Initial evidence for pharmacological modulation of observational threat learning by the GABAergic, but not the noradrenergic system in humans

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Abstract

Threat responses are often shaped by social information, such as observation of aversive outcomes for others. Yet, the neurochemistry regulating observational learning of threats is largely unknown. Here, we examined the impact of the GABAergic and noradrenergic system, which are central in regulating threat learning from first-hand experiences, on observational threat learning in humans. To this end, 61 participants received either 1mg Lorazepam (enhancing GABAergic signalling N=18), 20mg Yohimbine (enhancing Noradrenergic transmission, N=16), Placebo (double blind and randomized control for Lorazepam and Yohimbine, N=13) or no treatment (N=15) prior to observational threat conditioning. Participants acquired conditioned threat responses by observation of another individual who is presented with a conditioned stimulus (CS) and an aversive unconditioned stimulus (US). Participants' threat responses were tested by direct exposure to the CSs immediately after learning, as well as two days later (drug free). Our results indicate decreased fear ratings to socially acquired CSs by enhanced GABAergic transmission as compared to the control group (placebo and no treatment) during the immediate test. We could not provide evidence for noradrenergic modulation of socially acquired threat responses. Further, we found no differences in psychophysiological responses (Skin conductance responses) or long-term persistence of conditioned responses. Our results provide initial evidence for an impact of the GABAergic system on social acquisition of threats.

Key Words: Fear conditioning; social learning; noradrenergic and GABAergic system

1 INTRODUCTION

2 Threat learning is often investigated by employing protocols that use first-hand experiences of
3 aversive consequences (e.g., the experiment of “little albert” (Watson and Rayner, 1920), see
4 [this special issue](#)). However, we often learn about threats in our environment by observation
5 of others. As such, observational threat learning is a central ability across human development
6 (Askew et al., 2016; Kendal et al., 2018; Rodriguez Buritica et al., 2018) and an important
7 factor that contributes to the development of pathological threat responses (Askew and Field,
8 2007; Breslau and Kessler, 2001; Murphy et al., 1999; Neria et al., 2008). In fact, the Diagnostic
9 and Statistical Manual of Mental Disorders (DSM) version 5 includes witnessing and learning
10 about trauma in others as a diagnostic criterion A (Stressor) for Post-traumatic stress disorders
11 (PTSD) (American Psychiatric Association, 2013).

12 Yet, the knowledge about the basic mechanisms underlying observational threat learning is
13 limited. In particular, the neuropharmacological pathways that regulate how observation of
14 others shapes threat responses have not been identified yet.

15 One plausible mechanism is that threat responses that originate from observational
16 experiences are regulated by similar neurotransmitter systems that are also implied in learning
17 from direct aversive outcomes. In a previous study, we found that observational threat learning
18 is modulated by the endogenous opioid system, which plays a central role in regulating threat
19 responses that are acquired as a result of noxious, first-hand experiences (Haaker et al.,
20 2017b). Following this logic, neuropharmacological interventions that are effective in
21 modulating threat responses resulting from first-hand aversive experiences could presumably
22 exert similar effects on observational threat learning.

23 Across species, threat learning by first-hand experiences is often examined within classical
24 fear conditioning protocols (Haaker et al., 2019). These protocols include acquisition training,
25 in which a cue (conditioned stimulus, CS+) is followed by an aversive event (unconditioned
26 stimulus, US), for example an electric shock (Lonsdorf et al., 2017). Consequently, the CS is
27 associatively learned as a predictor of the US and the presentation of the CS evokes a

1 conditioned threat response. Studies in animals, including humans, suggest that conditioned
2 threat responses can be modulated by two opposing neurotransmitter systems: the gamma-
3 aminobutyric acid (GABA), which is important for inhibition of threat responses and the
4 noradrenergic (NA) system, which might enhance learned responses to threats (Ganella and
5 Kim, 2014; Griebel and Holmes, 2013; Singewald et al., 2015). Furthermore, studies in rodents
6 revealed that learned association between the CS and the US induce potentiation of neural
7 inputs to the amygdala (Bauer et al., 2001; Maren, 2000; Repa et al., 2001), as well as plasticity
8 of intra-amygdala connections (Quirk et al., 1995). These synaptic CS-US associations can
9 persist for a lifetime (of a rodent, see Fanselow and Gale, 2003) and are under inhibitory control
10 of GABAergic interneurons in order to dynamically suppress threat responses in situations that
11 are safe (Paré et al., 2003). In mammals, the amygdala is rich of GABA receptors of the A-
12 subtype mediating this inhibitory regulation of threat responses. In rodents, intra-amygdala
13 injection of benzodiazepines, which enhance the action of GABA by allosteric modulation of
14 the receptor subtype A, has been shown to produce anxiolytic-like effects in a number of animal
15 models of anxiety, including the elevated plus maze (EPM) (Green and Vale, 1992; Nunes-de-
16 Souza et al., 2000; Pesold and Treit, 1995). Similarly, we have recently shown that systemic
17 administration of the benzodiazepine Lorazepam in humans reduces anxiety-like behavior on
18 a virtual reality version of the EPM (Biedermann et al., 2017). These results fit to previous
19 studies, reporting that benzodiazepines reduces several types of threat responses in general,
20 such as eye-blink startle responses towards darkness (Baas et al., 2002) and towards
21 unpleasant pictures (Patrick et al., 1996). Moreover, administration of Diazepam during
22 acquisition training in fear conditioning protocols reduced later expression of conditioned threat
23 responses (measured as startle reflex, but not skin conductance responses (Scaife et al.,
24 2005)). The aforementioned studies further implied that this inhibitory effect of
25 benzodiazepines on conditioned threat responses is potentially mediated by a reduction of the
26 general arousal evoked by the context, rather than cue-specific effects on threat responses
27 (Baas et al., 2002; Scaife et al., 2005).

1 The enhancement of the NA system, in turn, has been suggested to enhance emotional
2 memory processes (McGaugh, 2004). In particular, emotional learning from first-hand aversive
3 experiences during classic fear conditioning protocols in animals involves the NA system,
4 which connects the Locus Ceruleus with brain regions that are important for threat learning,
5 e.g., amygdala, hippocampus, medial prefrontal cortex (for a recent review see Giustino and
6 Maren, 2018). The enhancement of NA (by blocking alpha-2 auto-receptors) through the
7 administration of Yohimbine in humans was found to enhance retrieval of emotionally arousing
8 information (Rombold et al., 2016). A similar effect was observed for administration of
9 Yohimbine during acquisition training of classical fear conditioning, which led to enhanced
10 expression of conditioned threat responses (measured as startle reflex) towards CS alone
11 presentation (i.e., extinction training, Soeter and Kindt, 2012, 2011), but see Visser et al.,
12 2015).

13 In order to investigate the effect of enhanced GABAergic and NAergic tone on observational
14 threat learning, we employed a protocol in which participants observe (through video-clips)
15 another demonstrating individual who is exposed to a CS that is followed by a US (Berger,
16 1962; Haaker et al., 2017a; Olsson and Phelps, 2007). Importantly, the participants never
17 make first-hand experiences of the aversive US themselves, but learn the association between
18 the CS and the US only by observation. Comparable to classical fear conditioning, conditioned
19 threat responses in this observational threat learning protocol can be assessed by direct
20 presentation of the CS (without the US) to the participants.

21 Based on the previously reported effects on direct threat learning, we expected that enhanced
22 GABAergic and NAergic tone during observational threat learning would decrease and
23 increase expression of threat responses, respectively.

MATERIALS AND METHODS

Participants

In total 61 healthy adults (33 females; mean age = 25.5, SD = 4.74) gave written informed consent and were compensated with 25 EURO for their participation. Exclusion criteria were self-reported neurological or psychiatric disorders, the use of prescription drugs up to two months before taking part in the experiment and/or illegal drug abuse.

46 of the 61 subjects took part in a virtual reality version of an EPM (Biedermann et al., 2017) before participating in this experiment (groups: Placebo, Yohimbine, Lorazepam). 15 subjects participated solely in this experiment and received no medication (no treatment control).

Participants in the placebo group differed from the no treatment controls in the experimental sequence. After drug administration, placebo controls (as well as the two active pharmacological groups) had to wait while answering questions regarding their side-effects (see *Pharmacological manipulation* below), whereas the participants that received no treatment started immediately. There were no differences in any outcome measure between the no treatment control and the Placebo group (group or factor*group interaction: all $p > .138$, see supplementary materials and table S1), and both groups were pooled as a control group for further analyses. No differences were observed regarding STAI trait anxiety and STAI state anxiety between participants in the control group (no treatment and Placebo combined), Lorazepam and Yohimbine group (all $p > .71$). The final sample included 18 participants in the Lorazepam group, 27 in the control group and 16 in the Yohimbine group.

The inclusion of sex as a covariate in all analyses did not conceptually change the reported effects of the pharmacological modulation of threat responses (see supplementary materials for the effect of sex on the main effects).

Pharmacological manipulation

Participants that received study medication were randomly assigned to receive Placebo (N = 13), 1 mg Lorazepam (N = 18) or 20 mg Yohimbine (N = 16) in a double-blind design 120 min before behavioral testing (Greenblatt, 1981). Drug dosage was determined in a pilot study in order to ensure that participants are not affected by side-effects (e.g., sedation after administration of Lorazepam).

Stimuli and experimental procedure

The exact experimental procedure has been described before (see Haaker et al., 2017a). In brief, prior to the experimental task, participants were attached to a shock electrode with the instruction that they might receive an aversive electrical stimulation shock throughout the experimental time-course. The participants were further instructed that video sequences, as well as geometric symbols, will appear on the screen.

During observational threat learning, participants watched video sequences that depicted a male demonstrator, who was visibly attached to a shock electrode at the right wrist and placed in front of a computer screen, in a similar manner as the participants. In each video-clip the demonstrator was confronted with one of two differently colored rectangular (yellow and blue), serving as observational CSs for the participant (see figure 1 for an illustration). Each observational CS was presented 6 times with a duration of 8 s. During the inter-trial-interval (ITI) a fixation cross was presented on the participants screen (no demonstrator visible). One of the two observational CSs (i.e., observational CS+) was followed by an aversive electrical stimulation for the demonstrator 6.5 s after CS+ onset in 75% of the trials. The demonstrator reacted with a slight twitch of the arm and face distorted with pain (i.e., social US for the participants). The observational CS- was never followed by an aversive stimulation for the demonstrator. Participants' threat responses were subsequently tested within an immediate expression test, which consisted of a presentation of the CSs on the participants' screen (i.e.,

direct CSs to the participants) in 9 trials. There were no instructions before the immediate expression test. Long-term expression of threat responses was tested, two days after learning (drug-free, approx. 48-54 hours after learning), consisting of 6 trials for each CS (long-term expression test). Beforehand, participants were instructed that the experiment will continue, similarly to what they experienced on the first day. In both testing stages, the direct CSs had a duration of 8 sec and a fixation cross served as the ITI. Importantly, no electrical stimulation was delivered to the participant in any of the phases. Throughout the experiment, all CSs were presented in pseudorandomized order and all ITIs were jittered between 6-10 s. All stimuli were depicted using Presentation software (NeuroBehavioral Systems, Albany California, USA).

Table 1: Illustration of the experimental design. Participants received either Lorazepam, Placebo or no treatment (control) or Yohimbine prior to an observational learning phase. Expression of conditioned responses was tested immediately after learning (immediate expression test) and after two days (long term expression test).

Subjective ratings

During both testing stages, participants were intermittently asked (after 3 trials of each CS) to rate the fear/stress evoked by each CS and the expectancy of the US on a visual analog scale [0 (none) to 100 (maximum)]. The long-term expression test was preceded by an additional fear and US expectancy rating. To account for individual differences in using this visual analog

scale, the maximal rating value in the immediate expression test was included as a covariate in all analyses of variance. The results did not differ conceptually to those not including this covariate. Participants that rated no US expectancy to all CSs within the immediate and long-term expression test were excluded from rating analyses (Total N=6, Control group: N=5; Yohimbine: N=1). The results did not conceptually change when including these participants (see supplement). Additionally, participants reported their affective evaluation of the demonstrators' experiences (similar to a previous experiment of observational threat learning, Haaker et al., 2017b) by estimating 1) how unpleasant the demonstrator felt when he received a shock and 2) how unpleasant the demonstrator felt when he was exposed to the CS+ and CS-, as well as 3) how uncomfortable it was for the participants observing the demonstrator receiving a shock and 4) how uncomfortable it was for the participants observing the demonstrator being exposed to the CS+ and CS-. All questions were answered on a visual analog scale [0 (none) to 100 (maximum)].

Skin conductance responses

Skin conductance was measured by two self-adhesive Ag/AgCl electrodes, which were attached on the palmar side of the left hand and placed on the distal and proximal hypothenar. Physical signals were recorded via BIOPAC MP-150 amplifier (BIOPAC Systems Inc, Goleta, California, USA) with AcqKnowledge 3.9 software. Phasic skin conductance responses (SCRs) were scored as an increase between .9 and 4.5 s after CSs onsets. SCR amplitudes was logarithmized and range-corrected by the maximal CS-response per day [$\ln(\text{SCR}/\text{SCR}_{\max}(\text{day}))$]. Since no SCRs to the first-hand US experiences could be used to identify non/responder, only participants with insufficient data quality (signal inspection of the experimenter during the experiment) were excluded from the analyses. Two participants were excluded on day 1 (Control group: N= 2) and three participants on day 2 (Control group: N= 1, Lorazepam: N=1, Yohimbine: N=1). SCRs were averaged over blocks of 3 trials on day 1 and 2 trials on day 2, resulting in 3 blocks each day.

Questionnaires

Before the pharmacological manipulation, the German version of the State-Trait-Anxiety-Inventory was completed (STAI; Laux, Glanzmann, Schaffner&Spielberger, 1981). No differences were observed regarding STAI trait anxiety and STAI state anxiety between participants in the control group (no treatment and Placebo combined), Lorazepam and Yohimbine group (all $p > .71$). After finishing the long-term expression test, each participant completed a post-experimental survey assessing awareness for the contingency between the observational CS and the social US.

Data analysis

Data analysis was performed using JASP 0.9.2 (JASP Team 2019), in order to test for differences between the two active pharmacological groups (Yohimbine and Lorazepam) and the control group (Placebo and no treatment). Therefore, repeated measures ANOVAs (rmANOVAs) with stimulus (2, CS-type) and block (day 1: 3 for ratings and SCR; day 2: 4 for ratings and 3 for SCR) as within-subject factors and group (3, Yohimbine, Lorazepam, Control) as between-subjects factor were performed. Significant interactions with the factor group were followed by post-hoc independent t-tsts to compare CS differentiation (CS+>CS-) between each the active pharmacological group (Yohimbine and Lorazepam separately) with the control group.

Statistical analysis was conducted in two steps: First, rmANOVAs were executed using p-value based hypothesis testing with an alpha-level of 0.05, which was corrected for sphericity violation using Greenhouse-Geisser, when appropriate. Second, Bayesian statistics were used to evaluate the results of the rmANOVA. Combination of two statistical evaluation of the results via p-value based and Bayesian statistics (Quintana and Williams, 2018) was carried out to check for the robustness our results (i.e., that results are not contingent on a particular statistical approach). Bayesian statistics in particular allow to quantify uncertainty about effect

sizes (Wagenmakers et al., 2018) and provide the ability to obtain evidence in favor of the null hypothesis and discriminate between “absence of evidence” and “evidence of absence” (Dienes, 2014). The Bayesian rmANOVAs were based on comparisons between models that include the factor “group” against a null model, which only includes the effects of task (stimulus, block, and stimulus*block interaction). The ratio of the likelihood of the data in the null model and in the model that includes the factor group was quantified as a Bayes Factor (BF_{10}). The likelihood for the data in all models with the factor group, compared to all models without was further quantified as the $BF_{inclusion}$. Prior distributions were modeled with an r for fixed effects=0.5, for random effects =1 and covariates =0.354 (JASP defaults). Effects were followed-up by using Bayesian equivalent of independent, one-sided, t-tests in which the null hypothesis states that only positive or negative (depending on the hypothesis) effect sizes are possible, and assigns more prior mass to values closer to 0 than extreme values (quantified by BF_{+0}) or BF_{-0}).

In order test if observational threat learning is associated with the affective evaluation of aversive experiences of others, we explored the association between conditioned threat responses and the rating of the participants about the experience of the demonstrator. To this end, six multiple linear regressions were calculated separately for the immediate (day 1) and long-term (day 2) expression test and for each outcome-measure. Each regression included CS discrimination ($CS+ > CS-$) as the dependent variable and tested for the association with differential ratings ($CS+ > CS-$) of 1) how unpleasant the demonstrator felt when he received a shock and 2) how unpleasant the demonstrator felt when he was exposed to the $CS+$ and $CS-$, as well as 3) how uncomfortable it was for the participants observing the demonstrator receiving a shock and 4) how uncomfortable it was for the participants observing the demonstrator being exposed to the $CS+$ and $CS-$. The group-factor was entered to weight least squares.

Additional supplementary analysis was carried out to test, if observational threat learning is associated with anxiety-like behavior investigated in the preceding study (Biedermann et al.,

2017). To this end we explored an association between the conditioned threat responses and the approach and avoidance behavior in the virtual reality version of the EPM in which some of the participants took part (see supplement for data analyses strategy). We found an association between conditioned responses and approach and avoidance behavior on the EPM (see supplement for result).

Sensitivity Analysis

The sample size in this experiment has not been determined *a priori* for testing our hypothesis, since participants were shared with another experiment. Hence, we calculated post-hoc sensitivity for the effects reported in this experiment. The whole sample (taking 50 participants as the smallest sample size for the SCR measurement) would provide sufficient power (0.95) to detect an interaction of within-subject effects (two measurements, i.e., CS+ and CS-) and between subject effects (three pharmacological groups) with an effect size $f = 0.28$ (moderate effect size, Cohen, 1988) that corresponds a critical F of 3.19 (using an alpha level of 0.05).

RESULTS

GABAergic and noradrenergic modulation of socially acquired threat responses immediately after learning

Successful observational threat conditioning was indicated by enhanced responses to the CS+ as compared to the CS- (see Table 1) in all outcome measures in the immediate expression test.

Immediate expression test						
Measure	N	effect	F	p	Eta ²	Post-hoc (corrected)
fear ratings	Co = 22 Lo = 18 Yo = 15	CS-type	72.326	< .001	.547	CS+ >CS- <.001
		Block	32.239	< .001	0.356	
		CS-type* Block	22.251	< .001	0.282	
US expectancy	Co = 22 Lo = 18 Yo = 15	CS-type	72.500	< .001	.557	CS+ >CS- < .001
		Block	76.670	< .001	0.526	
		CS-type* Block	32.952	< .001	0.330	
SCR	Co = 25 Lo = 18 Yo = 16	CS-type	8.212	.006	.124	CS+ >CS- = .006
		Block	17.155	< .001	0.228	
		CS-type* Block	0.877	0.412	0.015	

Table 1: Successful differentiation between CS-types across all outcome measures was found for the immediate expression test. Sphericity violation was corrected using Greenhouse-Geisser and post-hoc calculated p-values were corrected for multiple

comparisons using the Holm-Bonferroni Method. Abbreviations: **Control group (Co, Placebo and no treatment), Lorazepam group (Lo), and Yohimbine group (Yo).**

Fear ratings

According to our hypothesis, we tested if Lorazepam decreased and Yohimbine enhanced differential (i.e., CS+>CS-) ratings of fear compared to control group. Indeed, differences in fear ratings between groups were reflected by an CS-type*group interaction ($F(2, 52) = 3.685$, $p = 0.032$, $\eta^2 = 0.043$, see table S2 for effects of task). Bayesian model comparisons (when compared to a null model: $P(M|data)$: 0.884, BF_{10} : 15.58; analysis of effects: stimulus*group interaction, $P(incl|data) = 0.933$, $BF_{inclusion} = 14.011$, see table S5 for full model comparisons) further supported the inclusion of the factor group and the interaction stimulus*group. In accordance with our hypothesis, a decreased discrimination between CS+ and CS- was found in the Lorazepam group when compared to the control group (post-hoc, unpaired, one-sided t-tests: $t(38) = -2.378$, $p = 0.011$, $BF_{-0} = 5.275$; Mean CS-discrimination Lorazepam group = 12.167, Standard deviation = 13.425, Mean CS-discrimination Control group = 27.015, Standard deviation = 23.500, see figure 2a and table S3 for p-value based statistics and table S6 for Bayesian statistics). However, in contrast to our expectations, we found no enhancement of CS-discrimination in the Yohimbine group when compared to the control group (post-hoc unpaired one-sided t-tests: $t(35) = -1.829$, $p = .962$, $BF_{+0} = 0.180$, mean CS-discrimination Yohimbine group = 14.222, Standard deviation = 16.20, Mean CS-discrimination Control group = 27.015, Standard deviation = 23.500, see figure 2a and table S3 for p-value based statistics and table S6 for Bayesian statistics).

US expectancy

In coherence with the results for fear ratings, the analyses of US expectancy for CSs revealed a difference between the pharmacological groups as well. P-value based statistics indicated a difference in the temporal dynamics across blocks (Block*group interaction: $F(4, 102) = 3.889$, $P = 0.007$, $\eta^2 = .044$, see table S2 for effects of task). Results from Bayesian statistics favored

models that included the factor group and interaction between stimulus and group (when compared to the Null model: $P(M|data) = 0.634$, $BF_{10} = 3.198$; analysis of effects: $BF_{Inclusion} = 3.679$; see table S5 for full model comparisons). Hence, the follow-up t-tests focused group comparisons of CS discrimination (i.e., CS+>CS-) on average, as well as for each block. As expected, we found that differential US expectancy was decreased in the Lorazepam group on average, when compared to the control group (post-hoc, unpaired, one-sided t-tests: $t(38) = -2.237$, $p = 0.016$, $BF_{-0} = 4.122$; mean CS-discrimination Lorazepam group = 13.741, Standard deviation = 20.40, Mean CS-discrimination Control group = 30.379, Standard deviation = 25.58; see figure 2a, table S3 for p-value based statistics and table S6 for Bayesian statistics). However, we could not find evidence for enhanced US expectancy after administration of Yohimbine, when compared to placebo (post-hoc, unpaired, one-sided t-tests: $t(38) = -1.032$, $p = 0.845$, $BF_{+0} = 0.180$; mean CS-discrimination Yohimbine group = 22.667, Standard deviation = 16.28, Mean CS-discrimination Control group = 30.379, Standard deviation = 25.58; see figure 2a, table S3 for p-value based statistics and table S6 for Bayesian statistics). The block-wise comparisons further supported these results, revealing decreased differential US expectancy in the Lorazepam group, when compared to the control group, in Block 2 and 3 (post-hoc, unpaired, one-sided t-tests, Block 2: $t(38) = 2.431$, $p = 0.010$, $BF_{10} = 5.792$; Block 3: $t(38) = 2.432$, $p = 0.010$, $BF_{10} = 5.807$, see figure 1b, table S4 for p-value based statistics and table S7 for Bayesian statistics) and no support for enhanced US expectancy in the Yohimbine when compared to the control group in any of the blocks (post-hoc, unpaired, one-sided t-tests $ps > 0.6$, $BF_{+0} < 0.27$, see figure 2b, table S4 for p-value based statistics and table S7 for Bayesian statistics).

SCR

Unexpectedly, the group comparisons of SCRs in the immediate expression test, revealed no effect of the pharmacological intervention (no main effect of group or factor*group interaction, all $ps > .452$, see table S2 for effects of task). Consistent with these p-value based analyses,

all Bayesian statistics models including the factor group were rejected ($BF_{10} < 0.1$; analysis of effects: factor group, $P(\text{incl}|\text{data})$ all $< .130$, $BF_{\text{inclusion}}$ all $< .03$, see table S5 for full model comparisons) and therefore add further support that there is no evidence that GABAergic or noradrenergic enhancement affect SCRs.

Interestingly, we found differences between groups in SCRs towards the observational US, albeit weak evidence from our statistical models (ANOVA, main effect of group: $F(3, 56)=3.102$; $p=0.053$; $\eta^2=0.1$; Bayesian ANOVA: $P(M|\text{data})$: 0.576, BF_{10} : 1.358; analysis of group effect, $P(\text{incl}|\text{data}) = 0.576$, $BF_{\text{inclusion}} = 1.358$). Post-hoc test revealed that participants that received Lorazepam exhibited lower SCRs when observing the demonstrator receiving an US, as compared to the control group (post-hoc, one-sided, unpaired t-test: $t(41)=-2.533$, $p=0.008$; $BF_{-0}=7.046$, $\text{error}=1.923e-4$). There was no support for differences between the Yohimbine and the Placebo group (post-hoc, one-sided, unpaired t-test: $t(39)=0.823$, $p=0.008$; $BF_{+0}=0.181$, $\text{error}=0.002$). However, SCRs to the observational US were not associated with any differential SCRs towards the CSs (pairwise correlation with observational US responses: mean differential (CS+>CS-) SCRs during immediate expression test: Pearson's $r = -0.11$, $p=0.4$; mean differential (CS+>CS-) SCRs during long-term expression test: Pearson's $r = 0.07$, $p=0.6$).

Figure 1: a) Averaged differential (CS+>CS-) fear rating and US expectancy in the immediate expression test in the Lorazepam (red), Yohimbine (yellow) and control (green) group. b) Differential (CS+>CS-) rating after a block of 6 trials (3 CS+, 3 CS-). Error bars display standard error of the mean.

No evidence for long-term effects of GABAergic and noradrenergic modulation of socially acquired threat responses

Analysis of the drug-free long-term expression revealed persistent conditioned threat responses, indicated as higher responses towards the CS+ as compared to the CS- in all outcome measures (see table 2).

Immediate expression test						
measure	N	effect	F	p	Eta ²	Post-hoc (corrected)
fear ratings	Co = 22 Lo = 15 Yo = 16	CS-type	61.793	< .001	.583	CS+ > CS- < .001
		Block				
		CS-type* Block				
US expectancy	Co = 22 Lo = 15 Yo = 16	CS-type	75.186	< .001	.615	CS+ > CS- < .001
		Block				
		CS-type* Block				
SCR	Co = 26 Lo = 17 Yo = 15	CS-type	7.221	0.010	0.114	CS+ > CS- = .009
		Block	25.405	< .001	0.312	
		CS-type* Block	0.416	0.647	0.007	

Table 2: Successful differentiation between CS-types across all outcome measures in the long-term expression test. Sphericity violations are corrected using Greenhouse-Geisser and post-hoc test p-values were corrected for multiple comparisons using the Holm-Bonferroni Method. Abbreviations: Control group (Co, Placebo and no treatment), Lorazepam group (Lo), and Yohimbine group (Yo).

Nonetheless, we found no differences between pharmacological groups in any of the outcome measure: All interaction with factor group in the repeated measures ANOVAs yielded a $p > 0.1$ and only the main effect of group reached significance for the SCRs (see table S8). The Bayesian statistics concordantly provided no evidence to include the factor group in the model ($BF_{inclusion} < 0.09$, see table S9) in any of the outcome measures.

Hence, the results of all analyses render a long-lasting effect on learned threat responses by the pharmacological intervention in the noradrenergic and GABAergic system rather unlikely.

Association between conditioned threat responses and unpleasantness of observing the demonstrator

Next, we explored if differential responding (CS+>CS-) was associated with the unpleasantness for the participants observing the demonstrator being exposed to the US, CS+ or the CS- as well as the inferred unpleasantness for the demonstrator being exposed to these stimuli.

Multiple regression revealed a positive association between each outcome measure in the immediate expression test (mean difference between the CS+ and the CS-) and the unpleasantness for the participants to observe the demonstrator being confronted with the CS+ and the CS- (Model for Fear Ratings: $F(2,54)=12.28$, $p<0.001$; US expectancy: $F(3,54)=12.05$, $p<0.001$; SCR $F(2,49)=9.86$, $p<0.001$; regressor coefficients see table S10). Each model included a positive association with the unpleasantness to observe the demonstrator being confronted with the CS+, as well as negative association to observe the demonstrator being confronted with the CS- . Additionally, US expectancy ratings were associated with the inferred unpleasantness for the demonstrator being confronted with the CS+.

Similarly, fear ratings and US expectancy in the long-term expression test were associated with the inferred unpleasantness for the demonstrator being exposed to the CS+ and the CS- (Model for Fear Ratings: $F(2,52)=12.13$, $p<0.001$; US expectancy: $F(3,54)=16.51$, $p<0.001$; regressor coefficients see table S12).

All these associations were supported by Bayesian statistics (see table S11 for the immediate and table S13 for the long-term expression test).

DISCUSSION

Our results provide initial evidence that GABAergic enhancement decreases expression of socially acquired conditioned threat responses, measured as ratings of fear and US expectancy. Administration of Lorazepam before observational threat learning reduced differential responding (CS+>CS-) when participants were exposed to the socially acquired CSs. Our results fit to previous reports of decreased threat responses (fear-potentiated startle) after administration of benzodiazepines (Baas et al., 2002; Grillon et al., 2006; Scaife et al., 2005). These studies suggested that enhancement of GABAergic transmission by Benzodiazepines diminishes contextual arousal rather than cue-specific conditioned responses. In our experiment, we could show that decreasing CS-differentiation after the administration of Lorazepam (in comparison to placebo and no treatment control) was driven by decreased responses to the CS+ and a general decrement in arousal would rather affected CS+ and CS- responses. However, since we have no measure of the contextual responses, we cannot examine if the CS+ specific decrease was due to modulation of arousal of the context in which observational learning and/or expression test took place. Nevertheless, our finding that the administration of a benzodiazepine decreases threat responses that are shaped by observational experiences mirrors the effect on threat responses that result from direct, first-hand experiences. Interestingly, we found no effect of benzodiazepines on SCRs toward CSs, which is in line with a previous classical fear conditioning study (Scaife et al., 2005), which reported no effect of benzodiazepine administration on SCRs, as well. Instead, we only found an effect of Lorazepam on SCRs towards the observational US, which were reduced as compared to the control group. However, we could not observe any association between the responses towards the observational US and differential SCRs to the CSs during the immediate or long-term expression test. As a consequence, we suggest that the effect of Lorazepam on the observational US might be related to the aforementioned reduction of responses within the whole experimental context, rather than a specific decrement of aversive learning by reduction of the responses towards the US.

1 Taken together, our results suggest that systemic enhancement of GABAergic
2 neurotransmission results in reduced threat responses that are learned by social observation,
3 in a similar fashion as found for threat responses that are shaped by first-hand aversive
4 experiences.

5 In contrast to our hypothesis, we found no evidence for an effect of Yohimbine on observational
6 threat learning. Our result might suggest that there is a limited involvement of the noradrenergic
7 system in observational threat learning, when compared to threat responses that are acquired
8 by first-hand aversive experiences, since previous studies have reported an effect of
9 Yohimbine on threat responses in classical conditioning experiments (Soeter and Kindt, 2012,
10 2011). It should, however be noted that there are reports of no effect of Yohimbine on classical
11 fear conditioning (startle responses), as well (Visser et al., 2015). A recent study in patients
12 with major depression could only find a main effect of higher startle responses (irrespectively
13 of CS-type) after administration of Yohimbine (Kuehl et al., 2020). This study furthermore
14 reported an effect of Yohimbine on differential SCRs during acquisition training, but suggested
15 that this effect of Yohimbine is dependent on adverse childhood experience. Hence, the effect
16 of Yohimbine on direct threat learning might not be as clear as initially expected. One
17 explanation for the lack of an effect in our study might be that observational threat learning
18 does not trigger noradrenergic arousal in a similar manner as first-hand aversive experiences
19 (of electric shocks). The enhancement of memory processes by stress-related
20 neuromodulators (i.e., NA and glucocorticoids) is most pronounced on inherently arousing
21 learning situations (Kuhlmann and Wolf, 2006; Schwabe et al., 2012) and observational
22 learning by video-clips might not be sufficient for a noticeable effect of noradrenergic
23 enhancement. It might well be that observational learning from a live-interaction with another
24 individual would boost noradrenergic arousal and hence could be modulated by noradrenergic
25 drugs.

26 There are several other alternative explanations for the lack of the effect of Yohimbine on
27 observational threat learning, which should further be considered. For example, the receptor

1 profile of Yohimbine is not specific for the noradrenergic system, but involves action in the
2 serotonergic system (e.g., Winter and Rabin, 1992). Hence, our result should not be
3 interpreted as being specific for the noradrenergic system, but might be specific to the effect
4 of Yohimbine. The sample size in our experiment provides only sufficient power for moderate
5 effect sizes and might have failed to provide sufficient power to detect the effect of Yohimbine
6 on observational threat learning. Future studies that employ more specific pharmacological
7 agents for the noradrenergic system and larger sample sizes might be able to delineate the
8 noradrenergic involvement in social threat learning in a more detailed fashion.

9 We found the most pronounced effects of Lorazepam within the ratings of fear and US
10 expectancy, rather than the physiological read-out of skin-conductance. These dependent
11 measurements draw on different underlying processes (Klucken et al., 2009; Taschereau-
12 Dumouchel et al., 2019, for a detailed discussion see Lonsdorf et al., 2017) and it might well
13 be that GABAergic enhancement rather affects affective and cognitive evaluation of threats
14 (i.e., fear and US expectancy, respectively).

15 In sum, our results provide initial evidence that the GABAergic system (administration of
16 Lorazepam), but not the noradrenergic system (administration of Yohimbine) modulates
17 observational threat learning in humans. Hence, it might well be that the neurotransmitter
18 systems that are involved in learning from first-hand differ from neuromodulators that regulate
19 observational experiences. This would allow for a pharmacological dissection of direct, as
20 opposed to social learning processes, which is often not possible by behavioral or physiological
21 read-outs. Our results further align with the view that learning from first-hand and observational
22 experiences involves different neural processes, albeit activity in overlapping brain regions
23 (Krishnan et al., 2016; Lindström et al., 2017).

References

- American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (DSM) 5. American Psychiatric Association.
- Askew, C., Field, A.P., 2007. Vicarious learning and the development of fears in childhood. *Behaviour Research and Therapy* 45, 2616–2627. <https://doi.org/10.1016/j.brat.2007.06.008>
- Askew, C., Reynolds, G., Fielding-Smith, S., Field, A.P., 2016. Inhibition of vicariously learned fear in children using positive modeling and prior exposure. *J Abnorm Psychol* 125, 279–291. <https://doi.org/10.1037/abn0000131>
- Baas, J.M.P., Grillon, C., Böcker, K.B.E., Brack, A.A., Morgan, C.A., Kenemans, J.L., Verbaten, M.N., 2002. Benzodiazepines have no effect on fear-potentiated startle in humans. *Psychopharmacology (Berl.)* 161, 233–247. <https://doi.org/10.1007/s00213-002-1011-8>
- Bauer, E.P., LeDoux, J.E., Nader, K., 2001. Fear conditioning and LTP in the lateral amygdala are sensitive to the same stimulus contingencies. *Nature Neuroscience* 4, 687.
- Berger, S.M., 1962. Conditioning through vicarious instigation. *Psychological Review* 69, 450–466.
- Biedermann, S.V., Biedermann, D.G., Wenzlaff, F., Kurjak, T., Nouri, S., Auer, M.K., Wiedemann, K., Briken, P., Haaker, J., Lonsdorf, T.B., Fuss, J., 2017. An elevated plus-maze in mixed reality for studying human anxiety-related behavior. *BMC Biol.* 15, 125. <https://doi.org/10.1186/s12915-017-0463-6>
- Breslau, N., Kessler, R.C., 2001. The stressor criterion in DSM-IV posttraumatic stress disorder: an empirical investigation. *Biol. Psychiatry* 50, 699–704.
- Cohen, J., 1988. *Statistical Power Analysis for the Behavioral Sciences*. Routledge. <https://doi.org/10.4324/9780203771587>
- Dienes, Z., 2014. Using Bayes to get the most out of non-significant results. *Front Psychol* 5, 781. <https://doi.org/10.3389/fpsyg.2014.00781>
- Fanselow, M.S., Gale, G.D., 2003. The amygdala, fear, and memory. *Ann. N. Y. Acad. Sci.* 985, 125–134. <https://doi.org/10.1111/j.1749-6632.2003.tb07077.x>
- Ganella, D.E., Kim, J.H., 2014. Developmental rodent models of fear and anxiety: from neurobiology to pharmacology. *Br. J. Pharmacol.* 171, 4556–4574. <https://doi.org/10.1111/bph.12643>
- Giustino, T.F., Maren, S., 2018. Noradrenergic Modulation of Fear Conditioning and Extinction. *Front Behav Neurosci* 12, 43. <https://doi.org/10.3389/fnbeh.2018.00043>
- Green, S., Vale, A.L., 1992. Role of amygdaloid nuclei in the anxiolytic effects of benzodiazepines in rats. *Behav Pharmacol* 3, 261–264.
- Greenblatt, D.J., 1981. Clinical pharmacokinetics of oxazepam and lorazepam. *Clin Pharmacokinet* 6, 89–105. <https://doi.org/10.2165/00003088-198106020-00001>
- Griebel, G., Holmes, A., 2013. 50 years of hurdles and hope in anxiolytic drug discovery. *Nat Rev Drug Discov* 12, 667–687. <https://doi.org/10.1038/nrd4075>
- Grillon, C., Baas, J.M.P., Pine, D.S., Lissek, S., Lawley, M., Ellis, V., Levine, J., 2006. The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle. *Biol. Psychiatry* 60, 760–766. <https://doi.org/10.1016/j.biopsych.2005.11.027>
- Haaker, J., Golkar, A., Selbing, I., Olsson, A., 2017a. Assessment of social transmission of threats in humans using observational fear conditioning. *Nat. Protocols* 12, 1378–1386. <https://doi.org/10.1038/nprot.2017.027>
- Haaker, J., Maren, S., Andreatta, M., Merz, C.J., Richter, J., Richter, S.H., Meir Drexler, S., Lange, M.D., Jüngling, K., Nees, F., Seidenbecher, T., Fullana, M.A., Wotjak, C.T., Lonsdorf, T.B., 2019. Making translation work: Harmonizing cross-species methodology in the behavioural neuroscience of Pavlovian fear conditioning. *Neurosci Biobehav Rev* 107, 329–345. <https://doi.org/10.1016/j.neubiorev.2019.09.020>

- 1 Haaker, J., Yi, J., Petrovic, P., Olsson, A., 2017b. Endogenous opioids regulate social threat
2 learning in humans. *Nature Communications* 8, 15495.
3 <https://doi.org/10.1038/ncomms15495>
- 4 Kendal, R.L., Boogert, N.J., Rendell, L., Laland, K.N., Webster, M., Jones, P.L., 2018. Social
5 Learning Strategies: Bridge-Building between Fields. *Trends in Cognitive Sciences* 22,
6 651–665. <https://doi.org/10.1016/j.tics.2018.04.003>
- 7 Klucken, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., Stark, R., 2009. Neural,
8 electrodermal and behavioral response patterns in contingency aware and unaware
9 subjects during a picture-picture conditioning paradigm. *Neuroscience* 158, 721–31.
- 10 Krishnan, A., Woo, C.-W., Chang, L.J., Ruzic, L., Gu, X., López-Solà, M., Jackson, P.L.,
11 Pujol, J., Fan, J., Wager, T.D., 2016. Somatic and vicarious pain are represented by
12 dissociable multivariate brain patterns. *eLife Sciences* 5, e15166.
13 <https://doi.org/10.7554/eLife.15166>
- 14 Kuehl, L.K., Deuter, C.E., Hellmann-Regen, J., Kaczmarczyk, M., Otte, C., Wingenfeld, K.,
15 2020. Enhanced noradrenergic activity by yohimbine and differential fear conditioning in
16 patients with major depression with and without adverse childhood experiences.
17 *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 96, 109751.
18 <https://doi.org/10.1016/j.pnpbp.2019.109751>
- 19 Kuhlmann, S., Wolf, O.T., 2006. A non-arousing test situation abolishes the impairing effects
20 of cortisol on delayed memory retrieval in healthy women. *Neuroscience Letters* 399,
21 268–272. <https://doi.org/10.1016/j.neulet.2006.02.007>
- 22 Lindström, B., Haaker, J., Olsson, A., 2017. A common neural network differentially mediates
23 direct and social fear learning. *Neuroimage* 167, 121–129.
24 <https://doi.org/10.1016/j.neuroimage.2017.11.039>
- 25 Lonsdorf, T.B., Menz, M.M., Andreatta, M., Fullana, M.A., Golkar, A., Haaker, J., Heitland, I.,
26 Hermann, A., Kuhn, M., Kruse, O., Meir Drexler, S., Meulders, A., Nees, F., Pittig, A.,
27 Richter, J., Römer, S., Shiban, Y., Schmitz, A., Straube, B., Vervliet, B., Wendt, J., Baas,
28 J.M.P., Merz, C.J., 2017. Don't fear "fear conditioning": Methodological considerations
29 for the design and analysis of studies on human fear acquisition, extinction, and return of
30 fear. *Neurosci Biobehav Rev* 77, 247–285.
31 <https://doi.org/10.1016/j.neubiorev.2017.02.026>
- 32 Maren, S., 2000. Auditory fear conditioning increases CS-elicited spike firing in lateral
33 amygdala neurons even after extensive overtraining. *Eur. J. Neurosci.* 12, 4047–4054.
34 <https://doi.org/10.1046/j.1460-9568.2000.00281.x>
- 35 McGaugh, J.L., 2004. The amygdala modulates the consolidation of memories of emotionally
36 arousing experiences. *Annu.Rev.Neurosci.* 27, 1–28.
- 37 Murphy, S.A., Braun, T., Tillery, L., Cain, K.C., Johnson, L.C., Beaton, R.D., 1999. PTSD
38 among bereaved parents following the violent deaths of their 12- to 28-year-old children:
39 A longitudinal prospective analysis. *J. Traum. Stress* 12, 273–291.
40 <https://doi.org/10.1023/A:1024724425597>
- 41 Neria, Y., Olfson, M., Gameroff, M.J., Wickramaratne, P., Gross, R., Pilowsky, D.J., Blanco,
42 C., Manetti-Cusa, J., Lantigua, R., Shea, S., Weissman, M.M., 2008. The mental health
43 consequences of disaster-related loss: findings from primary care one year after the 9/11
44 terrorist attacks. *Psychiatry* 71, 339–348. <https://doi.org/10.1521/psyc.2008.71.4.339>
- 45 Nunes-de-Souza, R.L., Canto-de-Souza, A., da-Costa, M., Fornari, R.V., Graeff, F.G., Pelá,
46 I.R., 2000. Anxiety-induced antinociception in mice: effects of systemic and intra-
47 amygdala administration of 8-OH-DPAT and midazolam. *Psychopharmacology (Berl.)*
48 150, 300–310. <https://doi.org/10.1007/s002130000428>
- 49 Olsson, A., Phelps, E.A., 2007. Social learning of fear. *Nat Neurosci* 10, 1095–1102.
50 <https://doi.org/10.1038/nn1968>
- 51 Pare, D., Royer, S., Smith, Y., Lang, E.J., 2003. Contextual inhibitory gating of impulse traffic
52 in the intra-amygdaloid network. *Ann. N. Y. Acad. Sci.* 985, 78–91.
53 <https://doi.org/10.1111/j.1749-6632.2003.tb07073.x>
- 54 Patrick, C.J., Berthot, B.D., Moore, J.D., 1996. Diazepam blocks fear-potentiated startle in

- humans. *J Abnorm Psychol* 105, 89–96.
- Pesold, C., Treit, D., 1995. The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines. *Brain Res.* 671, 213–221. [https://doi.org/10.1016/0006-8993\(94\)01318-c](https://doi.org/10.1016/0006-8993(94)01318-c)
- Quintana, D.S., Williams, D.R., 2018. Bayesian alternatives for common null-hypothesis significance tests in psychiatry: a non-technical guide using JASP. *BMC Psychiatry* 18, 178. <https://doi.org/10.1186/s12888-018-1761-4>
- Quirk, G.J., Repa, C., LeDoux, J.E., 1995. Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* 15, 1029–1039. [https://doi.org/10.1016/0896-6273\(95\)90092-6](https://doi.org/10.1016/0896-6273(95)90092-6)
- Repa, J.C., Muller, J., Apergis, J., Desrochers, T.M., Zhou, Y., LeDoux, J.E., 2001. Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat. Neurosci.* 4, 724–731. <https://doi.org/10.1038/89512>
- Rodriguez Buritica, J.M., Heekeren, H.R., Li, S.-C., Eppinger, B., 2018. Developmental differences in the neural dynamics of observational learning. *Neuropsychologia* 119, 12–23. <https://doi.org/10.1016/j.neuropsychologia.2018.07.022>
- Rombold, F., Wingenfeld, K., Renneberg, B., Hellmann-Regen, J., Otte, C., Roepke, S., 2016. Influence of the noradrenergic system on the formation of intrusive memories in women: an experimental approach with a trauma film paradigm. *Psychol Med* 46, 2523–2534. <https://doi.org/10.1017/S0033291716001379>
- Scaife, J.C., Langley, R.W., Bradshaw, C.M., Szabadi, E., 2005. Diazepam suppresses the acquisition but not the expression of “fear-potentiation” of the acoustic startle response in man. *J. Psychopharmacol. (Oxford)* 19, 347–356. <https://doi.org/10.1177/0269881105053285>
- Schwabe, L., Joëls, M., Roozendaal, B., Wolf, O.T., Oitzl, M.S., 2012. Stress effects on memory: An update and integration. *Neuroscience & Biobehavioral Reviews, Memory Formation* 36, 1740–1749. <https://doi.org/10.1016/j.neubiorev.2011.07.002>
- Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., Ressler, K.J., 2015. Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol. Ther.* 149, 150–190. <https://doi.org/10.1016/j.pharmthera.2014.12.004>
- Soeter, M., Kindt, M., 2012. Stimulation of the noradrenergic system during memory formation impairs extinction learning but not the disruption of reconsolidation. *Neuropsychopharmacology* 37, 1204–1215. <https://doi.org/10.1038/npp.2011.307>
- Soeter, M., Kindt, M., 2011. Noradrenergic enhancement of associative fear memory in humans. *Neurobiology of Learning and Memory* 96, 263–271. <https://doi.org/10.1016/j.nlm.2011.05.003>
- Taschereau-Dumouchel, V., Kawato, M., Lau, H., 2019. Multivoxel pattern analysis reveals dissociations between subjective fear and its physiological correlates. *Mol. Psychiatry*. <https://doi.org/10.1038/s41380-019-0520-3>
- Visser, R.M., Kunze, A.E., Westhoff, B., Scholte, H.S., Kindt, M., 2015. Representational similarity analysis offers a preview of the noradrenergic modulation of long-term fear memory at the time of encoding. *Psychoneuroendocrinology* 55, 8–20. <https://doi.org/10.1016/j.psyneuen.2015.01.021>
- Wagenmakers, E.-J., Marsman, M., Jamil, T., Ly, A., Verhagen, J., Love, J., Selker, R., Gronau, Q.F., Šmíra, M., Epskamp, S., Matzke, D., Rouder, J.N., Morey, R.D., 2018. Bayesian inference for psychology. Part I: Theoretical advantages and practical ramifications. *Psychon Bull Rev* 25, 35–57. <https://doi.org/10.3758/s13423-017-1343-3>
- Watson, J.B., Rayner, R., 1920. Conditioned emotional reactions. *Journal of Experimental Psychology* 3, 1–14. <https://doi.org/10.1037/h0069608>
- Winter, J.C., Rabin, R.A., 1992. Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination. *J. Pharmacol. Exp. Ther.* 263, 682–689.

1

2 **CONFLICT OF INTEREST STATEMENT**

3 None of the authors has a financial relationship with a commercial entity that has an interest
4 in the subject of this manuscript.

5

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Supplementary Materials

No difference between placebo and no treatment groups

Successful observational fear conditioning was indicated in the immediate expression test in the pooled control group (placebo group and no treatment control) by higher subjective fear and US-expectancy ratings towards the CS+ as compared to the CS- and a trend for SCR when comparing CS+ to CS- (see table S1). Furthermore, we found no differences in these outcome measures between the placebo and no treatment control group (no main effect of group or factor*group interaction: all $P > .138$; CS-discrimination, i.e., CS+>CS-, unpaired t-test: all $P > .434$). Hence, all further analyses were calculated with a pooled control group.

Table S1: No differences in outcome measures between Placebo and No treatment group (No treat). Post hoc tests are corrected for multiple comparisons using the Bonferroni-Holm Method (Holm, 1979)

Placebo group and no treatment control immediate expression test							
effect	measure	N	F	df	p	Eta ²	Post-hoc (Holm)
CS-type	fear ratings	Placebo N = 11 No treat N = 11	90.288	1,20	<0.001	.819	CS+>CS- < .001
	US expectancy	Placebo N = 10 No treat N = 12	61.072	1,20	<0.001	.753	CS+>CS- < .001
	SCR	Placebo N = 9 No treat N = 11	3.965	1,18	.065	.177	CS+>CS- = .055

Influence of the participants' sex on outcome measures

Analyses of the immediate expression test revealed no influence of the participants sex in any outcome measure (all $p > 0.1$, $t < 2.1$, $BF_M < 1.4$). In the long-term expression test the interaction between sex and block reached significance in the analyses of the fear ratings ($F(3,162)=3.577$, $p=0.032$, $n=0.044$), however the Bayesian model statistics revealed only anecdotal evidence for the factor sex ($P(M|data) = 0.186$, $BF_M = 2.57$; analysis of effects: $BF_{Inclusion} = 0.267$). This corresponded with no significant difference in CS discrimination when comparing men against woman in each block separately (all $ps > 0.2$).

A similar effect was evident in the analyses of the US expectancy ratings in the long-term expression. There was a significant interaction between stimulus, block and sex ($F(3,162)=2.686$, $p=0.048$, $n=0.035$), including a significant simpler interaction between block and sex ($F(3,162)=5.761$, $p<0.001$, $n=0.071$). However, the Bayesian model statistics revealed only anecdotal evidence for the factor sex ($P(M|data) = 0.183$, $BF_M = 2.46$; analysis of effects: $BF_{Inclusion} = 0.204$) and again, there was no significant difference in CS discrimination when comparing men against woman in each block separately (all $ps > 0.2$).

Importantly, inclusion of the covariate sex did not conceptually change all reported effects of the pharmacological modulation of threat responses.

Inclusion of all participants for rating analyses

Inclusion of all participants for the rating analyses did not change the results conceptually: Analyses of the fear rating in the immediate expression test revealed an CS-type*group interaction ($F(2,57)=4.125$;

p=0.021). Similarly, analyses of the US expectancy revealed an CS-type*group interaction (F(2,57)=3.345; p=0.42).

Table S2: Repeated measurement ANOVAs of outcome measures in the Immediate expression test using p-value based hypothesis testing

Effect	df	F	p	η^2
Fear Ratings Immediate Expression test				
CS-type	1.000	83.036	< .001	0.489
CS-type * group	2.000	3.685	0.032	0.043
CS-type * Max rating	1.000	28.449	< .001	0.167
Residual	51.000			
Block	1.701	32.239	< .001	0.356
Block * group	3.403	1.277	0.287	0.028
Block * Max rating	1.701	4.700	0.016	0.052
Residual	86.776	32.952		
CS-type * Block	1.564	22.251	< .001	0.282
CS-type * Block * group	3.129	14.508	< .001	0.005
Stim * Block * Max rating	1.564	32.952	0.600	0.067
Residual	79.784	0.650		
Between subject effect				
group	2	0.567	0.571	0.012
Max rating	1	43.976	< .001	0.458
Residual	51	0.567		
US Expectancy Immediate Expression test				
CS-type	1.000	130.745	< .001	0.529
CS-type * group	2.000	2.311	0.109	0.019
CS-type * Max rating	1.000	60.835	< .001	0.246
Residual	51.000			
Block	1.821	76.670	< .001	0.526
Block * group	3.642	3.889	0.007	0.053
Block * Max rating	1.821	10.321	< .001	0.071
Residual	92.865			
CS-type * Block	1.662	32.952	< .001	0.330
CS-type * Block * group	3.324	0.650	0.600	0.013
Stim * Block * Max rating	1.662	14.508	< .001	0.145
Residual	84.761	130.745	< .001	0.529
Between subject effect				
group	2	0.157	0.855	0.002
Max rating	1	78.726	< .001	0.605
Residual	51			
SCRs Immediate Expression test				
CS-type	1.000	8.212	0.006	0.124
CS-type * group	2.000	1.023	0.366	0.031
Residual	56.000			
Block	1.767	17.155	< .001	0.228
Block * group	3.534	1.091	0.365	0.029
Residual	100.658			
CS-type * block	1.797	0.877	0.412	0.015
CS-type * block * group	4.0	0.793	0.524	0.027
Residual	100.658			

Between subject effect				
group	2	0.021	0.979	0.001
Residual	56			

Table S3: Follow-up t-tests of group comparisons in outcome measures in the Immediate expression test using p-value based hypothesis testing (N= 18/22/15)

	df	t	p
Fear Ratings			
Lorazepam < control			
CS+	38	2.334	0.012
CS-	38	0.151	0.441
CS+>CS-	38	2.378	0.011
Yohimbin > control			
CS+	35	-1.044	0.848
CS-	35	0.977	0.168
CS+>CS-	35	-1.829	0.962
US expectancy			
Lorazepam < control			
CS+	38	-1.929	0.031
CS-	38	0.742	0.769
CS+>CS-	38	-2.237	0.016
Yohimbin > control			
CS+	35	1.173	0.124
CS-	35	-0.394	0.652
CS+>CS-	35	-1.032	0.845
SCRs			
no effect of group			

Note. For all tests, the alternative hypothesis specifies that Lorazepam is less than control and Yohimbin is higher than control.

Table S4: Follow-up t-tests of Block-wise group comparisons in US expectancy ratings in the Immediate expression test using p-value based hypothesis testing

	df	t	p
US expectancy			
Lorazepam < control, CS+>CS-,			
Block 1	38	1.284	0.103
Block 2	38	2.431	0.010
Block 3	38	2.432	0.010
Yohimbin > control, CS+>CS-,			
Block 1	35	-0.270	0.606
Block 2	35	-1.270	0.894
Block 3	35	-1.422	0.918

Note. For all tests, the alternative hypothesis specifies that Lorazepam is less than control and Yohimbine is higher than control.

Table S5: Repeated measurement ANOVAs of outcome measures in the Immediate expression using Bayesian statistics

Model Comparisons					
Fear Ratings Immediate Expression test	P(M)	P(M data)	BF_M	BF₁₀	error %
Null model (incl. CS-type, Block, Max rating, CS-type * Block, subject)	1.000	0.057	0.301	1.000	
Group	0.167	0.009	0.048	0.166	12.535
Group + Group * CS-type	0.167	0.884	38.007	15.578	4.888
Group + Group * Block	0.167	4.618e -4	0.002	0.008	3.219
Group + Group * CS-type + Group * Block	0.167	0.047	0.246	0.827	2.964
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	0.003	0.014	0.048	16.533
Effects	P(incl)	P(incl data)	BF_{Inclusion}		
Group	0.833	0.943	3.325	x	x
CS-type* group	0.500	0.933	14.011	x	x
Block * group	0.500	0.050	0.053	x	x
CS-type* block* group	0.167	0.003	0.014	x	x
US Expectancy Immediate Expression test	P(M)	P(M data)	BF_M	BF₁₀	error %
Null model (incl. CS-type, Block, Max rating, CS-type * Block, subject)	0.167	0.198	1.237	1.000	
Group	0.167	0.013	0.066	0.066	3.296
Group + Group * CS-type	0.167	0.634	8.672	3.198	6.881
Group + Group * Block	0.167	0.002	0.012	0.012	4.470
Group + Group * CS-type + Group * Block	0.167	0.145	0.845	0.729	8.622
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	0.007	0.037	0.037	8.227
Effects	P(incl)	P(incl data)	BF_{Inclusion}		
Group	0.833	0.802	0.808		
CS-type* group	0.500	0.786	3.679		
Block * group	0.500	0.154	0.182		
CS-type* block* group	0.167	0.007	0.037		
SCR Immediate Expression test	P(M)	P(M data)	BF_M	BF₁₀	error %
Null model (incl. CS-type, Block, CS-type * Block, subject)	0.167	0.814	21.865	1.000	
Group	0.167	0.139	0.805	0.170	7.619
Group + Group * CS-type	0.167	0.025	0.130	0.031	10.286
Group + Group * Block	0.167	0.018	0.092	0.022	12.959
Group + Group * CS-type + Group * Block	0.167	0.004	0.020	0.005	23.374
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	1.649e -4	8.244e -4	2.026e -4	32.828
Effects	P(incl)	P(incl data)	BF_{Inclusion}		
Group	0.833	0.186	0.046		
CS-type* group	0.500	0.029	0.030		
Block * group	0.500	0.022	0.023		

CS-type* block* group	0.167	1.649e -4	8.244e -4		
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Note. All models for rating values include Stimulus, Block, Max_rating_d1, Stimulus * Block, subject.

Table S6: Follow-up t-tests of group comparisons in outcome measures in the Immediate expression test using **Bayesian statistics**

	BF_{-0/+0}	error %
Fear Ratings		
Lorazepam < control		
CS+	4.874	~ 2.473e -4
CS-	0.346	~ 2.878e -4
CS+>CS-	5.275	~ 1.803e -4
Yohimbin > control		
CS+	0.806	~ 2.143e -4
CS-	0.185	~ 0.002
CS+>CS-	2.180	~ 4.328e -4
US expectancy		
Lorazepam < control		
CS+	2.500	~ 6.196e -4
CS-	0.198	~ 0.003
CS+>CS-	4.122	~ 3.953e -4
Yohimbin > control		
CS+	0.251	~ 0.002
CS-	0.930	~ 8.730e -5
CS+>CS-	0.180	~ 0.001
SCRs		
no effect of group		

Note. For all tests, the alternative hypothesis specifies that Lorazepam is less than control (i.e., BF₋₀) and Yohimbine is higher than control (i.e., BF₊₀).

Table S7: Follow-up t-tests of Block-wise group comparisons in US expectancy ratings in the Immediate expression test using **Bayesian statistics**

	BF_{-0/+0}	error %
US expectancy		
Lorazepam < control, CSP>CSM,		
Block 1	1.032	~ 2.554e -4
Block 2	5.792	~ 1.098e -4
Block 3	5.807	~ 1.080e -4
Yohimbin > control, CSP>CSM,		
Block 1	0.270	~ 8.709e -4
Block 2	0.163	~ 7.832e -4
Block 3	0.153	~ 4.638e -4

Note. For all tests, the alternative hypothesis specifies that Lorazepam is less than control (i.e., BF₋₀) and Yohimbine is higher than control (BF₊₀).

Table S8: Repeated measurement ANOVAs of outcome measures in the long-term expression test using p-value based hypothesis testing

Effect	df	F	p	η^2
Fear Ratings				
CS-type	1.000	31.047	< .001	0.329
CS-type * group	2.000	0.213	0.809	0.005
CS-type * Max rating	1.000	14.037	< .001	0.149
Residual	49.000			
Block	1.982	13.515	< .001	0.193
Block * group	3.964	1.897	0.118	0.054
Block * Max rating	1.982	3.859	0.025	0.055
Residual	97.117			
CS-type * Block	1.964	4.989	0.009	0.084
CS-type * Block * group	3.928	1.809	0.134	0.061
Stim * Block * Max rating	1.964	2.104	0.128	0.035
Residual	96.247			
Between subject effect				
group	2	0.032	0.969	0.001
Max rating	1	16.407	< .001	0.251
Residual	49	0.032	0.969	0.001
US Expectancy				
CS-type	1.000	37.329	< .001	0.362
CS-type * group	2.000	0.332	0.719	0.006
CS-type * Max rating	1.000	16.242	< .001	0.157
Residual	49.000			
Block	1.765	18.599	< .001	0.262
Block * group	3.531	0.381	0.798	0.011
Block * Max rating	1.765	2.622	0.085	0.037
Residual	86.509			
CS-type * Block	1.616	9.291	< .001	0.147
CS-type * Block * group	3.232	0.897	0.453	0.028
Stim * Block * Max rating	1.616	3.105	0.061	0.049
Residual	79.191			
Between subject effect				
group	2	0.186	0.831	0.006
Max rating	1	15.326	< .001	0.237
Residual	49			
SCRs				
CS-type	1.000	7.221	0.010	0.114
CS-type * group	2.000	0.692	0.505	0.022
Residual	55.000			
Block	1.903	25.405	< .001	0.312
Block * group	3.806	0.518	0.710	0.013
Residual	102.720			

CS-type * block	1.854	0.416	0.647	0.007
CS-type * group * group	3.707	0.416	0.784	0.015
Residual	102.499			
Between subject effect				
group	2	1.842	0.168	0.063
Residual	46			

Note. Type II Sum of Squares, corrected for sphericity by using Greenhouse-Geisser

Table S9: Repeated measurement ANOVAs of outcome measures in the Immediate expression test using bayesian statistics

Model Comparisons					
Fear Ratings Immediate Expression test	P(M)	P(M data)	BF _M	BF ₁₀	error %
Null model (incl. CS-type, Block, Max rating, CS-type * Block, subject)	0.167	0.870	33.328	1.000	
Group	0.167	0.113	0.638	0.130	2.692
Group + Group * CS-type	0.167	0.008	0.039	0.009	7.376
Group + Group * Block	0.167	0.009	0.045	0.010	2.260
Group + Group * CS-type + Group * Block	0.167	6.168e -4	0.003	7.093e -4	6.157
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	2.754e -5	1.377e -4	3.167e -5	4.041
Effects	P(incl)	P(incl data)	BF _{Inclusion}		
Group	0.833	0.130	0.030		
CS-type* group	0.500	0.008	0.008		
Block * group	0.500	0.010	0.010		
CS-type* block* group	0.167	2.754e -5	1.377e -4		
US Expectancy Immediate Expression test	P(M)	P(M data)	BF _M	BF ₁₀	error %
Null model (incl. CS-type, Block, Max rating, CS-type * Block, subject)	0.167	0.898	44.158	1.000	
Group	0.167	0.093	0.515	0.104	6.905
Group + Group * CS-type	0.167	0.007	0.036	0.008	7.563
Group + Group * Block	0.167	0.001	0.006	0.001	7.202
Group + Group * CS-type + Group * Block	0.167	6.737e -5	3.369e -4	7.50e -5	5.016
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	2.251e -6	1.126e -5	2.50e -6	4.861
Effects	P(incl)	P(incl data)	BF _{Inclusion}		
Group	0.833	0.130	0.030		
CS-type* group	0.500	0.008	0.008		
Block * group	0.500	0.010	0.010		
CS-type* block* group	0.167	2.754e -5	1.377e -4		
SCR Immediate Expression test	P(M)	P(M data)	BF _M	BF ₁₀	error %
Null model (incl. CS-type, Block, CS-type * Block, subject)	0.167	0.693	11.279	1.000	
Group	0.167	0.267	1.823	0.386	3.487
Group + Group * CS-type	0.167	0.027	0.137	0.039	4.131
Group + Group * Block	0.167	0.012	0.061	0.017	9.791
Group + Group * CS-type + Group * Block	0.167	0.001	0.005	0.001	3.258
Group + Group * CS-type + Group * Block + Group * CS-type * Block	0.167	8.706e -5	4.353e -4	1.257e -4	6.251
Effects	P(incl)	P(incl data)	BF _{Inclusion}		
Group	0.833	0.307	0.089		

CS-type* group	0.500	0.028	0.029		
Block * group	0.500	0.013	0.013		
CS-type* block* group	0.167	8.706e -5	4.353e -4		

Note. All models for rating values include Stimulus, Block, Max_rating_d1,
Stimulus * Block, subject.

**S10: Linear Regression of conditioned threat responses in the Immediate Expression
Test and empathic appraisals using p-value based hypotheses testing (N=
18/22/15)**

Fear Ratings Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.566	0.321	0.295	23.42	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	13467	2	6733.3	12.281	< .001
residual	28510	52	548.3		
total	41976	54			
Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	12.101	3.223		3.755	< .001
Unpleasantness to observe the demonstrator being exposed to CS+	0.471	0.095	0.678	4.947	< .001
Unpleasantness to observe the demonstrator being exposed to CS-	-0.399	0.147	-0.361	-2.719	0.009
US Expectancy Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.644	0.415	0.380	23.82	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	20502	3	6834.0	12.048	< .001
residual	28929	51	567.2		
total	49431	54			
Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	15.034	3.445		4.364	< .001
Unpleasantness to observe the demonstrator being exposed to CS+	0.344	0.112	0.434	3.063	0.003
Unpleasantness to observe the demonstrator being exposed to CS-	-0.596	0.152	-0.473	-3.929	< .001
Inferred unpleasantness for the demonstrator being exposed to CS+	0.245	0.087	0.371	2.812	0.007
SCR Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.544	0.296	0.266	0.129	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	0.330	2	0.165	9.865	< .001
residual	0.787	47	0.017		
total	1.118	49			

Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	0.041	0.018		2.328	0.024
Unpleasantness to observe the demonstrator being exposed to CS+	0.002	4.763e -4	0.480	3.255	0.002
Unpleasantness to observe the demonstrator being exposed to CS-	-0.003	7.161e -4	-0.593	-4.345	< .001

S11: Linear Regression of conditioned threat responses in the Immediate Expression Test and empathic appraisals using bayesian statistics

Fear Ratings Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²
Unpleasantness to observe the demonstrator being exposed to CS+ + Unpleasantness to observe the demonstrator being exposed to CS-	0.010	0.080	9.095	6.913	0.321
US Expectancy Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²
Unpleasantness to observe the demonstrator being exposed to CS+ + Unpleasantness to observe the demonstrator being exposed to CS- + Inferred unpleasantness for the demonstrator being exposed to CS+	0.007	0.127	20.235	4688.374	0.415
SCRs Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²
Unpleasantness to observe the demonstrator being exposed to CS+ + Unpleasantness to observe the demonstrator being exposed to CS-	0.017	0.105	6.939	37.358	0.257

S12: Linear Regression of conditioned threat responses in the long-term Expression Test and empathic appraisals using p-value based hypotheses testing

Fear Ratings Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.668	0.446	0.424	15.93	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	10211	2	5105.4	20.127	< .001
residual	12683	50	253.7		
total	22894	52			
Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	4.921	1.930		2.550	0.014
Inferred unpleasantness for the demonstrator being exposed to CS+	0.414	0.066	0.805	6.292	< .001
Inferred unpleasantness for the demonstrator being exposed to CS-	-0.373	0.106	-0.403	-3.505	< .001
US Expectancy Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.631	0.398	0.374	18.49	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	11296	2	5647.9	16.513	< .001
residual	17102	50	342.0		
total	28397	52			
Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	8.005	2.241		3.572	< .001
Inferred unpleasantness for the demonstrator being exposed to CS+	0.423	0.076	0.732	5.537	< .001
Inferred unpleasantness for the demonstrator being exposed to CS-	-0.472	0.124	-0.454	-3.820	< .001
SCR Immediate Expression test					
No Model with p<0.05					

S13: Linear Regression of conditioned threat responses in the Long-term Expression Test and empathic appraisals using Bayesian statistics

Fear Ratings Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²

Inferred unpleasantness for the demonstrator being exposed to CS+ + Inferred unpleasantness for the demonstrator being exposed to CS-	0.010	0.298	44.242	40570.436	0.446
US Expectancy Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²
Inferred unpleasantness for the demonstrator being exposed to CS+ + Inferred unpleasantness for the demonstrator being exposed to CS-	0.010	0.256	35.734	5984.367	0.398
SCRs Immediate Expression test					
No Model with BF ₁₀ > 0.3					

1 *Association between socially acquired threat responses and behavior on an elevated plus maze test*

2 *Data analysis strategy*

3 In exploratory analyses, we tested for an association between conditioned threat responses and
4 behavioural measures within a virtual reality version of an elevated plus maze (Biedermann et al., 2017),
5 which some of the participants have been part of. In brief, behavioural measures describe approach and
6 avoidance behavior of participants that explored a cross-shaped platform with two (safe) closed arm and
7 two open arms that were high above the ground. To this end, linear regressions were calculated
8 separately for each outcome-measure during immediate (day 1) and long-term (day 2) expression test.
9 Each regression includes CS discrimination (CS+ > CS-) as the dependent variable and behavioural
10 readouts of the elevated plus maze as regressors. These behavioural read outs include the numbers of
11 entries into open arms, entries into closed arms, latency to enter open arms, latency to enter closed arms,
12 time spent in the open arms, time in closed arms, time in safe compartments (closed arms & middle of
13 the platform), average velocity in open arms, velocity in closed arms and the total distance covered. The
14 group-factor was entered to weight least squares.

15 *Results*

16 The regression models revealed that fear ratings (difference between the CS+ and the CS-) in the
17 immediate expression test were associated with two behavioral measures in a virtual reality version of
18 an elevated plus maze (Whole model $F(2,28) = 3.966$; $P = 0.031$, see table S14). There was a positive
19 association with the latency to explore the open arms (approach behavior of a potential danger,
20 standardized beta: 0.574; $p = 0.028$) and there was a negative relationship with time spend in safe
21 compartments (avoidance of a potential danger, standardized beta: -0.651; $p = 0.011$). This result was
22 underlined by a Bayesian multiple regression that favored inclusion of the two regressors, as well
23 ($BF_M = 5.716$, $P(M|data) = 0.016$, compared to a null model, see table S15).

24 Analyses of US expectancy in the immediate expression test did not reach the defined significance level
25 or provided sufficient evidence by Bayesian analyses, given the data (all models $p > 0.19$, $BF_M < 0.7$).
26 Additionally, the regression model for SCRs in the immediate expression test only reached trend-level
27 ($F(2,22) = 3.265$; $P = 0.059$) with two positive regressors (Time spent in immobility: $p = 0.035$ and the
28 velocity on open arms: $p = 0.020$). However, these associations could not be supported by the Bayesian
29 statistics (Time spent in immobility: $BF_M = 1.6$ $P(M|data) = 0.012$ and velocity on open arms $BF_M = 1.0$,
30 $P(M|data) = 0.018$).

31 The analyses of fear ratings and SCRs in the long-term expression test did not reach significance level
32 or provided sufficient evidence, given the data (fear ratings: all models $p > 0.21$, $BF_M < 2.9$; SCRs, all
33 models $p > 0.17$, $BF_M < 1.88$). The analyses of US expectancy reached only trend level ($F(1,26) = 4.026$,
34 $p > 0.059$) providing a positive association with the time in immobility ($p = 0.059$), which was generally
35 supported by Bayesian statistics ($BF_M < 4.992$, $P(M|data) = 0.053$, compared to a null model).

Hence, given the exploratory character of these analyses, the association of socially acquired fear ratings in the immediate expression test with the approach and avoidance behavior on the elevated-plus maze might be regarded the central findings of these analyses.

S14: Linear Regression of conditioned threat responses in the Immediate Expression Test and behavioural measures on the EPM using p-value based hypotheses testing

Fear Ratings Immediate Expression test					
Model Summary	R	R ²	Adjusted R ²	RMSE	
	0.484	0.234	0.175	19.12	
ANOVA	Sum of Squares	df	Mean Square	F	p
regression	2899	2	1449.4	3.966	0.031
residual	9501	26	365.4		
total	12400	28			
Coefficients	Unstandardized	Standard Error	Standardized	t	p
Intercept	33.439	9.278		3.604	0.001
Latency to explore open arms	0.073	0.031	0.574	2.330	0.028
Time spend on safe compartments	-0.154	0.056	-0.651	-2.748	0.011

S15: Linear Regression of conditioned threat responses in the Immediate Expression Test and behavioural measures on the EPM using Bayesian statistics

Fear Ratings Immediate Expression test					
Model	P(M)	P(M data)	BF _M	BF ₁₀	R ²
Latency to explore open arms + Time spend on safe compartments	0.003	0.016	5.716	2.244	0.234

- 1
- 2
- 3
- 4