 0.89 ± 0.05 in treated (A) and 0.52 ± 0.05 in control (B). These data demonstrate that ET-1 induced ischemia compromises long-term functional hyperemia and that GABA antagonist treatment results in the partial normalization of the ipsi-to-contra-lesional perfusion response ratio.

Discussion: The present work builds upon the exciting finding that continuous, low dose treatment with a novel GABA antagonist in the chronic stage of stroke recovery may ameliorate some of the deleterious effects of ischemic injury.³ The current data show beneficial effects of this treatment in rats on behavioural recovery, in agreement with prior work done in mice,³ and further suggests that the improved behavioural recovery is accompanied by the partial normalization of functional hyperemia. Further studies will focus on the identification of long term effects this treatment may have on the peri-infarct tissue and a detailed characterization of the underlying changes in neurovascular unit function so as to effect further improvements in behavioural recovery.

¹ Hill, Buchan, *Thrombolysis for acute ischemic stroke: results of the Canadian Alteplase for Stroke Effectiveness Study*. CMAJ 2005;172(10):1307-1312

² Evenson, Rosamond, Morris, *Prehospital and in-hospital delays in acute stroke care*. Neuroepidemiology 2001;20(2):65-76

³ Clarkson, Huang, Macisaac, Mody, Carmichael, *Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke*. Nature 2012;468(7321):305-309

⁴ Windle, Szymanska, Grant-Butt, White, Buist, Peeling, Corbett, *An analysis of four different methods of producing focal cerebral ischemia with endothelin-1 in the rat*. Exp Neurol 2006;210(2):324-334

⁵ Biernaskie, Corbett, Peeling, Wells, Lei, *A serial MR study of cerebral blood flow changes and lesion development following endothelin-1-induced ischemia in rats*. Magn Reson Med 2001;46(4):827-830

⁶ Montoya, Campbell-Hope, Pemberton, Dunnett, *The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats*. J. Neurosci. Methods 36, 219-228 (1991).

⁷ Atack, Pike, Clarke, *Rat pharmacokinetics and pharmacodynamics of a sustained release formulation of the GABA α -5-selective compound L-655,708*. Drug Metab. and Disposition 2006;34(5):887-893