Interim grant report

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 sensory deficits, with one third of migraineurs also suffering from migraine aura, which often precedes headache and presents as further sensory disturbances. We have evidence that targeting the stress regulator FKBP51 would be a suitable approach for the treatment of migraine and this project will provide further evidence that inhibiting FKBP51 can result in the clinical management of migraine.

This project is divided into 2 main parts:

1. The investigation of the underlying mechanisms of the regulation of migraine hypersensitivity by the stress regulator FKBP51.
2. Investigation the role of FKBP51 in cortical spreading depression.

While the second part of the project will take place in collaboration with Dr Anna Andreou when she returns from maternity leave, we have currently focused on the first part.

1. **Analysis of underlying mechanisms using molecular approaches.**

   Here, we use a model of repeated injection of glyceryl trinitrate GTN (5 injections, every other day for 10 days).

A) Behavioural outcome of inhibition of migraine-like pain using FKBP51 inhibition.

Following repeated injection of GTN, both male and female mice develop significant mechanical hypersensitivity that can be rescued by pharmacological inhibition of the stress regulator FKBP51 with SAFit2 (Fig.1).

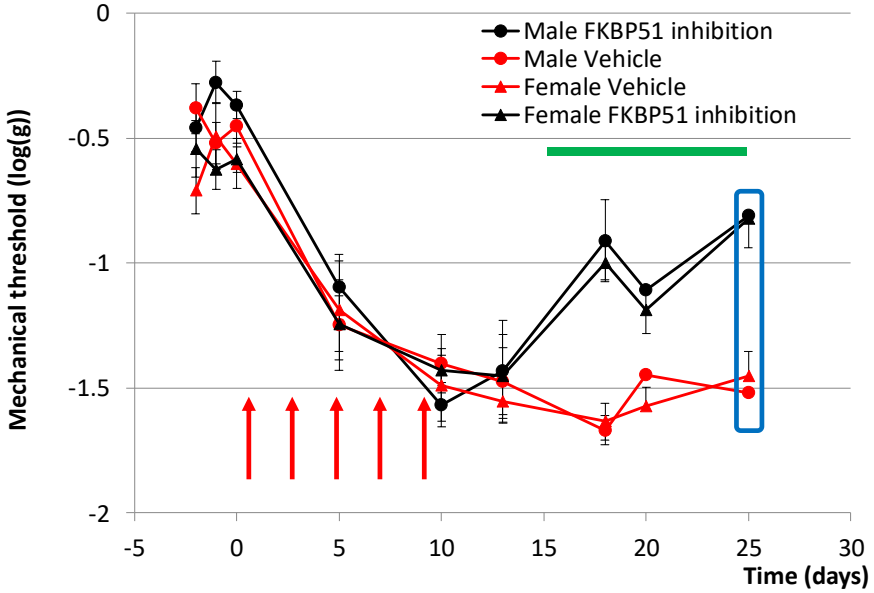

Figure 1: FKBP51 inhibition reduces GTN induced mechanical hypersensitivity in both male and female mice. Red arrows: GTN injections; green bar: SAFit2 (FKBP51 inhibition). Blue square: time point of tissue dissection for molecular analysis**P<0.01

We have dissected the spinal cord, cervical and brain tissue at a time point where the rescue by SAFit2 was still significant (highlighted in Fig.1 by the blue square) and we are now starting the analysis of the differences in gene expression between the SAFit2 and vehicle treated animals.

B) Analysis of molecular pathways involved in the regulation of migraine hypersensitivity by FKBP51.

Tissue of male mice is currently being processed for RNAseq analysis. We will identify genes significantly regulated in the male subset and validate our findings in females using RTqPCR approaches.

C) Mapping of neuronal activation in the pain circuits following the induction of migraine with single injection of GTN and impact of FKBP5 deletion.

We have started mapping the impact of GTN injection on neuronal activation using the immediate

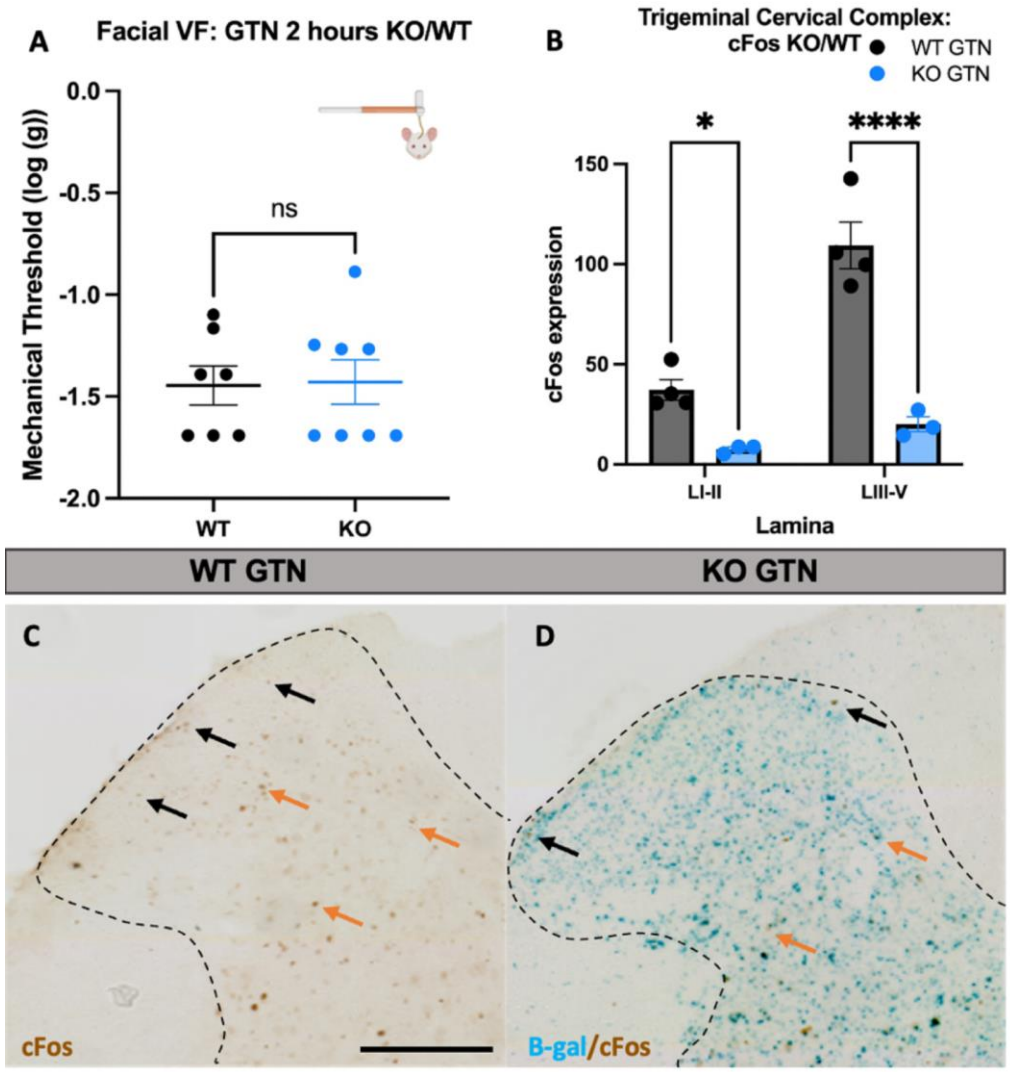

Figure 2. *FKBP5* KO mice develop acute facial hypersensitivity following GTN injection, but dorsal TCC cfos expression is significantly reduced. *FKBP5* KO and WT facial mechanical withdrawal thresholds (A) and TCC cFos expression (B-D) were measured at two hours post GTN injection. There were no significant differences in facial mechanical thresholds between *FKBP5* KO and WT mice. Collated cFos counts of the three TCC regions reveal a significant decrease in overall expression in KO mice, compared to WTs. C-D, representative images of WT (C) and KO (D) TCC (C2 shown). Brown stain: cFos, blue stain: ß-gal. Black arrows: lamina i-ii, orange arrows: lamina iii-v. Grey: WT; Blue: KO. Scale bar=200um, (Post hoc analysis (Sidak's MC): *P>0.05, ***P>0.001). A: n=6/8, B: n=4/3.

early gene cFos and looked at the impact of *FKBP5* deletion. Results are shown below (Fig.2-4).

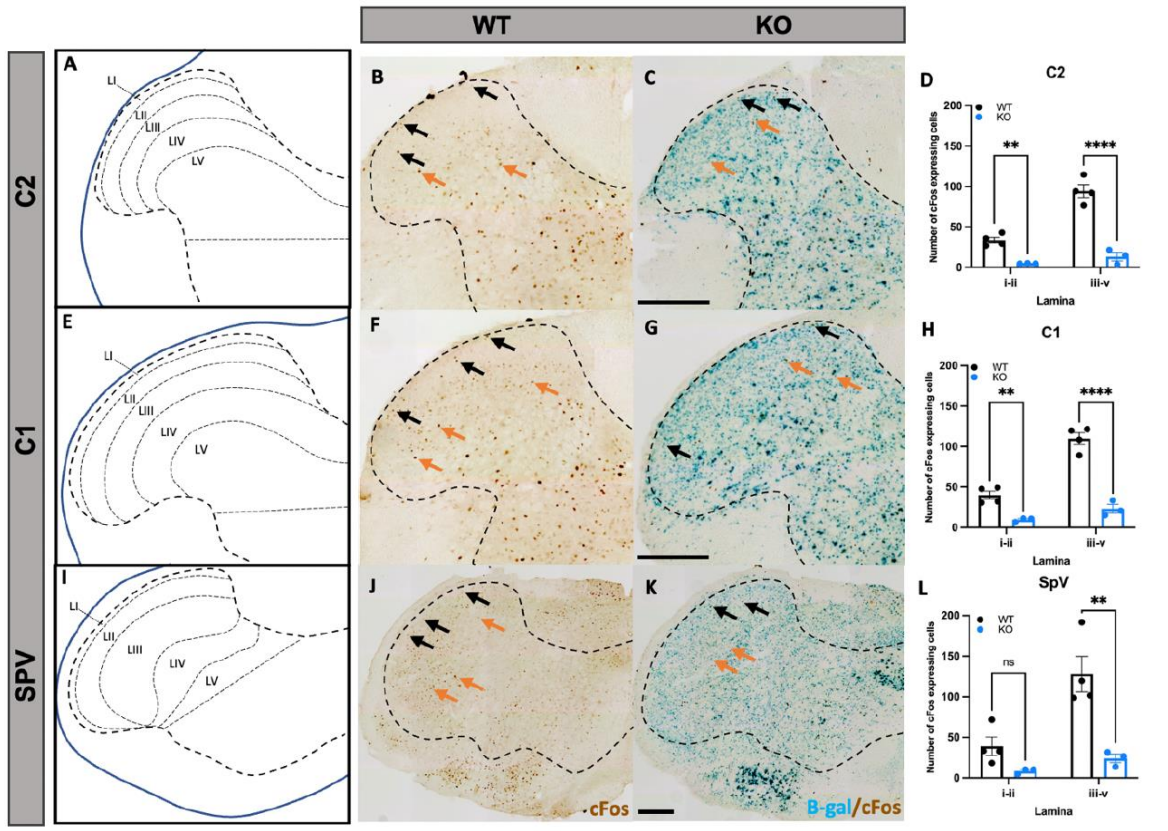

Figure 3. cFos expression is significantly reduced in the TCC dorsal horn of *FKBP5* KO mice at 2 hours post injection. A-C, lamina division (A) and representative images of spinal cord region C2 from WT (B) and KO (C) mice following GTN injection. E-G, lamina division (E) and representative images of spinal cord region C1 from GTN-injected WT (D) and KO (E) mice. I-K, representative images of spinal cord region SpV from GTN-injected WT (G) and KO (H) mice. Raw counts for cFos expression C1 (D), C2 (H) and SpV (L). There were significant differences between genotype in laminae i-ii and iii-v of C1 and C2, but in iii-v only in SpV. Brown: cFos, Blue: B-gal. Black arrows: Li-ii, orange arrows: Liii-v. Grey: WT; Blue: KO. Scale bar: 250um. (Post hoc analysis (Sidak's MC): ***p*<0.01, *****p*<0.0001). n=4/3.

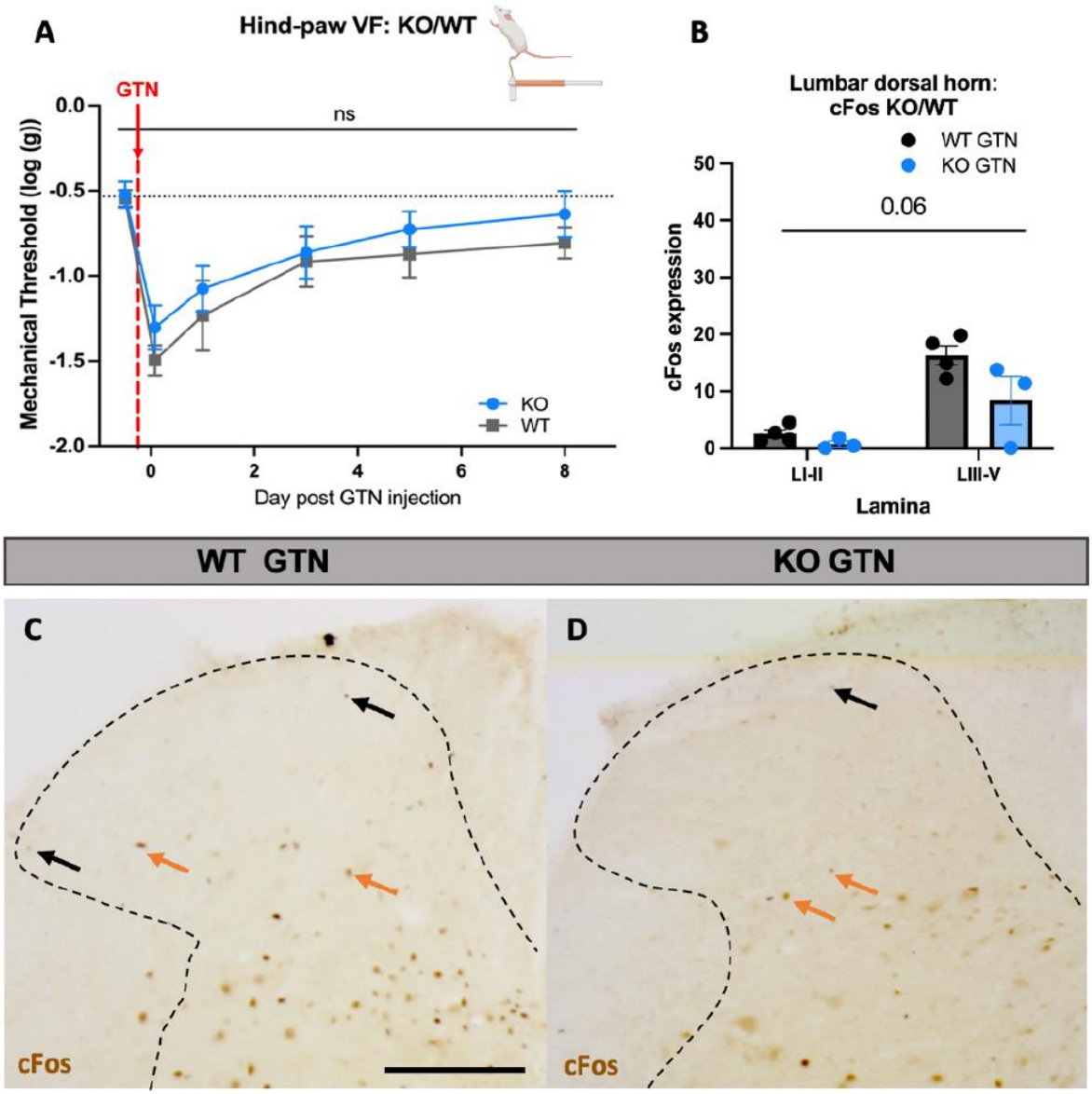

Figure 4. *FKBP5* KO mice develop mechanical hypersensitivity in the hind paw following GTN injection like WT mice, while lumbar dorsal horn cfos expression is reduced. Mechanical withdrawal thresholds were measured in *FKBP5* KO and WT mice, before and after a single systemic GTN injection (A) and cFos expression was measured in the lumbar dorsal horn (B-D). There were no significant differences in mechanical hypersensitivity between *FKBP5* KO and WT -GTN mice. There were trends towards a reduced cFos expression in lumbar dorsal horn of *FKBP5* KO mice. C-D: representative images of FKBP5 WT (C) and KO (D) lumbar spinal cord. Red line: GTN injection. Grey: WT; Blue: KO. Black arrows: Li-ii, orange arrows: Liii-v. Black line: ANOVA significance, genotype. Grey: WT; Blue; KO. Scale bar: 200um. A: n=6/6, B: n=4/3.

We will continue characterising cFos expression after GTN injection in CNS tissue in WT and *FKBP5* KO mice looking at higher brain centres involved in headaches.

2. **Investigation of the role of FKBP51 in cortical spreading depression.**

This part of the project will commence as soon as Dr Anna Andreou returns from maternity leave.