Role of mGluR7 in Fragile X syndrome

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INTRODUCTION

Fragile X syndrome (FXS):

- FXS is the leading cause of inherited autism and intellectual disabilities.
- FXS is caused by silencing of *FMR1* gene that codes for protein FMRP, a repressor of protein synthesis.
- Lack of FMRP leads to excessive protein synthesis in the brain that is responsible for most of the disease symptoms.

Metabotropic Glutamate Receptor 7 (mGluR7):

- mGluR7 is a member of Group III mGluRs, and it is linked with idiopathic autism and developmental delay.
- Mice deficient in mGluR7 show spontaneous seizures and impaired neuronal plasticity and working memory.
- mGluR7 is localized in presynaptic active zones of GABAergic neurons and in the postsynaptic terminals of glutamatergic neurons.

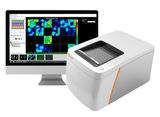
METHODS

1. Animals
WT and *Fmr1* KO mice (C57BL/6J background) and *Grm7* KO mice (MMRRC, B6.129P2-Grm7tm1Dgen/ Mmnc, stock No. 011626-UNC).

2. Primary cortical neuron cultures
Prepared from postnatal day 0-1 pups. Cultures were treated on 12-14th day (DIV 12-14).

3. Western blotting

4. Multielectrode Array recordings
Maestro Edge™ multiwell microelectrode array (MEA) with Cytoview MEA6 (6- well) plates from Axion Biosystems used to perform extracellular recordings.



5. Whole cell patch clamp recordings
Action potential (AP) firing was recorded in cultured neurons via whole-cell current clamp recording.

6. Audiogenic seizure monitoring
Fmr1 KO mice (P20-22) - saline or AMN082 (1mg/kg, IP). 110 dB auditory stimulus was presented for 2 minutes. Seizure was scored.

7. Behavioral tests
Behavioral tests were conducted on 6-8-week-old WT and *Fmr1* KO mice.

- Marble Burying test (MB)
- Novel Object Recognition test (NOR)
- Contextual Fear Conditioning test (CFC)

RESULTS

1. mGluR7 surface expression is altered in *Fmr1* KO neurons

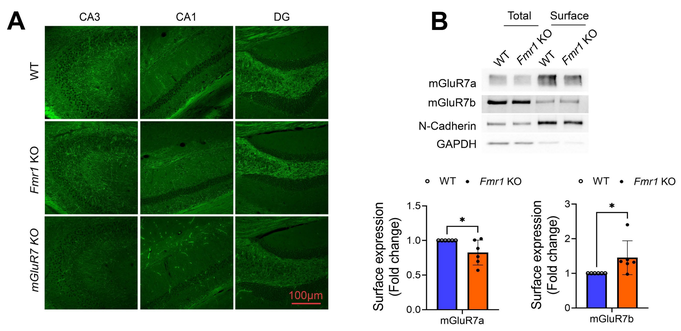


Figure 1. (A) Immunohistochemical images showing expression of mGluR7a in hippocampus of WT, *Fmr1* KO and *mGluR7* KO mice (n=4).

(B) Surface expression of mGluR7a and mGluR7b in WT and *Fmr1* KO cortical primary neurons (n=5-8).

Student's t-test, mean ± SEM with *p< 0.05, and NS: non-significant.

RESULTS

2. Activation of mGluR7 reduces protein synthesis in both WT and *Fmr1* KO neurons via ERK1/2 and eIF4E signaling

In vitro

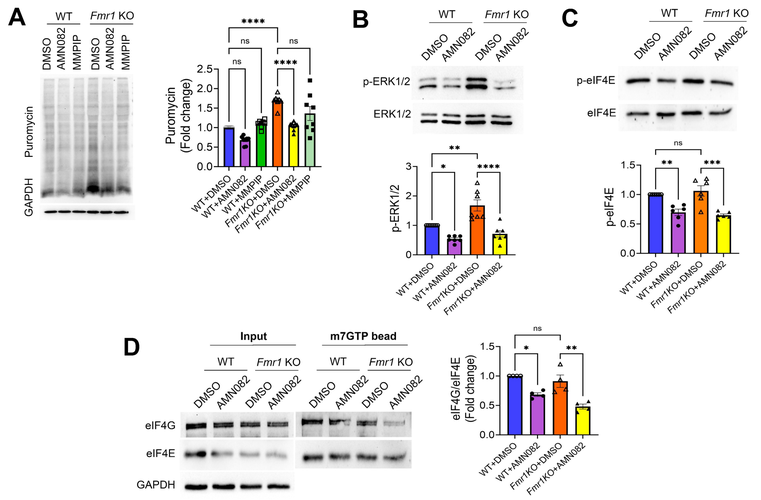


Figure 2. (A) Western blots and quantification showing puromycin labelling in WT and *Fmr1* KO primary cortical neurons. Neurons were treated with DMSO, AMN082 (1 μM), or MMPIP (1 μM) for 2 h and with puromycin (10 μg/ml) for another hour (n=8).

(B-C) Western blots showing p-ERK1/2 and eIF4E levels in WT and *Fmr1* KO primary cortical neurons. Neurons were treated with DMSO or AMN082 (1 μM) for 2 h (n=6).

(D) Western blots showing levels of eIF4E and eIF4G pulled down by m7GTP beads from WT and *Fmr1* KO primary cortical neurons treated with AMN082 (1 μM) for 2 h (n=4).

Two-way ANOVA (A, C-D) with Tukey test, mean ± SEM with *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and NS: non- significant.

In vivo

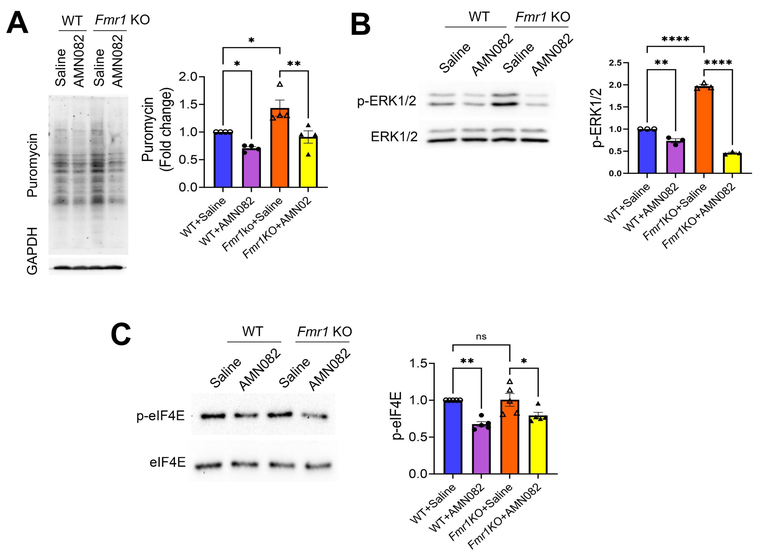
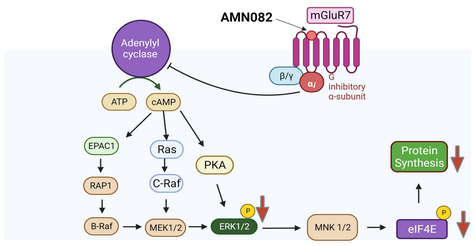


Figure 3. (A) Western blots showing hippocampal protein synthesis in vivo after puromycin labelling. WT and *Fmr1* KO mice injected with saline or AMN082 (1 mg/kg) and puromycin (200 mg/kg) for 1 h (n = 4). Student's t-test, mean± SEM with *p < 0.05, **p < 0.01.

(B, C) Western blots showing p-ERK1/2 and p-eIF4E levels in hippocampus of WT and *Fmr1* KO mice (n = 5). Two-way ANOVA with Tukey test, mean ± SEM with *p < 0.05, **p < 0.01, ****p < 0.0001 and NS: non-significant.



Proposed signaling pathway in mediating effect of AMN082 on reduction of protein synthesis.

RESULTS

3. Activation of mGluR7 reduces neuronal excitability

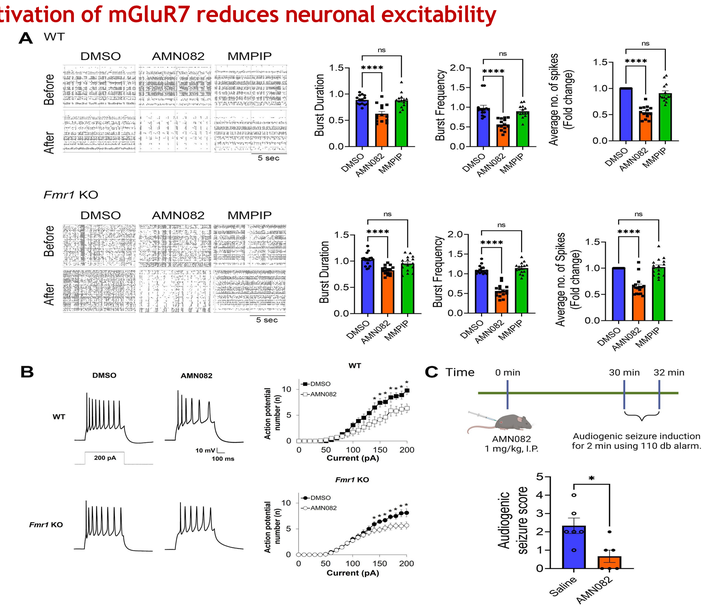


Figure 4. (A) Assessment of neuronal network activity in WT and *Fmr1* KO primary cortical neurons using MEA (n=5).

(B) Whole-cell patch clamp recordings indicating reduction of AP numbers in AMN082 treated WT and *Fmr1* KO primary cortical neurons (n=12-14).

(C) AMN082 treatment reduces audiogenic seizure susceptibility in *Fmr1* KO mice (n=6). One-way ANOVA with Tukey test (A, B) or two-tailed Mann-Whitney test (C), mean ± SEM with *p < 0.0001 and NS: non-significant.

4. mGluR7 activation reduces autism-like behavior and improves learning and memory in *Fmr1* KO mice

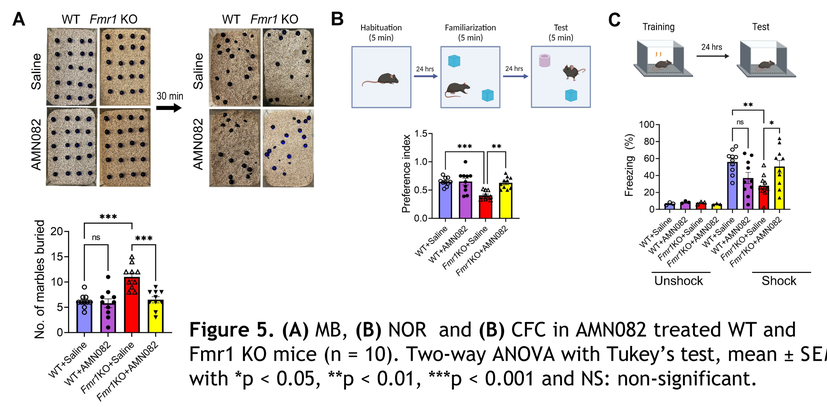
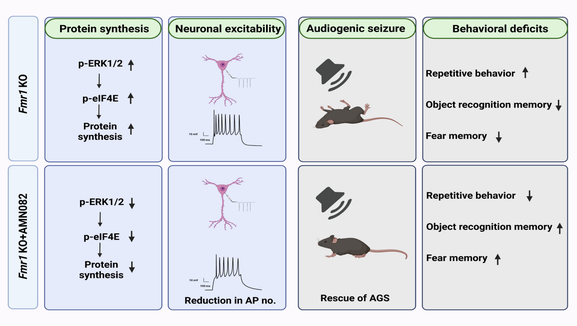


Figure 5. (A) MB, **(B)** NOR and **(B)** CFC in AMN082 treated WT and Fmr1 KO mice (n = 10). Two-way ANOVA with Tukey's test, mean ± SEM with *p < 0.05, **p < 0.01, ***p < 0.001 and NS: non-significant.

CONCLUSION

Summary of the effects of mGluR7 activation on *Fmr1* KO mice



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ACKNOWLEDGEMENTS

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