5200 words / 2 tables / 3 figures

Title: Reduction of aversive learning rates in Pavlovian conditioning by angiotensin II antagonist losartan

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Abstract

The angiotensin receptor blocker losartan has been linked to aspects of aversive learning such as fear acquisition and extinction, inhibition of aversive learning rates and reduced post-traumatic stress disorder (PTSD) symptoms. Here, we investigate the influence of losartan on aversive Pavlovian conditioning using a probabilistic learning paradigm. In a double-blind, randomised placebo-controlled design, we tested 45 healthy volunteers during a baseline session (session 1), after application of losartan or placebo (session 2) and during a follow-up session (session 3). On each session, participants engaged in a task where they had to predict the likelihood of an electrical stimulation on every trial while the true shock contingencies repeatedly switched between phases of high and low shock threat. Computational reinforcement learning models were used to investigate learning dynamics. Acute administration of losartan significantly reduced participants' adjustment during both low-to-high and high-to-low threat changes. This was driven by reduced aversive learning rates on the drug session compared to baseline. The 50mg drug dose did not induce reduction of blood pressure or change in reaction times, ruling out general reduction in attention and engagement. Decreased adjustment of aversive expectations to low-to-high, but not high-to-low, threat change was maintained on a follow up session 24hrs later, suggesting a possible role of losartan in prevention of formation of aversive associations on longer time scales.

Keywords: angiotensin receptor, anxiety, aversive learning fear, losartan, reinforcement learning

Introduction

With a life-time prevalence of 15-30%, anxiety disorders represent the most prevalent mental health problem (Alonso et al., 2007; Kessler, 2012; Kessler et al., 2009). These disabling conditions are associated with significant individual and economic costs, they tend to take a chronic course if untreated, and they are one of the most severe risk factors for developing depression (Andlin-Sobocki & Rehm, 2005; Kessler et al., 2009; Meier et al., 2015). However, little is currently known about the factors contributing to anxiety onset, even though such knowledge is crucial for the development of early strategies that may prevent the development of a disorder.

Recent research has increasingly implicated a key role of the renin-angiotensin system (RAS) in the development and treatment of anxiety disorders. The RAS is a key neuroendocrine circuit involved in blood pressure regulation. However, its receptors are also expressed in brain regions relevant to anxiety, including amygdala, hippocampus and the prefrontal cortex (Jackson et al., 2018; von Bohlen und Halbach & Albrecht, 2006). In line with this overlapping neural topography, the RAS has also been identified as a key player in hypothalamus-pituitary adrenocortical (HPA) axis modulation, inflammatory processes and neuroplasticity, all processes known to play a key role in anxiety (Chrissobolis et al., 2020).

In rodent models, increased angiotensin II levels are seen in response to stress (Kosunen et al., 1976). Drugs blocking angiotensin II activity, including angiotensin II receptor blockers (ARB) and angiotensin-converting enzyme inhibitors (ACEI), have been shown to reduce stress responses, to produce anxiolytic effects, and to facilitate fear extinction (Marvar et

al., 2014; Pavel et al., 2008; Ranjbar et al., 2018). In humans, ARB have been reported to improve symptoms of anxiety in type 2 diabetes patients (Pavlatou et al., 2008). Observational data from a large patient cohort indicate that antihypertensive use of ACEI or ARB such as losartan is linked to reduced traumatic symptoms in the aftermath of a traumatic event (Khoury et al., 2012). In line with such clinical effects, we have recently shown that a single dose of the ARB losartan prevents a physiological stress response and facilitates contextual processing during experimental trauma, two processes known to be relevant to the development of posttraumatic stress disorder (Shkreli et al., 2020). Similarly, administration of losartan has been associated with reductions in subjective fear during a fear-inducing aversive task (Zhang et al., 2022) and encoding of negative memories (Xu et al., 2022).

Taken together, such findings point to a prominent role of the RAS in threat processing and memory. However, no study has yet directly investigated the effect of RAS manipulation on Pavlovian threat learning, one of the basic learning mechanisms underlying the development of an anxiety disorder (VanElzakker et al., 2014; Wolpe & Rowan, 1988). Direct evidence for interference of RAS-modulating drugs with Pavlovian learning would have important implications for developments in the early detection of anxiety-risk and the need for preventative strategies. This study investigates the effect of angiotensin receptor blockade (i.e., a reduction in angiotensin II activity) on aversive learning in a probabilistic learning task, using shock expectancy ratings as primary outcome. Following recent criticism of the traditional fear extinction paradigm (Ojala & Bach, 2020) we used a paradigm where phases of high and low threat are matched in uncertainty. We employed computational modelling to understand which aspect of learning is impacted by losartan. While recent work has highlighted the use of computational models in understanding the mechanisms underlying learning in health and disease (Browning et al., 2015; Dubois & Hauser, 2022; Gillan et al., 2016; Lawson et al., 2017; Pulcu et al., 2019; Schlagenhauf et al., 2014), only two studies to date have employed this approach to study the impact of losartan on learning (Pulcu et al., 2019; Xu et al., 2023). Both studies used an instrumental learning paradigm involving monetary gains and losses. The authors reported reduction in loss but not gain learning rates following a single dose of losartan. Here, we extend this previous work by focusing on mechanisms of aversive Pavlovian learning involving primary reinforcers (shocks), which are more directly relevant for formation of anxiety disorders. Additionally, to improve precision and sensitivity of our analyses, we collect aversive expectancy ratings on each trial. We draw on the recent advances in computational modelling to develop a more detailed understanding of the specific learning subprocess impacted by drugs blocking angiotensin II activity. In addition to standard RL models, we also consider a number of plausible aspects of learning that might be impacted by the drug: differential learning from shocks and shock omissions (Jepma et al., 2018), context-dependent updating (Zika et al., 2022) and accelerated learning following large prediction errors (Li et al., 2011). Based on previous research, we hypothesized that a single dose of the ARB losartan will lead to a reduction in aversive learning rates.

Methods and Materials

Registration

Prior to the start of data collection, the study was registered with Clinical Trials and Research Governance at the University of Oxford, publicly available via OSF: osf.io/e3zrk.

Participants

Forty-five healthy volunteers (age 18–39 years) were recruited through local advertisements. Sample size was estimated based on the two available studies that investigated the impact of losartan on aversive learning rates (Pulcu et al., 2019; Xu et al., 2023). Means and standard deviations were extracted from the placebo>losartan effect on learning rates (Placebo M=0.25, SD=0.54; Losartan M=0.12, SD=0.35). Next, a power analysis by simulation was performed by generating data using a beta sampler and fitting the beta regression model used in the main analysis across 5000 simulations. This resulted in sample size estimate of forty-four participants (22 per group) at 80% power (alpha=0.05). To allow for potential exclusions we collected forty-five participants in total.
Participants were included in the study if they had no history of a DSM-V Axis I disorder as assessed using the Structured Clinical Interview for DSM-V (First et al., 2016). Participants also had to have been free from CNS-active medication for at least six weeks, have a body mass index between 18 and 30 kg/m^2, and have no first-degree family member with a history of a severe psychiatric disorder. The full list of inclusion and exclusion criteria is included in the Supp. Mat. The study was approved by the Oxford University Research Ethics Committee (R29583), and all participants gave written informed consent. Five participants had to be excluded from the study at the point of data analysis: one due to technical failure of the equipment, and four because even at the end of a third visit they failed to learn the distinction between consistently safe and threatening cues. In line with recent recommendations for exclusion criteria in aversive learning studies (Lonsdorf et al., 2019), we aimed to retain as many participants in the analyses as possible. Therefore, even participants who failed to show any learning in the main (reversal, see below) cue were included in all analyses. However, four participants failed to tell apart the stable-low and stable-high cues (assessed by a t-test on submitted ratings) even on the third visit which indicates a general lack of understanding of the task. Data of these four participants were therefore excluded from analyses. This left twenty participants in the losartan group (6 female, mean age 25.5 years) and twenty in the placebo group (10 female, mean age 24.1 years). Using the same simulation approach as above, the power in the final sample was 77.1%.

Materials and Study Design

The study involved three visits to the Department of Psychiatry at the University of Oxford. *Visit 1* (Baseline visit) included a medical and psychiatric screening for inclusion and exclusion criteria, followed by an introduction and completion of a shorter version of the aversive learning task to familiarise participants with the task (described below). *Visit 2* (Drug visit) included completing a battery of psychological questionnaires for group description, administration of one dose of losartan (50mg) or placebo one hour before

working on the full version of the aversive learning task. *Visit 3* (Follow-up visit) took place one day after the Drug visit to assess any potential next-day effects. Participants also completed a shorter version of the aversive learning task.

Prior to the Drug visit, participants were randomly allocated to one of two groups in a double-blind design, either receiving a 50mg single oral dose of losartan (Cozaar, Merck Sharp & Dohme Ltd.) or a placebo capsule that was matched to the active drug in appearance (microcrystalline cellulose; Rayotabs, Rayonex GmbH). Dosing of losartan was guided by the intention to assess its impact on aversive learning without triggering hypotensive effects, which had been achieved in previous studies using 50mg losartan (Pulcu et al., 2019; Reinecke et al., 2018a; Shkreli et al., 2020). Testing started one hour after capsule intake, when drug peak plasma levels are reached (Lo et al., 1995; Ohtawa et al., 1993). Before and one hour after drug intake, mood and physiological symptoms were assessed using self-report visual analogue scales recorded using paper and pen (VAS; 0-100; Anxious, Tearful, Hopeless, Sad, Depressed, Sleepy, Nauseous, Dizzy, Heart racing, Alert) and measuring heart rate and blood pressure (Omron 705IT sphygmomanometer), to capture transient effects of the drug. At the end of the Drug visit, participant and experimenter indicated independently whether they thought losartan or placebo had been administered during the session.

Data collection took place at the Warneford Hospital, Department of Psychiatry, Oxford. Initial screening, questionnaire completion and waiting periods (e.g., between drug administration and task) took place in a preparation room with natural illumination. The task itself took place in a testing room equipped only with artificial light which was kept on during testing. Participants were positioned 40 cm from a computer screen, provided with task instructions after which they could ask clarifying questions. Following this, participants completed a few practice trials and calibration of the electrical stimulations. They were also provided with a bell to call the researcher if they had any issues or questions throughout the task. During the task itself, the experimenter was not present in the same room.

Questionnaires

At the beginning of the Drug visit, participants completed a battery of psychological questionnaires to assess personality traits, anxiety, depression and attention regulation strategies (State-Trait Anxiety Inventory STAI; Spielberger, 2012; Beck Depression Inventory BDI; Beck et al., 1996; Attentional Control Scale ACS; Derryberry & Reed, 2002). Participants also completed the National Adult Reading Test NART, estimating verbal intelligence (Nelson, 1982).

Aversive stimuli

Electrical stimuli were applied using a commercial electric stimulation device (Constant Current Stimulator, model DS7A; Digitimer, Hertfordshire, UK), delivering a 2 monopolar square waveform pulse via a concentric silver chloride electrode attached to the back of the left hand. The stimuli were calibrated individually at the beginning of the task and during each break (every 10-12 minutes). The target intensity was 8 on a scale ranging from 0 (= not painful) to 10 (= too painful to take part) scale. The 8/10 pain level was defined as a sensation that is painful but tolerable for a given number of trials (visit-specific number corresponding to 50% of trials). Three qualitative anchor points were defined to help standardize the calibration across participants and studies: 1/10 which was defined as the

intensity at which the sensation starts to be moderately painful (i.e., it feels like a pin prick); 8/10 is a sensation that is clearly painful but tolerable; and 10/10 which would be the level of pain which is too strong to be tolerated. The calibration followed the Method of Limits (see e.g., Ploner et al., 2010). The stimulus intensity started at the pre-calibrated 1/10 level and changed after each rating in an increasing trend (individual stimuli could, however, get stronger or weaker). Upon each stimulus delivery, participants were asked to report how painful the sensation was on a 1-10 rating scale. If a rating was higher than 8, the stimulation intensity was decreased for the next calibration trial. The calibration terminated once three out of the most recent five stimuli were rated as 8.

Aversive Learning Task

A probabilistic aversive learning paradigm was employed to measure to how participants learned from environments changing between periods of high and low threat, which was manipulated by changing the probability of receiving an electrical shock (Figure 1). A session consisted of 150 (visits 1 and 3) or 300 (visit 2) trials. On each trial, participants were presented with one of three visual cues (neutral abstract fractals, randomized across participants) and asked to rate their subjective shock probability (rated on a 0% to 100% scale, increments of 1%, collected using a slider operated by left and right keyboard arrows and down arrow to submit the final answer). Participants had 4 seconds to provide a rating. If no rating was provided on time, the trial was restarted. After a inter-stimulus interval of 1 s, a short electrical impulse was either delivered (shock) or omitted (no shock). Unbeknownst to the participants, one of the cues switched between a 75% chance of shock (high-threat phase) and a 25% chance of shock (low-threat phase) in phases of 30 +/- 5 trials (“reversal” cue, presented on 50% of trials). Additionally, on half of the trials two other cues were presented which never changed their probability of shock, one remained always high (75%, stable-high-threat, cue) and one always low (25%, stable-low-threat cue). To ensure that participants pay attention throughout the experiment, they were instructed that shock probabilities signalled by the three visual cues could change at any time. Each session started randomly with either a high- or low-threat phase (i.e., either with 75% or 25% chance of shock in the reversal cue). No information was given regarding the number of cues or the number of switches. The task was paused every 10-12 minutes (one break on visits 1 and 3, three breaks on visit 2) to allow participants to rest and to re-calibrate the electrical stimuli. Instructions were delivered in a standardized (written) form, clarification questions were answered according to a curated answer list.

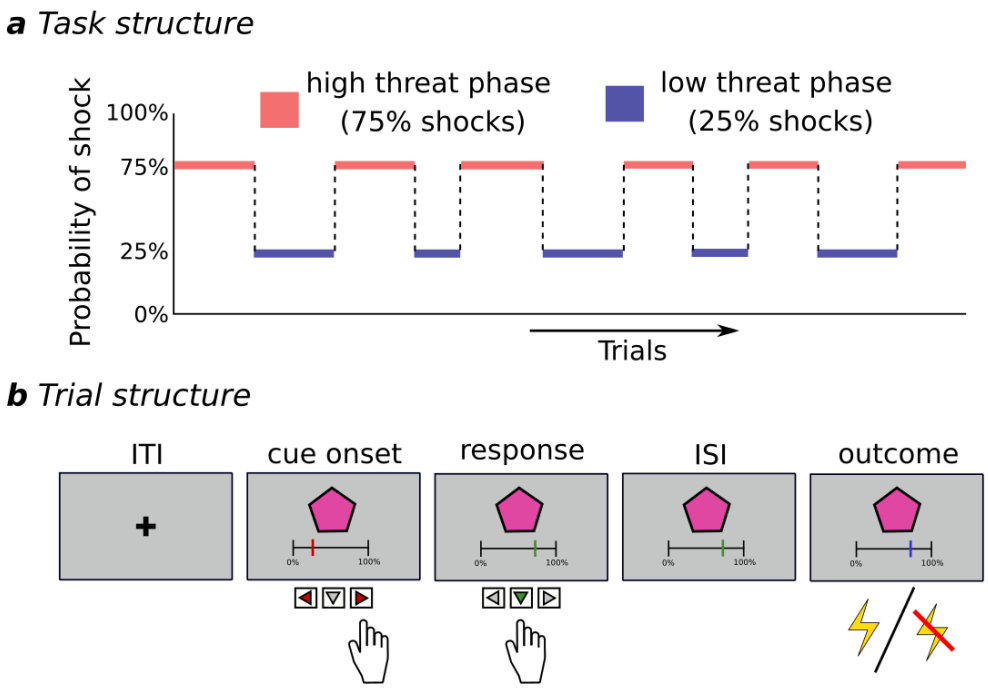

Figure 1: (**a**) Task structure: The objective probability of shock changed in semi-regular intervals between phases of high (red) and low (blue) threat. Visits 1 and 3 included 6 phases on average (short version, ~150 trials) while on Visit 2 there were 11 phases (long version, ~300 trials). Each participant could start either with high or low probability of shock – the depicted schedule starts with high shock probability. (**b**) Each trial started with an inter-trial interval (ITI; 2s) during which a fixation cross was shown. When the cue appeared on the screen, participants had 4s to submit their shock probability rating on a scale from 0% to 100% using a slider. After a variable inter-stimulus interval (ISI; 1s), the outcome was delivered (shock or no-shock). The colour of the slider changed when a rating was submitted, and when the outcome was delivered.

Behavioural measures

Estimated switch points

Reversals between high and low probability of shock were not signalled. Participants therefore had to infer that a change had occurred from the received binary outcomes (i.e., shock or no shock). To avoid false conclusions that can arise during averaging of temporal trajectories (Haider & Frensch, 2002), we used a data-driven approach to estimate the time point when the participant switched their beliefs after each reversal. Specifically, we extracted 5 trials before and 15 trials after each reversal (20 trials in total), calculated the cumulative sum of shock probability ratings and demeaned the time series (Page, 1954). The peak/trough of this series represents the point of fastest updating. For each reversal we labelled this point an estimated switch point.

Shock probability ratings

Participants provided a shock probability rating on each trial, ranging between 0% and 100%. To investigate the impact of losartan on learning, we focused on the change in ratings compared to baseline. The data were re-aligned to the estimated switch point, the baseline

(three trials before switch) was subtracted and the first five trials after switch were excluded as ratings only stabilised after about five trials following the reversal (see Supp. Fig. 1). This allowed us to assess changes in shock probability ratings before and after learning. Due to the baseline correction, the values in high-threat phase are positive while values in the low-threat phase are negative.

Error from true reinforcement

To directly assess whether participants under- or over-estimate the objective threat, we calculated their deviation from the true reinforcement rate (similarly to Zika et al. 2022). This was done by calculating the running mean of the binary outcomes (shocks=1 and noshock=0 outcomes) for each phase-type separately. This measure serves as an estimate of the true shock probability under the assumption that the agent knows which phase they are currently in. To obtain a directional measure of error, the true reinforcement rate was subtracted from the expectancy ratings. Therefore, negative values represent an underprediction of objective threat while positive values represent overprediction.

Computational Modelling

Fitting procedure and model comparison

Models were fitted to the trial-by-trial shock probability data using Bayesian Adaptive Direct Search (BADS; Acerbi & Ma, 2017) by minimizing the negative log likelihood of the data given a model (under Normal distribution with SD=0.2). To assess model fit across all trials, BIC (Schwarz, 1978) scores were calculated. To prevent convergence to local extremes, fitting was performed using random starting value 200 times for each participant, visit and cue, ensuring that computational resources were identical across models. From the 200 draws, the fit with lowest BIC was selected for model comparison. Model comparison was performed by calculating the mean BIC score per model and by computing the percentage of participants best fitted by each model.

Computational models

In line with similar experimental approaches (Browning et al., 2015; Jepma et al., 2018; Pulcu et al., 2019), we employed a modelling framework based on reinforcement learning (Sutton & Barto, 2018). Specifically, we built three models that were variations of the Rescorla-Wagner learning rule (Rescorla & Wagner, 1972), and one model with an adaptive learning rate (Pearce-Hall). Under RW, an agent holds a belief about the current probability of shock *P*. On each trial, this belief is updated using a prediction error (PE), that is, the difference between the current expectation P_t (continuous values between 0 and 1) and the outcome O_t (coded as 1=shock, 0=shock omission). Positive PEs lead to an increase in expected probability of a shock, while negative prediction errors lead to its decrease. Additionally, the PE is weighted by a free parameter α (continuous value between 0 and 1), which controls how much of the error is incorporated into the belief about probability of shock

on the next trial. Large values of α lead to rapid updating, while small values of alpha lead to slower learning. See Equation 1.

$$\text{Eq. 1 } P_{t+1} = P_t + \alpha(O_t - P_t)$$

In all models, the first value was estimated as a free parameter ($P_1 \in [0,1]$).

Outcome-sensitive model (RW-outcome-3)
Previous studies found faster learning from shocks compared to no-shocks (e.g., Jepma et al., 2018). To distinguish between learning from shocks and no-shock, we specified a model with separate learning rates for the two event types: $\alpha_{\mathrm{sh}} \in [0,1]\ and\ \alpha_{nosh} \in [0,1]$). See Equation 2.

$$\text{Eq. 2} \quad \begin{aligned} P_{t+1} &= P_t + \alpha_{sh}(O_t - P_t) \text{ if shock } (O_t = 1) \\ P_{t+1} &= P_t + \alpha_{nosh}(O_t - P_t) \text{ if no-shock } (O_t = 0) \end{aligned}$$

Phase-sensitive model (RW-phase-3)
Alternatively, participants may only be sensitive to the current context (high- versus low-threat phase). We therefore specified a model with separate learning rates for the two phases: $\alpha_{high} \in [0,1]$ and $\alpha_{low} \in [0,1]$. See Equation 3

$$\text{Eq. 3} \quad \begin{aligned} P_{t+1} &= P_t + \alpha_{low}(O_t - P_t) \text{ if low-threat-phase} \\ P_{t+1} &= P_t + \alpha_{high}(O_t - P_t) \text{ if high-threat-phase} \end{aligned}$$

Outcome- and phase-sensitive model (RW-both-5)
To account for the possibility that outcomes (shocks/no-shocks) are treated differently depending on the current context (high- versus low-threat phase), we combined the previous two models (RW-outcome-3 and RW-phase-3), which resulted in four learning rates: $\alpha_{(sh,high)} \in [0,1], \alpha_{(sh,low)} \in [0,1], \alpha_{(nosh,high)} \in [0,1]$ and $\alpha_{(nosh,low)} \in [0,1]$. See Equation 4.

$$\text{Eq. 4} \quad \begin{aligned} P_{t+1} &= P_t + \alpha_{(sh,low)}(O_t - P_t) \text{ if low-phase and shock} \\ P_{t+1} &= P_t + \alpha_{(nosh,high)}(O_t - P_t) \text{ if high-phase and no-shock} \\ P_{t+1} &= P_t + \alpha_{(sh,low)}(O_t - P_t) \text{ if low-phase and shock} \\ P_{t+1} &= P_t + \alpha_{(nosh,high)}(O_t - P_t) \text{ if high-phase and no-shock} \end{aligned}$$

Pearce-Hall (PH-5)
All previous models assume a fixed learning rate across all trials within a condition. However, surprising outcomes (i.e., large PEs) have been shown to increase the learning rate (Li et al. 2011). Therefore, to test for the possibility that learning rates are dynamically adjusted depending on recent prediction errors, we adopted the Pearce-Hall model used in a number of previous studies (Li et al., 2011; Norbury et al., 2018; Tzovara et al., 2018). Similar to the RW rule (Eq. 1), the model updates current shock probability estimates by a weighted PE. However, unlike in RW, the learning rate α can change from trial to trial (Eq. 5). This trial-specific learning rate α_{t+1} is updated by a weighted combination of the current absolute prediction error $|PE_t|$ and the learning rate α_t. The parameters $\eta_{\mathrm{sh}} \in [0,1]$ and $\eta_{\mathrm{nosh}} \in [0,1]$ control the degree to which the current absolute PE influences the learning rate

on the next trial. The sum is then scaled using the parameter. Note that if $\eta = 0$ the model is identical to Rescorla-Wagner model with fixed learning rate.

$$\text{Eq. 5 } P_{t+1} = P_t + \alpha_t(O_t - P_t)$$

$$\text{Eq. 6 } \alpha_{t+1} = \kappa[\eta_{sh}|PE_t| + (1-\eta_{sh})\alpha_t] \text{ if shock}$$
$$\alpha_{t+1} = \kappa[\eta_{nosh}|PE_t| + (1-\eta_{nosh})\alpha_t] \text{ if no-shock}$$

Parameter recovery

In order to ensure identifiability and interpretability of model estimates, the models were subjected to a parameter recovery procedure. First, each model was used to generate artificial datasets, such that the full range of parameter values and their combination was represented. Second, the model was fitted to the artificial data. Third, the original parameters were correlated with the recovered values.
Parameters of all four models recovered well – the lowest recovery rate was *r=.91* (the starting value of η in the Pearce-Hall model). All three parameters of the winning model recovered perfectly (*r=1.00*). The full parameter recovery matrices can be found in the Supplementary Material.

Statistical Analyses

Data were collected using custom MATLAB 2016 and PsychToolbox 3 code. All analyses were performed using MATLAB 2019b and R version 3.6. Full list and versions of used packages is provided in the associated GitHub repository.

Questionnaire, Physiological and Sociodemographic Data

All questionnaires were analysed according to their respective manual. Group differences on sociodemographic and questionnaire data were tested using t-tests (continuous variables) or χ^2(frequency variables) tests. The effect of losartan on physiological and VAS measures between baseline and drug-peak level was assessed using a linear effects models and subsequent ANOVA.

Behavioural data

Statistical analyses of ratings-derived measures (change in probability, estimated switch point) were performed using linear mixed effects models (LMM) with participant as a random effect (*lmer* package in R; Bates et al., 2015) and a subsequent ANOVA (from the *lmerTest* package). Post-hoc t-tests were corrected for multiple comparisons using the Holm (Holm, 1979) correction (as implemented in the *emmeans* package). Each session started randomly with either a high- or low-threat phase (i.e., either with 75% or 25% chance of shock). To control for any effect of starting probability, it was included in all statistical models as a random intercept. Since the random effects are identical for all analyses, we only mention what fixed effects were included in each analysis.

Model Parameters

Learning rates estimated by the winning model naturally follow a beta distribution (i.e., they range between 0 and 1, can be zero or one-inflated, and they are often negatively skewed). To respect this property and to maximise statistical sensitivity, we analysed learning rates using a generalized beta regression (Ferrari & Cribari-Neto, 2004) with logit link function (*glmmTMB* package; Brooks et al., 2017). Additionally, we also fitted a Gaussian GLM with linear mapping (identity link function) and compared the two models. The BIC (and root mean squared error; RMSE) scores suggest sizeable improvement in model fit using beta regression: BIC_{beta} = -602 (RMSE=0.066), BIC_{linear}= -352 (RMSE=0.208). Identical to the behavioural data, participant and starting probability were included as random effects. The statistical test of the model parameters was performed using the Type II Wald Chi-squared test (using the *car* package).

Results

Group Matching and Drug Side Effects

The two groups were well-matched on sociodemographic and questionnaire parameters (Table 1). There were no group differences in heart rate, blood pressure, mood and physiological symptoms VAS rating changes from baseline to drug peak level, all $F(1,38) < 2.21$, $p>.15$. (Table 2). Furthermore, neither the participants nor the experimenter were able to indicate above chance whether the participant had been allocated to the drug or the placebo group (experimenter: 40% correct, patients: 50%; both $\chi^2 < 0.98$, $p>.32$). These findings suggest that double-blindness towards drug randomisation was maintained throughout the study.

Table 1: *Sociodemographic, clinical and personality characteristics in the losartan versus placebo group (M, SD, and t-test/ X^2-test p-scores).*

	Losartan (N=20)		*Placebo (N=20)*	
	M	*SD*	*M*	*SD*
Sociodemographic Data				
Gender female	30%		50%	
First language English	75%		85%	
Age in years	25.6	4.7	24.2	4.3
Verbal intelligence (NART)	115	6.9	111	9.9
Years of education	16.8	2.6	17.4	2.2
Clinical and Personality Measures				
Trait Anxiety (STAIT)	34.9	8.5	37.0	7.28
Beck Depression Inventory (BDI)	4.0	6.19	5.05	6.46
Attentional Control (ACS)				
Total	58.2	7.9	56.6	9.5

Focusing	26.0	5.20	25.1	4.41
Shifting	32.2	4.7	31.5	5.84

Note: NART = National Adult Reading Test; STAIT = State-Trait Anxiety Inventory; BDI = Beck Depression Inventory; ACS = Attentional Control Scale.

Table 2 *Heart rate, blood pressure and visual analogue scale ratings in the two groups before drug intake and at drug peak-level.*

	Baseline				Drug Peak				
	Losartan		Placebo		Losartan		Placebo		
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*p*
Physiological Measures									
Heart rate	75	12	73	10	66	8	66	8	.83
Systolic blood pressure	124	16	125	14	119	16	119	14	.83
Diastolic blood pressure	71	9	74	10	69	8	73	11	.70
Visual Analogue Ratings									
Anxious	7	7	11	12	4	4	7	10	.95
Tearful	2	2	4	8	2	2	3	6	.73
Hopeless	4	9	5	11	3	5	4	8	.95
Sad	3	5	6	9	4	7	4	5	.27
Depressed	2	3	5	8	2	3	4	7	.65
Sleepy	17	14	18	17	18	17	21	17	.80
Nauseous	2	3	5	11	3	4	4	8	.67
Dizzy	4	7	5	6	7	12	6	11	.66
Heart racing	7	11	7	9	3	3	5	7	.56
Alert	45	32	52	29	44	33	45	30	.71
Flushed	10	9	16	21	4	7	6	9	.45

Note: The *p*-values in the right-most column correspond to the interaction between visit and group.

Behavioural results

Data quality and control measures

To investigate any task-related differences between groups, we compared both groups with respect to objective shock intensity, reaction times, initial aversive bias (expected probability reported on the first trial), association with objective starting probability of the reversal cue (25% or 75%) and the ability to learn shock probabilities in control stable cues from data assessed during the critical drug session. There was no difference in the calibrated shock intensity between the groups ($I_{losartan}$ = 1010 mA; $SD_{losartan}$ = 1850; $I_{placebo}$ = 514 mA; $SD_{placebo}$ = 673), t(36)=-.96, p=.34, starting probability, χ^2 = 0.13, p =.72 or initial bias ($B_{losartan}$ = 44%; $SD_{losartan}$ = .14; $B_{placebo}$ = 53%; $SD_{placebo}$ = .22), t(32)=-1.60, p=.12. Additionally, the drug did not impact reaction times during the drug visit in relation to baseline visit, χ^2 < 2.61, *p*>.27 (assessed by Gamma GLM). The mean shock probability reported for the control stable

cues in the drug session did not differ significantly from the true contingencies (assessed by one-sample t-test, *p*-values corrected): In the stable-low-threat cue (true probability: 25%), the mean probability ratings were 22.4% (losartan), $t(19)=-2.34$, p=.12, and 27.4% (placebo), $t(19)=1.05$, p=.49, in the stable-high-threat cue (true probability: 75%), they were 75.4% (losartan), $t(19)=-2.34$, p=.49, and 73.5% (placebo), $t(19)=-2.16$, p=.13. There were no significant differences in probability ratings between groups $t_{low}(35)=-1.09$, p=.28, $t_{high}(38)=.34$, p=.73. These quality checks confirm that starting probability did not relate to the group assignment, that participants in both groups did not have any initial bias or difference in shock intensity, and that both groups were able to learn shock contingencies well.

To check whether drug-induced changes were due to mechanisms of aversive learning or general decrease in attention/physiological relaxation we analysed any changes in blood pressure between baseline and peak on the drug visit in relation to ratings and learning rates (the two main outcome measures). There was no main effect or interaction with drug in either systolic or diastolic blood pressure in ratings or learning rates on either drug or follow-up visit, all *F*s<1.27, *p*s>.26.

Estimated switch points

The estimated switch points were used to realign behavioural data to the point when participants switched their beliefs from high- to low-threat and vice versa. The mean switch point value was 4.52 trials (SD=3.40) after the true change in contingencies. To assess any differences in the estimated switch points we constructed a LMM (DV: switch point; fixed effects: group, phase and visit). The model found no significant main effect or interaction (all *p*s > .18), suggesting that losartan had no impact on the time-point at which participants indicated a switch in contingencies. The estimated switch points were used to realign raw data (see Figure 2a).

Probability ratings

Baseline-corrected probability ratings were modelled using LMM with phase (low/high threat), visit (baseline, drug, follow-up) and group (losartan/placebo) as fixed effects (Figure 2b). The ANOVA test found significant main effects of phase and group. As expected, the ratings were positive in the high-threat phase (30.2%) and negative in the low-threat phase (-28.1%), F(1, 2197)=5112, p<.001. Across high and low threat phases, the probability ratings were lower for losartan (-0.01) compared to placebo (2.6%), F(1, 2135)=13.78, p<.001. Furthermore, there were significant interactions between group and visit, group and phase, and group, phase and visit. Post-hoc tests revealed no significant group difference for either low- or high-threat phase ratings on the baseline visit (v1). On the drug visit (v2), mean ratings were significantly lower in the losartan than the placebo group in the high-threat phase, $t_{high}(3841)=-6.559$, p<.001 (losartan: 22.6%; placebo: 32%), and significantly higher than placebo in the low-threat phase, $t_{low}(3849)=4.533$, p<.001 (losartan: -23.5%; placebo: -30.3%), suggesting that participants in the losartan group adjusted their shock probability ratings less through learning. The high-threat-phase group difference (but not the low-threat difference) remained significant on follow-up visit (v3), $t_{high}(3786)=-6.69$, p<.001 (losartan: 22.2%; placebo 39.2%), $t_{low}(3819)=2.15$, p=.50 (losartan: 26.9%; placebo 30.6%). Next, we tested whether the changes observed on the drug and follow-up visits relative to the baseline visit are driven by losartan or placebo. To answer this question, we directly

compared differences between visits. First, we focused on the low-to-high-threat switches. There was a decrease in ratings during high threat phase in the losartan group (baseline=30.7%, drug=22.6%, follow-up=22.2%), the ratings were decreased in both drug visit, t(2867)=4.11, p<.001, and follow-up visit, t(3428)=3.53, p=.001, compared to the baseline visit. In the placebo group (baseline=34.2%, drug=32%, follow-up=39.2%), there was no significant difference in either session compared to baseline, both *p*s > 0.08. Second, we analysed the high-to-low-threat switches in the same way. In the losartan group (baseline=-28.1%, drug=-23.5%, follow-up=-26.9%), the high-to-low ratings adjustment in the drug visit was significantly slower compared to the baseline visit, t(3141)=-2.75, p=.018. There was no difference in ratings on the follow-up visit, t(2719)=-.68, p=.50. In the placebo group (baseline=-29%, drug=-30.3%, follow-up=-30.6%), neither session after drug administration was significantly different from baseline, both *p*s > 0.96.

As a next step, we formally tested whether the change between the visits differed between groups (formalized as a contrast of contrasts). The change in adjustment between baseline and drug visits was significantly reduced in the losartan group, in both low-to-high, t(3864)=-2.31, p=.042, and high-to-low, t(3863)=2.48, p=.040, switches. Comparing the follow-up visit with baseline, losartan was found to significantly reduce probability adjustment in low-to-high, t(3865)=-3.73, p<.001 but not high-to-low adjustment, t(3859)=1.01, p>.31 compared to placebo.

Finally, we tested whether losartan selectively impacted either low-to-high or high-to-low switches differed compared to baseline visit. There was no difference in the magnitudes on the drug visit, t(3537)=.53, p>.59. On the follow up visit, losartan was found to reduce ratings significantly more in the low-to-high condition compared to high-to-low condition, t(3775)=3.76, p=.001.

In summary, these results suggest losartan reduces ratings adjustment compared to placebo. Upon acute administration of the drug, this drug-specific effect affects both high-to-low and low-to-high switches, i.e., reducing learning overall. However, on the follow-up visit, only low-to-high threat ratings are reduced, indicating selective prolonged reduction in learning in relative threat, but not in relative safety.

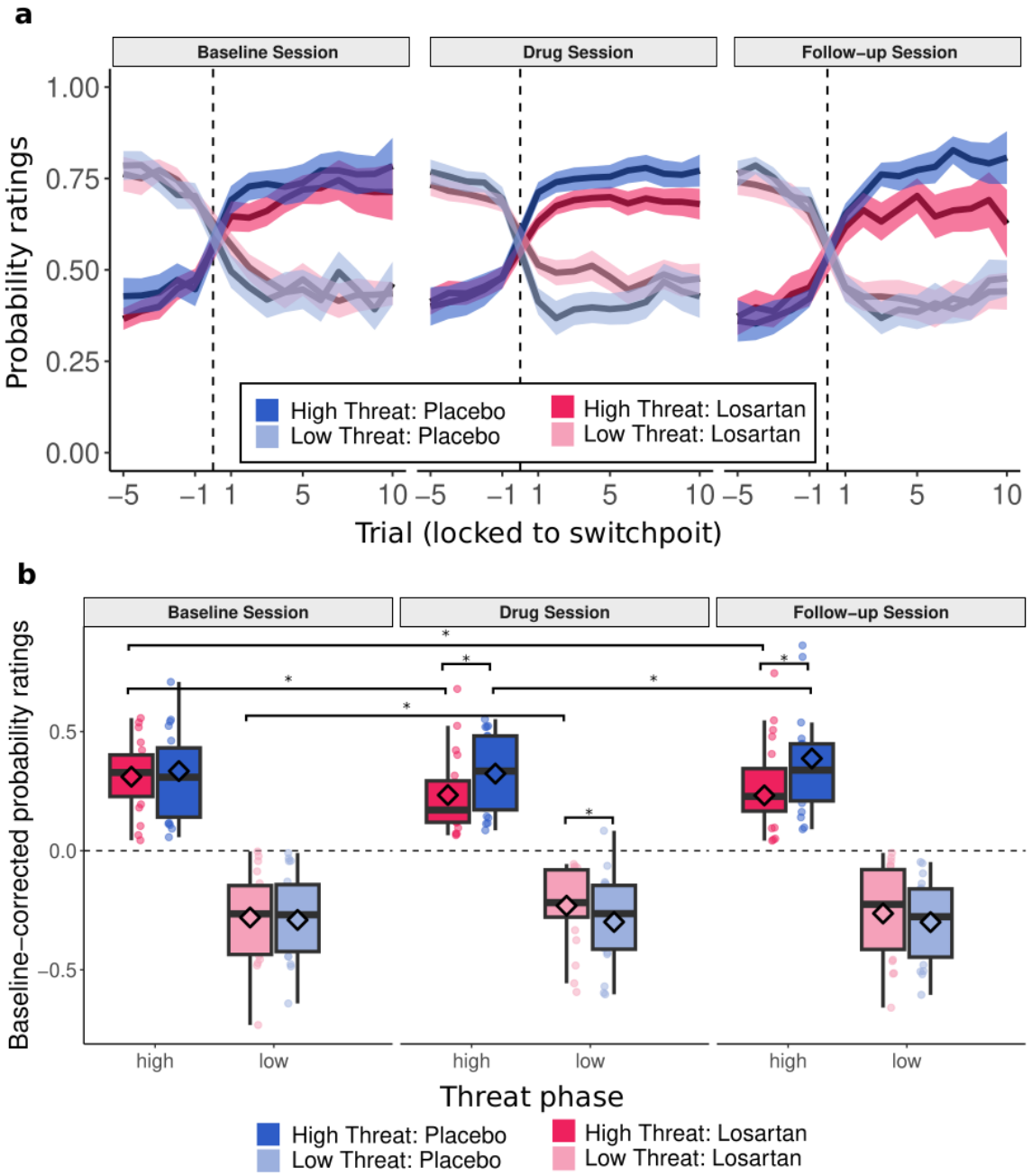

Figure 2 (**a**) Shock probability ratings on each trial split by drug group and threat phase. Data were aligned to the estimated switch point. Thick lines show mean while shaded areas show standard error of the mean. (**b**) Baseline-corrected probability rating change for each visit and threat phase. Values on the y-axis represent the change in ratings between baseline (trials 1-3 prior to switch) and after learning (trials 5-15 after the switch). Therefore, positive values reflect an increase in shock probability ratings (i.e., increase in shock expectancy), while negative values reflect a decrease in shock probability ratings. The central line on each summary box represents the median, the box itself reflects median +/- 1.58*IQR/sqrt(n), while the whiskers show the range of the data excluding outliers (for further details see the default settings of the ggplot2:geom_boxplot() function). Angled rectangles represent predictions of the fitted LMM model. The figure shows data for N=40 participants, N=20 in each drug group.

Error from true reinforcement

The previous analyses focused on the differences between groups. Here, we specifically ask whether participants in the two groups under- or over-predicted threat in each phase. To assess this, we analysed the error from the true reinforcement measure described above. Participants in both groups significantly over-predicted the probability of shock in the low-threat phase. In the placebo group, the over-prediction rates across the three sessions were: 25.3%, 21.9% and 20.5%; in the losartan group, they were: 24.9%, 25.7% and 22.1%, all *p*s < .002. In the high-threat phase, there were differences between sessions. During baseline, ratings of both groups did not deviate significantly from the true reinforcement rate: $M_{(v1,\ placebo)}$=-5.4%, $t_{(v1,\ placebo)}(19)$=-1.15, *n. s.*; $M_{(v1,\ losartan)}$=-7.2%, $t_{(v1,\ losartan)}(19)$=-1.60, *n. s.* During the drug and follow-up visits, ratings of the placebo group were not different from the true reinforcement level, $M_{(v2,\ placebo)}$=-2.24%, t(19)=-.61, *n. s.*, $M_{(v2,\ placebo)}$=-5.0%, t(17)=-1.30, *n. s.* The losartan group, on the other hand, showed underprediction of threat on the drug and follow-up visits, $M_{(v2,\ losartan)}$=-12.8%, $M_{(v3,\ losartan)}$=-13.3%. After correction for multiple comparisons this effect only remained significant for the drug visit, t(19)=-3.67, p=.010, but not the follow-up visit, t(19)=-2.63, p=0.082. In summary, these results suggest that the application of losartan leads to an underprediction of threat.

Taken together, an acute administration of losartan was found to reduce adjustment of ratings in switches from low-to-high and high-to-low threat. This reductive effect was still present in a follow-up session one day later but only in low-to-high threat switches. Zooming in specifically on threat estimation in relation to objective shock contingencies, both groups overpredicted probability of shock in the low-threat context. Following acute administration, losartan led to an underprediction of threat in the high-threat context.

Modelling results

Model comparison

Both model comparison methods agreed on the Rescorla-Wagner model with separate learning rates for shocks and no-shocks as the best fitting model. The BIC scores were: -65 (PH-5), **-82** (RW-outcome-3), -69 (RW-phase-3) and -72 (RW-both-5) (Figure 3a). **65%** of participants (N=26) were best fitted by the RW-outcome-3 model, 12.5% (N=5) by the PH-5, 12.5% (N=5) by the RW-both-5 and 10% (N=4) by the RW-phase-5 model. There were no differences in fit between the two groups (assessed by LMM with model and group as fixed effects). These results suggest that differential learning from shocks and no-shocks is the most prominent feature in the way participants in both groups updated their beliefs. Importantly, neither dynamic learning rates (Pearce-Hall) nor the current phase (low- vs. high-threat) were found to improve fit over the outcome-based model. Therefore, we next focus on analysing the parameters of the winning model in relation to the drug manipulation.

Learning rates

The learning rates for shock (α_{sh}) and no-shock (α_{nosh}) outcomes of the winning model were analysed together using beta GLM with visit (baseline/v1, drug/v2, follow-up/v3), group (losartan, placebo) and outcome type (shock, no-shock) as fixed effects. The model found a significant main effect of outcome: learning from shocks (α_{sh}=0.12) was significant faster than learning from no-shocks (α_{nosh}=0.08), $\chi^2(1)$=31.05, p<.001 (Figure 3b). Furthermore,

group was found to significantly interact with visit, $\chi^2(2)$ =17.44, p<.001. There was no change in learning rates in the placebo group ($\alpha_{v1,plac}$= 0.12 $\alpha_{v2,plac}$= 0.13 $\alpha_{v3,plac}$= 0.12), $t_{v1>v2}(225)$=-2.21, *n.s.*, $t_{v1>v3}(225)$=-1.52, *n.s.*, $t_{v2>v3}(225)$=0.72, *n.s.*. In the losartan group, learning rates were significantly lower during the drug (v2) and follow-up visits (v3) compared to the baseline visit (v1)($\alpha_{v1,losartan}$= 0.12 $\alpha_{v2,losartan}$= 0.08 $\alpha_{v3,losartan}$= 0.08), $t_{v1>v2}(225)$=3.155, p=.011, $t_{v1>v3}(225)$=3.013, p=.014. Learning rates did not differ between visits 2 and 3, $t_{v1>v3}(225)$=-.126, *n.s.* (Figure 3c) in this group. Next we tested whether the reduction in learning rates was significantly different between losartan and placebo by investigating contrast of contrasts using the emmeans package, e.g., for v1 and v2: $(\alpha_{v2,\mathrm{los}} - \alpha_{v1,los}) - (\alpha_{v2,plac} - \alpha_{v1,plac})$. This analysis revealed that the between-session reduction of learning rate was larger in the losartan compared to the placebo group on both drug, t(225)=-3.77, p=.001, and follow-up, t(225)=p-.001, visits.

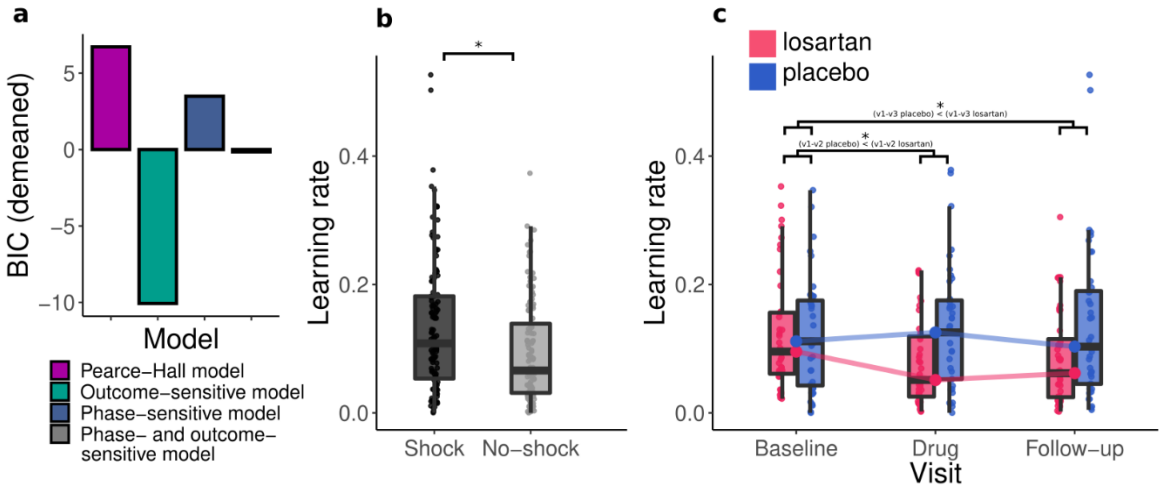

Figure 3: (**a**) Model comparison results showing demeaned BIC scores for the four models; lower values indicate better fit. Statistically significant effects of the model-estimated learning rates: (**b**) learning from shocks was overall faster than learning from no-shocks; (**c**) losartan reduced the learning rates on drug and follow-up visits compared to the baseline visit; there was no difference in learning rates in the placebo group.

Next, we tested how the parameters of the winning model relate to the behavioural effects. In particular, the behavioural results suggest that losartan decreases shock probability adjustment in high- and low-threat phases on the drug visit. Additionally, the decrease in the high-threat phase remained significant at the follow-up visit. However, the model comparison revealed that the data are best explained by learning rates sensitive to specific outcomes (shock/no-shock), not high-/low-threat phases. Therefore, to investigate how differential learning from shocks and no-shocks relates to shock probability adjustment, we performed a correlation analysis on data averaged across the visits. In the low-to-high-threat phase switches, learning rates for both outcomes were positively correlated with the adjustment in probability ratings, $r_{(\text{shock, high})}(38)$=.57, p<.001, $r_{(\text{no-shock, high})}(38)$=.66, p<.001. In the high-to-low-threat switches (where probability change is negative, Figure 2), both learning rates were negatively correlated with the behavioural marker, $r_{(\text{shock, low})}(38)$=.-.47, p=.002, $r_{(\text{no-shock, low})}(38)$=-.77, p<.001.

In summary, the modelling analysis revealed that losartan reduces aversive learning rates. This effect was present upon acute administration as well as on a follow up visit 24 hrs later.

Importantly, losartan did not reduce learning from a specific outcome type, i.e., learning from both shock and no-shock events was reduced.

Discussion

Our findings show that a small dose of the angiotensin II receptor antagonist losartan dampens learning in aversive environments. Acutely, this results in underprediction of threat in high-threat context (i.e., reduction in threat learning) and overprediction of threat in low-threat context (i.e., reduction in safety learning). One day later, aversive learning rates in the losartan group remain lowered compared to the baseline visit. Importantly, on the follow up visit, only the reduction in threat learning is maintained, while safety learning no longer differs from that seen in the placebo group. These results suggest a potential role of losartan in the prevention of developing fear-related memory associations.

Interestingly, while learning rates were generally higher for shocks compared to no-shocks (similarly to Cazé & van der Meer, 2013; Jepma et al., 2018), this difference was not modulated by the drug. Instead, losartan resulted in reduction of learning from all events across both high- and low-threat contexts. Taken together, these results suggest that a single dose of losartan reduces learning in aversive environments rather than from aversive events.

In the task, participants had to follow switches between high and low probability of receiving a shock (i.e., threat). Our analyses focused on the way in which individuals adjust their subjective belief about the probability of shock and how these adjustments are modulated by losartan. We found that when the objective shock probability changed from low to high (i.e., high threat context), the losartan group increased their expectations to a lesser degree than the placebo group. In turn, such a reduction in the perception of threat might be one of the mechanisms underlying reduced PTSD symptoms development, which has been associated with ARB intake (Khoury et al., 2012; Seligowski et al., 2021), autonomic stress response (Shkreli et al. 2020) and negative memory encoding (Xu et al., 2022). When the probability of shock changed from high to low (i.e., low threat context), the losartan group also showed less adjustment to reflect the now lower shock probability. Instead, they reported higher shock probabilities than the placebo group. Importantly, only the reduction in adjustment of subjective aversive estimates to high threat was maintained throughout a 24-hour follow-up period, highlighting the lasting impact of losartan on disrupting aversive acquisition beyond acute drug effects. While these findings highlight a potential long-term preventive effect of ARBs on aversive learning, it is important to consider general reduction in learning, including safety learning, upon acute administration.

Our modelling analyses revealed that the decrease in shock probability adjustment was associated with a reduction in aversive learning rates. This aligns with previous work that found a link between losartan and a reduction in aversive, but not appetitive, learning rates (Pulcu et al., 2019; Xu et al., 2023). Our results extend the previous finding in several ways. First, the trial-by-trial readouts of participants' subjective shock probability allows us to directly link the observed behaviour to model estimates. As pointed out by Palminteri et al. (2017), learning rates can reflect a variety of underlying behaviours and should therefore be

linked back to relevant behavioural readouts. We show that the decrease in learning rates is related to reduced adjustment of aversive expectations. This indicates that the model meaningfully captures the behavioural effect. Second, we used a Pavlovian conditioning task with primary reinforcers (as opposed to an instrumental task with secondary reinforcers). This is an important distinction, as Pavlovian learning is believed to underlie the formation of a number of anxiety and stress-related disorders (Fanselow & Sterlace, 2014; VanElzakker et al., 2014).

Taken together, these findings provide preliminary evidence that angiotensin II receptor blockade may be effective in preventing the development of anxiety disorders, by specifically interfering with learning under threat. However, such effects need to be replicated in large prospective studies, looking at the link between RAS variation or manipulation and the onset of DSM-V anxiety disorders. Specifically, future studies may identify a link between increased endogenous angiotensin II levels and an increased risk of prospective anxiety onset, similarly to observations in rodents (Duchemin et al., 2013; Gao et al., 2021). Such findings would have implications for the development of a simple blood test screening for anxiety risk that may inform triage to early preventative strategies.
Another important avenue would be to investigate whether reduction in learning changes the strength of context-dependence of acquisition and extinction. For example, individuals high in trait anxiety have been recently associated with increased context-dependent learning, which is believed to increase the rates of return of fear (Zika et al., 2022). More gradual adjustment of aversive beliefs has been associated with overriding previous aversive memories, rather creation of separate internal safety context (Gershman et al., 2013). In our data, losartan reduced learning in both high-to-low and low-to-high switches. While one interpretation is that this reflects a decrease in safety/threat learning, it may also reflect a tendency to gradually update existing associations rather than creating a separate internal context. A specific testable prediction of this alternative hypothesis would be that losartan decreases the rates of return of fear (e.g., spontaneous recovery or reinstatement; see Gershman and Hartley, 2015).

There are some limitations to this study. First, the task does not contain a control condition with appetitive or neutral stimuli (e.g., statistical learning). In turn, it remains inconclusive whether the reduction in Pavlovian learning is specific to aversive contexts or whether the drug reduces learning overall. Previous work identifying an aversion-specific role of losartan employed an instrumental, rather than a Pavlovian, learning task. Second, while a follow-up was performed 24 hours after the original drug session, long-term retention was not assessed. Investigating the duration of the reductive aversive learning effect would be useful in assessing preventive effects of losartan on formation of aversive associations. Further research is therefore needed to assess the duration of the reductive learning effect of losartan, particular in relation to the prevention of development of excessive fear and to return of fear phenomena such as reinstatement or spontaneous recovery. Nevertheless, our results highlight the potential preventative role of losartan in the formation of aversive associations, especially at longer time scales.

Author contributions

The following list of author contributions is based on the CRediT taxonomy. Conceptualization: O.Z., K.W., A.R.; Data curation: O.Z., J.A., C.K., L.S.; Formal analysis: O.Z..; Funding acquisition: A.R.; Investigation: A.R., O.Z., J.A., C.K., L.S. and M.B.; Methodology: A.R., O.Z., K.W.; Project administration: O.Z., J.A., L.S., A.R. C.K.; Resources: K.W., A.R.; Software: O.Z.; Supervision: A.R., K.W.; Validation: O.Z.; Visualization: O.Z.; Writing - original draft: O.Z., K.W., A.R., J.A., C.K., L.S., M.B.; Writing - review & editing: O.Z., K.W., A.R., J.A., C.K., L.S., M.B.

Data Availability

The behavioural data generated in this study have been deposited to GitHub and are openly accessible here: https://github.com/ozika/aversive-learning-losartan-zika2023.

Code Availability

The code used to derive statistical results is stored in the associated GitHub repository together with the data: https://github.com/ozika/aversive-learning-losartan-zika2023. The repository includes instructions to reproduce the results, including dedicated computational virtual environment in R.

Registration

The study was registered with Clinical Trials and Research Governance of the University of Oxford prior to the start of data collection and later made public via OSF: https://osf.io/e3zrk

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Competing Interests Statement

M.B. has received travel expenses from Lundbeck for attending conferences and has acted as a consultant for J&J, Novartis and CHDR. The remaining authors declare no competing interest.

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