How Brain Plasticity Underpins Sensory Experience and Perceptual Learning

Marianna Hronská, Dr Leena Eve Williams, Sarah Bendová, Jamie Elliott

Simons Initiative for the Developing Brain

The University of Edinburgh

CDBS Centre for Discovery Brain Sciences

Introduction

Touch sensitivities, often induced by aversion to non-harmful touch, are prevalent in neurodevelopmental disorders like Fragile X Syndrome (FXS) — a leading monogenically inherited form of intellectual disability, with up to 60% of individuals being co-diagnosed with autism. *Fmr1*-KO mice, genetic rodent models of FXS, were used to study sensory cortex mechanisms as the rodent whisker system is a tactile equivalent of human hands and this mouse model also exhibits tactile impairments. It is becoming increasingly evident that synaptic plasticity — a change in the connections between neurons, underpins sensory experience and perceptual learning. Initial findings suggest impaired synaptic plasticity *ex vivo* in living brain slices from *Fmr1*-KO mice.

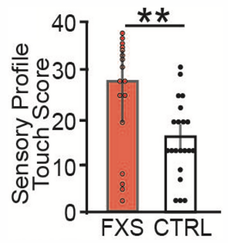


Figure 1: Preliminary data showing Sensory Profile Touch Score of FXS and typically developing children. (p = 0.009**) Based on Tomchek & Dunn.

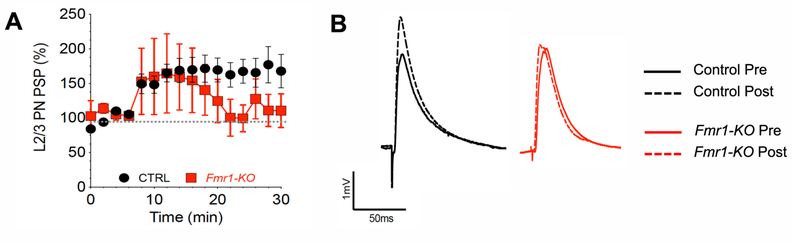


Figure 2: **A)** Post-synaptic potentials in layer 2/3 (L2/3) pyramidal neurons after paired stimulation of thalamic and L4 inputs at 8Hz for 1 minute in *Fmr1*-KO and control mice. **B)** Example post-synaptic potentials before and after rhythmic paired stimulation of inputs onto L2/3 PNs in *Fmr1*-KO and control mice. Figures based on preliminary data.

Aims

1. Develop a **2-photon (2P) calcium (Ca2+) imaging analysis pipeline** based on proxy neuronal activity recordings via the readout of **GCaMP6s fluorescence**. The pipeline will then be used for analysis of **synaptic plasticity** *in vivo* in awake, head-restrained mice.
2. Analyse behavioural recordings of mice undergoing tasks testing **whisker-based somatosensory function in freely moving *Fmr1*-KO mice**.

Methods - 2P Ca2+ imaging pipeline

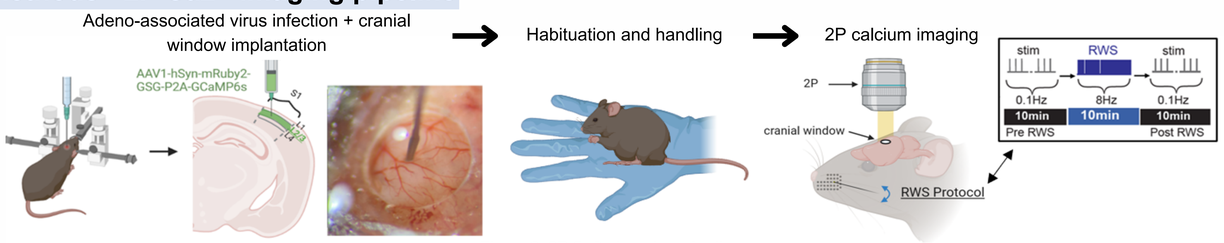


Figure 3: **2P Ca2+ imaging pipeline.**

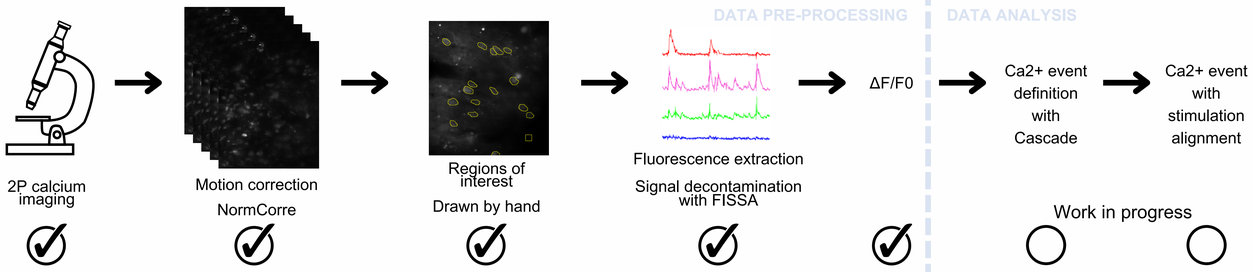


Figure 4: **2P Ca2+ imaging data pre-processing and analysis pipeline.** The pipeline was developed using MATLAB and Python.

Methods - Whisker-based somatosensory behaviour analysis

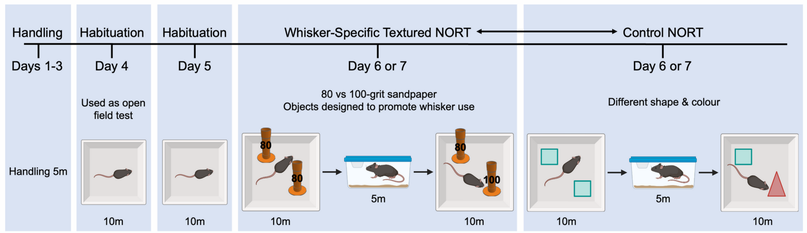


Figure 5: Pilot behavioural experiment to test whisker somatosensation in awake, moving mice. Novel object recognition test (NORT) was used.

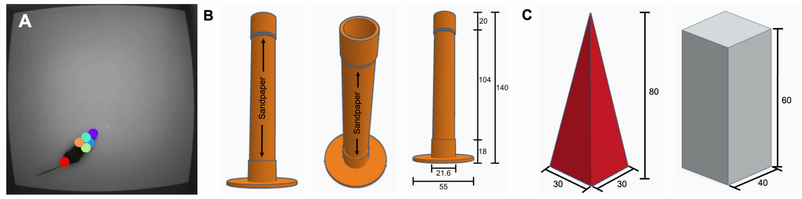


Figure 6: **A)** Anxiety-related behavioural testing analysis using the BORIS software. **B) and C)** final designs for test whisker-specific textured NORT (B) and control NORT (C) objects; dimensions in mm.

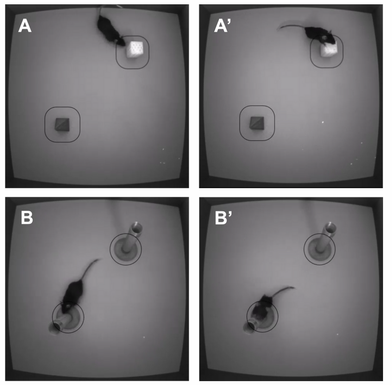


Figure 7: Images from BORIS (Behavioural Observation Research Interactive Software) taken during video scoring of control (A) and test whisker (B) experiments. **A) and B)** Examples of exploration behaviour. **A') and B')** Examples of pre-climbing exploration behaviour (excluded from analysis).

Results - 2P Ca2+ imaging pipeline

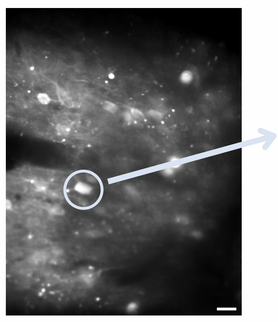


Figure 8: GCaMP6s-expressing neurons in L2/3 of somatosensory cortex.

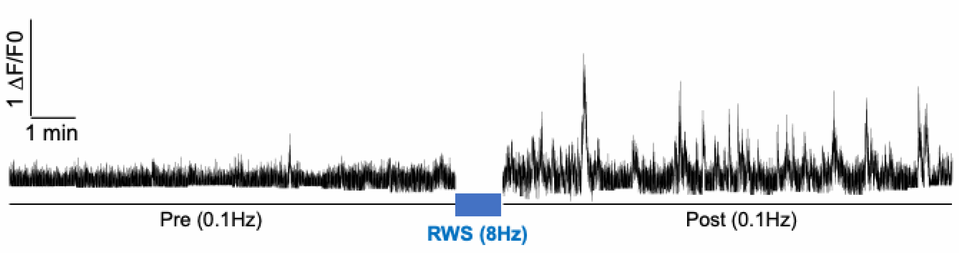


Figure 9: Ca2+ trace of a neural cell circled in figure 8 depicted as ΔF/F in pre-rhythmic whisker stimulation (pre-RWS), compared to post-RSW.

Results - Whisker-based somatosensory behaviour analysis

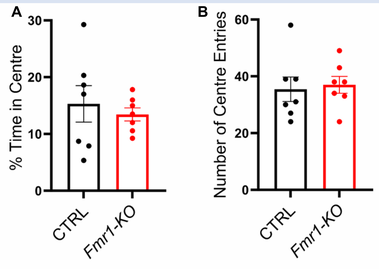


Figure 10: **A)** The percentage of time mice spent in the centre of the arena. **B)** The number of times mice entered the centre of the arena.

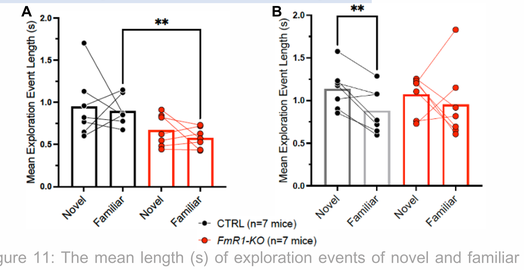


Figure 11: The mean length (s) of exploration events of novel and familiar object in the whisker (A) and control (B) NORT. *Fmr1*-KO mice explored familiar wNORT objects for significantly shorter periods of time per event (control = 0.90 ±0.061s, *Fmr1*-KO = 0.58 ±0.043s, p = 0.0018). Control mice exhibited significantly shorter bouts of exploration of the familiar object in NORT (novel = 1.14 ± 0.092s, familiar = 0.88 ± 0.99s, p = 0.01).

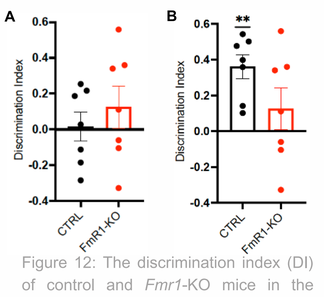


Figure 12: The discrimination index (DI) of control and *Fmr1*-KO mice in the whisker-based (A) and control (B) NORT. NORT experiment identified significant discrimination of the novel object using DI in control mice (DI = 0.36 ± 0.07, p = 0.0016).

Limitations

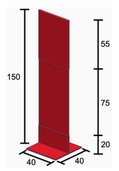
Whisker-based NORT was insufficient for detecting texture discrimination. This could be due to the fact that the mice have learnt to climb the experimental objects, and were trying to exit the arena, thus not performing the task correctly and skewing the results.

Summary

The data pre-processing pipeline is now finished and used in 2P Ca2+ imaging data analysis. Next steps will include inferring spikes and aligning them with the stimulation protocol. The analysis of behavioural videos did not show any whisker novelty recognition. However, *FmR1*-KO mice had significantly shorter mean exploration time of the familiar object than control mice in whisker-specific NORT.

Future work

1. Neuronal activity and thus synaptic plasticity will be assessed using the pipeline in *Fmr1*-KO, compared to wildtype, mice.
2. The behavioural experiment will be continued and replicated with refined objects.



References

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