# Progress Report 19th May 2015

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**Title of Project:** Targeting astrogliosis and brain stimulation after stroke to promote plasticity and functional recovery.

***Summary:*** Stroke often results in permanent brain damage which is ultimately due to a failure of nerve cells to re-grow across the injury site. Although the brain generates new stem cells in response to injury very few of these cells end up replacing damaged circuitry. Indeed, studies now show that the majority of migrating stem cells after stroke differentiate into astrocytes that become rapidly activated and contribute further to glial scar formation. Over activation of pre-existing astrocytes also disrupts neuronal signalling through reduced neurotransmitter turnover and energy transfer.

We therefore hypothesised that targeting over-activation of astrocytes to reduce the glial scar would facilitate recovery of neuronal pathways affected by stroke.

Our first study explored the potential effect of delayed treatment with the Rho-kinase inhibitor, fasudil, on reducing astrocyte reactivity after stroke. These studies were performed by an honours student, Ms Ellie Phillips, supervised by Dr Carli Roulston, as part of the Biomedical Science degree at the University of Melbourne.

Stroke was induced through application of the potent vasoconstrictor Endothelin-1 (60pmol in 3µL saline) to constrict the middle cerebral artery in conscious male hooded Wistar rats. Fasudil treatment was delayed until 3 days after stroke (50 mg/kg, intraperitoneal injections, daily) with vehicle (saline) and sham controls. Rats were treated for 28 days (n=9 per treatment group), over which time functional outcomes were assessed using various tests including the neurological deficit score, cylinder test, tape test and staircase test. After 28 days histological analysis of brain sections were conducted to observe the effect of fasudil treatment on astrocytes and other brain remodeling events. A smaller cohort (n=5 per treatment group) was recovered to 14 days and quantitative proteomics conducted using stable isotope dimethyl labelling in order to assess early changes in protein expression that may lead to functional recovery.

Stroke resulted in observable brain injury in all rats with no difference in infarct size detected between treatment groups (Figure 1A). Neurological deficits were detected in all groups after stroke and were significantly recovered following treatment with Fasudil, in comparison to vehicle controls (Figure 1B). Given that treatment with Fasudil was delayed until after damage had occurred, the results of this study suggest that Fasudil can promote functional recovery in the absence of neuroprotection. Further histological analysis confirmed that fasudil treatment significantly reduced the number of astrocytes present in the damaged striatum and cortex and astrocytes that were detected showed a less reactive morphology (Figure 2D) similar to that of trophic astrocytes. Since recovery to the contralateral forepaw

was rapidly observed upon treatment, we suggest that the effects of Fasudil are likely mediated through restored neurotransmission in surviving pathways, rather than axonal regeneration or remodelling. As such we have identified a new target for Fasudil, astrocytes, to retain neuronal-astrocyte metabolic coupling for restoration of neurotransmission.

Quantitative proteomic analysis revealed that fasudil treatment resulted in changes in protein expression levels. The most significant change was the up-regulation of dihydrolipoyllysine-residue succinyltransferase (DLST) following Fasudil treatment (12.29 ± 4.48 fold compared to vehicle). As an important component of the tricarboxylic acid cycle, DLST up-regulation may help astrocytes to maintain energy supplies, thereby allowing them to maintain neurotransmission without morphological transition into a diffuse, scar forming astrocyte.

The results of this study support the use of Fasudil to promote post-stroke recovery through reduced astrocyte activation for retained astrocyte support.

**Treatments that reactivate the depressed brain after stroke:**

Parallel to the above studies, we also hypothesized that direct stimulation of the stroke affected brain might also promote recovery of signalling pathways interrupted by stroke. Loss of electrical activity in otherwise structurally intact pathways now more accurately accounts for clinical findings and strategies that target this pathology represent an approach likely to enhance brain rescue and be accessible to a broader patient population. The use of electrical brain stimulation to treat stroke is an emerging concept. This study therefore explored the use of a minimally invasive epdidural device implanted over the rat motor cortex to promote recovery after stroke.

Following endothelin-1 induced stroke and neurological screening in conscious rats 2 separate groups (n=5 each) underwent either stimulation at functional threshold (6hr/day), or no-stimulation (where the stimulator is implanted but not turned on) for 5 days. Neurological outcome was assessed in all groups using a neurological deficit score and postural support cylinder test. After 5 days of stimulation immunohistochemical analysis was conducted to observe the effects on brain pathology.

Epidural stimulation of the rat motor cortex commencing 3 days after stroke promoted recovery of neurological deficits within 5 days (n=5) (Figure 2D & E). This is a marked improvement compared to reports using externalised devices and rats without stimulation showed no evidence of recovery over the same period.

Stimulation resulted in accelerated angiogenesis within the damaged cortex in comparison to non-stimulated controls (Figure 3B). Stimulation also altered the astrocyte response to stroke (Figure 3D). Over activation of astrocytes into a diffuse morphology (Figure 3C) appeared to be reduced with epidural stimulation (Figure 3D) which is similar to that reported with deep brain stimulation. Epidural stimulation resulted in activation of astrocytes with a stellated morphology with spindly, elaborate processes extending outwards into the brain (Figure 3D). Importantly astrocytes within the cortex retained end feet connections with blood vessels (Figure 3F) to potentially support reversal of functional deficits via neurovascular recoupling. These results suggest that electrical stimulation to reactive the sleeping brain after stroke may importantly involve retained astrocyte support.

***The results of these two studies have now been combined into a new application to the NHRMC for funding to investigate the role of astrocytes in brain recovery after stroke.***

### *Unanswered Questions*

*Our endpoints for this project were:*

1. Reduce scar formation and maintain important astroglial support. ✓
2. Promote new blood vessels to support brain repair. ✓
3. Promote stem cell migration into the damaged brain to support nerve regeneration and outgrowth.
4. Stimulate new and surviving nerve cells into organization of functional pathways
5. **Accelerate functional recovery**. ✓

Due to time constraints the effect of each treatment on neurogenesis and nerve regeneration after stroke are still currently under investigation.

### *What these research outcomes mean*

New targeted treatments to promote recovery after stroke are desperately needed for the majority of stroke survivors that do not get any form of neuroprotection. Targeting the astrocyte provides an alternative therapeutic strategy that may be instituted after stroke to improve outcomes. We now show that astrocytes can be therapeutically directed to support brain rescue with reduced scar formation, whilst at the same time retaining their trophic phenotype that is capable of supporting neurotransmission to reverse functional deficits.

Whilst each treatment approach is currently in clinical trial for use in other neurological diseases, neither is used for stroke. Future studies therefore aim to learn more about these treatments, in particular how and when they should be applied, as well as their effects on other brain remodelling events in order to expedite their use for rehabilitation after stroke.

Please include any appropriate photos or diagrams.

**Figure 1:** Treatment with Fasudil did not affect stroke volume (A) but significantly improved contralateral forepaw deficit by 14 days (B). Diffuse astroctyes (GFAP; green, DAPI; blue) within the peri-infarct cortex of vehicle rats by 28 days (C) are reduced by Fasudil (10mg/kg/i.p./daily) (D). *P<0.05 vs 0hr scores; #P<0.01 vs vehicle treatment; n=9/group.

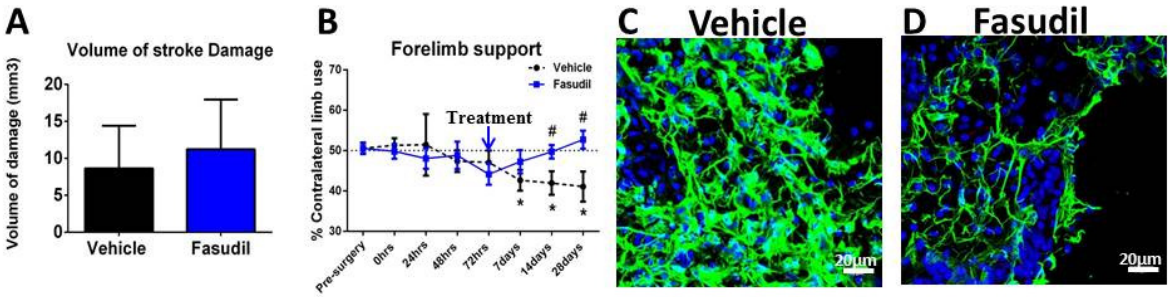


**Figure 2.** (A) Stimulating electrodes connect to fully implantable cochlear undergoing safety and efficacy profiling for the treatment of Epilepsy (NHMRC APP1075347); (B) Small animal stimulator fully implantable in rats; (C) brain activity and stimulation is fully recordable; (D) stimulation commencing 3 days post-stroke significantly improves neurological deficit and (E) contralateral forepaw use after 5 days of stimulation. * P<0.05 vs 0hr; # P<0.05 vs 24 hr; n=5/group

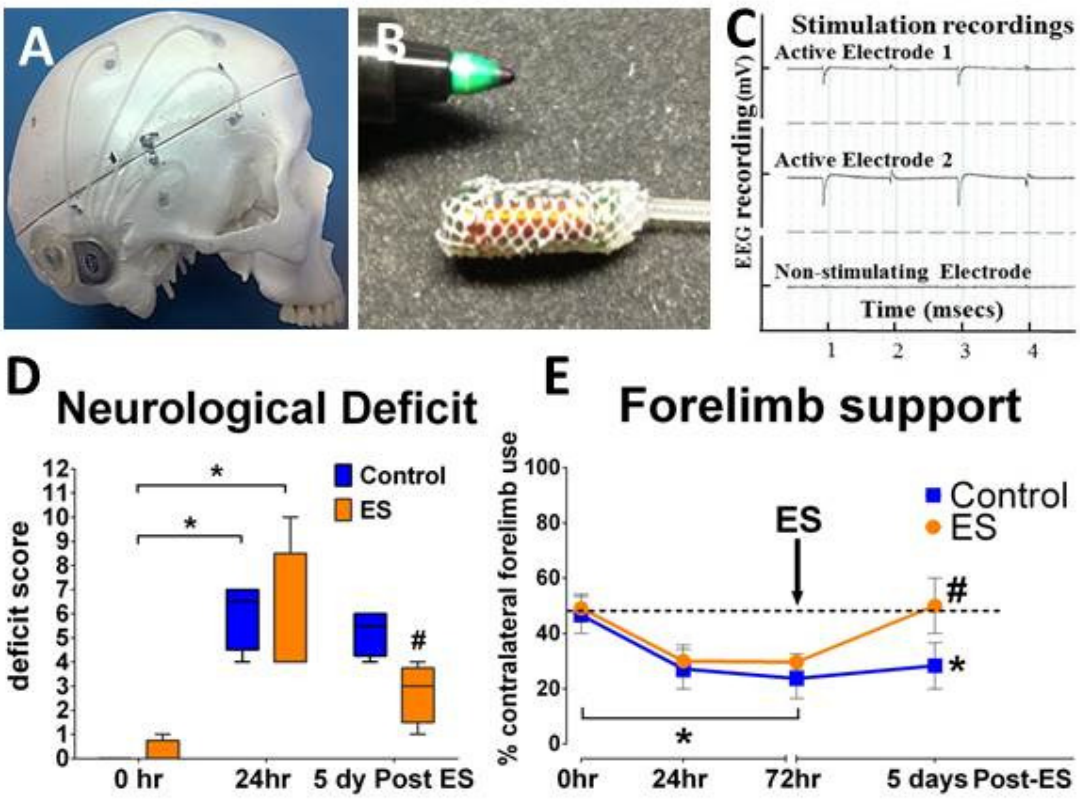

**Figure 3** (A) Stroke induced Angiogenesis (vWF green; DAPI blue) is increased with epidural stimulation (B). (C) Many diffuse astrocytes (GFAP red; DAPI blue) are detected within the neurogenic niche after stroke; (D) Astrocyte activation with ES involves extension of spindly processes; (E) Astrocytes within the motor cortex retract their end-feet from vessels in control rats (GFAP green; vWF red); (F) Electrical stimulation activates astrocytes but maintains neurovascular coupling. Scale bar = 20µm