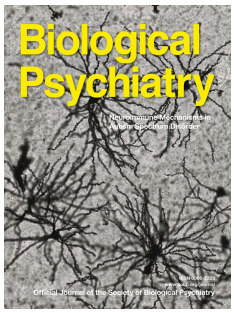


Reduction of aversive learning rates in Pavlovian conditioning by angiotensin II antagonist losartan: a randomised controlled trial

Ondrej Zika, Judith Appel, Corinna Klinge, Lorika Shkreli, Michael Browning, Katja Wiech, Andrea Reinecke

PII: S0006-3223(24)00063-5

DOI: https://doi.org/10.1016/j.biopsych.2024.01.020

Reference: BPS 15412

To appear in: *Biological Psychiatry*

Received Date: 16 May 2023

Revised Date: 12 January 2024

Accepted Date: 22 January 2024

Please cite this article as: Zika O., Appel J., Klinge C., Shkreli L., Browning M., Wiech K. & Reinecke A., Reduction of aversive learning rates in Pavlovian conditioning by angiotensin II antagonist losartan: a randomised controlled trial, *Biological Psychiatry* (2024), doi: https://doi.org/10.1016/j.biopsych.2024.01.020.

4063 words / 2 tables / 3 figures

Title: Reduction of aversive learning rates in Pavlovian conditioning by angiotensin II antagonist losartan: a randomised controlled trial

Running title: Losartan and Pavlovian aversive learning

Authors: Ondrej Zika[1], Judith Appel[2], Corinna Klinge[3], Lorika Shkreli[3], Michael Browning[3,4], Katja Wiech[5], Andrea Reinecke[3,4]

Affiliations:
[1] Max Planck Institute for Human Development, Lentzeallee 94, 14195, Berlin, Germany
[2] Behavioural Science Institute, Radboud University Nijmegen, the Netherlands
[3] Department of Psychiatry, University of Oxford, UK
[4] Oxford Health NHS Trust, Warneford Hospital, Oxford, UK
[5] Wellcome Centre for Integrative Functional Neuroimaging, Nuffield Department of Clinical Neurosciences, University of Oxford, UK

Corresponding author:
Andrea Reinecke, University of Oxford, Department of Psychiatry, Warneford Hospital, Oxford OX37JX, UK, e-mail: andrea.reinecke@psych.ox.ac.uk, phone: +44 1865 618320.

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

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, highlighting a potential role of the renin-angiotensin system in anxiety development.

The study was registered on OSF (osf.io/e3zrk).

Main text

Introduction

With a life-time prevalence of 15-30%, 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 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 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 (8) or the 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, ARBs 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 angiotensin antagonists such as losartan is linked to reduced traumatic symptoms following 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 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 losartan on learning rates (19,20). 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 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 using computational models (29–31), we employ a probabilistic learning paradigm to test the effect of RAS antagonist losartan on aversive learning. 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 the only two related studies (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^2 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. Repeating the power simulations, 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 task. Follow-up session took place one 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 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). The randomization sequence was generated by a researcher not in direct contact with participants using a random number generator and was based on blocked randomisation (blocks of four), stratifying for gender.

Treatments were sealed in sequentially numbered containers and administered to participants according to the randomisation sequence. Dosing of losartan was guided by the intention to assess its impact on aversive learning without triggering hypotensive effects, similar to previous studies (16,19,24,38). 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 (0-100% 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.

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)

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 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 α 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: $\alpha_{\text{sh}} \in [0,1]$ and $\alpha_{nosh} \in [0,1]$ (Eq. 2).

Eq. 2 $P_{t+1} = P_t + \alpha_{sh}(O_t - P_t)$ if shock ($O_t = 1$), otherwise α_{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 ξ. Here,

the probability prediction for next trial is a mixture of updated previous prediction and random rating. See Eq. 3 (Lapse model).

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

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 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 $\kappa \in [0,1]$.

Eq. 4 $$P_{t+1} = P_t + \alpha_{\boldsymbol{t}}(O_t - P_t)$$

Eq. 5 $$\alpha_{t+1} = \kappa[\eta_{sh}|PE_t| + (1 - \eta_{sh})\alpha_t]$$ if shock, otherwise η_{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 (LMMs) and ANOVAa. Behavioural analyses were performed using LMMs (*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 χ^2 < 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 dissociated 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)=-.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. The drug did not impact reaction times during the Drug session in relation to baseline session, χ^2 < 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 Fs<1.27, ps>.26.

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.

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 ($s1_{losartan,\ high}$: 37.1%, $s2_{losartan,\ high}$: 25.0%, $s3_{losartan,\ high}$: 27.9%). In the low-threat phase losartan was found to increase ratings 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 ($s1_{losartan,\ low}$: -36.8%, $s2_{losartan,\ low}$: -25.0%, $s3_{losartan,\ low}$: 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] ($s1_{placebo,\ high}$: 33.6%, $s2_{placebo,\ high}$: 36.6%, , $s3_{placebo,\ high}$: 41.6%), in the low-threat phase neither s2, $(46.2)=.39$, $p=1.00$, $\eta^2_p=.00$ [.00 .10], nor s3, $(46)=.85$, $p=1.00$, $\eta^2_p=.01$ [.00 .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 ($s1_{los}$-$s2_{los}$) - ($s1_{placebo}$-$s2_{placebo}$), 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. sessions2s3These 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.

Focusing on the winning model, we assessed parameter consistency within participants, we calculated inter-class correlations (ICC) for α_{sh} and α_{nosh}. 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 (α_{sh}=0.15) was significant faster than learning from no-shocks (α_{nosh}=0.10), $\chi^2(1)$=28.78, p<.001, η^2_p=.34 [.12 .54] (Fig. 3b). Furthermore, group was found to significantly interact with session, $\chi^2(2)$ =7.45, p=.024, η^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, η^2_p=.00 [.00 .02]., $t_{s1>s3}(216)$=.274,p=1.00, η^2_p=.00 [.00 .02], $t_{s2>s3}(216)$=.61, p=1.00, η^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, η^2_p=.04 [.00 .10]. There was no difference between s1 and s3, $t_{s1>s3}(216)$=.97, p=.34, η^2_p=.00 [.00 .04], or s2 and s3, t(216)=-1.93, p=.010, η^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: $(\alpha_{s2,\mathrm{los}} - \alpha_{s1,los}) - (\alpha_{s2,plac} - \alpha_{s1,plac})$. 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, η^2_p=.02 [.00 .08] but not the follow-up sessions, t(216)=.51, p=.614, η^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 role of losartan in the development of fear-related associations via a reduction in aversive learning rates. While this mechanism might 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 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, one might

wonder whether these types of drugs might also impair extinction in a clinical context. While the used paradigm was not designed to answer this question, previous studies in rodents and humans support an overall augmentative role of losartan on fear extinction(11–13).

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 (26,74). While learning rates were generally higher for shocks compared to no-shocks (33,75), 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). Dopaminergic cells express angiotensin type 1 and 2 (AT1 and AT2) receptors across a range of regions (76) including the striatum and substantia nigra (77,78), regions closely associated with learning and prediction error processing (21,22). Activation of AT1 receptors was shown to lead to release of dopamine, which was inhibited by AT1R receptor blockade (8). Further, AT1R were found in striatal projection neurons, suggesting an additional indirect modulatory role of angiotensin in dopaminergic transmission (79). Recent work in humans reported increased reward-related processing by a single dose of losartan in the midbrain dopamine system (24,25). 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)(80), which should be considered by future work. Adding further to the discussion on 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). Further, 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 (81).

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 (82,83). 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 (87).

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.

Acknowledgements

This study was funded by a fellowship from MQ: Transforming Mental Health (MQ14F192) awarded to AR and supported by the NIHR Oxford Health Biomedical Research Centre. OZ was funded by scholarships from the Medical Research Council, UK and The Engineering and Physical Sciences Research, Council, UK. KW was supported by an MRC UK New Investigator grant (MR/L011719/1) and the National Institute for Health and Care Research (NIHR) Oxford Health Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. MB was supported by the Oxford Health NIHR Biomedical Research Centre and Oxford Health Clinical Research Facility. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

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.

Disclosures

M.B. has received travel expenses from Lundbeck for attending conferences and has acted as a consultant for J&J, Novartis and CHDR. All other authors report no biomedical financial interests or potential conflicts of interest.

The manuscript will be deposited on publicly accessible preprint server: URL.

References

1. Alonso J, Lépine JP, ESEMeD/MHEDEA 2000 Scientific Committee. Overview of key data from the European Study of the Epidemiology of Mental Disorders (ESEMeD). J Clin Psychiatry. 2007;68 Suppl 2:3–9.

2. Kessler RC. The Costs of Depression. Psychiatr Clin North Am. 2012 Mar;35(1):1–14.

3. Kessler RC, Ruscio AM, Shear K, Wittchen HU. Epidemiology of Anxiety Disorders. In: Stein MB, Steckler T, editors. Behavioral Neurobiology of Anxiety and Its Treatment [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009 [cited 2023 Jan 28]. p. 21–35. (Current Topics in Behavioral Neurosciences; vol. 2). Available from: http://link.springer.com/10.1007/7854_2009_9

4. Andlin-Sobocki P, Rehm J. Cost of addiction in Europe. Eur J Neurol. 2005 Jun;12(s1):28–33.

5. Meier SM, Petersen L, Mattheisen M, Mors O, Mortensen PB, Laursen TM. Secondary depression in severe anxiety disorders: a population-based cohort study in Denmark. Lancet Psychiatry. 2015 Jun;2(6):515–23.

6. Jackson L, Eldahshan W, Fagan S, Ergul A. Within the Brain: The Renin Angiotensin System. Int J Mol Sci. 2018 Mar 15;19(3):876.

7. von Bohlen und Halbach O, Albrecht D. The CNS renin-angiotensin system. Cell Tissue Res. 2006 Nov;326(2):599–616.

8. Narayanaswami V, Somkuwar SS, Horton DB, Cassis LA, Dwoskin LP. Angiotensin AT1 and AT2 receptor antagonists modulate nicotine-evoked [3H]dopamine and [3H]norepinephrine release. Biochem Pharmacol. 2013 Sep;86(5):656–65.

9. Balthazar L, Lages YVM, Romano VC, Landeira-Fernandez J, Krahe TE. The association between the renin-angiotensin system and the hypothalamic-pituitary-adrenal axis in anxiety disorders: A systematic review of animal studies. Psychoneuroendocrinology. 2021 Oct;132:105354.

10. Kosunen KJ, Pakarinen AJ, Kuoppasalmi K, Adlercreutz H. Plasma renin activity, angiotensin II, and aldosterone during intense heat stress. J Appl Physiol. 1976 Sep 1;41(3):323–7.

11. Marvar PJ, Goodman J, Fuchs S, Choi DC, Banerjee S, Ressler KJ. Angiotensin Type 1 Receptor Inhibition Enhances the Extinction of Fear Memory. Biol Psychiatry. 2014 Jun;75(11):864–72.

12. Pavel J, Benicky J, Murakami Y, Sanchez-Lemus E, Saavedra JM. Peripherally Administered Angiotensin II AT $_1$ Receptor Antagonists Are Anti-stress Compounds *in Vivo*. Ann N Y Acad Sci. 2008 Dec;1148(1):360–6.

13. Ranjbar H, Aghaei I, Moosazadeh M, Shabani M. Angiotensin II type 1 receptor blocker losartan attenuates locomotor, anxiety-like behavior and passive avoidance learning deficits in a sub-chronic stress model. Iran J Basic Med Sci [Internet]. 2018 Jun [cited 2023 Feb 13];(Online First). Available from: https://doi.org/10.22038/ijbms.2018.27113.6632

14. Pavlatou MG, Mastorakos G, Lekakis I, Liatis S, Vamvakou G, Zoumakis E, et al. Chronic administration of an angiotensin II receptor antagonist resets the hypothalamic–pituitary–adrenal (HPA) axis and improves the affect of patients with diabetes mellitus type 2: Preliminary results: Research Report. Stress. 2008 Jan;11(1):62–72.

15. Khoury NM, Marvar PJ, Gillespie CF, Wingo A, Schwartz A, Bradley B, et al. The Renin-Angiotensin Pathway in Posttraumatic Stress Disorder: Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers Are Associated With Fewer Traumatic Stress Symptoms. J Clin Psychiatry. 2012 Jun 15;73(06):849–55.

16. Shkreli L, Woud ML, Ramsbottom R, Rupietta AE, Waldhauser GT, Kumsta R, et al. Angiotensin involvement in trauma processing—exploring candidate neurocognitive mechanisms of preventing post-traumatic stress symptoms. Neuropsychopharmacology. 2020 Feb;45(3):507–14.

17. Zhang R, Zhao W, Qi Z, Xu T, Zhou F, Becker B. Angiotensin II regulates the neural expression of subjective fear in humans. 2022;20.

18. Xu T, Zhou X, Jiao G, Zeng Y, Zhao W, Li J, et al. Angiotensin Antagonist Inhibits Preferential Negative Memory Encoding via Decreasing Hippocampus Activation and Its Coupling With the Amygdala. Biol Psychiatry Cogn Neurosci Neuroimaging. 2022 Oct;7(10):970–8.

19. Pulcu E, Shkreli L, Holst CG, Woud ML, Craske MG, Browning M, et al. The Effects of the Angiotensin II Receptor Antagonist Losartan on Appetitive Versus Aversive Learning: A Randomized Controlled Trial. Biol Psychiatry. 2019 Sep;86(5):397–404.

20. Xu T, Zhou X, Kanen JW, Wang L, Li J, Chen Z, et al. Angiotensin blockade enhances motivational reward learning via enhancing striatal prediction error signaling and frontostriatal communication. Mol Psychiatry [Internet]. 2023 Feb 21 [cited 2023 Mar 29]; Available from: https://www.nature.com/articles/s41380-023-02001-6

21. Glimcher PW. Understanding dopamine and reinforcement learning: The dopamine reward prediction error hypothesis. Proc Natl Acad Sci. 2011 Sep 13;108(supplement_3):15647–54.

22. Schultz W. Dopamine reward prediction error coding. Dialogues Clin Neurosci. 2016 Mar 31;18(1):23–32.

23. Li D, Scott L, Crambert S, Zelenin S, Eklöf AC, Di Ciano L, et al. Binding of Losartan to Angiotensin AT1 Receptors Increases Dopamine D1 Receptor Activation. J Am Soc Nephrol. 2012 Mar;23(3):421–8.

24. Zhou X, Xu T, Zeng Y, Zhang R, Qi Z, Zhao W, et al. The Angiotensin Antagonist Losartan Modulates Social Reward Motivation and Punishment Sensitivity via Modulating Midbrain-Striato-Frontal Circuits. J Neurosci. 2023 Jan 18;43(3):472–83.

25. Xu T, Zhou X, Kanen JW, Wang L, Li J, Chen Z, et al. Angiotensin blockade enhances motivational reward learning via enhancing striatal prediction error signaling and frontostriatal communication. Mol Psychiatry [Internet]. 2023 Feb 21 [cited 2023 Apr 14]; Available from: https://www.nature.com/articles/s41380-023-02001-6

26. VanElzakker MB, Kathryn Dahlgren M, Caroline Davis F, Dubois S, Shin LM. From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. Neurobiol Learn Mem. 2014 Sep;113:3–18.

27. Wolpe J, Rowan VC. Panic disorder: A product of classical conditioning. Behav Res Ther. 1988;26(6):441–50.

28. Beckers T, Hermans D, Lange I, Luyten L, Scheveneels S, Vervliet B. Understanding clinical fear and anxiety through the lens of human fear conditioning. Nat Rev Psychol. 2023 Feb 16;2(4):233–45.

29. Browning M, Behrens TE, Jocham G, O'Reilly JX, Bishop SJ. Anxious individuals have difficulty learning the causal statistics of aversive environments. Nat Neurosci. 2015 Apr;18(4):590–6.

30. Pike AC, Robinson OJ. Reinforcement Learning in Patients With Mood and Anxiety Disorders vs Control Individuals: A Systematic Review and Meta-analysis. JAMA Psychiatry. 2022 Apr 1;79(4):313.

31. Deserno L, Boehme R, Mathys C, Katthagen T, Kaminski J, Stephan KE, et al. Volatility estimates increase choice switching and relate to prefrontal activity in schizophrenia [Internet]. Neuroscience; 2017 Dec [cited 2021 Feb 28]. Available from: http://biorxiv.org/lookup/doi/10.1101/227967

32. Aylward J, Valton V, Ahn WY, Bond RL, Dayan P, Roiser JP, et al. Altered learning under uncertainty in unmedicated mood and anxiety disorders. Nat Hum Behav. 2019 Jun 17;3(10):1116–23.

33. Jepma M, Koban L, van Doorn J, Jones M, Wager TD. Behavioural and neural evidence for self-reinforcing expectancy effects on pain. Nat Hum Behav. 2018 Nov;2(11):838–55.

34. Zika O, Wiech K, Reinecke A, Browning M, Schuck NW. Trait anxiety is associated with hidden state inference during aversive reversal learning. Nat Commun. 2023 Jul 14;14(1):4203.

35. Li J, Schiller D, Schoenbaum G, Phelps EA, Daw ND. Differential roles of human striatum and amygdala in associative learning. Nat Neurosci. 2011 Oct;14(10):1250–2.

36. Gershman SJ, Guitart-Masip M, Cavanagh JF. Neural signatures of arbitration between Pavlovian and instrumental action selection. Marinazzo D, editor. PLOS Comput Biol. 2021 Feb 10;17(2):e1008553.

37. First M, Williams J, Karg R, Spitzer R. tructured Clinical Interview for DSM-5 Disorders, Clinician Version (SCID-5-CV). Arlington: American Psychiatric Association; 2016.

38. Reinecke A, Browning M, Klein Breteler J, Kappelmann N, Ressler KJ, Harmer CJ, et al. Angiotensin Regulation of Amygdala Response to Threat in High-Trait-Anxiety Individuals. Biol Psychiatry Cogn Neurosci Neuroimaging. 2018 Oct;3(10):826–35.

39. Tsunoda K, Abe K, Hagino T, Omata K, Misawa S, Imai Y, et al. Hypotensive Effect of Losartan, a Nonpeptide Angiotensin II Receptor Antagonist, in Essential Hypertension. Am J Hypertens. 1993 Jan;6(1):28–32.

40. Webb NJA, Lam C, Loeys T, Shahinfar S, Strehlau J, Wells TG, et al. Randomized, Double-Blind, Controlled Study of Losartan in Children with Proteinuria. Clin J Am Soc Nephrol. 2010 Mar;5(3):417–24.

41. Lo MW, Goldberg MR, McCrea JB, Lu H, Furtek CI, Bjornsson TD. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. Clin Pharmacol Ther. 1995 Dec;58(6):641–9.

42. Ohtawa M, Takayama F, Saitoh K, Yoshinaga T, Nakashima M. Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans. Br J Clin Pharmacol. 1993 Mar;35(3):290–7.

43. Spielberger CD. State-Trait Anxiety Inventory for Adults [Internet]. American Psychological Association; 2012 [cited 2021 Jan 15]. Available from: http://doi.apa.org/getdoi.cfm?doi=10.1037/t06496-000

44. Beck AT, Steer RA, Brown GK. BDI-II, Beck depression inventory: manual. 2nd ed. San Antonio, Tex. : Boston: Psychological Corp. ; Harcourt Brace; 1996. 38 p.

45. Derryberry D, Reed MA. Anxiety-related attentional biases and their regulation by attentional control. J Abnorm Psychol. 2002;111(2):225–36.

46. Nelson HE. National Adult Reading Test (NART): For the assessment of premorbid intelligence in patients with dementia: Test manual. Windsor: NFER-Nelson.; 1982.

47. Ploner M, Lee MC, Wiech K, Bingel U, Tracey I. Prestimulus functional connectivity determines pain perception in humans. Proc Natl Acad Sci. 2010 Jan 5;107(1):355–60.

48. Haider H, Frensch PA. Why aggregated learning follows the power law of practice when individual learning does not: Comment on Rickard (1997, 1999), Delaney et al. (1998), and Palmeri (1999). J Exp Psychol Learn Mem Cogn. 2002;28(2):392–406.

49. Page ES. CONTINUOUS INSPECTION SCHEMES. Biometrika. 1954;41(1–2):100–15.

50. Carpenter B, Gelman A, Hoffman MD, Lee D, Goodrich B, Betancourt M, et al. *Stan* : A Probabilistic Programming Language. J Stat Softw [Internet]. 2017 [cited 2023 Oct 28];76(1). Available from: http://www.jstatsoft.org/v76/i01/

51. Vehtari A, Gelman A, Gabry J. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Stat Comput. 2017 Sep;27(5):1413–32.

52. Sutton RS, Barto AG. Reinforcement learning: an introduction. Second edition. Cambridge, Massachusetts: The MIT Press; 2018. 526 p. (Adaptive computation and machine learning series).

53. Rescorla RA, Wagner AR. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Classical Conditioning II: Current Research and Theory. Appleton-Century-Crofts, New York, NY; 1972. p. 64–99.

54. Norbury A, Robbins TW, Seymour B. Value generalization in human avoidance learning. eLife. 2018 May 8;7:e34779.

55. Tzovara A, Korn CW, Bach DR. Human Pavlovian fear conditioning conforms to probabilistic learning. Gershman SJ, editor. PLOS Comput Biol. 2018 Aug 31;14(8):e1006243.

56. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using **lme4**. J Stat Softw [Internet]. 2015 [cited 2022 Mar 7];67(1). Available from: http://www.jstatsoft.org/v67/i01/

57. Kuznetsova A, Brockhoff PB, Christensen RHB. **lmerTest** Package: Tests in Linear Mixed Effects Models. J Stat Softw [Internet]. 2017 [cited 2023 Oct 27];82(13). Available from: http://www.jstatsoft.org/v82/i13/

58. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. 1979. 1979;2(6):65–70.

59. Ferrari S, Cribari-Neto F. Beta Regression for Modelling Rates and Proportions. J Appl Stat. 2004 Aug;31(7):799–815.

60. Brooks M E, Kristensen K, Benthem K J ,van, Magnusson A, Berg C W, Nielsen A, et al. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. R J. 2017;9(2):378.

61. Fox J, Weisberg S. An R companion to applied regression. Third edition. Los Angeles: SAGE; 2019. 577 p.

62. Lüdecke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. J Open Source Softw. 2021 Apr 21;6(60):3139.

63. Wickham H, Henry L. tidyr: Tidy messy data. R package version 1.1.4. 2020 [Internet]. Available from: https://CRAN.R-project.org/package=tidyr

64. Wickham H. The Split-Apply-Combine Strategy for Data Analysis. J Stat Softw [Internet]. 2011 [cited 2023 Oct 27];40(1). Available from: http://www.jstatsoft.org/v40/i01/

65. Lüdecke D, Ben-Shachar M, Patil I, Makowski D. Extracting, Computing and Exploring the Parameters of Statistical Models using R. J Open Source Softw. 2020 Sep 9;5(53):2445.

66. Wickham H, Francois R, Henry L, Müller K, Vaughan D. dplyr: A Grammar of Data Manipulation. 2023 [Internet]. Available from: https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr.

67. Ushey K, Wickham H. renv: Project Environments. Available from: https://rstudio.github.io/renv/, https://github.com/rstudio/renv.

68. Stan Development Team. RStan: the R interface to Stan. [Internet]. 2023. Available from: https://mc-stan.org/

69. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2nd ed. 2016. Cham: Springer International Publishing : Imprint: Springer; 2016. 1 p. (Use R!).

70. Lenth R. Estimated marginal means, aka least-squares means [Internet]. 2020. Available from: https://cran.r-project.org/package=emmeans, https://github.com/rvlenth/emmeans

71. Ben-Shachar M, Lüdecke D, Makowski D. effectsize: Estimation of Effect Size Indices and Standardized Parameters. J Open Source Softw. 2020 Dec 23;5(56):2815.

72. McGraw KO, Wong SP. Forming inferences about some intraclass correlation coefficients. Psychol Methods. 1996 Mar;1(1):30–46.

73. Seligowski AV, Duffy LA, Merker JB, Michopoulos V, Gillespie CF, Marvar PJ, et al. The renin–angiotensin system in PTSD: a replication and extension. Neuropsychopharmacology. 2021 Mar;46(4):750–5.

74. Fanselow MS, Sterlace SR. Pavlovian Fear Conditioning. In: McSweeney FK, Murphy ES, editors. The Wiley Blackwell Handbook of Operant and Classical Conditioning [Internet]. Oxford, UK: John Wiley & Sons, Ltd; 2014 [cited 2023 Jan 29]. p. 117–41. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781118468135.ch6

75. Cazé RD, van der Meer MAA. Adaptive properties of differential learning rates for positive and negative outcomes. Biol Cybern. 2013 Dec;107(6):711–9.

76. Saavedra JM. Beneficial effects of Angiotensin II receptor blockers in brain disorders. Pharmacol Res. 2017 Nov;125:91–103.

77. Zhao HR, Jiang T, Tian YY, Gao Q, Li Z, Pan Y, et al. Angiotensin II Triggers Apoptosis Via Enhancement of NADPH Oxidase-Dependent Oxidative Stress in a Dopaminergic Neuronal Cell Line. Neurochem Res. 2015 Apr;40(4):854–63.

78. Martínez-Pinilla E, Rodríguez-Pérez AI, Navarro G, Aguinaga D, Moreno E, Lanciego JL, et al. Dopamine D2 and angiotensin II type 1 receptors form functional heteromers in rat striatum. Biochem Pharmacol. 2015 Jul;96(2):131–42.

79. Garrido-Gil P, Rodriguez-Perez AI, Fernandez-Rodriguez P, Lanciego JL, Labandeira-Garcia JL. Expression of angiotensinogen and receptors for angiotensin and prorenin in the rat and monkey striatal neurons and glial cells. Brain Struct Funct. 2017 Aug;222(6):2559–71.

80. Pandi V, Nagappa AN, Thakurdesai P. Effect of Losartan Potassium on Central Dopaminergic System in Mice. Journal of Pharmacology and Toxicology. 2007;2(6):551–8.

81. Dunlop BW, Wong A. The hypothalamic-pituitary-adrenal axis in PTSD: Pathophysiology and treatment interventions. Prog Neuropsychopharmacol Biol Psychiatry. 2019 Mar;89:361–79.

82. Duchemin S, Belanger E, Wu R, Ferland G, Girouard H. Chronic perfusion of angiotensin II causes cognitive dysfunctions and anxiety in mice. Physiol Behav. 2013 Jan;109:63–8.

83. Gao N, Wang H, Xu X, Yang Z, Zhang T. Angiotensin II induces cognitive decline and anxiety-like behavior via disturbing pattern of theta-gamma oscillations. Brain Res Bull. 2021 Sep;174:84–91.

84. Waltmann M, Schlagenhauf F, Deserno L. Sufficient reliability of the behavioral and computational readouts of a probabilistic reversal learning task. Behav Res Methods. 2022 Feb 15;54(6):2993–3014.

85. Brown VM, Zhu L, Wang JM, Frueh BC, King-Casas B, Chiu PH. Associability-modulated loss learning is increased in posttraumatic stress disorder. eLife. 2018 Jan 9;7:e30150.

86. Donegan KR, Brown VM, Price R, Pringle A, Gallagher E, Hanlon A, et al. Using smartphones to optimise and scale-up the assessment of model-based planning [Internet]. PsyArXiv; 2023 Feb [cited 2023 Oct 29]. Available from: https://osf.io/hpm4s

87. Wise T, Emery K, Radulescu A. Naturalistic reinforcement learning. Trends Cogn Sci. 2023 Sep;S1364661323002127.

Figure legends

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.

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 +/- 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). 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.

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 +/- 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). 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.

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*
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.

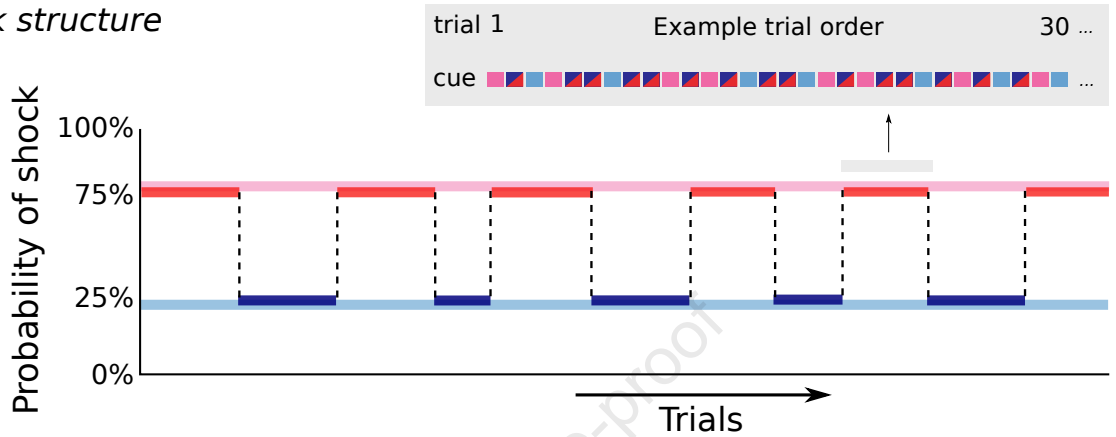
Table 2 *Heart rate, blood pressure and visual analogue scale ratings in the two groups before drug intake and at drug peak-level.*

	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 (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

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

A *Task structure*



Reversal (switching) cue

high threat phase (75% shocks)

low threat phase (25% shocks)

Stable cues

stable high threat cue (always 75% shocks)

stable low threat cue (always 25% shocks)

B *Trial structure*