

Gamma oryzanol impairs alcohol-induced anxiety-like behavior in mice via upregulation of central monoamines associated with *Bdnf* and *Il-1 β* signaling

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Supplemental Table S1 Summary results of statistical analysis

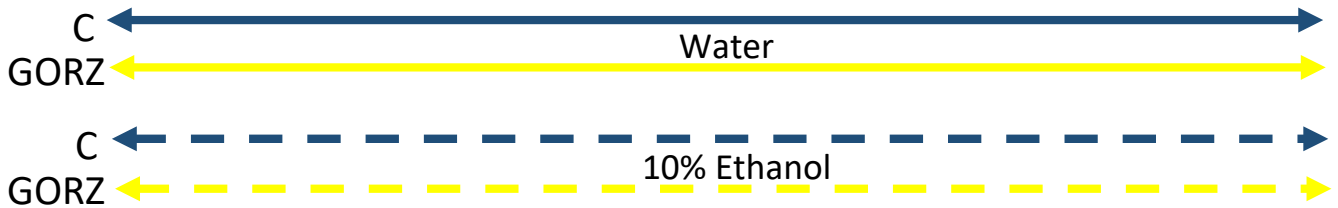
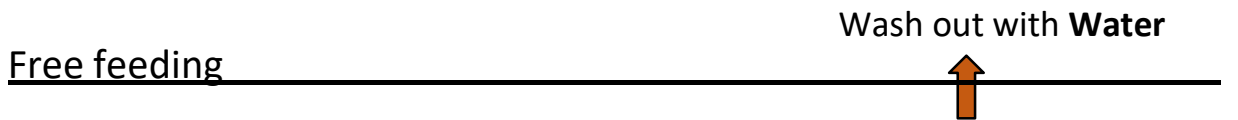
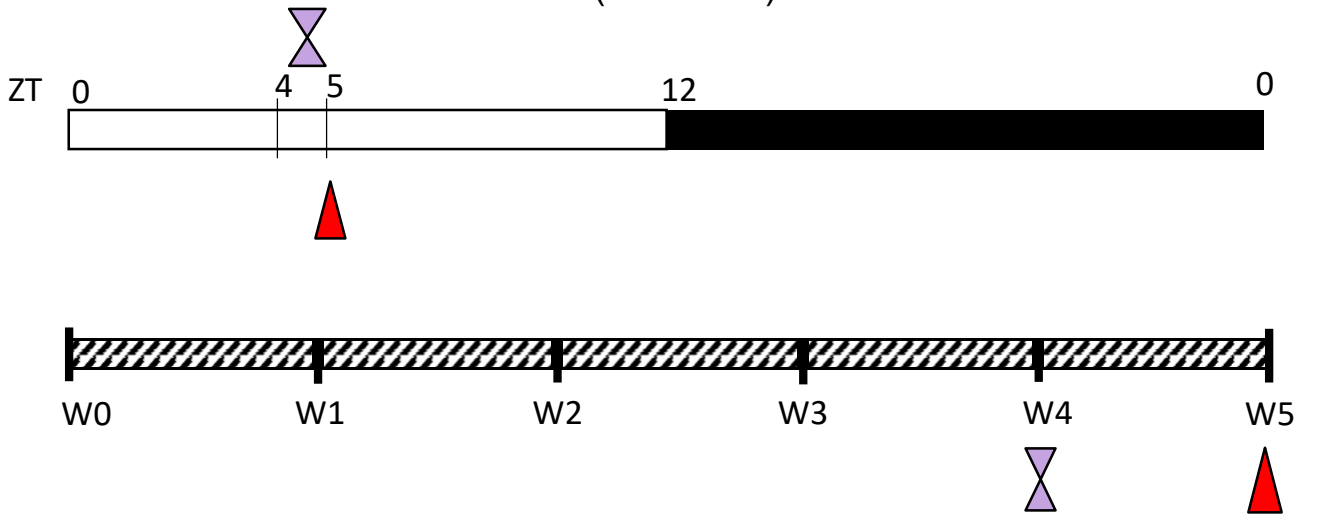
Figure	Test	Detail	Parametric Non-parametric	Analysis	Factor	F value	P value
1 (A)	Body weight	W0 body weight (g)	Non-parametric	Kruskal-Wallis	-	-	0.1898
1 (A)	Body weight	W1 body weight (g)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 3.845 F (1, 41) = 0.3748 F (1, 41) = 0.1094	0.1848 0.6759 0.8212
1 (A)	Body weight	W2 body weight (g)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 9.997 F (1, 41) = 6.337 F (1, 41) = 0.1994	0.1486 0.2479 0.8363
1 (A)	Body weight	W3 body weight (g)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 0.002959 F (1, 41) = 0.02172 F (1, 41) = 0.626	0.9859 0.9619 0.7975
1 (A)	Body weight	W4 body weight (g)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 0.06366 F (1, 41) = 0.01126 F (1, 41) = 1.559	0.9472 0.9778 0.7432
1 (C)	Food intake	Food intake for 2 weeks (g)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 13) = 0.003252 F (1, 13) = 1.801 F (1, 13) = 0.09367	0.8597 0.2025 0.7644
1 (E)	Drink intake	Drink intake for 2 weeks (g)	Non-parametric	Kruskal-Wallis	-	-	0.5011
2 (A)	Elevated plus maze test	Distance travelled in the open arm (m)	Non-parametric	Kruskal-Wallis	-	-	0.0012
2 (B)	Elevated plus maze test	Time in the open arm (s)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 4760 F (1, 41) = 806.2 F (1, 41) = 10585	0.0106 0.2770 0.0003
2 (C)	Elevated plus maze test	No. of entries into the open arm	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 127.2 F (1, 41) = 13.28 F (1, 41) = 117.7	0.0567 0.5300 0.0664
2 (D)	Elevated plus maze test	Distance travelled in the closed arm (m)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 0.8924 F (1, 41) = 0.001496 F (1, 41) = 0.7428	0.3672 0.9704 0.4103
2 (E)	Elevated plus maze test	Time in the closed arm (s)	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 41) = 6047 F (1, 41) = 1079 F (1, 41) = 7886	0.0450 0.3873 0.0230
2 (F)	Elevated plus maze test	No. of entries into the closed arm	Non-parametric	Kruskal-Wallis	-	-	0.4439
3 (A)	HPLC	5-HT in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.0125
3 (B)	HPLC	5-HIAA in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.0002
3 (C)	HPLC	HVA in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.1429
3 (D)	HPLC	NE in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.5048
3 (E)	HPLC	MHPG in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.0027
3 (F)	HPLC	5-HT in cerebral cortex	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1, 49) = 0.5519 F (1, 49) = 0.3085 F (1, 49) = 0.4373	0.4611 0.5811 0.5115
3 (G)	HPLC	5-HIAA in cerebral cortex	Non-parametric	Kruskal-Wallis	-	-	0.0087
3 (H)	HPLC	HVA in cerebral cortex	Non-parametric	Kruskal-Wallis	-	-	0.0061
3 (I)	HPLC	NE in cerebral cortex	Non-parametric	Kruskal-Wallis	-	-	0.5423
3 (J)	HPLC	MHPG in cerebral cortex	Non-parametric	Kruskal-Wallis	-	-	0.0223
4 (A)	RT-PCR	<i>Bdnf</i> expression in hippocampus	Non-parametric	Kruskal-Wallis	-	-	0.0118
4 (B)	RT-PCR	<i>Il1-β</i> expression in hippocampus	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1,37) = 6.438 F (1,37) = 0.2536 F (1,37) = 4.723	0.0155 0.6175 0.0362
4 (C)	RT-PCR	<i>Bdnf</i> expression in cerebral cortex	Non-parametric	Kruskal-Wallis	-	-	0.0805
4 (D)	RT-PCR	<i>Il1-β</i> expression in cerebral cortex	Non-Parametric	Kruskal-Wallis	-	-	0.2073
5 (A)	ELISA	BDNF concentration in serum	Non-parametric	Kruskal-Wallis	-	-	0.4873
5 (B)	ELISA	IL1-β concentration in serum	Parametric	ANOVA Factor1 Factor2 Interaction factor	Food Drink Food × Drink	F (1,21) = 0.1322 F (1,21) = 0.823 F (1,21) = 0.758	0.6697 0.2926 0.3119

Supplemental Table S2 Primer sequence for reverse transcription polymerase chain reaction

Gene		Sequences
<i>Bdnf</i>	Forward	5'-gcggcagataaaaaga-3'
	Reverse	5'-tcagttggcctttgga-3'
<i>IL1-β</i>	Forward	5'-tccatgagctttgtacaagg -3'
	Reverse	5'-ggtgctgatgtaccagttgg -3'
<i>Gapdh</i>	Forward	5'-tggtgaaggtcgggtgaac-3'
	Reverse	5'-aatgaaggggtcgttgatgg-3'



Supplementary Fig. 1 Experiment Design

5 W old ICR male mice
(n = 10-12)



C (Control, 14% casein, AIN 93 M)
GORZ (γ -Oryzanol, 14% casein, AIN 93 M + 0.5% GORZ)
W (Week)
ZT (Zeitgeber time)

Sample: Blood, Hippocampus, cerebral cortex

 Elevated plus maze test  Sampling