End of grant report

Summary (suitable for sharing on Pain Relief Foundation website)

Chronic migraine causes considerable distress to the sufferer and is a significant burden to society. As a result, migraine has a substantial negative impact on quality of life and large social and financial implications. In the UK alone, chronic migraine affects 6 million people and remains a major clinical challenge despite the significant advances made in the context of newly developed CGRP (calcitonin gene-related peptide) focused therapies. This is because chronic migraine is a complex neurological disorder, characterised by recurrent unilateral headaches and a multitude of other sensory deficits, with one third of migraineurs also suffering from migraine aura, which often precedes headache and presents as further disturbances. We have evidence that targeting the stress regulator FKBP51 could be a novel approach for the treatment of chronic migraine and this project has provided further evidence that blocking FKBP51 can result in the clinical management of persistent migraine states.

With this project, we have continued to characterise the impact of the deletion of FKBP51 on the hypersensitivity seen in migraine. For this, we have assessed mouse behaviour in well-established rodent models of migraine. While we had previously reported that the protein FKBP51 was a key driver of persistent migraine, we found that its deletion did not reduce short-lasting headache. More importantly, we found that the deletion of this protein prevented the transition from acute to chronic migraine. All together, the behavioural data we collected supported our original findings suggesting that FKBP51 is a key driver of chronic migraine states. This suggests that its inhibition would prevent the transition from acute to chronic migraine, which would be an invaluable approach for the management of migraine in the clinics.

We next used the same animal model of migraine to investigate the changes in signalling cascades in the central nervous system associated with migraine and assessed how this may be modified in the absence of the protein FKBP51. Here, we found that the absence of the stress driver FKBP51 significantly reduced neuronal activation in regions of the central nervous system known to be activated by migraine, strengthening the idea that FKBP51 was a molecular driver of migraine. To further our understanding of the role of FKBP51 in migraine, we ran some sequencing studies that allowed us to look at the overall impact of FKBP51 deletion on gene expression in migraine state. This approach is likely to lead to the discovery of new treatment approaches for the clinical management of migraine.

Next steps

With this project, we have raised data that will be included in a manuscript currently in preparation. We were also able to raise some pilot data to apply for further funding to other charities (Rosetrees Trust and Brain Research UK) so we can continue our research on migraine with more substantial funding. This would not have been possible without the support from PRF.

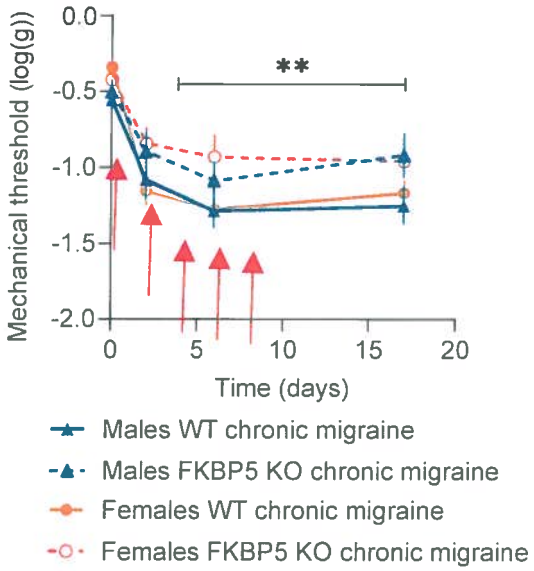
More information (not suitable for sharing on Pain Relief Foundation website as this is unpublished data)

Animal model: to induce migraine in mice, we injected a compound called GTN, which induces hypersensitivity in mice all over the body, including the hindpaws. We then evaluate the mechanical threshold of mice, *i.e.* their level of sensitivity, as a correlate for migraine.

Result 1: We have established that genetic knock-down of *Fkbp5* significantly reduces mechanical hypersensitivity in chronic but not acute migraine and prevents the transition from acute to chronic migraine.

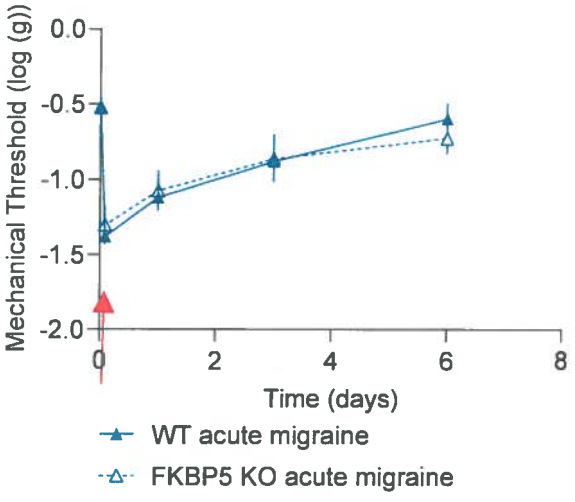
A. Chronic migraine

Mechanical hypersensitivity
Fkbp5 KO



B. Acute migraine

Mechanical hypersensitivity
Fkbp5 KO



C. Transition from acute to chronic migraine

Mechanical hypersensitivity
Fkbp5 KO

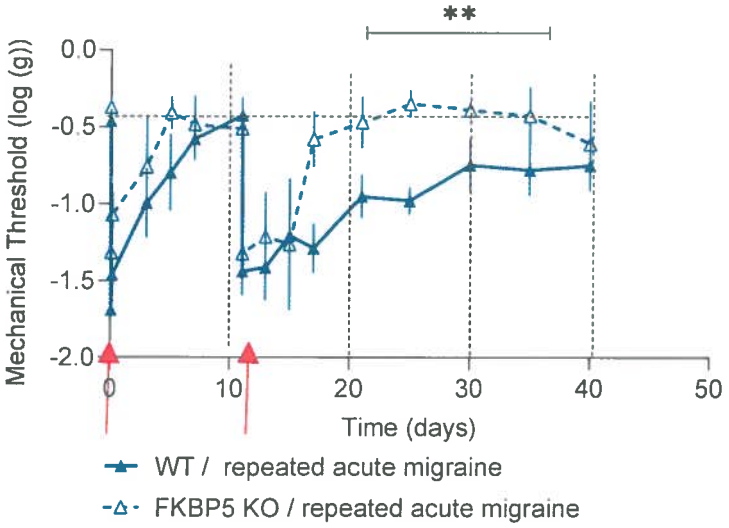


Figure 1: Genetic knock down of *Fkbp5* significantly reduces mechanical hypersensitivity in chronic but not acute migraine. (**A**) *Fkbp5* KO mice show reduced mechanical hypersensitivity in the model of chronic migraine. N=10-12 per group. (**B**) There was no difference to the response to a single GTN injection (at day 0) between WT and *Fkbp5* KO mice. N=6/6. (**C**) GTN injection does not exacerbate the subsequent response to another GTN injection in *Fkbp5* KO mice. (**A, B, and C**) Mechanical thresholds were measured in the hindpaw using Von Frey filaments. Red arrows: GTN injections. *P<0.5; **P<0.01, SAFit2 *vs* Vehicle or KO *vs* WT, as appropriate. Straight line indicates result of ANOVA. ***Data unpublished.***

Result 2: We have established that genetic knock down of *Fkbp5* reduces neuronal excitation in the central nervous system induced by GTN injection.

We have continued to map the impact of GTN injection on neuronal activation using the expression of the immediate early gene cFos as a correlate of neuronal activation and looked at the impact of *Fkbp5* deletion.

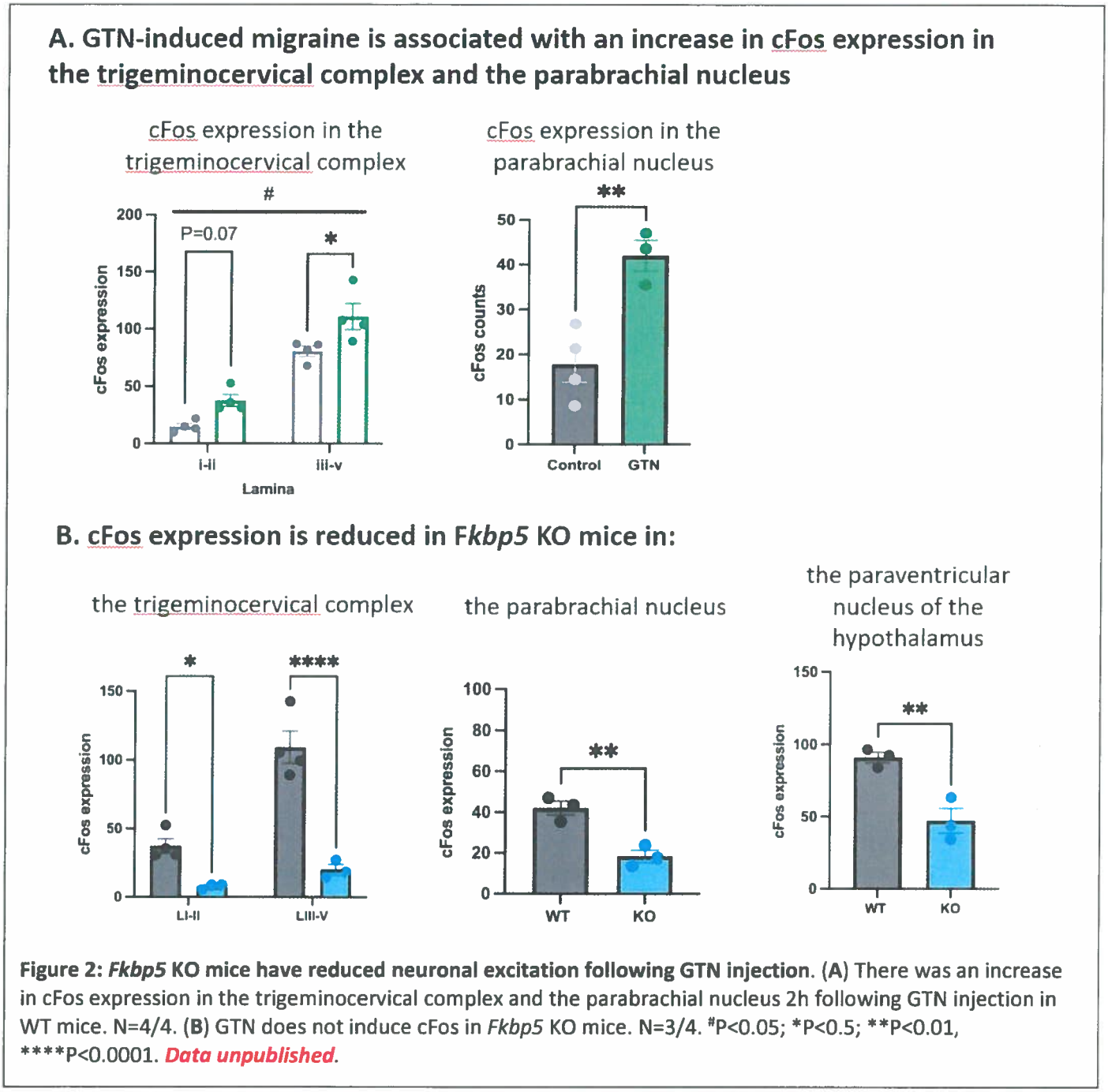


Figure 2: *Fkbp5* KO mice have reduced neuronal excitation following GTN injection. (**A**) There was an increase in cFos expression in the trigeminocervical complex and the parabrachial nucleus 2h following GTN injection in WT mice. N=4/4. (**B**) GTN does not induce cFos in *Fkbp5* KO mice. N=3/4. #P<0.05; *P<0.5; **P<0.01, ****P<0.0001. ***Data unpublished***.

Result 3: Detailed sequencing studies suggest that FKBP51 drives migraine through modulation of various signalling pathways.

We were able to identify molecular pathways modified by FKBP51 deletion and this included inhibitory signalling as we had predicted. More work is now needed to further validate the identified targets.