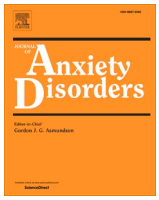
Journal of Anxiety Disorders 104 (2024) 102870



Contents lists available at ScienceDirect

Journal of Anxiety Disorders

journal homepage: www.elsevier.com/locate/janxdis





No harmful effect of propranolol administered prior to fear memory extinction in rats and humans

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ARTICLE INFO

Original content: No harmful effect of propranolol administered prior to fear memory extinction in rats and humans (Original data)

Keywords:
Extinction
Propranolol
Exposure therapy
Benzodiazepine
Midazolam
Fear conditioning
Anxiety

ABSTRACT

Exposure therapy is an evidence-based treatment option for anxiety-related disorders. Many patients also take medication that could, in principle, affect exposure therapy efficacy. Clinical and laboratory evidence indeed suggests that benzodiazepines may have detrimental effects. Large clinical trials with propranolol, a common beta-blocker, are currently lacking, but several preclinical studies do indicate impaired establishment of safety memories. Here, we investigated the effects of propranolol given prior to extinction training in 9 rat studies (N = 215) and one human study (N = 72). A Bayesian meta-analysis of our rat studies provided strong evidence against propranolol-induced extinction memory impairment during a drug-free test, and the human study found no significant difference with placebo. Two of the rat studies actually suggested a small beneficial effect of propranolol. Lastly, two rat studies with a benzodiazepine (midazolam) group provided some evidence for a harmful effect on extinction memory, i.e., impaired extinction retention. In conclusion, our midazolam findings are in line with prior literature (i.e., an extinction retention impairment), but this is not the case for the 10 studies with propranolol. Our data thus support caution regarding the use of benzodiazepines during exposure therapy, but argue against a harmful effect of propranolol on extinction learning.

1. Introduction

Anxiety-related disorders are a considerable burden on many people's lives and on society in general. They affect at least 300 million people (estimated global prevalence) and are responsible for more than 35 million disability-adjusted life years globally, numbers which have increased considerably since the start of the COVID-19 pandemic (Santomauro et al., 2021). Fortunately, several treatment options exist, mostly consisting of pharmacological or behavioral interventions. The latter include exposure-based methods, referred to as 'exposure therapy'. These are typically integrated into a cognitive behavioral therapy trajectory and entail exposing an individual to the object of their fears, e.g., by confronting them with a spider if they suffer from arachnophobia or by performing real-life, virtual or imaginary exposure to a feared social situation in case of social anxiety disorder, with the aim of reducing the unwanted fear or anxiety. Extensive empirical evidence supports the effectiveness of exposure therapy for a range of anxiety (-related) disorders (Carl et al., 2019; Carpenter et al., 2018; McLean et al., 2022; Sars & van Minnen, 2015).

Among those individuals who start an exposure therapy trajectory, there are many who take medication, often on a daily basis. These drugs may have been prescribed for their anxiety symptoms or for other, unrelated medical issues. Typical examples of drugs with widespread use, especially among individuals suffering from severe anxiety, are benzodiazepines (Hirschtritt et al., 2021; Olfson et al., 2015). These drugs act on GABA-A receptors, have acute anxiolytic and hypnotic effects, and are often used to alleviate sleeping problems. Prior research has already shown that taking benzodiazepines can negatively influence the (long-term) effectiveness of exposure therapy (Marks et al., 1993; Rothbaum et al., 2014; van Minnen et al., 2002), although sometimes no

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https://doi.org/10.1016/j.janxdis.2024.102870
Received 2 October 2023; Received in revised form 15 March 2024; Accepted 29 April 2024
Available online 5 May 2024
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significant effects were found (Rosen et al., 2013; Wardle et al., 1994). The aforementioned studies have directly examined the effect of benzodiazepines on therapy outcome, whereas others have used extinction learning following fear conditioning in the lab as a tool to investigate exposure therapy in more controlled circumstances. Note that we use the term 'fear' to refer to the whole range of conscious and unconscious responses to a threat (including subjective, physiological and behavioral reactions) that together make up this emotional state (Beckers et al., 2023). Fear memory extinction in the lab is not identical to exposure therapy in a clinical setting, but it does tap into the inhibitory learning process that is thought to be a key ingredient of exposure therapy. Extinction learning is thus widely considered to be a valid model to study exposure (Beckers et al., 2023; Scheveneels et al., 2016). Evidence from clinical and rodent research with benzodiazepines converges, as rat fear conditioning studies show that extinction learning under the influence of benzodiazepines results in worse extinction memory retention (Bouton et al., 1990; Hart et al., 2009, 2014). One explanation for this negative effect is that the internal context of having the drug in the system during extinction learning (i.e., a context that is clearly characterized by the benzodiazepine's sedative effects) may become associated with the extinction memory, resulting in state dependency and, eventually, fear renewal if the benzodiazepine is no longer present when subjects are again confronted with the object of their fears at a later timepoint (Bouton et al., 1990). Furthermore, patients may attribute the anxiolytic effects of the exposure therapy to the benzodiazepine, which may interfere with the eventual therapeutic effect (Başoğlu et al., 1994). At present, the consensus among clinicians is that there is not enough evidence to exclude benzodiazepine users from exposure therapy, but that caution is advised (Melani et al., 2020).

A different drug that may be relevant in the context of exposure – and which is the focus of the current study – is propranolol. As for benzodiazepines, there is preclinical evidence suggesting that propranolol may impair extinction learning. However, in contrast with benzodiazepines, these preclinical propranolol findings have not yet trickled down to large, controlled clinical trials, and their potential implications for clinical practice are unclear. Yet, this β-adrenergic receptor antagonist is widely prescribed, primarily to treat hypertension, but also to reduce tremor, mostly related to performance anxiety (e.g., for musicians). In 2020, the number of propranolol users in the US alone was estimated at almost two million (Kane, 2022). As mentioned above, the potential effects of propranolol use on the outcome of exposure therapy have received much less attention than those of benzodiazepines. However, research on reminder-dependent amnesia suggests that noradrenergic blockade may interfere with memory storage (Debiec & Ledoux, 2004; Ouyang & Thomas, 2005; Soeter & Kindt, 2012; pp, 2011). Therefore, it is not unlikely that, despite its potentially pleasant acute effects during exposure (e.g., feeling less aroused), propranolol may in the end undermine the effect of exposure therapy. Only a few (very) small-scale, controlled clinical studies are available that have looked at the effects of propranolol given prior to exposure therapy, and they support this overall picture: acute beneficial effects (e.g., lower distress during exposure), but a (slight) return of symptoms at follow-up (Meyerbröker et al., 2018; Morissette et al., 2008). Human fear conditioning studies in the lab obtained mixed findings, showing fear-increasing or -decreasing effects of propranolol (40 mg), both acutely during extinction learning, as well as on a drug-free retention test (Bos et al., 2012; Kroes et al., 2016; pp, 2015). Fear conditioning studies with rats, mice and rabbits have repeatedly found acute fear-reducing effects of propranolol (typically 10 mg/kg) during extinction learning (Burhans et al., 2018; Cain et al., 2004; Leal Santos et al., 2021; Rodriguez-Romaguera et al., 2009). On a subsequent drug-free retention test, some of these studies found no evidence for a significant difference between propranolol and control conditions (Burhans et al., 2018; Cain et al., 2004; Rodriguez-Romaguera et al., 2009), whereas several others reported negative effects (Cain et al., 2004; Fitzgerald et al., 2015; Leal Santos et al., 2021; Mueller et al., 2008), suggesting that having propranolol in the system during extinction learning might be detrimental to the retention of learned safety. All in all, prior clinical and preclinical findings appear to advise against combining propranolol use and exposure therapy. However, at this moment it is unclear whether there are sufficient grounds for a formal recommendation for clinicians to ask patients to stop or suspend propranolol use when starting exposure therapy.

In the current study, we investigated the effect of propranolol administration prior to extinction learning on a later drug-free memory retention test. In two parallel research lines, consisting of 9 preregistered rat experiments and one preregistered experiment with healthy human participants, we studied this effect using cued fear conditioning. Our procedures were based on prior research describing acute fear-reducing effects of propranolol during extinction training. For the studies with rats, we based our protocol on Rodriguez-Romaguera et al. (2009) (who found significant acute decreases in freezing and suppression of bar pressing) and for the human study, we used the procedure of Chalkia et al. (2019) (who described significant attenuating effects on fear-potentiated startle, heart rate and blood pressure). Alongside the group receiving 40 mg of propranolol, the current human study also included a group in which participants received an 80-mg dose, which is twice the dose that has often been used in human research on propranolol and fear memory attenuation, but which is more in line with the clinical dosage used for the treatment of hypertension and essential tremor.

Here, we thus aimed to test if propranolol given before extinction training would have an effect on fear responding during a drug-free retention test. Prior literature provides evidence for a harmful effect of propranolol (i.e., impaired extinction retention), and additional support for such an effect may argue against the continued use of propranolol during exposure therapy. Two of the rat experiments simultaneously examined the effect of the benzodiazepine midazolam, as an active control condition. Whereas our findings with midazolam are in line with prior literature (i.e., a detrimental effect on extinction learning), this is not the case for our 10 studies with propranolol.

2. Methods

2.1. Rat experiments

We present a series of experiments with non-naïve animals (Experiments 1–2, non-naïve for practical reasons) and with naïve animals (Experiments 3–9) using a standard fear conditioning and extinction procedure, which was in some cases extended with an additional test session (Experiments 5–9), all comparing the effects of propranolol and saline. Experiment 7 investigated the effect of propranolol after stronger fear conditioning, whereas Experiment 8 examined the effect of propranolol in combination with longer extinction training. Experiment 9 used the standard procedure, but in female animals. Two experiments (Experiments 5–6) included a third group of animals receiving midazolam.

2.1.1. Preregistration and data availability

All experiments, including protocols, planned sample sizes and analysis plans, were registered on the Open Science Framework (OSF) before the start of data collection (https://osf.io/ydwba). All data can be found there as well.

2.1.2. Ethics approval

This project was approved by the animal ethics committee at KU Leuven (P234–2015, P188–2020) and was conducted in accordance with the Belgian and European laws, guidelines and policies for animal experimentation, housing and care (Belgian Royal Decree of 29 May 2013 and European Directive 2010/63/EU on the protection of animals used for scientific purposes of 20 October 2010).

2.1.3. Subjects

Two hundred fifteen rats were used (male Wistar (N = 97), male Sprague-Dawley (N = 92) and female Sprague-Dawley (N = 26), 7–8 weeks old upon arrival in the lab) (Janvier Labs, Le Genest-Saint-Isle, France). They were housed in plastic cages with bedding, cage enrichment (Dura-Chew, Bio-Serv, Flemington, NJ, USA), food and water ad libitum, in groups of 4 in Experiments 1–2 and individually in Experiments 3–9. Behavioral testing started 4–5 days after arrival of the animals in the lab, except for Experiments 1–2 which used non-naïve animals that had undergone prior testing (including contextual fear conditioning) in our lab. Animals in Experiments 1–2 were therefore 13 and 10 weeks old, respectively, when entering the current study, whereas animals in Experiments 3–9 were naïve and about 8–9 weeks old. Animals were kept on a 12 h/12 h day-night cycle, with experiments starting at least 1 h after the beginning of the light phase. Rats were brought from the housing facility to a room adjacent to the testing room approximately 23 min before each behavioral session in their home cage, carried by the experimenter (Experiments 1–2) or transported on a wheeled cart (all other experiments). Immediately before the start of behavioral testing, animals were carried to the testing room in their home cage and then placed in the testing chambers.

2.1.4. Equipment

Four identical chambers (Contextual NIR Video Fear Conditioning System for Rats, Med Associates Inc., St. Albans, VT, USA) were used, with built-in ventilation fans providing background noise (± 67 dB) in all chambers. The same contextual configuration was used for all sessions and consisted of a standard grid floor and a white plastic curved back wall insert, illuminated by infrared light only, and cleaned and scented with a window cleaning product.

2.1.5. Procedure

Experimental designs, including the number of tones (conditioned stimulus, CS) and shocks (unconditioned stimulus, US) presented during each session, are shown in Fig. 1*A*. Details regarding the timing of the tone and shock presentations can be found in the ***Supplement***. In all experiments, freezing was scored manually from videos by an experienced observer blinded to group allocation (Luyten et al., 2014), and expressed as a percentage of the time under evaluation (i.e., the 3-min acclimation period ("PreCS" measurement) or during the 30-s CSs). The preregistered criterion for exclusion was less than 20% freezing on average during the last 4 Training trials, and 7 rats (i.e., 3% of the total sample) were excluded for this reason. In addition, one rat was never tested because it fell sick before the start of the experiment and 6 rats were excluded from all data analyses either because Extinction data were lost due to technical issues (N = 1) or because the predefined sample size had been reached (N = 5).

2.1.5.1. Habituation. Animals were habituated to the experimental context for 38 min.

2.1.5.2. Training. One day after Habituation, rats were first presented with 5 tones without shocks. Next, to acquire a cued fear memory, rats received 7 tone-shock pairings. Conditioned stimuli (CSs) were 30-s 4000-Hz, 78-dB tones and unconditioned stimuli (USs) were 0.5-s, 0.55-mA shocks, except in Experiment 7 for which shock intensity was increased to 0.8 mA. Animals were matched into equivalent groups (block-randomization) based upon average freezing to the tone during the last 4 (Experiments 1–4) or last 2 (Experiments 5–9) conditioning trials.

2.1.5.3. Extinction. One day later, an intraperitoneal injection was given with propranolol, midazolam or saline, and 20 min later, the behavioral session took place, during which rats were given 12 tones without shocks, in order to induce extinction learning. In Experiment 8, the number of Extinction trials was doubled to 24 tones, with the aim of producing (more) complete extinction learning.

Test 1. One day later, retention of the extinction memory was tested, by presenting 4, 8 or 12 tones (depending on the Experiment), without shock. In all experiments, the first 4 tones were considered to assess extinction memory retention, but in Experiments 5–9 more tones were added to induce additional extinction learning, which could then be evaluated on Test 2.

Test 2. In Experiments 5–9, a second test day took place, one week after the first one. During this session, 4 tones were presented, without shock, to assess retention of the extinction memory and any spontaneous recovery of the fear memory.

2.1.6. Drugs

2.1.6.1. Propranolol. In Experiments 1–9, propranolol (PROP) (Product P0884, Sigma-Aldrich, Overijse, Belgium) was dissolved in saline on the day of injection to obtain a solution of 10 mg/ml, administered intraperitoneally at 1 ml/kg (10 mg/kg dose). Control animals received saline (SAL) (1 ml/kg). Injections were given in a room adjacent to the behavioral testing room.

2.1.6.2. Midazolam. In Experiments 5–6, midazolam (MDZ) (Mylan, 15 mg/3 ml ampoules) was diluted with saline on the day of injection to obtain a solution of 3 mg/ml, administered intraperitoneally at 1 ml/kg (3 mg/kg dose).

2.1.7. Statistical analyses

All statistical analyses were conducted using JASP 0.16.3 (JASP Team, 2022). Significance levels for frequentist statistics were set at $p < .05$ and Greenhouse-Geisser corrections were applied when necessary. All graphs were created using GraphPad Prism 7.02 (GraphPad Software, La Jolla, CA, USA).

2.1.8. Preregistered analyses

2.1.8.1. Extinction. We hypothesized that animals that had received PROP before Extinction would show less freezing to the Extinction tones than SAL animals. This was tested with a Group by Trial Repeated-Measures ANOVA (a main effect of Group and/or interaction of Group x Trial was considered to assess differences between PROP and SAL rats) and a Group by Block Repeated-Measures ANOVA (an interaction of Group x Block was considered to assess differences between PROP and SAL rats). Block refers to the first (CS1-6) and second (CS7-12) half of Extinction. Note that the Block analysis was not preregistered for Experiments 1 & 3, but is nevertheless included in Table 1 for consistency. For Experiment 8, which contained 24 instead of 12 Extinction trials, an additional analysis was conducted including all Extinction trials (see ***Supplement***). The hypothesis for MDZ rats (in Experiments 5–6) was the same (i.e., less fear during the Extinction tones than SAL animals), although we expected that the acute sedative effects of the drug might interfere with the proper evaluation of freezing.

Test 1. In the first Experiments (1−4), the preregistered hypothesis for PROP versus SAL groups at test was no group difference, in line with Rodriguez-Romaguera et al. (2009). In light of the findings in Experiments 2 & 4, this was adapted to a hypothesis of lower freezing to the Test tones in PROP versus SAL animals, which may indicate better retention of the extinction memory in PROP animals in Experiments 5–9. In all experiments, a group difference at Test 1 was assessed with a Group by Trial Repeated-Measures ANOVA including the first four Test 1 trials (a main effect of Group and/or interaction of Group x Trial was considered to assess differences between groups). A separate analysis only including the first CS of Test 1 was also preregistered and can be found in the ***Supplement***. The analyses for the comparison of MDZ and SAL groups were similar, but for Experiment 5, we preregistered that we

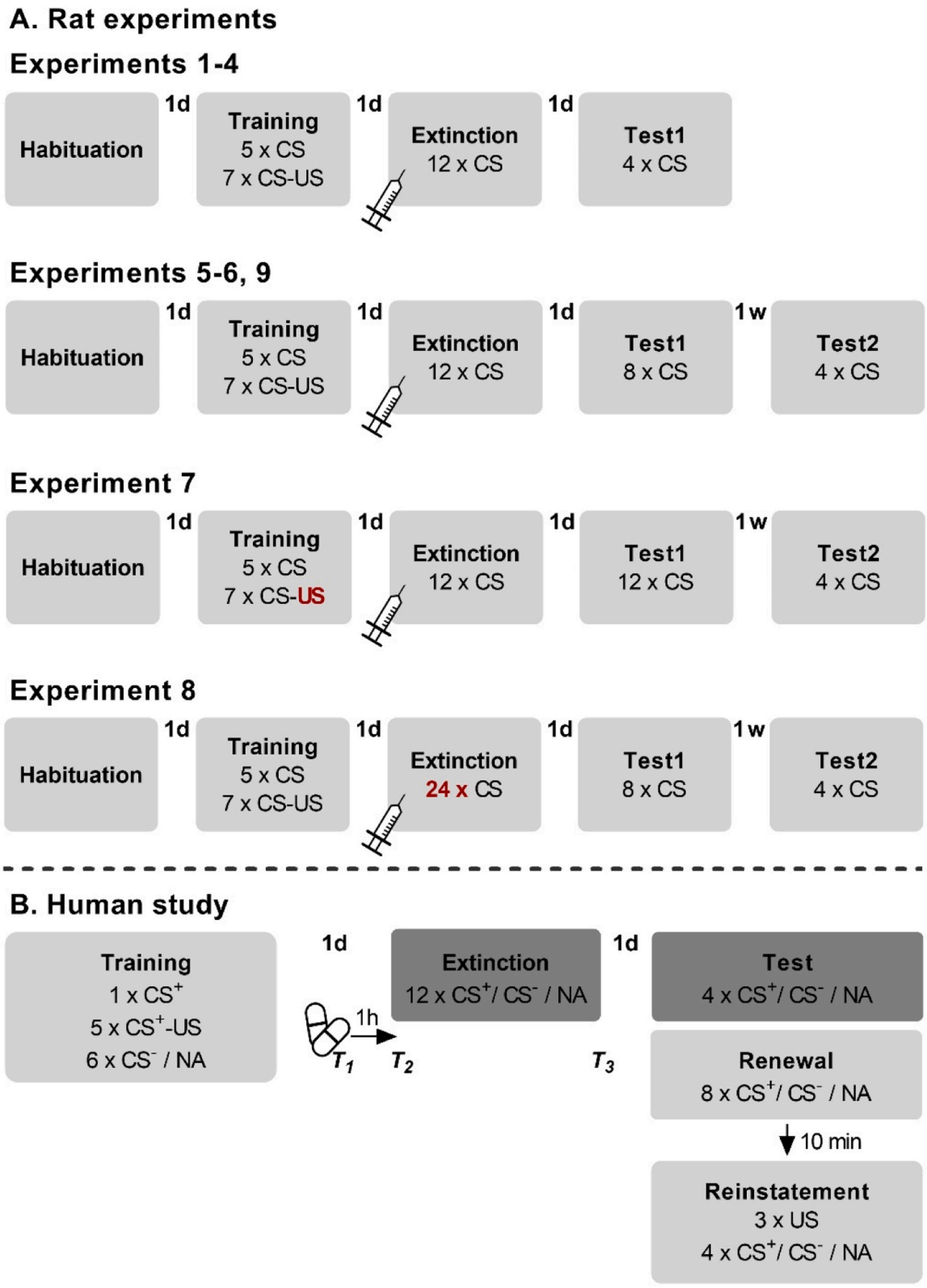

Fig. 1. Experimental designs. A. All rat experiments included propranolol and saline groups. In addition, Experiments 5 and 6 included a midazolam group. The syringe indicates that an intraperitoneal drug injection was given 20 min before the start of the Extinction session. Red indicates deviations from the standard procedure (i.e., stronger shocks, more extinction trials). B. For the human study, two contexts were used, shown as different shades of gray. At the start of each day, 10 noise-alone trials were presented (not shown in the figure). The pills (propranolol or placebo) were given 1 h before the start of the Extinction session. $T_{1/2/3}$ indicates the time points at which saliva samples, heart rate, blood pressure and arousal measurements were taken. CS: conditioned stimulus, NA: noise-alone trials, US: unconditioned stimulus.

Table 1
Results of the Repeated-Measures ANOVAs and Bayesian meta-analysis comparing PROP with SAL during Extinction trials 1–12 in Experiments 1–9. In each experiment, there were main effects of Trial and of Block during Extinction (not shown here for brevity, see OSF).

Exp	Extinction (per trial)		Extinction (per block)	Bayesian Meta-analysis on average of 12 Extinction trials
	Group	Trial by Group	Block by Group	
1	$F(1,14) = 7.194$, $p = .018$ $\eta^2_p = 0.339$	$F(5.36,75.09) = 1.058$, $p = .393$ $\eta^2_p = 0.070$	$F(1,14) = 0.096$, $p = .762$ $\eta^2_p = 0.007$	
2	$F(1,14) = 21.560$, $p < .001$ $\eta^2_p = 0.606$	$F(5.25,73.46) = 2.490$, $p = .036$ $\eta^2_p = 0.151$	$F(1,14) = 1.383$, $p = .259$ $\eta^2_p = 0.090$	
3	$F(1,15) = 0.250$, $p = .624$ $\eta^2_p = 0.016$	$F(5.32,79.75) = 1.449$, $p = .213$ $\eta^2_p = 0.088$	$F(1,15) = 7.510$, $p = .015$ $\eta^2_p = 0.334$	
4	$F(1,21) = 6.992$, $p = .015$ $\eta^2_p = 0.250$	$F(11,231) = 0.793$, $p = .647$ $\eta^2_p = 0.036$	$F(1,21) = 1.496$, $p = .235$ $\eta^2_p = 0.066$	
5	$F(1,13) = 0.690$, $p = .421$ $\eta^2_p = 0.050$	$F(11,143) = 0.676$, $p = .759$ $\eta^2_p = 0.049$	$F(1,13) = 0.276$, $p = .608$ $\eta^2_p = 0.021$	The average effect size of the difference between PROP and SAL rats during Extinction is estimated to be − 0.84 [− 1.20, − 0.48], with a corresponding BF_{10} of 179.027, indicating extreme evidence in favor of a group difference during Extinction, with PROP rats showing less freezing than SAL rats.
6	$F(1,22) = 8.904$, $p = .007$ $\eta^2_p = 0.288$	$F(5.82,128.12) = 1.124$, $p = .352$ $\eta^2_p = 0.049$	$F(1,22) = 3.214$, $p = .087$ $\eta^2_p = 0.127$	
7	$F(1,22) = 1.540$, $p = .228$ $\eta^2_p = 0.065$	$F(11,242) = 1.545$, $p = .116$ $\eta^2_p = 0.066$	$F(1,22) = 2.980$, $p = .098$ $\eta^2_p = 0.119$	
8	$F(1,22) = 15.441$, $p < .001$ $\eta^2_p = 0.412$	$F(11,242) = 0.746$, $p = .693$ $\eta^2_p = 0.033$	$F(1,22) = 1.007$, $p = .327$ $\eta^2_p = 0.044$	
9	$F(1,21) = 0.037$, $p = .850$ $\eta^2_p = 0.002$	$F(5.68,119.24) = 1.135$, $p = .346$ $\eta^2_p = 0.051$	$F(1,21) = 3.500$, $p = .075$ $\eta^2_p = 0.143$	

expected no difference between MDZ and SAL rats, and for Experiment 6, we hypothesized more freezing to the Test 1 tones in MDZ than SAL rats (based on prior literature and the results of Experiment 5).

Test 2. In the Experiments that included a second test session (Experiments 5–9), the hypotheses and preregistered analyses for Test 2 were the same as for Test 1. In light of the findings on Test 1, the results of Test 2 are shown in the ***Supplement***.

2.1.9. Non-preregistered analyses

2.1.9.1. Extinction and Test 1. Given the results of the preregistered frequentist analyses described above, we conducted additional Bayesian meta-analyses using the 9 individual experiments' effect sizes to quantify the overall evidence for the presence or absence of drug effects on average freezing during the tones of Extinction and Test 1. The absence of a significant difference using the preregistered frequentist statistics should be interpreted as inconclusive. On the contrary, Bayesian statistics can provide evidence for (or against) the absence of a group difference. In these Bayesian analyses, we used default priors as set in JASP 0.16.3. For each Bayesian meta-analysis, we computed a model-averaged inclusion Bayes factor for an effect. In the null model, it is assumed that there is no effect. When we assumed in the alternative model that there is an effect, we denote the Bayes factor comparing the null model and the alternative model with BF_{01} or BF_{10}, where BF_{01} quantifies (relative) evidence in favor of the absence of a group difference, and BF_{10} (relative) evidence in favor of a group difference. When we assumed in the alternative model that the effect is positive, we denote the Bayes factor comparing the null model and the alternative model with BF_{0+} or BF_{+0}, where BF_{0+} quantifies (relative) evidence in favor of the absence of an effect, and BF_{+0} (relative) evidence in favor of a positive effect. This one-sided analysis was used when investigating the (drug-free) extinction memory retention, where a positive effect indicates that retention is worse in subjects that underwent extinction training with propranolol or midazolam on board as compared to vehicle, and BF_{+0} quantifies the (relative) evidence that propranolol or midazolam rats show more freezing than saline rats. Besides the Bayes factors, we report the model-averaged posterior mean and 95% credible interval for the overall effect size. These estimates are conditional on the assumption that an effect is present, without further restrictions on the sign of the effect, so should be interpreted with caution when the Bayes factor indicates evidence for the absence of an effect.

2.2. Human study

2.2.1. Study information and preregistration

This study was registered as a clinical trial in the EU Drug Regulating Authorities Clinical Trials Database (EudraCT; 2018–003409-25) and the results are publicly available at https://www.clinicaltrialsregister.eu/. All procedures were carried out in accordance with the Declaration of Helsinki and full ethical approval was granted by the UZ/KU Leuven Ethics Committee Research. Experimental procedures and planned analyses were preregistered on Aspredicted.org (https://aspredicted.org/vu7y4.pdf). All data and analyses are available on https://osf.io/ydwba.

2.2.2. Subjects

One hundred and fifty-two human subjects were recruited from the university subject pool to participate in this study. The final sample included 72 subjects (59 women), between the ages of 18 and 39 years old (20.21 ± 3.14, mean ± standard deviation), which were equally divided over 3 groups. See ***Supplement*** for details regarding the reasons for exclusion. All subjects gave written informed consent and were reimbursed with €50 or partial course credits.

2.2.3. Fear conditioning paradigm

The fear conditioning paradigm employed in this study was a modified version of the widely-used protocol introduced by Milad et al. (2005). Images of an office room and a room with a shelf served as two contexts (CTX) A and B, respectively. An identical desk lamp was included in each CTX that lit up in either blue or yellow light, and the different lights indicated the conditioned stimuli (CS+/CS-; counterbalanced). The US was a 200-ms electrical stimulus generated by a constant-current stimulator (DS7A Digitimer, Hertfordshire, United Kingdom). The US was delivered through a stimulating bar electrode, composed of two 8-mm stainless steel electrodes with an inter-electrode distance of 30 mm (Digitimer). The bar electrode was attached to the top of the wrist of the dominant hand with a Velcro strap.

Subjects completed a 3-day protocol (Fig. 1*B*, for a detailed description see ***Supplement***), with a Training (i.e., fear acquisition) phase in CTX A on Day 1, Extinction learning in CTX B on Day 2, a memory retention Test in CTX B on Day 3, followed by a Renewal and Reinstatement test in CTX A. At the start of each experimental trial, the CTX was presented alone for 3 s and then in combination with the CS for another 8 s. On all trials, startle probes were presented 1 s prior to CS offset, and on CS+ trials, the US (individually selected to have an intensity that was uncomfortable, but not painful) was presented 800 ms after the startle probe and co-terminated with CTX/CS offset. A fixation cross was presented during intertrial intervals, which had a duration of 15 – 25 s (20 s on average). Interspersed between CS trials, noise-alone (NA) trials were presented as to have a measure of baseline startle responding. NA trials had a duration of 40 ms, i.e., the duration of the startle probe. Throughout the experiment, order of trial presentations was randomized in blocks of 3 stimuli (CS+, CS-, NA), so that no trial type was presented more than two times in a row. Stimulus presentation and data acquisition were controlled by Affect 4.0 (Spruyt et al., 2010).

2.2.4. Drug administration

Propranolol HCl and placebo tablets were administered on Day 2 of the study. Placebos were manufactured (Wolfs, Zwijndrecht, Belgium) to be identical in shape, size, and color as the propranolol tablets. Each subject received two tablets in a single, oral administration: those in the 80 mg group received two 40-mg propranolol tables, those in the 40 mg group received one 40-mg propranolol and one placebo tablet, and subjects in the Placebo group received two placebo tablets. At the end of the session, subjects were asked whether they believed that they had received propranolol or placebo.

2.2.5. Subjective assessments

2.2.5.1. Questionnaires & ratings. The Anxiety Sensitivity Index (ASI; (Peterson & Heilbronner, 1987)) was used to assess subjects' anxiety sensitivity, or the degree to which they fear the subjective experience of sensations associated with anxiety. State and trait anxiety were measured using the State and Trait Anxiety Inventory (STAI-S/STAI-T; (Spielberger et al., 1977)). In addition, participants were asked to rate the US, the startle probes, and their subjective arousal at several time points (see ***Supplement***).

2.2.5.2. US expectancies. Throughout the experiment, subjects were asked to indicate their online US expectancy on an 11-point scale ranging from "certainly no electric stimulus" (−5), over "uncertain" (0), to "certainly an electric stimulus" (5). This scale was presented upon CS onset on all trials and subjects were allowed 7 s to respond.

2.2.6. Physiological measures

As a manipulation check for the physiological effects of propranolol, heart rate (HR), blood pressure (BP) and salivary α-amylase (sAA) were measured during Day 2 (see ***Supplement*** for details).

2.2.6.1. Fear-potentiated startle. Electromyography (EMG) of the right orbicularis oculi muscle was used to capture fear-potentiated startle (FPS) responses. Two reusable, 4-mm Ag/AgCl electrodes filled with highly conductive, electrolyte gel (Signa Gel, Parker Labs Inc, Fairfield, NJ, United States) were placed 1 cm below the pupil and 1 cm below the lateral canthus, and a third (ground) electrode was placed on the forehead (Blumenthal et al., 2005). To elicit startle responding, acoustic probes (40 ms, white noise, 100 dBA) were presented through headphones (Sennheiser HD 202; Wedemark, Germany). The signal was measured at 1000 Hz and amplified using an isolated bioamplifier with band-pass filter (Lablinc v75–04, Coulbourn Instruments; Holliston, MA, United States), with a high pass filter of 13 Hz and a low pass filter of 500 Hz. A 4-channel integrator (Lablinc v76–24, Coulbourn Instruments) rectified and smoothed the signal online, at a time constant of 20 ms. The analog output was then digitized by a 16-bit AD converter (National Instruments, NI-6221; Austin, TX, United States). Data extraction was completed offline using the software package Psychophysiological Analysis (PSPHA) (De Clercq et al., 2006). Blink amplitude was determined by subtracting a 20-ms baseline (0–20 ms following probe onset) from the peak response in a 21–200 ms window following probe onset. Trials where a spontaneous blink or excessive movement occurred at the time of probe presentation were excluded, leading to a loss of 7.1% of the data. To standardize the data, means and standard deviations from the first day were used to calculate within-participant *z*-scores. Observations with a *z*-score > 4 or < -4 were considered outliers and scored as 4 or − 4, respectively, and kept in the analyses.

2.2.6.2. Skin conductance. Skin conductance responses (SCR) were recorded using an isolated skin conductance coupler (LabLinc v71–23, Coulbourn Instruments) and obtained using two disposable, pre-gelled 8-mm Ag/AgCl electrodes (Biopac Systems, Goleta, CA, United States) attached to the palm of the non-dominant hand. The signal was sampled at 1000 Hz with an identical analogue-to-digital conversion as FPS. Offline data extraction was completed with a custom-made MATLAB toolbox (R2021a, MathWorks; Natick, MA, United States). SCR amplitudes were determined by subtracting the average of a 2-s baseline (prior to CS onset) from the maximum response in a 0–7 s window following CS onset. We did not employ a minimum response criterion and kept all responses in the analysis. SCR data were *z*-transformed analogously to FPS responses, and outliers were similarly determined and treated.

2.2.7. Statistical analyses

2.2.7.1. Preregistered analyses. FPS and SCR data were first averaged over blocks of two trials (except for the memory retention Test, Renewal and Reinstatement tests, where we also looked at the first trial only) and then analyzed with Repeated-Measures ANOVAs with Group (80 mg, 40 mg, Placebo) as between-subjects factor and Cue [CS+ , CS-, (NA)] and Time as within-subjects factors. Note that the factor Time was not clearly specified in the preregistration. For Training and Extinction, the factor Time comprised a comparison between the first and last blocks of each phase. For the memory retention Test, Renewal, and Reinstatement, ANOVAs included a Time comparison between the end of the previous phase and the beginning of the current phase. US expectancy data were not averaged over blocks, but single-trial data were analyzed in an identical manner to FPS and SCR. Interactions were followed up

with Bonferroni-corrected *t*-tests. The group comparison for within-session Extinction and for the first trial of the memory retention Test are shown in the main text, whereas all other fear conditioning analyses are shown in the ***Supplement***. Day 2 arousal ratings, HR, BP, and sAA, were analyzed using Repeated-Measures ANOVAs with Group as between-subjects factor and Time (1, 2, 3) as within-subjects factor. Missing values (in FPS and US expectancies) were replaced by linear trend at point. Greenhouse–Geisser corrections were applied in case of sphericity violations. An alpha level of 0.05 was set for all analyses, which were executed using JASP 0.16.3 (JASP Team, 2022).

2.2.8. Non-preregistered analyses

Test. In light of the results of the Bayesian meta-analysis of the rodent experiments, we conducted additional Bayesian t-tests to quantify the overall evidence for the absence (or presence) of propranolol effects on US expectancy, FPS and SCR on the first Test trial. For each of these three outcomes, we used a difference score (CS+ minus CS-), to correct for responding to the CS-, and examined the contrast of each Propranolol group with the Placebo group. Descriptives can be found in the ***Supplement***. We computed Bayes factors for a Student independent samples t-test. In the null model, it is assumed that there is no difference between Propranolol and Placebo, whereas in the alternative model, it is assumed there is a difference. We assumed the default priors as set in JASP 0.16.3, using sqrt(2)/2 as the scale for the prior distribution on the effect size. The Bayes factor comparing the null model and the alternative model, BF_{01}, quantifies the (relative) evidence in favor of the

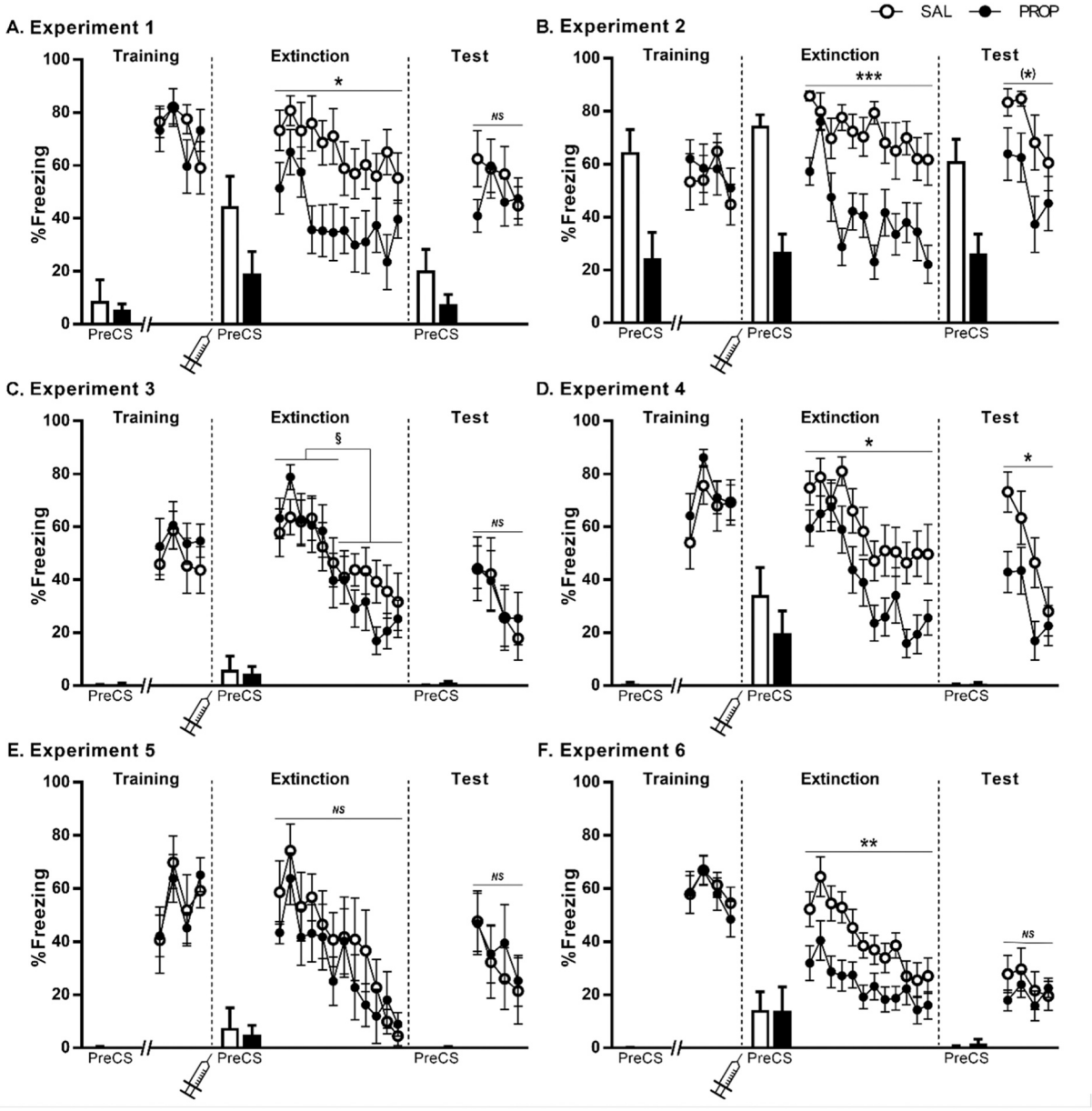

Fig. 2. Percentage freezing (mean and SEM) is shown during the 3-min acclimation phase (PreCS) and during the 4 last tones of Training, all 12 tones of Extinction and the (first) 4 tones of Test (i.e., Test 1 for the experiments that had two tests). All experiments were carried out using male rats. Experiments 1 (N = 16) and 2 (N = 16) were conducted using non-naïve Wistar rats, Experiments 3 (N = 17), 4 (N = 23) and 5 (N = 22) with naïve Sprague-Dawley rats and Experiment 6 (N = 36) with naïve Wistar rats. Note that Experiments 5 and 6 also contained a MDZ group which is presented in Fig. 4. Saline (SAL) groups are shown in white, propranolol (PROP) groups in black. Propranolol or saline was injected 20 min before the start of Extinction. Asterisks indicate main effects of Group (i.e., PROP ‡ SAL, *p < .05, **p < . 01, ***p < .001, whereas (*) indicates a trend, p = .05). The section sign denotes a significant interaction between Group and Block (i.e., PROP and SAL differ when comparing the first and second half of Extinction, §p < .05. NS indicates no significant main Group effect nor interaction.

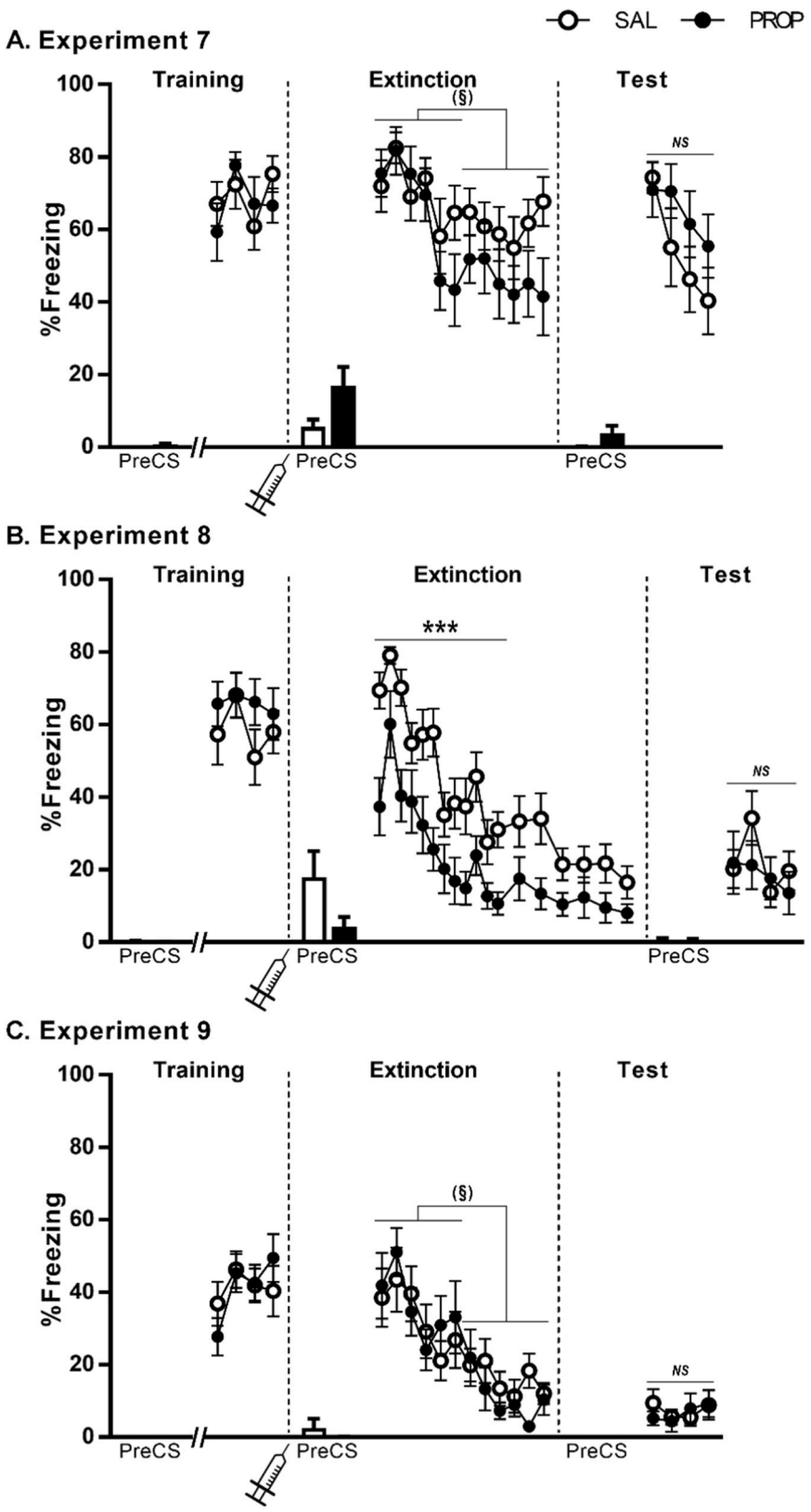

Fig. 3. Percentage freezing (mean and SEM) is shown during the 3-min acclimation phase (PreCS) and during the 4 last tones of Training, all tones of Extinction and the first 4 tones of Test (i.e., Test 1). Experiment 7 (N = 24) was conducted with naïve, male Sprague-Dawley rats and used more intense shocks during Training. Experiment 8 (N = 24) was carried out with naïve, male Wistar rats and entailed more Extinction trials (24 instead of 12). Experiment 9 (N = 23) was conducted with naïve, female Sprague-Dawley rats and used the standard procedure. Saline (SAL) groups are shown in white, propranolol (PROP) groups in black. Propranolol or saline was injected 20 min before the start of Extinction. Asterisks indicate a main effect of Group (i.e., PROP ‡ SAL, ***$p < .001$). The section sign denotes the interaction between Group and Block (i.e., comparing the first and second half of Extinction, with (§) indicating a trend, $p < .10$). NS indicates no significant main Group effect nor interaction.

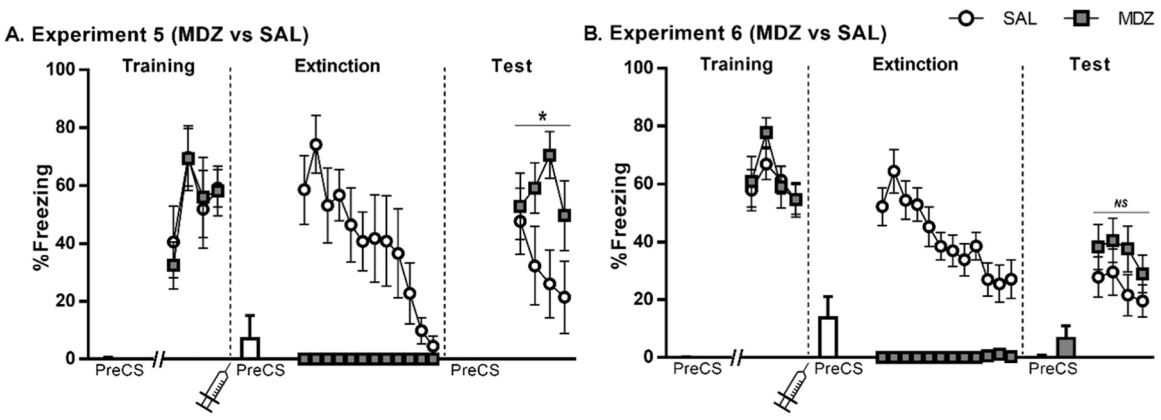

Fig. 4. Percentage freezing (mean and SEM) is shown during the 3-min acclimation phase (PreCS) and during the 4 last tones of Training, all 12 tones of Extinction and the first 4 tones of Test (i.e., Test 1). Experiment 5 was conducted with male Sprague-Dawley rats, Experiment 6 with male Wistar rats. Note that the SAL groups shown here are the same as those in Fig. 2. Saline (SAL) groups are shown in white, midazolam (MDZ) groups in gray. Midazolam or saline was injected 20 min before the start of Extinction. The asterisk indicates a significant interaction between Group and Trial (*p < .05). NS indicates no significant main Group effect nor interaction.

absence of a group difference.

3. Results

3.1. Rat experiments

Given the number of studies included in this manuscript, the main text will focus on the main results (Figs. 2–4), being the acute drug effects during Extinction, as well as any effects during the drug-free retention Test one day later (i.e., Test 1 in the studies with two retention tests). Additional analyses, e.g., regarding group comparisons during Training, during the longer Extinction session (in Experiment 8) and at Test 2 (in Experiments 5–9) can be found in the ***Supplement***. Below, preregistered frequentist analyses are presented first, followed by the non-preregistered (Bayesian) meta-analyses.

3.1.1. Extinction

Table 1 shows the results for the comparison of the PROP and SAL conditions in Experiments 1–9 (Figs. 2–3). In line with our hypothesis, we found acute freezing-reducing effects of PROP in the majority of the experiments. Freezing during the 12 Extinction trials was significantly lower in PROP than SAL rats in Experiments 1, 2, 4, 6 and 8. In addition, there was a faster decline in freezing in PROP than SAL rats over the 12 trials in Experiment 2, and when comparing the first and last half of the trials also in Experiment 3. Trends for a faster decline were seen in Experiments 6, 7 (which entailed stronger fear conditioning) and 9 (the experiment with females).

A Bayesian meta-analysis including all 9 experiments indicated extreme evidence for a group difference, with PROP rats showing less freezing than SAL rats during Extinction.

Experiments 5 and 6 included a third group in which rats were given midazolam prior to Extinction training. The planned statistical analyses (comparing MDZ and SAL) were not carried out because none of the MDZ animals showed any freezing during the Extinction session due to the drug's strong sedative effects (Fig. 4).

3.1.2. Test 1

Table 2 summarizes the analyses comparing the PROP and SAL conditions in Experiments 1–9 during the first 4 trials of the drug-free retention test, one day after Extinction (Figs. 2–3). In most experiments, there was no evidence for a group difference. Exceptions to this overall image are Experiment 4 (naïve, male Sprague-Dawley rats, Fig. 2*D*), in which we found significantly less freezing to the tones in PROP than SAL rats, and Experiment 2 (non-naïve, male Wistar rats, Fig. 2*B*) where we found a trend for the same effect.

Note that Experiment 7 (Fig. 3*A*) entailed a fear conditioning procedure with stronger shocks because exploratory analyses had suggested that the freezing-reducing effect at Test might be larger if there was more freezing at Test in the control condition (i.e., more room for improvement with the drug), but no effect was found in Experiment 7. In addition, Experiment 8 (Fig. 3*B*) was designed to have a longer Extinction protocol (24 instead of 12 trials) to evaluate a potential freezing-reducing effect of PROP at Test if extinction learning was more complete (actually implying less room for improvement), and again no effect of PROP was found. Experiment 9 (Fig. 3*C*) was conducted to test whether the (marginally) significant effect seen in male rats (in Experiments 2 and 4) could also be observed in females, but no freezing-reducing effect of PROP at Test was found.

A Bayesian meta-analysis including all 9 experiments indicated weak evidence in favor of the absence of a group difference at Test. Furthermore, a one-sided analysis was carried out in light of several reports in the literature of impaired extinction memory retention when propranolol was administered before extinction learning. The one-sided Bayesian meta-analysis indicated strong evidence against such a harmful effect (i.e., against PROP rats freezing more than SAL rats at Test).

In addition, analyses were conducted to evaluate the effect of midazolam given before extinction training on retention of the extinction memory during the drug-free Test (Fig. 4 and Table 3). Freezing at Test was numerically higher in MDZ than SAL rats in both Experiments 5 and 6, but the difference (interaction effect of Trial by Group) was only statistically significant in Experiment 5 (Fig. 4***A***). A Bayesian meta-analysis including both experiments indicated very weak evidence in favor of a group difference, with MDZ rats showing more freezing than SAL rats at Test. Furthermore, a one-sided analysis was carried out in light of several reports in the literature of impaired extinction memory retention when midazolam was administered before extinction learning. The one-sided Bayesian meta-analysis indicated weak evidence in favor of such a harmful effect (i.e., MDZ rats freezing more than SAL rats at Test).

3.1.3. Test 2

Given the absence of significant effects of PROP on Test 1 in Experiments 5–9, Test 2 is relatively uninformative. Details of the analyses can therefore be found in the Supplement (Tables S5-S7). Generally, no significant group differences were found on Test 2 in Experiments 5–9.

Table 2
Results of the Repeated-Measures ANOVAs and Bayesian meta-analyses comparing PROP with SAL during Test 1 trials 1–4 in Experiments 1–9.

Exp	Test 1 (per trial)			Bayesian Meta-analysis on average of 4 Test 1 trials
	Group	Trial	Trial by Group	
1	$F(1,14) = 0.499$, $p = .492$ $\eta^2_p = 0.034$	$F(3,42) = 1.306$, $p = .285$ $\eta^2_p = 0.085$	$F(3,42) = 1.435$, $p = .246$ $\eta^2_p = 0.093$	
2	$F(1,14) = 4.484$, $p = .053$ $\eta^2_p = 0.243$	$F(1.87,26.14) = 8.013$, $p = .002$ $\eta^2_p = 0.364$	$F(1.87,26.14) = 0.593$, $p = .549$ $\eta^2_p = 0.041$	
3	$F(1,15) = 0.011$, $p = .920$ $\eta^2_p < 0.001$	$F(3,45) = 5.445$, $p = .003$ $\eta^2_p = 0.266$	$F(3,45) = 0.202$, $p = .894$ $\eta^2_p = 0.013$	
4	$F(1,21) = 4.375$, $p = .049$ $\eta^2_p = 0.172$	$F(3,63) = 18.717$, $p < .001$ $\eta^2_p = 0.471$	$F(3,63) = 2.436$, $p = .073$ $\eta^2_p = 0.104$	The average effect size of the difference between PROP and SAL rats during Test is estimated to be − 0.19 [− 0.49, 0.10], with a corresponding BF_{01} of 2.536, indicating weak evidence in favor of the absence of a group difference during Test. BF_{0+} is 12.936, indicating strong evidence against PROP rats freezing more than SAL rats at Test.
5	$F(1,13) = 0.093$, $p = .765$ $\eta^2_p = 0.007$	$F(3,39) = 7.656$, $p < .001$ $\eta^2_p = 0.371$	$F(3,39) = 0.744$, $p = .532$ $\eta^2_p = 0.054$	
6	$F(1,22) = 0.416$, $p = .526$ $\eta^2_p = 0.019$	$F(3,66) = 1.783$, $p = .159$ $\eta^2_p = 0.075$	$F(3,66) = 1.143$, $p = .338$ $\eta^2_p = 0.049$	
7	$F(1,22) = 1.145$, $p = .296$ $\eta^2_p = 0.049$	$F(3,66) = 7.676$, $p < .001$ $\eta^2_p = 0.259$	$F(3,66) = 1.438$, $p = .240$ $\eta^2_p = 0.061$	
8	$F(1,22) = 0.235$, $p = .633$ $\eta^2_p = 0.011$	$F(3,66) = 3.019$, $p = .036$ $\eta^2_p = 0.121$	$F(3,66) = 1.480$, $p = .228$ $\eta^2_p = 0.063$	
9	$F(1,21) = 0.035$, $p = .853$ $\eta^2_p = 0.002$	$F(2.12,44.61) = 0.952$, $p = .398$ $\eta^2_p = 0.043$	$F(2.12,44.61) = 0.664$, $p = .529$ $\eta^2_p = 0.031$	

Table 3
Results of the Repeated-Measures ANOVAs and Bayesian meta-analyses comparing MDZ with SAL during Test 1 trials 1–4 in Experiments 5 and 6.

Exp	Test 1 (per trial)			Bayesian Meta-analysis on average of 4 Test 1 trials
	Group	Trial	Trial by Group	
5	$F(1,12) = 3.399$, $p = .090$ $\eta^2_p = 0.221$	$F(3,36) = 2.357$, $p = .088$ $\eta^2_p = 0.164$	$F(3,36) = 3.614$, $p = .022$ $\eta^2_p = 0.231$	The average effect size of the difference between MDZ and SAL rats during Test (first 4 trials) is estimated to be 0.54 [− 0.13, 1.24], with a corresponding BF_{10} of 1.389, indicating, if anything, very weak evidence in favor of a group difference during Test, with MDZ rats showing more freezing than SAL rats. BF_{+0} is 2.626, indicating weak evidence in favor of MDZ rats freezing more than SAL rats at Test.
6	$F(1,22) = 1.583$, $p = .221$ $\eta^2_p = 0.067$	$F(3,66) = 3.654$, $p = .017$ $\eta^2_p = 0.142$	$F(3,66) = 0.356$, $p = .785$ $\eta^2_p = 0.016$	

3.2. Human study

3.2.1. Acute physiological effects of propranolol

Table 4 shows the results for the comparison of the 80 mg propranolol, 40 mg propranolol and placebo conditions in the human fear conditioning study (N = 72) (Fig. 5). As expected, propranolol acutely lowered salivary α-amylase (marker of noradrenergic activity), heart rate, systolic and diastolic blood pressure. There were no significant differences between the 40-mg and 80-mg dose.

In addition to these physiological measurements, we also asked the participants to rate their levels of arousal at the same time points. In contrast with the clearly significant effects on α-amylase, heart rate and blood pressure, there was no significant effect of propranolol on subjective arousal. The mean arousal ratings (± standard deviations) on T_1, T_2 and T_3 were 3.0 (± 1.3), 2.1 (± 0.9) and 2.3 (± 1.3) on a 9-point scale. There was a main effect of Time, but no main effect of Group nor interaction (Table 4).

3.2.2. Extinction and its retention

Here, we focus on the group comparisons of fear memory indices during Extinction and the memory retention Test (Table 5). These data, as well as the Training data, are shown in Fig. 6. Detailed results for the Training, Renewal and Reinstatement sessions are presented in the ***Supplement***.

For Extinction on Day 2 (Table 5), significant effects of Time and Cue by Time were observed in all measurements (US expectancy, FPS and SCR), supporting extinction learning in all groups (see also Fig. 6). The absence of significant Group effects and of interactions with Group, however, indicate that there were no significant differences in extinction learning between the 3 groups. Note that FPS and SCR responses to the CS- were relatively high at the start of Extinction, particularly in the 80 mg group, and might reflect uncertainty about the meaning of the CS- at the start of a new experimental day. Importantly, conditioned fear responding to the CS+ was evident and comparable across groups. When comparing the 80 mg, 40 mg and Placebo groups at the first trial of the memory retention Test on Day 3, there was again no evidence for group differences in any of the outcome measures (no main effects of Group and no interactions with Group, Table 5).

The lack of significant group differences at Test was further evaluated using Bayesian analyses to quantify the strength of the evidence for the absence of differences between the 80 mg, 40 mg and Placebo groups. In all cases, we found more evidence for the 'no difference' hypothesis than for the alternative hypothesis, in varying degrees of strength (see Table 5). In all cases, increasing the scale increased BF_{01}. While some Bayes factors indicate that the evidence is inconclusive or very weak, they indicate that, overall, if we would be willing to draw a conclusion, we would conclude there is no effect of propranolol, which is consistent with the results described above. The strongest evidence for the absence of an effect of propranolol was found for SCR.

4. Discussion

In this series of experiments (9 in rats and 1 in humans), we investigated the effects of systemic propranolol administration prior to extinction training in a cued fear conditioning procedure. We assessed the acute effects of propranolol during extinction learning, as well as its subsequent effects on extinction memory during drug-free retention testing, one or more days later.

Overall, we found evidence for acute effects of propranolol on freezing behavior in rats and on physiological measurements (cardiovascular parameters and salivary α-amylase, a marker for noradrenergic

Table 4
Results of the Repeated-Measures ANOVAs and subsequent posthoc tests comparing the 80 mg propranolol, 40 mg propranolol and Placebo groups at three time points (T_1, T_2 and T_3) on Day 2, for salivary α-amylase, heart rate, systolic and diastolic blood pressure (BP), and subjective arousal.

RM ANOVAs	Group	Time	Time by Group
Salivary α-amylase	F (2,69) = 2.624, p = .080 η^2_p = 0.071	F (1.48,102.35) = 7.754, p = .002 η^2_p = 0.101	F (2.97,102.35) = 3.139, p = .029 η^2_p = 0.083
Heart rate	F(2,69) = 3.766, p = .028 η^2_p = 0.098	F(1.57,108.12) = 206.643, p < .001 η^2_p = 0.750	F(3.13,108.12) = 2.078, p = .105 η^2_p = 0.057
Systolic blood pressure	F(2,69) = 4.238, p = .018 η^2_p = 0.109	F(2,138) = 88.463, p < .001 η^2_p = 0.562	F(4,138) = 4.935, p < .001 η^2_p = 0.125
Diastolic blood pressure	F(2,69) = 1.599, p = .210 η^2_p = 0.044	F(2,138) = 7.777, p < .001 η^2_p = 0.101	F(4,138) = 4.132, p = .003 η^2_p = 0.107
Subjective arousal	F(2,69) = 0.448, p = .640 η^2_p = 0.013	F(1.79,123.48) = 20.081, p < .001 η^2_p = 0.225	F(3.58,123.48) = 0.734, p = .556 η^2_p = 0.021
Posthoc tests (T1)	80 mg vs Placebo	40 mg vs Placebo	80 mg vs 40 mg
Salivary α-amylase	t(46) = −0.748, p = .458 d = −0.216	t(46) = −0.051, p = .960 d = −0.015	t(46) = −0.728, p = .470 d = −0.210
Heart rate	t(46) = −0.999, p = .323 d = −0.288	t(46) = −0.570, p = .572 d = −0.164	t(46) = −0.518, p = .607 d = −0.150
Systolic blood pressure	t(46) = −0.892, p = .377 d = −0.257	t(46) = −0.470, p = .640 d = −0.136	t(46) = −0.435, p = .666 d = −0.125
Diastolic blood pressure	t(46) = 0.047, p = .963 d = 0.014	t(46) = 0.662, p = .511 d = 0.191	t(46) = −0.557, p = .580 d = −0.161
Subjective arousal	t(46) = 0.464, p = .645 d = 0.134	t(46) = 0.615, p = .541 d = 0.178	t(46) = −0.219, p = .828 d = −0.063
Posthoc tests (T2)	80 mg vs Placebo	40 mg vs Placebo	80 mg vs 40 mg
Salivary α-amylase	t(46) = −2.495, p = .016 d = −0.720	t(46) = −2.258, p = .029 d = −0.652	t(46) = −0.348, p = .729 d = −0.101
Heart rate	t(46) = −3.213, p = .002 d = −0.927	t(46) = −2.718, p = .009 d = −0.784	t(46) = −0.319, p = .751 d = −0.092
Systolic blood pressure	t(46) = −2.819, p = .007 d = −0.814	t(46) = −2.430, p = .019 d = −0.701	t(46) = −0.084, p = .934 d = −0.024
Diastolic blood pressure	t(46) = −2.513, p = .016 d = −0.726	t(46) = −1.914, p = .062 d = −0.553	t(46) = −0.729, p = .469 d = −0.211
Subjective arousal	t(46) = 1.519, p = .136 d = 0.438	t(46) = 1.011, p = .317 d = 0.292	t(46) = 0.142, p = .887 d = 0.041
Posthoc tests (T3)	80 mg vs Placebo	40 mg vs Placebo	80 mg vs 40 mg
Salivary α-amylase	t(46) = −2.037, p = .047 d = −0.588	t(46) = −2.453, p = .018 d = −0.708	t(46) = 0.606, p = .547 d = 0.175
Heart rate	t(46) = −2.504, p = .016 d = −0.723	t(46) = −2.748, p = .009 d = −0.793	t(46) = 0.125, p = .901 d = 0.036
Systolic blood pressure	t(46) = −3.950, p < .001 d = −1.140	t(46) = −2.976, p = .005 d = −0.859	t(46) = −0.486, p = .629 d = −0.140
Diastolic blood pressure	t(46) = −1.755, p = .086 d = −0.507	t(46) = −1.251, p = .217 d = −0.361	t(46) = −0.416, p = .680 d = −0.120
Subjective arousal	t(46) = −0.571, p = .571 d = −0.165	t(46) = 0.690, p = .494 d = 0.199	t(46) = −1.051, p = .299 d = −0.303

activity) in humans, both suggesting lower fear or arousal compared to the placebo control condition during the extinction training phase. This finding is in line with prior research and with the expected physiological effects of propranolol at this dose (10 mg/kg for the rats, 40 or 80 mg for the human participants) (Cain et al., 2004; Chalkia et al., 2019; Kroes et al., 2016; pp, 2015; Leal Santos et al., 2021; Rodriguez-Romaguera et al., 2009). Note that prior research in humans, using similar behavioral procedures, in addition to these cardiovascular changes, also found effects on fear memory indices, i.e., an attenuation of fear-potentiated startle during extinction learning (Chalkia et al., 2019), but such effects were not found in the current sample, not even with the higher 80-mg dose.

Our main research question, however, focused on drug-free testing of extinction memory retention. Prior research indicated that propranolol can have detrimental effects on extinction memories (Bos et al., 2012; Cain et al., 2004; Fitzgerald et al., 2015; Leal Santos et al., 2021; Mueller et al., 2008), a finding with potential clinical implications for people receiving exposure therapy for anxiety-related disorders. Our own data, however, suggest otherwise. A Bayesian meta-analysis of the 9 rat studies presented here provided strong evidence against such a harmful effect, and in the human study there was no significant difference between the propranolol-treated groups and placebo. An additional Bayesian t-test on the human data confirmed that there was evidence against an effect of propranolol on extinction memory. Moreover, two of the rat studies actually suggested improved extinction memory retention with propranolol (statistically significant ($p < .05$) in Experiment 4 and a trend ($p = .05$) in Experiment 2). The effects were small, but nevertheless suggested a durable fear-reducing effect of propranolol.

The early promising findings with propranolol in two of our rat studies fueled further experiments within this line of research. In the end, the full set of 9 experiments (10, including the human fear conditioning study) did not corroborate that propranolol improves extinction memory retention. It should be noted that the idea of drug-enhanced psychotherapy is not new. There is no doubt that many therapists are wary of using medication aimed at enhancing behavioral therapy, given that such a drug may unwantedly take on the role of a safety cue, which most therapists prefer to steer clear of (Blakey & Abramowitz, 2016; Rosen et al., 2013). Nevertheless, quite some research efforts have been devoted to drug enhancement of psychotherapy, but, to date, their translation to clinical successes has been limited. Examples of drugs that have been put forward as ways to 'boost' exposure therapy are D-Cycloserine (DCS) and yohimbine (Singewald et al., 2015). DCS may improve extinction by acting on the glutamate receptors required for learning (Davis et al., 2006; Rosenfield et al., 2019), whereas yohimbine has been suggested to enhance emotional memories through increased (forebrain) levels of noradrenalin (Holmes & Quirk, 2010; Meyerbröker et al., 2018). But what about propranolol? If this beta-blocker would improve extinction memory retention (and, likewise, the outcome of exposure therapy), its working mechanism would presumably be quite different. Our initial findings in Experiments 2 and 4 led us to speculate that such beneficial effects of propranolol may occur through its mild arousal-reducing effects during extinction learning. We hypothesized that these may bring about a re-evaluation of the threat, which is integrated into the fear memory, resulting in permanent lower fear responding, even when the drug is no longer in the system. In contrast with benzodiazepines, which typically have clearly discernable, acute physical effects, the effect of propranolol may be more subtle and hit a sweet spot that precludes the detrimental state dependency effect

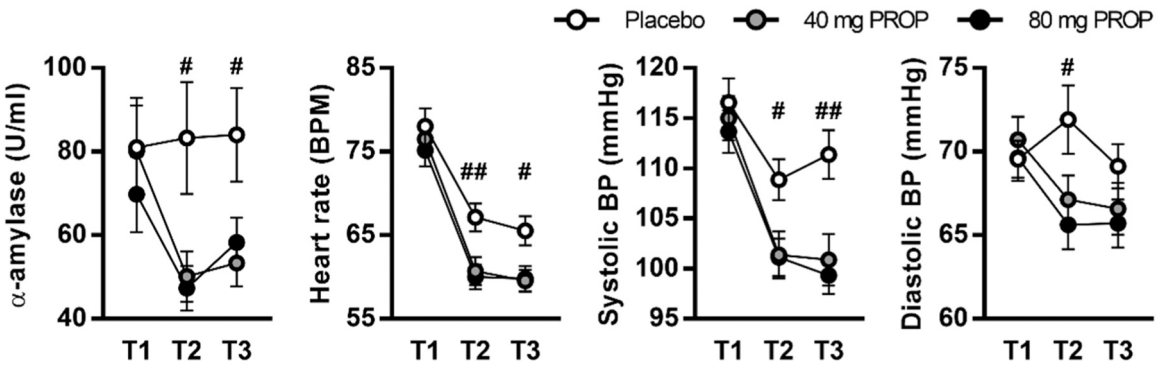

Fig. 5. Acute physiological effects of propranolol. Measurements (mean and SEM) at three time points (T_1, T_2 and T_3) on Day 2 are shown for salivary α-amylase, heart rate (in beats per minute, BPM), systolic and diastolic blood pressure (BP). T_1 is immediately before pill intake, T_2 is one hour later (immediately before the start of Extinction) and T_3 is again one hour later (immediately after the end of Extinction). Placebo groups are shown in white, 40-mg propranolol groups in gray and 80-mg propranolol groups in black (N = 24 per group). Hashtags denote significant posthoc tests (pairwise group comparisons) indicating a difference between each Propranolol group and the Placebo group ($^{\#}p < .05$, $^{\#\#}p < .01$). Note that for the measurement of diastolic blood pressure on time point T_2, the difference with the Placebo group was significant for the 80-mg PROP group, but only showed a trend for the comparison of the Placebo and 40-mg PROP groups (p = .06).

Table 5
Results of the Repeated-Measures ANOVAs comparing US expectancies (US EXP), fear-potentiated startle (FPS) and skin conductance responding (SCR) between the 80 mg propranolol, 40 mg propranolol and Placebo groups on Day 2 (Extinction) and Day 3 (Test, first trial). The bottom section shows the results (Bayes factors, BF) of the Bayesian t-tests for the difference scores (CS+ minus CS-) on the first trial of the Test on Day 3. Qualitative interpretations of the strength of the evidence in favor of the absence of a group difference (80 mg or 40 mg propranolol versus Placebo) are included.

Extinction – RM ANOVA with factors Group, Cue (CS+, CS-, [NA]) and Time (first and last block of Extinction)				
	Group	**Time**	**Cue**	
US EXP	F(2,69) = 0.339 p =.714, η^2_p = 0.010	**F(1,69) = 385.300** **p <.001, η^2_p = 0.848**	**F(1,69) = 110.240** **p <.001, η^2_p = 0.615**	
FPS	F(2,69) = 0.213 p =.809, η^2_p = 0.006	**F(1,69) = 300.899** **p <.001, η^2_p = 0.813**	**F(2,138) = 86.245** **p <.001, η^2_p = 0.556**	
SCR	F(2,69) = 0.179 p =.837, η^2_p = 0.005	**F(1,69) = 55.790** **p <.001, η^2_p = 0.447**	**F(1,69) = 14.828** **p <.001, η^2_p = 0.177**	
	Cue by Time	**Group by Cue**	**Group by Time**	**Group by Cue by Time**
US EXP	**F(1,69) = 151.459** **p <.001, η^2_p = 0.687**	F(2,69) = 1.688 p =.192, η^2_p = 0.047	F(2,69) = 0.742 p =.480, η^2_p = 0.021	F(2,69) = 2.027 p =.140, η^2_p = 0.055
FPS	**F(2,138) = 19.799** **p <.001, η^2_p = 0.223**	F(4,138) = 1.703 p =.153, η^2_p = 0.047	F(2,69) = 1.187 p =.311, η^2_p = 0.033	F(4,138) = 1.946 p =.106, η^2_p = 0.053
SCR	**F(1,69) = 5.951** **p =.017, η^2_p = 0.079**	F(2,69) = 2.331 p =.105, η^2_p = 0.063	F(2,69) = 1.245 p =.294, η^2_p = 0.035	F(2,69) = 1.591 p =.211, η^2_p = 0.044
First trial of Test – RM ANOVA with factors Group and Cue (CS+, CS-, [NA])				
	Group	**Cue**	**Group by Cue**	
US EXP	F(2,69) = 0.238 p =.789, η^2_p = 0.007	**F(1,69) = 47.137** **p <.001, η^2_p = 0.406**	F(2,69) = 0.508 p =.604, η^2_p = 0.015	
FPS	F(2,69) = 1.715 p =.187, η^2_p = 0.047	**F(2,138) = 51.396** **p <.001, η^2_p = 0.427**	F(4,138) = 1.004 p =.408, η^2_p = 0.028	
SCR	F(2,69) = 0.149 p =.862, η^2_p = 0.004	**F(1,69) = 4.510** **p =.037, η^2_p = 0.061**	F(2,69) = 0.306 p =.738, η^2_p = 0.009	
First trial of Test – Bayesian t-test using the difference score (CS+ minus CS-)				
	80 mg vs Placebo	**40 mg vs Placebo**		
US EXP	BF_{01} = 2.756 weak evidence	BF_{01} = 2.476 weak evidence		
FPS	BF_{01} = 1.164 inconclusive	BF_{01} = 1.863 very weak evidence		
SCR	BF_{01}= 3.238 moderate evidence	BF_{01} = 3.289 moderate evidence		

associated with benzodiazepines. Moreover, propranolol leaves alertness intact (hence its popularity among musicians with performance anxiety) and may – in contrast with strong sedatives – still allow for proper learning during extinction training.

The procedural variations that we introduced throughout the rat experiments were intended to examine the conditions required for observing a long-term improvement of extinction memory retention with propranolol and were based on preliminary, exploratory analyses of the data of the first 4 experiments. These seemed, for example, to suggest differences between rat strains, and a stronger effect in more fearful animals. Our subsequent experiments eventually did not unambiguously determine the prerequisites of a beneficial long-term effect of propranolol on extinction memory retention, but combining the information obtained in all experiments may nevertheless give some hints for future research. Therefore, we looked at Experiments 2 and 4, i.e., the 'successful' experiments where we did find (a trend for) improved extinction retention with propranolol, and compared these with the 'unsuccessful' experiments, where we found no effect of propranolol at Test. In both successful experiments, saline animals (see ***Supplement***) showed evidence for within-session extinction (freezing on first CS of Extinction > last CS of Extinction), but a significant return of fear at Test (freezing on last CS of Extinction < first CS of Test 1). On top of that, both experiments showed an acute effect of propranolol on freezing during extinction training (PROP < SAL during Extinction). In these two

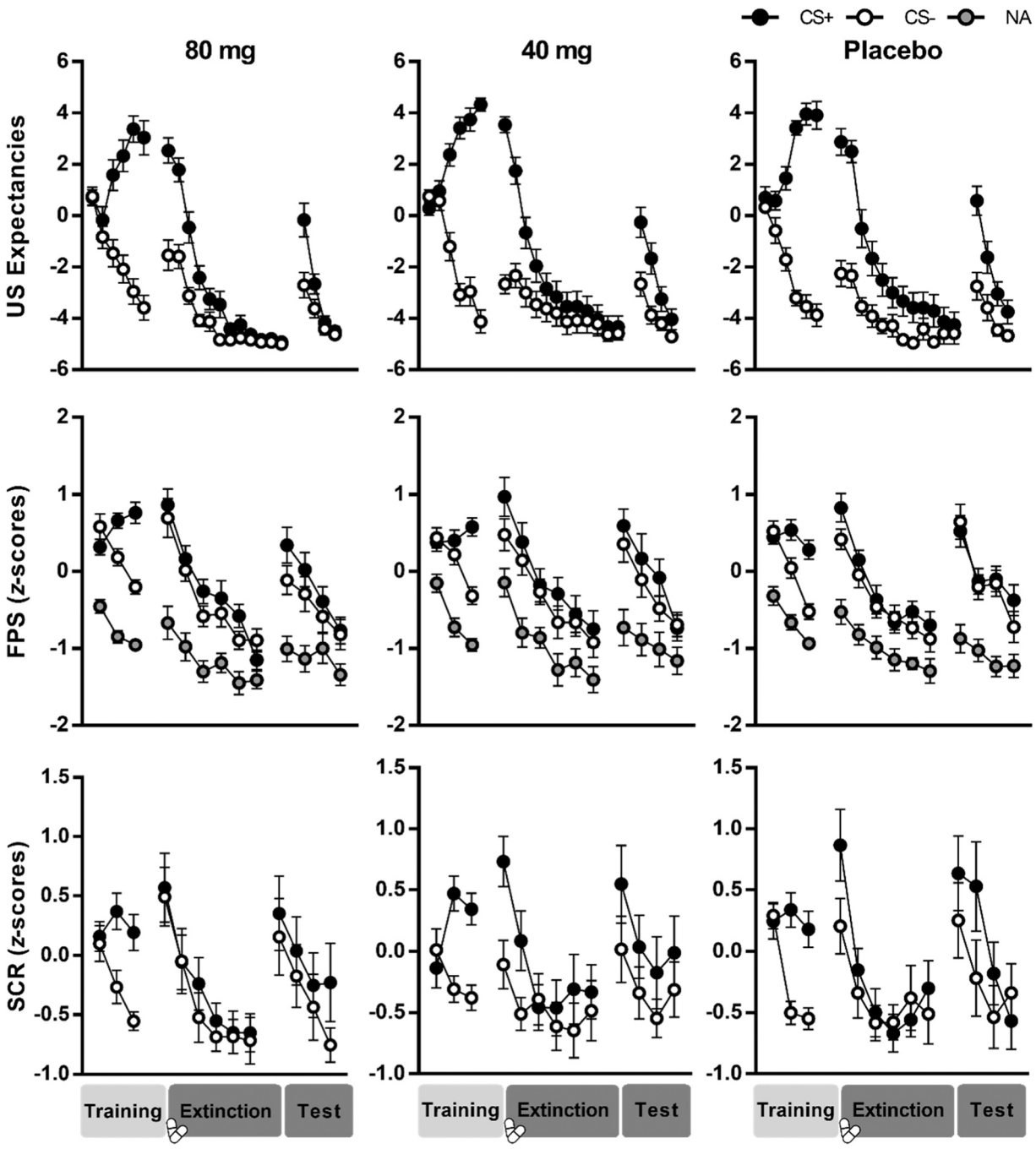

Fig. 6. US expectancies (upper panel), fear-potentiated startle (FPS, middle panel) and skin conductance responding (SCR, bottom panel) are shown for the 3 groups, which received either 80 mg of propranolol, 40 mg of propranolol or placebo pills 1 h before the start of Extinction. Data are presented as means and SEM. US expectancies are shown per trial. FPS and SCR are expressed as z-scores and are shown in blocks of 2 trials during Training (i.e., the acquisition phase on Day 1) and Extinction (i.e., the extinction learning phase on Day 2), and per trial for Test (i.e., the memory retention test on Day 3). CS+ is shown in black, CS- in white and noise-alone (NA) trials (in the FPS graphs) in gray. Overall, the data indicate adequate fear learning and extinction learning, with initial return of fear at Test in the extinction context, and no group differences throughout.

experiments, we thus observed (a trend toward) a mitigating effect of propranolol during the drug-free extinction memory retention test. Conversely, experiments without evidence for within-session extinction in the saline condition (Experiment 7), or without an acute effect of propranolol on behavioral indices of fear during extinction learning (Experiment 5 and the human study), or without return of fear at Test in the control group (Experiments 1, 3, 6–9) did not show any benefit of the use of propranolol. Notably, in many prior studies (but see (Kroes et al., 2016, pp. 2015)), these proposed prerequisites for a beneficial effect of propranolol (i.e., improving extinction retention) may not have been met, as several studies did not obtain clear within-session extinction in the control group and/or did not find acute fear-reducing effects of propranolol during extinction learning (Bos et al., 2012; Cain et al., 2004; Fitzgerald et al., 2015; Leal Santos et al., 2021; Mueller et al., 2008). All in all, our observations are in line with the idea that propranolol could be particularly useful if patients are very fearful or aroused during extinction learning (Meyerbröker et al., 2018; Morissette et al., 2008) or if it may be difficult for them to reach full extinction otherwise. We do acknowledge that our rodent studies provide little insight in any sex differences that may exist (Soldin & Mattison, 2009)

(we conducted only one study with female rats) and did not include contextual changes that may be clinically relevant (all rodent studies used AAA designs). Further research (also in clinical populations) will be necessary to uncover if propranolol may have a place in a subpopulation of patients who are too anxious to even begin with exposure therapy or who have difficulties completing the treatment due to unbearable levels of distress during exposure. Evidently, the conclusion from our data that propranolol may not be detrimental in combination with exposure therapy awaits confirmation through well-powered double-blind, controlled clinical trials with patients who are being treated with exposure therapy. Such future research should also address whether therapy outcome might be affected differently by acute versus chronic use of (different doses of) the drug.

Although we can speculate about potential benefits of having propranolol in the system during extinction learning, and by extension during exposure therapy, it is important to stress that the current set of studies primarily supports the absence of impaired extinction memory retention with propranolol, in contrast to prior literature. Our Bayesian meta-analysis provided strong evidence against such a harmful effect. Conversely, our two studies that included a benzodiazepine group did show results in line with the literature, with the meta-analysis indicating some evidence for impaired extinction retention with midazolam. Our data thus support caution regarding the use of a benzodiazepine during exposure therapy, but suggest that there is less concern when it comes to the use of propranolol during such therapy.

CRediT authorship contribution statement

Laura Luyten: Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anastasia Chalkia:** Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition. **Anna Elisabeth Schnell:** Investigation, Writing – review & editing. **Burcu Özcan:** Writing – review & editing, Investigation. **Lu Leng:** Writing – review & editing, Investigation. **Natalie Schroyens:** Writing – review & editing, Investigation. **Lukas Van Oudenhove:** Writing – review & editing, Methodology. **Wolf Vanpaemel:** Writing – review & editing, Validation, Methodology, Formal analysis. **Tom Beckers:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

None.

Data availability

Data are available on the Open Science Framework:
No harmful effect of propranolol administered prior to fear memory extinction in rats and humans (Original data)

Acknowledgments

WWe would like to thank Jonas Zaman and Angelos Krypotos for their help with our initial exploration of the Bayesian methods. This work was supported by ERC Consolidator Grant 648176 (to Tom Beckers) and by the KU Leuven Research Council (Project 3H190245, to Tom Beckers and Laura Luyten). Anastasia Chalkia was supported by a personal fellowship awarded by the KU Leuven Research Council (PDM/20/061). Additionally, the human study was supported by an infrastructure grant from the Research Foundation - Flanders (FWO) and the Research Fund of KU Leuven (I011320N, AKUL/19/06).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.janxdis.2024.102870.

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