The effects of angiotensin-receptor blockade on mood and vigilance behaviour in a gamified foraging task.

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Abstract

Losartan, an angiotensin-receptor blocker, has previously been shown to have anxiolytic effects. Previous studies have suggested losartan may enhance threat processing via reduced attentional avoidance of threat. We investigated this suggestion, using the Gamified Foraging Task, to examine how losartan alters vigilance and avoidance behaviour in response to threats. We hypothesized that losartan would act anxiolytically, increasing vigilant and decreasing avoidant behaviours. As this task has not previously been used in smaller scale studies, we also aimed to characterize the viability of this task for use in smaller samples.

60 healthy participants were randomised to receive a single dose of losartan (50mg) or placebo. 45-minutes after drug administration, participants completed a battery of computerised tasks, including the Gamified Foraging Task. Task behaviours were extracted from raw data and input into a validated regression model from Trier et al. (2023) to compute an anxiety-related compound measure. We regressed this compound measure against group, and additional predictors of no interest, to determine if losartan impacts behavioural markers of anxiety. We additionally replicated the previous study hypotheses testing analyses, investigating how environmental factors impact mood, to compare results and aid in task viability assessments.

Pre-drug diastolic blood pressure (BP) predicted behavioural markers of anxiety in linear regression models. We suggest that changes in diastolic BP may underpin this relationship, such that decreases in BP may decrease anxiety-related behaviours. In post-hoc analysis, we found group also predicted behavioural measures of anxiety. We therefore suggest that losartan may act anxiolytically to reduce threat-related worries

and increase vigilant checking behaviours. This supports suggestions that losartan may be useful as an adjunct to exposure therapy, acting synergistically to improve clinical outcomes. Additionally, we replicated the finding that task environment impacts mood ratings and suggest this task may therefore be useful for investigating mood in future studies.

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List of Abbreviations

ACC: Anterior Cingulate Cortex

ACE: Angiotensin Converting Enzyme

ACS: Attentional Control Scale

ARB: Angiotensin Receptor Blocker

ASI-R: Anxiety Sensitivity Index - Revised

AT1: Angiotensin II Type 1

AT2: Angiotensin II Type 2

BDI-II: Beck's Depression Inventory II

BIS/BAS: Behavioural Inhibition System / Behavioural Activation System

BP: Blood Pressure

CBT: Cognitive Behavioural Therapy

CR: Conditioned Response

CRH: Corticotropin-Releasing Hormone

CS: Conditioned Stimuli

DSM: Diagnostic and Statistical Manual (of Mental Disorders)

EPQ: Eysenck Personality Questionnaire

fMRI: Functional Magnetic Resonance Imaging

HPA: Hypothalamic-Pituitary-Adrenal

HR: Heart Rate

ICV: Intracerebroventricular

PFC: Prefrontal Cortex

PTSD: Post-Traumatic Stress Disorder

RAS: Renin-Angiotensin System

SCID: Structured Clinical Interview for DSM

SCR: Skin Conductance Response

STAI-S: State-Trait Anxiety Inventory - State

STAI-T: State-Trait Anxiety Inventory - Trait

STICSA: State-Trait Inventory of Cognitive and Somatic Anxiety

STW-2: Spot the Word 2

US: Unconditioned Stimuli

VAS: Visual Analogue Scale

vmPFC: Ventromedial Prefrontal Cortex

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Background

Anxiety and threat-processing

Anxiety disorders are highly prevalent mental health conditions. In 2019 the Global Burden of Disease report estimated the worldwide prevalence of anxiety disorders at 4% (Dattani et al., 2021). This has only increased in recent years, with an estimated 26% rise in 2020 (Santomauro et al., 2021). Cognitive behavioural therapies (CBT) are widely used to treat anxious disorders (National Institute for Health and Care Excellence., 2011). CBT for anxiety disorders has an estimated rate of remission of around 50% (Loerinc et al., 2015; Springer et al., 2018). The high prevalence of anxiety disorders, along with this low rate of remission, highlights the need for improved treatment options.

Anxiety disorders generally involve feelings of fear and unease in situations with no real, tangible threat. These feelings of fear and worry are difficult to control, excessive, and impair daily functioning (American Psychiatric Association, 2013). Research investigating threat-processing and fear neurocircuitry has therefore been essential to the understanding of anxiety disorders. Determining how these systems function and how they may change in anxiety disorders can provide a more complete picture of anxiety, as well as highlight potential new targets for treatment.

Anxiety itself is adaptive in function, signalling the presence of threat which focuses the attention and coordinates physiological and psychological responses, enabling rapid responses to avoid harm. Anxiety becomes maladaptive when these processes are overused, chronic, and sensitised so that they are applied in the absence of

threat, or in the presence of safety stimuli. This maladaptive state is thought to be accompanied by changes in threat-detection, attention, and fear-processing.

Research suggests that anxious individuals are hypervigilant to threat, and their attention rapidly orientates toward threatening stimuli (Bar-Haim et al., 2007; MacLeod et al., 1986; Mathews et al., 1997; Mathews & MacLeod, 2005; Reinecke et al., 2011; Wermes et al., 2018). This hypervigilant response is then postulated to be followed by a pattern of avoidance for threatening stimuli (Derakshan et al., 2007; Mogg et al., 2004; Mogg & Bradley, 2006; Schwerdtfeger & Derakshan, 2010), reflecting an avoidance of deeper threat processing. This hypervigilance-avoidance pattern of threat response is posed to maintain anxiety as it prevents individuals learning from the absence of their feared outcomes and forming inhibitory connections that reduce anxiety (Bögels & Mansell, 2004; Foa & Kozak, 1986; Reinecke & Harmer, 2016).

These biases are suggested to be underpinned by changes in a myriad of brain regions, including the pre- frontal cortex (PFC), amygdala, anterior cingulate cortex (ACC), and hippocampus (Daffre et al., 2020). The amygdala, a brain region central to threat-detection, is proposed to be hyperactive in many anxiety disorders (Etkin & Wager, 2007; Öhman, 2005; for review: Shin & Liberzon, 2010) including PTSD (El Khoury-Malhame et al., 2011), specific phobia (Larson et al., 2006; Lipka et al., 2011), and social anxiety (Brühl et al., 2014; Stein et al., 2002). Furthermore, anxiety and amygdala hyperactivity has been shown to decrease after exposure (Goossens et al., 2007).

Changes to amygdala reactivity have been suggested to be due to changes in PFC top-down control. In line with this theory, the PFC has been shown to be hypoactive in generalised anxiety disorder (Ball et al., 2013; Mochcovitch et al., 2014), panic disorder

(Ball et al., 2013), and OCD (Bijanki et al., 2021). Further, increasing PFC activity has been shown to alter amygdala outputs (Quirk et al., 2003) and reduce amygdala responses to threat (Ironside et al., 2019). Decreased PFC activity may represent decreased cognitive control, leading to amygdala hyperactivity and anxious behaviour. Contrary to this view, PFC activity has also been shown to be hyperactive in anxiety disorders such as specific phobia (Linares et al., 2012). Though this seems in direct opposition with previous findings, it has been suggested that PFC hyperactivity represents maladaptive avoidant strategies (Hofmann et al., 2012), and so these results may represent different cognitive mechanisms.

CBT, including exposure therapy, aims to reduce anxiety and restore normative functioning through correction of these psychological and neurological biases. Indeed, changes in threat biases have been suggested to precede symptom improvement in exposure therapy (Reinecke et al., 2013), and changes in amygdala threat responsivity have been associated with greater clinical improvements after single-session exposure (Reinecke et al., 2020). Thus, correction of cognitive and neurobiological biases may form essential mechanisms underpinning symptom improvement and recovery with exposure. Adjunct treatments that target and correct for these biases may therefore improve exposure therapy efficacy and increase the low response rate seen with CBT for anxiety.

Exposure therapy, inhibitory learning, and avoidance

Exposure therapy usually involves several sessions where patients are actively encouraged to engage with feared situations and stimuli, in the absence of any safety

strategies. Patients learn over repeated sessions that these feared stimuli do not predict negative outcomes and thus their presence should not trigger an anxious response. Over time, patients' fear responses and anxiety during these sessions reduce. This reduction in the expression of fear is thought to be due to the extinction of previously conditioned fear responses.

Fear responses can be learnt through the process of fear conditioning, which pairs a neutral conditioned stimuli (CS) with an aversive unconditioned stimulus (US+). Presentation of the US+ triggers a fear response referred to as the conditioned response (CR). Through repeated presentations of the CS and US+ together, the CS gains predictive value and can therefore trigger the CR when presented in isolation. In this way, a previously neutral stimulus becomes associated with a feared outcome and can thus trigger anxious behaviour. Extinction is the process of reducing this CR by repeatedly presenting the CS in the absence of any aversive outcome (US-) (Bouton et al., 2021; Milad & Quirk, 2012).

Repeatedly presenting the CS without the US has been suggested to form new CS-US- connections in a process referred to as inhibitory learning (Bouton, 1993; Craske et al., 2014). The new CS-US- associations are inhibitory to the conditioned fear memory (CS-US+). With repeated reinforcement the strength of these inhibitory connections increases, therefore increasing inhibition of the CR. In exposure therapy, this translates to a reduction in fear and anxiety with successive successful sessions, due to increased inhibitory learning.

Avoidance or distraction may lead to reduced inhibitory learning. Failures to fully engage with threats and feared stimuli, reduces individuals' opportunities to learn of the

non-occurrence of aversive outcomes. Avoidance may therefore act to undermine exposure treatments. For example, experiential avoidance has been shown to negatively impact fear reduction during group-based transdiagnostic CBT (Espejo et al., 2017). Moreover, individuals with social phobia demonstrating greater avoidant biases showed worse treatment responses during exposure-based CBT than those with positive attentional biases (Price et al., 2011). Further, distraction has been shown to negatively impact fear and symptom reduction with exposure for claustrophobic fear (Kamphuis & Telch, 2000; Telch et al., 2004).

Safety strategies can be considered a form of avoidance or distraction. These behaviours are commonly used by anxious individuals to alleviate fear in the short-term, but they may maintain anxiety long-term. Safety behaviours have been shown to negatively impact fear reduction after exposure (see Blakey & Abramowitz, 2016 for review). This is best documented in panic disorder with agoraphobia (Helbig-Lang et al., 2014; Salkovskis et al., 1999), and social anxiety (Piccirillo et al., 2016). Additionally, engaging in safety strategies has been shown to reduce extinction learning (Lovibond et al., 2009; Rattel et al., 2017), a central underlying mechanism for exposure therapy. Thus, removal of safety strategies appears to be key to successful exposure.

As avoidance negatively impact exposure success, improving engagement with threatening stimuli may improve exposure treatment by providing more opportunities for inhibitory learning.

Use of pharmacological agents in exposure therapy

CBT, including exposure, is a primary treatment for anxiety disorders. Though exposure has been successful at reducing anxiety in several disorders (Butler et al., 2006; Hofmann et al., 2012), a suitable number of patients fail to respond or reach remission (Loerinc et al., 2015; Springer et al., 2018). Research has thus focused on improving the success rate for exposure therapy. Recent research has investigated the possibilities of enhancing exposure therapy through a range of conjunctive pharmacological manipulations (Singewald et al., 2015). These manipulations should work synergistically with the mechanisms of action of exposure therapy to improve or accelerate responses. Potential targets for pharmacological agents have been identified through investigating anxiety neurocircuitry and mechanisms of action. One target that has been identified for potential pharmacological manipulation is the renin-angiotensin system.

Renin-Angiotensin System

The renin-angiotensin system (RAS; Figure 1) is a complex hormonal system with many physiological roles. The RAS plays an important role in regulating blood pressure, cardiac output, and inflammation (Chrissobolis et al., 2020; Forrester et al., 2018; Stegbauer & Coffman, 2011). Angiotensinogen is a precursor peptide which is converted by the enzyme renin into angiotensin I which is subsequently converted into angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II can bind to both AT1 and AT2 receptors. These receptors are located in several regions throughout the body, including

brain regions pertinent to anxiety such as the PFC, hippocampus, and amygdala (Johren & Saavedra, 1996; Tsutsumi & Saavedra, 1991; Wright & Harding, 1994, 1995).

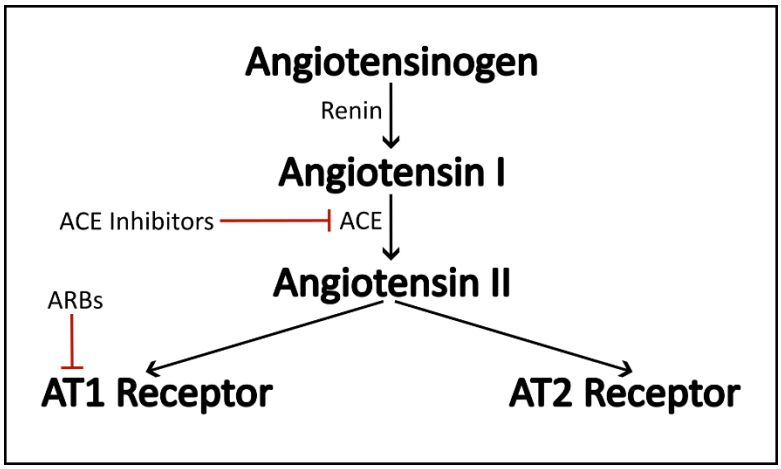


Figure 1 The Renin-Angiotensin System

This diagram shows part of the renin-angiotensin system (RAS). Angiotensinogen is converted into angiotensin-I by the enzyme renin. Angiotensin-I is converted into angiotensin-II via angiotensin-converting enzyme (ACE); this can be inhibited using ACE inhibitors. Angiotensin-II can bind to either angiotensin-II type 1 (AT1) or angiotensin-II type 2 (AT2) receptors. Angiotensin receptors blockers (ARBs) can prevent angiotensin from binding to AT1 receptors. Inhibition is shown in red.

The RAS has been suggested to play a role in anxiety (for review see: Chrissobolis et al., 2020). Injection or infusion of angiotensin-II (Braszko et al., 2003; Duchemin et al., 2013), a reduction in AT2 receptors (Okuyama et al., 1999), and angiotensinogen deficiency (Voigt et al., 2005) have all been shown to promote anxiety-like behaviour in rodents. Blockade of AT1 receptors with angiotensin receptor blockers (ARBs) has additionally been shown to reduce anxiety-like behaviours (Llano López et al., 2012; Pavel et al., 2008; Ranjbar et al., 2018; Rocha dos Santos et al., 2022; Salmani et al., 2020; for review: Saavedra et al., 2011). Therefore, the RAS is suggested to enact anxiogenic effects

via activation of AT1 receptors or inhibition of AT2 receptors, and anxiolytic effects via the activation of AT2 receptors or inhibition of AT1 receptors.

Furthermore, the RAS has been shown to play a role in the stress response (Jindra & Kvetnanský, 1982; Saavedra & Benicky, 2007; Yang et al., 1994, 1996). AT1 receptors play a role in regulating the hypothalamic-pituitary-adrenal (HPA) axis (Aguilera et al., 1995; Armando et al., 2001) and associated stress hormones such as corticotropin-releasing hormone (CRH) (Armando et al., 2007; Saavedra & Pavel, 2005; Sumitomo et al., 1991). Indeed, AT1 receptors on CRH-releasing neurons have been shown to be important to the expression of conditioned fear such that their deletion resulted in decreased fear expression in transgenic mice (Hurt et al., 2015). Thus, AT1 receptors may play a role in anxiety via changes in CRH and the stress response.

Targeting the RAS, using pharmacological agents, for anxiety treatment has been of particular interest due to findings suggesting the RAS may alter aspects of fear learning and extinction. In animals, deletion of AT1 receptors in the central amygdala of transgenic mice has been shown to enhance fear extinction (Yu et al., 2023), a central mechanism for exposure therapy. Further, activation of AT2 receptors has been shown to reduce fear expression following fear conditioning in mice (Yu et al., 2019). Importantly, in a human observational study, reduced PTSD symptom development was reported for trauma-exposed individuals taking ACE inhibitors or ARBs (Khoury et al., 2012). This presents the potential for ARBs to alter underlying mechanisms of fear learning and exposure therapy, such that they work synergistically to improve exposure treatment outcomes.

Losartan and Anxiety

Losartan, an angiotensin-II type 1 (AT1) receptor antagonist, has been highlighted as a potential agent for conjunctive use with exposure therapy. Consistent with the proposed role of AT1 receptor activation, losartan has been shown to exert an anxiolytic effect. Braszko et al. (2003) found that an intracerebroventricular (ICV) injection of angiotensin-II induced an anxiety-like phenotype in rats but this was abolished by a subsequent ICV injection of losartan. Losartan additionally had an anxiolytic effect when injected into the amygdala of rats prior to completion of the Elevated Plus Maze (Llano López et al., 2012). Further, Ranjbar et al., (2018) found losartan decreased the anxiety-like behaviours induced by Forced Swim Tests in rats.

Importantly, losartan has been shown to enhance fear extinction in mice (Marvar et al., 2014). Mice were conditioned to acquire a tone-shock (CS-US+) pairing. A single administration of losartan was injected the following day, prior to extinction training. Losartan mice demonstrated improved extinction retention compared to vehicle mice when tested 24hrs after extinction training. Chronic administration of losartan, over a two-week period, was additionally shown to enhance extinction retention. Losartan may therefore improve exposure therapy through enhancing aspects of fear extinction memories.

This is furtherer supported by Zhou et al (2019) who conducted a similar study in humans. Seventy participants were subjected to a Pavlovian fear conditioning paradigm to acquire a CS-US+ association between a visual stimulus and a mild electric shock. Participants were then given either placebo or losartan 90-minutes prior to extinction training. Losartan treated participants showed reduced skin conductance responses

(SCR), a physiological marker for fear arousal, during early extinction learning compared with placebo participants. The authors suggest losartan may therefore accelerate extinction learning. Additionally, they found that the ventromedial PFC (vmPFC) showed increased threat-specific engagement during this early extinction phase for losartan but not placebo, suggesting this region facilitates the improvement in extinction learning (Zhou et al., 2019).

Other human studies have shown losartan can also improve prospective memory (Mechaeil et al., 2011), improve learning from positive relative to negative outcomes in reinforcement learning paradigms (Pulcu et al., 2019), improve immediate and delayed memory (Fogari et al., 2003), and facilitate contextual processing of trauma images (Shkreli et al., 2020). This collection of research provides a very promising outlook for the potential of losartan to improve aspects of learning and memory that may be utilised in exposure therapy. Further research is needed to determine what mechanisms mediate these improvements.

One recent study suggests losartan affects emotional processing via changes to amygdala activation patterns (Reinecke et al., 2018). High-trait anxious participants were presented with fearful and happy faces, with presentations split for analysis into the first 40 seconds (early) and last 40 seconds (late). Individuals on losartan showed sustained amygdala activation during both early and late presentation of fearful faces whereas placebo showed a decrease in activation with time. The authors suggest this prolonged engagement may represent additional processing of fearful stimuli. It was additionally shown that in response to the early presentation of happy faces, losartan participants showed significantly reduced amygdala activity compared with placebo. This was

suggested to represent faster habituation to happy faces which may act as a safety signal. It was suggested that this improved distinction and increased engagement with threatening stimuli whilst taking losartan could enable greater processing of threats and more opportunities for inhibitory learning. As previously discussed, avoidance can have a negative impact on exposure therapy outcomes and reinforces anxious behaviour (see: Exposure therapy, inhibitory learning, and avoidance section). Therefore, losartan may have clinical efficacy by reducing avoidance of threats.

The Gamified Foraging Task

Due to the complex nature of threat and reward behaviour, classical tasks often involve simplistic designs with many assumptions. These tasks have great value however their simplistic nature may prevent more complex decision-making behaviours from being captured. Decisions are rarely made in isolation with no associated cost (e.g. time, energy, etc) and the inclusion of costs and trade-offs in everyday decisions impacts our behaviour. These behaviours are missed or simplified in classical tasks.

More complex computerised tasks offer the possibility of representing more ecologically relevant scenarios (Scholl & Klein-Flügge, 2018). For example, the spontaneous appearance of a threat in the environment may recruit different processes to those relevant for searching and subsequently finding a threat in the environment. The Gamified Foraging Task (Trier et al., 2023) is a computerised decision-making game that accounts for this more complex scenario through its inclusion of trade-offs between rewards and threats.

In the Gamified Foraging Task participants make decisions as a fish avatar in an ocean environment. There are three actions participants can undertake: diving for food, checking for hidden predators, and hiding from predators. Diving increases the fish's energy level and translates into game lives; these lives can be lost if the fish is caught by a predator. Three predators of different speeds, and therefore threat, can enter the environment. These distinct threat levels enable variations in threat-related behaviour to be captured. Predators are initially hidden, forcing participants to check for predators to avoid being caught. As each behaviour incurs a time cost, participants must trade-off foraging, checking, and hiding behaviour to optimise performance.

Trier et al., (2023) analysed data from over 700 participants who completed the Gamified Foraging Task, along with a range of psychiatric questionnaires, remotely. They found that participants with high trait-somatic anxiety displayed increased threat-avoidance and increased threat-related worry. Higher trait anxious participants checked for predators less often (as a percentage of all actions) and reported feeling more nervous after seeing a predator in self-report compared to lower trait anxious participants. They concluded that more anxious participants showed decreased vigilance and a greater sensitivity to threat-related worries. Trier et al., (2023) additionally found that environmental task factors impacted participants' feelings of stress and excitement.

As losartan has been shown to have anxiolytic effects (see Losartan and Anxiety section) and has been suggested to reduce attentional avoidance via prolonged engagement with threatening stimuli (Reinecke et al., 2018), we predicted losartan would reduce threat-avoidant behaviour resulting in an increase in checking behaviour and more optimal task outcomes. We additionally hypothesised that losartan would reduce

the impact of environmental task factors on stress ratings, due to the role of the RAS in stress physiology, but not on excitement ratings.

Study Aims and Rationale

Losartan, an angiotensin-receptor blocker, has been highlighted as a potential adjunct to anxiety treatment. Losartan has been shown to reverse anxiogenic induction (Braszko et al., 2003), reduce anxiety-like behaviours (Llano López et al., 2012; Ranjbar et al., 2018) and enhance fear extinction (an analogue for human CBT) (Marvar et al., 2014). Furthermore, an observational study in humans has reported reduced PTSD symptom development in trauma-exposed individuals taking antihypertensive drugs such as losartan (Khoury et al., 2012). This evidence suggests losartan may be useful for enhancing the efficacy of exposure therapy.

Anxiety is characterised by cognitive biases, including hypervigilance for threat and avoidance of deeper attentional processing (Reinecke & Harmer, 2016). Exposure therapy for anxiety disorders works by encouraging patients towards extended attention and processing of fear stimuli, and research shows that those with higher levels of avoidance bias will benefit less from treatment (Price et al., 2011). A study in high-trait anxious participants has suggested losartan may enhance threat processing through prolonged amygdala activation. This activation may represent reduced avoidance of threat stimuli, implying that losartan may have beneficial effects on exposure therapy via this mechanism (Reinecke et al., 2018). The present study aims to investigate this possibility by determining how losartan alters vigilance and avoidance behaviours in response to threats during a decision-making task. This may improve our understanding of the effect of losartan on threat processing. The Gamified Foraging Task (Trier et al., 2023) has not previously been used in smaller scale laboratory studies. As such, this study

also aimed to characterize the viability of this task for use in smaller sample studies by examining the placebo group for replications of the mood results found in Trier et al.

This study hypothesized that losartan would act anxiolytically, thus increasing vigilant and decreasing avoidant behaviours. We predicted that this would be evident through increased checking behaviours during the task and decreased threat-related worry ratings in the post-task debrief. Furthermore, we hypothesised that losartan may alter the relationship between task environment and mood. We predicted that losartan participants' stress levels will be less affected by task environment, as demonstrated through lower stress ratings between task blocks.

Method

Participant data collection started on 13.04.2021 and ended on 03.11.2022. On 31.03.2023, prior to data analysis, we pre-registered our analysis methods using OSF (https://osf.io/). A copy of the pre-registration document is provided in Annex 1: Pre-registration document.

Participants

This project received ethical approval from the Central University Research Ethics Committee of the University of Oxford. Additional approval was granted from Oxford Brooked University for advertising purposes.

Sixty healthy volunteers aged between 18 to 50 years old were recruited. Power calculations based on Mechaeil et al. (2011) suggested sample sizes of 26 per group to achieve power of 0.8 and effect sizes of at least d=0.80 at an α-level of 0.05. This sample size of 30 per group was chosen to ensure this power would be maintained despite potential task failures or performance-based exclusions.

Recruitment strategies included online advertisements via Facebook and Daily Information, emails to college MCR and JCR mailing lists, posters in departments and colleges of both the University of Oxford and Oxford Brookes University, and flyers handed out to members of the local community. Participants were excluded from participation if they met criteria for a current DSM-5 Axis I Disorder assessed using the Structured Clinical Interview for DSM-5 (SCID) (First, 2015). Participants additionally needed to be light (<5 cig/day) or non-smokers, have no first-degree family member with

a history of severe psychiatric illness, be medication free for 6 weeks, have a BMI between 18-30kg/m², and be physically healthy such that they may take losartan (Table 1).

Criterion	Exclusion
Age	<18 or >50
BMI	<18 or >30
English Skills	Not fluent in English
Smoking	>5 cigarettes per day
Pregnancy Status	Pregnant, trying to become pregnant, or breastfeeding
Medication	CNS-active medication within last 6 weeks Taking steroid medication Taking blood pressure medication
Psychiatric Illness	Previous or present diagnoses for a psychological disorder First-degree family member with history of severe psychiatric illness
Physical Health	History of epilepsy or another neurological disorder Current diagnosis for intravascular fluid depletion Severe disease affecting the kidneys, liver, heart, or respiratory system Renal insufficiency or systemic infection
Participation in Other Studies	Participated in a study involving CNS-active medication during last 6 weeks Tasks completed in other studies overlap with current study
Coronavirus Risk*	High risk for COVID, based on NHS checklist for coronavirus vulnerability

Table 1 Participant Exclusion Criteria

List of criteria assessed during the screening stage of the study to determine participant eligibility. *This criterion was removed Feb 2022, after the relaxation of COVID restrictions.

Testing Procedure

Participants initially completed a short set of questions through either a survey hosted on Qualtrics or via email (Figure 2A). These questions assessed basic eligibility for the study (Appendix A).

Participants who passed this initial screening were then invited to an online screening session where they were asked an extended set of eligibility questions based on Table 1. Sociodemographic data, including age, gender, first language, years of education, and employment status, was collected from participants at this stage. Participants then answered a series of medical screening questions to ensure they would be safe to take losartan if allocated (Appendix B). This medical information was reviewed by the study doctor if necessary. A psychological screening was then completed using the Structured Clinical Interview for DSM-5 (First, 2015) to assess for any psychiatric conditions. This was followed by the Spot the Word Task (STW-2; Baddeley & Crawford, 2012) to assess verbal intelligence. This session took approximately 40 minutes (Figure 2B).

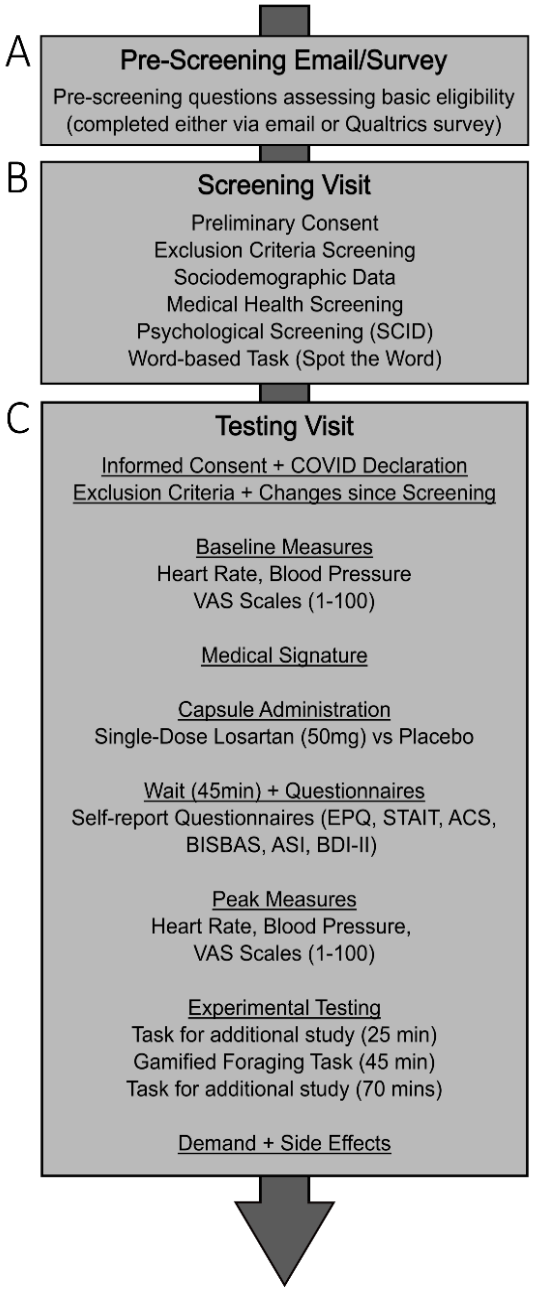


Figure 2 Procedure Flow Diagram

Flow diagram showing the entirety of the study procedure that included participants. Participants completed pre-screening sessions online or by email to assess basic eligibility prior to invitation for full screening (A). The full screening sessions assessed complete medical and psychiatric eligibility (B). If eligible, participants were invited to the in-person testing visit (C).

Eligible participants were then invited to an in-person testing session at the Department of Psychiatry's Neuroscience facility (Figure 2C). This session took approximately 3.5-4 hours. Participants were randomly allocated in a double-blind design to either losartan (50mg) or placebo (microcrystalline cellulose) tablets which were over-encapsulated to look identical. Randomisation, which followed a 1:1 blocked randomisation design with gender stratification, was conducted by a researcher with no direct contact with participants to maintain blindness.

All participants provided written informed consent and completed a COVID declaration stating they did not have any symptoms on the day of testing. Participants were asked to have their last meal 2 hours prior to testing to ensure homogeneous drug absorption across the participants.

Visual analogue ratings of mood and wellbeing and physiological measures were taken prior to capsule administration and at drug-peak level (≈45 minutes after intake), to rule out noticeable acute effects of the drug that may induce response bias. Physiological measures of heart rate and blood pressure were taken using a blood pressure machine (Omron 705IT sphygmomanometer). The Visual Analogue Scales at both time points asked participants to rate their feelings of the following: "anxious", "stressed", "sleepy", "flushed", "tearful", "nauseous", "hopeless", "tremor", "sad", "dizzy", "depressed", "heart racing", and "alert". These were later scored from 0-100 by the experimenter or by Qualtrics.

During the 45-minute waiting period for the drug to reach peak plasma levels, participants were asked to complete a battery of questionnaires including: the Eysenck Personality Questionnaire (EPQ; Eysenck & Eysenck, 1975), the trait form of the State-

Trait Anxiety Inventory (STAI-T; Spielberger et al., 1983), the Attentional Control Scale (ACS; Derryberry & Reed, 2002), the Behavioral Inhibition and Behavioral Activation Scales (BIS/BAS; Carver & White, 1994), the Anxiety Sensitivity Index Revised (ASI-R; Taylor & Cox, 1998), and the Beck Depression Inventory (BDI; Beck et al., 1996). Experimental testing begun after this 45-minute wait period, when peak drug levels are obtained. Participants completed three experimental tasks, including the Gamified Foraging Task (Trier et al., 2023). After finishing all tasks, participants completed a questionnaire to indicate to what degree they experienced the following side-effects: “nausea”, “dizziness”, “dry mouth”, “headache”, “alert”, and “agitation”. Both the experimenter and participant were then asked to guess the group allocation.

Task Procedure

The Gamified Foraging Task (Trier et al., 2023) was designed to ecologically capture vigilance and checking behaviour. The game consists of 18 blocks of 90 seconds each, where participants make decisions as a fish avatar in an ocean environment. The time remaining in the block is represented by an animated sun or moon moving across the sky. There are three actions participants can undertake: foraging for food, checking for hidden predators, and hiding from predators. Each action incurred a time cost: foraging 1.5s, checking 0.5s, hiding 0.5s, return from hiding 2s. Participants thus had to trade-off foraging, checking, and hiding behaviours if they were to perform optimally.

There were two practice rounds prior to the start of the main task to allow participants to become familiar with the game. Additionally, a questionnaire was

presented, after the practice rounds but prior to the main task, which asked about the task rules to ensure the instructions were fully understood.

Participants could choose to forage by clicking the “EAT” arrow which caused their fish to dive for food (Figure 3B). Foraging increased the fish’s energy level and translated into game lives; these lives could be lost if the fish was caught by a predator (Figure 3I). The amount of food available varied randomly (represented by a changing green bar) and thus the energy gained from each foraging dive could vary.

Participants could avoid the predator by clicking the “HIDE” arrow which hid their fish in a safe space (Figure 3 F-G). They could leave the hiding position whenever they chose by clicking the “SWIM” arrow (Figure 3H). Whilst hidden, the participant could see when the predator entered the centre of the display and wait until the predator exited the environment (Figure 3G). To prevent participants from hiding for very long periods of time, a warning message was displayed when participants hid for longer than 15s.

Predators were initially hidden, forcing participants to check for predators to avoid being caught. The area surrounding the fish was split into a varying number of segments (between 1-6) (Figure 3 C-D). Each segment had an eye icon which participants would need to click if they wanted to reveal and check the segment for a predator (Figure 3E). Thus, as the number of segments increased, the area of each segment decreased meaning a greater number of checking actions would be required to check the entire environment. Checking enabled participants to decide when to forage or hide based on how close the predator was to their central position.

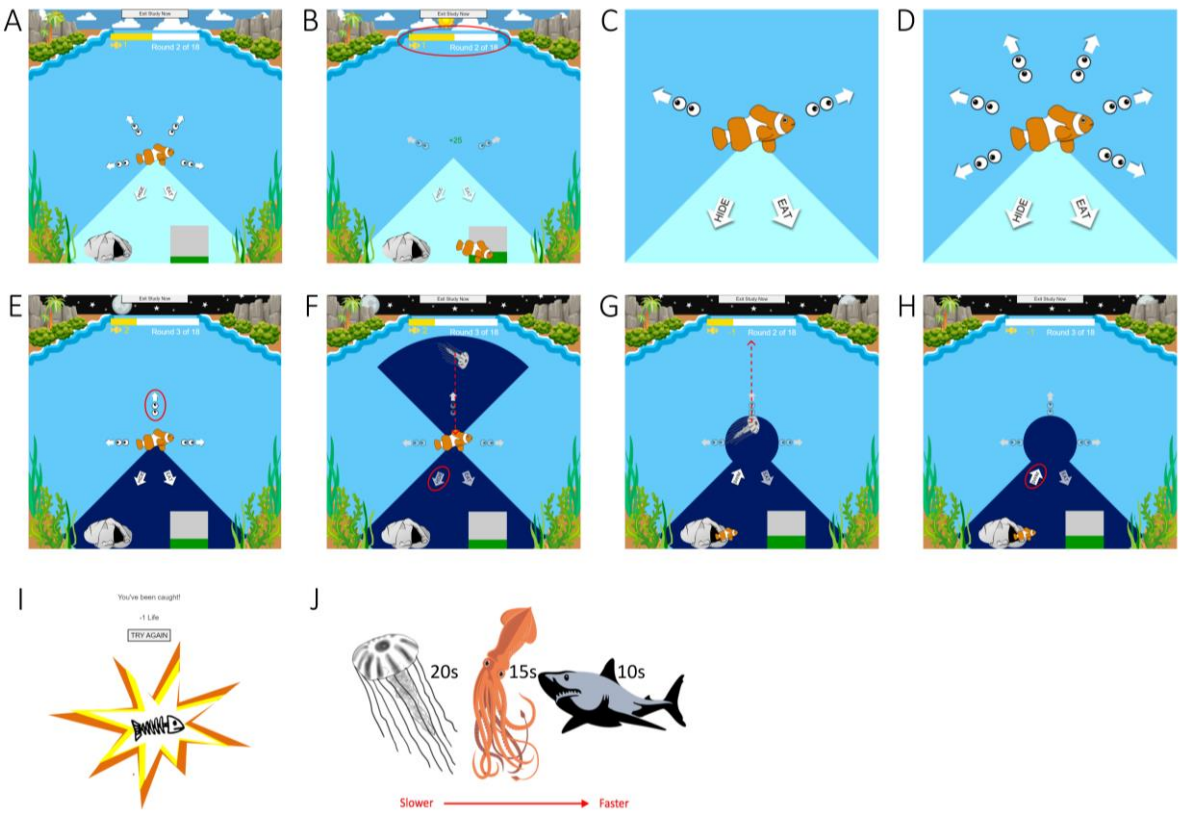


Figure 3 Gamified Foraging Fish Task

A) The fish starts each block in the center. Two arrows, “EAT” and “HIDE”, denote the foraging and hiding actions. Eye icons may be clicked to reveal sections of the environment to check for predators.

B) The “EAT” action causes the fish to dive for food, and gain energy. Food availability varies randomly and is represented by the green bar. The current energy level is represented by the yellow bar (circled in red) and increases when energy is gained. Energy gained is displayed in green text (i.e. +25). Once the energy bar is full, an additional life is added to the counter and the bar resets.

C-D) The environment is divided into a variable (1-6) number of segments. The greater the number of segments, the smaller the area covered by each set of eyes.

E) Selecting eye icons (circled in red) reveals a portion of the surroundings to be checked for predators.

F) Predators in the environment move towards the center where they may catch the fish. To avoid being caught, participants must use the “HIDE” arrow (circled in red).

G) Hiding moves the fish to a safe space where it can wait until the predator reaches the centre. Once hidden the “HIDE” arrow switches to “SWIM”. In the absence of a fish, the predator will promptly leave the environment.

H) Once the predator exits, participants can leave the hiding space using the “SWIM” arrow (circled in red).

I) If the predator reaches the fish before it is hidden, a life is lost and the life counter at the top of the display reduces by 1.

There were three predators of different speeds, and therefore threat, which could enter the environment: jellyfish (slowest), squid (medium), shark (fastest) (Figure 3J). Including predators of differing speeds, allows for variations in threat-related behaviour to be captured as each predator represents a distinct threat-level. Predators approached the centre of the display, where they may catch the fish if it is not hidden. Once the predator reached the centre, they promptly exited the environment if no fish was present.

Only one type of predator was present in each block, and these appeared one at a time. Participants were told which predator would be present prior to starting the block. Predators entered the environment after a random delay of 1-10 seconds between the start of the block or the previous predator exit, and the new predator's appearance.

After completing each block, participants were asked four questions about the round: How much food was available, how difficult was it to balance between eating food and checking for predators, how stressful was the last round, and how exciting was the last round. Once all 18 blocks were finished, 31 self-report questions were presented in a debrief questionnaire (Appendix C). These questions aimed to capture participants metacognitive awareness of their behaviour.

Task Processing

Pre-processing

Raw task data was processed in R (R Core Team, 2023) by Dr Scholl. This processing was conducted using a script adapted from their previous, fully independent study (Trier et al., 2023). These methods were pre-registered on OSF (Annex 1: Pre-registration document).

Data was pre-processed to remove participants who displayed poor task behaviour. Participants were excluded for poor task quality if they met one of the following criteria:

- they checked for threat less than 50 times over the entire course of the task
- their final life counter was less than 5
- they were caught more than 9 times
- there was any issue with the displaying of reward during the task

Participants who passed these additional exclusion criteria were then used for further processing and analyses. Task-based measures were computed for each participant from their task responses (e.g. number of times the fish was caught by the predator). These were computed using the same method as in Trier et al., 2023. A list of the computed measures and their interpretation can be found in Appendix D.

After task-based measures were extracted for each participant, missing data was imputed using the MICEafter package in R (Heymans, 2022). The following sociodemographic and physiological measures were additionally included for imputation: age, gender, education, weight, systolic and diastolic blood pressure pre-capsule administration, and group assignment.

All data was then standardized using the mean and standard deviation of the corresponding measures from the previous study (Trier et al., 2023). Regression models were then applied to each participant to compute behavioural measures that related task features with behaviour or mood. Full details of the modelling used can be found in Trier et al., 2023. All regressors were z-scored for each participant.

Mood-related measures

Regression weights for behavioural measures relating task features (environment factors) with mood were then combined (with signs flipped based on coding, see Trier et al., 2023) to give each participant a value representing the general effect of environment on mood (H1), on excitement (H1A), and on stress (H1B) (Table 2). These methods were not included in the pre-registration and are a replication of the method used in Trier et al. (2023).

Hypothesis	Sub-hypothesis	Regressor
Task environment is linked with mood (H1)	Task features affect excitement (H1A)	Effect of maximum reward on excitement
		Effect of predator speed on excitement
		Effect of number of directions to check on excitement
	Task features affect stress (H1B)	Effect of maximum reward on stress
		Effect of minimum reward on stress
		Effect of predator speed on stress
		Effect of number of directions to check on stress
		Effect of fish caught on excitement
		Effect of fish caught on stress

Table 2 List of mood-related hypotheses

Regressors that form the basis for each hypothesis are listed in the regressor column. The overall hypothesis (H1) is divided into two sub-hypotheses (H1A, H1B). Hypotheses are from Trier et al., 2023.

Vigilance and threat-related worry measures

Further regression models (from Trier et al., 2023) were then applied to compute "compound measures". The regression models from Trier et al., were formed by relating participant behavioural measures and debrief questionnaire responses to their scores on psychiatric questionnaires. The models thus contain combinations of task-behaviours and debrief responses (Table 3) that best predict the clinical score of interest. The validated models were then applied to the current data set (using participant task-behaviour and debrief responses as inputs) to compute "predicted clinical scores" which we refer to as "compound measures". These compound measures can thus be considered task-based measures with validated links to psychiatric dimensions.

Trier et al., 2023 found that sensitivity of vigilance to threat-related worry, avoidance, and task outcome (fish being caught) was related to somatic anxiety (as measured by the State-Trait Inventory for Cognitive and Somatic Anxiety [STICSA; Grös et al., 2007]). This compound measures was chosen based on the proposed effect of losartan to alleviate anxiety (Chrissobolis et al., 2020).

Compound Measure (Previous Association)	Component behavioural measures	Behaviour measure description
Sensitivity of vigilance to threat-related worry and avoidance and the resultant task outcome (Somatic Anxiety - STICSA)	Fish caught	Number of times the fish was caught
	Checking as a % of all actions (intercept)	Percentage of time spent checking
	Avoided checks because indecisive about hiding (SR2)	Self-report debrief Q2: "I avoided checking for the predator because I didn't want to make a decision about when to hide"
	Felt more nervous after seeing predator (SR13)	Self-report debrief Q13: "After seeing the predator I felt more nervous than before"

Table 3 List of behavioural measures combined for compound measure

Component behavioural measures that form the basis for the vigilance and threat-related worry compound measure. These behavioural measures were combined in a regression model to calculate a compound measure ("predicted STICSA score") for each participant.

Additional compound measures were computed using machine-learning (regularized Bayesian regression) models trained on data in the previous study (Trier et al., 2023). These models selected measures that best predicted the clinical score of interest (STICSA), without any assumed knowledge of how these variables may interact psychologically or within the task. The models were then applied to the current data set to derive estimates for the related clinical score. These estimates were then analysed for group differences the same as the other primary outcome compound measures. The following machine-learning measures were calculated: cognitive trait anxiety (without post-task debrief), somatic trait anxiety (without task debrief).

Full details for the computational processing of the task can be found in Trier et al., 2023. A summary of the task processing method is provided in Figure 4A. These methods were included in the pre-registration (Annex 1: Pre-registration document).

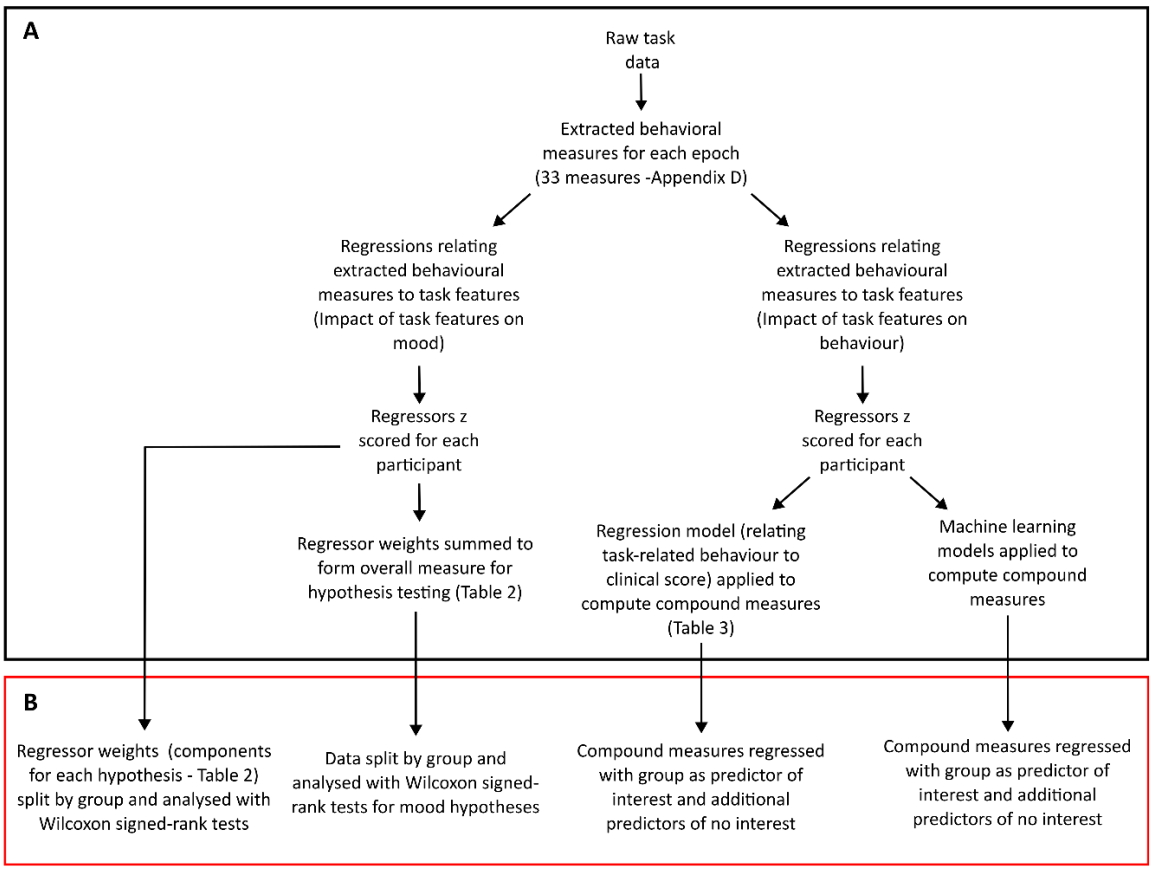


Figure 4 Task Processing and Analysis Summary

This diagram contains a summary of the processing and analyses methods used for this study. Task processing, completed by Dr. Jacqueline Scholl (A), was adapted from their fully independent study (Trier et al., 2023). Analyses (B), using the outputs from the task processing, was completed by the candidate.

N.B. This diagram does not contain all analyses conducted.

Analyses

All statistical analyses were carried out using IBM SPSS Statistics 28 (IBM Corp, 2021). Statistical tests were two-tailed with alpha-level of significance 0.05. Figures were edited using Inkscape (Inkscape Project, 2022).

Control Analyses

Sociodemographic and psychiatric questionnaire data was analysed using independent samples T-test and chi-squared analyses. Visual analogue ratings and physiological measures of heart rate and blood pressure were analysed using 2x2 mixed model ANOVAs with time (baseline, peak) as the within-subjects factor and group (placebo, losartan) as the between-subjects factor. Reported side effects and demand guesses for group allocation were analysed using chi-squared and Fisher exact tests.

Mood-related measures

Computed values for the effects of environment on mood (for the regressor weights that were relevant and combined for each hypothesis, see Table 2) were split into two groups based on drug allocation (placebo, losartan). For each hypothesis and sub-hypothesis, Wilcoxon signed rank tests were conducted for each group's median, against a predicted median of zero. Hypotheses were tested hierarchically, as in Trier et al., such that a significant result in the main hypothesis, triggered further analysis of sub hypotheses. A Mann-Whitney U test was performed to compare group differences. These non-parametric tests were chosen as data did not meet normality assumptions (Shapiro-Wilk tests significant).

Component regressor weights that formed the basis of the hypothesis test values for each group (Table 2) were then analysed using further Wilcoxon signed rank tests. Mann-Whitney U tests were then performed to determine if losartan significantly altered participants' mood response to each of the task variables.

Vigilance and threat-related worry measures

Computed compound measures (Table 3) and machine-learning derived compound measures were assessed for group differences using multiple linear regression models. Group was used as the predictor of interest, with additional predictors of no interest added for control. The following regression model was pre-registered:

Compound Measure ~ Group + Gender + Pre-drug Systolic BP + Pre-drug Diastolic BP + Weight + Age + Education.

Further regression models were run after conducting correlational (Pearson's r) analysis between predictor variables (group, gender, pre-drug systolic BP, pre-drug diastolic BP, weight, age, and education) to aid in model choice. This was to reduce issues of interpretability, overfitting, and collinearity. Collinearity diagnostic analysis was additionally run alongside all regression analyses. The following two regression models, not included in the pre-registration, were run:

Compound Measure ~ Group + Gender + Weight + Pre-drug Diastolic BP

Compound Measure ~ Group + Gender + Weight + Pre-drug Diastolic BP + Interaction (pre-drug diastolic BP x group)

No corrections were made for multiple comparisons. A summary of the analysis conducted can be found in Figure 4B.

Independent sample t-tests were then run for the component behavioural measures (those used for the compound measure related to STICSA, see Table 3) to compare group means.

Post-hoc Exploratory Analysis

These analyses took place after those listed above and were not part of any pre-registration. Two further independent samples t-tests were conducted to compare the change in diastolic blood pressure over time (between pre- and post-drug administration) between the losartan and placebo groups. One t-test included the potential outlier, the other excluded this participant. Additionally, both the reduced and interaction regression models were run without the potential outlying participant, for the anxiety-related compound measure (STICSA) only. Machine learning model estimates were not regressed for this analysis.

Results

Control Analysis

Sixty participants were successfully recruited for this study. Of these, 3 participants were excluded from analysis due to errors with task data saving (2) and having completed the task in a previous study (1). The remaining data was sent to Dr. Scholl for processing. A further 5 participants were then excluded from further analysis for poor task quality as listed in methods. The remaining 52 participants (N=26 placebo, N=26 losartan) were analysed fully.

Sociodemographic, clinical, and personality characteristics

Sociodemographic, clinical, and personality measures were well matched across the two groups as all t-tests (chi-squared for gender) were non-significant (p>0.11) (Table 4).

	Placebo (N=26)		Losartan (N=26)		
	M	SD	M	SD	p
Sociodemographic Data					
Gender	16F/10M		17F/9M		0.77
Age	25.1	4.5	24.2	6.3	0.56
Years of Education	17.3	2.8	17.3	3.6	0.98
STW (FSIQ)	104.2	7.3	100.8	6.9	0.11
Clinical and Personality Measures					
Trait Anxiety (STAIT)	39.9	8.2	40.4	10.0	0.84
Anxiety Sensitivity (ASI)	25.3	16.8	26.8	17.6	0.75
Attentional Control Scale (ACS)	50.9	8.9	54.5	8.7	0.15
Behavioural Activation (BAS)	39.7	6.4	40.5	5.5	0.63
Behavioural Inhibition (BIS)	20.5	3.3	20.5	4.4	1.00
Beck Depression Inventory (BDI)	5.9	6.3	7.4	7.2	0.44
Neuroticism (EPQ)	8.7	3.4	8.6	5.3	0.94
Psychosis (EPQ)	3.4	2.6	3.3	2.4	0.90
Lie (EPQ)	7.5	3.8	8.9	3.0	0.14
Extraversion (EPQ)	11.6	5.4	12.7	5.9	0.50

Table 4 Sociodemographic, Clinical, and Personality Measures

Table of group means and standard deviations for sociodemographic, clinical, and personality measures. P-values are from independent samples t-tests for all measures other than gender (chi-squared test). Sample sizes were n=26 for all measures other than for Years of Education (placebo n=25), and for STW (placebo n=22). Significance level α=0.05.

Physiological measures and mood ratings

Baseline and drug peak physiological and VAS measures are summarized in Table 5.

	Baseline				Drug Peak			
	Placebo		Losartan		Placebo		Losartan	
	M	SD	M	SD	M	SD	M	SD
Physiological Measures								
Heart rate	78.2	13.7	77.1	14.4	66.8	9.7	66.8	11.3
Systolic blood pressure	120.0	9.3	120.3	13.4	113.2	8.6	110.8	12.1
Diastolic blood pressure	72.3	7.8	75.6	8.4	68.5	7.8	67.9	10.1
Visual Analogue Ratings								
Anxious	17.4	19.2	14.3	17.4	8.4	10.1	10.1	13.8
Stressed	20.4	24.2	19.8	22.0	11.7	17.3	14.2	19.7
Sleepy	26.1	25.3	21.4	22.3	29.2	27.7	26.0	25.0
Flushed	12.0	17.4	8.4	11.7	3.0	6.6	5.4	11.1
Tearful	4.1	7.4	4.6	7.2	1.8	3.5	3.1	6.4
Nauseous	1.5	3.4	4.2	9.0	1.2	3.0	3.2	6.0
Hopeless	6.1	12.2	4.4	7.8	4.8	14.0	2.6	4.6
Tremor	2.1	3.0	3.8	7.4	1.4	2.8	1.9	3.6
Sad	7.3	10.1	7.8	11.7	5.1	9.6	3.3	5.6
Dizzy	2.2	4.5	3.1	7.5	2.1	3.5	8.1	15.5
Depressed	4.4	10.7	3.2	5.7	3.0	5.2	2.8	5.8
Heart racing	8.3	14.2	5.8	12.8	2.1	3.3	5.6	11.5
Alert	43.1	23.7	31.3	27.2	28.2	22.3	29.2	29.7

Table 5 Physiological and VAS measures summary

Group means and standard deviations for physiological measures and visual analogue ratings. Baseline measures were taken prior to drug administration, drug peak measures were taken 45-minutes after drug administration.

Two-way mixed ANOVAs (Table 6) revealed significant main effects of time for heart rate ($p<0.001$; Figure 5A), systolic blood pressure ($p<0.001$; Figure 5B), and diastolic blood pressure ($p<0.001$; Figure 5C), with all measures decreasing with time. In addition, a significant time x group interaction effect was found for diastolic blood pressure ($p=0.019$), where losartan participants blood pressure decreased by a greater degree over time than placebo (Figure 5C).

Measure	Factor	F Statistic	p value	η_p^2
Heart rate	Time	90.33	<0.001	0.644
Systolic blood pressure	Time	43.55	<0.001	0.466
Diastolic blood pressure	Time	50.81	<0.001	0.504
	Time x Group	5.87	0.019	0.105

Table 6 Significant HR and BP ANOVA results

Summary of significant ANOVA results for physiological measures. Significance level $\alpha=0.05$.

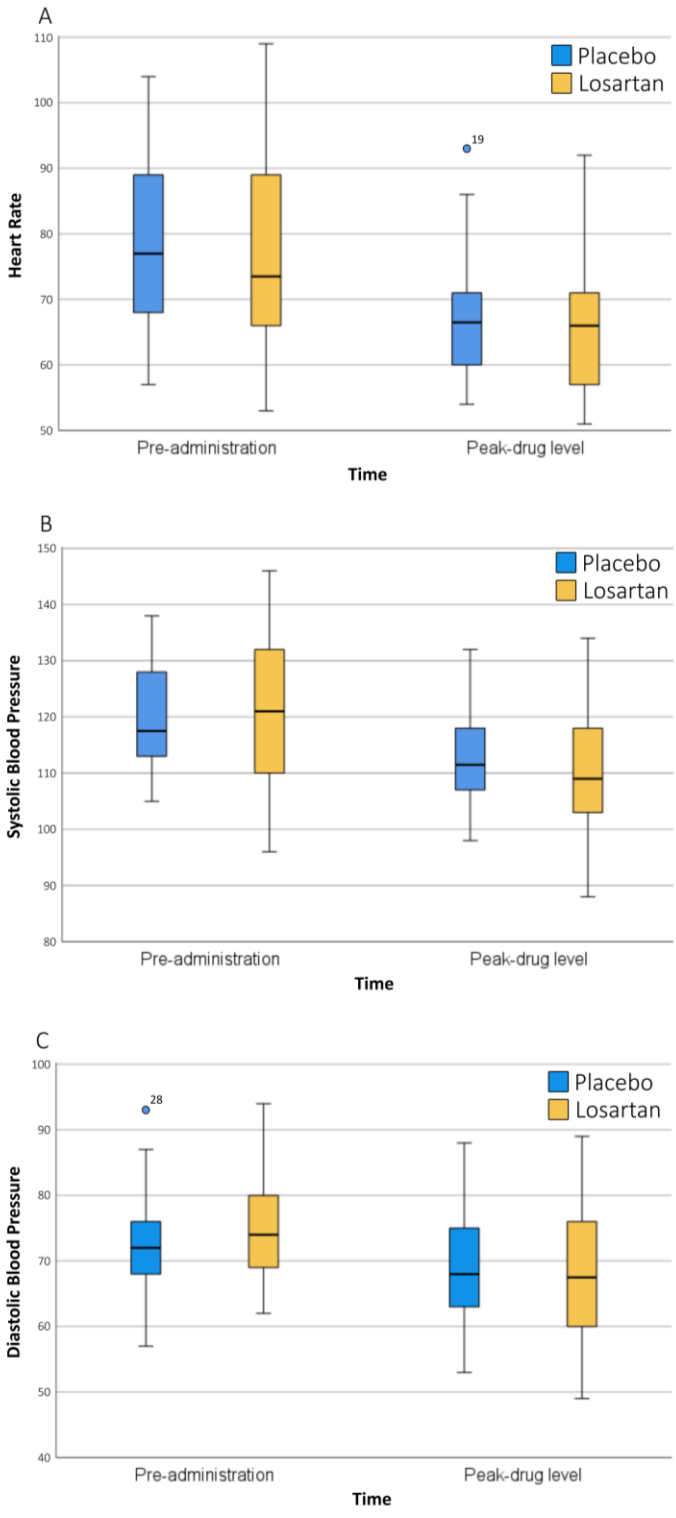


Figure 5 HR and BP Box Plots

Box plots for HR (A), systolic BP (B), and diastolic BP (C) at both time points. Blue indicates placebo, yellow indicates losartan. Labeled points are outliers (classified as above Q3 or below Q1 by at least 1.5 * IQR). Pre-administration measures were taken prior to drug administration, peak drug level measures were taken 45-minutes after drug.

A significant main effect of time was additionally found for the following visual analogue rating measures: Anxious, Stressed, Flushed, Tearful, Sad, Dizzy, Heart Racing, and Alert (all p<0.034). Each of these measures generally decreased over time except for the Dizzy measure, which increased.

A group x time interaction effect was significant for the Alert scale (p=0.009) where ratings for placebo participants decreased over time whilst losartan participants' ratings did not change. Further, a significant interaction effect (p=0.037) was also found for Heart Racing measures, with placebo ratings decreasing more over time than losartan ratings. Finally, a significant interaction for the Dizzy ratings (p=0.030) was found with increased ratings over time for losartan participants, whilst placebo participant ratings maintained a low level. (Table 7; Figure 6).

Measure	Factor	F Statistic	p value	η_p^2
Anxious	Time	12.21	0.001	0.196
Stressed	Time	13.50	<0.001	0.213
Flushed	Time	15.85	<0.001	0.241
Tearful	Time	5.11	0.028	0.093
Sad	Time	12.81	<0.001	0.204
Dizzy	Time	4.77	0.034	0.087
	Time x Group	4.97	0.030	0.090
Heart racing	Time	5.08	0.029	0.092
	Time x Group	4.61	0.037	0.084
Alert	Time	13.12	<0.001	0.208
	Time x Group	7.35	0.009	0.128

Table 7 Significant VAS ANOVA results

Summary of significant results for visual analogue ratings from ANOVAs. Significance level α=0.05.

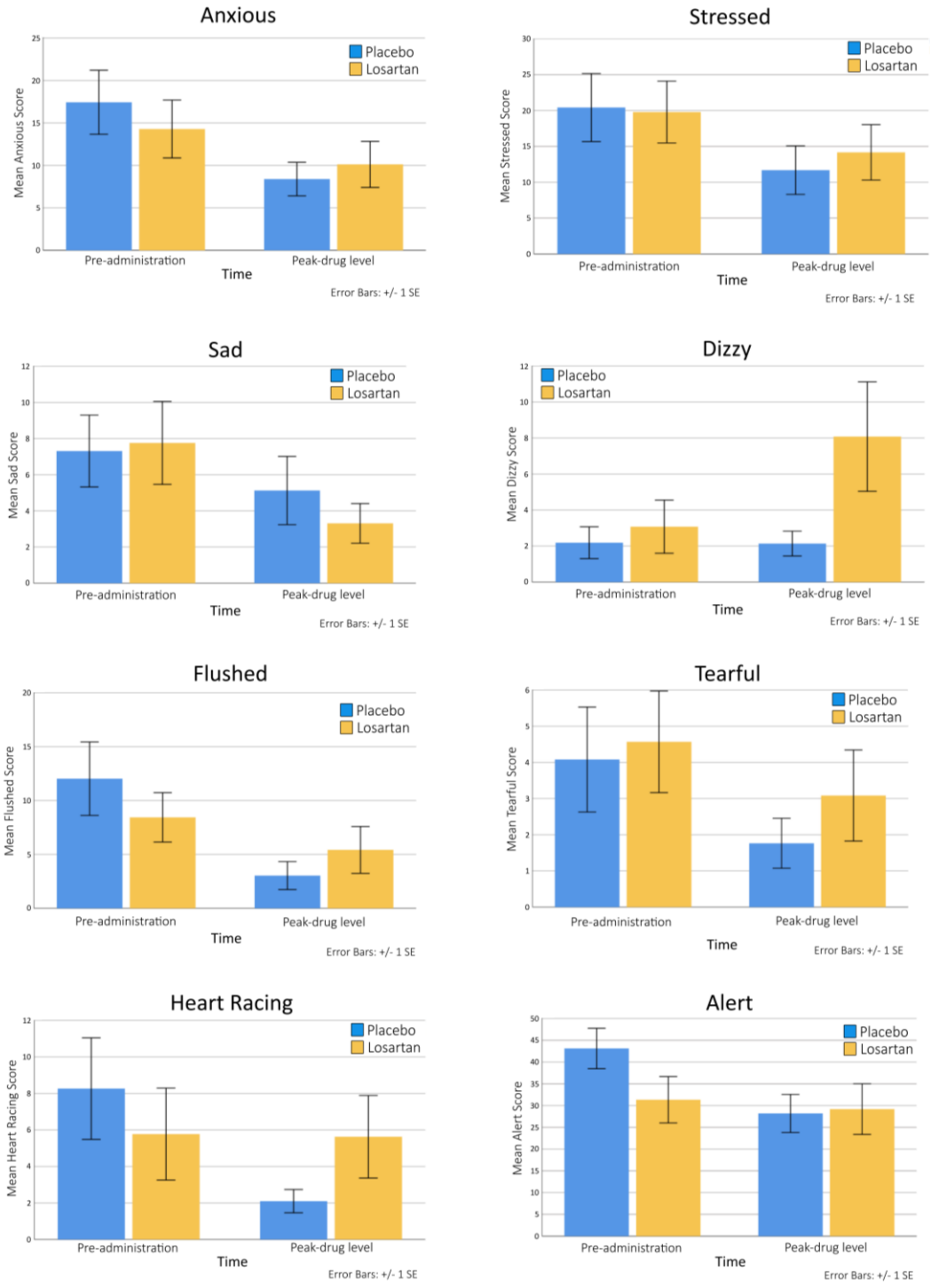


Figure 6 Bar Graphs for significant VAS measures

Bar graphs for VAS measures found significant during ANOVA. Each graph consists of the mean VAS ratings for each group (placebo, losartan), plotted for each measurement (pre-administration, peak-drug level). Error bars show +/- 1 standard error. Blue indicates placebo, yellow indicates losartan.

Blindness and Side Effects

Blindness was maintained on behalf of both the experimenter and the participants. The experimenter was equally likely to be correct for both placebo and losartan (% correct: experimenter: placebo=53.85%, losartan=53.85%; χ^2 (1) =0, p=1).

Although there was a significant difference for participants guessing the group allocation correctly (% correct: participants: placebo=80.77%, losartan=26.92%; χ^2 (1) =15.17, p<0.001), this reflects a bias in participant guessing patterns. Participants guessed placebo more often than losartan (76.9% of guesses were placebo; χ^2 (1) =15.08, p<0.001), and were therefore more likely to be correct when allocated to placebo. As participants were not more likely to be correct in their guesses generally than experimenters (χ^2 (1) =0, p=1), blindness was maintained.

A Fisher's exact test was run for side-effect comparisons due to small (<5) count sizes. A significant effect was found for the side effect dizziness (p=0.050), with losartan participants reporting higher rates of dizziness. No other significant effects were found.

Mood-related measures

Overall Effects

Regression weights for behavioural measures relating task environment features with mood states were combined to form compound measures (Table 2). The compound measures, calculated for each participant, reflect the effect of environment on mood generally (H1), on excitement (H1A), and on stress (H1B). These compound measures were then used in hypothesis testing, as described in analysis methods, to determine if task environment impacts participant mood. Data was split by group allocation for analysis.

One-sample Wilcoxon signed rank tests were conducted for each group's (placebo, losartan) compound measures linking task features with mood. Mood for both placebo ($M=0.903$, $Z=226$, $p<0.001$) and losartan ($M=0.643$, $Z=202$, $p=0.003$) participants was found to be significantly impacted by task-features, confirming H1 (Table 2).

Due to a significant main hypothesis, sub hypotheses were tested. Wilcoxon signed rank analysis revealed a significant effect of task-features on excitement (Table 2; H1A) for placebo ($M=0.121$, $Z=281$, $p=0.007$) but not for losartan ($M=0.080$, $Z=225$, $p=0.209$). A significant effect was additionally found for task-features on stress (Table 2; H1B) for both placebo ($M=0.362$, $Z=328$, $p<0.001$) and losartan ($M=0.419$, $Z=338$, $p<0.001$).

Mann-Whitney U tests were performed to determine whether the effects of task-features on mood significantly differed by group. The results indicated that group did not significantly impact mood overall ($U=173$, $p=0.232$) nor excitement ($U=295$, $p=0.431$), or

stress (U=377, p=0.475). Therefore, losartan did not impact the effects of task features on subjective mood ratings.

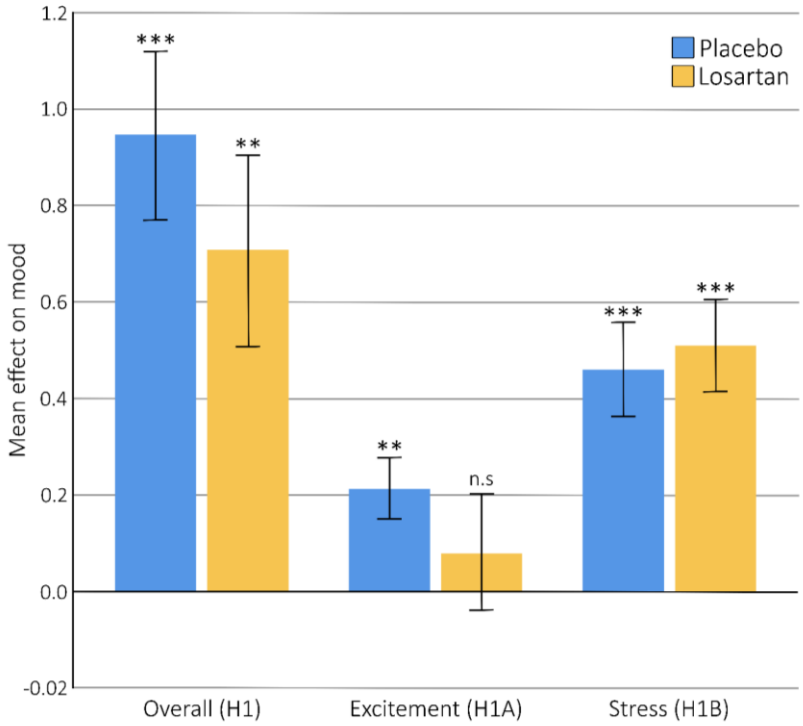


Figure 7 General effects of environment on mood

Mean values for the compound measure for each mood hypothesis are graphed for each group (placebo, losartan). Hypotheses and their component measures can be found in Table 2. Blue indicates placebo, yellow indicates losartan.

Error bars show +/- 1 Standard Error. n.s = non-significant, * p<0.05, ** p<0.01, *** p<0.001

Component effects

The components of these compound measures were further analysed using Wilcoxon signed-rank tests (Table 3). The effect of predator speed on excitement was found to be statistically significant for placebo (p=0.043) but not losartan (p=0.341). Predator speed, for both placebo (p<0.001) and losartan (p<0.001), was found to significantly affect participant stress. The number of check directions (number of segments into which the display is split) was found to significantly affect stress in both

placebo (p=0.003) and losartan (p=0.001). Maximum reward was found to significantly affect excitement in placebo (p=0.026) but not losartan (p=0.751) participants. Being caught by a predator significantly affected excitement for placebo participants (p=0.025), but not losartan (p=0.566), and significantly affected stress ratings in both the placebo (p=0.005) and losartan (p=0.005) group. All other tests were non-significant (p>0.101). A representation of this is shown in Figure 8.

Variable	Emotion	Group	N	Effect on Emotion	p-value
Predator Speed	Excitement	Placebo	26	0.106	0.043
		Losartan	26	0.017	0.341
	Stress	Placebo	26	0.329	<0.001
		Losartan	26	0.406	<0.001
Number of Check Directions	Excitement	Placebo	26	0.026	0.292
		Losartan	26	0.040	0.101
	Stress	Placebo	26	0.102	0.003
		Losartan	26	0.143	0.001
Maximum Reward	Excitement	Placebo	26	0.081	0.026
		Losartan	26	0.022	0.751
	Stress	Placebo	26	0.011	0.909
		Losartan	26	0.052	0.218
Minimum Reward	Excitement	Placebo	26	-0.057	0.182
		Losartan	26	-0.052	0.517
	Stress	Placebo	26	-0.018	0.585
		Losartan	26	0.091	0.107
Caught by Predator	Excitement	Placebo	21	-0.085	0.025
		Losartan	21	-0.027	0.566
	Stress	Placebo	21	0.101	0.005
		Losartan	21	0.158	0.005

Table 8 Summary of the effects of environmental factors on mood by group

Table shows the effects of each task environmental feature each mood (excitement, stress). Environmental variables form the basis for the compound measure relating task-features with mood (Table 2). Effect on emotion is equivalent to the mean for each measure. Significance level α=0.05.

Mann-Whitney U tests were conducted to compare the impact of each task-feature on mood (stress, excitement) between losartan and placebo group (Table 9). There were no significant differences between the impact on mood for losartan compared with placebo.

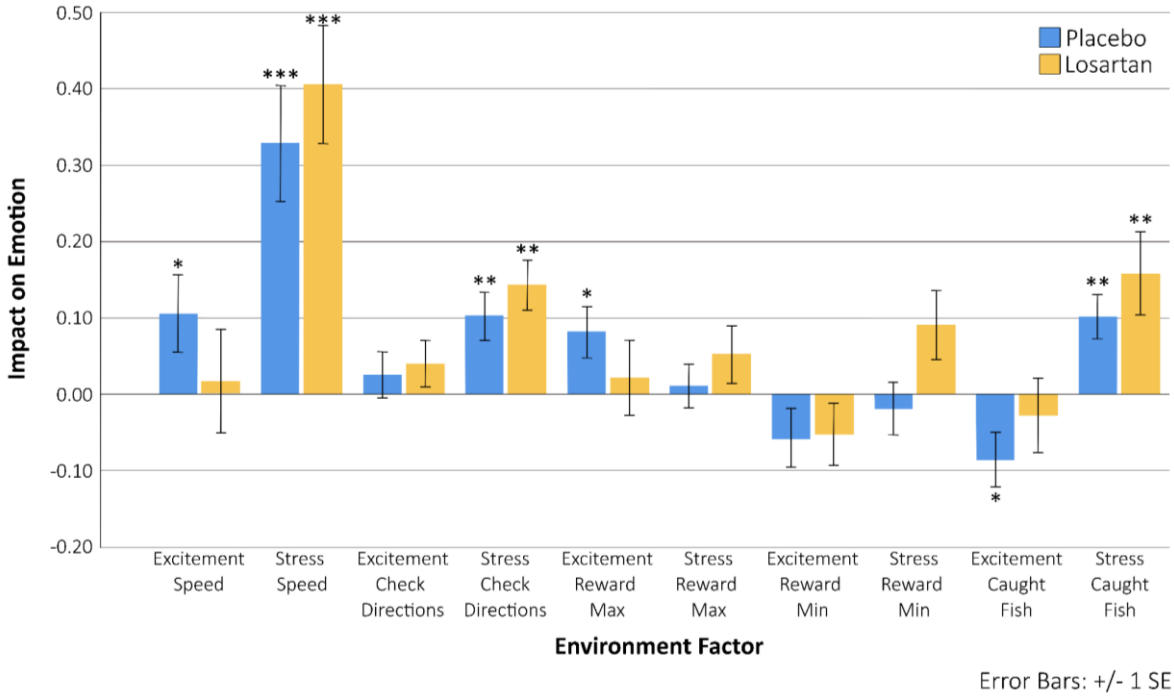


Figure 8 Impact of component environmental factors on mood by group

Bars show the effect of each environmental factor on excitement or stress. Blue indicates placebo, yellow indicates losartan. Error bars indicate +/- 1 standard error.
* p<0.05, ** p<0.01, *** p<0.001

Variable	Emotion	U Statistic	N	p-value
Predator Speed	Excitement	304	52	0.534
	Stress	377	52	0.475
Number of Check Directions	Excitement	367	52	0.596
	Stress	409	52	0.194
Maximum Reward	Excitement	274	52	0.241
	Stress	384	52	0.400
Minimum Reward	Excitement	355	52	0.756
	Stress	437	52	0.070
Caught by Predator	Excitement	270	42	0.213
	Stress	238	42	0.660

Table 9 Mann-Whitney results summary

Summary for Mann-Whitney U tests comparing the effects of environmental variables on mood for placebo and losartan. Significance level α=0.05.

Vigilance and threat-related worry measures

Regression - Full model

A multiple linear regression model was conducted as defined in the pre-registration (see Annex 1: Pre-registration document):

Compound Measure ~ Group + Gender + Pre-drug Systolic BP + Pre-drug Diastolic BP + Weight + Age + Education

Primary outcome measures

For the compound measure related to trait somatic anxiety (STICSA; Table 3) the overall regression was statistically significant (R^2=0.310, F(7,44)=2.821, p=0.016). The predictor variables systolic (β=0.081, p=0.019) and diastolic blood pressure (β=-0.098, p=0.003) were found to significantly predict behavioural markers of anxiety (anxiety-related compound measure). Group was not a significant predictor (p=0.199). All other predictors were non-significant.

Secondary outcome measures

For the machine-learning derived measures related to trait anxiety (STICSA) neither the regression for somatic anxiety nor cognitive anxiety was significant (trait somatic anxiety without debrief: R^2=0.124, F(7,44)=0.893, p=0.520; trait cognitive anxiety without debrief: R^2=0.109, F(7,44)=0.766, p=0.619).

Regression – Exploratory Analyses

The full pre-registered model above includes 8 parameters including the intercept. With an increasing number of predictors there can be issues of overfitting the data. Estimates for the minimum sample size required for a given number of predictor variables vary between 8-20 per predictor but a general rule-of-thumb is around N=10 per predictor (Harrell, 2015). Thus, additional regression models were run removing excess predictors. Gender was maintained as a predictor as this was used as a stratification factor during drug allocation. Further, due to the results of the control analyses diastolic BP was also maintained in models for consideration.

Correlations between group, gender, systolic BP, diastolic BP, weight, age, and education were conducted to aid in model choice. Highly correlated predictor variables may introduce issues with interpretability and collinearity and add little unique predictive value to models. Models were then devised based on correlational information, prior knowledge of the biological relevance and relationships of predictor variables, and results from the control analyses. The resultant correlations are presented in Figure 9.

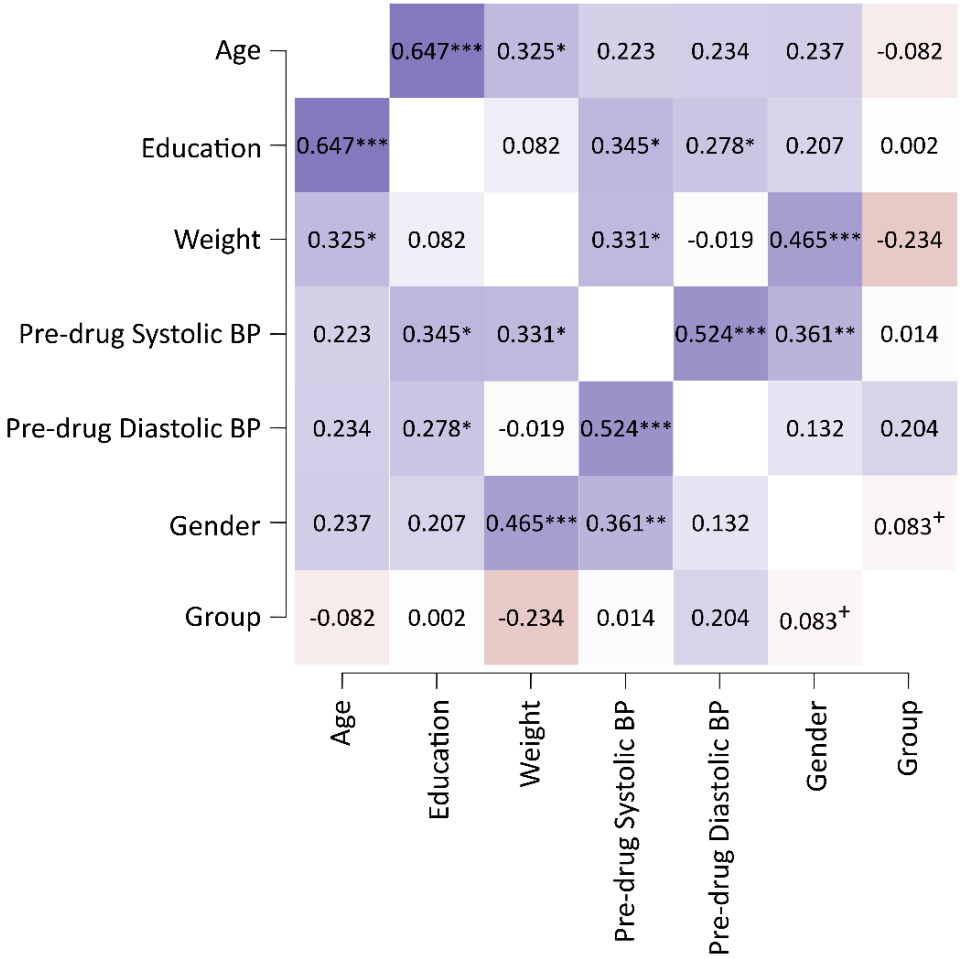


Figure 9 Pearson's R correlation heatmap for predictor variables

Pearson's R heatmap showing correlations between predictor variables. Red indicates a positive association and blue indicates a negative association.

* p<0.05, ** p<0.01, *** p<0.001. + value is Chi-Squared value.

Weight was the only additional predictor included for regression analysis. This was chosen due to the prior knowledge that each participant received the same dose of losartan, not scaled doses. Despite a significant correlation with gender ($r(50)=0.465$, $p<0.001$), it was felt this was an important parameter to maintain. Collinearity diagnostic checks were run to ensure this would not affect the interpretability of any results. Age was discarded as it added little predictive value to the models.

The following multiple linear regressions were then applied for each compound measure:

1. Reduced model:

Compound Measure ~ Group + Gender + Weight + Pre-drug Diastolic BP

2. Interaction model:

Compound Measure ~ Group +Gender + Weight + Pre-drug Diastolic BP + Interaction (group X Pre-drug Diastolic BP)

A group x pre-drug diastolic BP interaction was added as this was found to be significant in the control analysis. These were classified as exploratory as they were additional regression analyses that were not pre-registered.

Primary outcome measures

The reduced model (1) multiple linear regression was statistically significant (R^2=0.213, F(4,47)=3.181, p=0.022). Diastolic blood pressure was found to be a significant predictor of behavioural markers of anxiety (β=-0.057, p=0.032). Group was not a significant predictor (p=0.180). All other predictors were non-significant.

The interaction model (2) multiple linear regression was statistically significant (R^2=0.286, F(5,46)=3.687, p=0.007). Diastolic blood pressure (β=-0.226, p=0.008) and the group x diastolic BP interaction (β=0.110, p=0.035) were found to be significant predictors for behavioural markers of anxiety (anxiety-related compound measure). Group was not a significant predictor (p=0.160). All other predictors were non-significant.

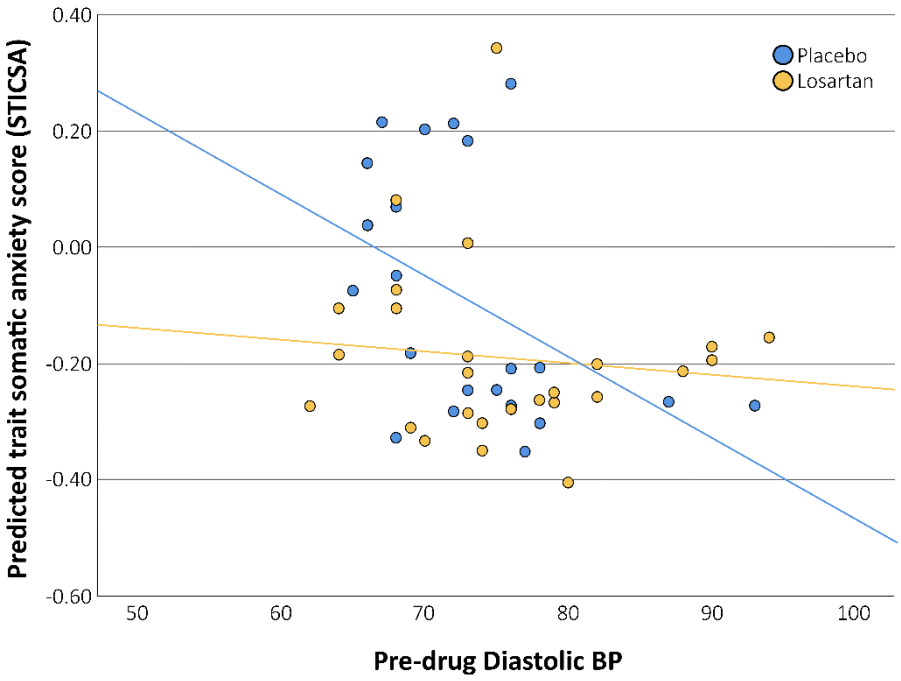


Figure 10 Diastolic BP plotted against anxiety-related compound measure

"Predicted STICSA score", the compound measure related to vigilance behavior and threat-related worry, plotted against pre-drug administration diastolic BP. Blue indicates placebo, yellow indicates losartan. Plotted trend lines illustrate general relationships.

Secondary outcome machine-learning derived measures

The reduced model (1) regression for trait somatic anxiety (without debrief) was insignificant (R^2=0.117, F(4,47)=1.557, p=0.201). The interaction model (2) regression for trait somatic measures (without debrief) was also insignificant (R^2=0.121, F(5,46)=1.262, p=0.296).

Both regressions with machine-learning derived estimates for trait cognitive anxiety measures (computed without task debrief) were also non-significant [Reduced model (1): R^2=0.075, F(4,47)=0.947, p=0.445; Interaction model (2): R^2=0.075, F(5,46)=0.743, p=0.595.]

A full summary of these results is shown in Table 10.

Outcome Measure	Related Clinical Measure	Regression Model	R^2	F value	p-value	Significant Regressors
Compound measures (Primary)	Trait Somatic Anxiety (STICSA)	Full	0.310	2.821	0.016	Systolic BP Diastolic BP
		Reduced	0.213	3.181	0.022	Diastolic BP
		Interaction	0.286	3.687	0.007	Diastolic BP Diastolic x Group Interaction
Machine-learning derived measures (Secondary)	Trait Somatic Anxiety (STICSA)	Full	0.124	0.893	0.520	-
		Reduced	0.117	1.557	0.201	-
		Interaction	0.121	1.262	0.296	-
	Trait Cognitive Anxiety (STICSA)	Full	0.109	0.766	0.619	-
		Reduced	0.075	0.947	0.445	-
		Interaction	0.075	0.743	0.595	-

Table 10 Regression results summary

Summary of regression results. Only significant predictor variables are listed, all other entered predictor variables were non-significant.

Component Behavioural Measures

Components measures, that form the basis for the compound anxiety-related measure (Table 3), were split by group (placebo, losartan) for analysis. Independent samples t-tests comparing groups were insignificant for all behavioural measures ($p>0.064$). This component analysis was conducted to allow a deeper understanding of how significant predictors in the regression models may relate to the compound measure ("predicted STICSA score"). Means of each component measure are plotted in Figure 11.

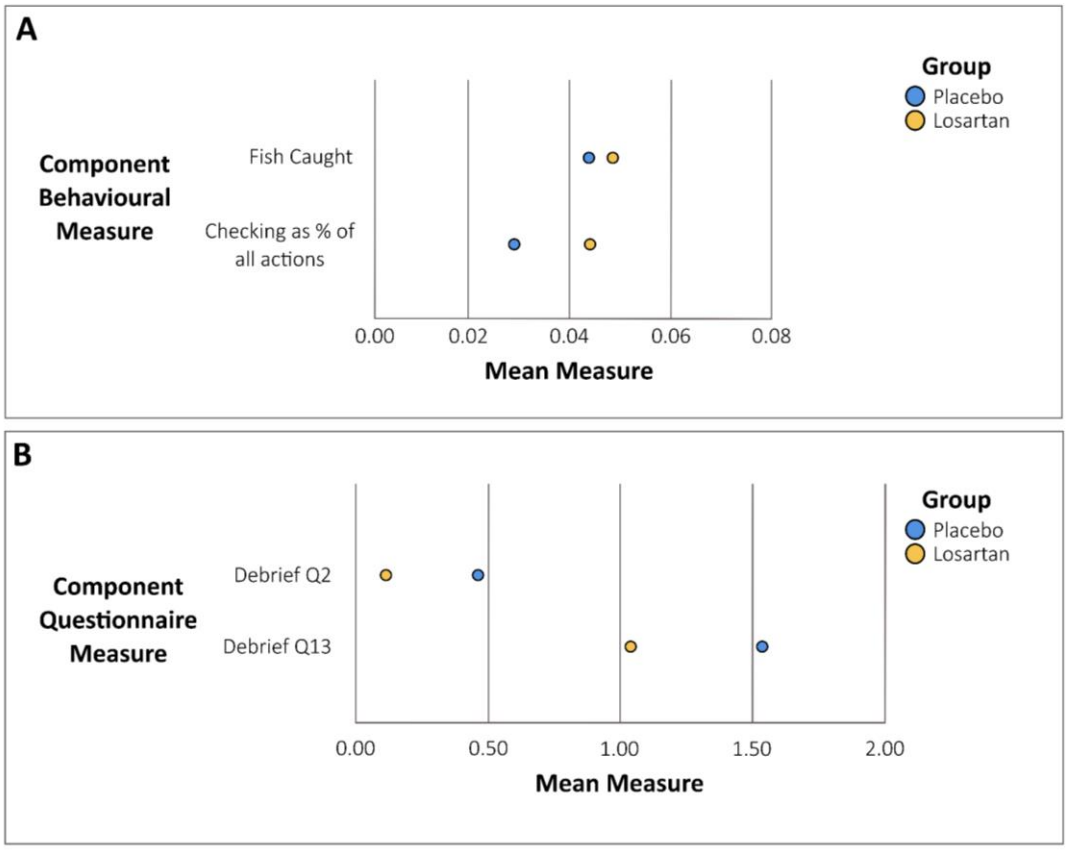


Figure 11 Component Behavioural Measures for vigilance and threat-related worry compound measure

Means for the component behavioral (A) and questionnaire (B) measures that form the basis for the vigilance and threat-related worry compound measure (previously related to STICSA).

Post-hoc exploratory results

A single participant was highlighted as having a larger than expected change in diastolic BP whilst graphing results. This point was classified as an extreme outlier when compared to all diastolic blood changes together, but only a standard outlier when this was split by group. This potential outlier has been maintained in the previous analysis as we cannot rule out the possibility this is a true effect. However, as exploratory analysis, a subset of analyses were run without the inclusion of this data point.

A t-test comparing the change in diastolic blood pressure (pre-drug minus post-drug) across groups was significant both with ($t(50)=-2.42$, $p=0.019$) and without ($t(49)=-2.17$, $p=0.035$) the potential outlier. Losartan participants therefore had a significantly greater decrease in diastolic blood pressure over time (Mean = 7.00, no outlier) than placebo (Mean = 3.81).

The reduced (1) regression model was significant ($R^2=0.253$, $F(4, 46)=3.900$, $p=0.008$). Pre-drug diastolic blood pressure ($\beta=-0.058$, $p=0.019$) and intercept ($\beta=-0.080$, $p=0.042$) were both significant predictors of the "predicted STICSA score", otherwise referred to as our anxiety-related compound measure. All other predictors, including group ($p=0.058$), were non-significant.

The interaction (2) regression model was additionally significant ($R^2=0.335$, $F(5, 45)=4.525$, $p=0.002$). Pre-drug diastolic blood pressure ($\beta=-0.225$, $p=0.004$), intercept ($\beta=-0.082$, $p=0.029$), group ($\beta=-0.096$, $p=0.046$), and diastolic x group interaction ($\beta=0.109$, $p=0.023$) were significant predictors for behavioural markers of anxiety. All other predictors were non-significant.

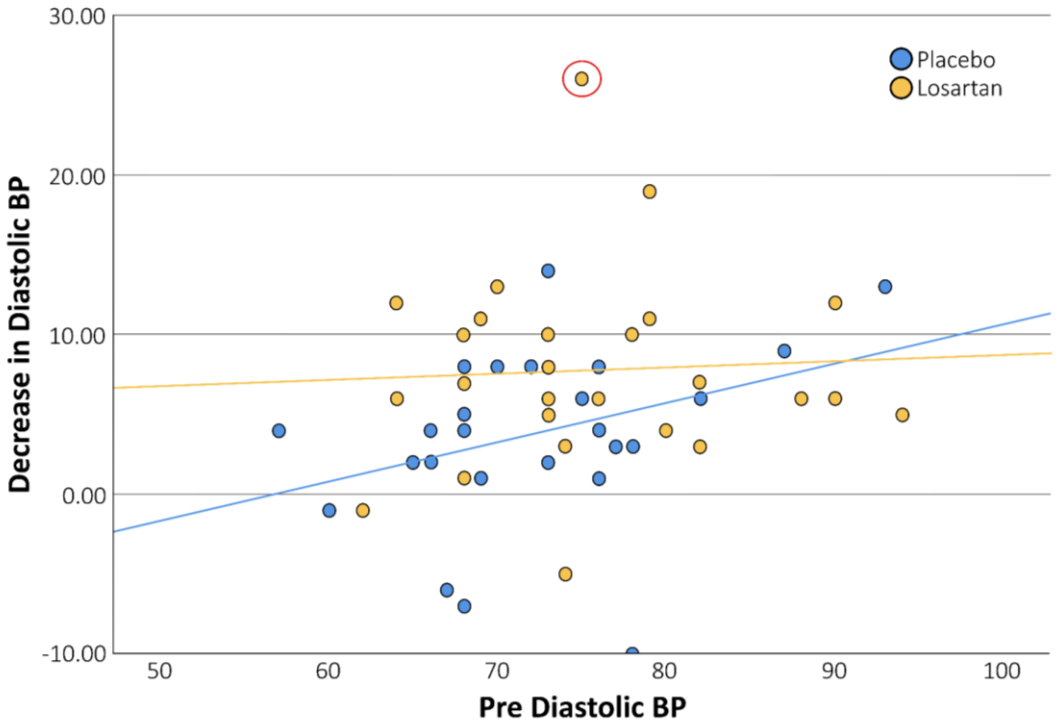


Figure 12 The relationship between pre-drug diastolic BP and change in BP over time

Pre-drug administration diastolic BP is plotted against the decrease in diastolic BP between pre-drug administration and peak-drug level measurements. Blue indicates placebo, yellow indicates losartan. Plotted trend lines illustrate general relationships. Red circled point highlights the potential outlier that was removed for the post-hoc exploratory analysis.

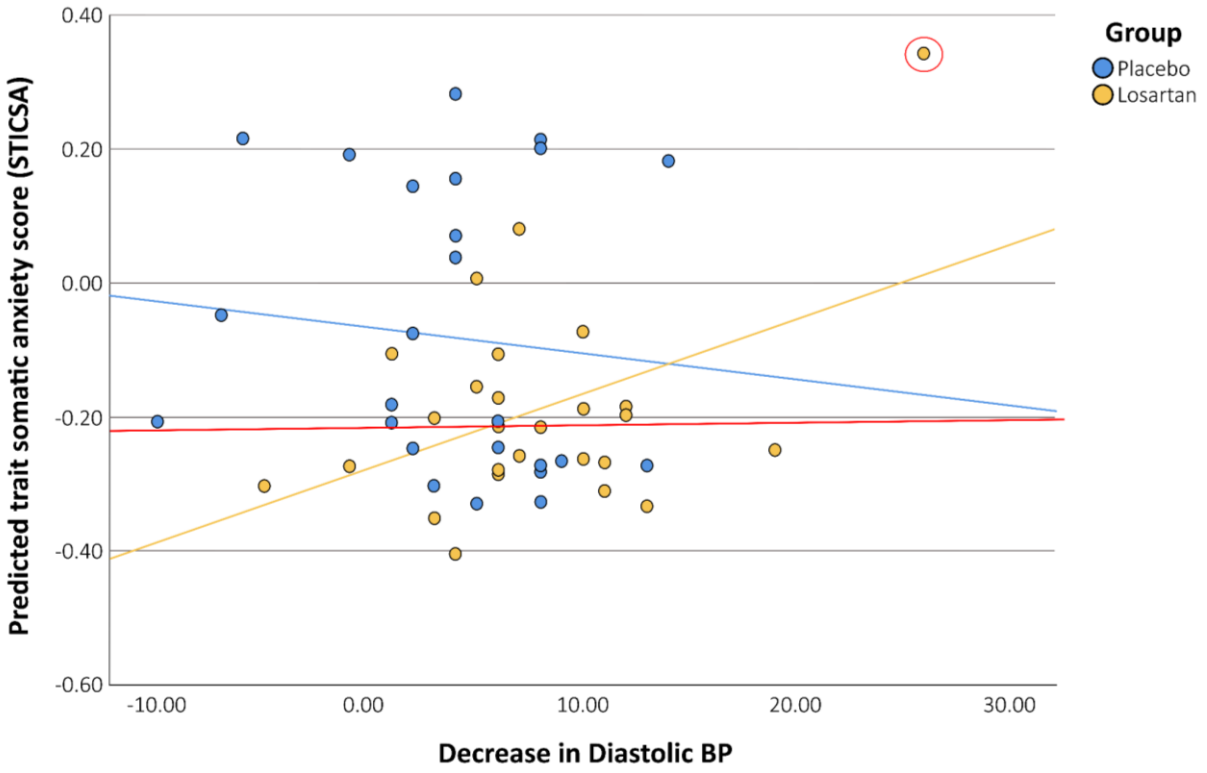


Figure 13 The relationship between change in diastolic BP and "predicted STICSA score"

Change in diastolic BP (between pre-drug and peak-drug measurements) is plotted against the "predicted STICSA score" compound measure. This compound measure relates to vigilance behavior and threat-related worry. Blue indicates placebo, yellow indicates losartan. Plotted blue (placebo) and yellow (losartan) trend lines illustrate general relationships. Red circle indicates the potential outlier that was removed for the exploratory analysis. Red line illustrates the relationship for losartan data without the potential outlier.

Discussion

We used the Gamified Foraging Task to determine how losartan alters threat-related behaviour in healthy participants. A compound measure (Table 3), previously related to STICSA scores in the previous study (Trier et al., 2023), was regressed against group and other potential predictors. We found that this anxiety-related compound measure was predicted by pre-drug administration diastolic BP. Our findings suggest that changes in diastolic blood pressure may underpin this relationship, such that decreases in blood pressure may decrease anxiety-related behaviours. In a further post-hoc analysis, we found that group also predicted behavioural measures of anxiety. We therefore suggest that losartan may act anxiolytically to reduce threat-related worries and increase vigilant checking behaviours.

An additional aim of this study was to determine the validity of the Gamified Foraging Task for use in smaller laboratory studies. For this, we replicated the previous studies hypotheses testing analyses investigating how environmental factors impact mood. Our findings replicate that task environment impacts both stress and excitement mood ratings (Trier et al., 2023), and suggest this task may therefore be useful for investigating mood in future studies.

Control analyses

The interpretation of these results is somewhat complicated by the significant results for diastolic BP. The significant diastolic BP decrease between pre-drug and post-administration, as additionally found for systolic BP and HR (Table 6), may reflect participants relaxing into the testing session. This is supported by the VAS “Anxious”

measure which additionally significantly decreased over time. This decrease differs significantly depending on group, suggesting an acute effect of the drug which may induce response bias. This drop in diastolic BP may have additionally increased feelings of dizziness for losartan participants, with higher VAS ratings and greater reporting of dizzy side effects. To account for these effects, pre-drug diastolic blood pressure was included in regression analysis.

The significant decreases over time for VAS “Flushed”, “Sad”, “Stressed”, and “Tearful” measures may additionally reflect participants relaxing into the session (Table 7). “Heart Racing” ratings decreased over time for placebo but did not significantly change for losartan participants. This decrease for placebo but not losartan suggests the drug may marginally increase feelings of a racing heart. This result is unexpected given the decrease in HR over time found in both groups as demonstrated by the significant HR ANOVA result.

Placebo participants’ ratings for “Alert” scales decreased between pre- and post-drug, but losartan participants’ ratings did not change. Thus, losartan may counteract a decrease in alertness over time.

Task features impact mood ratings

Studies with clinical patients often include small sample sizes with testing occurring in-person in controlled laboratory settings – thus one aim of this study was to characterize the feasibility and sensitivity of the Gamified Foraging Task under these conditions. This analysis aimed to replicate the findings from Trier et al. that task-features impact participant mood ratings for both stress and excitement. Further, as this current study differs from the one previous due to the inclusion of a drug allocation, we were particularly interested in how losartan may alter the relationship between environment and mood.

Firstly, looking at placebo participants only, our results (Figure 14A) replicate the finding from Trier et al. 2023 that mood ratings are impacted by task features. This effect was replicated for both stress and excitement. Further, individual environment factors impacted mood with the same directionality as found in the previous study (Figure 14B). Faster predator speed, increased number of directions to check, and increased maximum available reward were all linked to increased excitement and stress ratings whilst increasing reward minimum decreased excitement and stress ratings. Our results additionally replicate the finding that being caught by a predator impacted excitement and stress in opposite directions, with excitement ratings decreasing and stress ratings increasing after being caught. The pattern of task impact on each emotion is very similar to the previous study, despite some differences in the pattern of significance. This is likely due to the smaller sample size, leading to a larger variability. Despite these differences, these findings suggest that the Gamified Foraging Task is sufficiently sensitive to replicate these effects in smaller scale studies. The task could therefore be used in future studies,

with different manipulations, to investigate changes in subjective stress and excitement ratings.

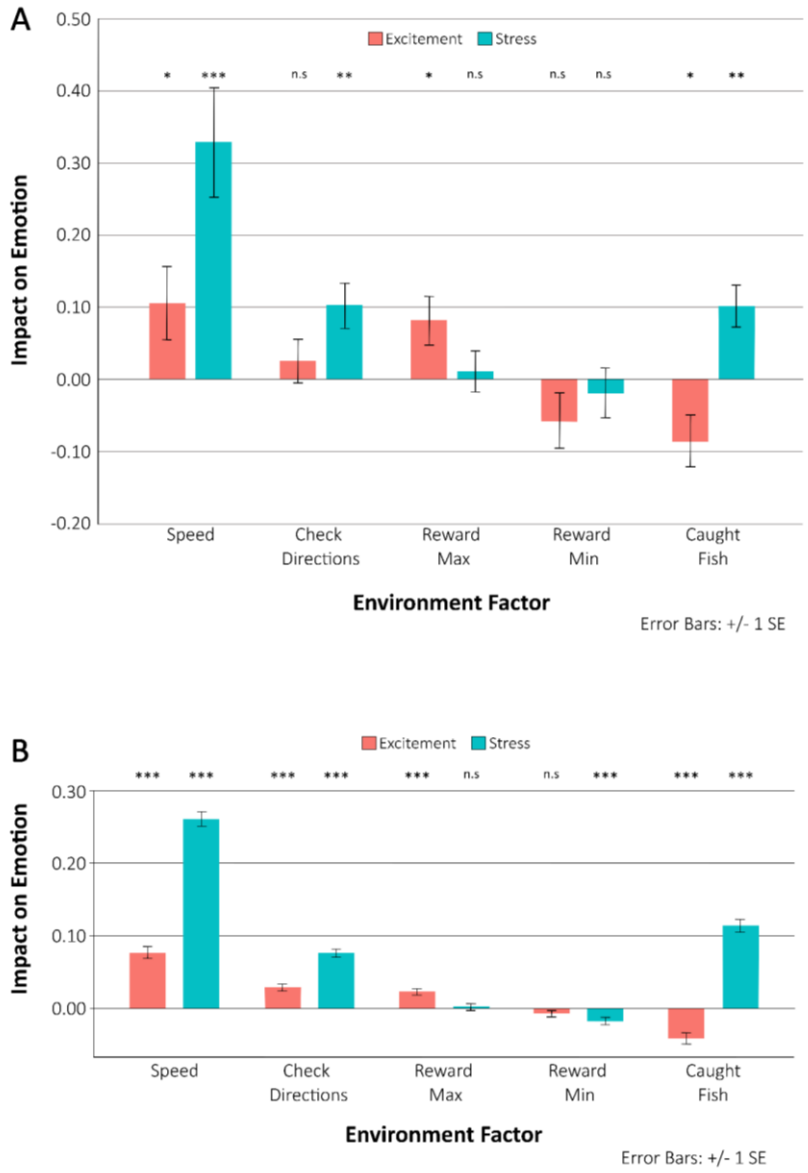


Figure 14 Comparison of placebo results with results of previous study (Trier et al. 2023).

Bar charts show the impact of each task environmental factor on each emotion for the placebo group from this current study (A) and the previous study by Trier et al. (2023) (B).

In the current study, losartan did not significantly modulate the effects of task-features on mood. Although all statistical comparisons between placebo and losartan were non-significant ($p>0.070$), examining the differences between groups highlights some potential drug-induced changes. Firstly, minimum reward appears to affect stress in opposite directions for losartan vs placebo. Secondly, comparing the impact of task-features on excitement for placebo vs losartan suggests losartan may have a slightly dampening effect. The impact on excitement of increased predator speed, maximum reward, and being caught by a predator are all decreased for losartan participants such that the impact is significant for placebo but non-significant for losartan. Furthermore, the overall hypothesis test for the impact of task factors on excitement is significant for placebo (H1A: $p=0.007$) but not losartan (H1A: $p=0.209$). Though this difference was statistically non-significant, it could reflect decreased arousal in losartan participants. There is little research on the effect of losartan on subjective feelings of excitement and, given the non-significance of the comparison effects between placebo and losartan, this potential dampening effect of losartan on excitement ratings should be considered carefully and as merely one possible interpretation. Further research investigating the effects of losartan on a range of subjective mood measurements would be needed before this interpretation could confidently be made.

Decreases in blood pressure increase vigilance and reduced threat-related worry

Trier et al., (2023) found that trait somatic anxiety impacted participants vigilance behaviour, such that higher anxiety predicted reduced vigilance via fewer checking actions. As losartan has been shown to have an anxiolytic effect (for review: Chrissobolis et al., 2020; see Losartan and Anxiety section), we were interested in how losartan may impact these behaviours and predicted that losartan participants should more closely resemble participants with low somatic anxiety.

A compound measure, which combined task behaviours relevant to vigilance, represented a "predicted STICSA score" which we regressed against a range of predictor variables. Pre-drug diastolic BP was found to be a significant predictor for "predicted STICSA score" in all 3 regression models. Higher pre-drug diastolic BP predicted a lower STICSA score (Figure 10) therefore suggesting they demonstrated increased vigilance behaviour and decreased threat-related worry, similar to those with lower trait anxiety in the previous study.

Generally, those with a higher diastolic blood pressure pre-drug, experienced the greatest decrease in diastolic BP over time (Figure 12). Blood pressure has been shown to be linked with anxiety such that acute increases in anxiety increases blood pressure (Lacy et al., 1995; for review: Huang et al., 2013), blood pressure decreases accompany reductions in anxiety during a relaxation intervention (Feinstein et al., 2018), and higher trait anxiety is associated with blood pressure elevations (Ifeagwazi et al., 2018). Furthermore, stress, a factor impacting the precipitation and maintenance of anxiety, has

additionally been associated with increases in BP (Gasperin et al., 2009; Puzserova & Bernatova, 2016; Tomitani et al., 2021). Further, decreases in diastolic BP have previously been associated with increased subjective ratings for wellbeing (Wiklund et al., 1997). Lower blood pressures therefore reflect calmer, low-anxiety states. Decreases in diastolic BP may therefore induce a calmer cognitive state for the participant, such that they engage in fewer anxiety-related behaviours, leading to a reduced "predicted STICSA score" compound measure. The role of pre-drug diastolic BP in predicting behaviour in regression models may therefore be due to the BP change it represents, rather than the baseline measure itself.

Losartan participants experienced a greater diastolic BP decrease on average than placebo. This additional decrease in diastolic BP for losartan participants is likely an acute effect of the drug, as supported by the significant diastolic BP x group interaction in both control and regression analyses. The decrease in BP may contribute to lower predicted STICSA scores as suggested above, however these scores remain lower for losartan when matched with placebo for blood pressure change (Red trend line: Figure 13). This suggests a specific anxiolytic drug effect, as supported by the significant effect of group found during post-hoc analysis.

As "predicted STICSA score" acts as a proxy measure for task-behaviour, a reduced STICSA score can be interpreted in regard to behavioural responses. Reduced "predicted STICSA scores" suggest losartan participants are less sensitive to threat-related worry and act more vigilantly. This is somewhat reflected in the component behavioural measures (Figure 11). Although these component behavioural measures that constitute the

predicted STICSA score did not significantly differ between groups, exploring the data still provides some insight into how predicted STICSA score varied with treatment.

Trier et al. 2023 found that participants with higher somatic anxiety were less vigilant and experienced greater threat-related worry, as reflected by their reduced number of checking actions, as well as rating higher for debrief questions 2 and 13. In this study, losartan may be reducing anxiety surrounding the predator's arrival such that they experience fewer threat-related worries, as suggested by lower ratings on post-task debrief questions (Figure 11B). Further, losartan has been speculated to decrease threat avoidance (Reinecke et al., 2018). As participants need to actively search the environment for threats during the task, checking actions may elicit fear due to the anticipation of confrontation with a threat. Losartan may reduce this fear response, resulting in reduced threat-avoidance. Participants therefore more readily attend to incoming predators, as seen through increased checking and vigilance behaviour (Figure 11A). This suggests losartan participants in our study more closely resemble lower somatic anxiety individuals and supports an anxiolytic action for losartan. However, the lack of significant differences when comparing individual component measures, suggests the task may not be sensitive enough to pick up these effects in this smaller scale study.

Finally, we cannot ignore the possibility that the participant removed during the post-hoc exploratory analyses represents a real effect. At this small dose, it is unusual to see blood pressure effects; the large change in diastolic blood pressure observed for this participant may represent a specify sensitivity to the blood pressure effects of losartan. This participant may belong to a subset of individuals for whom losartan would have no anxiolytic effect. Further, building on the suggestions above, this participant may have

interpreted such a large change in BP as a worrying side-effect of the drug, inducing anxiety or panic.

Determining how losartan effects vigilance and avoidance under threat for anxious individuals provides a good translational framework for its use in exposure therapy. As individuals are confronted with their fears, and need to actively attend to them, a positive influence of losartan to decrease threat-avoidance could improve the outcomes for exposure therapy. This reduced threat-avoidance may increase opportunities for inhibitory learning, and therefore improve or accelerate responses to exposure treatment.

Limitations

As previously mentioned, the effect of losartan on diastolic blood pressure makes the interpretation of these results somewhat difficult. This finding is unusual as peripheral drug effects on blood pressure are not usually observed at this lower losartan dose. Though this study tries to account for blood pressure through its inclusion in regression models, this factor was not controlled during analysis of effects on mood.

Moreover, the sample size for this study may be too small for detecting true effects in this task. The removal of a single participant producing changes to analyses outcomes suggests the effects reported are small such that they are not reliably demonstrated in the presence of one outlier. Further research investigating on what scale the Gamified Foraging Task can detect changes in compound measures would be necessary to determine if this study sample is too small to detect these effects.

Further, the previous study on which much of the pre-processing and analysis relies, uses a much larger sample and has not previously been run on smaller scales. This limits the interpretability of our losartan results as there is little precedent set for how placebo participants should behave under these lab conditions, within a smaller sample. The Gamified Foraging Task was additionally adapted for use in this study, with changes to the task formatting, length, and the removal of monetary reward for good performance. These changes may further impact the validity of using regression models from the previous study for compound measures in this study. Moreover, changes in motivation due to the lack of monetary incentive may have impacted task behaviour. As this task has not previously been used without monetary incentives for good performance, we cannot quantify how this may have impacted participant actions. Participant behaviour may have begun to reflect the minimal effort required to finish the task rather than true decision-making behaviours. Although pre-processing steps were taken to remove participants with poor task quality, decreased motivations and subsequent changes in behaviour that impact result interpretations cannot be fully ruled out.

Additionally, the omission of the STICSA in this study as an anxiety measure prevents further control analyses which could have been useful for checking that the models used for compound measures act as expected within this sample and environment.

Finally, many of our results were insignificant, even those which are replication analysis of the previous independent study (Trier et al., 2023). This could be due to a reduced sensitivity of the task when used with smaller samples. The interpretation of

these results should therefore be carefully considered as it relies upon several assumptions.

Future Directions

To build upon this current work, further studies with the inclusion of STICSA measurements, a range of anxiety scores, or a stratification for low- and high-trait anxious individuals would expand our understanding of how vigilance behaviours are demonstrated in this task, and how losartan may alter task outcomes. Future studies investigating the efficacy of the Gamified Foraging Task for use in both non-clinical and clinical smaller scale studies should be conducted with the aim of characterising how this task translates for in-person laboratory studies. Validation for models of task behaviour in such studies would provide greater confidence that any effects of drug manipulations reflect real changes in behaviour. Further, a high-trait anxious or a clinical sample may provide a clearer picture of the effects of losartan within this task as it is possible the sensitivity of this task to these potentially small effects cannot be detected with the current task format and sample. Indeed, a replication of this study with high-trait anxious individuals could provide additional support to the findings of Trier et al., as well as provide insight into how losartan may alter these anxiety-related behaviours. This would additionally be of great relevance when considering the use of losartan for exposure therapy for anxiety. Finally, research investigating changes in blood pressure with losartan and how this influences mood, interoception, and threat-related behaviours would be of further benefit. Adding this to the current body of work, would allow for a more critical interpretation of these findings.

Conclusions

In summary, this study successfully replicated the result that task environment impacts subjective excitement and stress (Trier et al., 2023). Losartan had no impact on this relationship between environment and mood. This demonstrates that the Gamified Foraging Task can be used on a smaller laboratory scale to detect the impacts of task features on mood. In future studies, this task could be used to investigate the effects of manipulating environmental features on this relationship between environment and mood, and how different pharmacological agents affect this relationship.

Blood pressure was found to predict task-behaviour. Increased pre-diastolic blood pressure was correlated with increased threat-related worry and decreased vigilance behaviour, as demonstrated via increases in our proxy compound measure, “predicted STICSA score”. By considering the relationship between pre-diastolic BP and the change in BP over time, decreases in BP may be the true predictor for this effect such that greater decreases in BP lead to decreased compound scores via reductions in anxiety-related behaviour. Blood pressure decreases may therefore underpin reductions in threat-related worry and increases in checking actions. Losartan may have an additional anxiolytic effect, distinct from its impact on BP. Post-hoc removal of one data point, revealed a potential effect of group on vigilance and threat-related worry. Losartan may thus decrease worry and increase vigilant checking behaviour. This may be due to a decrease in threat-avoidant behaviour. Further studies are needed to confirm the effect of losartan on checking behaviour and determine its influence. Additionally, further research is necessary to dissect the relative contribution of increased vigilance or decreased avoidance to these results, as they rely on different cognitive mechanisms.

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Appendices

Appendix A

Recruitment basic eligibility questions

- PHONE/EMAIL SCREENING	Date: ☐☐ ☐☐ ☐☐	Researcher:

General inclusion and exclusion criteria * If any grey shadowed boxes are ticked then the patient may not be eligible for the study.

		Y	N
1	Is the participant aged 18 – 50 years?	☐	☐
2	Light or non-smoker (< 5 cigarettes per day)?	☐	☐
3	Has the patient taken CNS-active medication (e.g. antidepressants) during the last 6 weeks?	☐	☐
4	Has the patient taken steroid medication?	☐	☐
6	Current or past psychosis, depression, substance abuse?	☐	☐
6	First-degree family member with history of psychiatric disease?	☐	☐
7	History serious or relevant illness (e.g. heart, lungs; epilepsy, intravascular fluid depletion)?	☐	☐
8	BMI 18-30 (kg/m²)?	☐	☐
9	Does the patient have sufficient English skills?	☐	☐
10	If female: Is the patient pregnant or breast-feeding?	☐	☐
11	Participation in medication studies in last 6 weeks?	☐	☐

These questions were asked via Qualtrics survey or email to assess basic eligibility prior to invitation for full screening. During the screening session, these questions were repeated to ensure participants' eligibility before commencing the longer screening session.

Appendix B

Medical screening questionnaire

MEDICAL SCREENING FORM	Date: ☐☐ ☐☐ ☐☐	Signature Medic:

MEDICAL SCREENING		
Height: ___ ___ ___ cm	Smoking: ___ ___ cig/ day	Illegal drugs last 3 m: ☐ NO ☐ YES
Weight: ___ ___ ___ kg	Drinking: ___ ___ units/ week	Allergies/asthma (esp. lactose): ☐ NO ☐ YES .....
How is your general health?		
Ever had a serious medical problem (heart, lungs), or an operation?		
Current intravascular fluid depletion/ dehydration diagnosis?		
Currently suffering from any medical conditions, e.g. asthma, diabetes, migraine, epilepsy, high BP?		
Have you ever had a fit, a blackout, or fainted?		
Ever suffered from palpitations or a racing heart?		
Ever suffered from a persistent cough, or lung pains?		
Ever suffered from dizziness when standing up?		
Pregnant/ breastfeeding? Trying to become pregnant?		
Do you have a family history of any medical problems, such as cardiovascular disease?		

MEDICATION				
NAME ROUTE/ DAILY DOSE START DATE REASON FOR TAKING	1)	2)	3)	4)

VITAL SIGNS	
Heart rate:	Sitting: __ __ __ beats / min
Blood Pressure:	Sitting: __ __ __ / __ __ __ mmHg

Any concerns regarding the participant's enrolment in the study: ☐ NO ☐ YES

This form was completed with the participant during the screening session to assess their medical suitability to take losartan, if allocated. Heart rate (HR) and blood pressure (BP) were taken on the day of the in-person session using an Omron 705IT sphygmomanometer. This form was then signed by the study doctor on the day of testing.

Appendix C

Gamified Foraging Task debrief questionnaire

The following questions ask you to think about your experience playing the ocean adventure game. Please mark how often the following statements were true for you:

Q1. I hid for longer than necessary because I was afraid to leave the hiding place.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q2. I avoided checking for the predator because I didn't want to make a decision about when to hide.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q3. I avoided checking for the predator because I wanted to keep diving for food.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q4. It was hard to focus on gathering food in a block with a fast predator.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q5. I got caught by the predator because I was distracted by gathering food.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q6. I avoided checking altogether and went straight to hiding instead.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q7. I hid earlier than necessary because I was stressed.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q8. Before a round with a fast predator, I took a longer break than usual.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q9. After a round with a fast predator, a took a longer break than usual.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q10. I thought a round was too stressful, so I tried not to take it too seriously and didn't try hard to gain more lives.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q11. I didn't dive for food until I knew where the predator was located.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q12. I only dove for food when there was no predator in the area.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q13. After seeing a predator I felt more nervous than before.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q14. After seeing a predator I felt more relaxed than before.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q15. After seeing a predator I felt more excited than before.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q16. After seeing a predator I felt more stressed than before.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q17. I hid earlier than necessary because I was tired of diving for food.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q18. I avoided checking for a predator to save time.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q19. I avoided checking for a predator because I was afraid to see one.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q20. I sometimes checked again for a predator even though I had just finished checking in all directions?

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q21. I tried to find the predator as early as possible.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q22. I tried to find the predator as late as possible.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q23. I tried to gather as much food as possible right before hiding.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q24. I tried not to check on the predator when it was very close because it felt threatening.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q25. I was not worried about how soon the predator would arrive.

0 (Never)	1	2	3	4	5	6 (Very often)
○	○	○	○	○	○	○

Q26. Please explain your answer to Question 6 ("I avoided checking altogether and went straight to hiding instead"). If you sometimes did this, please explain why:

_____

_____

_____

_____

How much time did you spend performing each of these actions?

Q27. Eating food:

0 (None of the time)	1	2	3	4	5	6 (All of the time)
○	○	○	○	○	○	○

Q28. Checking for a predator:

0 (None of the time)	1	2	3	4	5	6 (All of the time)
○	○	○	○	○	○	○

Q29. Hiding from a predator:

0 (None of the time)	1	2	3	4	5	6 (All of the time)
○	○	○	○	○	○	○

Q30. Did you have any technical issues in playing the game or completing the questionnaires? Please circle one: Yes / No

Q31. If you answered "yes" to the previous question, what technical issues did you experience? We appreciate any feedback as we are trying to improve the study.

This questionnaire was provided within the Gamified Foraging Task, after the completion of all task blocks, to assess participants' metacognitive awareness of their task behaviour.

Appendix D

Computed task-based measures

Variable name	Variable description
caughtFish	Number of times the fish was caught by the predator
preDiscovery.firstAction.type	First action in the epoch before predator discovered
preDiscovery.firstForage.time	Time (s) to first forage before predator discovered
preDiscovery.firstCheck.time	Time (s) to first check before predator discovered
preDiscovery.firstStopHidingAction.time	Time (s) to stop hiding at beginning of epoch before predator discovered
fullEpoch.perc.forage	Foraging as a percentage of all actions across the epoch
fullEpoch.perc.hide	Hiding as a percentage of all actions across the epoch
fullEpoch.perc.checkRelCheckPlusForage	Checking as a percentage of number of checks plus number of forages, across the epoch
fullEpoch.checks.any	Whether any checks occurred in the epoch
preDiscovery.checks.transition.forageToCheck.rateSeconds	Rate of transitioning from foraging to checking per second before predator discovered
preDiscovery.checks.time.between.checkSequences	Time (s) in between checking sequences before predator discovered
preDiscovery.checkSeqFrecIndepLength	Frequency of checking sequences before predator discovered
preDiscovery.checks.anyPartialChecks	Whether any partial checks (not checking in every direction) occurred before predator discovered
postDiscovery.checks.any	Whether any checks occurred after predator discovered
postDiscovery.checks.ratePerSec	Rate of checking per second after predator discovered
postDiscovery.lastCheckBeforeHide.secUntilPredArrival	Seconds until predator arrival at time of last check before hiding
postDiscovery.lastCheckBeforeHide.percDistanceUntilPredArrival	Percentage of predator journey remaining at time of last check before hiding
postDiscovery.lastActionBeforeHide.type	Type of last action before hiding
postDiscovery.checks.secBetweenChecks	Time (s) in between checks after predator discovery
discovery.secPredatorUntilPredArrival	Time (s) until predator arrival at time of predator discovery
discovery.percPredatorDistanceUntilArrival	Percentage of predator journey remaining at time of predator discovery
hiding.secPredatorUntilPredArrival	Time (s) until predator arrival at time of hide
hiding.timeSinceBeginEpoch	Time (s) since epoch began at time of hide
hide.moreThanOnce	Whether the participant hid more than once in the epoch
preDiscovery.forage.rate	Rate of foraging before predator discovered
preDiscovery.forage.any	Whether any foraging occurred before predator discovered
preDiscovery.forage.seqLength.max	Maximum number of forages in a sequence (uninterrupted by checking) before predator discovered
postDiscovery.forage.seqLength.max	Maximum number of forages in a sequence (uninterrupted by checking) after predator discovered
postDiscovery.forage.rate	Rate of foraging after predator discovered
postDiscovery.forage.any	Whether any foraging occurred after predator discovery
nonspec.rate.inactive.forages	Rate of inactive forage button presses across the epoch
nonspec.rate.inactive.check	Rate of inactive check button presses across the epoch
nonspec.hide.inactive.number	Rate of inactive hide button presses across the epoch

This table lists all task-based measures that are computed from participant task responses and their interpretation. This table is from Trier et al., (2023).

Annex 1: Pre-registration document

Study title: The effects of losartan on threat and reward processing

Principal Investigators: Andrea Reinecke

Co-Investigators: Jacqueline Scholl, Lauren Morse, Hailey Trier, Lorika Shkreli

Sponsor: University of Oxford

Ethics committee and reference number: University of Oxford Central University Research Ethics committee (CUREC), R65978/RE003

Study actual start date: 13.04.2021

Study end date: 03.11.2022

Study design: Double-blind placebo-controlled experimental medicine study

Masking: Double (Participant, Investigator) – study medication and placebo over-encapsulated to look identical

Randomisation: 1:1 blocked randomisation with gender stratification

Arms and interventions:

Arm 1/ Experimental: Losartan; single dose of 50mg losartan (Cozaar; Merck Sharp & Dohme Ltd.), tablet, over-encapsulated, to be taken orally

Arm 2/ Placebo Comparator: Placebo/ non-active sham intervention; single dose of placebo (Rayotabs, Rayonex GmbH; main ingredient microcrystalline cellulose), tablet, over-encapsulated, to be taken orally

Number of participants: 60

Procedure:

Blood pressure, heart rate, and subjective visual-analogue measures of physiological and mood symptoms were measured at baseline and at losartan drug-peak level, 45 minutes after capsule administration. Participants started working on a battery of experimental tasks after peak-level assessment, with the task described here occurring approximately 70 minutes after capsule administration.

Eligibility criteria

Inclusion criteria:

- Willing and able to provide informed consent
- Male or female, aged 18-50 years
- Body mass index (BMI) of 18-30 kg/m2
- Non- or light-smoker (< 5 cigarettes a day)

Exclusion criteria:

- Female participant who is pregnant or breast-feeding
- Central nervous system (CNS)-active medication during the last 6 weeks
- Current blood pressure or other heart medication (especially aliskiren or beta blockers)
- Current steroid medication (e.g. cortisone, prednisone)
- Diagnosis of intravascular fluid depletion or dehydration
- Past or present DSM-5 axis-I diagnosis or suspected diagnosis (from SCID results at screening)
- Alcohol or substance abuse
- First-degree family member with a history of a severe psychiatric disease
- Impaired liver or kidney function
- Lifetime history of epilepsy or other neurological disease, systemic infection, or clinically significant hepatic, cardiac, obstructive respiratory, renal, cerebrovascular, metabolic, endocrine or pulmonary disease or disorder which, in the opinion of the investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.
- Insufficient English skills
- Participated in another study involving CNS-active medication during the last 6 weeks

Additional exclusion criteria based on quality of the task data:

- Participants were excluded if throughout the whole experiment they checked for threat less than 50 times (n=2)
- Participants were excluded whose final life counter was below 5 (n=2)
- Participants were excluded who got caught more often than 9 times (n=2)
- Participants were excluded if there was a problem with displaying of reward at some point during the task (n=2)

Combining these criteria, overall, 5 participants were excluded based on task quality and 3 based on having previously done the task (1) or errors during data saving (2). Exclusions were done blinded to the group assignment.

Baseline assessments

- Self-report questionnaire battery to assess personality traits, anxiety, and depression: Eysenck Personality Inventory, Eysenck & Eysenck, 1975; Beck Depression Inventory BDI, Beck et al., 1996, Trait form of the State-Trait Anxiety Inventory STAIT, Spielberger et al., 1983; Attentional Control Scale ACS, Derryberry & Reed, 2002; Anxiety Sensitivity Index Revised ASI-R, Taylor & Cox, 1998; Behavioural Inhibition Scale BIS, Carver & White 1998
- Visual Analogue Scales before administration of capsule and at peak level
- Basic physiological parameters (blood pressure, heart rate) before capsule administration and at peak level

Data analysis timeline

Participants were tested by LM and LS. LM then sent the task data to JS who extracted and pre-processed the data. JS did not extract information about participant group assignment. After pre-registration, LM will send group assignment (as well as age, gender and education) to JS for combination with task-based measurements.

Experimental task

Gamified foraging task

At drug-peak level, participants work on a 'gamified' task designed to ecologically capture vigilance, where participants freely make a series of choices with the goal of earning as many lives as possible. During the task, participants play a fish avatar that has to decide how to split up its time between gathering food (later translated into game lives), checking the environments for hidden predators (leading to life loss if they catch them), and hiding from the predators, which have varying threat levels, so that variations in threat-related behaviour can be measured (e.g. how often participants check for threats). This is a version of a task described in a previous pre-registration: https://osf.io/3gb8n. It diverges from the previous task version in the following points:

- Participants used a track pad, rather than keyboard to make choices
- Reward was not blocked but drifted continuously. To account for this, in regression analyses the reward entered was not the average for the whole predator epoch, but either the average before the predator was (for actions happening before the predator was seen), after the predator was seen, before hiding (for actions happening after discovery of the predator) or the average of the full epoch (for actions referring to the full epoch)
- Participants received a warning if they hid for too long (I.e. 15s or longer)
- Number of blocks: 18
- Time remaining was indicated by an animated sun or moon instead of a wheel
- After each 90s block, participants responded to two additional questions ("How much food was available in the last round?" and "In the last round, how difficult was it to balance between eating food and checking for predators?") using a sliding scale
- Participants did not gain any extra financial reimbursement from performing well in the task (I.e. gaining extra lives).

Statistical analysis plan

All statistical analyses will be carried out using R (see code included with pre-registration). Statistical tests will be two-tailed and based on an alpha-level of significance of 0.05. Based on the results of the previous, fully independent study (https://osf.io/3gb8n), we derived compound behavioural measures from the experimental task (and the post-task debrief about the task), see below in the section 'primary outcome markers', 'secondary outcome markers'. All of these have in a separate study been found to relate to individual difference in markers such as behavioural and emotional apathy (from the Apathy Motivation Index, [AMI; Ang et al., 2017]), anhedonia (from the Snaith-Hamilton Pleasure Scale [SHAPS; Snaith et al., 1995]), cognitive and somatic anxiety (from the State-Trait Inventory for Cognitive and Somatic Anxiety [STICSA; Grös et al., 2007]), intolerance to uncertainty (from the Intolerance of Uncertainty Scale Short Form [IUS-12; Carleton et al., 2007]) and compulsivity (from the Obsessive Compulsive Inventory (Revised) 'Checking' subscale [OCI-RC; Foa et al., 2002]). Group differences on the below outcome markers will be assessed using linear regression models, with "group" (losartan, placebo) as a predictor of interest and gender, age, education, weight and systolic and diastolic blood pressure before capsule administration as regressors of no interest.

Task_derived_measure ~ Group + Age + Gender +Education + Weight + pre_bloodpressure_systolic + pre_bloodpressure_diastolic

Analyses will be done using the MICEafter package [Heymans, 2022] which allows combining the results across the different imputed data sets to a single statistical value per regressor.

No correction for multiple comparisons within the primary or secondary outcomes.

Primary outcome markers:

- Sensitivity of vigilance to threat-related worry and avoidance and resultant task outcome [being caught] (**previous association: somatic anxiety – STICSA [Grös et al., 2007]**). This measure is composed of the behavioural measures post-task debrief Q2 ("I avoided checking for the predator because I didn't want to make a decision about when to hide"), post-task debrief Q13 ("After seeing a predator I felt more nervous than before"), average times having been caught, percentage of time spent checking relative to the total percentage of active time (checking or foraging).
- Sensitivity of vigilance/forage balance to reward and self-reported task engagement (**previous association: anhedonia – SHAPS [Snaith et al., 1995]**). This measures is composed of a weighted sum of behaviours measuring the impact of reward on when the predator is discovered, the impact of reward on when participants hid, the impact of reward on frequency of checking, the impact of reward on when participants came out of hiding to start a new epoch, the impact of reward on the forage rate (pre discovery of the predator), the impact of reward on the maximum length of forages (pre discovery of predator), the impact of reward on the percentage of time spent foraging, post-task debrief Q21 ("I tried to find the predator as early as possible"), post-task debrief Q22 ("I tried to find the predator as late as possible"), post-task debrief Q23 ("I tried to gather as much food as possible right before hiding"), the overall excitement rating (intercept of regression model)

Models are included in the pre-registration in the files:
List_individual_measures_fromOnlineStudyDiscoverySample.xlsx (see tabs Anxiety (5B) and Apathy-anhedonia (6B)) and summarised in
RegressionModels_HypothesisBasedModels_onlineStudy_summaries.txt.

Secondary outcome measures:

Machine-learning derived measures of the relationship of task-behaviour, post-task self-report and scales of anxiety and anhedonia. This is based on models trained and validated in the previous studies. Without any theoretical considerations, the models (regularized Bayesian regressions) select measures that together can predict the clinical score of interest. The models are uploaded with the pre-registration [filenames:
OnlineStudy_PredictorWeights_TaskAndDebiref.docx,
OnlineStudy_PredictorWeights_TaskOnly.docx]. Table 1 contains a list of the task measures that had the highest contributions to the predictive model, but note that we will apply the full model, not just a selection of the most predictive measures.

- Cognitive trait anxiety
    - Without post-task debrief
- Somatic trait anxiety
    - Without task debrief
- Anhedonia
    - Without task debrief

Table 1: Table of correlations between the final included measures (primary or secondary interest)

	sticsa.trait.cognitive_machineLearning_WithoutTaskDebrief	*sticsa.trait.somatic_machineLearning_WithTaskDebrief*	*sticsa.trait.somatic_machineLearning_WithoutTaskDebrief*	*shaps_machineLearning_WithoutTaskDebrief*	*shaps.predicted.H3B*	*sticsa.trait.somatic.predicted.H2B*
sticsa.trait.cognitive_machineLearning_WithoutTaskDebrief		0.606***	0.711***	0.565***	0.373**	0.169
sticsa.trait.somatic_machineLearning_WithTaskDebrief	0.606***		0.698***	0.278*	0.316*	0.558***
sticsa.trait.somatic_machineLearning_WithoutTaskDebrief	0.711***	0.698***		0.244	0.150	0.150
shaps_machineLearning_WithoutTaskDebrief	0.565***	0.278*	0.244		0.727***	-0.050
shaps.predicted.H3B	0.373**	0.316*	0.150	0.727***		0.062
sticsa.trait.somatic.predicted.H2B	0.169	0.558***	0.150	-0.050	0.062	
Computed correlation used pearson-method with listwise-deletion.						

Exploratory analyses:

1) We'll compare all other behavioural compound measures obtained in Trier et al. study (https://osf.io/3gb8n) between the groups (including compound measures that were related to clinical questionnaire scores, as well as compound measures related to emotions and homeostatic regulation of emotions).
2) We'll perform a nested cross-validated machine learning analysis that tests whether the task features overall are changed by group.

Data analysis:

Task-based measures will be extracted as in the previous study. Then, the following steps will be applied (see also included code):

1. Missing data will be imputed (MICE package in R [Heymans, 2022]; 20 imputations). For this, of the possible 436 task-based measures, only those were retained that showed significance at the group-level in the online study (e.g. impact of reward on rate of foraging), leaving n=203. Age, gender, education, weight, systolic and diastolic blood pressure pre-capsule administration, and group assignment will additionally be included for imputation.

2. All data were standardized by removing the mean and dividing by the standard deviation of the corresponding measures in the previous online study.

3. Compound measures were computed by applying the regressions models (see defined above) derived from the previous study (models trained on combined discovery and replication sample).