

SUPPLEMENTARY MATERIAL

Supplementary Figures

Supplementary Figure S.1. YAC genetic rescue strategy.

Male WT, *Fmr1* KO and *Fmr1* KO/YAC (or YAC rescue) mice were obtained by crossing females heterozygous for the *Fmr1* mutation (*Fmr1*^{+/-}), to male WT/YAC transgenic mice²⁷. + and – indicate presence or absence of the *Fmr1* mutation or YAC transgene. Y represents the Y chromosome.

Supplementary Figure S.2. *In silico* identification of quadruplex forming G-rich sequences in the 3'UTR of GABA_A receptor α_1 and δ subunit mRNA.

Putative quadruplex forming G-rich sequences (QGRS) were identified using an *in silico* tool (<http://bioinformatics.ramapo.edu/QGRS2/index.php>) and are indicated in grey background. The G-rich part of the GABA_A receptor α_1 3'UTR is indicated with a grey box.

Supplementary Figure S.3. Ganaxolone modulates explorative behaviour in the open field

Vehicle (0 mg/kg body weight, VEH) or ganaxolone (10 mg/kg, GAN) was administered to *Fmr1* KO mice (grey bars) and WT littermates (white bars). Significant differences between the groups were detected in (A) the total distance travelled in the open field ($p=0.003$, one-way ANOVA; post-hoc Bonferroni t-test), (B) the distance travelled in the periphery ($p=0.008$, Kruskal-Wallis; post-hoc Dunn's test), (C) the distance travelled in the corners ($p=0.008$, Kruskal-Wallis; post-hoc Dunn's test), (D) the duration in the corners ($p=0.035$, Kruskal-Wallis; post-hoc Dunn's test). Overall, ganaxolone treatment did not have a significant effect on explorative behaviour in *Fmr1* KO and WT mice. No significant differences were observed for the other parameters (i.e. number of entries in the centre circle and number of entries in the corners, data not shown). ** $p<0.01$; * $p<0.05$. $n=17-21$ /group.

Supplementary Figure S.4. Ganaxolone does not affect overall cage activity

Vehicle (0 mg/kg body weight) or ganaxolone (10 mg/kg) was administered to *Fmr1* KO mice (A and B, respectively) and WT littermates (C and D, respectively). No significant differences were found in total activity counts ($p=0.768$, Kruskal-Wallis), nor during the first 30 min ($p=0.497$, Kruskal-Wallis), nor during the last 30 min ($p=0.718$, Kruskal-Wallis test). Analysis of the activity pattern indicates a significant decline in activity over time (effect group $p=0.739$, effect time $p<0.001$; effect group x time $p=0.073$, two-Way repeated-measures ANOVA). Error bars indicate SEM. $n=17-21$ /group.

Supplementary Tables

Supplementary Table S.1. Primer sequences used in quantitative real-time PCR experiments with SYBR Green

Gene	F-primer (5' → 3')	R-primer (5' → 3')
<i>Gabra1</i> (α_1)	GCCCTCCCAAGATGAACTTA	CTCTCCCAAACCTGGTCTCA
<i>Gabra2</i> (α_2)	ACCATGAGGCTTACAGTCCA	TTTCAGTGGGCATGAATGAG
<i>Gabra3</i> (α_3)	GATCTCACAGGTCTTCAAGTTGC	ACAACCTCTGGTCACATAAAAAGTGC
<i>Gabrb1</i> (β_1)	GAGTTCACAACAGGGGCATA	GACACCCAGGACAGGATTGT
<i>Gabrb2</i> (β_2)	GTGTCAATGACCCTAGTAATATGTCC	TCTGGTCTCAGACGAATGTCA
<i>Gabrd</i> (δ)	GGAGGTGCTCCTGTGAATGT	GGTGTATTCCATGTTTGCCTCT
<i>Gabrg1</i> (γ_1)	CCCTGGGTATCACTACGGTTT	AGAGATCCATTGCTGTCACG
<i>Gabrg2</i> (γ_2)	AGTGAAGACAACCTTCTGGTGAC	GACAGTAGTGATTCTAAAGATG
<i>Abat</i> (GABA-T)	CTCGTTCAACAGCCTCAGAA	GACTCCTGGAGCTTGCCAC
<i>Gad2</i> (Gad65)	AACCAGTCTGCTGCTAATCCA	GCAATTAACAGGGCATCC
<i>Gad1</i> (Gad67)	GGTGGAGCGATCAAATGTCT	GCACAGAGACCGACTTCTCC
<i>Fmr1</i>	CCGAACAGATAATCGTCCACG	ACGCTGTCTGGCTTTTCCTTC

Supplementary Table S.2. Reference gene stability in *Fmr1* KO experiments

Brain region	Reference gene	CV ^A	M (geNorm) ^B
Cortex	<i>Ap3d1</i>	0.142	0.327
	<i>Cdc40</i>	0.096	0.249
	<i>Zfp91</i>	0.092	0.239
	Mean	0.110	0.271
Cerebellum	<i>Ap3d1</i>	0.048	0.135
	<i>Cdc40</i>	0.059	0.146
	<i>Gapdh</i>	0.074	0.168
	Mean	0.061	0.150

^A The coefficient of variation (CV) represents the variation of the normalised relative quantities of a reference gene across all samples. CV should be lower than 0.25 for stable reference genes.

^B M is a value for the gene expression stability and should be lower than 0.5 for stable reference genes).

Supplementary Table S.3. Assays on demand real-time PCR YAC

Gene	Assay ID
Reference genes	
<i>Gapdh</i>	Mm99999915_g1
<i>Hprt</i>	Mm00446968_m1
<i>Hmbs</i>	Mm006660262_g1
Genes of interest	
<i>Gabra1</i> (α_1)	Mm00439040_m1
<i>Gabra2</i> (α_2)	Mm00433435_m1
<i>Gabra3</i> (α_3)	Mm00433440_m1
<i>Gabra4</i> (α_4)	Mm00802631_m1
<i>Gabrb1</i> (β_1)	Mm00433461_m1
<i>Gabrb1</i> (β_2)	Mm00433467_m1
<i>Gabrd</i> (δ)	Mm00433476_m1
<i>Gabrg1</i> (γ_1)	Mm00439047_m1
<i>Gabrg2</i> (γ_2)	Mm00433489_m1
<i>Gad1</i> *	Mm00725661_s1
<i>Slc6a1</i> *	Mm00618601_m1
<i>Slc6a11</i> *	Mm00556476_m1
<i>Aldh5a1</i> *	Mm00553115_m1
<i>Gphn</i> *	Mm00556895_m1
Controls	
<i>Map1b</i>	Mm00485261_m1
<i>Fmr1</i>	Mm00484415_m1

**Aldh5a1* (*Ssadh*), *Gad1* (GAD67), *Gphn* (*gephyrin*), *Slc6A1* (GAT1), *Slc6a11* (GAT4)

Supplementary Table S.4. Reference gene stability in YAC rescue experiments

Reference gene	CV ^A	M (geNorm) ^B
<i>Gapdh</i>	4.52	0.128
<i>Hprt</i>	4.36	0.128
Mean	4.44	0.128

^A The coefficient of variation (CV) represents the variation of the normalised relative quantities of a reference gene across all samples. CV should be lower than 0.25 for stable reference genes.

^B M is a value for the gene expression stability and should be lower than 0.5 for stable reference genes.