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# Reduction of Aversive Learning Rates in Pavlovian Conditioning by Angiotensin II Antagonist Losartan: A Randomized Controlled Trial

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## ABSTRACT

**BACKGROUND:** Angiotensin receptor blockade has been linked to aspects of aversive learning and memory formation and to the prevention of posttraumatic stress disorder symptom development.

**METHODS:** We investigated the influence of the angiotensin receptor blocker losartan on aversive Pavlovian conditioning using a probabilistic learning paradigm. In a double-blind, randomized, 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. During each session, participants engaged in a task in which they had to predict the probability of an electrical stimulation on every trial while the true shock contingencies switched repeatedly 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 during the drug session compared with baseline. The 50-mg drug dose did not induce reduction of blood pressure or change in reaction times, ruling out a general reduction in attention and engagement. Decreased adjustment of aversive expectations was maintained at a follow-up session 24 hours later.

**CONCLUSIONS:** This study shows that losartan acutely reduces Pavlovian learning in aversive environments, thereby highlighting a potential role of the renin-angiotensin system in anxiety development.


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With a lifetime prevalence of 15% to 30%, significant economics costs, and increased depression risk, anxiety disorders represent an impactful mental health problem (1–5). However, little is currently known about the factors that contribute to anxiety onset, even though such knowledge is crucial for the development of 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 etiology of anxiety disorders. The RAS is a neuroendocrine circuit involved in blood pressure regulation. However, its receptors are also expressed in brain regions relevant to anxiety, including the amygdala, midbrain, hippocampus, and prefrontal cortex (6,7), where they interact with other neuroendocrine systems including dopamine (8) or the hypothalamic-pituitary-adrenal axis (9). Increased angiotensin II levels have been reported as a response to stress in rodent models (10). Drugs that block angiotensin II activity, including angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors, have been shown to reduce stress responses, produce anxiolytic effects, and facilitate fear extinction (11–13). In humans, ARBs have been reported to improve symptoms of anxiety in patients with type 2 diabetes (14). Observational data from a large patient cohort indicate that antihypertensive use of angiotensin antagonists such as losartan is linked to reduced traumatic symptoms following a traumatic event (15). Consistent with such clinical effects, we recently showed that a single dose of the ARB losartan prevented a physiological stress response and facilitated contextual processing—two processes known to be relevant to the development of posttraumatic stress disorder (PTSD) (16) during experimental trauma. Similarly, administration of losartan has been associated with reductions in subjective fear during an aversive task (17) and encoding of negative memories (18). Identifying specific aspects of learning (e.g., prediction errors or learning rates) that are affected by RAS-modulating drugs can help us understand the function that they play in the prevention of anxiety development. Two studies have used a learning approach to study the impact of losartan on learning rates (19,20). Both studies reported reduction in loss but not gain





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learning rates following a single dose of losartan. Given the known link between the dopaminergic system and prediction error processing (21,22), this may also suggest RAS modulation of learning via interaction with dopamine (8,23). Recent work with humans has reported modulation reward–related processing by a single dose of losartan in the midbrain dopamine system (20,24).

Such findings point to a prominent role of the RAS in aversive learning. However, the only 2 studies reported to date that have investigated the role of losartan in learning used an instrumental conditioning paradigm. No study has directly investigated the effect of the RAS on Pavlovian threat learning, the key learning mechanism that underlies the development of anxiety disorders (25–27).

Direct evidence for RAS-modulating drug effects 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 using computational models (28–30), we employed a probabilistic learning paradigm to test the effect of the RAS antagonist losartan on aversive learning. In the task, periods of relative threat and safety alternated, going beyond traditional classical conditioning paradigms. Similar tasks have been used to identify learning differences in clinical anxiety (31). Here, we extended previous work by focusing on mechanisms of Pavlovian learning from primary reinforcers. We considered a number of plausible aspects of learning, such as differential learning from shocks and shock omissions (32), context-dependent updating (33), uncertainty-driven learning (34), and biases in probability perception (35).

Building on previous work (19,20), we tested the hypothesis that a single dose of the ARB losartan would lead to acute reduction in learning during aversive Pavlovian learning.

## METHODS AND MATERIALS

Sample size was estimated based on the only 2 related studies reported to date (19,20). A power analysis by simulation was performed using beta likelihood for the main effect of drug at session 2. The estimated sample size was 44 participants (22 per group) at 80% power (alpha = .05) (see the Supplement). Forty-five healthy volunteers (ages 18–39 years) were recruited.

Participants without a history of DSM-5 Axis I disorder (36), who had been free from central nervous system–active medication for at least 6 weeks, had no first-degree family member with a history of a severe psychiatric disorder, and had a body mass index between 18 and 30 were included (full selection criteria are provided in the Supplement). 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: 1 due to technical failure of the equipment and 4 because they failed to dissociate between the stable cues at the follow-up session. This left 20 participants in the losartan group ($n_{female}$ = 6, mean age = 25.5 years) and 20 participants in the placebo group ($n_{female}$ = 10, mean age = 24.1 years). When the power simulations were repeated, the power in the final sample was 77.1%.

The study involved 3 sessions at the Department of Psychiatry at Oxford University. The baseline session (s1) included a medical and psychiatric screening followed by instructions and completion of a short version of the task. The drug session (s2) included completing a battery of psychological questionnaires, with administration of a single dose of losartan or placebo 1 hour before completion of the task. The follow-up session (s3) took place 1 day later to assess any potential next-day effects. Participants also completed a shorter version of the task.

Prior to the drug session, participants were randomly assigned to one of the 2 groups in a double-blind design, receiving either a single 50-mg oral dose of losartan (Cozaar; Merck Sharp & Dohme Limited) or a placebo capsule that was matched to the active drug in appearance (microcrystalline cellulose; Rayotabs, Rayonex GmbH). The randomization sequence was generated by a researcher who was not in direct contact with participants using a random number generator and was based on blocked randomization (blocks of 4) stratifying for gender. Treatments were sealed in sequentially numbered containers and administered to participants according to the randomization sequence. Dosing of losartan was guided by the intention to assess its impact on aversive learning without triggering hypotensive effects, similar to previous studies (16,19,24,37). Robust hypotensive effects occur in humans only after a 3- to 4-week period of daily 50-mg intake rather than following a single administration (38). Even after 2 weeks, no blood pressure changes were found in normotensive individuals (39). To monitor potential confounding effects of losartan on acute changes in blood pressure, heart rate, or mood, we assessed these variables using self-report visual analog scales (range 0–100) and an Omron 705IT sphygmomanometer 1 hour before and just before drug administration. Testing started 1 hour after capsule intake, when drug peak plasma levels are reached (40,41). At the end of the drug session, the participant and the experimenter independently indicated whether they thought that losartan or placebo had been administered during the session.

During the drug session, participants completed a battery of psychological questionnaires assessing personality traits, anxiety, depression, and attention regulation strategies [State-Trait Anxiety Inventory (42), Beck Depression Inventory (43), Attentional Control Scale (44), and National Adult Reading Test (45)].

Electrical stimuli were applied using an electric stimulation device (Digitimer DS7A) that delivered 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 10 minutes approximately to the 8/10 level, ranging from a 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 (46).

A 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. During each trial, participants were presented with one of 3 visual cues (abstract fractals, randomized) and asked to provide a subjective shock probability rating (0–100% scale) within 4 seconds. After an

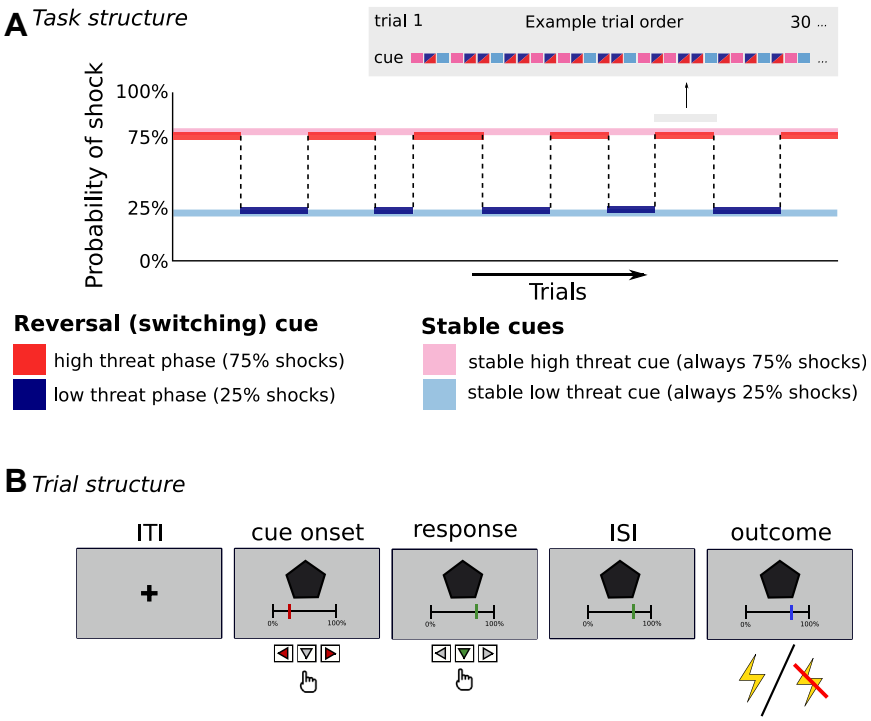


**Figure 1.** **(A)** Task structure. Three cues were presented sequentially (example in gray box). Two cues had a fixed objective probability of resulting in shock that was either high (pink) or low (light blue) throughout. The objective shock probability of the main cue changed at semi-regular intervals between phases of high (red) and low (blue) threat. Sessions 1 and 3 included 6 phases on average (short version, ~150 trials) and for session 2 there were 11 phases (long version, ~300 trials). Each participant could start either with a high or low probability of shock; the depicted schedule starts with high shock probability. **(B)** Each trial started with an intertrial interval (ITI) (2 seconds) during which a fixation cross was shown. When the cue appeared on the screen, participants had 4 seconds to submit their shock probability rating on a scale from 0% to 100% using a slider. After a variable interstimulus interval (ISI) (1 second), the outcome was delivered (shock or no shock). The color of the slider changed when a rating was submitted and when the outcome was delivered.

interstimulus interval (1 second), a short electrical impulse was either delivered (shock) or omitted (no shock). One of the cues switched between 75% and 25% chance of shock (high- vs. low-threat phases) every 30 ± 5 trials (reversal cue, presented on 50% of trials). The starting level was randomized. During the remaining trials, one of the 2 control cues was presented: either stable high-threat or stable low-threat cue (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 to 12 minutes for stimuli recalibration and to allow participants to rest. Instructions were delivered on a standardized form.

Reversals between the 2 levels were not signaled. 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 (47), we used a data-driven approach to estimate the point in time 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 (48). The peak/trough of this series represents the point of fastest updating. For each reversal, we labeled this point an estimated switch point (see Supplement).

Participants provided a probability rating (0–100%) on each trial. To investigate the impact of losartan on learning, we focused on the change in ratings compared with baseline. The data were realigned to the estimated switch point, the baseline (3 trials before switch) was subtracted, and the first 5 trials after the switch were excluded because ratings only stabilized after about 5 trials following the reversal (see Figure 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 (49). To assess model fit, the leave-one-out information criterion was computed for each model (50). Posterior samples were estimated using Markov chain Monte Carlo sampling using no-U-turn sampling across 4 chains, with 2000 samples per chain (600 warmup).

Consistent with similar studies (19,28,32), we employed a modeling framework based on reinforcement learning (51). Four models were variations of the Rescorla-Wagner (RW) learning rule (52), and 1 model had an adaptive learning rate (Hybrid RW–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 (equation 1).

$$P_{t+1} = P_t + \alpha(O_t - P_t) \quad (1)$$

The starting value was estimated as a free parameter ($P_1 \in [0,1]$). The first 2 models captured differential learning from shock/no-shock outcomes (Outcome model) or during high-/low-threat phases (Phase model). The third model was a combination of the two, i.e., it had learning rates for all combinations of shock/no shock and high/low threat (Outcome-phase model). Differential learning was specified as separate learning rates for shocks (*sh*) and no shocks (*nosh*): $\alpha_{sh} \in [0,1]$ and $\alpha_{nosh} \in [0,1]$ (see equation 2).

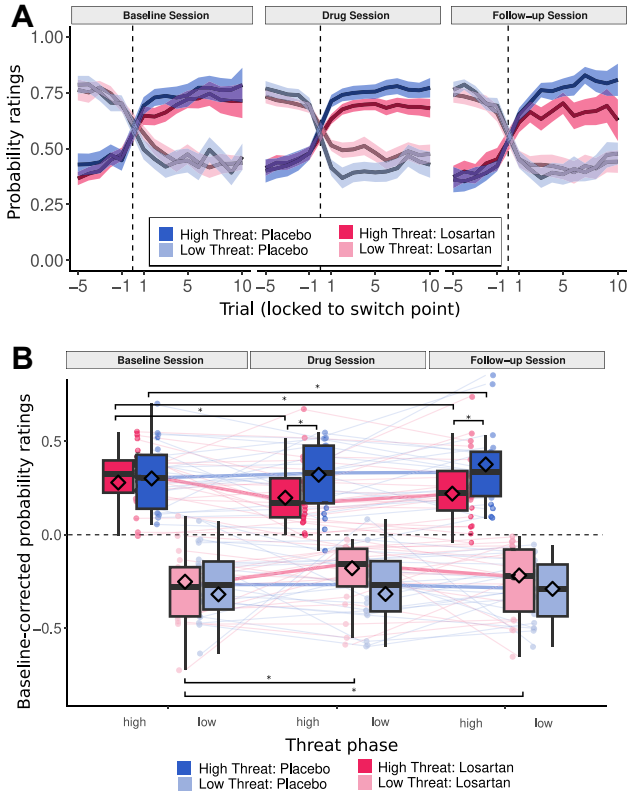


**Figure 2.** **(A)** Shock probability ratings for each trial split by drug group and threat phase. Data were aligned to the estimated switch point. Thick lines show the mean while shaded areas show the standard error of the mean. **(B)** Baseline-corrected probability rating change for each session 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, and the box itself reflects the median ± 1.58 × IQR/sqrt(n), while the whiskers show the range of the data excluding outliers [for additional details, see the default settings of the ggplot2:geom_boxplot() function]. Individual thin lines connect data points for a specific participant across the 3 sessions. Angled rectangles represent predictions of the fitted model. The figure shows data for 40 participants, with 20 in each drug group. *Significant statistical finding at $p < .05$.

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

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

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

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

The final model was a hybrid of the RW and PH models (Hybrid RW-PH) (35,53,54). It dynamically adjusts the learning rate on each trial (equations 4 and 5). The 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 $\kappa \in [0,1]$.

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

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

Data were collected using custom MATLAB 2016 (The MathWorks, Inc.) 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 visual analog scale measures between baseline and drug peak level was assessed using linear mixed-effects models and analysis of variance. All effect size ranges are reported as 95% confidence intervals. Behavioral analyses were also performed using linear mixed-effects models (*lmer*) (55) and analyses of variance (*lmerTest*) (56). Post hoc *t* tests were corrected for multiple comparisons using the Holm (57) correction. Learning rates were analyzed using a generalized beta regression (58) with logit link function (*glmmTMB*) (59). The statistical test was performed using the type II Wald $\chi^2$ test (using the *car* package) (60). Participant ID and starting probability were included as random intercepts in all models. Session and either phase (behavioral model) or outcome type (learning rates model) were included as random slopes to account for within-participant variability. Other R packages used for the analyses were loo (50), performance (61), tidyr (62), plyr (63), parameters (64), dplyr (65), renv (66), rstan (67), ggplot (68), emmeans (69), and effectsize (70).

## RESULTS

The 2 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, or visual analog scale 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% correct; both $\chi^2_1 < 0.98$, $p > .32$), suggesting that double-blindness was maintained throughout the study.

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 distinguish between stable cues during the drug session. There was no group difference in the calibrated shock intensity ($I_{losartan}$ = 1010 mA, $SD_{losartan}$ = 1850, $I_{placebo}$ = 514 mA, $SD_{placebo}$ = 673; $t_{36} = -0.96$, $p = .34$), starting probability ($\chi^2 = 0.13$, $p = .72$), or initial bias ($B_{losartan}$ = 44%, $SD_{losartan}$ = 0.14, $B_{placebo}$ = 53%, $SD_{placebo}$ = 0.22; $t_{32} = -1.60$, $p = .12$). The drug did not impact reaction times during the drug session in relation to the baseline session, $\chi^2 < 2.61$, $p > .27$. The

**Table 1. Sociodemographic, Clinical, and Personality Characteristics in the Losartan and Placebo Groups**

| | Losartan, *n* = 20 | Placebo, *n* = 20 |
|---|---|---|
| Sociodemographic Data | | |
| Gender, female, *n* (%) | 6 (30%) | 10 (50%) |
| First language, English, *n* (%) | 15 (75%) | 17 (85%) |
| Age, years | 25.6 (4.7) | 24.2 (4.3) |
| Verbal intelligence (NART) | 115 (6.9) | 111 (9.9) |
| Education, years | 16.8 (2.6) | 17.4 (2.2) |
| Clinical and Personality Measures | | |
| Trait anxiety (STAI-T) | 34.9 (8.5) | 37.0 (7.28) |
| Depression (BDI) | 4.0 (6.19) | 5.05 (6.46) |
| 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) |

Values are presented as % or mean (SD).

ACS, Attentional Control Scale; BDI, Beck Depression Inventory; NART, National Adult Reading Test; STAI-T, State-Trait Anxiety Inventory-Trait.

ratings for the stable cues did not differ significantly from the true contingencies or 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 $< 1.27$ and $p$s $>.26$.

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

Probability rating changes are shown in Figure 2B. Statistical tests 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%) ($F_{1,38.1} = 166.33$, $p < .001$, $\eta_p^2 = 0.81$ [95% CI, 0.70–0.88]). Furthermore, there was a significant interaction between group, session, and phase ($F_{2,3903} = 49.06$, $p < .001$, $\eta_p^2 = 0.02$ [0.02–0.03]). In the high-threat phase, losartan was found to decrease ratings at s2 ($t_{54.4} = 4.03$, $p = .001$, $\eta_p^2 = 0.23$ [0.06–0.41]) and s3 ($t_{66.5} = 2.93$, $p = .009$, $\eta_p^2 = 0.11$ [0.01–0.27]) compared with the baseline session ($s1_{losartan}$, high: 37.1%; $s2_{losartan}$, high: 25.0%; $s3_{losartan}$, high: 27.9%). In the low-threat phase, losartan was found to increase ratings at s2 ($t_{49.9} = -4.07$, $p = .001$, $\eta_p^2 = 0.25$ [0.07–0.43]) and s3 ($t_{48} = -3.79$, $p = .001$, $\eta_p^2 = 0.23$ [0.06–0.42]) compared with baseline ($s1_{losartan}$, low: −36.8%; $s2_{losartan}$, low: −25.0%; $s3_{losartan}$, low: 25.8%). In the placebo group in the high-threat condition, ratings at s2 were not different from baseline ($t_{50.4} = -1.02$, $p = .314$, $\eta_p^2 = 0.02$ [0.00–0.15]), while ratings at s3 were higher ($t_{64.1} = -2.57$, $p = .038$, $\eta_p^2 = 0.09$ [0.00–0.25]) ($s1_{placebo}$, high: 33.6%; $s2_{placebo}$, high: 36.6%; $s3_{placebo}$, high: 41.6%); in the low-threat phase, neither s2 ($t_{46.2} = 0.39$, $p = .99$, $\eta_p^2 = 0.00$ [0.00–0.10]) nor s3 ($t_{46} = 0.85$, $p = 1.00$, $\eta_p^2 = 0.01$ [0.00–0.11]) differed from baseline ($s1_{placebo}$, low: −30.1%; $s2_{placebo}$, low: −31.2%; $s3_{placebo}$, low: −32.5%). Contrasting these effects between groups, for example ($s1_{losartan} - s2_{losartan}$) − ($s1_{placebo} - s2_{placebo}$), the ratings decrease in the high-threat phase was larger in the losartan group at both s2 ($t_{52.7} = -3.68$, $p = .002$, $\eta_p^2 = 0.20$ [0.05–0.39]) and s3 ($t_{65.1} = -3.91$, $p = .001$, $\eta_p^2 = 0.19$ [0.05–0.35]) compared with placebo. The ratings increase in the low-threat phase was also significant for both s2 ($t_{48.5} = 3.21$, $p = .004$, $\eta_p^2 = 0.18$ [0.03–0.36]) and s3 ($t_{41} = -3.31$, $p = .004$, $\eta_p^2 = 0.19$ [0.03–0.38]) sessions compared with placebo. All percentages in this section correspond to the model-estimated marginal means. These results suggest that unlike placebo, losartan acutely reduced rating adjustment in the

**Table 2. Heart Rate, Blood Pressure, and Visual Analog Scale Ratings in the Losartan and Placebo Groups Before Drug Intake and at Drug Peak Level**

| | Baseline | | Drug Peak | | |
|---|---|---|---|---|---|
| | Losartan | Placebo | Losartan | Placebo | *p* |
| Physiological Measures | | | | | |
| Heart rate, bpm | 75 (12) | 73 (10) | 66 (8) | 66 (8) | .83 |
| Systolic blood pressure, mm Hg | 124 (16) | 125 (14) | 119 (16) | 119 (14) | .83 |
| Diastolic blood pressure, mm Hg | 71 (9) | 74 (10) | 69 (8) | 73 (11) | .70 |
| Visual Analog Ratings (0–100) | | | | | |
| 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 |

Values are presented as mean (SD). The *p* values correspond to the interaction between visit and group.

bpm, beats per minute.

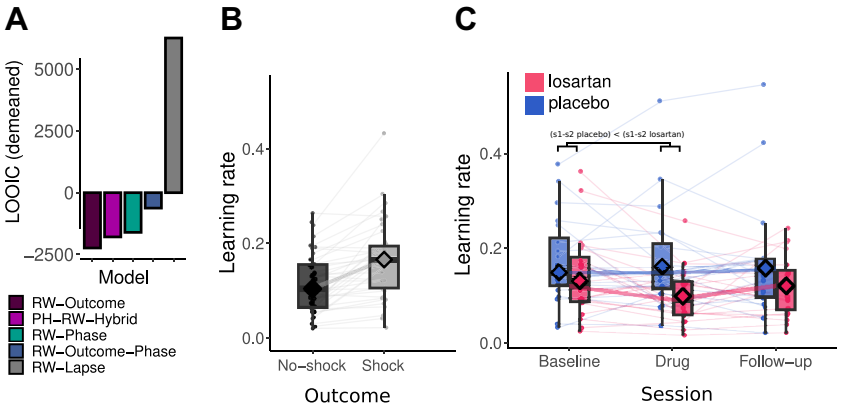


**Figure 3.** **(A)** Model comparison results showing demeaned leave-one-out information criterion (LOOIC) scores for the 5 models; lower values indicate better fit. Statistically significant effects of the model-estimated learning rates: **(B)** learning from shocks was overall faster than learning from no shocks; **(C)** losartan reduced the learning rates for the drug session compared with the baseline session; there was no difference in learning rates in the placebo group. Panels **(B)** and **(C)** contain data for 40 participants, 20 in each drug group. The central line on each summary box represents the median, and the box itself reflects the median ± 1.58 × IQR/sqrt(n), while the whiskers show the range of the data excluding outliers [for additional 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. PH, Pearce-Hall; RW, Rescorla-Wagner.

low-to-high as well as high-to-low threat switches both at acute administration (s2) and one day later (s3).

Model comparison using the leave-one-out information criterion found the Outcome model to fit best. The leave-one-out information criterion scores for the 5 models were 39,460.16 (Outcome model), 39,908.11 (Hybrid RW-PH), 40,092.65 (Phase model), 41,075.22 (Outcome-phase model), and 47,960.11 (Lapse model) (Figure 3A). The model fit ranking was identical for the losartan and placebo groups (see the Supplement). 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.

Focusing on the winning model, we assessed parameter consistency within participants by calculating interclass correlations (ICCs) for $\alpha_{sh}$ and $\alpha_{nosh}$. Specifically, we calculated ICC(A,1), 2-way mixed, single-measure, absolute agreement (71). 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 analyzed together using generalized beta regression and Wald-II test. The model found a significant main effect of outcome: learning from shocks ($\alpha_{sh} = 0.15$) was significantly faster than learning from no shocks ($\alpha_{nosh} = 0.10$) ($\chi^2_1 = 28.78$, $p < .001$, $\eta_p^2 = 0.34$ [0.12–0.54]) (Figure 3B). Furthermore, group was found to interact significantly with session ($\chi^2_2 = 7.45$, $p = .024$, $\eta_p^2 = 0.01$ [0.00–0.16]). There was no change in learning rates in the placebo group ($\alpha_{s1,placebo} = 0.150$, $\alpha_{s2,placebo} = 0.154$, $\alpha_{s3,placebo} = 0.146$) (s1 > s2: $t_{216} = -0.222$, $p = 1.00$, $\eta_p^2 = 0.00$ [0.00–0.02]; s1 > s3: $t_{216} = 0.274$, $p = 1.00$, $\eta_p^2 = 0.00$ [0.00–0.02]; s2 > s3: $t_{216} = 0.61$, $p = 1.00$, $\eta_p^2 = 0.00$ [0.00–0.03]). In the losartan group, learning rates were significantly lower during the drug (s2) compared with the baseline session (s1) ($\alpha_{s1,losartan} = 0.120$, $\alpha_{s2,losartan} = 0.085$, $\alpha_{s3,losartan} = 0.106$) (s1 > s2: $t_{216} = 2.90$, $p = .012$, $\eta_p^2 = 0.04$ [0.00–0.10]). There was no difference between s1 and s3 (s1 > s3: $t_{216} = 0.97$, $p = .34$, $\eta_p^2 = 0.00$ [0.00–0.04]) or s2 and s3 ($t_{216} = -1.93$, $p = .010$, $\eta_p^2 = 0.02$ [0.00–0.07]) (Figure 3C). Next, we tested whether the reduction in learning rates was significantly different between losartan and placebo by investigating the contrast of contrasts, e.g., for s1 and s2: $(\alpha_{s2,losartan} - \alpha_{s1,losartan}) - (\alpha_{s2,placebo} - \alpha_{s1,placebo})$. This analysis revealed that the between-session reduction in learning rate was larger in the losartan group than the placebo group for the drug session ($t_{216} = 2.29$, $p = .046$, $\eta_p^2 = 0.02$ [0.00–0.08]) but not for the follow-up session ($t_{216} = 0.51$, $p = .614$, $\eta_p^2 = 0.00$ [0.00–0.03]).

Lastly, we examined correlations between learning rates and rating adjustment. Model-estimated learning rates were correlated with probability adjustment both in high-to-low ($r_{38} = -0.40$, $p = .04$) and low-to-high ($r_{38} = 0.53$, $p = .003$) conditions.

## DISCUSSION

Our findings show that a 50-mg 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 overprediction of threat remains; however, it is no longer supported by a between-group difference in learning rates. These results suggest a role of losartan in the development of fear-related associations via a reduction in aversive learning rates. While this mechanism may play a role in the development of anxiety and PTSD, we also note potential implications for reduction in extinction learning.

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 1 day later, while there were no differences during a baseline visit. We showed that this decrease in overall learning was driven by a reduction in aversive learning rates. Such a global reduction in threat learning may be one of the mechanisms underlying reduced PTSD symptom development, which has previously been associated with ARB intake (15,72), 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 may include reduced safety learning. This raises the question of whether these types of drugs may also impair extinction in a clinical context. While the paradigm used was not designed to answer this question, previous studies conducted with rodents and humans support an overall augmentative role of losartan on fear extinction (11–13).

Our modeling found reduction in aversive learning rates by losartan, similar to previous work that found that losartan reduced aversive, but not appetitive, learning rates (19,20). We extended this work in several ways. First, trial-by-trial ratings allowed us to directly link the observed behavior to model estimates. This is important because learning rates can reflect a variety of cognitive processes. Second, unlike previous studies, we used a Pavlovian conditioning task with primary reinforcers that are believed to underlie the formation of anxiety and stress-related disorders (26,73). While learning rates were generally higher for shocks than no shocks (33,74), 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 that underlie the observed learning effects are unclear, previous work in animals has shown close interaction between the RAS and the dopaminergic system (8,23). Dopaminergic cells express angiotensin type I and II receptors across a range of regions (75) including the striatum and substantia nigra (76,77), regions closely associated with learning and PE processing (21,22). Activation of angiotensin type I receptors was shown to lead to release of dopamine, which was inhibited by angiotensin type I receptor blockade (8). Furthermore, angiotensin type I receptors were found in striatal projection neurons, suggesting an additional indirect modulatory role of angiotensin in dopaminergic transmission (78). Recent work with humans reported increased reward-related processing in the midbrain dopamine system after a single dose of losartan (20,24). There is some evidence that the observed effects on the dopaminergic system are not due to losartan per se but instead arise due to its active metabolite (EXP 3174) (79), which should be considered in future work. Adding further to the discussion about potential underlying mechanistic pathways of the effects reported here, losartan has also been shown to reduce encoding of negative, but not positive, memories via reduced hippocampus-amygdala connectivity (18). Furthermore, cognitive and anxiolytic effects of drugs that interfere with the RAS receptor may be related to calming effects on the hypothalamic-pituitary-adrenal axis (9,16), a neuroendocrine system implicated in PTSD etiology (80).

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 that examine 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, similar to observations in rodents (81,82). This would inform the development of preventive 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 that identified an aversion-specific role of losartan employed an instrumental, rather than a Pavlovian, learning task. Second, while the observed behavioral effect persisted at 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 for assessing preventive effects of losartan on the formation of aversive associations.

The probabilistic learning paradigm that was used was designed to identify changes in aversive learning rates. The task has previously been shown to provide reliable learning estimates via computational readouts (77), which was also supported by relatively high ICC scores in our sample. While similar approaches have been fruitful in understanding psychiatric conditions (78,79), recent work has also called for more naturalistic and ecologically valid paradigms (83).

## Conclusions

Taken together, our results provide behavioral and modeling evidence for reduction of aversive learning by the angiotensin II antagonist losartan. Hopefully, this finding will contribute to improvements in prevention of the development of anxiety and trauma disorders.

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OZ, KW, and AR were responsible for study conceptualization; OZ, JA, CK, and LS were responsible for data curation; OZ was responsible for formal analysis; AR was responsible for funding acquisition; AR, OZ, JA, CK, LS, and MB were responsible for investigation; AR, OZ, and KW developed the methodology; OZ, JA, LS, AR, and CK were responsible for project administration; KW and AR were responsible for resources; OZ was responsible for software; AR and KW supervised the work; and OZ was responsible for validation and visualization. All authors were responsible for writing the original draft, reviewing, and editing the manuscript.

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