

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

**Running title:** Losartan and Pavlovian aversive learning

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## Abstract

**Background:** Angiotensin receptor blockade (ARB) has been linked to aspects of aversive learning and memory formation, and to the prevention of post-traumatic stress disorder symptom development.

**Methods:** We investigate the influence of the ARB losartan on aversive Pavlovian conditioning using a probabilistic learning paradigm. In a double-blind, randomised placebo-controlled design, we tested 45 (18 female) healthy volunteers during a Baseline session, after application of losartan or placebo (Drug session) and during a Follow-up session. On each session, participants engaged in a task where they had to predict the probability 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.

**Results:** 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 in the losartan group 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 was maintained on a follow up session 24hrs later.

**Conclusions:** This study shows that losartan acutely reduces Pavlovian learning in aversive environments. Such decreased learning may explain a previously reported preventive role of ARB in the development of anxiety symptoms.

The study was registered on OSF ([osf.io/e3zrk](https://osf.io/e3zrk)).

# Main text

## Introduction

With a life-time prevalence of 15-30%, significant individual economics costs and increased depression risk, anxiety disorders represent one of the most impactful mental health problems (1–5). 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 aetiology 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, the midbrain, hippocampus and the prefrontal cortex (6,7), where they interact with other neuroendocrine systems including dopamine function(8) or the stress-related hypothalamic-pituitary-adrenal (HPA) axis (9)

Increased angiotensin II levels have been reported as a response to stress in rodent models (10). 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 (11–13). In humans, ARB have been reported to improve symptoms of anxiety in type 2 diabetes patients (14). 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 (15). 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 (PTSD, 16). Similarly, administration of losartan has been associated with reductions in subjective fear during a fear-inducing

aversive task (17) and encoding of negative memories (18). Identifying specific aspects of learning (e.g., prediction errors or learning rates) impacted by RAS-modulating drugs can help us understand the function they play in the prevention of anxiety development. Two studies have used an learning approach to study the impact of RAS antagonist losartan on learning rates (19,20). Both used a learning paradigm involving gains and losses. The authors reported reduction in loss but not gain learning rates following a single dose of losartan. Given the known link between the dopaminergic system and prediction error processing (21,22), this might also suggest RAS modulation of learning via interaction with dopamine (8,23). Indeed, recent work in humans has reported modulation reward-related processing by a single dose of losartan in the midbrain dopamine system (24,25).

Such findings point to a prominent role of the RAS in aversive learning. The only two studies investigating the role of losartan in learning used an instrumental conditioning paradigm. No study has directly investigated the effect of RAS on Pavlovian threat learning, the key learning mechanism associated underlying the development of anxiety disorders (26–28). Direct evidence for RAS-modulating drug effect on Pavlovian learning would provide an important insight into mechanisms of anxiety development with implications for preventive strategies and improvements in the early detection of anxiety risk.

Building on a growing literature investigating learning mechanisms in psychiatry using computational models (29–31), we employ a probabilistic learning paradigm to test the effect of RAS antagonist losartan on aversive learning mechanisms. In the task, periods of relative threat and safety alternated, going beyond traditional classical conditioning paradigm. Similar tasks have been used to identify learning differences in clinical anxiety (32). Here, we extend previous work by focusing on mechanisms of Pavlovian learning from primary reinforcers. We consider a number of plausible aspects of learning, such as differential learning from shocks and shock omissions (33), context-dependent updating (34), uncertainty-driven learning (35) and biases in probability perception (36).

Building on previous work (19,20), we test the hypothesis that a single dose of the ARB

losartan leads to acute reduction in learning during aversive Pavlovian learning.

## Methods and Materials

Sample size was estimated based on two previous studies investigating the impact of losartan on aversive learning rates (19,20). A power analysis by simulation was performed using beta likelihood for the main effect of drug on Session 2. The estimated sample size was 44 participants (22 per group) at 80% power ( $\alpha=0.05$ ). See SI. 45 healthy volunteers (18–39 years) were recruited.

Participants without history of DSM-V Axis I disorder (37), free from CNS-active medication for at least six weeks, no first-degree family member with a history of a severe psychiatric disorder and with body mass index between 18 and 30 kg/m<sup>2</sup> were included (full selection criteria in SI). The study was approved by the Oxford University Research Ethics Committee (R29583). All participants gave written informed consent. Five participants had to be excluded from the study: one due to technical failure of the equipment, four because they failed to dissociate between the stable cues on the Follow-up session (see below). This left twenty participants in the losartan group (N(female)=6, M=25.5 years) and twenty in the placebo group (N(female)=10, M=24.1 years). Using the same simulation approach as above, the power in the final sample was 77.1%.

The study involved three sessions to the Department of Psychiatry at the Oxford University. Baseline session included a medical and psychiatric screening, followed by instructions and completion of a short version of the task. Drug session included completing a battery of psychological questionnaires, administration of one dose of losartan or placebo one hour before completion of the full version of the task. Follow-up session took place one day after

the Drug session to assess any potential next-day effects. Participants also completed a shorter version of the task.

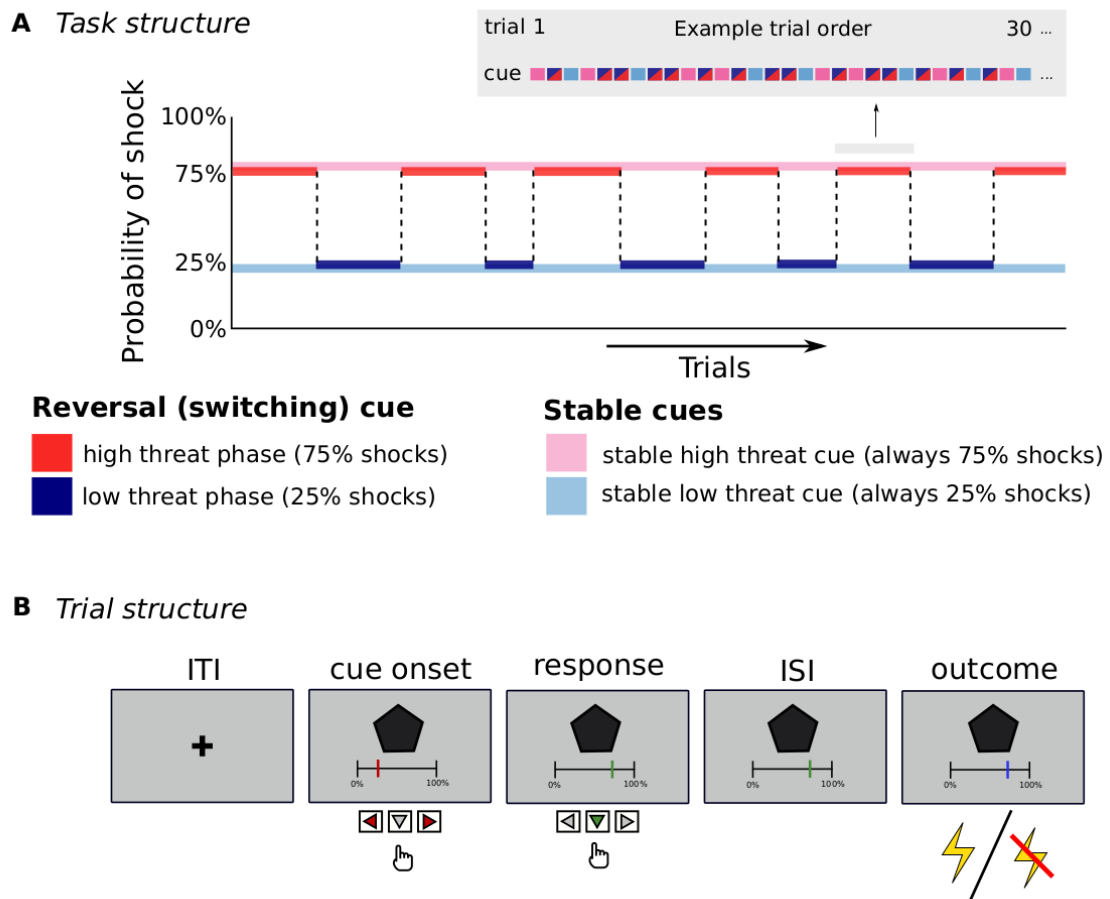
Prior to the Drug session, participants were randomly allocated to one of two groups in a double-blind design, either receiving a 50mg single oral dose of losartan (Cozaar, Merck Sharp & Dohme Ltd.) or a placebo capsule that was matched to the active drug in appearance (microcrystalline cellulose; Rayotabs, Rayonex GmbH). Dosing of losartan was guided by the intention to assess its impact on aversive learning without triggering hypotensive effects, similar to previous studies (16,19,24,38). Robust hypotensive effects in humans occur only after a three to four week period of daily 50mg intake, rather than following a single administration (39). Even after two weeks, no blood pressure changes were found in normotensive individuals (40). To monitor potential confounding effects of losartan on acute changes in blood pressure, heart rate or mood, we assessed these measures using self-report visual analogue scales (VAS; 0-100) and Omron 705IT sphygmomanometer one hour and just before drug administration. Testing started one hour after capsule intake, when drug peak plasma levels are reached (41,42). At the end of the Drug session, participant and experimenter indicated independently whether they thought losartan or placebo had been administered during the session.

On the Drug session participants completed a battery of psychological questionnaires assessing personality traits, anxiety, depression and attention regulation strategies (State-Trait Anxiety Inventory STAI; (43); Beck Depression Inventory BDI; (44); Attentional Control Scale ACS; (45) and the National Adult Reading Test (46).

Electrical stimuli were applied using an electric stimulation device (Digitimer 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 every approx. ten minutes to the 8/10 level, 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 expected stimulations. The calibration followed the Method of Limits (47).

Pavlovian aversive learning paradigm with repeated changes between periods of high and low threat was employed (Figure 1a). Each session consisted of 150 (short version) or 300 (long version) trials. On each trial, participants were presented with one of three visual cues (abstract fractals, randomized) and asked to provide subjective shock probability rating (0% to 100% scale) within 4 seconds. After an inter-stimulus interval (1s), a short electrical impulse was either delivered (shock) or omitted (no-shock). One of the cues switched between a 75% and 25% chance of shock (high- vs low-threat phases). every 30 +/- 5 trials (reversal cue, presented on 50% of trials). Starting level was randomized. On the remaining trials one of two control cues was presented: stable-high- and stable-low-threat cues (fixed chance of shock, 75% and 25% respectively). No information was given regarding the number of cues or the number of switches. The task was paused every 10-12 minutes for stimuli recalibration and to allow participants to rest. Instructions were delivered in a standardized form.



**Figure 1** (a) Task structure: Three cues were presented sequentially (example in grey box). Two cues had fixed objective probability of resulting in shock which was either high (pink) or low (light blue) throughout. The objective shock probability of the main cue changed in semi-regular intervals between phases of high (red) and low (blue) threat. Sessions 1 and 3 included 6 phases on average (short version, ~150 trials) while on Session 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.

Reversals between the two levels were not signalled. Participants had to infer that a change had occurred from the received binary outcomes. To avoid false conclusions that can arise during averaging of temporal trajectories (48), 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, demeaned the time series and calculated the cumulative sum of probability ratings(49). The peak/trough of this series



represents the point of fastest updating. For each reversal we labelled this point an estimated switchpoint (See SI for robustness checks of this method)

Participants provided a probability rating between 0% and 100% on each trial. 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 switchpoint, 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 Fig. 2a). This allowed us to assess changes in probability ratings before and after learning.

Models were specified and fitted to probability ratings of the reversal cue using Stan (50) . To assess model fit , the leave-one-out (LOO) information criterion was computed for each model (51) . Posterior samples were estimated using Markov Chain Monte Carlo sampling using No U-Turn Sampling across 4 chains, 2000 samples per chain (600 warmup). session

In line with similar studies (19,29,33), we employed a modelling framework based on reinforcement learning (52). Four models were variations of the Rescorla-Wagner (RW) learning rule (53), one model had an adaptive learning rate (Hybrid-Rescorla-Wagner-Pearce-Hall; Hybrid RW-PH). 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), i.e., the difference between the expectation  $P_t$  and the outcome  $O_t$  (1=shock, 0=no-shock). The PE is weighted by a free parameter  $\alpha \in [0,1]$ . Large values of  $\alpha$  lead to rapid updating, while small values of alpha lead to slower learning (Eq. 1).

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

The starting value was estimated as a free parameter ( $P_1 \in [0,1]$ ). Two models varied in regard to differential learning from shock/no-shock (Outcome model) or high/low threat phase (Phase model). The third model was a combination of the two, i.e., it had learning rates for all combinations of shock/no-shock high/low threat (Outcome-phase model). The Outcome model distinguished between learning from shocks and no-shocks. This was specified as separate learning rates for the two outcomes:  $\alpha_{sh} \in [0,1]$  and  $\alpha_{nosh} \in [0,1]$  (Eq. 2).

$$\text{Eq. 2} \quad P_{t+1} = P_t + \alpha_{sh}(O_t - P_t) \text{ if shock } (O_t = 1), \text{ otherwise } \alpha_{nosh}$$

Phase and Outcome-phase models followed the same logic (see SI for a full description).

To account for the possibility that the behaviour was driven by generally more uncertain prediction (i.e., values closer to 0.5) we specified a model with a lapse parameter  $\xi$ . Here, the probability prediction for next trial is a mixture of updated previous prediction and random rating. See Eq. 3 (Lapse model).

$$\text{Eq. 3} \quad P_{t+1} = (1 - \xi)[P_t + \alpha(O_t - P_t)] + \xi 0.5$$

The last model was a hybrid of the Rescorla-Wagner and Pearce-Hall models (Hybrid-RW-PH) (35,54,55). This model dynamically adjusts learning rate on each trial (Eqs. 4 and 5). Trial-specific learning rate  $\alpha_{t+1}$  is updated by a weighted combination of the current absolute prediction error  $|PE_t|$  and the learning rate  $\alpha_t$ . The parameters  $\eta_{sh} \in [0,1]$  and  $\eta_{nosh} \in [0,1]$  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  $\kappa \in [0,1]$ .

$$\text{Eq. 4} \quad P_{t+1} = P_t + \alpha_t(O_t - P_t)$$

$$\text{Eq. 5} \quad \alpha_{t+1} = \kappa[\eta_{sh}|PE_t| + (1 - \eta_{sh})\alpha_t] \text{ if shock, otherwise } \eta_{nosh}$$

Data were collected using custom MATLAB 2016 and Psychtoolbox3 code. All analyses were performed using MATLAB 2019b and R 3.6.3. The data and code with reproduction instructions are openly accessible at <https://github.com/ozika/aversive-learning-losartan-zika2023>.

The effect of losartan on physiological and VAS measures between baseline and drug-peak level was assessed using a linear mixed effects models and ANOVAa. Behavioural analyses were performed using linear mixed effects models (LMM; *lmer* 46) and a ANOVAs (*lmerTest* 47). Post-hoc t-tests were corrected for multiple comparisons using the Holm (58) correction. Learning rates were analysed using a generalized beta regression (59) with logit link function (*glmmTMB*; 50). The statistical test was performed using the Type II Wald Chi-squared test (using the *car* package(61). Participant ID and starting probability were included in all models as random intercepts. Session and either phase (behavioural model) or outcome type (learning rates model) were included as random slopes to account for within-subject variability. Other R packages used for the analyses were: *loo* (51), *performance* (62), *tidyr* (63), *plyr* (64), *parameters* (65), *dplyr* (66), *renv* (67) *rstan* (68), *ggplot* (69), *emmeans* (70) and *effectsize* (71).

## Results

The two groups were well-matched on sociodemographic and questionnaire parameters (Table 1). As expected, there were no group differences in heart rate, blood pressure, mood and physiological symptoms VAS rating changes from baseline to drug peak level (Table 2). Furthermore, neither the participants nor the experimenter was able to indicate the true group allocation (experimenter: 40% correct, patients: 50%; both  $\chi^2 < 0.98$ ,  $p > .32$ ),

suggesting that double-blindness was maintained throughout the study.

**Table 1:** Sociodemographic, clinical and personality characteristics in the losartan versus placebo group (M, SD).

	Losartan (N=20)		Placebo (N=20)	
	M	SD	M	SD
<i>Sociodemographic Data</i>				
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
<i>Clinical and Personality Measures</i>				
Trait Anxiety (STAIT)	34.9	8.5	37.0	7.28
Beck Depression Inventory (BDI)	4.0	6.19	5.05	6.46
<i>Attentional Control (ACS)</i>				
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.

To investigate any task-related differences between groups, we compared objective shock intensity, reaction times, initial aversive bias, starting probability of the reversal cue and the ability to dissociated between stable cues during the Drug session. There was no group difference in the calibrated shock intensity ( $I_{\text{losartan}}=1010$  mA;  $SD_{\text{losartan}}=1850$ ;  $I_{\text{placebo}}=514$  mA;  $SD_{\text{placebo}}=673$ ),  $t(36)=-.96$ ,  $p=.34$ , starting probability, = 0.13,  $p=.72$  or initial bias ( $B_{\text{losartan}}=44\%$ ;  $SD_{\text{losartan}}=.14$ ;  $B_{\text{placebo}}=53\%$ ;  $SD_{\text{placebo}}=.22$ ),  $t(32)=-1.60$ ,  $p=.12$ . The drug did not impact reaction times during the Drug session in relation to baseline session,  $<2.61$ ,  $p>.27$ .

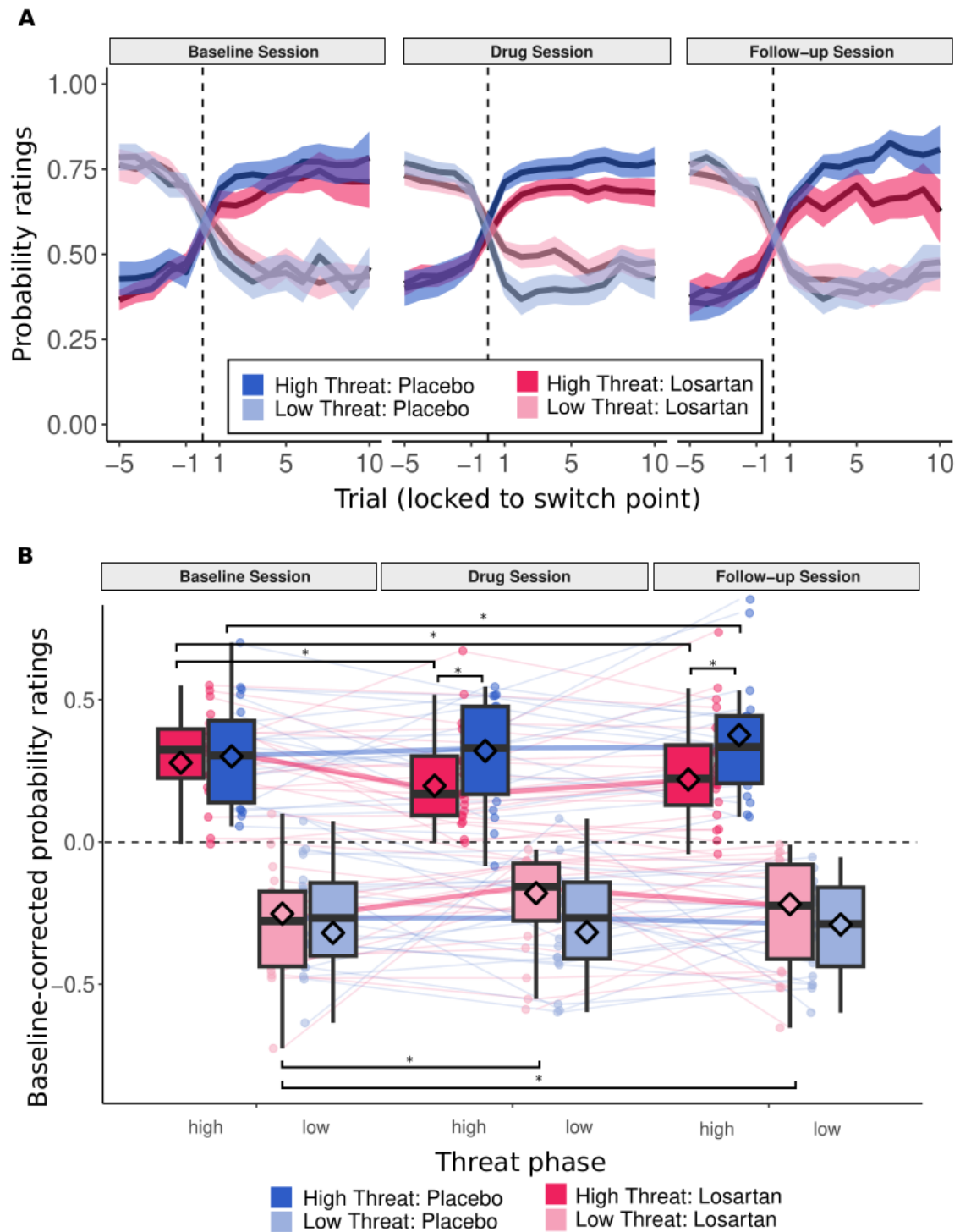
The ratings for the stable cues did not differ significantly from the true contingencies nor between groups. Lastly, there was no main effect or interaction with drug in either systolic or diastolic blood pressure in ratings or learning rates , all  $F_s < 1.27$ ,  $p_s > .26$ .

**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				<i>p</i>
	Losartan		Placebo		Losartan		Placebo		
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	
<i>Physiological Measures</i>									
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
<i>Visual Analogue Ratings (0-100)</i>									
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.

The behavioural data were realigned using the estimated switchpoints (Figure 2a). The mean switch point value was 4.52 trials (SD=3.40) after reversal. There was no difference in switchpoints between groups or sessions.

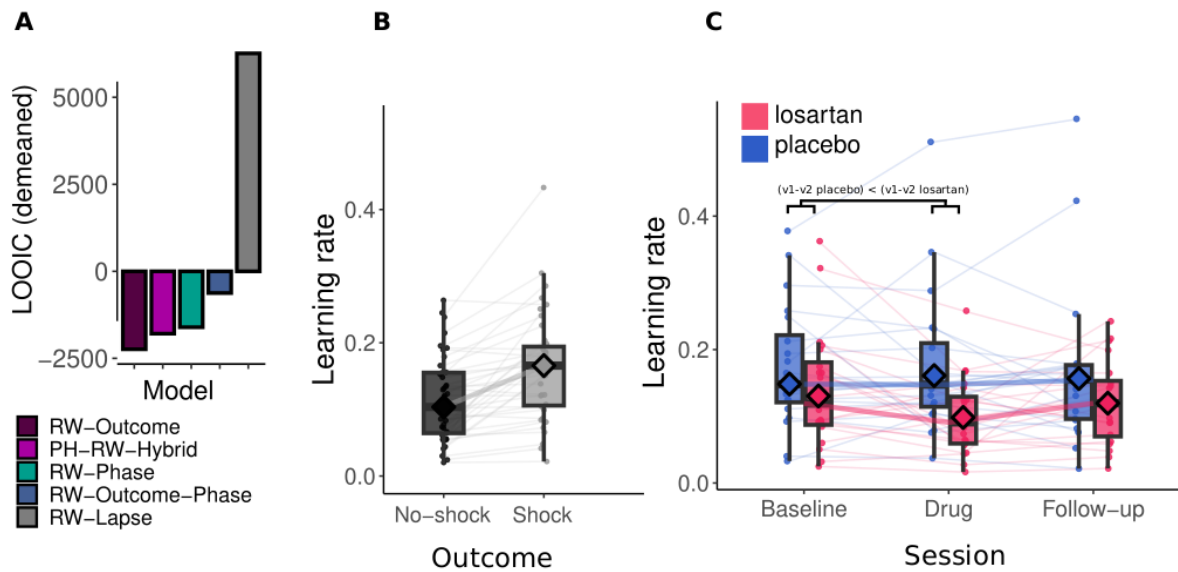


**Figure 2** (a) Shock probability ratings on each trial split by drug group and threat phase. Data were aligned to the estimated switch point. Thick lines show mean while shaded areas show standard error of the mean. (b) Baseline-corrected probability rating change for each session and threat phase. 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  $\pm 1.58 \cdot \text{IQR} / \sqrt{n}$ , while the whiskers show the range of the data excluding outliers (for further details see the default settings of the `ggplot2::geom_boxplot()` function). Individual thin lines connect data point for a specific participant across the three sessions. Angled rectangles represent predictions of the fitted LMM model. The figure shows data for  $N=40$  participants,  $N=20$  in each drug group.

Probability ratings changes are shown in Figure 2b. Statistical test found significant main effects of phase. The ratings were positive in the high-threat phase (33.6%) and negative in the low-threat phase (-30.2%),  $F(1, 38.1)=166.33$ ,  $p<.001$ ,  $\eta^2_p=.81$  [.70 .88]. Furthermore, there was a significant interaction between group, session and phase,  $F(2, 3903)=49.06$ ,  $p<.001$ ,  $\eta^2_p=.02$  [.02 .03]. In the high-threat phase, losartan was found to decrease ratings on s2,  $t(54.4)=4.03$ ,  $p=.001$ ,  $\eta^2_p=.23$  [.06 .41], and s3,  $t(66.5)=2.93$ ,  $p=.009$ ,  $\eta^2_p=.11$  [.01 .27], compared to baseline session ( $s1_{\text{losartan, high}}: 37.1\%$ ,  $s2_{\text{losartan, high}}: 25.0\%$ ,  $s3_{\text{losartan, high}}: 27.9\%$ ). In the low-threat phase losartan was found to increase ratings on s2,  $t(49.9)=-4.07$ ,  $p=.001$ ,  $\eta^2_p=.25$  [.07 .43], and s3,  $t(48)=-3.79$ ,  $p=.001$ ,  $\eta^2_p=.23$  [.06 .42], compared to baseline ( $s1_{\text{losartan, low}}: -36.8\%$ ,  $s2_{\text{losartan, low}}: -25.0\%$ ,  $s3_{\text{losartan, low}}: 25.8\%$ ). In the placebo group in high-threat condition ratings on s2 were not different from baseline s1,  $t(50.4)=-1.02$ ,  $p=.314$ ,  $\eta^2_p=.02$  [.00 .15], while ratings on s3 were higher,  $t(64.1)=-2.57$ ,  $p=.038$ ,  $\eta^2_p=.09$  [.00 .25] ( $s1_{\text{placebo, high}}: 33.6\%$ ,  $s2_{\text{placebo, high}}: 36.6\%$ ,  $s3_{\text{placebo, high}}: 41.6\%$ ), in the low-threat phase neither s2,  $t(46.2)=-.39$ ,  $p=1.00$ ,  $\eta^2_p=.00$  [.00 .10], nor s3,  $t(46)=-.85$ ,  $p=1.00$ ,  $\eta^2_p=.01$  [.00 .11], differed from baseline ( $s1_{\text{placebo, low}}: -30.1\%$ ,  $s2_{\text{placebo, low}}: -31.2\%$ ,  $s3_{\text{placebo, low}}: -32.5\%$ ). Contrasting these effects between groups, for example ( $s1_{\text{los}}-s2_{\text{los}}$ ) - ( $s1_{\text{placebo}}-s2_{\text{placebo}}$ ), the ratings decrease in high-threat phase was larger in the losartan group on both s2,  $t(52.7)=-3.68$ ,  $p=.002$ ,  $\eta^2_p=.20$  [.05 .39], and s3,  $t(65.1)=-3.91$ ,  $p=.001$ , compared to placebo. The ratings increase in the low-threat phase was also significant on both s2,  $t(48.5)=3.21$ ,  $p=.004$ ,  $\eta^2_p=.18$  [.03 .36], and s3,  $t(41)=-3.31$ ,  $p=.004$ ,  $\eta^2_p=.19$  [.03 .38] sessions compared to placebo. All percentages in this section correspond to the model-estimated marginal means. sessions2s3 These results suggest that unlike placebo losartan

acutely reduces ratings adjustment in the low-to-high as well as high-to-low threat switches upon both acute administration (s2) and one day later (s3). Model comparison using leave-one-out information criterion (LOOIC) found the Outcome model to fit best. The LOOIC scores for the five models were: 39460.16 (Outcome-model), 39908.11 (Hybrid-RW-PH), 40092.65 (Phase-model), 41075.22 (Outcome-phase-model) and 47960.11 (Lapse-model), Fig 3a. The model fit ranking was identical for losartan and placebo groups (see SI). These results suggest that differential learning from shocks and no-shocks determined learning rather than dynamic learning rates (Hybrid RW\_PH) or current threat context.



**Figure 3** (a) Model comparison results showing demeaned LOOIC (leave-one-out cross validated information criterion) 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 session compared to the baseline session; there was no difference in learning rates in the placebo group. Panels b) and c) contain data for N=40 participants, 20 in each drug group. The central line on each summary box represents the median, the box itself reflects median  $\pm 1.58 \times \text{IQR} / \sqrt{n}$ , while the whiskers show the range of the data excluding outliers (for further details see the default settings of the `ggplot2::geom_boxplot()` function). Individual thin lines connect data points for a specific participant (i.e., within-subject effect). Angled rectangles represent predictions of the fitted beta regression model.

Focusing on the winning model, we assessed parameter consistency within participants, we calculated inter-class correlations (ICC) for  $\alpha_{sh}$  and  $\alpha_{no-sh}$ . Specifically, we calculated ICC(A,1), two-way mixed, single-measure, absolute agreement (72). The ICC(A,1) for no-



shock learning rate was  $ICC=0.645$ ,  $F(39,79.2)=6.38$ ,  $p<.001$  and for shock learning rate  $ICC=0.657$ ,  $F(39,78)=6.94$ ,  $p<.001$ .

The learning rates of the winning model were analysed together using generalized beta regression and Wald test. The model found a significant main effect of outcome: learning from shocks ( $\alpha_{sh}=0.15$ ) was significant faster than learning from no-shocks ( $\alpha_{nosh}=0.10$ ),  $\chi^2(1)=28.78$ ,  $p<.001$ ,  $\eta^2_p=.34$  [.12 .54] (Fig. 3b). Furthermore, group was found to significantly interact with session,  $\chi^2(2) = 7.45$ ,  $p=.024$ ,  $\eta^2_p=.01$  [.00 .16]. There was no change in learning rates in the placebo group ( $\alpha_{v1,plac}= 0.150$   $\alpha_{v2,plac}= 0.154$   $\alpha_{v3,plac}= 0.146$ ),  $t_{s1>s2}(216)=-0.222$ ,  $p=1.00$ ,  $\eta^2_p=.00$  [.00 .02],  $t_{s1>s3}(216)=-.274$ ,  $p=1.00$ ,  $\eta^2_p=.00$  [.00 .02],  $t_{s2>s3}(216)=.61$ ,  $p=1.00$ ,  $\eta^2_p=.00$  [.00 .03]. In the losartan group, learning rates were significantly lower during the drug (s2) compared to the baseline session (s1) ( $\alpha_{v1,losartan}=0.120$ ,  $\alpha_{v2,losartan}=0.085$ ,  $\alpha_{v3,losartan}=0.106$ ),  $t_{s1>s2}(216)=2.90$ ,  $p=.012$ ,  $\eta^2_p=.04$  [.00 .10]. There was no difference between s1 and s3,  $t_{s1>s3}(216)=.97$ ,  $p=.34$ ,  $\eta^2_p=.00$  [.00 .04], or s2 and s3,  $t(216)=-1.93$ ,  $p=.010$ ,  $\eta^2_p=.02$  [.00 .07] (Figure 3c).. Next, we tested whether the reduction in learning rates was significantly different between losartan and placebo by investigating contrast of contrasts, e.g., for s1 and s2:  $(\alpha_{s2,los} - \alpha_{s1,los}) - (\alpha_{s2,plac} - \alpha_{s1,plac})$ . This analysis revealed that the between-session reduction of learning rate was larger in the losartan compared to the placebo group on the drug,  $t(216)=2.29$ ,  $p=.046$ ,  $\eta^2_p=.02$  [.00 .08] but not the follow-up sessions,  $t(216)=.51$ ,  $p=.614$ ,  $\eta^2_p=.00$  [.00 .03].

Lastly, we correlated learning rates with ratings adjustment. Model-estimated learning rates correlated with probability adjustment both in high-to-low,  $r(38)=-.40$ ,  $p=.04$ , and low-to-high,  $r(38)=.53$ ,  $p=.003$ .

## Discussion

Our findings show that a 50mg dose of the angiotensin II receptor antagonist losartan dampens learning in aversive environments. Acutely, this results in underprediction of threat in high-threat contexts (i.e., reduction in threat learning) and overprediction of threat in low-threat contexts (i.e., reduction in learning of relative safety), driven by reduced aversive learning rates. One day later, the under- and over-prediction of threat remains, however it is no longer supported by a between-group difference in learning rates. These results suggest a potential role of losartan in the development of fear-related associations via a reduction in aversive learning rates. This mechanism might play a role in the development of anxiety and PTSD.

In our analyses we found that when the shock probability changed from low-to-high (high-threat context) or high-to-low (low-threat context), the losartan group exhibited slower adjustment following drug administration and one day later, while there were no differences during a baseline visit. We show that this decrease in overall learning was driven by a reduction in aversive learning rates. Such a global reduction in threat learning might be one of the mechanisms underlying reduced PTSD symptoms development, which has previously been associated with ARB intake (15,73), autonomic stress response (16) and negative memory encoding (18). While these findings highlight a potential long-term role of ARBs on aversive learning and anxiety/trauma development, it is important to consider that a general reduction in aversive learning might include reduced safety learning. Therefore, via reducing learning, these drugs might also impair extinction in a clinical context. However, it is important to note that our low-threat condition still includes some level of threat and therefore does not directly translate to extinction learning, which is driven by a 100% safe environment.

Our modelling found reduction in aversive learning rates by losartan, similarly to previous work which found losartan reduce aversive, but not appetitive, learning rates (19,20). We extend this work in several ways. First, trial-by-trial ratings allowed us to directly link the observed behaviour to model estimates. This is important since learning rates can reflect a

variety of cognitive processes. Second, unlike previous studies, we used a Pavlovian conditioning task with primary reinforcers which is believed to underlie the formation of anxiety and stress-related disorders (26,75). While learning rates were generally higher for shocks compared to no-shocks (33,76), 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 specific aversive events.

While the neurobiological mechanisms underlying the observed learning effects are unclear, previous work in animals has shown close interaction between the renin-angiotensin and the dopaminergic (DA) systems (8,23). This connection might be of particular relevance considering the established role of midbrain DA in prediction error processing (21,22). Crucially, recent work in humans reported modulation of reward-related processing by a single dose of losartan in the midbrain dopamine system (24,25). Relatedly, losartan has been shown to reduce encoding of negative, but not positive, memories via reduced hippocampus-amygdala connectivity (18). Alternatively, cognitive and anxiolytic effects of drugs interfering with the RAS receptor might be related to calming effects on the hypothalamic-pituitary-adrenal axis (HPA) (9,16), a neuroendocrine system implicated in PTSD aetiology (77).

These findings provide evidence that angiotensin II receptor blockade may play a role in 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 anxiety disorders or development of PTSD. Future work may investigate whether increased endogenous angiotensin II levels pose an increased risk of prospective anxiety onset, similarly to observations in rodents (78,79). This would inform development of preventative strategies related to anxiety risk.

In this study, there was no appetitive or neutral condition. Therefore, it remains inconclusive whether the reduction in Pavlovian learning is specific to aversive contexts. Previous work identifying an aversion-specific role of losartan employed an instrumental, rather than a Pavlovian, learning task. Second, while the observed behavioural effect persisted on the Follow-up visit, this was not matched by the learning rates (i.e., there was no difference between losartan and placebo). While this indicates that losartan has a prolonged effect on aversive learning, this result was not conclusive. Additionally, 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.

The used probabilistic learning paradigm was designed to identify changes in aversive learning rates. The task has previously been shown to provide reliable learning estimates via computational readouts (78), which was also supported by relatively high ICC scores in our sample. While similar approaches have been fruitful in understanding psychiatric conditions (79,80), recent work has also called for more naturalistic and ecologically valid paradigms (83).

Taken together, we provide behavioural and modelling evidence for reduction of aversive learning by the angiotensin II antagonist losartan. This finding will hopefully contribute to improvements in prevention of development of anxiety and trauma disorders.

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## Disclosures

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. The manuscript will be deposited on publicly accessible preprint server upon submission.

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