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An investigation of propranolol as an agent for the experimental manipulation of interoception.

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Abstract

Interoception has recently come under research focus as a potential influence on emotional and epistemic feelings. However, existing means to manipulate it experimentally have conceptual or logistical drawbacks. We investigated whether 20 mg of propranolol is a viable agent for experimentally manipulating interoception. Thirteen participants completed a double-blind, placebo-controlled crossover study, performing two heartbeat perception tasks, control tasks and measures of anxiety and alertness. All measures were obtained at the beginning and end of both sessions. Propranolol significantly decreased heart rate and systolic blood pressure. Heartbeat detection performance numerically decreased under propranolol, although this effect failed to reach statistical significance. Heartbeat tracking exhibited a practice effect in both sessions. There were no significant effects on the control tasks. State anxiety was unchanged within either session, and alertness decreased in both. These findings validate the propranolol paradigm, and the numerical change in heartbeat detection warrants follow-up with a larger sample.

Keywords

Interoception, propranolol, heartbeat detection, heartbeat tracking

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Chapter 1

1 Introduction

The physiological state of the body influences the brain along two major dimensions. The first is metabolic and physiological support necessary for the maintenance of brain health and functioning. The second is moment-to-moment afferent signalling, used for homeostatic regulation, affective response, and, it has been argued, in generating and informing cognitive states (Critchley, Mathias, & Dolan, 2002). This second role relies on interoception, the ability to sense the physiological state of the body. Interoception subsumes the detection of any sensation arising from within the body, including "subtle changes in bodily systems including muscles, skin, joints and viscera" (Dunn et al, 2010). It can be contrasted with exteroception, which includes senses for the world outside the body, such as taste, smell and touch. It has been suggested that one function of interoception is to give rise to the subjective states we know as "feelings", which can be defined as "mental experiences of body states" (Damasio & Carvalho, 2013). In bringing the current state of the organism to conscious awareness, it may aid in motivating appropriate action and inform decision making (Damasio & Carvalho, 2013).

To date, research on the functional role of interoception has been largely confined to the domain of emotion. In particular, it is thought to be integral to the subjective experience of "feelings". In a commonly held view, "feelings" are defined as a purely subjective experience, and are differentiable from "emotions". The latter can be viewed as "innate physiological actions" in response to stimuli, for example, the changes in heart rate, breathing, and facial muscles constituting part of the overall emotion of fear (Damasio & Carvalho, 2013). In contrast, feelings are produced when interoceptive sensation of the physiological changes associated with emotion reaches conscious awareness (Damasio & Carvalho, 2013). Empirical research has implicated individual differences in interoception in modulating the intensity of conscious emotional experience. For example, Wiens, Mezzacappa & Katkin (2000) showed that good performers on a heartbeat perception task, an index of interoceptive ability, experienced emotional film

clips as being more arousing, as indexed by a self-report measure. Other studies have corroborated these findings (Pollatos, Kirsch & Schandry, 2005; Dunn et al., 2010).

Although interoception need not be accompanied by conscious awareness, when it is, the resulting subjective experience, or "feeling", has been proposed to play a role in the modulation or selection of the response to the stimulus precipitating the physiological change. In this way, feelings are thought to provide more flexibility in responding to one's environment (Damasio & Carvalho, 2013; Wiens, 2005). However, "feelings" also play a role in non-emotional states, for example, as feelings of familiarity or feelings-of-knowing in memory. Memory research has termed such states "epistemic feelings", and evidence is mounting that they are functionally and mechanistically akin to the feelings we experience in emotion (Moulin & Souchay, 2013). Indeed, like emotional feelings, epistemic states are regarded as being fast, involuntary and subjective, as opposed to deliberative (Koriat, 2000). Additionally, a motivational framework has been described in which epistemic feelings can inform subsequent action, for example, in increasing search effort to retrieve information that is believed to be known, but cannot be accessed at the moment (e.g. the tip-of-the-tongue phenomenon) (Koriat, 2000). Given these parallels, the question of whether interoception can inform epistemic feelings presents a promising direction for future research.

Interoception has been implicated in non-emotional cognition, in support of an "embodied cognition" view (Garfinkel, Seth, Barrett, Suzuki & Critchley, 2015). Preliminary evidence exists for a connection between interoception and memory judgments. Fiacconi et al. (2017) demonstrated a link between cardiac reactivity in response to novel and familiar faces, and the degree of change in participants' feeling-of-knowing judgments for the names associated with the faces. Critically, this relationship was moderated by individual differences in interoceptive ability. Interoception has also been linked to meta-memory confidence judgments (Garfinkel et al. 2013, Chua & Bliss-Moreau, 2016) and decision-making (Dunn et al., 2010). These findings suggest that interoceptive perception of changes in bodily state can inform cognition, and epistemic feelings in particular, in addition to emotional experience. However, most of the work done to date has been correlational, linking inter-individual variation in interoceptive

ability to performance on cognitive tasks. To extend such findings, and establish a causal role for interoception in mediating between body states and epistemic feelings, it is necessary to both measure and manipulate interoceptive ability experimentally. The goal of the present study is to establish an experimental paradigm for the manipulation of interoceptive accuracy, which can in the future be used in conjunction with cognitive tasks to probe the role of interoception in memory and epistemic feelings.

Interoception plays an important part in autonomic system regulation. Interoceptive pathways are involved in both sympathetic arousal and parasympathetic regulation, and play a role in diverse autonomic functions such as monitoring and regulation of blood pressure (Strigo & Craig, 2016), thermoregulation (Fealey, 2013) and physiological response to stress (Schulz & Vogele, 2015). Interoceptive signals are relayed to the human brain along two major pathways: the lamina I (spinothalamocortical) pathway and the vagus nerve. Both pathways ultimately project through the brainstem and thalamus to the insula and thereafter to diverse cortical structures. The lamina I pathway carries information about diverse body systems, including the organism's thermoregulation, pain, blood flow and chemical balance. Afferent fibers reach the lamina I of the spinal cord grey matter, and from there projections carry information to structures in the brainstem, notably the nucleus of the solitary tract (NTS), parabrachial nucleus (PBN) and periaqueductal grey (PAG). These structures are involved in homeostatic regulation and are believed to be essential to interoceptive sensation, as their lesioning can result in coma (Damasio & Carvalho, 2013). This group of posterior brainstem structures projects to the thalamus, which in turn relays information to the insula, believed to be the main hub of integration of interoceptive and exteroceptive sensations (Critchley & Harrison, 2013). As the insula is widely cortically connected, interoceptive information can interface with diverse brain regions, notably the somatosensory cortices, anterior cingulate cortex, amygdala and ventromedial prefrontal cortex. These structures are intimately linked to phenomena such as emotion, cognitive control, decision-making and salience processing, and recent evidence has substantiated a link between interoception and these cognitive domains (Dunn et al., 2010; Chong, Ng, Lee & Zhou, 2017).

The vagus nerve, i.e., the second major afferent pathway for interoception, carries information primarily about pulmonary, cardiovascular and gastrointestinal systems (Damasio & Carvalho, 2013). Projections carry information from the vagus nerve to the NTS, and subsequently to the higher brainstem structures such as PAG and PBN and the hypothalamus. Thereafter they are once again integrated in the insular cortex. A third pathway exists, bypassing the brainstem, directly through the area postrema, which is a chemosensory structure dealing mainly with homeostatic regulation of metabolic processes, and which has been implicated in cardiovascular regulation.

Measurement of interoceptive ability can take many forms, ranging from self-report questionnaires to objective measures of interoceptive accuracy. Perhaps the most widespread are heartbeat detection and tracking tasks, which have the advantage of being concerned with cardiac processes, a class of physiological response that has been experimentally and intuitively implicated in emotional experience. A cardiac response is a well documented consequence of viewing emotional stimuli, and interoceptive ability in particular has been found to mediate the intensity of perceived emotions (Wiens et al., 2000). Heartbeat tasks have several advantages over other tasks that probe interoception, primarily ease of measurement, as well as cardiac activity being amenable to experimental manipulation. These tasks can be broken down into two distinct types. Heartbeat *detection* involves comparing the presentation of auditory tones with the heartbeat to determine whether the two are coincident or offset (Whitehead, Drescher, Heiman, & Blackwell, 1977). It has been widely used (Critchley & Garfinkel, 2015), performance on it well characterized, and has the advantage of being immune to participants' knowledge of typical heart rates. The major drawback of heartbeat detection tasks is consistently low performance, which typically rises only slightly above chance level (Khalsa et al, 2009).

A second category of cardiovascular interoceptive tasks includes heartbeat *tracking*. This type of task involves participants counting their heartbeats (without feeling their pulse) during a predetermined time window not disclosed to the participant. Typical administration parameters involve time windows of 25, 35 and 45 seconds. This type of task, although easier to administer and amenable to better performance, suffers from

several major drawbacks, including few trials and the possibility of a participant using prior knowledge of their heart rate to estimate, rather than individually perceive, their heartbeat (Khalsa et al., 2009). The link between interoception and memory judgments reported by Fiacconi et al. (2017) was obtained using a heartbeat counting task. In the present study, we utilized both tasks in order to allow for a more comprehensive assessment of individual variation in interoceptive ability, and to determine whether our experimental manipulation has differential effects on the different types of heartbeat tracking measures.

The approach of correlating individual differences in interoception with other cognitive processes, although widely used, suffers from the typical drawbacks of correlational research. Although it presents a promising way of identifying areas in which interoception may be involved, this approach falls short in establishing whether interoception plays a causal role in the processes being studied. In order to make strong claims about the directionality, and indeed the presence of a causal relationship, interoception must be experimentally manipulated.

Several methods for manipulating interoception have been proposed and used in the past. Common methods include utilizing a tilt table to change body position between horizontal and vertical, as well as engaging participants in dynamic and isometric exercise, such as stationary cycling or isometric hand grip. Position changes from vertical to horizontal and physical exertion activate the sympathetic nervous system, and have been associated with an increase in interoceptive accuracy as indexed by both heartbeat detection and heartbeat tracking tasks (Pollatos et al., 2007; Schandry, Bestler & Montoya, 1993; Ring, Liu & Brener, 1994). Although these methods are widely used and non-invasive, they do not give insight into the neurochemistry or specific pathways involved in the relationship between influences on cardiac response and interoceptive ability.

Perhaps the paradigm most amenable to careful experimental control to date, reported by Khalsa et al. (2009), employs isoproterenol, a beta-adrenergic agonist that raises heart rate and is used in clinical settings primarily to treat bradycardia and heart block via its

action to increase heart rate by stimulation of beta-adrenergic receptors in the heart. By adapting an established clinical threshold test for experimental settings, Khalsa and colleagues were able to achieve an incremental and carefully controlled dose-dependent heart rate increase. This increase was transient, lasting only as long as intravenous isoproterenol was administered, and could be maintained by continued administration. Khalsa and colleagues observed an increase in interoceptive awareness, indexed by real-time self-report ratings of heartbeat and breathing sensation intensity, corresponding to increasing doses of isoproterenol (and thus to increasing heart rate).

In addition to being amenable to very precise control over the resulting physiological effect, this pharmacological manipulation is distinguished from the aforementioned methods by its ability to probe not just the possible effect of interoception on a cognitive process, but also the physiological means through which this effect may be carried out. By considering which receptors and signalling pathways are affected by the drug, it is possible to make inferences about the physiological substrate and neurochemistry of interoception's involvement in cognition. However, despite its strengths, this paradigm suffers from the major drawback of being difficult to administer. Intravenous infusion requires the supervision of a nurse, and increased risk to participants. We therefore sought to pursue less invasive pharmacological means of heart rate manipulation.

A promising pharmacological candidate is propranolol, a beta-adrenergic antagonist. Propranolol was chosen for this initial study for several reasons. First, it is considered to be safe for administration in non-clinical research settings; second, it has documented robust and controllable effects on heart rate (Chamberlain et al., 2006); and third, it is thought to have limited effects on overall cognitive performance (Chamberlain & Robbins, 2013).

Propranolol is typically prescribed for high blood pressure, and off label for performance anxiety. With the administration of a single dose, it induces a drop in heart rate and blood pressure. Propranolol reaches peak plasma concentration at around 90 minutes after administration, making it a viable candidate for use with research studies. Its effects are relatively short lived, with a half life of 3-4 hours (Hurlemann et al., 2005). Side effects

are not typically associated with a one-time dose as used in research, and in a wide array of studies, research participants were not able to reliably identify the drug and placebo conditions. In the context of research studies, propranolol is typically administered in doses of 20-80 mg, with the resulting drop in heart rate of around 5 bpm for 20 mg and increasing with the dose (Chamberlain & Robbins, 2013). We used a 20mg dose because preliminary piloting revealed a robust reduction in heart rate even at this low dose.

One of the major advantages of this drug for future use with cognitive paradigms is the rich literature documenting its lack of effect on general cognitive function. Multiple studies have reported negative results on attentional and memory tasks (Chamberlain & Robbins, 2013; Chamberlain et al., 2006). In a meta-analysis (Chamberlain & Robbins, 2013), only one study found that a higher (40 mg) dose impaired attention on an attentional blink paradigm (De Martino, Strange & Dolan, 2008), while most showed negative results. Results on working memory are mixed, although there is evidence that propranolol in particular (as opposed to peripherally acting beta-blockers) has detrimental effects, especially in participants with low anxiety (Chamberlain et al., 2006). Because propranolol is a beta-adrenergic antagonist, in contrast to the agonist isoproterenol, we expect that by lowering heart rate it will decrease interoceptive awareness, thus lowering interoceptive accuracy. To control for potential effects of the drug on global cognitive processes, we included two cognitive control tasks that mimic the interoception tasks in structure without invoking their interoceptive component.

In the current study, we hypothesized that i) a single 20 mg dose of propranolol will result in a measurable physiological response, indexed by a drop of heart rate by about 5 bpm; ii) performance on the heartbeat interoception tasks will be lower in the drug than the placebo condition; iii) performance on non-interoceptive cognitive control tasks will not be different between drug and placebo conditions.

Chapter 2

2 Methods

2.1 Session structure and drug administration

We conducted a double-blind, randomized, placebo-controlled crossover study, in which each participant completed two 2.5 hour sessions, one under propranolol and the other under placebo. Drug/placebo order was counterbalanced across participants. The drug condition involved ingesting a 20 mg oral capsule of propranolol; the placebo capsule contained corn starch. In keeping with the double-blind nature of the study, the pill packets were prepared and coded by a separate member of the lab. Sessions were conducted at the same time of day, with a washout period of 7 days in-between. The washout period was chosen to be in keeping with other cognitive propranolol studies, as well as be at least 10x the elimination half-life of propranolol (3-4 hours; Hurlemann et al., 2005).

Session structure is illustrated in Figure 1. The procedure for both sessions was identical except where noted. Each session started with consent (1st session only), ECG setup and Questionnaire Set 1 (State anxiety, Bond-Lader visual analogue scale, and in the 1st session only, Trait anxiety). This was followed by ingestion of the capsule, the first of the physiological measurements, and immediately the first block of tasks. The task block included both interoception tasks and both control tasks. ECG recordings were made during the interoception tasks. The interoception task order was randomized, and each interoception task was followed by its corresponding control task. The second task block repeated the 4 tasks from the first block, in the same order, and was timed to be centered around the time of peak drug effect at 90 minutes after ingestion. The order of tasks was kept constant between the two sessions for any given participant. The 2nd task block was followed by Questionnaire Set 2 (State anxiety and Bond-Lader visual analogue scale). Physiological measurements (blood pressure and heart rate) were obtained every 10 minutes over the course of 2 hours following ingestion, for a total of 13 measurements. Additional measures were included near the middle of the session (“Additional

Measures" in Figure 1) for exploratory purposes, and are not considered in the current investigation.

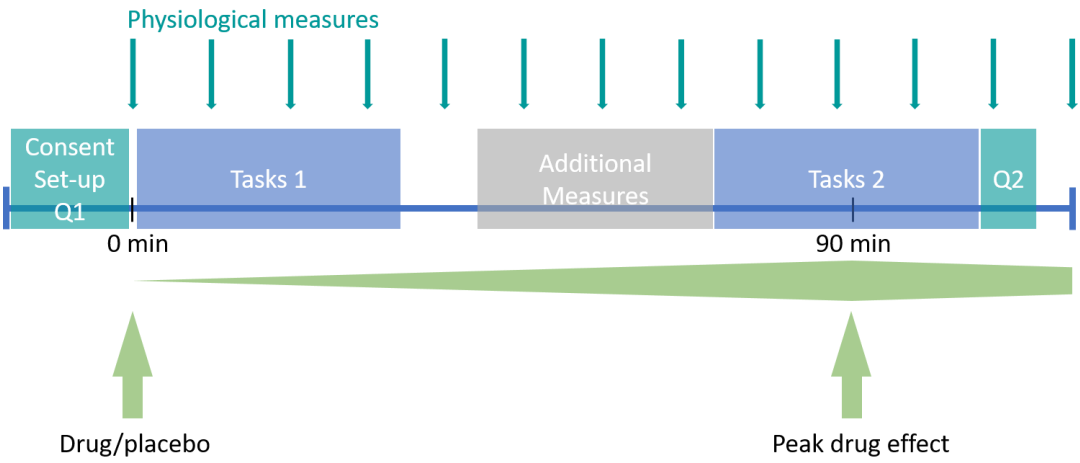

Figure 1: Session structure. Each participant completed both a drug session and a placebo session. Both sessions followed the same format (except where noted). Following set-up, each session began with Questionnaire Set 1 ("Q1": Alertness, State anxiety and, in the first session only, Trait anxiety) and administration of the capsule (designated as time=0 minutes). Capsule ingestion was immediately followed by Task Block 1 ("Tasks 1"), which consisted of both interoception tasks and both control tasks. Around the time of peak drug effect (at time=90 minutes), all behavioural tasks were repeated in Task Block 2 ("Tasks 2"), followed by State anxiety and Alertness in Questionnaire Set 2 ("Q2"). Additional measures ("Additional Measures") were included in the middle of both sessions for exploratory purposes and are not considered in the present investigation. The light green bar represents the course of the drug effect in the drug session, starting with ingestion at t=0 and peaking at t=90 minutes. Physiological measures consisted of heart rate and blood pressure, and were taken every 10 minutes (indicated by teal arrows) starting at capsule ingestion, for a total of 13 measurements.

2.2 Participants

Thirteen young adults (23.8 (SD=3.1) years of age, with 18.8 (SD=3.1) years of education; 5 female) were recruited by poster from the Western University community. Participants were monetarily compensated for their time. Exclusion criteria included any

history of cardiovascular, neurological or psychiatric conditions, any contraindications to propranolol, resting blood pressure below 90/60 mmHg and resting heart rate below 60 bpm. The study was approved by the Health Sciences Research Ethics Board at Western University. Not all tasks were completed by every participant (n=12 for heartbeat tracking; n=9 for seconds counting; n=8 for one-back; n=13 for heartbeat detection and all self-report measures)

2.3 Experimental setup

ECG recordings were made using three Ag/AgCl electrodes in a Lead II configuration, and a BIOPAC MP150 MRI-compatible system connected to a BIOPAC ECG100C-MRI amplifier (BIOPAC Systems, Goleta, CA). The signal was acquired at a sampling rate of 2000 Hz and bandpass filtered at 1-35 Hz. AcqKnowledge software (Biopac Systems Inc.) was used to record the ECG signal and, for the tone-matching task, calculate R-wave onsets in real time. The R-wave onsets were then relayed to the testing computer running E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA) in order to trigger heartbeat-dependent acoustic tone presentation.

2.4 Behavioural tasks

2.4.1 Interoception tasks

2.4.1.1 Heartbeat detection

A tone matching (Whitehead et al., 1977) task was used, in which a series of tones were presented through a speaker that matched the participant's heart rate in speed and pattern. Tones were either coincident with each heartbeat or were offset by 500ms. This was achieved by simultaneous recording of the ECG signal. AcqKnowledge software was used to detect R-peaks in real time and this signal was relayed back to the testing computer in order to trigger an acoustic tone, with or without delay. Each trial consisted of a series of ten tones, after which the participant was prompted to indicate whether the series was synchronous or asynchronous with the heartbeat. There were 60 trials in total, and performance was assessed as the percent of trials correctly classified (chance is 50%).

2.4.1.2 Heartbeat tracking

A commonly used heartbeat counting paradigm was employed (Schandry, 1981). Participants were asked to count their heartbeats within a certain period of time (without feeling for their pulse). There were 6 trials in total, with durations of 25s, 35s and 45s, twice each. A veridical heartbeat count during each period was obtained via ECG. Performance was assessed as Percent Accuracy = (counted heartbeats)/(actual heartbeats), averaged over all 6 trials.

2.4.2 Cognitive control tasks

A concern with any apparent change in performance on interoception tasks is whether the effect is due to a change in interoceptive ability, or some other cognitive process, for example, a decrease in global attentional capacity. In order to rule out this alternative interpretation, we included two cognitive control tasks, performed as close in time as possible to the interoception tasks. Any global effects on cognition as a result of drug administration or other effects over the course of the sessions should be reflected in performance on these tasks.

2.4.2.1 One-back task

An auditory one-back task was administered in which participants listened to a series of letters of the alphabet spoken on a recording, and were asked to press a button each time the same letter appeared on two consecutive trials. There were 10 different letters presented over 90 trials in total. Back-to-back repeats were considered target trials and represented 25% of all trials. Accuracy was recorded as percent targets detected. The One-back task was administered on a Dell XPS 13 laptop running Windows 10 and custom Matlab code using Psychtoolbox (Brainard, 1997; Pelli, 1997; Kleiner et al, 2007). This task was included as a control task for the tone-matching interoception task. It was intended to probe auditory vigilance and sustained attention.

2.4.2.2 Seconds counting task

This procedure was identical to the heartbeat counting task, but participants were asked to count the number of seconds (without the aid of a clock) instead of heartbeats.

Performance was assessed as for the heartbeat counting task. This task was meant to mimic the cognitive demands of the heartbeat counting task as closely as possible, without engaging interoceptive processes.

2.5 Self-report measures

2.5.1 Anxiety

To assess individual differences in anxiety, as well as to track changes in anxiety over the course of testing, we administered the State and Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). This is a widely used inventory consisting of two scales: trait anxiety, meant to assess stable individual differences in anxiety, and state anxiety, which indexes anxious disposition at a given moment and can be administered several times over the course of testing. This measure was included because both propranolol's effects and individual differences in interoception have been linked to anxiety (Steenen et al., 2016; Critchley et al., 2004). Including these scales allowed us to assess the impact of individual differences in this important characteristic on our tasks and manipulation, both in terms of stable differences in personality, and potential differences in how participants may react to the drug or the experimental paradigm itself.

2.5.2 Alertness

To index alertness throughout the testing sessions we used the Alertness subscale of the Bond-Lader Visual Analogue Mood Scale (BL-VAS; Bond & Lader, 1974). Because decreases in alertness can be a side effect of clinical propranolol use, as well as of lengthy experimental paradigms, including a measure of alertness around the time of task performance allowed us to assess the contribution of this factor to any change in behavioural performance in our study.

2.6 Processing of physiological data

ECG recordings and e-Prime data files were processed using custom Matlab R2014b code (The Mathworks, Natick, MA). ECG recordings obtained during the heartbeat counting tasks were run through custom Matlab code that counted the number of

heartbeats by identifying local maxima (R-peaks) above a certain amplitude threshold (which was manually determined for each participant to ensure clean separation of R-peaks from the rest of the ECG signal).

2.7 Statistical analyses

Statistical analyses were performed using Matlab and R (R Development Core Team, 2015). Time-dependent trends in physiological measurements (heart rate and blood pressure) were assessed by fitting a linear model to the measurements obtained during the sessions. T-tests were used to check for differences between conditions at the time of peak drug effect and averaged over a 40-minute window centered around same. The 40-minute window was selected because it encompassed the fairly lengthy battery of behavioural tasks meant to be performed around the time of peak drug effect.

To determine whether propranolol or the session structure had any effect on outcomes for the self-report measures and behavioural tasks, we asked two questions: 1. Did drug treatment result in different performance at the 2nd measure (outcome), when accounting for baseline performance? 2. Was there a systematic difference between conditions, or a systematic change in performance over the course of both sessions?

To determine whether any differences in outcome scores existed between drug and placebo conditions, we performed an ANCOVA analysis by fitting a model of the form: $(Y_{drug}-Y_{placebo})\sim(X_{drug}-X_{placebo})$, where Y_{drug} and $Y_{placebo}$ are outcome measures in the drug and placebo conditions, respectively, and X_{drug} and $X_{placebo}$ are baseline measures for same. This approach has been shown to have the most sensitivity in taking advantage of the strengths of a 2x2 crossover design, and is especially relevant for studies with small samples (Metcalfe, 2010; Mehrotra, 2014). The ANCOVA was performed in conjunction with stepwise regression using the Akaike Information Criterion to identify covariates that contributed to outcome performance beyond the contribution of baseline measures included in the base model. For the behavioural tasks, the covariates considered were: task order (indexing the order of interoception tasks), session order (indexing drug/placebo order assignment), trait anxiety scores and difference in state anxiety outcome scores. For the self-report measures, only trait anxiety scores and session order

were considered. ANCOVA results are reported only for the model identified by stepwise regression as having the best fit.

To determine whether performance changed over the course of the sessions, and to confirm that performance did not differ systematically between drug and placebo conditions, a repeated measures ANOVA was conducted to assess main effects of time and condition.

Chapter 3

3 Results

3.1 Physiological response

3.1.1 Heart rate

Mean heart rate, recorded at 13 time points during each session, is presented in Figure 2. Linear regression was performed to assess the effect of the drug manipulation on heart rate (Figure 3). Propranolol reliably lowered participants' heart rate over time (β=-1.22, $p=1.59\times10^{-11}$). A weaker negative relationship was also observed in the placebo condition (β=-0.43, p=0.0462), indicative of participants settling into their resting heart rate over the course of a sedentary testing session. The degree of change reported for propranolol is thus likely a combination of the settling effect in addition to the action of the drug. Baseline heart rate, as measured at the time of ingestion, did not differ between conditions (placebo M=69.4, SD=12.1; drug M=70.8, SD=9.6; t(12)=-0.41, p=0.69). Important for the present study, a significant difference in heart rate was confirmed between the placebo and drug conditions after the drug had time to take effect. This was confirmed at the time of peak drug effect 90 minutes after ingestion (placebo M=65.9, SD=10.3; drug M=60.1, SD=8.9; t(12)=2.83, p=0.0151, Figure 4A), and over a time window of 70-110 minutes after ingestion (placebo M=65.4, SD=10.0; drug M=59.5, SD=7.7; t(12)=2.83, p=0.0068, Figure 4B). The latter time window was isolated for analysis because it fully encompasses the battery of tasks performed in the second half of the session. The presence of a significant difference in heart rate during this time window indicates that the drug manipulation was effective while the tasks were being performed.

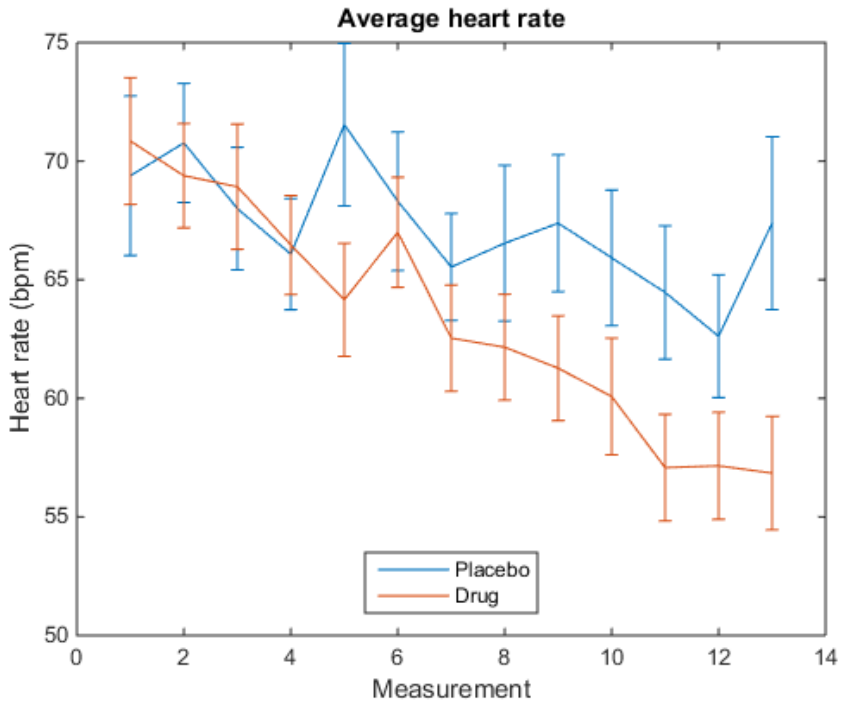

Figure 2: Heart rate measurements. Thirteen measurements were taken in total. Measurement 1 was taken at the time of ingestion, and measurement 10 was timed to the peak of drug activity, 90 minutes after ingestion. Error bars are standard error of the mean. Baseline heart rate did not differ between conditions (placebo M=69.4, SD=12.1; drug M=70.8, SD=9.6; t(12)=-0.41, p=0.69).

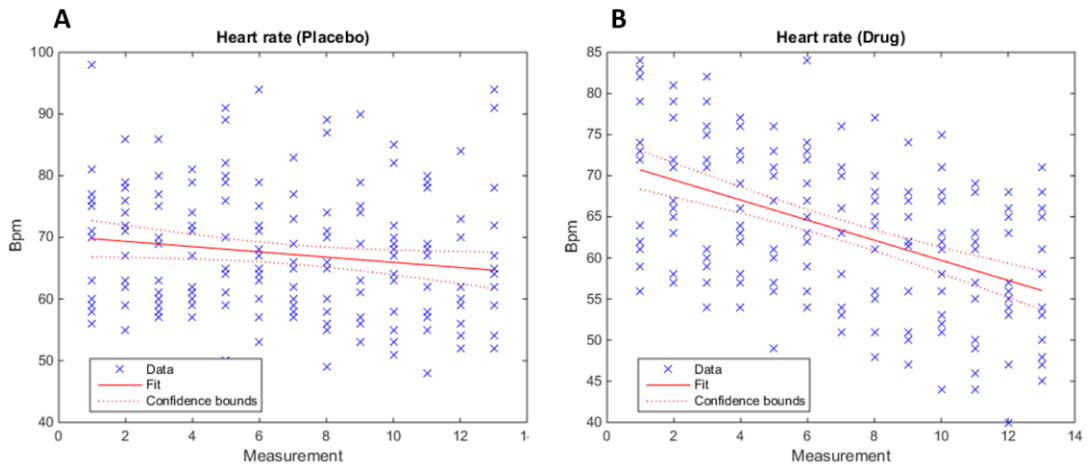

Figure 3: Linear regression of heart rate over time for the placebo (A) and drug (B) conditions. A significant negative trend was observed for both conditions (β=-0.43, p=0.0462 for placebo; β=-1.22, $p=1.59\times10^{-11}$ for drug).

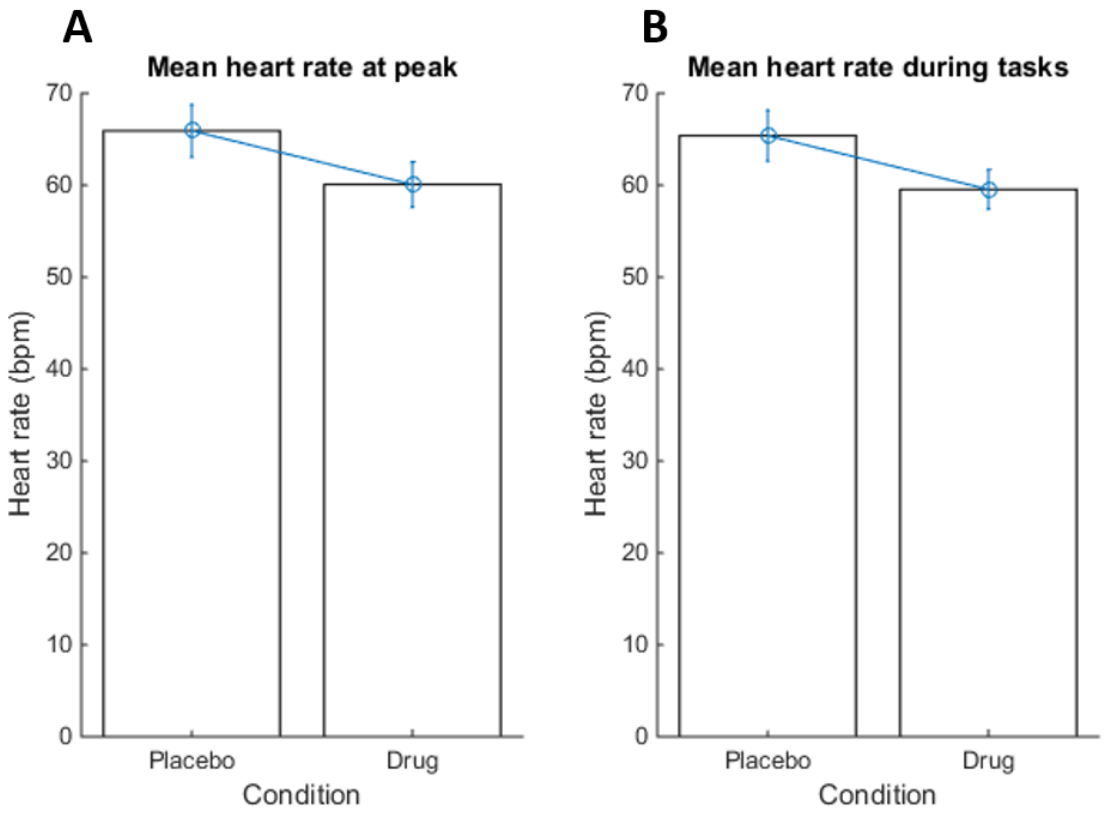

Figure 4: Heart rate at and around peak drug effect. Heart rate was found to be significantly lower in the drug, compared to the placebo, condition at both (A) the time of

peak drug effect (placebo M=65.9, SD=10.3; drug M=60.1, SD=8.9; t(12)=2.83, p=0.0151) and (B) during the 70-110 minute time window encompassing the second battery of tasks (placebo M=65.4, SD=10.0; drug M=59.5, SD=7.7; t(12)=2.83, p=0.0068).

3.1.2 Blood pressure

Mean systolic and diastolic blood pressure measurements are presented in Figure 5. Systolic blood pressure was found to decrease over the course of the session in the drug (β=-0.529, p=0.0236, Figure 6B), but not the placebo condition (β=0.088, p=0.684, Figure 6A). However, even at the time of peak drug effect, a significant difference could not be statistically confirmed for systolic blood pressure (t(12)=0.316, p=0.757). A significant decrease in diastolic blood pressure was not detected in either the drug (β=-0.27, p=0.0833, Figure 6D) or the placebo condition (β=0.103, p=0.54, Figure 6C).

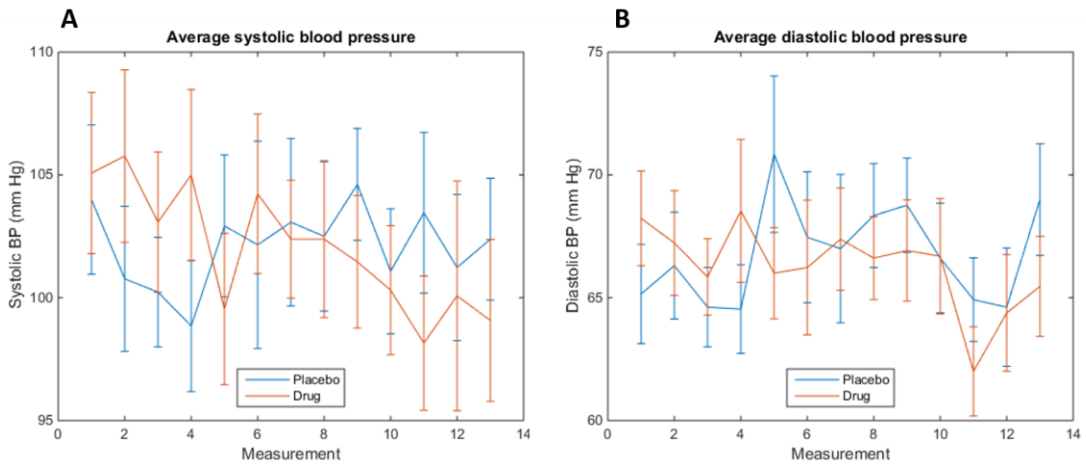

Figure 5: Average systolic (A) and diastolic (B) blood pressure over the course of both sessions. Error bars are standard error of the mean (SEM).

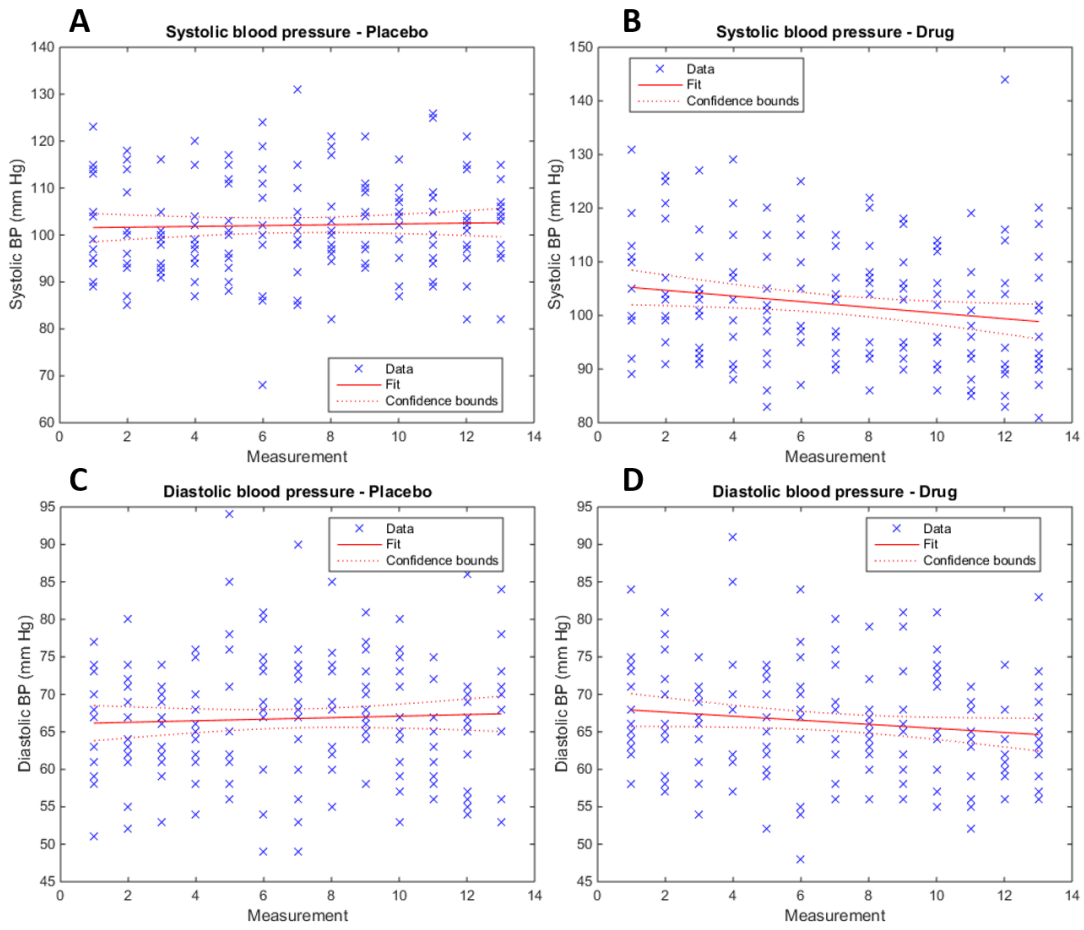

Figure 6: Linear regression of blood pressure over time for the placebo (A, C) and drug (B, D) conditions. Systolic blood pressure was found to decrease over the course of the session in the drug (β=-0.529, p=0.0236), but not the placebo condition (β=0.088, p=0.684). Diastolic blood pressure was not found to decrease in the drug (β=-0.27, p=0.0833) or the placebo condition (β=0.103, p=0.54).

3.2 Self-report measures

3.2.1 Trait and state anxiety

Self-report and behavioural measure outcomes are presented in Table 1. Trait anxiety was assessed at the beginning of the first session (Figure 7). The average score reported by the participants in the present study was 38.15 (SD 11.19), which is in agreement with norms for this measure based on samples of healthy adults (M=34.8, SD=9.2 for women and M=34.9, SD=9.2 for men; Spielberger et al., 1983). Baseline measures of state anxiety

(Figure 8) were similarly typical, 30.85 (SD 8.64) for the placebo session and 31.85 (SD 10.7) for the drug session (typical means are M=35.2, SD=10.6 for women and M=35.7, SD=10.4 for men; Spielberger et al., 1983). An ANCOVA was performed on the difference between outcome scores, with baseline score difference as a covariate, in order to assess differences in outcome between conditions. Outcome state anxiety did not differ between conditions (intercept=2.97, p=0.119), and stepwise regression did not detect a significant contribution of either trait anxiety or session order as covariates. No effect of condition ($F(1, 12)=2.683$, $p=0.127$) or time ($F(1, 2)=0.048$, $p=0.831$) on state anxiety measures was found, and no time x condition interaction was observed ($F(1, 12)=0.582$, $p=0.46$) using repeated measures ANOVA.

Table 1: Baseline and outcome scores on self-report and behavioural measures.

	Placebo		Drug	
	Baseline Mean(SD)	Outcome Mean(SD)	Baseline Mean(SD)	Outcome Mean(SD)
State anxiety (total)	30.85 (8.64)	30.15 (10.06)	31.85 (10.7)	33.08 (9.3)
Alertness (total)	61.21 (17.83)	49.05 (22.46)	61.17 (20.48)	56.21 (19.41)
Tone matching (% accuracy)	57.05 (11.02)	56.28 (11.75)	57.05 (10.43)	54.23 (8.21)
Heartbeat counting (% accuracy)	71.26 (15.44)	77.2 (11.24)	71.34 (15.32)	79.95 (10.37)
One-back (% accuracy)	82.39 (20.1)	89.2 (5.92)	97.73 (2.43)	88.64 (12.86)
Seconds counting (% accuracy)	69.48 (12.59)	72.24 (14.15)	72.59 (14.91)	70.53 (14.09)

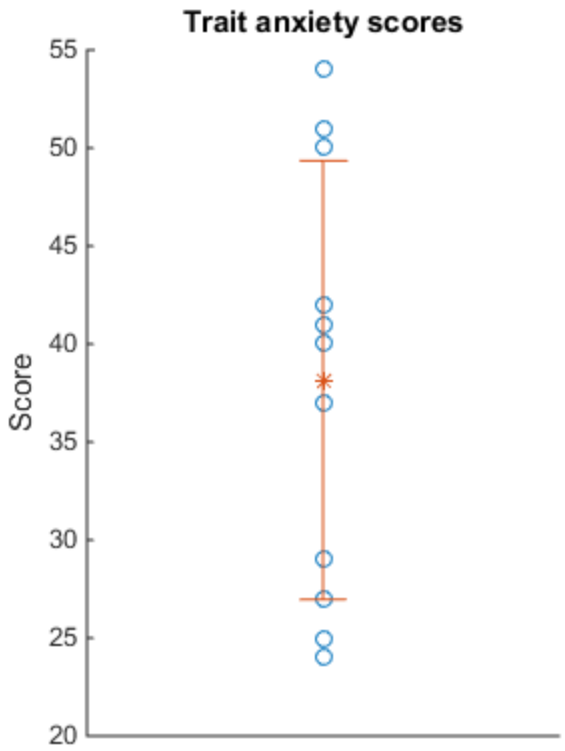

Figure 7: Trait anxiety. Participants reported mean trait anxiety scores of 38.15 (SD 11.19). Trait anxiety was assessed once, at the beginning of the first session for each participant. The scores obtained in this sample are in agreement with norms based on samples of healthy adults (M=34.8, SD=9.2 for women and M=34.9, SD=9.2 for men; Spielberger et al., 1983).

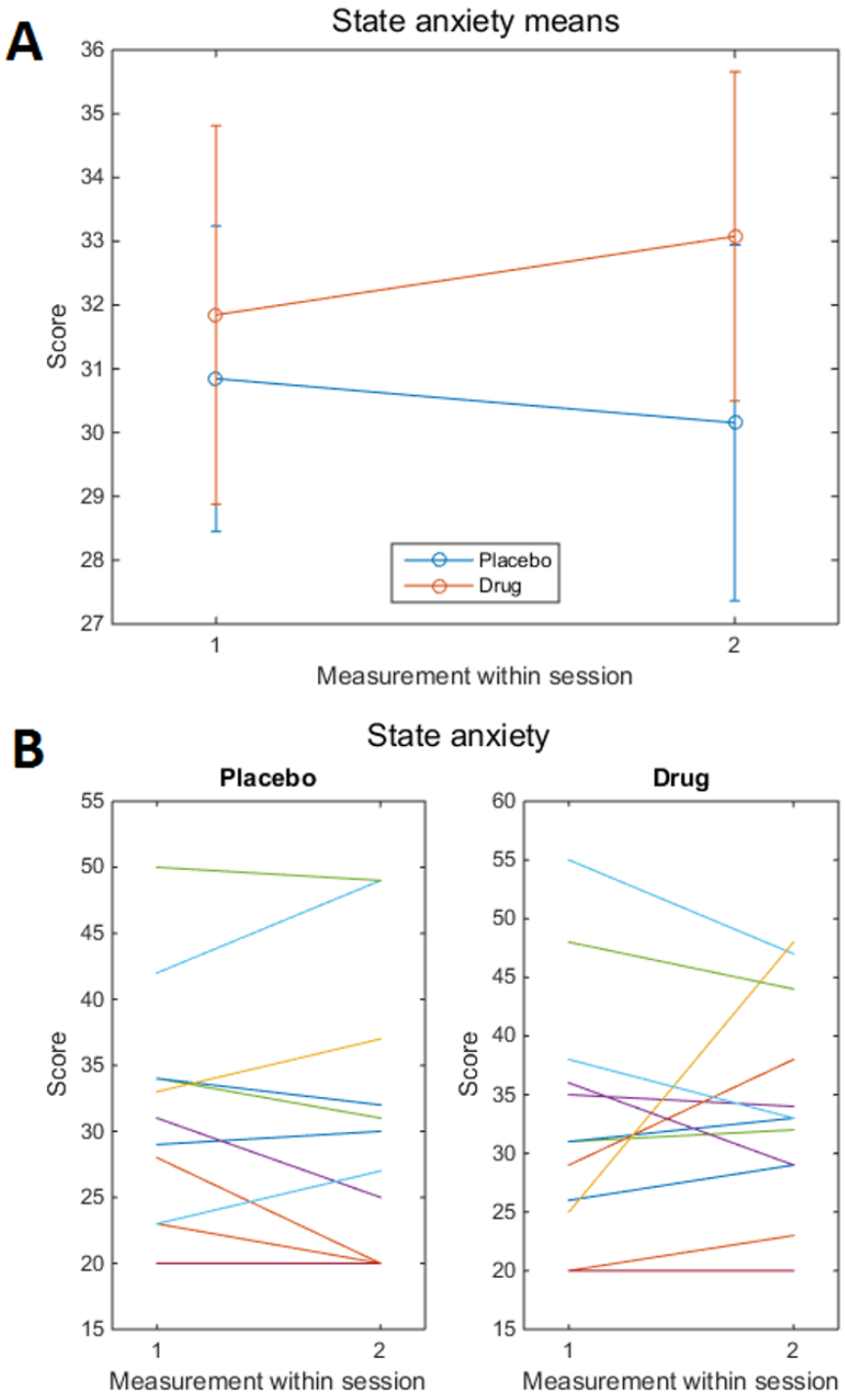

Figure 8: State anxiety mean scores (A) and individual paired-data plots (B). Error bars are standard error of the mean. There was no effect of condition ($F(1, 12)=2.683$, $p=0.127$) or time ($F(1, 2)=0.048$, $p=0.831$) on state anxiety measures, and no time x condition interaction ($F(1, 12)=0.582$, $p=0.46$).

3.2.2 Alertness

ANCOVA analysis using a model that included only baseline score differences as a covariate found that outcome alertness scores were not significantly different between conditions (intercept=7.1912, p=0.0987). Stepwise regression confirmed that models including trait anxiety scores and session order as covariates did not predict the outcome variable better (AIC=71.13 for best model), and the best model was found to explain 51.87% of the variance in outcome measures (coefficient=0.8305, R^2=0.5187, p=0.005). Repeated measures ANOVA revealed that alertness decreased over the course of both testing sessions (main effect of time, F(1, 12) = 6.11, p=0.0236), and no Time x Condition interaction was observed (F(1, 12) = 3.403, p=0.0899), Figure 9).

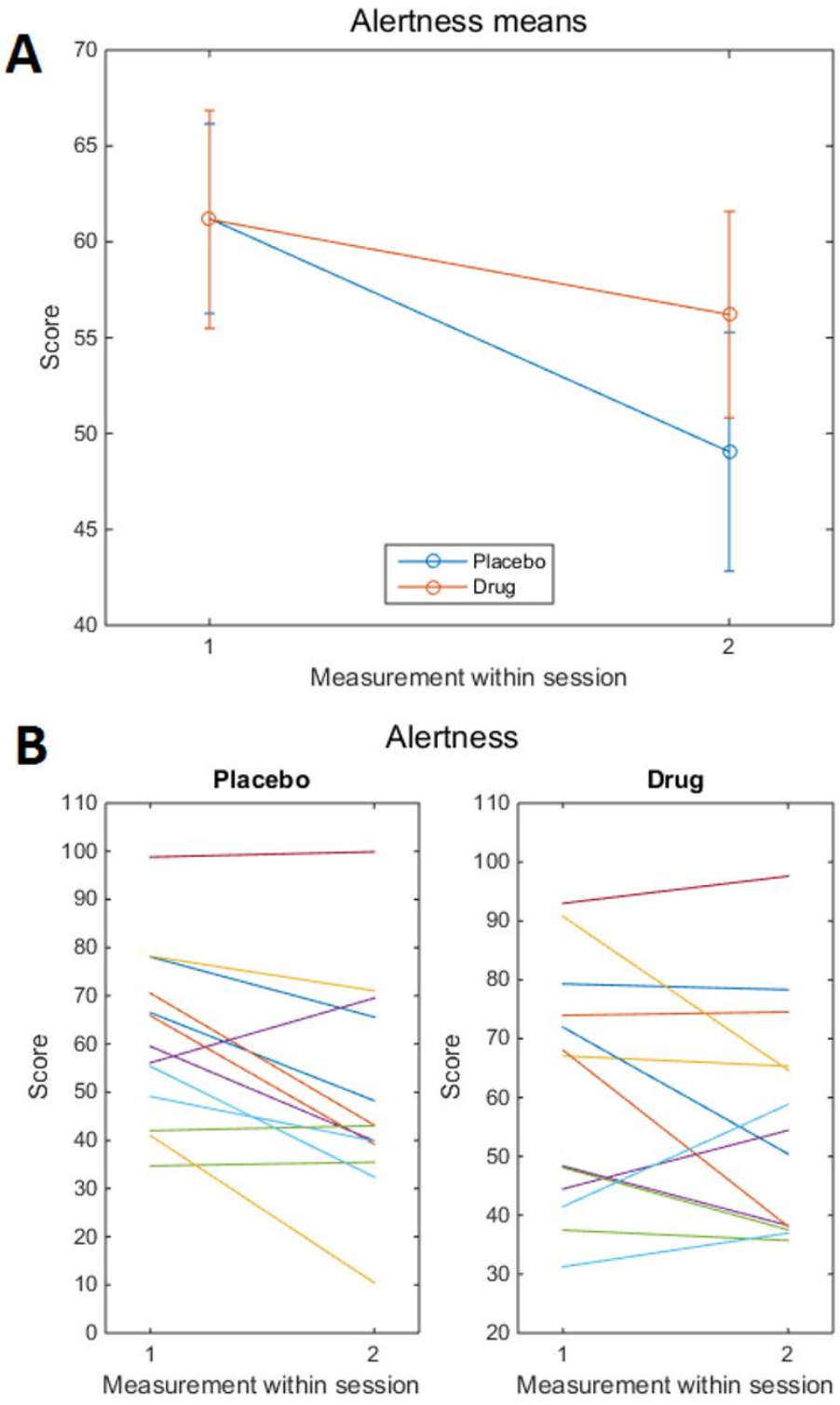

Figure 9: Alertness mean scores (A) and individual paired-data plots (B). Errors bars are standard error of the mean. Alertness decreased over the course of both testing

sessions (main effect of time, F(1, 12) = 6.11, p=0.0236). No time x condition interaction was observed (F(1, 12) = 3.403, p=0.0899)

3.3 Behavioural tasks

3.3.1 Interoception tasks

3.3.1.1 Heartbeat detection

Outcome performance on the tone matching task was not found to differ significantly between the drug and placebo conditions (intercept=-20.07, p=0.113), as assessed by ANCOVA; however, performance in the drug session was numerically lower (Figure 10). Stepwise regression assessed the contribution of task order, session order, outcome state anxiety and trait anxiety as covariates. The best performing model included outcome state anxiety and trait anxiety in addition to baseline task performance (AIC=63.55). However, the overall model did not predict outcome tone matching scores (R^2=0.4132, p=0.1688). There was no main effect of either time (F(1, 12)=0.649, p=0.436) or condition (F(1, 12)=0.196, p=0.666), and no significant interaction (F(1, 12)=0.244, p=0.63), as assessed by repeated measures ANOVA.

It should be noted that 8 of 13 subjects failed to achieve above-chance baseline performance in both conditions. When these subjects are excluded, visual inspection of the resulting plot (Figure 10C) shows that every remaining subject exhibited a decrease in performance over the course of the drug condition, but not the placebo condition. Statistical analyses are not presented for this small sub-sample.

A post-hoc power analysis revealed that, based on the effect size observed in our limited sample (f2=0.7), a sample of 25 participants would be needed to detect a difference in outcome scores between conditions at an alpha level of 0.05, with a power of 0.9.

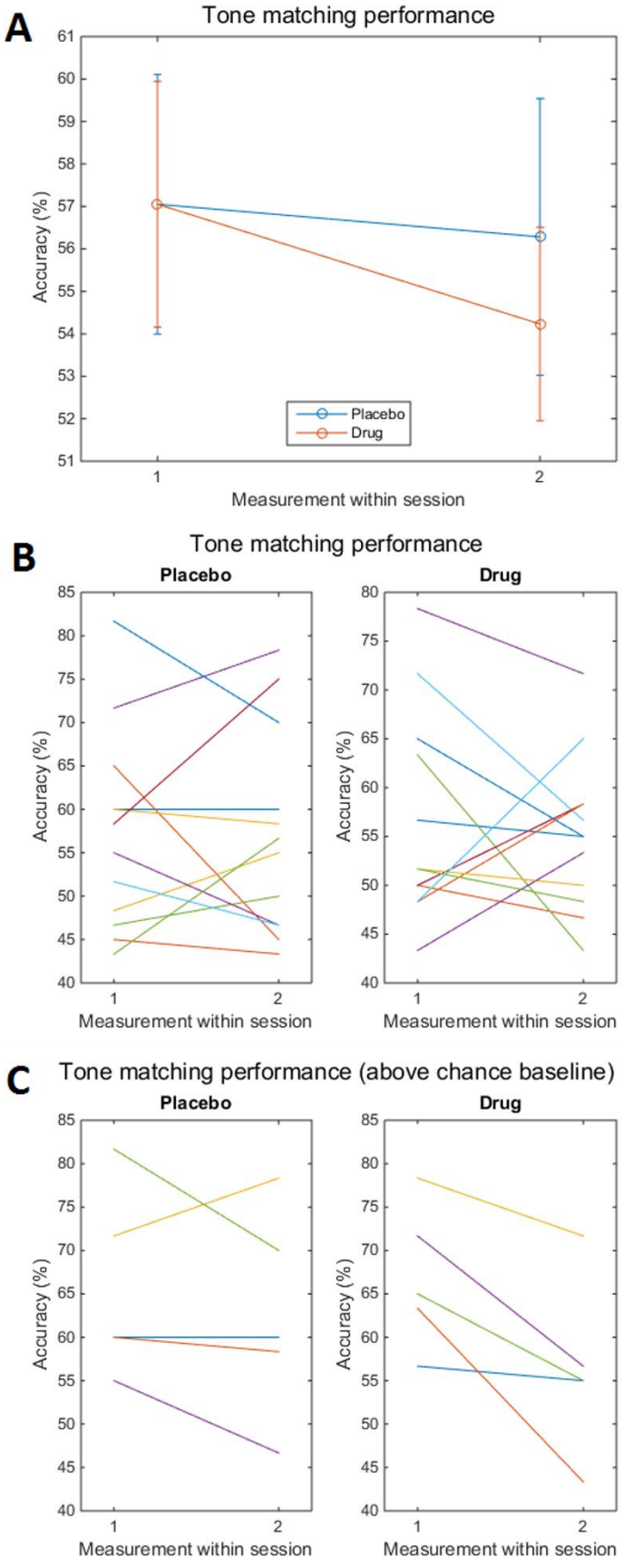
A
Tone matching performance
Accuracy (%)
Measurement within session
Placebo
Drug
B
Tone matching performance
Placebo
Drug
Accuracy (%)
Measurement within session
C
Tone matching performance (above chance baseline)
Placebo
Drug
Accuracy (%)
Measurement within session

Figure 10: Tone matching (heartbeat detection) mean performance (A), individual paired-data plots (B) and individual paired-data plots for good performers (C). Errors bars are standard error of the mean. Outcome performance on the tone matching task was found not to differ significantly between the drug and placebo conditions (intercept=-20.07, p=0.113); however, performance in the drug session was numerically lower. Only 5 subjects achieved above-chance performance at baseline in both conditions, and their data are plotted separately (C). The numerical trend observed in the overall sample appears to be more pronounced in the sub-sample of good performers, in that decreases in performance appear to be stronger in the drug condition. Statistical analyses were not performed on this sub-sample.

3.3.1.2 Heartbeat tracking

Heartbeat counting performance did not differ significantly between conditions (ANCOVA; intercept=0.027, p=0.437). Stepwise regression did not identify task order, session order, trait anxiety or outcome state anxiety as additional covariates beyond baseline task performance (best model AIC= -49.66). The resulting model did not significantly predict heartbeat counting outcome scores (R^2=0.057, p=0.453).

A repeated measures ANOVA revealed a significant main effect of time on outcome heartbeat counting scores (F(1, 11)=10.21, p=0.009), indicating that for both conditions, performance improved at the second measurement (Figure 11). This is in opposition to the tone matching results, which, although not statistically significant, were numerically lower under the influence of the drug than at the beginning. No significant main effect of condition (F(1, 11)=0.255, p=0.624), nor a condition x time interaction (F(1, 11)=0.363, p=0.559), was observed.

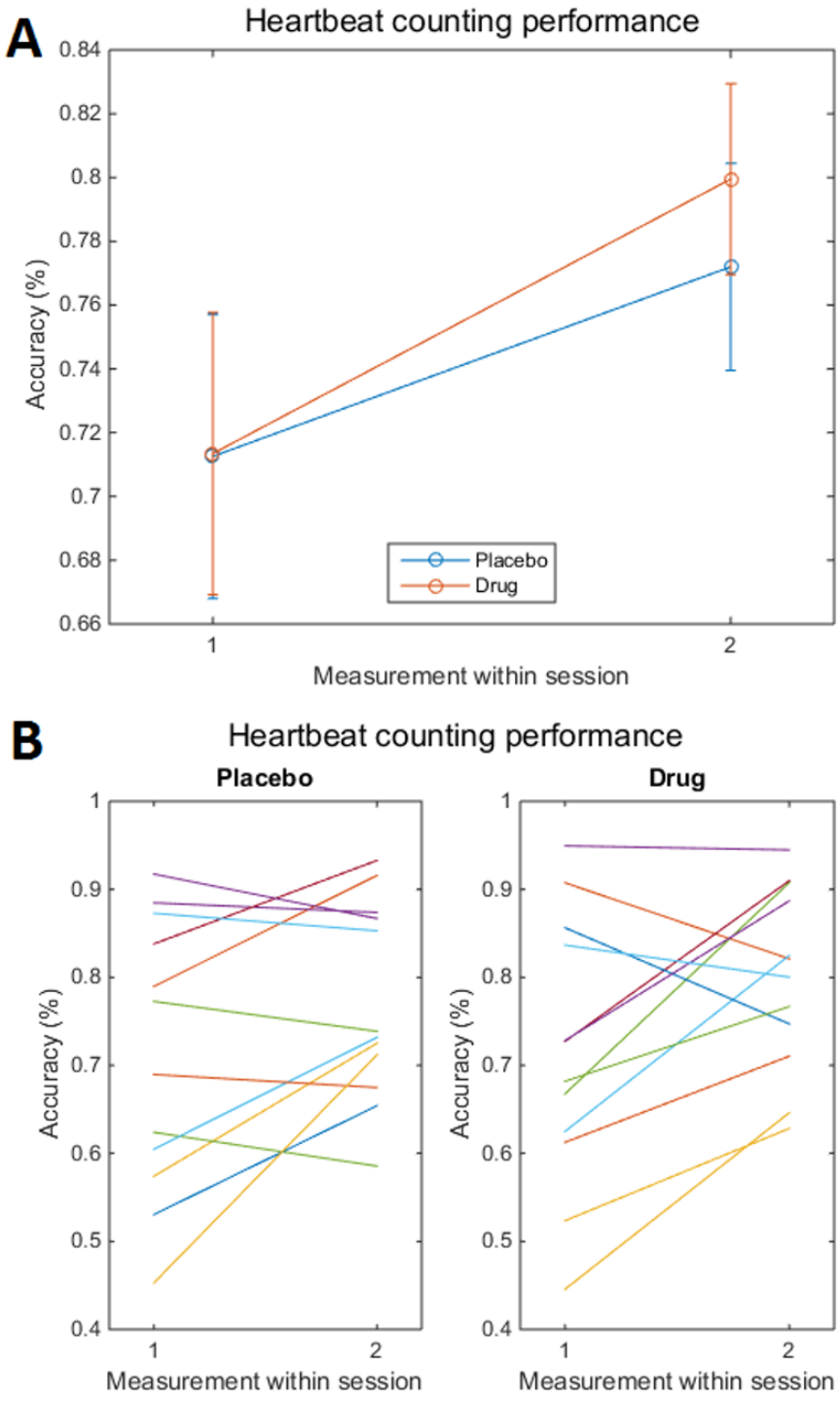

Figure 11: Heartbeat counting (tracking) task performance mean performance (A) and individual paired-data plots (B). Errors bars are standard error of the mean. A significant main effect of time on outcome heartbeat counting scores was observed ($F(1, 11)=10.21$, $p=0.009$), indicating that performance increased over time in both sessions.

No significant main effect of condition (F(1, 11)=0.255, p=0.624), nor a condition x time interaction (F(1, 11)=0.363, p=0.559), was observed. Heartbeat counting performance did not differ significantly between conditions (ANCOVA; intercept=0.027, p=0.437).

3.3.2 Cognitive control tasks

3.3.2.1 One-back task

ANOVA did not identify a main effect of either condition(F(1,7)=2.8, p=0.138) or time(F(1,7)=0.054, p=0.822) on task performance, and no significant interaction of time x condition was found (F(1, 7)=4.455, p=0.0727). However, it should be noted that performance on this task was frequently at or near ceiling (Figure 12), which can mask potential effects on performance. In particular, the homogeneity of slopes assumption is violated when the sample includes baseline scores at ceiling. ANCOVA was thus not performed on data from this task. Additionally, there was no difference in baseline scores (t(7)=-2.04, p=0.0812).

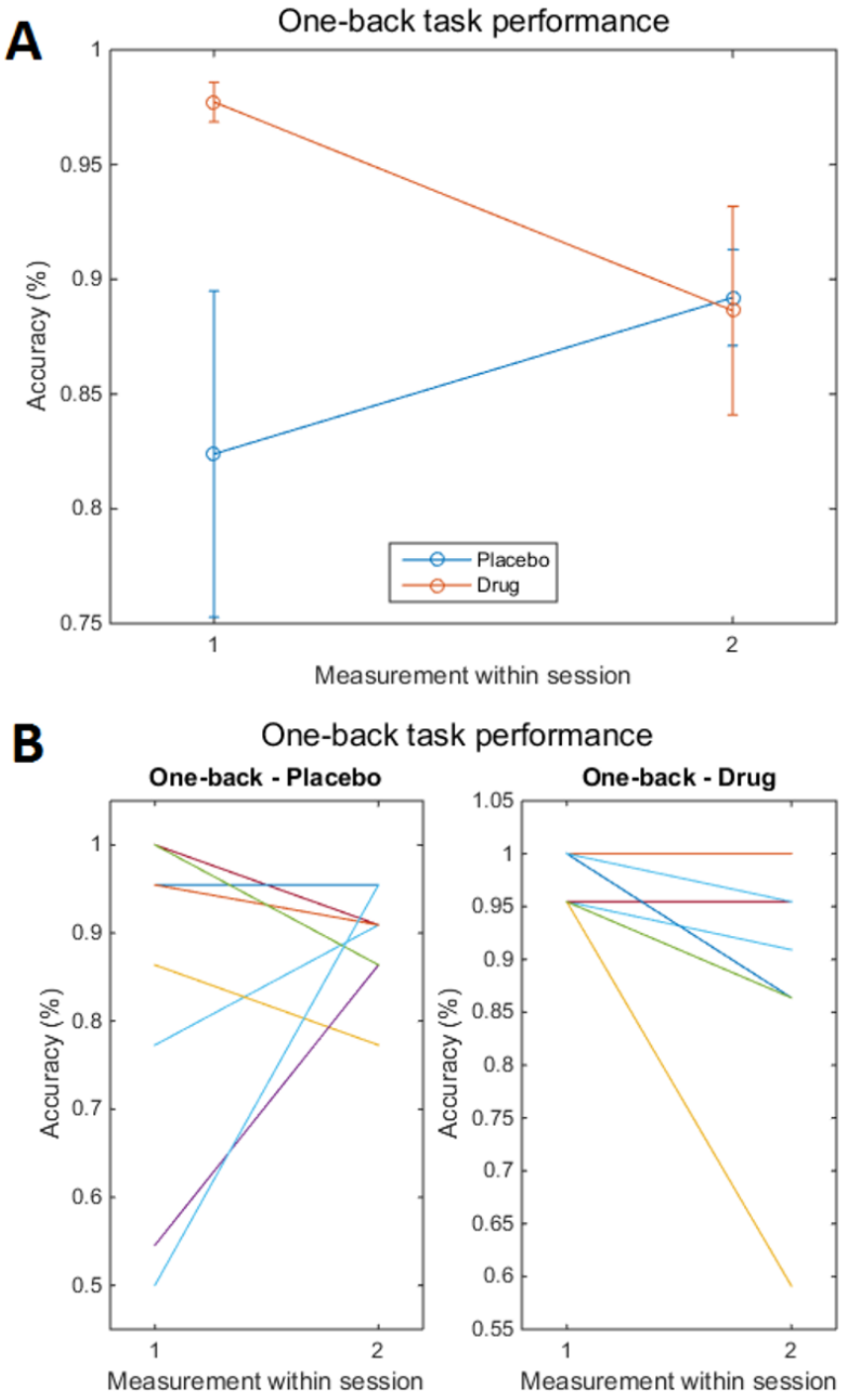

Figure 12: One-back task performance mean performance (A) and individual paired-data plots (B). Errors bars are standard error of the mean. Several participants exhibited near-ceiling performance. There was no main effect of either condition ($F(1,7)=2.8$, $p=0.138$) or time ($F(1,7)=0.054$, $p=0.822$) on task performance, and no

significant interaction of time x condition (F(1, 7)=4.455, p=0.0727). There was no difference in baseline scores (t(7)=-2.04, p=0.0812).

3.3.2.2 Seconds counting

Performance on the seconds counting task did not appear to be affected by the drug manipulation, or significantly change throughout the session (Figure 13). No significant difference in outcome scores for the seconds counting task was observed via ANCOVA using the base model that included a covariate of baseline performance only (intercept=-0.029, p=0.123). Stepwise regression did not identify a significant contribution from task order, session order, outcome state anxiety or trait anxiety, beyond baseline performance (best model AIC=-52.78). The final model, which included baseline performance only, accounted for 49.23% of the variance in the outcome measure (R^2=0.4923, p=0.03515). A repeated measures ANOVA revealed no main effects of time (F(1, 8)=0.19, p=0.895) or condition (F(1, 8)=0.065, p=0.805), or time x condition interaction (F(1, 8)=3.115, p=0.116).

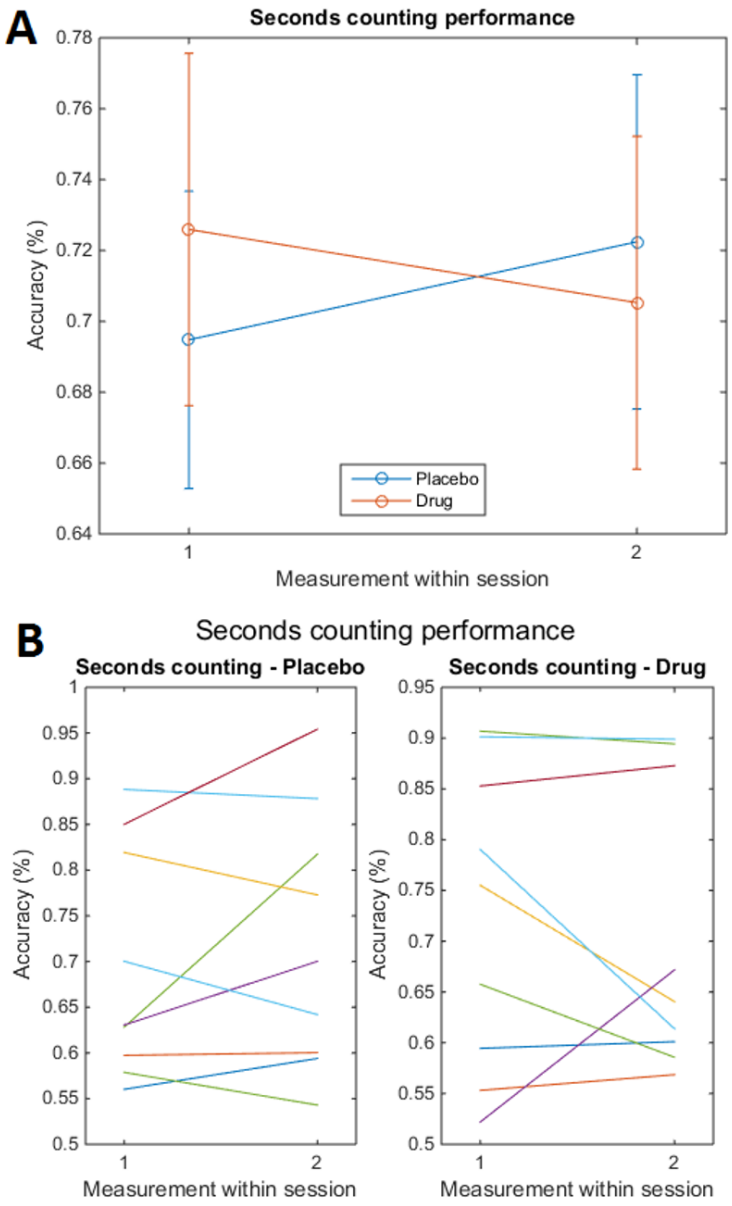

Figure 13: Seconds counting task performance mean performance (A) and individual paired-data plots (B). Errors bars are standard error of the mean. No significant difference in outcome scores for the seconds counting task was observed (intercept=-0.029, p=0.123). The final regression model, which included baseline performance only, accounted for 49.23% of the variance in the outcome measure (R^2=0.4923, p=0.03515). A repeated measures ANOVA revealed no main effects of time (F(1, 8)=0.19, p=0.895) or condition (F(1, 8)=0.065, p=0.805), or time x condition interaction (F(1, 8)=3.115, p=0.116).

Chapter 4

4 Discussion

The primary goal of the present study was to identify whether propranolol is a promising agent for the manipulation of interoceptive processes. We conducted a double-blind crossover study, in which we assessed the effects of 20 mg of propranolol on physiological response, two measures of interoceptive ability, self-reported anxiety and alertness, as well as two cognitive control tasks. We found that propranolol produced a reliable decrease in heart rate, compared to placebo, as well as a decrease in systolic blood pressure. Propranolol numerically decreased interoceptive performance on the tone matching interoception task, although this effect did not reach statistical significance. In contrast, on the heartbeat counting task performance improved with time in both conditions. No effect of drug on self-report measures was found, but alertness was observed to decrease over time in both conditions. Finally, propranolol had no effect on performance on either cognitive control task.

4.1 Physiological effects

Propranolol, at a dose of 20 mg administered orally, elicited an expected and reliable physiological response. Participants demonstrated a significant drop in heart rate of nearly 6 bpm in the drug, compared to the placebo, condition, thus confirming the effectiveness of our pharmacological manipulation. This finding is in line with other studies reporting an approximately 5 bpm decrease for 20 mg, and somewhat higher decreases (10-15 bpm) for 40 and 80 mg (Chamberlain & Robbins, 2013; Chamberlain et al., 2006). Although doses of 40 and 80 mg are much more common in the cognitive propranolol literature, our findings demonstrate that even a 20 mg dose produces reliable heart rate changes, statistically detectable even in a small sample. Our findings thus confirm a reliable effect of propranolol at low dose, which is in line with its medicinal use, where a dosage of 10 mg is common. We further confirmed that heart rate displayed a linear downward trend over time, and that the drop in heart rate was reliable not just at the time of peak drug effect, but in a 40-minute window around the peak, which encompassed administration of our tasks of interest. This finding suggests that the drug

effect is reliable over the time course of reasonably long experimental paradigms, and that this is achievable even with a dose considerably smaller than what is typically used.

In addition to an effect on heart rate, we found an effect of the drug on systolic blood pressure. This is in line with propranolol's known action and concordant with findings from other studies, which frequently report drops in systolic blood pressure, and occasionally diastolic (Kroes et al., 2010; Kroes et al., 2015; Tollenaar et al., 2009; Schwabe et al., 2012; Hurlemann et al., 2005; van Stegeren, Evaraerd & Gooren, 2002). It is worth noting that although our findings are similar, the dose used in our study (20 mg) is considerably smaller than those used in most other studies (40-80 mg), and in fact, a study conducted using a 20 mg dose reported minimal drops in blood pressure (De Martino et al., 2008). This discrepancy may largely be due to differences in protocol. Our conclusions are based on regression analysis, made possible through frequent measurements that were taken every 10 minutes throughout the session. In contrast, most published studies collected physiological measurements at only a few time points, and performed a statistical contrast typically only at the time of peak drug effect. In fact, in our own analysis, t-tests failed to confirm a statistically reliable difference at peak, highlighting the benefits of repeated measurement over extended time periods for detection of small effects. These findings confirm the utility of incorporating frequent physiological measurements into any propranolol paradigm, and caution against interpreting any results as a consequence of heart rate changes alone in terms of physiological mechanisms. Even though the observed downward trends in blood pressure are small, and the differences at any particular time point likely of minimal clinical significance, the presence of these trends confirms that propranolol's mechanisms of action on blood pressure are engaged even by a small dose. In light of this, care should be taken in deriving mechanistic conclusions that separate the drug's effects on heart rate from its effects on blood pressure.

4.2 Effects on interoception

Although we predicted that performance on both interoception tasks should decrease under the effect of propranolol, we found that outcome performance changed in different directions for the two tasks. Heartbeat counting performance improved over the course of

both the drug and the placebo sessions, whereas tone matching performance showed a numerical decrease for the drug session alone. If borne out in a larger sample, this finding would be indicative of the presence of different mechanisms of action, or different strategies used by participants. This would be in line with existing research showing that the two interoception tasks may engage different strategies. Furthermore, prior research on the link between beta-adrenergic mechanisms and interoception has focused on interoceptive awareness, rather than accuracy (Khalsa et al., 2009).

Propranolol, whose action is in opposition to agonists such as isoproterenol, known to intensify interoceptive sensations (Khalsa et al., 2009), can be reasonably expected to cause a decrease in interoceptive *awareness*; however, the resulting effect on interoceptive *accuracy* is not necessarily clear-cut. Although it is reasonable to suppose that decreasing sensitivity would lead to more interoceptive errors, it has been shown that interoceptive awareness only partially predicts interoceptive accuracy (Garfinkel et al., 2015). This could reflect a true partial independence between these processes, or possibly be a consequence of measurement strategies. Since interoceptive awareness is measured through self-report, whereas interoceptive accuracy is measured objectively, it is possible for the two not to concur. In fact, discrepancies have been observed in previous studies, which have noted that even good performance on objective measures of interoceptive accuracy is often accompanied by subjective reports to the contrary (Wiens, 2005).

It is also possible that a drop in interoceptive awareness could trigger compensatory mechanisms, or an over-reliance on alternative strategies, which can alter overall task performance in unexpected ways. In fact, using an estimate of one's heart rate, rather than engaging in beat-by-beat heartbeat counting, has been identified as an alternative strategy for the heartbeat counting task (Khalsa et al., 2009; Kleckner et al., 2015). While participants' ability to detect their heartbeat is assumed not to change throughout the placebo session, their estimate of their *heart rate* can certainly improve, as most of the experimental session consists of tasks that effectively gather heart rate information for the participant. Thus, the heartbeat counting task appears to be susceptible to within-session practice effects, and it is possible that this improvement can mask any changes in moment-to-moment interoceptive ability if participants switch over to estimation

strategies towards the end of the session. This would produce a pattern of results exactly as seen: an overall increase in heartbeat counting performance over time, regardless of condition, that does not align with tone matching performance (where estimation strategies are patently of no use).

Task order was not found to contribute significantly to explaining variability in heartbeat counting outcome scores. This indicates that the strategy used for heartbeat counting did not depend on having been explicitly exposed to heart rate information immediately before the task - i.e. hearing their heartbeat in the form of auditory tones as part of the tone matching task did not seem to affect participants' performance on heartbeat counting. This would be of particular concern if participants were relying on estimation strategies. This could be indicative that they are not, or, in light of the observed practice effect, that they could be hitting an information ceiling even without a specific contribution of the auditory feedback from the tone matching task. It is worth noting, also, that the tone matching task is notoriously difficult (Kleckner et al., 2015) and typically commands all of a participant's attention, making at least explicit additional strategies less likely.

Although the numerical decrease in performance on the tone matching task for the drug condition failed to reach statistical significance at the 5% alpha level, the numerical change and corresponding statistical reliability ($p=0.113$) of the effect suggests it could be worth exploring with a larger sample. Since this task is less susceptible to alternative strategies than heartbeat counting (Khalsa et al., 2009; Kleckner et al., 2015), it seems likely that this result is more indicative of the actual effect, if any, of propranolol on interoceptive ability. A post hoc power analysis revealed that, based on the effect size observed in this limited sample, a relatively modest sample of 25 participants would be needed to detect it with good statistical power. This result suggests that this effect is a good candidate for follow-up in a larger sample.

It is important to note that this task is known to be exceptionally difficult, resulting in high rates of below-chance performance (Khalsa et al., 2009). This seems to have been an issue in our study, as well, with 8 out of 13 participants failing to perform above chance

at the beginning of both sessions. With this in mind, it would be sensible to limit the analysis only to participants who achieved above-chance performance at baseline in both conditions. The present study's sample is too small for this to be a viable statistical query, however, visual inspection of the individual participant plots (Figure 10C) suggests that the decrease in performance for the drug condition may possibly be more pronounced in this subgroup. In fact, every subject who started above baseline in the drug condition experienced a drop in performance (whereas the same cannot be said for the placebo session). The present investigation used a relatively small sample size with the purpose of validating our proposed pharmacological manipulation and identifying avenues for future exploration. Therefore, the numerical effect seen in this task, as well as the trends observed within the subgroup of good performers, are taken as warranting further investigation, and given the reasonable sample size suggested by the power analysis, will be followed up by a larger study aiming for a total sample of 25.

4.3 Effects on state anxiety

A surprising finding in the present study was a lack of effect of propranolol on state anxiety, as assessed by pre- and post-drug self-report questionnaires. This finding runs in opposition to other studies that have found anxiolytic effects for propranolol, even at doses as low as 10 mg (Steenen et al., 2016; Mealy et al., 1996), as well as to its off-label use in treating performance anxiety. A potential explanation is that participants in the present study were specifically screened to have no history of clinical anxiety diagnosis, as confirmed by the finding that for our sample the trait and state scores were within the typical range. Additionally, the testing session was not an anxiogenic situation. Finally, it is possible that the nature of the tasks in the present study may have interacted with the anxiolytic effects of the drug (Domschke et al., 2010). It is even possible that detecting a change in one's own heart rate could have increased state anxiety for some individuals. (Indeed, the largest change from baseline to outcome was an increase observed for one participant in the drug condition, and was more than twice the magnitude of any other pre-post change on state anxiety in the study.) The lack of effect in the present study can be taken to indicate that a change in state anxiety is unlikely to account for the present findings, either on the interoception or the control tasks. It is worth noting that

interactions between anxiety and cognitive performance under propranolol have been noted with respect to other tasks (Chamberlain et al., 2006), so caution should be taken in extrapolating these findings to other paradigms.

4.4 Effects on alertness

Alertness was found to decrease over time in both conditions. This is an expected finding, since each testing session lasted 2.5 hours with minimal breaks. No effect of condition on alertness was found, indicating that any decreases in performance on the behavioural tasks for the drug condition cannot be explained as a result of lower alertness alone. From a methodological perspective, this finding also confirms that although decreased alertness is a known side-effect of propranolol used chronically, this is not an issue in the context of a single dose administered to healthy, low-risk individuals in the context of a research study. This finding further points to the viability of propranolol for future research work.

4.5 Effects on cognitive control tasks

Neither control task exhibited a reliable difference in outcome performance between conditions, confirming that any differences in interoception task performance cannot be simply due to a global detrimental or facilitatory effect of propranolol. Because the seconds counting task did not exhibit the same increase in performance over time as heartbeat counting, we can reasonably conclude that the mechanism responsible for the heartbeat counting performance increase could be specific to interoception, whether or not it is sensitive to the propranolol manipulation.

No significant effects were observed on the one-back task. However, this result should be interpreted with caution, as performance on this task was at or near ceiling for many participants, which can mask effects of practice or drug on performance. Although this task was originally included in the study as a conceptual parallel to the tone matching task, in that it functions effectively as an auditory vigilance control task, our findings indicate that it is not a suitable control. Especially considering the level of difficulty of the tone matching task, the near-ceiling performance on the one-back task renders it a

poor point of reference with respect to cognitive performance. If this or a similar task is to be included in future studies, the difficulty level will need to be increased substantially.

4.6 Mechanisms of action

If the numerical decrease observed on the tone matching task in the drug condition, and its independence of effects on cognitive control tasks, are confirmed in a larger sample, these results will indicate the involvement of beta-adrenergic signalling in interoception. While propranolol's dual action on both central and peripheral pathways ensures that neither route's potential effects are missed in this initial investigation, in order to elucidate the precise mechanism of beta-adrenergic signalling in interoception, further follow-up work utilizing more selective agents will be necessary. However, some speculation can be made even at this point, based on existing evidence. In particular, it does not seem to be the case that lower heart rate generally results in lower interoception performance. A relevant analysis was performed by Kleckner and colleagues (2015), who conducted a study in which 174 participants performed the tone matching task employed here. The authors found no correlation between resting heart rate and interoceptive ability, as indexed by the tone matching task. This suggests that a slow heart rate alone is not enough to limit interoceptive ability. It is, however, possible that a slower heart rate, *as deviation from a person's habitual baseline,* may still have an effect. This effect could be to decrease interoceptive ability by attenuating the heart rate signal, or on the contrary, to be perceived as being more salient, and result in an increase of interoceptive ability. Our preliminary results suggest that the former is more likely, however, more investigation is needed.

The mechanisms of propranolol's action on interoception, and more generally, that of beta-adrenergic blockade, are certain to be complex. The involvement of at least some peripheral mechanisms, i.e. action on the heart rate directly, which in turn affects downstream interoceptive processes in the brain, is very likely, possibly in addition to central mechanisms. The primary evidence for this suggestion is the effect of hydrophilic, peripherally acting isoproterenol on interoceptive awareness (Khalsa et al., 2009), and the predominance of beta-adrenergic receptors peripherally, relative to the brain (Szabadi, 2013). Additionally, the anxiolytic effects of propranolol are believed to be largely

peripheral, acting by blocking the autonomic arousal associated with specific anxiogenic triggers, rather than changing brain chemistry more chronically (Steenen et al., 2016).

Because propranolol does cross the blood-brain barrier, central mechanisms concerning the action of noradrenaline within the brain itself must also be considered. There is some evidence that the action of propranolol on working memory is due to such central mechanisms, as peripherally acting beta-blockers such as atenolol have not been shown to impair working memory. Similarly, while propranolol has been shown to affect encoding of emotional memories, hydrophilic beta-blockers do not exhibit the same influence, thus implicating propranolol's central effects in its influence on emotional memory (Rimmele et al., 2016; Chamberlain et al., 2006). This effect has been linked to the presence of beta-adrenergic receptors in the amygdala (Szabadi, 2013). Although the same is not known with certainty for the insula, it is important to note that beta-adrenergic receptors have been found in the human brain in other areas relevant to interoceptive signalling, such as the thalamus and basal ganglia (Reznikoff et al., 1986). Additionally, a recent study found that intra-insular injections of propranolol in rats affected arousal-related behaviour (Rojas et al., 2015). In the same study, intra-insular injections of norepinephrine were found to interact with the effects of oral propranolol, confirming central action in the insula following oral administration in rats. Thus, although strong empirical evidence exists for the modulation of interoception by peripheral effects of beta-adrenergic signalling, the possibility exists that propranolol's central effects could extend to interoceptive systems.

4.7 Limitations

The above conclusions must be considered in light of the present study's design and limited sample size. Although this limitation is mitigated by our use of a crossover design, which gives more power than the between-subjects designs used by the majority of cognitive propranolol studies, care must still be taken in interpreting these preliminary findings. In particular, nonsignificant, but interesting, results are presented here as indications that further investigation is warranted, rather than hard evidence in themselves. Similarly, the negative results presented must be also be interpreted with caution, keeping in mind that the present study can be underpowered to detect small

behavioural effects. This is especially important when null effects alter the interpretation of the main findings, as in the case of null effects of propranolol on cognitive control tasks

4.8 Future directions

Follow-up studies with agents more selective than propranolol are needed to disentangle potential mechanistic pathways involved in the influence of beta-adrenergic agents on interoception. A promising candidate agent is atenolol, whose effects, while similar to propranolol, are confined to peripheral pathways due to its nearly absent penetrance of the blood brain barrier. Although studies utilizing isoproterenol have already implicated peripheral mechanisms in the control of interoceptive sensitivity, employing atenolol would provide important evidence regarding the effects of attenuating, rather than increasing, cardiac activity. Furthermore, considerable insight is to be gained by conducting parallel studies with propranolol and atenolol. By utilizing identical paradigms while varying only the drug used, and calibrating the doses for identical degrees of physiological response, it would be possible to obtain a quantitative comparison of the differential contributions of central-cum-peripheral, versus solely peripheral, mechanisms to any observed effect on interoception. A quantitative contrast of this nature would thus give some idea of the degree of contribution of central mechanisms alone.

A robust and safe paradigm for manipulating interoception, as well as a mechanistic understanding of its action, would be a great asset in investigating the contribution of interoception to other cognitive processes. Such a paradigm would be invaluable in establishing a causal link between interoception and non-emotional cognition, such as metamemory. The present study represents a promising start, in terms of both establishing a viable paradigm for administering propranolol in the context of interoceptive accuracy measures and their associated control tasks, as well as providing tentative evidence for an effect of beta-adrenergic blockade on interoceptive accuracy. Although much work remains to be done to confirm these effects, the paradigm presented in this study represents a viable platform for this investigation, as well as a foundation for

follow-up work with more selective pharmacological agents and additional cognitive tasks.

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Appendices

Appendix A: Research ethics board approval for present study.

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Amendment Approval Notice

Principal Investigator: Dr. Penny MacDonald
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,London Health Sciences Centre

Review Type: Full Board
HSREB File Number: 102018
Study Title: Distinguishing the roles of ventral and dorsal striatum in cognition (REB #18517)
Sponsor: Canadian Excellence Research Chair

HSREB Amendment Approval Date: May 16, 2016
HSREB Expiry Date: November 29, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	State Trait Anxiety Inventory for Adults-April/2015	
Revised Letter of Information & Consent		2016/03/01
Revised Western University Protocol	Received Mar 23, 2016	

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]
Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information: Erika Basile ___ Katelyn Harris ___ Nicole Kaniki ___ Grace Kelly ___ Vikki Tran ___

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Curriculum Vitae

EDUCATION

2015–2017 **MSc Neuroscience**, Western University
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2005–2011 **HBSc Psychology**, University of Toronto

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2015–2017 Teaching Assistant, Western University

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PUBLICATIONS

Fiacconi, C.M., **Kouptsova, J.E.**, Köhler, S. (2017). A role for visceral feedback and interoception in feelings-of-knowing. *Consciousness and Cognition, 53,* 70-80.

Panchal, T., Chen, X., Alchits, E., Oh, Y., Poon, J., **Kouptsova, J.**, Laski, F., Godt, D. (2017). Specification and spatial arrangement of cells in the germline stem cell niche of the Drosophila ovary depend on the Maf transcription factor Traffic jam. *PLoS Genetics, 13*(5): e1006790

Kouptsova, J.E., Leung, R.C., Taylor, M.J. (2017) Stimulus exposure duration alters implicit processing of neutral and emotional faces. *Neuroscience, 341*, 154-159.

POSTERS and TALKS

1. **Kouptsova, J.** A role for interoception in Feelings-of-Knowing. Talk given at the Toronto Area Memory Group. Toronto, ON, 2017
2. **Kouptsova, J.**, Fiacconi, C., Köhler, S. Are There Feelings in Feelings-of-Knowing? Evidence from Psychophysiology and Measures of Interoception. Poster session presented at the Lake Ontario Visionary Establishment (LOVE) Conference. Niagara Falls, ON, 2017
3. Birca, A., Vakorin, V., Madathil, S., Miller, S., Doesburg, S., Seed, M., Chau, V., Nita, D., Duerden, E., Lim, J.,**Kouptsova, J.**, Thompson, A., De Petrillo, A., Hickey, E., Hahn, C. Structural-functional correlates of pre-operative brain development in term newborns with congenital heart disease. Poster session

presented at the Pediatric Academic Societies (PAS) Annual Meeting. San Diego, CA. 2015

4. Melo, H., **Kouptsova, J.**, Cunningham, W., Anderson, A. Dopamine-related genetic influences on cognitive flexibility. Poster session presented at the Medical Image Computing and Computer Assisted Intervention Conference (MICCAI). Cambridge, MA. 2014
5. **Kouptsova, J.**, Leung, R., Taylor, MJ. Exposure duration differentially affects processing of emotional and neutral faces. Poster session presented at the 19th International Conference on Biomagnetism (BIOMAG). Halifax, NS. 2014
6. Panchal T., **Kouptsova J.**, Alchits A., Godt D. Traffic Jam, a large Maf transcription factor, is required for germline stem cell niche formation. Poster session presented at the Toronto FlyGroup Meeting, Toronto, ON. 2012
7. Panchal T., **Kouptsova J.**, Alchits A., Godt D. Traffic Jam, a large Maf transcription factor, is required for capcell specification and organization into a cluster. Poster session presented at the Canadian Drosophila Research Conference, St. Catharines, ON. 2011