Investigating changes in metabotropic glutamate receptor 5 expression in a mouse model of motor neuron disease

Motor neuron disease (MND) is a fatal neurodegenerative disease. Riluzole is the only approved drug for MND but has only modest effects on survival. Riluzole is thought to exert its therapeutic effect through the indirect antagonism of glutamate. Glutamate-mediated excitotoxicity is a recognised mechanism of astrocyte and neuronal injury. Astrocytes expressing mutant *SOD1* (a mutation causing MND) are more vulnerable to glutamate toxicity suggested to be mediated by the metabotropic glutamate receptor 5 ($mGlu_5$). Elevated levels of $mGlu_5$ have been observed on reactive astrocytes and correlate with areas of motor neuron pathology, both in mouse and human MND. Research suggests that $mGlu_5$ receptor levels change throughout the disease course in *SOD1*^{G93A} mice (a robust and reproducible mouse model of MND) however, data is conflicting on whether receptor levels increase or decrease in brain and spinal cord (SC) (1,2). The aim of this study was to characterise $mGlu_5$ expression changes in *SOD1* MND.

SOD1^{G93A} transgenic and wild-type mice were perfuse-fixed under terminal anaesthesia (PBS followed by 4% paraformaldehyde). Tissue was dissected, cryoprotected (20% sucrose) and lumbar SC embedded in OCT (cryo-embedding media). Sections were serially cut (10 µm), stained for $mGlu_5$ (rabbit αmGlu5; Abcam,1:250) and GFAP (chicken αGFAP Abcam,1:500) (16h ;4^{0}C) followed by staining with secondary antibodies (donkey anti-rabbit Alexa 555,1:500; donkey anti-chicken Alexa 488, 1:500) (1.5 h; 20°C). Human derived *SOD1* and healthy control iAstrocytes were cultured as described in (3) and stained for $mGlu_5$ as above. All images were captured on the InCell Analyzer 2000 (GE) and analysed using InCell Developer Toolbox/ImageJ. Whole brain was collected from wild-type and *SOD1*^{G93A} mice at 30 and 120 days of age. Brains were homogenized (Polytron, 30s) in buffer (0.9% (w/v) NaCl, 10mM HEPES, 0.2% (w/v) EDTA, pH 7.4) and centrifuged (5 mins, 200 xg, 4°C). Supernatant was re-homogenised (30 s) then centrifuged (20 mins, 40,000 xg). This step was repeated, first re-suspending the pellet in 10mM HEPES, 10 mM EDTA (pH 7.4) then 10mM HEPES, 0.1 mM EDTA (pH 7.4). Membranes were stored at -80 °C. [^{3}H] M-MPEP saturation was performed and analysed as described in (4) (20µg membranes/well).

$mGlu_5$ expression as detected by IHC analysis was predominant in the substantia gelatinosa of the lumbar spinal cord in both wild-type and SOD1^{G93A} mice. Weaker staining in the grey matter parenchyma and in ventral motor neurons was apparent. No co-localisation between astrocytes and $mGlu_5$ positive processes was observed. Saturation binding showed a non-significant trend for higher receptor numbers at 30 days compared to 120 days, in both wild-type (Bmax (fmol/mg) 1929 ± 256 and 1799 ± 235; 30D and 120D respectively) and *SOD1*^{G93A} (Bmax (fmol/mg) = 1882 ± 234 and 1462 ± 206; 30D and 120D respectively) membranes ($p=0.14$; 1-way ANOVA).

This study is not consistent with literature reports of increased expression of $mGlu_5$ on astrocytes in *SOD1* MND. Whilst no significant difference in $mGlu_5$ receptor levels was found on cortical membranes this does not rule out significant changes in specific brain regions (e.g. cortex) (2) and will be further investigated.

1. Martorana et al., (2012) *Hum. Mol. Gen* 21:4:826–40.
2. Brownell et al., (2015) *J Neuroinflammation* 12:217.
3. Meyer et al., (2014) *PNAS* 112:2: 829–832.
4. Christopher et al., (2015) *J Med. Chem* 58:6653–6664.