Supplementary Materials

Influence of Stress Severity on Contextual Fear Extinction and Avoidance in a Posttraumatic-Like Mouse Model

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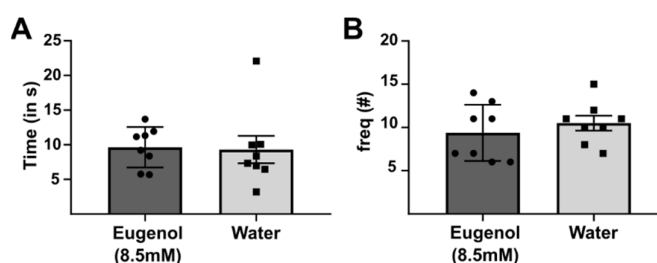* Correspondence: catherine.belzung@univ-tours.fr

Figure S1. Eugenol did not induce preference behaviors in naïve C57BL6N mice. The odor preference test was performed in a three-chamber arena with on one side an object cleaned with an 8.5mM solution of eugenol and on the other side the identical object but cleaned with water. Mice were placed at the center of the arena and were allowed to explore for 10 minutes (A) the time spent sniffing the objects was manually measured. Mice spent the same amount of time sniffing both the eugenol-scented object and the neutral-scented object (paired t-test: t=0.1394, p=0.8930). (B) They did not exhibit higher frequency (freq) of smelling event on either object (paired t-test: t=0.6854, p= 0.5151).

Table S1. Detailed statistical analysis and results for each behavioral outcome presented (ext: Extinction).

Behavioral Test	Statistical Test	p-Value	*post-hoc*
Distance moved during habituation to NOR arena	Shapiro-Wilk	p=0.1986	
	Bartlett's	p=0.3070	
	Brown-Forsythe	p=0.1238	
	One-way ANOVA	F(2, 55)=10.58; p=0.0001	Tukey CTRL vs PTSD2: p=0.0004 CTRL vs PTSD4: p=0.0007 PTSD2 vs PTSD4: p=0.9786
Correlation distance moved during habituation and freezing at the 1st re-exposure	Shapiro-Wilk	PTSD2: Distance: p=0.5666; freezing: p=0.7867 PTSD4: Distance: p=0.1865; freezing: p=0.6214	
	Pearson	PTSD2: r=-0.3551; p=0.1261 PTSD4: r=-0.5422; p=0.0135	
Familiarization during NOR learning	Shapiro-Wilk	p=0.0159	
	Bartlett's	p=0.6741	

			Dunn CTRL vs PTSD2: p=0.0042 CTRL vs PTSD4: p=0.0277 PTSD2 vs PTSD4: p>0.9999
	Kruskal-Wallis	H=11.39, p=0.0034	
Distance moved during NOR test	Shapiro-Wilk	p=0.8745	
	Bartlett's	p=0.1472	
	Brown-Forsythe	p=0.1221	
	One-way ANOVA	F(2, 55)=2.221; p=0.1182	Tukey CTRL vs PTSD2: p=0.9916 CTRL vs PTSD4: p=0.0545 PTSD2 vs PTSD4: p=0.0345
NOR test (gross)	2w RM ANOVA	Interaction: F(2, 55)=1.231; p=0.2999 grp: F(1, 55)=2.706, p=0.1057 obj: F(2, 55)=4.819, p=0.0118	Sidak on obj factor new vs known CTRL: p>0.999 PTSD2: p=0.9041 PTSD4: p=0.0868
NOR test (normalized)	Shapiro-Wilk	p=0.9820	
	Bartlett's	p=0.3019	
	Brown-Forsythe	p=0.5024	
	1w ANOVA	F(2, 55)=4.113; p=0.0216	
	One sample t test vs 50%	CTRL: t=0.05118; df=17; p=0.9598 PTSD2: t=0.1173; df=19; p=0.9078 PTSD4: t=3.539; df=19; p=0.0022	
Distance moved during the avoidance test	Shapiro-Wilk	p=0.0004	
	Bartlett's	p=0.0149	
	Kruskal-Wallis	H=10.21; p=0.0061	Dunn CTRL vs PTSD2: p=0.0133 CTRL vs PTSD4: p=0.0187 PTSD2 vs PTSD4: p>0.9999
Time spent on each object during avoidance test pre-ext	2w RM ANOVA	Interaction: F(2, 55)=2.352; p=0.1047 grp: F(2, 55)=26.25; p<0.0001 obj: F (1, 55) = 23.28; p<0.0001	Tukey (group factor): CTRL vs PTSD2: p<0.0001 CTRL vs PTSD4: p<0.0001 PTSD2 vs PTSD4: p=0.8335 Sidak (obj factor: neutral - reminder): CTRL: p=0.6481 PTSD2: p=0.0003 PTSD4: p=0.0077
Time spent in each room during avoidance test pre-ext	2w RM ANOVA	Interaction: F(2, 55)=12.83; p<0.0001 grp: F(2, 55)=18.67; p<0.0001 room: F(1, 55)=55.29; p<0.0001	Tukey (interaction): neutral: CTRL vs PTSD2: p=0.9891 CTRL vs PTSD4: p=0.9942 PTSD2 vs PTSD4: p=0.9660 reminder:CTRL vs PTSD2: p<0.0001 CTRL vs PTSD4: p<0.0001

			PTSD2 vs PTSD4: p=0.5417 Sidak (neutral vs reminder) CTRL: p=0.9987 PTSD2: p<0.0001 PTSD4: p<0.0001
Extinction acquisition	RM GLM	Day×Time×group: F(12, 330)=1.28; p=0.2261 Day×group: F(4, 110)=21.22; p<0.0001 Time×group: F(6, 165)=15.82; p<0.0001	Tukey (Day×group): CTRL: day 1 vs day 2 p=0.8741 day 2 vs day 3 p>0.9999 PTSD2: day 1 vs day 2 p<0.0001 day 2 vs day 3 p=0.0002 PTSD4: day 1 vs day 2 p<0.0001 day 2 vs day 3 p=0.0006 Day 1: CTRL vs PTSD2 p<0.0001 CTRL vs PTSD4 p<0.0001 PTSD2 vs PTSD4 p=0.9320 Day 2: CTRL vs PTSD2 p<0.0001 CTRL vs PTSD4 p<0.0001 PTSD2 vs PTSD4 p=0.9925 Day 3: CTRL vs PTSD2 p<0.0001 CTRL vs PTSD4 p<0.0001 PTSD2 vs PTSD4 p=0.8312 Tukey (time×group): CTRL: 5min vs 10min p>0.9999 5min vs 15min p>0.9999 5min vs 20min p>0.9999 PTSD2 : 5min vs 10min p=0.8679 5min vs 15min p<0.0001 10min vs 20min p<0.0001 PTSD4 : 5min vs 10min p>0.9999 5min vs 15min p<0.0001 10min vs 20min p<0.0001
Retrieval vs 1st re-exposure	2-way ANOVA	Interaction: F(2, 55)=13.24; p<0.0001 grp: F(2, 55)=115.3; p<0.0001 day: F(1, 55)=137.3; p<0.0001	Tukey (retention): CTRL vs PTSD2: p<0.0001 CTRL vs PTSD4: p<0.0001 PTSD2 vs PTSD4: p=0.6361 Sidak (day factor: re-expo 1 - retention): CTRL: p=0.0435 PTSD2: p<0.0001 PTSD4: p<0.0001
% of extinction: PTSD2 vs PTSD4	Shapiro-Wilk	p=0.2315	
	F test	p=0.1328	
	Unpaired t-test	p=0.3613	
Proportion of low extinction	Khi2	P=0.52	
Distance moved during the avoidance test post-extinction	Shapiro-Wilk	p=0.2863	
	Bartlett's	p=0.8603	
	Brown-Forsythe	p=0.7180	
	1w ANOVA	F(2, 27)=7.715; p=0.0022	Tukey CTRL vs PTSD2: p=0.0016 CTRL vs PTSD4: p=0.0652 PTSD2 vs PTSD4: p=0.2861

Time spent on each object during avoidance test post-ext	2w RM ANOVA	Interaction: F(2, 27)=6.899; p=0.0038 grp: F(2, 27)=2.995; p=0.0669 obj: F(1,27)=4.599; p=0.0411	Tukey (interaction): neutral: CTRL vs PTSD2: p=0.8097 CTRL vs PTSD4: p=0.0337 PTSD2 vs PTSD4: p=0.1334 reminder: CTRL vs PTSD2: p=0.0033 CTRL vs PTSD4: p=0.0870 PTSD2 vs PTSD4: p=0.4040 Sidak (neutral vs reminder) CTRL: p=0.2417 PTSD2: p=0.0684 PTSD4: p=0.0141
Time spent in each room during avoidance test post-ext	2w RM ANOVA	Interaction: F(2, 27)=7.020; p=0.0035 grp: F(2, 27)=2.641; p=0.0896 obj: F(1,27)=27.36; p<0.0001	Tukey (interaction): neutral: CTRL vs PTSD2: p=0.0765 CTRL vs PTSD4: p=0.0689 PTSD2 vs PTSD4: p=0.9988 reminder: CTRL vs PTSD2: p=0.0005 CTRL vs PTSD4: p=0.0038 PTSD2 vs PTSD4: p=0.7997 Sidak (neutral vs reminder) CTRL: p>0.9999 PTSD2: p=0.0002 PTSD4: p=0.0006
Latency to go to the lighted room (BCO)	Shapiro-Wilk	p<0.0001	
	Bartlett's	p<0.0001	
	Kruskal-Wallis	H=1.456; p=0.4828	
time spent in the lighted room (BCO)	Shapiro-Wilk	p<0.0001	
	Bartlett's	p=0.0177	
	Kruskal-Wallis	H=2.093; p=0.3512	