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Rat Model: SFARI *Fmr1* null allele

The X-linked disorder fragile X syndrome is the most common genetically inherited form of intellectual disability; its core symptoms include anxiety, cognitive challenges and inflexibility. It is caused by genetic silencing of the fragile X mental retardation 1 (*FMR1*) gene that results in the loss of the protein it encodes (FMRP).

To model this disorder in rats, a null mutant was generated using CRISPR/SpCas9 knockout (KO) of *Fmr1* on an outbred Long–Evans background. Briefly, a CRISPR targeting the *Fmr1* exon 8 sequence 5'-GGTCTAGCTATTGGTACTCATGG-3' [protospacer adjacent motif (PAM) in bold) was injected into Crl:LE embryos (Charles River Laboratories). A mutant strain was generated (LE-*Fmr1*^{em2Mcwi} RGDID: 11553873) harboring a net 2-bp insertion (Figure 1A). Complete loss of FMRP expression was confirmed by Western blot (Figure 1B). Rats lacking FMRP appear healthy, fertile and indistinguishable from wild-type (WT) littermates by bodyweight (Figure 1C). Knockout rats had significantly increased testicular weights at 30 days of age (Figure 1D). For all the data presented below, heterozygous females were mated with WT male rats. All data is from *Fmr1*$^{+/y}$ and *Fmr1*$^{-/y}$ male rats from several litters but all with littermate controls. Furthermore, the phenotypes presented below for this model are in good agreement with those found in two additional *Fmr1* null mutant lines, one generated by the Simons Foundation Autism Research Initiative (SFARI), the other generated by the Patrick Wild Centre, Edinburgh University.

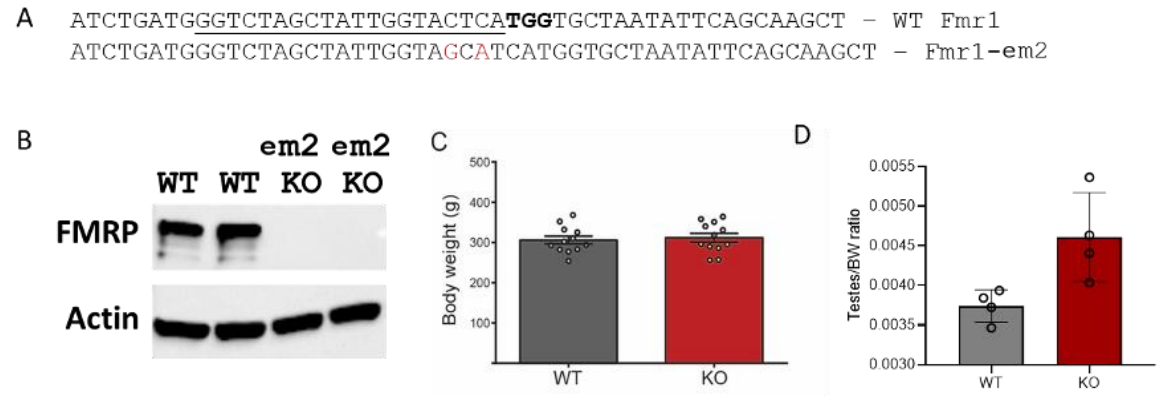

Figure 1. Generation and confirmation of *Fmr1* null rats. (A) The SpCas9 target site for mutagenesis of exon 8 is underlined and protospacer adjacent motif (PAM) in bold. The *Fmr1*-em2 mutant harbors 2-bp insertion in exon 8 (red sequence) compared to the wild-type sequence. (B) FMRP immunoblots of hippocampal homogenates confirming complete ablation of FMRP expression. (C) Comparable bodyweight in adult *Fmr1* KO rats and WT littermates (n= 12 *Fmr1*$^{+/y}$, 12 *Fmr1*$^{-/y}$). (D) Testes to bodyweight (BW) ratio is increased in *Fmr1* KO rats compared to WT littermates (n= 4 *Fmr1*$^{+/y}$, 4 *Fmr1*$^{-/y}$). Figure adapted from Miller E.A. *et al.*[1]

Compared to WT littermates, adult *Fmr1* KO rats exhibit similar locomotor activity in an open field (Figure 2A), but impaired motor coordination on the accelerating rotarod (Figure 2B). Interest in foreign objects as assessed in the marble interaction task was comparable between genotypes (Figure 2C).

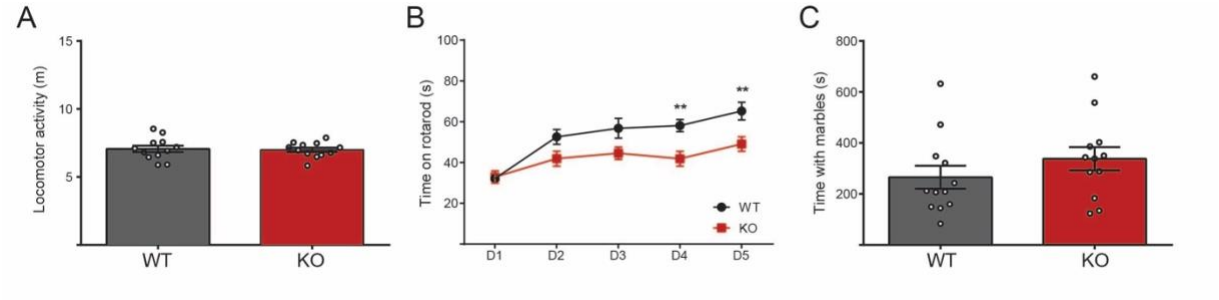

Figure 2. Locomotor function and object interaction. (A) Distance traveled in an open field (n= 12 *Fmr1*$^{+/y}$, 12 *Fmr1*$^{-/y}$). (B) Latency to fall from an accelerating rotarod over training days (n= 12 *Fmr1*$^{+/y}$, 12 *Fmr1*$^{-/y}$; two-way repeated measures ANOVA: Factor Genotype P<0.01; Days P<0.001; Interaction P<0.005). (C) Time interacting with marbles in a familiar cage (n= 12 *Fmr1*$^{+/y}$, 12 *Fmr1*$^{-/y}$).

To investigate the effect of the loss of FMRP on cognitive function and adaptive behaviours, we used an active place avoidance task to assess active acquisition and reacquisition of the shock zone in a circular rotating arena. Adult *Fmr1* KO rats appear to receive more number of shocks during trial one of both training sessions (Figures 3A and B) as well as during relearning of new shock zone during the conflict session (Figure 3C).

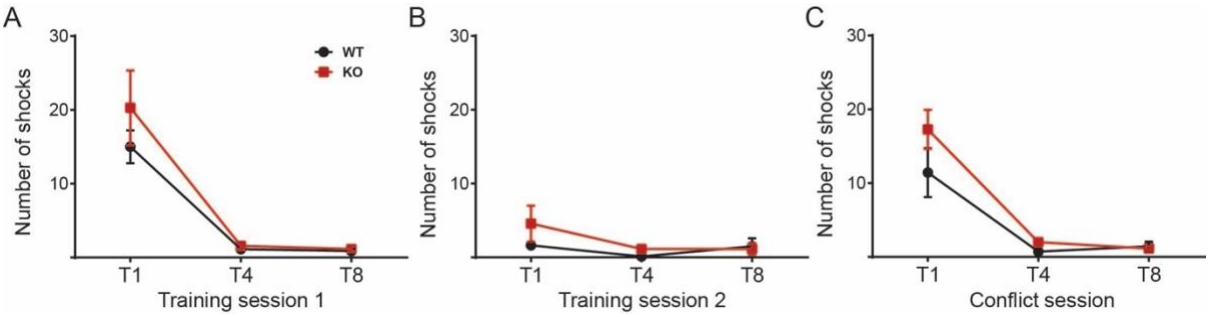

Figure 3. Preliminary data showing associative learning and recall in an active place avoidance task. (A-C) The number of shocks received during the training session one (A), training session two (B; 24 hours apart) and during relearning of new shock zone during conflict session (C). Data collection is ongoing (n= 8 *Fmr1*$^{+/y}$, 7 *Fmr1*$^{-/y}$) but current trends agree with findings from *Fmr1*$^{-y}$ null mutant rats generated in Edinburgh showing an increase number of shocks are necessary for learning the location of the shock zone. Protocols from O'Reilly K.C. *et al.*[2].

Abnormal response to sensory stimuli is commonly associated with fragile X syndrome in humans. Using the tail-flick paradigm to test reflexive response to thermal pain, we find that this model of *Fmr1* KO rats exhibits similar pain sensitivity compared to WT littermates (Figures 4A and B). To cope with anxiety, the perseverative or repetitive behavior in humans with fragile X syndrome has also been reported. We examined this behavioral phenotype with wood chew task and *Fmr1* KO rats showed high perseverative behavior compared to WT littermates (Figure 4C).

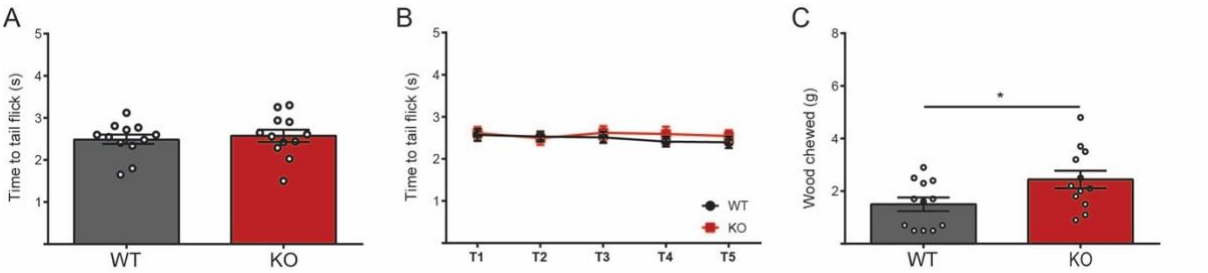

Figure 4. Thermal pain sensitivity and perseverative behavior. (A) Latency to withdraw tail in response to the heat source and (B) during five testing trials. (C) Mass of wood chewed from a woodblock given to animals (n= 12 *Fmr1*$^{+/y}$, 12 *Fmr1*$^{-/y}$), paired t-test, $p<0.05$).

References

1. Miller E.A. *et al*. *Mol. Psychiatry* Epub ahead of print (2020) PubMed

2. O'Reilly K.C. *et al. Front. Neurosci.* **8**, 153 (2014) PubMed

Acknowledgements and contacts

LE-Fmr1-em2 rats were generated by the Medical College of Wisconsin with support from SFARI.

Additional information on the LE-Fmr1-em2 rats, including genotyping and breeding protocols, can be found here.

Animals were phenotyped through a partnership with the Simons Initiative for the Developing Brain at the Center for Brain Developmental and Repair in Bangalore. Additional behavioral experiments are ongoing, and validated results will be periodically added to this data sheet. For questions or comments regarding behavioral protocols and data, please contact Peter Kind (P.Kind@ed.ac.uk) at the University of Edinburgh.

LE-Fmr1-m2 rats can be obtained from the Medical College of Wisconsin by contacting mcwcustomrats@mcw.edu. A standard MTA is required.