The effects of serotonergic manipulation on behavioural and neural correlates of reward processing

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Declaration of authorship: I Alexander Smith, confirm that the work presented in this thesis is my own. I state that no substantial part of my dissertation has already been submitted, or, is being currently submitted for any such degree, diploma or other qualification at the University of Oxford or other University or similar institution. A section of the introduction and chapter 2 have been published. None of my other work has been submitted or accepted for publication. All submitted papers are related directly to my field of study, and have been written whilst holding the status of a student for the DPhil.

Statement of Contribution: Chapters 2 and 3 present data from one study. I designed, set up and collected data for this study. Ms Sorcha Hamilton and Ms Monica Taing assisted in screening and data collection for this study. I analysed all data presented in these two chapters (under the supervision of my named supervisors) and with supervision from Dr Juliet Griffin for computational analysis of the reward learning task. Chapters 4 and 5 present data from a separate study. I set up this study, collected data and solely analysed all data presented in these two chapters (under the supervision of Dr Marieke Martens for the neuroimaging component and my named supervisors).

This thesis is under 50 000 words in length.

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Contents

Abstract

The serotonin system is implicated in a wide variety of mental illnesses; indeed, its modulation is utilised in treatments for such illnesses. However, such serotonergic-based treatments are not effective for all individuals, or perhaps more specifically for all symptoms an individual experiences. One such symptom is anhedonia, commonly defined as the loss of pleasure. Anhedonia is often studied through reward processing, that is a series of stages that include the experience of a reward (liking), motivation to acquire it (wanting) and learning about how to obtain the reward again (learning).

Contrasting evidence exists on the influence of serotonergic modulation on these stages, with some indicating the commonly used selective serotonin reuptake inhibitors (SSRI) may worsen reward processing, which would be of high clinical relevance.

The central serotonin system is anatomically widespread and diverse in its downstream effects. One key component of the system is the 5-HT$_{1A}$ receptor, whilst well characterised behaviourally in animals, there is a paucity of evidence in humans. The scarcity of evidence extends to not only anhedonia but also cognition and emotional processing. It is important to clarify as any serotonergic modulation will almost certainly be influenced by the response of the 5-HT$_{1A}$ receptor.

The goal of this thesis is to explore the effect of serotonergic interventions on the separate stages of reward processing both at behavioural and neural level in healthy volunteers using a randomised, double-blind, placebo-controlled, between-subjects design. It also investigates the effects of the 5-HT$_{1A}$ receptor agonist buspirone on cognition and emotional processing.

This thesis demonstrates the divergent role of different serotonergic interventions, used over different durations, on reward processing. This is of relevance to the transdiagnostic symptom of anhedonia. It may enable further work to identify what, and how, specific components of the serotonergic system can influence anhedonia in clinical populations and treatments therein.

Background & introduction

Anhedonia

How to define it, its presentation and prevalence

As psychiatric phenomenology began to formalise, anhedonia was classically understood as a 'loss of pleasure' (Ribot 1896). Such a conceptualisation stood the test of time for several decades and it was incorporated into the evolving diagnostic manuals accompanying clinical practice, with the most recent definition being "marked diminished interest or pleasure in all, or almost all, activities."(American Psychiatric Association and American Psychiatric Association 2013). Indeed, anhedonia is regarded as one of two core features of depression (the other being low mood) and its symptomatic scope has increased to incorporate not only a deficit in consummatory pleasure but also diminished motivational drive to seek out and experience pleasure. In parallel, an increase in clinical questionnaires to characterise anhedonia has emerged, such as the Snaith Hamilton Pleasure Scale (SHAPS) and the Fawcett Clark Pleasure Scale (FCPS), perhaps an acknowledgement of the many facets of anhedonia.

Why does it warrant investigation? – the impact and scale of the problem

As a core feature of depression, unsurprisingly the symptom of anhedonia is prevalent in depressed patients, with estimates ranging between 35 to 70% (Pelizza and Ferrari 2009; Cao et al. 2019).

Improvement in anhedonia is a strong predictor of psychosocial improvement and symptomatic benefit in Major Depressive Disorder (MDD) (Vinckier, Gourion, and Mouchabac 2017), consistent with the biopsychosocial approach to treating depression (Malhi and Mann 2018). Extrapolating from the view of ongoing anhedonia being a barrier to functional recovery from depression, it could be inferred that anhedonia presents a significant economic burden (Greenberg et al. 2015).

Alongside the greater severity of depression associated with anhedonia (Luca et al. 2024), it is worth noting that the most commonly used antidepressant medications do not reliably resolve anhedonia. As such anhedonia if often linked to treatment resistance in both adolescents

(McMakin et al. 2012) and adults at a variety of follow-up periods (Dunlop et al. 2020; Uher et al. 2012; Spijker et al. 2001). Anhedonia also responds relatively poorly to other treatment modalities including cognitive therapy (Dunn et al. 2020), behavioural activation (Sandman and Craske 2022) and neuromodulatory treatments (Downar et al. 2014; Nord et al. 2019).

A meta-analysis of observational studies indicate anhedonia is a risk factor for suicidal ideation, independent of depression (Ducasse et al. 2018). Whilst the link between anhedonia and suicidal behaviours is yet to be shown, the presence of anhedonia may contribute to the transition from thoughts to action (Auerbach, Pagliaccio, and Kirshenbaum 2022).

Here anhedonia will be viewed in the context of depression; however it is noteworthy that its presence extends beyond depression, with anhedonia being identified in various other mental illnesses such as schizophrenia, PTSD, anxiety disorders and eating disorders, as well as other neurodegenerative conditions under the guise of apathy and amotivational syndromes (Husain 2018; Pizzagalli 2022a). The transdiagnostic nature of anhedonia indicate the benefits of understanding anhedonia and improving its treatment could have value throughout psychiatry.

In summary, with its relatively high prevalence, economic impact, increased risk of suicidal ideation, increased symptom severity and duration, presence in treatment-resistant sub-populations and transdiagnostic relevance it is clear that anhedonia represents an unmet therapeutic need worthy of investigation.

Reward processing

Aligned with the broadening definition of anhedonia in the 20th century, the last 25 years have seen an evolution in the concept of anhedonia as a dysfunction in reward processing. As such reward processing is now a widely accepted framework for investigating the behavioural and neural correlates of anhedonia. An early and accessible conceptualisation of reward processing was developed by Berridge & Kringlebach (2008) (Berridge and Kringelbach 2008), separating reward processing into the broad but distinct psychological and neurobiological stages of liking (consummation), wanting (motivation for reward) and learning (associating and predicting future rewards with cues).

This concept aligns with the Research Domain Criteria (RDoC) initiative, which aims to move away from research involving symptomatic diagnostic categories towards one of transdiagnostic psychological processes that have molecular, behavioural and neural circuitry dimensions for investigation. It hopes to clarify research questions and subsequent answers, as well as facilitating greater translation between pre-clinical and clinical research. Reward processing can be found in the Positive Valence System of the RDoC, whose sub-components include reward responsiveness (anticipation, satiation and initial response), valuation (involving probability, delay and effort discounting) and learning (prediction error, habit and probabilistic reward learning) ('Positive Valence Systems'). Whilst RDoC broadly maps to the construct of liking, wanting and learning respectively, they do not map together precisely. For example, in terms of the wanting stage of reward processing, the RDoC approach separates anticipation (found in reward responsiveness in RDoC) and incentive salience (found in valuation in RDoC) in separate sub-components. However an updated RDoC approach has grouped these stages together in an ‘approach motivation’ domain, which includes reward valuation, effort valuation/willingness to work, prediction error and then action selection ('Positive Valence Systems: Workshop Proceedings' 2011).

A more recent view incorporates both RDoC approaches into sequential stages of option generation; cost benefit decision making (similar to reward valuation) & option selection; anticipation; initiating & sustaining action (or ‘appetitive behaviour’) and finally consummation & hedonic impact of goal (similar to reward responsiveness). This then feeds back to the option generation stage again through learning (Husain 2018).

All of these classification systems for reward and motivational processing have enabled greater insights into reward processing, facilitating more focussed & informative research questions and answers. For example, finer characterisation of anhedonia has enabled that anhedonia experienced in MDD is likely different to that experienced in psychosis (Culbreth, Moran, and Barch 2018; Lambert et al. 2018) and within MDD different anhedonia sub groups exist, in terms of self-report and neural functional connectivity (Ding et al. 2023).

Stages

For the purposes of this thesis, the stages of reward processing will be briefly defined with an overview of how they have been measured behaviourally in recent experimental studies.

Liking

Liking, or reward consummation, reflects the in the moment experience of a pleasurable goal (Husain 2018), which can be within conscious experience and thus subjective, or an unconscious experience (Berridge and Kringelbach 2008).

Consummatory pleasure can be evaluated with self-report questionnaires such as the Temporal Evaluation of Pleasure Scale (Gard et al. 2006). However, such questionnaires enquire about imagined pleasurable experiences, perhaps without salience to the individual or with a degree of recollection bias. Therefore, it is possible such questionnaires measure the internal value of a hypothetical rewarding experience as opposed measuring in the moment pleasure.

However, questionnaire inventories are often limited to measure only certain subcomponents of anhedonia. For instance, the SHAPs focusses on consummatory pleasure, being sensitive to hedonic capacity (Rizvi et al. 2016) and accordingly did not correlate with willingness to exert effort in a reward task (Treadway et al. 2009). The SHAPS also tends to focus more on a state' of anhedonia (as opposed to a trait) which would be or more value when testing the effects of interventions. However, it should be noted with all inventories there is a degree of subjectivity that may not be relevant to the individual. For example, the SHAPS enquires about a favourite meal, however many individuals may not derive pleasure/hedonic response from food particularly, making this type of question and possibly others somewhat redundant in ascertaining anhedonia at an individual level. More recent inventories include the TEPS, which has sub-scales for anticipatory and consummatory anhedonia (Gard et al. 2006). However, it has been noted that it focusses on physical or sensory aspects anhedonia, as opposed to more abstract contributions to anhedonia, such as financial gain, social relationships or past times (Rizvi et al. 2016). These questionnaires would often miss key motivational and learning aspects of reward processing. This gap can be partially filled with new questionnaires such as the Apathy motivation Index (Ang et al. 2017), which measures motivational apathy across behaviour, social motivation and emotional sensitivity.

Behaviourally the subjective experience of pleasure can be evaluated with simple taste tasks, such as the sucrose preference test in animals, whereby animals develop a preference for drinking a sucrose solution over plain water. This has valuable translational validity in the subjective rating of pleasurableness of various tastes in humans, as well as overcoming the risk of hypothetical reward seen with self-report scales. Taste tasks have been widely used in testing the effect of pharmacological interventions in healthy volunteers (Kaltenboeck et al. 2022; McCabe et al. 2010). With the aim to identify group differences between healthy volunteers and depressed individuals, other types of reward have been used, such as pleasant music or pleasant images (Osuch et al. 2009; Smoski, Rittenberg, and Dichter 2011). However, its noteworthy sweet taste tasks do not seem sensitive to MDD diagnosis or depression rating scales (Arrondo, Murray, et al. 2015; Berlin et al. 1998; Dichter et al. 2010). Although subjective pleasantness and perception threshold for sweet tastes did negatively correlate with anhedonia-specific scores in depressed patients indicting its potential utility in assessing anhedonia (Berlin et al. 1998).

Evidence indicates the nature of reward can influence choices in reward processing. The value of primary rewards such as food- or sex-based stimuli, may lose their subjective value quickly once satiety or arousal-limits have been reached respectively. However secondary reward, that rely on learnt associations, may not have such a rapid, satiety-based decay but in fact grow in subjective value with repeated receipt (Yang et al. 2021). This potentially enables greater generalisation for secondary rewards across contexts. This is somewhat supported by primary rewards activating more evolutionary conserved brain regions such as the amygdala and insula, whereas monetary rewards activate vmPFC/OFC regions (Sescousse et al. 2013). However there is evidence rewards of different nature may be subject to similar discounting behaviours (Markman et al. 2024) and possibly transposed to a common 'neural' currency (Sescousse et al. 2013).

Related to conscious experience of a reward or punishment is that of an unconscious, internal reward value, which whilst informing, is not identical to, the subjective rating of reward or punishment. Internal reward value is often a latent process that can be inferred through its neural correlates or the computational analysis of behavioural results. Recent perspectives are that reward value represents how much an individual expects to gain from an outcome (either pleasure or satisfying a social or physiological need) (Kieslich, Valton, and Roiser 2022), whilst being discounted either by effort required for, time until receipt of, or probability of receiving said reward. Reward value can be inferred from choices in behavioural tasks that implicitly or explicitly vary probability of an outcome (Pizzagalli, Jahn, and O'Shea 2005; Pessiglione et al. 2006), the effort required for an outcome (Treadway et al. 2009; Meyniel et al. 2016) or delay of an outcome (Dombrovski et al. 2012; Pulcu et al. 2014) to infer reward value. Other indices can also be used to judge reward value, such as reaction times of responses.

Behavioural tasks involving Pavlovian or instrumental tasks can be performed whilst undertaking neuroimaging to identify neural correlates associated with consummation of rewarding or aversive stimuli. One particular task developed for measuring consummation of reward in an fMRI setting is the Monetary Incentive Delay task (Knutson et al. 2001) in which a subject must respond within a specified time window, with outcomes involving win, loss or no change.

It is of note however that reward value is a latent construct that is not directly observable, therefore only its behavioural or neural correlates are measurable. Self-report questionnaires and reaction times can be the result of several processes. Self-report scales are vulnerable to aforementioned biases and reaction times are sensitive to aspects such as reward sensitivity, cost evaluation, learning rates and exploratory vs exploitative behaviours. Using tasks that incrementally change one component of a task such as magnitude, effort or probability (i.e. parametrically), one can reliably ascertain reward value based on the costs they are willing to expend to obtain it or how frequently they select one option over another (Kieslich, Valton, and Roiser 2022). Clarifying this would allow disambiguation as to the effect of an intervention on current value of a cue in the current state and its potential for use in the future, as opposed to pure hedonic capacity, learning and/or prediction error, motivation to obtain an outcome or simple fatigue. As these processes are all inter-dependent, the subjective experience of anhedonia may arise from unique impairments in each of these components to give a unique 'mechanistic signature' of an anhedonia.

Wanting

Motivation is key to reward processing; a recent conceptualisation of its dysfunction considers it as the overlap of the two syndromes of apathy and anhedonia (Husain & Rosier 2018). Aligned with this, clinical self-report scales to measure motivation include the anticipatory subscales of the Temporal Evaluation of Pleasure Scale and the Apathy Motivation Inventory.

Motivation can be separated into several stages, which can overlap with each other and other stages of reward processing. These stages include cost:benefit decision making, anticipation and appetitive behaviour (Husain 2018).

Measures of appetitive (or 'wanting') phases of behaviour can be used to measure motivation. These tasks typically involve the subject having to judge expenditure of effort for a given reward and then expend the effort to gain it.

Cost benefit decision making

A popular task to test this stage measures an individual's willingness to accept an offer of a monetary reward which would require an explicit amount of physical effort expenditure to obtain said reward. They then go on to expend the physical effort through gripping a hand-held dynamometer to obtain the reward offered (Bonnelle et al. 2015; Meyniel et al. 2016; Cléry-Melin et al. 2011). Another commonly used task is the effort expenditure task (EEfRT), which requires the physical effort of a pre-specified number of button presses (a few button presses is 'easy' or many button presses is 'hard'), paired with low or high reward respectively, but also with the added element of variable probability of the outcome (Treadway et al. 2009). Such tasks have been considered valuable in early stage drug development (Bilderbeck et al. 2020).

Other studies have used alternatives to monetary outcomes, such as a humorous cartoon and willingness to exert effort to view it, to demonstrate a dissociation of consummation & motivation in depressed individuals (Sherdell, Waugh, and Gotlib 2012).

As discussed previously the willingness to expend effort for a reward could be considered part of effort discounting in the valuation of reward (Kieslich, Valton, and Roiser 2022) or of a cost:benefit decision making stage (Husain 2018). After this stage further stages have been commonly described under the umbrella of wanting or motivation which include anticipation and appetitive behaviour or motor invigoration (i.e. actually exerting the effort for the outcome) (Husain 2018).

Anticipation

Anticipation is intertwined with the concept reward valuation and cost:benefit decision making. Behaviourally it can be challenging to measure, therefore it is often inferred from physiological measurements such as heart rate or pupil dilatation (Brehm and Self 1989). In the aforementioned Monetary Incentive Delay task, increased pupil dilatation has been broadly shown to indicate an increased anticipation response (Schneider et al. 2020; Guath et al. 2023).

The Monetary Incentive Delay task, as described earlier, is also commonly used to measure the neural correlates of anticipation, in addition to reward response, and has been shown to be sensitive to group effects (e.g. healthy volunteers vs MDD) (Stoy et al. 2012). The task has also demonstrated sensitivity to drug effects, specifically a 14-day course of the SNRI duloxetine in healthy volunteers led to an increased ventral striatal response during anticipation (Ossewaarde et al. 2011). Additionally, a 6-week course of the SSRI escitalopram was associated with increased ventral striatal response to loss anticipation in depressed individuals, to a degree that closer resembled healthy control volunteers (Stoy et al. 2012).

Appetitive behaviour

Appetitive behaviour can be defined as the exertion of effort to obtain an outcome. In animals this has been measured in various paradigms such as the T-maze test, whereby one option with greater reward has a small barrier to climb, whereas a lower reward option has no barrier. Another example would be a progressive ratio task in which the animal must press a lever for a reward, the number of level presses gradually increases until the animal no longer presses, assumed to be due to the effort outweighing the outcome. In humans, similar tasks have been developed to measure motor invigoration to pursue a task, such as button presses (e.g. EEfRT), grip force expended or response times in almost any instrumental task. In recognition that many daily tasks require more cognitive than physical effort, some tasks have used cognitive effort as a metric in decision making tasks, such as those requiring attention or memory (Chong et al. 2017; Barch et al. 2023). These tasks would often measure appetitive behaviour via choices in task, similar to cost:benefit decision stages. For example tasks have included a selection of options which require either an easy or harder numerical decision (Barch et al. 2023), an increasing number of shifts in attention (Chong et al. 2017) or a spatial working memory task with an increasing number of locations to remember (Ang, Gelda, and Pizzagalli 2023). The distinction in effort types is a valid one, namely whilst a common neural pathway for both types of effort likely exists, evidence indicates they can engage differing neural pathways, for instance unlike physical effort, cognitive effort engages the amygdala in healthy volunteers (Chong et al. 2017).

Learning

Learning, specifically the construct of reinforcement learning, entails associating a cue or action with an outcome. Based upon outcomes of choices as feedback, the internal value of the cue or action is updated so that choices can subsequently change over time. Measuring the changes in choices in response to feedback can indicate how well a subject is learning. Various aspects of the outcome can be altered such as reward magnitude or probability of outcome. Tasks developed to measure this include a probabilistic instrumental learning task, whereby implicit probabilities of outcomes are associated with separate symbols in a pair of symbols, and through trial and error the individual must learn which symbol has the highest probability of reward (if in a winning context) (Pessiglione et al. 2006). Another task, from which several adaptions have been made, is the Iowa Gambling task, which presents a set of four cards, each with differing magnitudes of monetary win and loss, with the individual having to learn which cards are most advantageous in winning the most amount of money in the long term (Bechara et al. 1994).

However, choices in tasks to probe learning can be influenced by several other stages in reward processing other than the ability to learn itself, such as the actual hedonic impact of an outcome. In an attempt to address this complexity, computational analysis of choices has been used to measure such latent processes such as rate of learning, sensitivity to outcome and a process called prediction error, which represents a situation when the actual outcome does not match the expected outcome, which can be positive (e.g. gain reward or avoid losing) or negative (e.g. not gain reward or lose) in valence (Huys et al. 2013). Prediction error can often be measured by changing the magnitude or probability of an outcome once learnt during a task (Rizvi et al. 2016). Prediction error can not only be captured computationally but also in terms of its neural correlates (Kumar et al. 2008; Gradin et al. 2011).

In summary there are several tasks that can be used to measure the various stages of reward processing and a single task can be used to measure several stages of reward processing although caution must be taken around the construct validity of such tasks e.g. assuming a task is measuring both appetitive behaviour and cost:benefit decision making when it is actually only measuring the latter.

Limitations in measurement of these constructs

It should also acknowledge inherent limitations with questionnaire measures to measure anhedonia and reward processing. This can include the subjective nature of responses in the form of biases, such as insight biases where they under-report difficulties due to lack of insight , or over report if they are highly self-critical or believe this is what the researcher may want or if they are simply having a particularly bad day or recent experience and feel they are unmotivated. The aforementioned questionnaires also do not measure learning components of reward processing, important as an individual may not be motivated for a reward as they are not learning to pair the outcome with the stimulus e.g. I feel better after a run.

Limitations also extend to behavioural tests measuring various stages of reward processing. Measurements may have inherent confounds that should be considered. These include sensitivity to concentration of taste in taste tasks or attention and coordination confounding reaction time tasks when used as a surrogate marker of motivation. Finally, prediction error, a latent variable, is subject to assumptions and confounds. These include aspects related to task, such as subject expectations being assumed, through training and instructions or cues, and never directly measured or the novelty of a stimulus varying during a task, particularly early in tasks, having an effect on expectations and predictions or finally the computational model used not capturing the real-world strategies of subjects for that particular task.

Are these stages in reward processing dysfunctional in depression or in those with anhedonia?

Liking

In animals the sucrose preference test appears sensitive the chronic mild stress model of depression, with the depressive phenotype selecting the sucrose solution less often, indicating lower hedonic capacity or liking of pleasurable stimuli (Willner 2017).

Much evidence indicates no significant difference in subjective pleasantness of sweet tastes between depressed patients and control groups (Thomsen 2015). However, in focussing on the symptom of anhedonia a difference has been observed. A healthy volunteer study of either high or low self-reported anhedonia found the high anhedonic group required a higher sucrose concentration for detection compared to the low anhedonic group, which was interpreted as a reduced sensitivity to a sweet taste, i.e. the consummatory aspect of reward in high anhedonic individuals (Slaney et al. 2023). However, it is not clear if the findings for pleasant sweet tastes are replicated using aversive stimuli, as such the absence of group differences for depressed patients, but not necessarily anhedonic patients, is unique to pleasant, rewarding tastes or is a more general impairment in consummation e.g. to aversive tastes too.

Some have suggested it may be that individuals with depression require more evidence to experience the same degree of pleasure as an otherwise healthy volunteer would or alternatively that the implicit internal reward valuation becomes dysfunctional in anhedonia (Kieslich, Valton, and Roiser 2022). As mentioned previously internal reward value must be inferred, either through choices, reaction times, computational modelling or from neural correlates in functional neuroimaging (Kieslich, Valton, and Roiser 2022). Through these measurements it seems depressed patients show a reduced sensitivity to reward (Henriques and Davidson 2000) and that this is associated with anhedonia specifically (Huys et al. 2013).

It appears that neural correlates during consummation of reward are blunted in MDD compared to healthy volunteers (Rupprechter et al. 2021; Pizzagalli et al. 2009). This appears to extend to neural responses tracking reward magnitude. A healthy volunteer study found ventral striatal activation increased proportionally to reward size in the Monetary Incentive Delay task (MID), a phenomena not observed in depressed patients (Takamura et al. 2017).

These group difference appear to extend to high vs low anhedonic individuals, as defined by self-report as well. In depressed subjects, a negative correlation with anhedonia was observed with hypoactivation in the ventral striatum, left dorsolateral prefrontal cortex and the right frontal gyrus when viewing positive words (Epstein et al. 2006). A young adult study using the Monetary Incentive Delay task observed that, during receipt of a large win outcome (vs. no win), self-reported anhedonia negatively correlated with medial orbito-frontal cortex (OFC) activation (Xie et al. 2021). A study of 12 medicated MDD participants recalling happy memories found anhedonia scores positively correlated with ventromedial prefrontal cortex (vmPFC), OFC and anterior cingulate gyrus (ACC) activation and negatively correlated with activation in the left insula, right amygdala and striatal regions (Keedwell et al. 2005).   In line with these findings, activation of the dorsal medial prefrontal cortex during pleasurable music was found to positively correlate with anhedonia severity in depressed subjects (Osuch et al. 2009).

Several studies, whilst not directly correlating anhedonia scores with behavioural or neural correlates of reward consummation, have reported group differences in anhedonia scores and neural correlates of reward consummation. In depressed patients with higher anhedonic scores than healthy volunteers, Pizzagalli et al. used the Monetary Incentive delay task to examine neural responses to receipt of reward and found that the depressed patient group exhibited reduced activation of left nucleus accumbens and bilateral caudate nuclei during reward feedback (Pizzagalli et al. 2009). Furthermore, the hedonic component of the BDI negatively correlated with caudate volume (Pizzagalli et al. 2009).

Wanting

A healthy volunteer study examined the relationship between self-reported anhedonia and the anticipated, experienced and recalled experience of tasting a pleasant chocolate taste

(Chentsova-Dutton and Hanley 2010). The study found anticipatory anhedonia predicted the anticipated enjoyment, but not the actual experience or recalled experience of the pleasant chocolate taste, indicating that reduced reward anticipation, relative to individuals without anticipatory anhedonia, may significantly contribute to motivational deficits and anhedonia (Chentsova-Dutton and Hanley 2010).

Studies of healthy volunteers using behavioural tasks have also found increased anhedonia is associated with a lower motivation to exert physical effort. In a seminal study in healthy volunteers, Treadway et al (2009) found that self-reported anhedonia negatively correlated with willingness to accept harder effort offers. Similarly, Geaney et al (2015) found in low probability trials of the EEfRT, self-rated anticipatory pleasure (i.e. lower anticipatory anhedonia) positively predicted the willingness to select high effort:high gain options. This was interpreted that the anticipation of a large but unlikely reward may 'mitigate perceived effort costs' during immediate cost:benefit decision making in individuals without anticipatory anhedonia (Geaney, Treadway, and Smillie 2015). However a study of healthy volunteers grouped as high or low anhedonia from anhedonic subscale score of the BDI, did not find a significant difference between groups in hard task choices accepted in another study (Lopez-Gamundi and Wardle 2018). Although the differences were in the same direction as aforementioned studies, the lack of significant difference between groups could be due to low rates of anhedonia in this study or insufficient power to detect group differences. Morris et al (2020) used a task that required subjects to indicate if they would be willing to accept an offer that varied in both the physical effort required and amount of monetary reward offered (Morris et al. 2020). They found that subjects high in anticipatory anhedonia were less likely to increase their effort expenditure as the size of the reward increased, indicating a bias against self-generated motivation to exert physical effort for reward in those with high anticipatory anhedonia (Morris et al. 2020).

Clinical studies using several different paradigms involving cost:benefit decision making and appetitive behaviour, also provide evidence that anhedonia correlates negatively with willingness to exert effort.

Treadway et al. (2012) found that higher anticipatory anhedonia negatively correlated with acceptance of high effort:high gain options in the EEfRT, which was cautiously interpreted as a lower expectation of reward, leading to a reduced willingness to expend effort for high rewards (Treadway et al. 2012). Likewise using the EEfRT in depressed subjects, lower anticipatory and consummatory TEPS scores predicted a reduced willingness to exert effort; however in sub-syndromal depressed subjects only a lower anticipatory TEPS score (but not consummatory) predicted a reduced willingness to exert effort (Yang et al. 2014).

Supporting these findings but using humorous cartoons as the rewarding stimuli and button clicks in a moving square as effort cost, Sherdell et al. (2012) found increased anticipatory anhedonia negatively correlated with willingness to accept offers (Sherdell, Waugh, and Gotlib 2012). Furthermore, in support of distinguishing the different stages of reward processing in different samples, they found the consummatory liking of cartoons predicted motivation to exert effort to view cartoons in healthy volunteers but not depressed subjects (Sherdell, Waugh, and Gotlib 2012).

The broad correlation of increased anticipatory anhedonia (or reduced anticipation of pleasure) with reduced willingness to exert physical effort can also be observed in tasks requiring cognitive effort. Using a progressive ratio task in a clinical sample, Hershenberg et al (2016) found higher anhedonia scores were associated with a reduced willingness to exert attentional effort (Hershenberg et al. 2016). In contrast, using a novel cognitive version of the EEfRT in a healthy volunteer sample, no significant correlation between anhedonia scores and willing to exert cognitive effort were observed (Lopez-Gamundi and Wardle 2018). Although as mentioned previously, this discrepancy could be due to insufficient power or low baseline anhedonia in the study by Lopez-Gamundi et al. (2018).

In summary higher anhedonia appears to be associated with a reduced willingness to exert physical effort for rewards in healthy volunteers and depressed subjects, however only in depressed subjects is a reduced willingness to exert cognitive effort for reward observed, as indicated by a recent meta-analysis (Horne, Topp, and Ferkauf 2021).

Neural Representations

Neuroimaging studies investigating motivation capture either anticipation of reward (time between option presentation and delivery) or incentive motivation (selection of options and appetitive behaviour leading to selection). Anticipation tasks tend to involve the MID & card guessing tasks, whilst incentive motivation can be investigated with cost:benefit decision tasks (varying reward, effort or probability) or a wheel of fortune task (varying reward & probability). Results from these tasks can further support the view that motivational dysfunction could contribute to anhedonia in depression.

Incentive motivation – striatal & frontal cortex regions

There is limited evidence directly linking subjective anhedonia ratings with neural correlates of incentive motivation, with most studies comparing depressed subjects, with presumed high anhedonic burden, with healthy controls. In an MDD sample undergoing the EEfRT, Yang et al. (2016) found caudate hypoactivation during high reward offer (vs low reward) contrasts and left superior temporal gyrus and right caudate hypoactivation during high (vs low) probability contrast (Yang et al. 2016). A study measuring healthy volunteer and depressed patients (the latter at baseline and after a 6 week course of escitalopram) found ventral striatal response to reward outcome correlated with reaction time in the MID task in healthy volunteers, which was not observed in depressed subjects at baseline, indicating a possible decoupling of reward sensitivity and incentive motivation in depression (Takamura et al. 2017). Reduced medial orbitofrontal-striatal connectivity in depressed subjects has also been demonstrated using an effort-based decision task in depressed subjects (Park et al. 2017). Intriguingly divergent findings for orbito-frontal cortex (OFC) and anterior cingulate cortex (ACC) activation have been found during tasks measuring incentive motivation. OFC hypoactivation was observed in an adolescent sample using the WoF task (Shad et al. 2011) and a probabilistic reward task (Forbes et al. 2006), whereas OFC hyperactivation was observed in an adult, depressed sample (Smoski et al. 2009). ACC hyperactivation was reported in an adolescent MDD sample (Shad et al. 2011), in contrast to ACC hypoactivation in an adult MDD sample (Smoski et al. 2009). This indicates the neural correlates of incentive motivation in depression may change with age.

In summary evidence to date is limited by the absence of direct measures of anhedonia in several studies, with divergent findings in different samples (e.g. adolescent vs adult). However, it does seem some neural correlates of incentive motivation are aberrant in depression. It seems adult depressed individuals, used as a proxy measure of high anhedonia, show hypoactivation of the striatum to high rewards (Yang et al. 2016; Takamura et al. 2017), OFC and ACC are hyper- and hypo-activated respectively during motivation (Smoski et al. 2009) and that this may reflect a decoupling of reward value and subsequent effortful action to obtain it (Takamura et al. 2017), possibly due to reduced connectivity between striatal and cortical regions (Yang et al. 2016).

Anticipation of reward – striatum, frontal and cingulate cortex regions

Similar to studies examining incentive motivation, many studies examining anticipation demonstrate a difference between MDD and healthy controls, but very few measure anhedonia directly.

Arrondo et al. (2015) found depressed subjects exhibited bilateral ventral striatum (VS) hypoactivation during reward anticipation phase of the Monetary Incentive Delay task (Arrondo, Segarra, et al. 2015). Extending this finding, Stringaris et al. (2015) dichotomised a mixed adolescent sample of depressed and healthy volunteers into high & low anhedonia groups, based on a single item in the Development and Well-Being Assessment questionnaire and observed the high anhedonia group exhibited VS hypoactivation during reward anticipation (Stringaris et al.

2015). A novel approach found three subtypes of VS response of depressed subjects to anticipation of win or loss during the MID (Misaki et al. 2016). Specifically, hyperactivation during win & loss anticipation; increased activation to win compared to loss and hypoactivation during anticipation of both win & loss (Misaki et al. 2016). Furthermore, the SHAPS score was associated the VS hyper- and hypoactivation subtypes but not the mixed subgroup, which could cautiously indicate a potential non-linear relationship between anhedonia and ventral striatal response during anticipation of win and loss (Misaki et al. 2016).

Using a modified version of the MID, medication-free depressed subjects demonstrated a positive correlation between SHAPS score and activity in the OFC and VS (Ubl et al. 2015). In the same study, a positive correlation was found between SHAPS score and VS activity in healthy volunteers, supporting the value distinguishing anhedonia as a transdiagnostic concept (Ubl et al. 2015).

Observations of the cingulate cortex response during anticipation are varied. When anticipating pleasant images in a modified MID task, MDD subjects exhibited paracingulate cortex hypoactivation (Smoski, Rittenberg, and Dichter 2011). The same group of researchers observed hypoactivation of dACC during the anticipation phase of a wheel of fortune task (Smoski et al. 2009). Consistent with this but using a card guessing task, Chase et al. (2013) observed significant ACC hypoactivation during anticipation of reward in MDD subjects (Chase et al. 2013).

In contrast a study using a passive slot machine task, found ACC hyperactivation during the anticipation of rewards in MDD subjects (Gorka et al. 2014). This is supported by a study using the MID task, which found the anticipation of increasing monetary reward correlated with increasing ACC activity in depressed subjects (Knutson et al. 2008). The discrepancy in findings may be attributable to task difference however the consensus of meta-analyses indicates depressed patients exhibit ACC hyperactivation during anticipation (Zhang et al. 2013; Borsini et al. 2020).

In summary, similar to incentive motivation, striatal hypoactivation seems to be a marker of reduced reward anticipation in depressed adults, although this is nuanced in that a non-linear relationship between striatal response during anticipation and subjective anhedonia could exist. Again, similar to incentive motivation, the OFC appears to be involved in reward expectation, with OFC hyperactivation during anticipation positively correlating with SHAPS scores. Unlike incentive motivation, ACC appears to exhibit hyperactivation during anticipation in depressed subjects, possibly indicating in anhedonia greater neural resources are used monitor and anticipate reward, even though motivation is reduced (Zhang et al. 2013; Borsini et al. 2020).

Learning

In healthy volunteers, a failure to develop a reward response bias (or the inclination to choose the more frequently rewarded option) in a probabilistic reward task was predictive of anhedonic symptoms at time of testing and 1 month later (Pizzagalli, Jahn, and O'Shea 2005). Similarly in a mixed sample of first degree relatives of depressed patients and healthy volunteers, SHAPS score negatively correlated with reward response bias across both groups (Liu et al. 2016). Consistent with healthy volunteer results, higher hedonic burden is associated with lower reward response bias in both a mixed sample of depressed, socially anxious and healthy subjects (Reilly et al. 2020) and a MDD-only sample (Vrieze et al. 2013).

Albeit in depression, rather than anhedonia specifically, a recent meta-analysis found a small to moderate worsening of performance in reinforcement learning and reward response bias tasks in depressed subjects (Halahakoon et al. 2020). It is noteworthy however that neither reward learning nor response bias correlated with anhedonia scores where available. In summary it seems that increases in subjective anhedonia are associated with impaired learning about reward, due to changes in reward response bias or reinforcement learning.

Neural correlates of learning

Broadly, in depressed patients, reduced activation can be observed in several fronto-striatal regions during reward learning (Kumar et al. 2018; Kumar et al. 2008; Gradin et al. 2011; Geugies et al. 2019; Rothkirch et al. 2017).

Depressed subjects exhibit striatal & midbrain hypoactivation during prediction errors in an instrumental learning task, importantly it seems the higher the anhedonia score the greater the reduction in midbrain & striatal activation during prediction errors (Gradin et al. 2011). This is supported by another study using an instrumental learning task in depressed subjects, which observed a negative correlation between medial orbitofrontal cortex and striatal activation during reward prediction error with anhedonia severity (Rothkirch et al. 2017).

Interestingly in remitted depressed patients, higher anhedonia was associated with lower ventral tegmental area (VTA) activation during prediction error, but in healthy volunteer's higher anhedonia was associated with higher VTA activation during prediction errors (Geugies et al. 2019). This could indicate that reduced VTA activity during prediction errors may be a vulnerability marker of those who may experience anhedonia and depression, or alternatively those who have responsive VTA activity during anhedonic states may be resilient to developing depression.

In summary it seems higher anhedonia is associated dysfunction across several regions of reward pathways. Activation of the VTA, the start of the dopaminergic mesolimbic pathway, during prediction error negatively correlates with anhedonia in remitted depressed subjects. Then hypoactivation of midbrain, striatal and orbito-frontal regions during prediction errors & value updating during reward learning has been observed. Finally, reduction in fronto-striatal connectivity could indicate an overall impairment in reward valuation and integrating into updating expectation about reward.

Reward processing shows a distinctive developmental trajectory.

In adolescents, subjective anhedonia could be *under-detected* because the main anhedonia scales were validated in adults and contain few items relatable to adolescents (McCabe 2018). Behaviourally, anhedonic adolescents maintain consummatory pleasure for sweet tastes and pleasant images, yet they (i) fail to scale behaviour to reward magnitude on high-probability trials (Forbes, Shaw, and Dahl 2007) and (ii) invest less effort to obtain reward or avoid aversion (Rzepa and McCabe 2019). Neuro-imaging supports this: ventral-striatal (VS) and pre-genual ACC hypoactivity is seen during the anticipation of both gains and losses, while insula and putamen hypoactivity is observed when effort is required; consummatory ventral striatum activity remains largely intact however and the caudate hyperactivity is seen to aversive taste—hinting at a possible negativity bias in adolescents (Rzepa and McCabe 2019; Stringaris et al. 2015).

By adulthood the deficit seems to broaden throughout reward processing. A small amount of evidence indicates a modest drop in subjective liking for various pleasant stimuli (Treadway and Zald 2011), although many studies using taste indicate no group differences (Rizvi et al. 2016; Thomsen 2015). Additionally, reliable impairments in reinforcement learning on probabilistic reward tasks can be found in depressed adults (Thomsen 2015; Rizvi et al. 2016). Correspondingly, VS and vmPFC activity is blunted across anticipation, consummation and feedback phases, and prediction-error encoding is weakened (Kumar et al. 2008; Gradin et al. 2011). The VS hypoactivation during consummation appearing to persist into remission (McCabe, Cowen, and Harmer 2009), suggesting a trait-like phenomenon. In later life the behavioural literature is scarce, but a cross -sectional questionnaire study indicates anhedonia becomes more prominent with age (Wuthrich, Johnco, and Wetherell 2015), and an EEG study indicates age-related reduction of the reward-positivity signal, especially in mid-life women with depressive symptoms (Harold et al. 2023).

In summary, adolescence is marked by a mainly motivational and anticipatory shortfall with sensitivity to aversive outcomes. By adulthood by additional consummatory and learning deficits emerge, and in older age by a global dampening of reward signals may occur, possibly independent of depression-specific effects.

Is the 5-HT system involved in reward processing?

Raphe nuclei and reward processing

The raphe nuclei (RN) are a rich source of serotonergic neurons, which project to many regions of the brain. These include regions key to reward processing, such as the ventral tegmental area (VTA) (Hervé et al. 1987; Vertes 1991; Watabe-Uchida et al. 2012; Courtiol, Menezes, and Teixeira 2021; Alex and Pehek 2007). However, the raphe is not exclusively serotonergic, with some raphe neurons also releasing the glutamate, thus having an excitatory effect on the dopaminergic (DA) meso-limbic pathway (Wang et al. 2019; Qi et al. 2014).

It seems anatomical linkage of serotonergic regions with reward related regions is necessary but not sufficient in the case for serotonergic involvement in reward processing. Measurement of the downstream effects of the various serotonin (5-HT) receptor subtypes increases in complexity when considering the probes used to modulate serotonergic action (route, localisation & duration of administration). The consensus that VTA dopaminergic neurons are under tonic inhibition by dorsal RN 5-HT neurons is supported by observation that selective 5-HT reuptake inhibition reduces VTA DA neuron firing (Di Mascio et al. 1998) and that inhibition of serotonergic neurons, through 5-HT$_{1A}$ agonism, increases VTA neuron firing rate (Prisco, Pagannone, and Esposito 1994). The direct action of 5-HT within the VTA diverges from this however, specifically direct action of 5-HT appears to be excitatory, in that it was found to depolarize VTA neurons *in vitro*, possibly through 5-HT$_2$ receptors (Pessia et al. 1994) , and lead to DA release in the nucleus accumbens, possibly through 5-HT$_{1B}$ receptors (Guan and McBride 1989).

Evidence indicates that raphe nuclei project to ventral striatum both indirectly, via the aforementioned VTA projections, and directly (Lechin, van der Dijs, and Hernández-Adrián 2006;

Brown and Molliver 2000; Hensler 2006). Serotonergic neurons may have contrasting effects on dopamine release in the ventral striatum however. For example 5-HT$_{2A}$ activation can elicit DA release in the ventral striatum however 5-HT$_{2B/C}$ activation seems to tonically inhibit DA release in striatal areas (De Deurwaerdère and Spampinato 1999).

In addition to midbrain projections, the raphe nuclei have strong connections with cortical regions involved in reward processing, such as the prefrontal (PFC) and orbitofrontal cortices (OFC) (Vertes 1991; O'Hearn and Molliver 1984). Similarly this relationship seems to be inhibitory, with raphe nucleus stimulation inhibiting vmPFC activity, possibly via 5-HT$_{1A}$ activation (Hajos et al. 2003).

However, caution should be taken in ascribing behavioural responses to electrophysiological results from interventions modulating raphe nucleus transmission e.g. optogenetics, as although it may activate a certain reward region, it may also serve and modulate several other brain regions which contribute to behavioural outcomes. For example, the raphe projects to several other regions implicated in reward, emotional and cognitive processing, such as amygdala & hippocampus (Haber and Knutson 2010), with distinct and possibly contrasting behavioural effects, which could confound conclusions regarding isolated signalling through specific raphe projections.

Evidence of 5-HT receptors within, thus influence, reward related areas

Evidence of serotonergic neurons projecting directly to reward regions has been presented; however, the effect of 5-HT release at projection sites is dependent on the unique blends of the 14 possible 5-HT receptors at post-synaptic terminals within these projection sites. For example, whilst most VTA neurons possess 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, non-dopaminergic neurons also express 5-HT$_{1B}$, 5-HT$_{4}$ and 5-HT$_{6}$ receptors (Hayes and Greenshaw 2011; De Deurwaerdère and Di Giovanni 2017).

Post-synaptic 5-HT_{1A} agonism appears to increase DA firing (Arborelius, Nomikos, et al. 1993), likely through 5-HT_{1A} receptors located in the midbrain (Arborelius, Chergui, et al. 1993). Furthermore, 5-HT_{1B} agonism in the VTA is thought to increase DA release in the nucleus accumbens by inhibiting inhibitory GABAergic interneurons (Alex and Pehek 2007). Opposing these actions is the 5-HT_{2C} receptors, found in both VTA and nucleus accumbens regions (Alex and Pehek 2007), which through constitutive activity, inhibit DA release in the mesolimbic system (De Deurwaerdère et al. 2004).

5-HT effects dependent on 5-HT receptor subtype

As described above, depending on the receptor subtype, 5-HT can facilitate or inhibit dopamine release, thus activation of different 5-HT receptor subtypes could elicit different behavioural effects in reward paradigms. 5-HT_{1A} agonism increases rewarding behaviours in rodents (Harrison and Markou 2001; Papp and Willner 1991), thought to be primarily due to pre-synaptic 5-HT_{1A} agonism in the raphe nucleus (Fletcher, Ming, and Higgins 1993), although evidence indicates post-synaptic 5-HT_{1A} agonism could also have reward enhancing effects (Depoortère, Auclair, and Newman-Tancredi 2021; Depoortere et al. 2019).

Moving to other 5-HT receptors, evidence indicates 5-HT_{1B} agonism can lower reward sensitivity (Hayes, Graham, and Greenshaw 2009; Cervo et al. 2002) but conversely have antidepressant effects in animal models of depression (Chenu et al. 2008). These divergent effects could be due to 5-HT_{1B} receptor localisation in different neuronal populations (Tiger et al. 2018). Similarly 5-HT_{2C} agonism appears to reduce rewarding behaviours (Hayes, Clements, and Greenshaw 2009), possibly due to receptors located with the mPFC and nucleus accumbens (Katsidoni, Apazoglou, and Panagis 2011). Evidence for the role of the remaining 5-HT receptor subtypes in pre-clinical reward behaviour paradigms remains limited (Hayes and Greenshaw 2011).

Evidence that manipulating 5-HT levels influences anhedonia and reward processing stages (at the behavioural and neural level)

Learning

A wide range of evidence implicates the serotonergic system in reward processing. Several studies from the same group highlighted the importance of 5-HT signalling in the prefrontal (PFC) and orbitofrontal cortex (OFC) in non-human primates (Clarke et al. 2006; Clarke et al. 2004; Clarke et al. 2005). These studies demonstrated that selectively destroying serotonergic neurons in the PFC and OFC made non-human primates more perseverative on a reversal learning task, possibly due to a failure to inhibit responding to a previously rewarded cue-outcome pairing (Clarke et al. 2006; Clarke et al. 2004). This indicates that cortical 5-HT could play a role in sensitivity to changing contingencies in learning tasks. This is supported by another non-human primate study that found tonic firing of dorsal raphe nucleus (DRN) neurones during a reward task, interpreted as DRN neurons keeping track of reward value by playing a role in both motivation for and receipt of rewards (Nakamura 2013). Furthermore it is postulated DRN neurones may actively incorporate several pieces of information regarding reward, such as reward magnitude, delay, uncertainty as well as prediction error (Feng, Bromberg-Martin, and Monosov 2024). Animal evidence also supports serotonergic manipulation influencing reward learning in nuanced ways. Using a probabilistic reversal learning study in rats, acute citalopram increased punishment sensitivity (lose-shift behaviour), whereas 5-7 day courses of citalopram increased reward sensitivity (win-stay behaviour) (Bari et al. 2010)

A healthy volunteer study using a Markov decision task found increased raphe activation to large, future financial gain but not small, immediate gain, interpreted as the DRN possibly playing a role in evaluating the magnitude and timescale of reward prediction (Tanaka et al. 2004). The same group, using intervention causing high, low and control tryptophan levels and the delivery of liquid as a reward, found differential activation of the striatum during reward prediction (Tanaka et al. 2007). The differential activation was associated with the degree of delay discounting, with ventral striatal regions activated during the greater discounting of future rewards and dorsal striatal regions activated during reduced discounting (Tanaka et al. 2007). Importantly this relationship was influenced by serotonin, with increased ventral striatum activity in tryptophan depleted states and increased dorsal striatal activity in tryptophan loaded states, indicating 5-HT may influence reward prediction of different time scales through action in different striatal regions (Tanaka et al. 2007). However, these neural changes did not translate into behavioural difference, with no difference in choices of short- vs. delayed-reward choices between states being observed (Tanaka et al. 2007).

In addition to influencing the time scale of reward prediction, serotonergic modulation may influence aversive learning too. A healthy volunteer study found an asymmetric behavioural change in an instrumental reward learning task after a 7-day course of citalopram, specifically a reduction in reward, but enhancement of punishment, learning (Michely et al. 2022), an opposing conclusion drawn from the aforementioned study in animals (Bari et al. 2010) . This asymmetry in learning in humans was interpreted as possibly beneficial in depressed people, as it would lead to stronger expectations of negative events (with fewer positive expectations), therefore when events are better than expected, more positive prediction errors occur, hopefully facilitating a more positive view of the world.

At this juncture it is valuable to highlight the divergent findings of acute versus chronic administration of serotonergic medications on reward processing. Acute vs chronic selective serotonin reuptake inhibitor (SSRI) administration has been shown to influence differences in serotonin reuptake transporter (SERT) occupancy. High SERT occupancy after acute SSRI, but even higher SERT occupancy after prolonged SSRI is observed in regions such as the raphe nucleus, dorsal striatum and anterior cingulate cortex in depressed patients (Baldinger et al. 2014). Interestingly only after prolonged use did SSRI plasma levels correlate with SERT occupancy in many of these regions (Baldinger et al. 2014). Behavioural evidence from reward learning studies indicate a detrimental effect of acute SSRI medication, either through increasing errors (Skandali et al. 2018) or reducing inhibition (Guitart-Masip et al. 2014). It seems this effect may be modulated by duration of treatment too, namely the aforementioned instrumental learning task in healthy volunteers using a citalopram intervention observed a change in reward learning rates after 7 days but not at after 1 day (Michely et al. 2022). Several reasons could account for the divergent effect from differing durations of SSRI treatment. This includes the view that acute activation of 5-HT$_{1A}$ receptor agonism during initial SSRI treatment may lead to reduced serotonergic tone (Kim et al. 2017; Nord et al. 2013) as opposed to the increased tone expected with sub-chronic SSRI administration, the delay for SSRI medication to reach steady state in the body and thus increase serotonin (Gutierrez and Abramowitz 2000) or the time required for neuroplastic changes to occur via neurotrophins such as Brain Derived Neurotrophic Factor (BDNF), which increase with sub-chronic SSRI treatment (Balu et al. 2008).

Scholl et al. used a reward-effort paradigm, that varied the effort, magnitude and uncertainty of reward, to study the effects of a 14-day course of citalopram in healthy volunteers on prediction error in reward and aversive (i.e. effort) learning (Scholl et al. 2017). To overcome the ceiling effect of learning in healthy volunteers (i.e. the control group are already very good at learning) they examined the degree to which interference from extraneous information can interrupt learning. The study found, compared to the placebo group, the citalopram group were more resilient in using reward prediction errors in learning in the face of interfering information (i.e. whether the effort cost would be surprisingly high or whether the reward would be genuine or hypothetical) (Scholl et al. 2017). Furthermore, the citalopram group demonstrated increased activation in regions implicated in reward learning during reward prediction error, such as the striatum, midcingulate cortex, vmPFC and parietal cortex, which the authors postulate could be due to synaptic plasticity from the prolonged course of SSRI. They interpreted their findings as suggesting that SSRIs would not improve the learning rate but rather enhance the precision of the learned information, i.e. using learned information more consistently or achieving a higher signal-to-noise ratio (Scholl et al., 2017).

Liking

Serotonergic modulation, particularly with SSRIs, appear to influence the hedonic response to reward. Following a 7-day course of SSRI a healthy male volunteer sample exhibited a reduction in striatal activation in response to viewing erotic stimuli (a ‘primary’ reward) (Abler et al. 2011). Similarly, following a 7-day course of SSRI in healthy volunteers, no change in the subjective liking of a rewarding chocolate taste was observed, however a reduction in striatal activation to the sight and taste of chocolate was found (McCabe et al. 2010). Another healthy volunteers study using a risk-related decision-making task found a 21-day course of SSRI reduced midbrain activation, including the raphe nuclei, during high reward (& high risk) outcomes (Macoveanu et al. 2014). This was interpreted as a prolonged SSRI course increasing the serotonergic inhibitory feedback from the frontal cortices to the raphe (Sharp et al. 2007). Somewhat counterintuitive to the presumed pharmacological goal of SSRI, this could result in a reduction in raphe activity, thus reducing serotonin release from its projections in several brain regions, including those involved in reward processing. This hypothetical effect could possibly be mitigated by reduced serotonin reuptake in these regions. The aforementioned task by Scholl and colleagues (2017), found a 14 day course of citalopram reduced the activation in the striatum and mPFC after receiving a reward outcome, albeit in a the delayed time window of 8-12 seconds after outcome receipt (Scholl et al. 2017).

Wanting (anticipation & motivation)

It seems sub-chronic and chronic SSRI increase the motivation to pursue reward, possibly through reducing the subjective cost of effort required. This is supported by two healthy volunteer studies, the first found an 8-week course of escitalopram increased willingness to exert effort for reward, which computational analysis attributed to reduced effort cost as opposed to increased reward valuation (Meyniel et al. 2016). The second study found a 7-day course of SSRI led to greater willingness to gather information before making a decision in an information gathering task, interpreted a reduction in subjective cost of cognitive effort (Michely et al. 2023).

The neural correlates of anticipation and appetitive behaviour also seem to be influenced by serotonergic modulation. A meta-analysis of healthy volunteer studies using the MID found the striatum, insula, amygdala & thalamus were activated during anticipation of either loss or reward (Oldham et al. 2018). A small study used the MID task to examine healthy volunteers and depressed patients (depressed patients were tested twice: at baseline and after a 6-week course of escitalopram) (Stoy et al. 2012). It found at baseline, depressed patients exhibited reduced bilateral ventral striatum activation during loss anticipation and reduced right ventral striatal activation during reward anticipation, the former correlating with BDI anhedonia scores (i.e. the greater the anhedonia the lower the VS response to anticipation) (Stoy et al. 2012). Importantly, after the 6-week course of escitalopram no significant differences in ventral striatal activation during anticipation were observed between depressed patients and the healthy volunteers, possibly indicating a possible normalising effect of SSRI on striatal response to anticipation (Stoy et al. 2012).

In depressed subjects performing the MID task, a significant positive correlation between left ventral striatal sensitivity to rewarding outcomes and score on the Hamilton rating scale for depression emerged after a 6-week course of escitalopram, indicating partial recovery of neural reward sensitivity with symptomatic improvement (Takamura et al. 2017). However, no improvement in incentive behaviour (i.e. reaction times) was observed as reward increased, indicating chronic SSRI may not be sufficient to ‘re-couple’ improved reward valuation at a neural level into increased motivation at a behavioural level.

In summary modulation of the serotonergic system appears to have contrasting influences on the behavioural and neural correlates of each stage of reward processing, as defined in this thesis. Contrasting behavioural effects of sub-chronic SSRI use on learning in healthy volunteers have been observed, with different tasks finding reduced reward learning but others finding reward learning more resilient to interference. In healthy volunteers, sub-chronic SSRI seems to increase activation of striatal and cortical regions during reward learning. During reward consummation it appears sub-chronic SSRI has little subjective or behavioural effects on consummation of reward, however it does seem to reduce striatal responses during receipt of reward. Finally, SSRI appear to reduce the subjective cost of physical and cognitive effort, improve appetitive behaviour and normalise the neural correlates of striatal response during anticipation.

Challenges in observing behavioural change when just manipulating 5-HT system

It is important to note that most neuroimaging studies cited, whilst observing neural changes do not always observe corresponding alterations in behavioural performance, a key goal for research aiming to improve psychiatric symptoms. This could be due to other neurotransmitter systems compensating for the induced changes in the 5-HT system, akin to a behavioural homeostasis (Macoveanu 2014). Thus, it could be difficult to elicit behavioural changes when, or attribute them to, modulating the 5-HT system alone. This is supported by a review by Kranz et al. suggesting effects of modulation of extracellular serotonin should be interpreted with caution owing to such manipulations almost certainly involving various feedback and re-adjustment of other neurotransmitter systems (Kranz, Kasper, and Lanzenberger 2010). Indeed, contrasting effects of SSRI on reward in animals alone have been observed. Reward enhancing effects were postulated to be due to the effect of serotonin on the dopaminergic mesolimbic system (Hoebel et al. 1989). However the reward reducing effect were thought to occur owing to an interplay between SSRI, opioid and dopamine reward networks (Subhan et al. 2000), which is aligned with the finding of increased beta endorphin in the striatum following direct SSRI injection (Zangen, Nakash, and Yadid 1999).

When considering the large body of evidence implicating the 5HT system and its modulation in reward processing, a potential line of investigation could be exploring the effects of modulating different 5-HT receptor subtypes, given the heterogeneity of their distribution throughout the brain (Sharp and Barnes 2020). On candidate subtype for further exploration is the 5-HT$_{1A}$ receptor, one of the most widespread and well-studied receptors in the 5-HT family.

The 5-HT_{1A} system

In 1979 two subtypes of serotonin (5-HT) receptors were identified and named 5-HT_1 and 5-HT_2 (Peroutka and Snyder 1979). Subsequent work showed that the 5-HT_1 receptor could itself be subdivided into further subtypes with the 5-HT_{1A} receptor being characterised by the binding of spiperone and 8-OH-DPAT (Peroutka 1988).

An important population of 5-HT_{1A} receptors are found on the cell bodies and dendrites of 5-HT neurons originating from the raphe nuclei (Kia et al. 1996; Verge et al. 1985). These ‘pre-synaptic’ 5-HT_{1A} receptors function as inhibitory autoreceptors which, when activated, decrease 5-HT neuron firing and diminish 5-HT release in terminal fields (Sharp et al. 2007).

However, 5-HT_{1A} receptors are widely distributed and found in several other regions of the brain including the frontal cortex, hippocampus, amygdala and hypothalamus – these receptors are described as ‘post-synaptic’ (Savitz, Lucki, and Drevets 2009; Celada et al. 2004; Celada, Bortolozzi, and Artigas 2013).

Like their pre-synaptic counterparts, post-synaptic 5-HT_{1A} receptors are inhibitory in nature and can modulate the release of other neurotransmitters such as GABA and acetylcholine (Halasy et al. 1992; Ogren et al. 2008; Meltzer and Sumiyoshi 2008). This can lead to a wide array of effects such as disinhibition of downstream neuronal activity. For example, stimulation of post-synaptic 5-HT_{1A} receptors on inhibitory GABAergic interneurons can lead to excitation of pyramidal neurons and increased glutamate release (Sprouse and Aghajanian 1988, 1987). This may be relevant to the intriguing observation that in animal models, the antidepressant-like effect of the NMDA receptor antagonist, ketamine is abolished by selective 5-HT_{1A} receptor blockade (Fukumoto et al. 2018).

Are 5-HT_{1A} receptors affected in depression?

The disturbances in 5-HT regulation present in depression are subtler and more complex than the originally proposed 5-HT deficit in depression, correctable with a simple increase in 5-HT levels (Menkes 2022; Jauhar, Cowen, and Browning 2023) . As such much work has focused on the possible role of specific receptor sub-types, such as the 5-HT$_{1A}$ receptor, in depression. Evidence implicating the 5-HT$_{1A}$ receptor in depression will be briefly summarised here.

Post-mortem studies

Post-mortem studies of 5-HT$_{1A}$ receptor binding in deceased individuals with depression were mainly conducted 10-20 years ago and the findings are rather contradictory (Kaufman et al. 2016). However, the overall picture is one of diminished 5-HT$_{1A}$ receptor binding in cortical and subcortical post-synaptic regions in depression (Lopez-Figueroa et al. 2004).

Neuroimaging studies

Most studies have found that 5-HT$_{1A}$ receptor binding exhibits a modest reduction in both post-synaptic and raphe regions in unmedicated patients with depression (Wang et al. 2016; Drevets et al. 2007). Nevertheless, this apparent consensus is complicated by one group who have consistently found unmedicated patients with depression to have higher 5-HT$_{1A}$ receptor binding in the raphe region, as well as cortical areas compared to controls subjects (Parsey et al. 2010; Parsey et al. 2006).

These contrasting findings could relate to technical and sample factors. The principal technical factor is believed to be the choice of reference tissue (usually the cerebellum) with which to compare 5-HT$_{1A}$ receptor binding in regions of interest (Shrestha et al. 2012). However, whilst the matter is unresolved, neuroimaging evidence indicates there is a disruption in 5-HT$_{1A}$ receptor binding in depression.

Pharmacological challenge studies

The availability of drugs that can be used in humans and possess some selectivity for 5-HT$_{1A}$ receptors has led to studies that have probed the function of brain 5-HT$_{1A}$ receptors in people with depression.

In studies of depression, a commonly-used agent has been buspirone, possibly because of its availability and familiarity in clinical settings (Kishi et al. 2014; Loane and Politis 2012). However, other agents, such as ipsapirone, gepirone and flesinoxan have also been employed (Kishi et al.

2014; Ansseau et al. 1993). Typical functional markers of 5-HT_{1A} receptor activation in humans include increased secretion of certain anterior pituitary hormones, such as ACTH (with a consequent increase in plasma cortisol) and growth hormone (Cowen 1993; Savitz, Lucki, and Drevets 2009; Pitchot et al. 2002), and a decrease in body temperature (Pitchot et al. 2002; Voronova 2021). Some agents, such as buspirone, also increase plasma prolactin, but this is not thought to be mediated by 5-HT_{1A} receptors (Anderson et al. 1992).

It is agreed that the ACTH and growth hormone responses to 5-HT_{1A} receptor challenge in humans is due to stimulation of post-synaptic 5-HT_{1A} receptors in the hypothalamus (Pan and Gilbert 1992).

In humans, the location of the receptors relevant to 5-HT_{1A} receptor-related hypothermic responses is not clearly resolved. However, in healthy participants, cortisol treatment attenuates 5-HT_{1A} autoreceptor function as measured by EEG changes (McAllister-Williams, Massey, and Fairchild 2007) and similar cortisol treatment blunts the hypothermic response, but not the growth hormone response, to buspirone challenge (Young et al. 1994). This suggest that in humans the hypothermic response to 5-HT_{1A} receptor agonist challenge at least involves 5-HT_{1A} autoreceptors.

In summary, the majority of studies investigating 5-HT_{1A}-mediated responses in unmedicated patients with depression have found blunted endocrine and/or hypothermic responses to pharmacological challenge though there is some disagreement (Lesch 1991; Meltzer and Maes 1995; Shapira et al. 2000; Riedel et al. 2002; Navines et al. 2007). Overall, therefore this impaired functional response is consistent with post-mortem and PET binding studies suggesting that depression is associated with deficient 5-HT_{1A} receptor activity, both pre- and post-synaptically.

What can be used as a pharmacological probe for 5-HT_{1A} receptor activation in humans

The most widely available probe for assessing the clinical effects of 5-HT_{1A} receptor agonism is buspirone, which has an aryl-piperazine linked to an azaspirodecanedione moiety (Loane and

Politis 2012). As the latter is a defining feature, buspirone is also referred to an azapirone. The 'azapirone' class includes gepirone, ipsapirone and tandospirone (Kishi et al. 2014).

Most of these drugs exhibit a degree of multimodality that result in challenges in attributing particular behavioural or therapeutic effects specifically to 5-HT$_{1A}$ receptor activation alone. For example, buspirone has affinity for dopamine D$_2$ and D$_3$ receptors (Loane and Politis 2012) while tandospirone exhibits dopamine D$_4$ receptor engagement (Newman-Tancredi et al. 2022).

What's the evidence 5-HT$_{1A}$ agents are effective treatments for depression

Broadly, 5-HT$_{1A}$ receptor agonists show antidepressant activity, alleviating depression more than placebo (RR 0.74, 95% CI 0.65-0.83) (Kishi et al. 2014). There may be within-class differences in antidepressant efficacy, with more robust effects seen with buspirone, gepirone & flesinoxan (Ansseau et al. 1993; Feiger et al. 2003; Bielski et al. 2008; Kishi et al. 2014), than agents such as ipsapirone (Heller et al. 1990; Lapierre et al. 1998; Stahl et al. 1998). Licensing does not reflect this directly, with buspirone and tandospirone approved for anxiety disorders rather than depression.

What's the evidence 5-HT$_{1A}$ agents influence emotional processing

Depression is associated with negative emotional biases which serve to maintain and reinforce the persistent low mood that characterise depression (Disner et al. 2011). Conventional antidepressant drugs such as SSRIs produce rapid effects to shift emotional processing in a positive direction in both healthy volunteers and patients (Harmer, Cowen, and Goodwin 2011; Harmer, Duman, and Cowen 2017). This shift in emotional processing has been used by industry to screen for novel antidepressant compounds (Harmer, Cowen, and Goodwin 2011).

There is little work on the effect of 5-HT$_{1A}$ receptor agonists on responses to emotional response bias. However, in a cross-over study of 15 healthy participants, Bernasconi and colleagues (2015) reported that, relative to placebo, a single dose of buspirone (15mg) impaired the discrimination of fearful but not happy faces (Bernasconi et al. 2015). This reduction in sensitivity to fear is consistent with an anxiolytic effect and is also seen after repeated SSRI treatment. Using EEG, Bernasconi et al (2015) found that buspirone reduced activity in the right dorsolateral prefrontal cortex (dlPFC) in response to fearful faces, consistent with a role for 5-HT$_{1A}$ receptor agonism in reducing cortical attention to negative stimuli (Bernasconi et al. 2015).

Using combined PET & fMRI imaging, two healthy volunteer studies (with a total of 35 participants), demonstrated an inverse correlation between 5-HT$_{1A}$ receptor density in the raphe and amygdala response to fearful faces (Fisher et al. 2006; Selvaraj et al. 2015). This suggests that higher availability of 5-HT$_{1A}$ autoreceptors is associated with reduced neural response to fear, which is consistent with an important role for pre-synaptic 5-HT$_{1A}$ receptors in the control of anxiety and fear. Interestingly, pre-clinical evidence indicates that mice with high 5-HT$_{1A}$ receptor expression in the raphe nuclei exhibit increased behavioural despair and poor response to antidepressants in behavioural models (Richardson-Jones et al. 2010). This could suggest the relative importance of post-synaptic 5-HT$_{1A}$ receptor mechanisms in facilitating responses to antidepressant treatment.

What's the evidence 5-HT$_{1A}$ agents influence reward processes

Anhedonia is key symptom in the diagnosis of depression and is not well managed by current pharmacological approaches (Pizzagalli 2022b). One reason for the great interest in the use of ketamine as an antidepressant is its ability to rapidly relieve anhedonic symptoms in patients with treatment resistant depression (Coyle and Laws 2015; Lally et al. 2015). In animal models, the antidepressant-like effect of ketamine can be reversed by drugs that block cortical 5-HT$_{1A}$ receptors (Fukumoto et al. 2018). In parallel to this line of investigation new compounds are being developed, termed biased agonists, that despite activating the same receptor as other agonists will signal through differing intracellular ('non-canonical') pathways. Such a compound has been developed for the 5-HT$_{1A}$ receptor, called NLX-101, which not only preferentially binds post-synaptic over pre-synaptic 5-HT$_{1A}$ receptors, signals through an alternative pathway to other common 5-HT$_{1A}$ receptor agonists. Excitingly NLX-101 has been shown to rapidly reverse anhedonic-like behaviour in the chronic mild stress test in rats (Depoortere et al. 2019). These results make the role of the 5-HT$_{1A}$ receptor, and possibly biased agonism towards it, in human reward & aversive processing of particular interest.

At present there does not seem to be any work in humans that has investigated the effect of 5-HT$_{1A}$ receptor agonism on behavioural or neural aspects of reward experience or reward learning.

What’s the evidence 5-HT$_{1A}$ agents influence cognition

There is an unmet need for pharmacological treatments that can improve cognition in depression as the common cognitive deficits in patients with depression often persist in remission (Douglas and Porter 2009). ‘Difficulty in concentrating’ is listed as a symptom contributing to the diagnosis of depression in ICD-11. In patients with depression, however, cognitive impairment is typically seen over a wide range of neuropsychological domains, including attention, learning, memory, and executive function, with some resolving as the patient enters remission and others persisting (Rock et al. 2014) . Such impairments can be assessed by cognitive and behavioural tests in humans including well-established test batteries such as the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Cognition 2019).

As noted above, conventional azapirone 5-HT$_{1A}$ receptor agonists such as buspirone and tandospirone, do not reliably affect cognition in healthy participants, compared to placebo (Yasuno et al. 2003; Chamberlain et al. 2007). However, the multimodal antidepressant agent, vortioxetine, does have some evidence for pro-cognitive effects in patients with depression (standardized effect size = 0.254) (Mahableshwarkar et al. 2015). This is of interest because among vortioxetine’s pharmacological properties is full agonism at 5-HT$_{1A}$ receptors (D'Agostino, English, and Rey 2015).

Summary

Serotonin & reward processing

What is not known

In the context of its widespread presence, and thus likely influence in the brain, very little is known about the role of 5-HT$_{1A}$ receptor agonism in reward & aversive processing. Greater evidence exists for the effect of SSRI on reward & aversive processing. However, the time course of such effects is intriguing, with a 2-week SSRI course leading to an increased resilience of reward learning but a 1-week SSRI course worsening reward, but improving aversive, learning. Furthermore, the neural correlates of any behavioural changes at one week of SSRI are yet to be evaluated with a monetary (i.e. secondary) outcome and probabilistic learning.

Anticipation, cost-benefit decision making and appetitive behaviour have been evaluated with different durations of SSRI. However, behaviourally cost-benefit decision making and appetitive behaviour are often inferred from one another, rather than measured directly as separate steps in motivation, an important distinction when searching for the specific stages of reward processing that are dysfunctional and how they may be remedied.

Finally, it appears 5-HT modulation has no significant effect on subjective rating of pleasant and aversive tastes at a group level, however this observation was taken in an MRI environment and possibly not controlling for the random effect of participant preference (McCabe et al. 2010). It is possible a difference emerges from 5-HT modulation in a more naturalistic environment i.e. outside of an MRI scan and when controlling for the random effect of individuals.

Why it matters that we do know

Using a relatively straightforward reinforcement learning task, without the confounds of hidden information and aspect of gambling, we can isolate the effects in probabilistic learning in win and loss scenarios more specifically. Furthermore, identifying any neural correlates that can corroborate any behavioural effects would also be valuable in contributing to potential behavioural and neural signature of subacute SSRI effects on probabilistic reward learning.

Serotonin is thought to mediate costs in decision making, as evidenced by 1 week of citalopram increasing the monetary reward won in an effort grip task e.g. computationally found to be a discounting of effort cost, however this cost-benefit decision is yet to studied in isolation. As the basis for subsequent stages in motivational drive and appetitive behaviour, it is important to identify if cost:benefit decision making is influenced by serotonergic modulation. This finding would complement the learning and probability discounting from the probabilistic instrumental learning task.

Finally measuring the subjective experience of various tastes, considering individual differences, can give insights into sensitivity to primary reward to complement behavioural sensitivity to a secondary, monetary reward. This is key to all subsequent stages in reward and aversive processing, therefore crucial to ascertain in order to make firmer conclusions of the effect of serotonergic modulation on reward and aversive processing, a key question in our understanding of SSRI effects on different sub domains of depression.

5-HT_{1A} agonism and emotional, cognitive and reward processing effects

What we do not know and why it is important

Whilst the cognitive effects of 5-HT_{1A} agonism have been studied in healthy male volunteers (Chamberlain et al. 2007), relatively less is known about its effects in a mixed sample, which is important for the generalizability of results. Less is known about the effects of 5-HT_{1A} agonism on emotional processing, with a single study identifying reduced sensitivity to fearful faces (Bernasconi et al. 2015). It is important to have a broader understanding of the emotional effects of 5-HT_{1A} agonism (e.g. emotional memory, wider range of emotions), because this receptor, due to its widespread presence throughout the brain, plays a role in the effects of many serotonergic drugs either indirectly through increased extracellular 5-HT levels or through direct agonism e.g. buspirone and vortioxetine. Finally, to date studies have not explored the effect of direct 5-HT_{1A} receptor agonism on reward processing in humans, however animal work indicates buspirone may have a mild detrimental effect on reward learning (Frick et al. 2015). This understanding will provide a richer understanding of its consequence, negative or otherwise, on each of the stages of reward processing.

The influence of time course of intervention in serotonergic system changes

The serotonergic system is complex with contrasting effects, which is reflected by serotonergic drugs having changing effects over the time course of their administration. Broadly one can divide time course of treatment into acute (< 24 hours), sub-acute (7-10 days) and chronic/therapeutic (> 2 weeks). A single dose, or isolated increases in 5-HT, would transiently reduce 5-HT levels by activating inhibitory 5-HT$_{1A}$ auto-receptors found on pre-synaptic serotonergic neurones, reducing tonic firing of serotonergic neurones (a 'handbrake' on 5-HT release) (Sharp et al. 1993). Persistent, stable increases in 5-HT, through repeated dosing in the sub-acute to chronic range, would desensitise 5-HT$_{1A}$ auto-receptors (removing the 'handbrake') leading to a net increase in tonic firing of 5-HT neurones (Dawson et al. 2000; Muraki et al. 2008; Smith et al. 2000; Lifschytz et al. 2004). This would soon lead to neuroplastic changes e.g. Brain Derived Neurotrophic Factor upregulation, as well.

This thesis would explore these temporal dynamics of serotonergic signalling: a single dose of the 5-HT$_{1A}$ agonist buspirone explores the acute receptor-level effects of reducing serotonergic tone, whereas a sub-acute course of the SSRI citalopram gives insights into early effects of restoring and increasing serotonergic tone.

Outline of thesis

In Chapter 2, I present the effects of 5-HT$_{1A}$ agonism, through a single dose of buspirone, on established emotional processing & cognitive tasks in a healthy volunteer samples of males & females. I hypothesize that a reduction in accuracy for emotional information of negative valence will occur, using a wider range of emotions than used in previous studies. I hypothesize also to observe no significant effect in cognitive tasks used. Importantly for both hypotheses this will extend current evidence to both males and females.

In chapter 3, I present the effects of 5-HT$_{1A}$ agonism, through a single dose of buspirone, on a battery of reward processing tasks to measure the behavioural correlates of reward and aversive consummation, probabilistic learning and physical effort cost:benefit decision making. I hypothesize acute buspirone will have an overall positive effect on reward processing, namely increasing the pleasurableness of sweet tastes, increase the willingness to expend effort for reward and improve learning about reward. I predict to observe minimal effect of buspirone on the aversive equivalents e.g. bitter taste, high effort-low reward offers or loss learning trials. This is the first study to explore the effects of 5-HT$_{1A}$ agonism on reward processing in humans, however animal indicates it may a mild detrimental effect on reward learning.

In chapter 4, I use the same battery of reward tasks as chapter 3 to examine the behavioural effects of a 7-day course of citalopram in reward processing. I hypothesize a 7-day course of citalopram will improve aversive learning but have no effect on reward learning; increase the willingness to expend effort and reduce the subjective pleasantness of rewarding tastes, but not unpleasant tastes.

In chapter 5, I examine the neural correlates of probabilistic learning, giving results for anticipation, consummation and learning (through prediction error). I hypothesised that a 7-day course of citalopram would reduce activation in reward related areas, such as striatum, during the anticipation and receipt of rewarding outcomes (e.g. win). However, I hypothesise an increase in activation of regions related to aversive processing, such as the insula and anterior cingulate cortex, during the anticipation and receipt of aversive outcomes (e.g. loss).

Chapter 2: The behavioural effects of acute buspirone on cognition and emotional processing

Introduction

The central serotonergic system projects widely throughout the brain and is functionally implicated in key aspects of mood, emotion and cognition. A critical node of control of the serotonergic system is the 5-HT$_{1A}$ receptor, which has complex pharmacology and effects. For example, pre-synaptic 5-HT$_{1A}$ agonism at 5-HT$_{1A}$ autoreceptors results in a reduction in serotonergic transmission via inhibition of 5-HT release in the terminal fields of serotonergic neurones. However, activation of post-synaptic 5-HT$_{1A}$ receptors can modulate downstream GABA, glutamatergic and cholinergic transmission (Meneses and Perez-Garcia 2007; Ślifirski, Król, and Turło 2021; Ogren et al. 2008). It is through these multifaceted mechanisms that 5-HT$_{1A}$ receptor modulation is implicated in the pathophysiology and treatment of depression (Smith et al. 2023; Sharp and Barnes 2020; Savitz, Lucki, and Drevets 2009).

5-HT$_{1A}$ receptors influence several brain regions relevant to cognition and emotional processing (Yasuno et al. 2003; Selvaraj et al. 2018), which are often implicated in depression (Sheline et al. 2001; Tartt et al. 2022). Various tasks examining these cognitive and emotional domains, such as N-back and facial expression recognition tasks, have demonstrated impairments in untreated individuals diagnosed with depression (Nikolin et al. 2021; Prado, Watt, and Crowe 2018; Talarowska et al. 2010; Harmer et al. 2009), while drugs that have clinical efficacy in Major Depressive Disorder (MDD), typically improve performance on these tasks (Prado, Watt, and Crowe 2018; Harmer et al. 2009).

There is a paucity of information on the effects of drugs with 5HT$_{1A}$ receptor agonist properties on emotional processing. A study using a single dose of the partial 5-HT$_{1A}$ agonist, buspirone, reported reduced accuracy in the recognition of fearful (but not happy) faces (Bernasconi et al. 2015). Interestingly, mixed fMRI/PET studies have reported a negative correlation between dorsal raphe (DRN) 5-HT$_{1A}$ binding and amygdala activity to negatively-valanced faces, suggesting that increased activation of DRN 5-HT_{1A} receptors may inhibit amygdala reactivity, and thus blunt neural responses to negatively-valanced emotional stimuli (Fisher et al. 2006; Selvaraj et al. 2015). Taken together, this evidence suggests that buspirone, through pre-synaptic 5-HT_{1A} agonism, could reduce sensitivity to negatively valanced stimuli.

Studies examining the effects of $5HT_{1A}$ receptor agonism on non-emotional cognition are inconsistent. Several studies report no impairing effect of buspirone or other 5-HT_{1A} agonists on verbal and working memory performance (Chamberlain et al. 2007; Barbee et al. 1991; Takahashi et al. 2010); however some smaller investigations, which combine cognitive assessment with neuroimaging, do find verbal memory impairment following buspirone administration (Grasby et al. 1992; Yasuno et al. 2003). This inconsistency of results could be attributed to the heterogenous nature of the studies, with some using small sample sizes and male-only participants, or to the use of different measures of memory performance (which vary for example in the number of trials, the inclusion of a reminder of forgotten words between trials and intermediary tasks between trials).

Buspirone is a readily available pharmacological probe for 5-HT_{1A} agonism in humans, however its complex pharmacology should be noted. Acutely buspirone demonstrates full agonism at the inhibitory 5-HT_{1A} receptors, located in pre-‘synaptic sites on serotonergic neurons in the raphe nuclei. In essence this action acutely attenuates serotonergic transmission to several brain regions served by the ascending pathways from the raphe such as the prefrontal cortex, hippocampus and amygdala (Vertes and Linley 2007; Artigas 2013). Additionally, buspirone has partial agonism at post-synaptic 5-HT_{1A} receptor sites throughout the brain, with lower efficacy than endogenous 5-HT. Thus the effect of the partial 5-HT_{1A} agonism of buspirone at post-synaptic 5-HT_{1A} receptors may be contextual, with some proposing it has antagonistic action in hyper-serotonergic states and agonist action in hypo-serotonergic states (Yocca 1990). In healthy participants, acute administration of buspirone reliably increases plasma levels of cortisol and growth hormone, indicating agonist action at post-synaptic 5-HT_{1A} receptors in the hypothalamus (Cowen et al, 1990).

Furthermore buspirone undergoes marked first pass metabolism to 1-(2-pyrimidinyl)-piperazine, a compound that blocks noradrenergic α_2-adrenoceptors (Cowen, Anderson, and Grahame-Smith 1990). α_2-adrenoceptors have been implicated in regulating several neurotransmitter systems (Langer 2015), particularly noradrenaline transmission, which influences working memory (Berridge and Spencer 2016). Furthermore, buspirone has antagonistic activity at both pre- and post-synaptic D_2 receptors (Loane and Politis 2012).

The pharmacokinetics of buspirone include a T_{max} of 40-60 minutes with a half-life of approximately 2.5 hours (however its metabolite, 1-PP, remains for 5-6 hours) (Mahmood and Sahajwalla 1999). In terms of pharmacodynamics of buspirone, EEG (Bernasconi et al. 2015) and neuroendocrine (Alexander et al. 2010) studies indicate it has effects at 5-HT_{1A} receptor by 1 hour, in spite of relatively low 5-HT_{1A} as detected by current PET neuroimaging approaches (Rabiner et al. 2000; Passchier et al. 2001)

Here we test the effects of a single 20mg dose of the 5-HT_{1A} receptor agonist buspirone on emotional processing and working and verbal memory in a large sample of healthy male and female volunteers. We use a battery of emotional processing tasks that are known to be sensitive to the effects of conventional antidepressants (Harmer, Bhagwagar, et al. 2003), as well as testing verbal and working memory using previously used and widely available cognitive tasks (Prado, Watt, and Crowe 2018).

We hypothesized that acute administration of buspirone would reduce accuracy for detection of emotional facial expressions, particularly those of negative valance and would have no effect, or a mild impairing effect, on verbal and working memory in healthy volunteers.

Methods

Recruitment, screening & randomisation

Sixty-three healthy participants were recruited (28 male, 35 female), age range 18-50 years, through local and social media advertising. Participants were screened for any contraindications to buspirone use. Other exclusion criteria included: previous or current mental illness; previous dependence or recent use of illicit drugs; current pregnancy or breastfeeding; prior completion of tasks used in the study; current use of psychoactive medication or medication likely to influence safe participation in the study. The study was approved by Local Research Ethics Committee (Oxford, MSD-IDREC reference R79236/RE006). Written informed consent was obtained from all participants.

Study design

The study had a double-blind, placebo controlled, between-subject design. Participants were randomly allocated to either placebo or buspirone, stratified by gender. Buspirone dose was 20mg, selected based on a balance of target engagement and tolerability (Bernasconi et al. 2015; Cowen et al. 1994; McAllister-Williams and Massey 2003). Participants received either encapsulated placebo (lactose) or buspirone (20mg). Participants began testing 1 hour post capsule administration as this is peak plasma concentration of buspirone (Mahmood and Sahajwalla 1999). The pharmacokinetics of buspirone include a T_{max} of 40-60 minutes with a half-life of approximately 2.5 hours (however its metabolite, 1-PP, remains for 5-6 hours) (Mahmood and Sahajwalla 1999). Encapsulation of buspirone was utilised to preserve participant and researcher blinding, this would have minimal effects on the pharmacokinetics of buspirone (Stegemann et al. 2015).

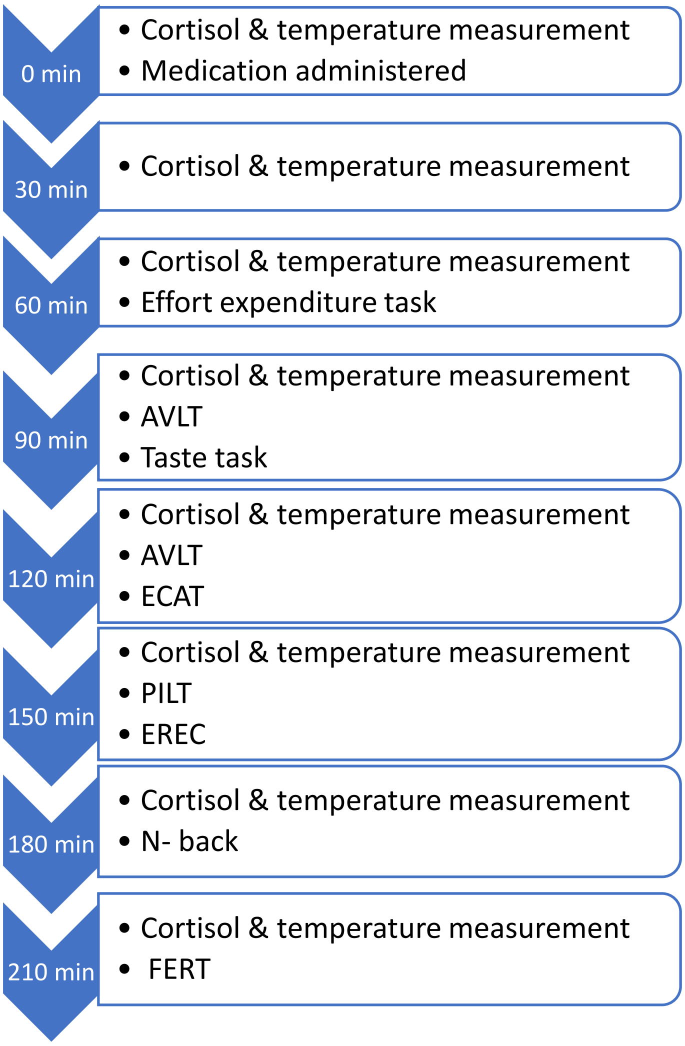
In terms of pharmacodynamics of buspirone, EEG and neuroendocrine studies indicate it has effects at $5\text{-}HT_{1A}$ receptor by 1 hour (Bernasconi et al. 2015; Alexander et al. 2010), in spite of relatively low $5\text{-}HT_{1A}$ as detected by current PET neuroimaging approaches (Rabiner et al. 2000; Passchier et al. 2001). This indicates commencing testing 1-hour post buspirone dose would be reasonable as it would have reached its maximum plasma concentration and engaged the $5\text{-}HT_{1A}$

receptor. It should be noted however two behavioural tasks may be outside the plasma half-life of buspirone (e.g. N-back and FERT), which is a limitation of the study design. Recruitment took place between May 2022-April 2023.

Primary analysis was a repeated measure ANOVA for accuracy of emotion identification (six emotions) between groups (two groups) to test if buspirone changed emotion-identification accuracy relative to placebo.

A meta-analysis of four trials measuring the response to buspirone in major-depressive disorder observed a risk-ratio of 0.71 (Kishi et al. 2014). Conversion of this to a standardised mean difference gives Cohen's d ≈ 0.55 (Chinn 2000). For two groups a mixed-ANOVA uses Cohen's f = d / 2 ≈ 0.28 (Cohen 1988). Because each participant classifies six emotions and these scores are moderately correlated (≈ ρ 0.40), the repeated-measures design roughly halves the error variance. Entering f = 0.28, ρ = 0.40, α = 0.05, power = 90 % into G*Power software (Faul et al. 2007) indicates that 22 per group were needed. I recruited 31 per arm to cover attrition, which provides over 90% power for the target effect and ≥ 80 % power for any effect size ≥ 0.40.

A limitation of the effect size calculation being based upon response of MDD subjects using clinical questionnaires, rather than healthy volunteers and behavioural tasks.



Cortisol & temperature measurements

To evaluate engagement of the 5-HT$_{1A}$ receptor, salivary cortisol (collected using Salivette® Cortisol, Sarstedt AG & Co.) and temperature measurements were taken at 30 minute intervals; these are recognised surrogate markers of post-synaptic and pre-synaptic 5-HT$_{1A}$ activation respectively (Smith et al. 2023).

A total of 8 cortisol and temperature measurements per participant were recorded. Saliva samples were rendered acellular and frozen on the day of collection. At the end of participant recruitment, saliva samples were assayed for cortisol using the salivary cortisol enzyme immunoassay kit from Salimetrics, LLC. Temperature was measured from the forehead using a non-contact infrared thermometer (Shenzen Pacom medical instruments).

Side effects and subjective mood measurements

Side effects and subjective mood were measured at baseline (- 30min), 1hr (start of testing) and 4hrs (end of testing) after capsule administration. Participants were required to rate their experience of side effects (nausea, light-headedness, restless and drowsy) and mood (happy, calm, energetic) on a visual analogue scale of 0-100 (not at all – very much). A composite score of side effects and mood was created for each participant at each timepoint e.g. side effects score = nausea + light-headedness + restless + drowsiness.

Questionnaire measures

Participants completed the Beck Depression Inventory (Beck et al. 1996) at baseline to ensure no incidental differences arose between groups at baseline.

Neuropsychological assessment

Tasks were completed in the same order for every subject. Thirty-minute testing windows, at the start of which temperature and salivary samples were taken and tasks commenced and the experimenter would leave the testing room. Participants would remain in the room and advised to relax between the end of a task and the start of the next 30-minute window.

Emotional Test battery (ETB)

The tasks used form part of the Emotional Test Battery (Harmer et al. 2009) which examines the processing of a variety of emotionally valanced stimuli through the use of computerised tasks. Here three tasks were used: Facial Expression Recognition Task (FERT), Emotional Categorisation Task (ECAT) and the Emotional Recall Task (EREC).

FERT

During the FERT participants were shown, one at a time, a human face (n = 250 trials) displaying one of seven emotions (anger, sad, disgust, fear, surprise, happy, neutral). Emotions were expressed at a range of intensities, morphed between neutral and full emotions in 10% steps. Participants were required to report the emotion of the face via a button press. Outcome measures were overall accuracy (when classified by emotion, emotion x intensity and valence) & reaction time. Furthermore, the number of misclassifications of emotions was measured, either broadly e.g. disgust to any other emotion; specifically, e.g. disgust to surprise, or by emotion valence e.g. any negative to any positive emotion.

ECAT

In the ECAT, participants were shown a series of 40 (20 positively and 20 negatively valanced) descriptive personality words, and asked to rate whether they would like or dislike being described as each descriptor e.g. empathetic. Words were displayed randomly on a computer screen for 500msec. The primary outcome measure was accuracy and reaction time.

EREC

The EREC was a surprise free recall task that required the participant to write as many words from the ECAT task (completed approximately 40 minutes previously) in 4 minutes. The primary outcome measure was the number of words correctly recalled.

Cognitive tasks

Rey Auditory Verbal Learning Task (AVLT)

Participants listened to a recording of 15 words (List A) and were asked to immediately recall the list verbally. This was repeated a further four times with the same words. Participants then listened to a separate, unrelated list of 15 words (List B) and asked to recall them verbally. Immediately after this (short delay) and after a 15-minute delay (long delay), participants were again asked to verbally recall as many words from List A as they could remember. Finally, participants were read a list of words (containing the 15 words from List A and 35 distractor words) and were asked to indicate if a word was from list A or not (recognition). The primary outcome measure was number of words correctly recalled.

N-back

In this task, adapted from (Mannie et al. 2010), participants were required to indicate (‘*same*’ or ‘*different*’) whether a letter presented on screen matched the letter presented *n* trials previously (where *n* equals one, two or three, representing 1-back, 2-back or 3-back respectively). Letters were presented for 500msec, with a fixation cross presented for 1500msec between letters. Participants underwent 160 trials in total (40 trials per condition) in blocks of 10 trials, a fixation cross was presented for 5000msec between each block. Each block was for one condition only e.g. all 1-back or all 2-back). Outcome measures were overall accuracy and average reaction time for each condition for each participant.

Statistical analysis

Data cleaning

All outlier removal was performed prior to unblinding. Rationale for outlier removal was on the basis of non-engagement with the trial or task as a whole.

Broad statistical approach

All statistical work was carried out in R 4.3 (Posit, 2023-09) with the *lme4*, *lmerTest*, *emmeans* and *effectsize()* functions. Because most outcomes were repeated measures with occasional missing cells, every analysis was framed as a linear mixed-effects model fitted with *lmer()*.Random intercepts for participants allowed us to retain every available row, even when a particular rating or time-point was missing. Fixed effects were tested with Type-III F-tests from *lmerTest*() function, which supply Satterthwaite or Kenward–Roger denominator degrees of freedom (Appendix: Chapter 2: Behavioural testing: Statistical testing and degrees of freedom note). Effect size was calculated using the

Significant, or trend-significant, interactions were followed up with estimated marginal means with the *emmeans()* function. Family-wise error in pairwise contrasts was adjusted using the Holm method. Effect sizes were calculated using the *eff_size()* function using the model's residual standard deviation. Tasks that involved only a single two-group comparison—such as AVLT long-delay recognition—were analysed with an ordinary least-squares model (*lm()*), but the same *emmeans*/Holm-corrected/Hedges effect size pipeline was applied.

Cortisol, temperature, mood and side effect analysis

A repeated measure analysis of variance (ANOVA) was performed to evaluate changes in mood and side effect burden during the study, with individual rating three for mood and four for side effects), allocation group and timepoint as predictor variables. Additionally, composite scores for each category of rating was taken e.g. mood = calm + energetic + happy, and compared between groups at the different timepoints.

The mean average cortisol measurement was calculated for each allocation group at each timepoint. The difference in temperature from baseline at an individual level was calculated. A mean average of the difference from baseline was calculated for each allocation group at each timepoint. Side effect score was calculated as a composite score of all side effect ratings (nausea, restless, sleepy and light headedness) at the start of testing (e.g. 1 hour).

Emotional Test Battery & cognitive task analysis

Behavioural data were analysed using a repeated measures ANOVA. Group allocation (buspirone or placebo) was used as a between subjects' factor in all tests. For FERT analysis the seven emotions were further classified into valence categories that were negative (anger, sad, disgust, fear), positive (surprise, happy) or neutral (neutral). Emotion, intensity or valence were then used as predictor variables for the FERT task in separate analyses. To investigate misclassification, the number of misclassifications was used as an outcome variable. Three separate approaches were used to analyse misclassifications. These were for broad misclassifications (an emotion misclassified to any other emotion, e.g. anger misclassified to happy, sad, disgust etc.), specific misclassification (an emotion misclassified to a specific emotion, e.g. anger misclassified to sad) and valence (a positive, negative or neutral emotion misclassified to an emotion of different valence or another emotion within the same valence e.g. a positive emotion misclassified to negative emotion).

In the EREC and ECAT analysis, word valence was a predictor variable and total number of words recalled an outcome variable.

The outcome variable for the AVLT was number of words recalled in the first five trials and the delayed recall (short & long) trials as well as for the long delay recognition of words. Acquisition block (AVLT) and condition (N-back) were additional predictor variables.

Results

Missing data and outlier removal

One participant was excluded from all analyses having received a half dose of the study medication in error (buspirone group).

All outlier removal, based upon accuracy and reaction time, was performed prior to unblinding. In the FERT dataset one participants accuracy was more than two standard deviation outside of the mean accuracy for the entire dataset, therefore presumed to have not engaged with the task to a sufficient degree. Therefore, all data from this participant was removed from the FERT dataset. This resulted in 31 participants in the placebo group and 30 participants in the buspirone group.

FERT & ECAT trials with reaction times that were either too fast (to register information & execute a motor response) or too slow (presumed to not have engaged with the trial) were removed. Trials with response time less than 200 msec were deemed too fast to register stimuli and enact a motor response. This resulted in removal 1 trial in the FERT and 1 trial in the ECAT. The upper limit for response time was defined on a participant-basis: if a participant's performance met the criteria as an extreme outlier (trials lying at more than three times the participants' interquartile range above their third quartile), it was removed, as per previous analysis of the tasks (Murphy et al. 2020). This resulted in removal 311 trials (2%) in the FERT and 44 trials (1.8%) in the ECAT.

In analysis of N-back data, the data of one participant was removed due to consistently being more than two standard deviations outside of the mean for accuracy, resulting in 31 participants in the placebo group and 30 participants in the buspirone group.

Three participants (all buspirone) were excluded from EREC analysis due to errors in data collection, leaving 29 participants in the buspirone group and 31 participants in the placebo group.

No participants or trials were excluded from the AVLT analysis.

Two participants (both in buspirone allocation group) were omitted from side effect analysis and subsequent sensitivity analysis due to an error in collection of side effect data.

Demographics, mood and side effects

The groups were well matched for age, gender and baseline mood (Appendix: chapter 2, Table 1). The mixed-effects ANOVA revealed a significant main effect of allocation, $F(1, 60.1) = 7.01$, $p = 0.010$, indicating lower composite mood in the buspirone group. A significant time-point × allocation interaction was observed, $F(2, 458.9) = 9.87$, $p < 0.001$, showing that the mood difference between groups emerged at 1-hour and persisted to 3 h post-dose. Estimated marginal means (Holms-corrected) confirmed that at 1-hour post-intervention the buspirone group rated themselves both less happy ($t(332) = –2.91$, $p = 0.004$) and less energetic ($t(318) = –5.23$, $p < 0.001$) than the placebo group. No other allocation differences reached significance after adjustment (all $p \geq 0.05$).

The mixed-effects ANOVA revealed a significant main effect of allocation on composite side effect score, ($F(1, 60.4) = 16.01$, $p < 0.001$), with higher side-effect burden in the buspirone group. A significant three-way interaction (allocation × time-point × rating) was observed, ($F(6, 648.5) = 3.11$, $p = 0.005$). Estimated marginal means (Holms-corrected) indicated the buspirone group experienced greater *light-headedness* ($t(593) = 7.40$, $p < 0.001$) and *sleepiness* ($t(593) = 4.71$, $p < 0.001$) than the placebo group at 1-hour post-intervention. Of note a small baseline difference in sleepiness was also present, with the buspirone group feeling slightly sleepier ($t(585) = 2.09$, $p = 0.038$). No significant allocation effects emerged for nausea or restlessness at any time-point, and by 3 h post-dose all side-effect ratings were not significantly different (all $p \geq 0.1$).

To explore the effect further Pearson's correlation coefficients were calculated for outcome measures and the composite side effect score and mood score separately. A significant negative correlation between composite mood and side effect scores at 1-hour post intervention was observed (r = - 0.44, p = 0.0096) (Appendix: Chapter 2: table 3). A significant correlation between timepoint and a subjective energetic feeling was observed across all participants (r = - 0.21, p = 0.022) (Appendix: Chapter 2: table 4).

Cortisol & temperature measurement

There was a significant interaction between group and timepoint for cortisol measurement [F(7, 427) = 2.54, p = 0.014]. Figure 1 illustrates the buspirone group maintained salivary cortisol levels whilst the placebo group showed lowered cortisol levels during the testing period. The difference in cortisol between groups became significant by 90 minutes post-intervention (t(216) = 2.60, p = 0.01), with the placebo group showing diminished cortisol levels. This difference remained until 3 ½ hours post intervention. This could represent engagement of post-synaptic hypothalamic 5-HT_{1A} receptors by buspirone (Cowen, Anderson, and Grahame-Smith 1990).

No significant effect allocation (F(1,59.17) = 0.021, p = 0.89) was observed for temperature. Visual inspection of change in temperature from baseline (Appendix: chapter 2, Figure 1) indicated a difference between allocation groups at certain timepoints however.

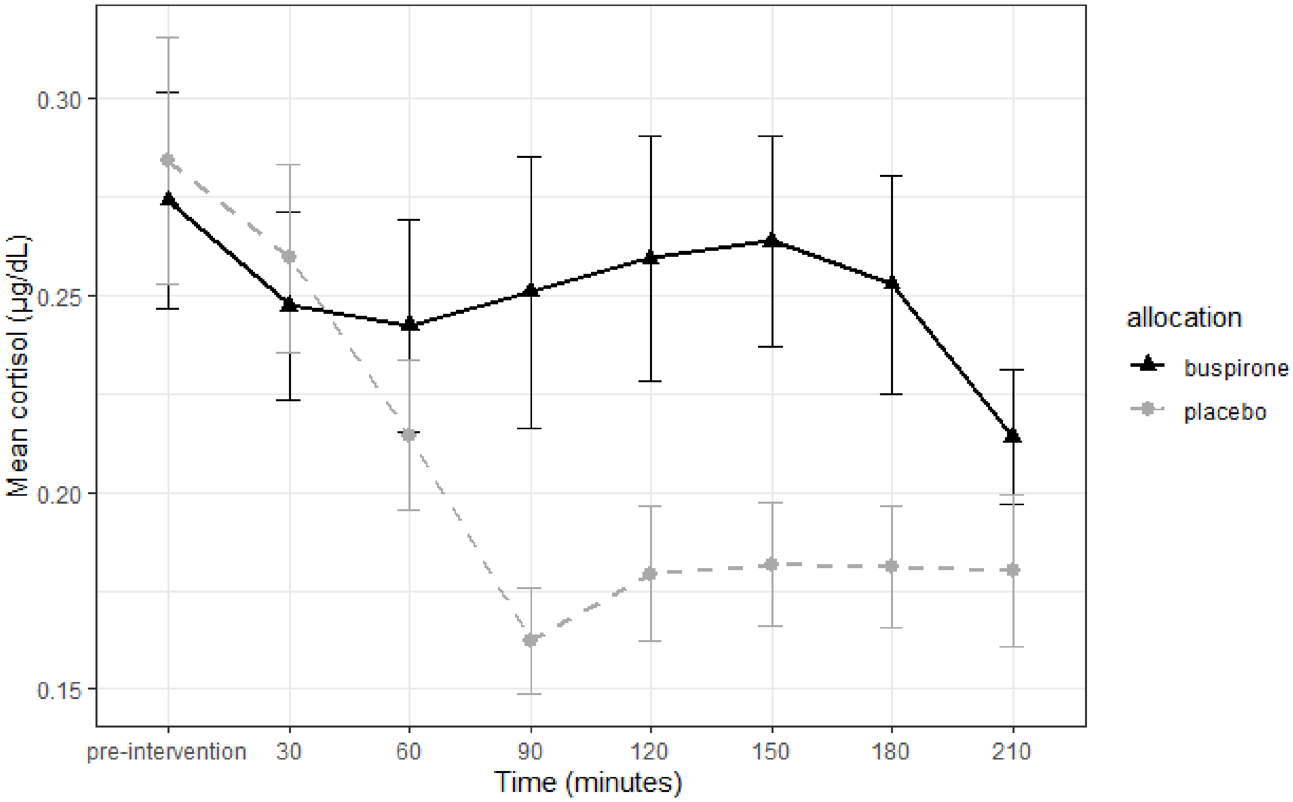


Figure 1 Mean salivary cortisol response following either buspirone or placebo. Error bars = +/- S.E.M

Emotional test battery

Facial Expression Recognition Test (FERT)

Accuracy

There was a significant group by emotion interaction [F(6, 326.55) = 3.08, p = 0.006, $\eta^2_p = 0.05$) on accuracy of facial expression recognition. Further analyses indicated that the buspirone group were less accurate in recognising disgust [t(357) = -3.14, p = 0.002, Hedges *g* = –0.87, 95 % CI [–1.42, –0.33], buspirone = 32.6, placebo = 42.6] and more accurate in recognising sad emotions t(357) = 1.99, p = 0.047, Hedges *g* = 0.55, 95 % CI [0.006, 1.09], buspirone = 59.1, placebo = 52.9) (Fig. 2). There was no significant main effect of group [F(1, 59.17) = 0.003, p = 0.96] or group by emotion interaction [F(6, 322.51) = 1.80, p = 0.099] for reaction time.

A non-significant main effect of allocation was observed for accuracy when including intensity of emotion in testing (F(1, 52.49) = 2.51, p = 0.12, $\eta^2_p = 0.05$).

When facial emotions were collapsed into valence categories (positive, neutral, negative), there was no significant main effect of group on accuracy [F(1,59) = 1.61, p = 0.21] or reaction time [F(1,59) = 0.015, p = 0.90]. There was no significant interaction effect between group and valence for accuracy [F(2, 118) = 0.32, p = 0.73) or reaction time [F(2, 118) = 0.84, p = 0.44).

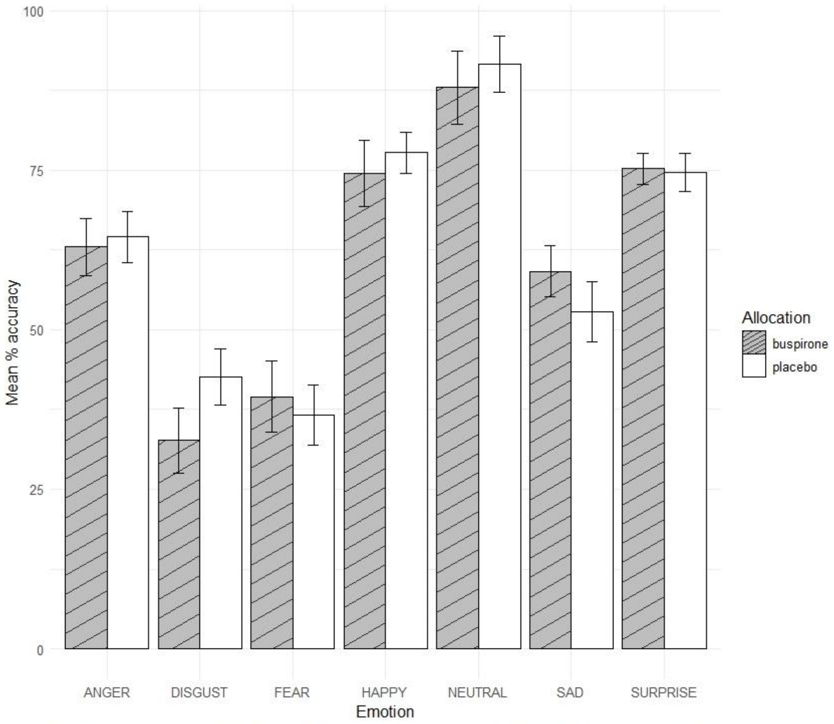


Figure 2. Mean percentage accuracy for emotional categories in the FERT. Error bars = 95% C.I.

Misclassification

Group allocation did not have a significant main effect on the total number of broad misclassifications (F(6, 381) = 0.20, p = 0.66), however a trend significant interaction between group and emotion was observed (F(6, 381) = 1.98, p = 0.068, η^2_p = 0.03), which was possibly driven by the buspirone group making fewer errors for neutral faces (*t*(381) = –2.31, *p* = 0.021, Hedges *g* = – 0.59, 95 % CI [–1.10, –0.09] but more errors for sad faces (*t*(381) = 2.38, *p* = 0.018, Hedges *g* = 0.61, 95 % CI [0.10, 1.13]).

There was a significant interaction between group and specific misclassification [F(41, 2419) = 1.64, p = 0.0069, η^2_p = 0.027). This was driven by buspirone significantly increasing misclassification of anger to sad (*t*(2, 471) = 2.78, *p* = 0.006, *g* = 0.71, 95 % CI [0.21, 1.22]), disgust to anger (*t*(2, 471) = 2.36, *p* = 0.018, *g* = 0.61 [0.10, 1.11]), disgust to sad (*t*(2, 471) = 4.66, *p* < 0.001, *g* = 1.20 [0.69, 1.70]) and happy to fear (*t*(2, 471) = 2.39, *p* = 0.017, *g* = 0.61 [0.11, 1.12]).

Conversely, buspirone reducing the misclassification of fear to neutral (*t*(2, 471) = -2.95, *p* = 0.003, *g* = -0.76 [-1.26, -0.25]) and sad to neutral (*t*(2, 471) = -2.70, *p* = 0.007, *g* = -0.69 [-1.20, -0.19]).

Broadly this could be interpreted as acute buspirone maintaining, or possibly inducing, a negative bias in emotional processing, compared to placebo.

When the face emotions were collapsed into valence categories (positive, neutral, negative) there was a significant group x valance misclassification interaction [F(7, 472) = 3.08, p = 0.0035, η^2_p = 0.04) (Fig. 3). This was possibly driven by a significantly increase in misclassification of negative to negative e.g. sad to anger or disgust to sad ($t(472) = 3.69$, $p < 0.001$; Hedges $g = 1.13$, 95 % CI [0.53, 1.73]), buspirone = 13.2%, placebo = 11.0) and reducing the negative to neutral misclassification ($t(472) = –2.68$, $p = .0077$; Hedges $g = –0.82$, 95 % CI [–1.42, –0.22]; buspirone = 16.6%, placebo = 18.2%), supporting the interpretation that acute buspirone may maintain, or possibly induce, a negative bias in emotional processing.

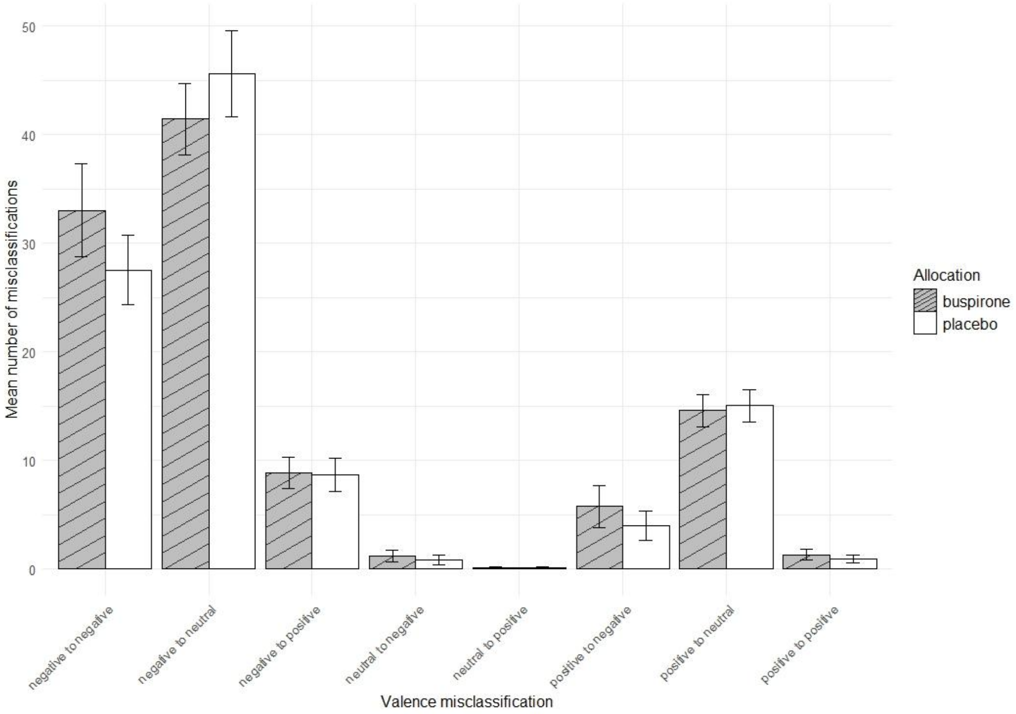


Figure 3. Mean number of misclassifications for valence categories in the FERT. Error bars = 95% C.I.

Emotional categorisation words (ECAT)

There was no significant effect of group on the percentage of words correctly classified (F(1, 58) = 0.00, p = 0.97) or on the average reaction time to words (F(1, 58) = 0.22, p = 0.64).

Emotional word recall task (EREC)

There was no significant effect of group on accurate recall of positive or negative words F[1, 228] = 1.21, p = 0.27).

Cognitive testing

AVLT

No significant effect of group was found on the average number of words recalled during all free recall trials (t(60) = -1.05, p = 0.3). There was a significant block by group interaction [F(6, 360) = 2.76, p = 0.012, η^2_p = 0.044) on the AVLT, which reflected poorer recall in the buspirone group compared to placebo. This group difference was due to a trend significant difference on block 3 (t(130) = - 1.95, p = 0.053, Hedges *g* = - 0.8, 95 % CI [-1.60, 0.012]; buspirone = 11.84, placebo = 12.84). There was no significant group difference in the long delay free recall (t(130) = -1.70, p = 0.09) and long delay recognition (t(60) = -1.10, p = 0.28) blocks, although the buspirone group again had numerically poorer performance (Fig. 4). There was no significant difference in recall of the distractor word list B (t(60) = 0, p =1).

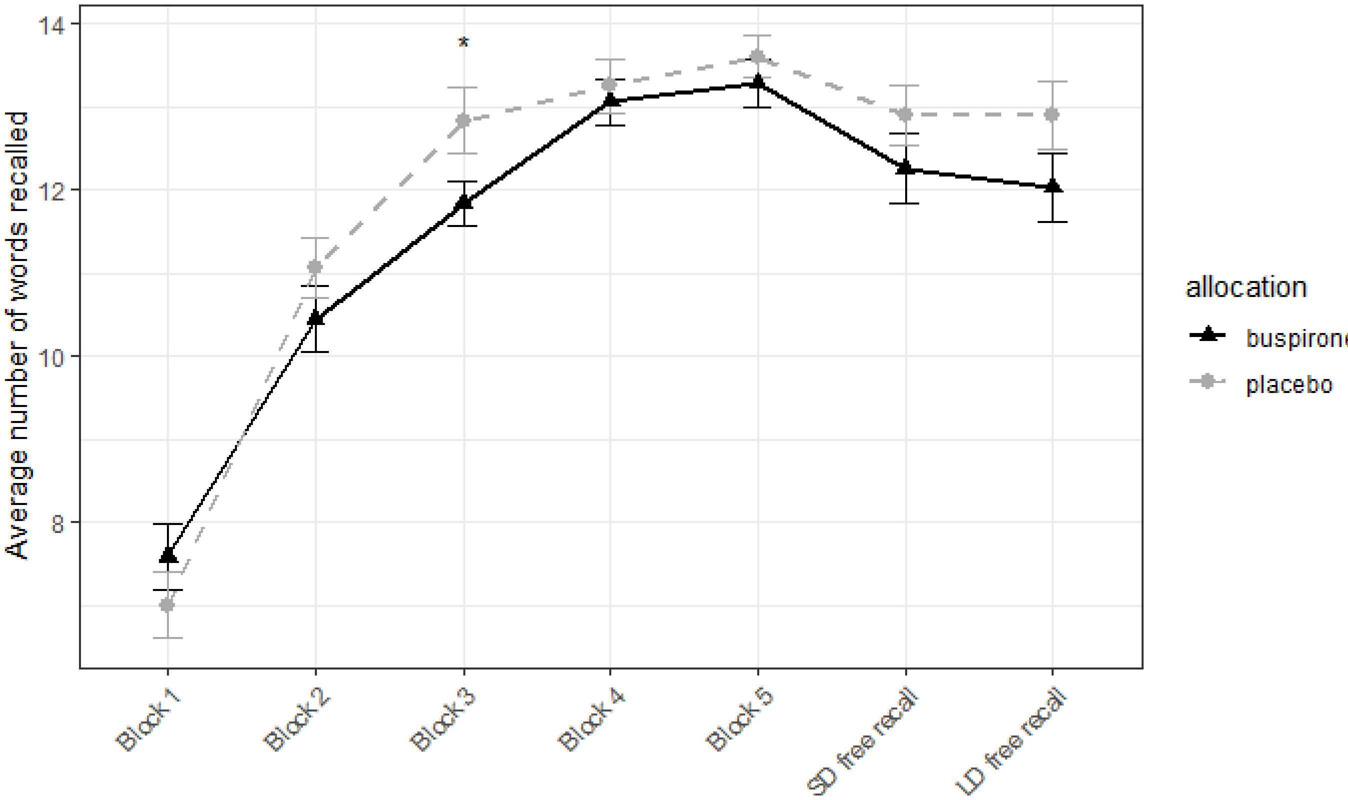


Figure 4. Performance on the AVLT. Mean number of words recalled by the placebo and buspirone groups immediately following presentation of the word list (five repetitions) and following a short delay (SD free recall) and long delay (LD free recall). Asterisks represent significant level of difference between groups *p<0.05. Error bars = +/- S.E.M

N back

There was no significant main effect of group allocation on overall accuracy [F(1,60) = 0.20, p = 0.66) or average reaction time [F(1,60) = 0.09, p = 0.77) (Fig. 5). Furthermore, there was no interaction between group allocation and condition for overall accuracy [F(3,180) = 0.20, p = 0.66) or average reaction time [F(3,180) = 0.57, p = 0.64).

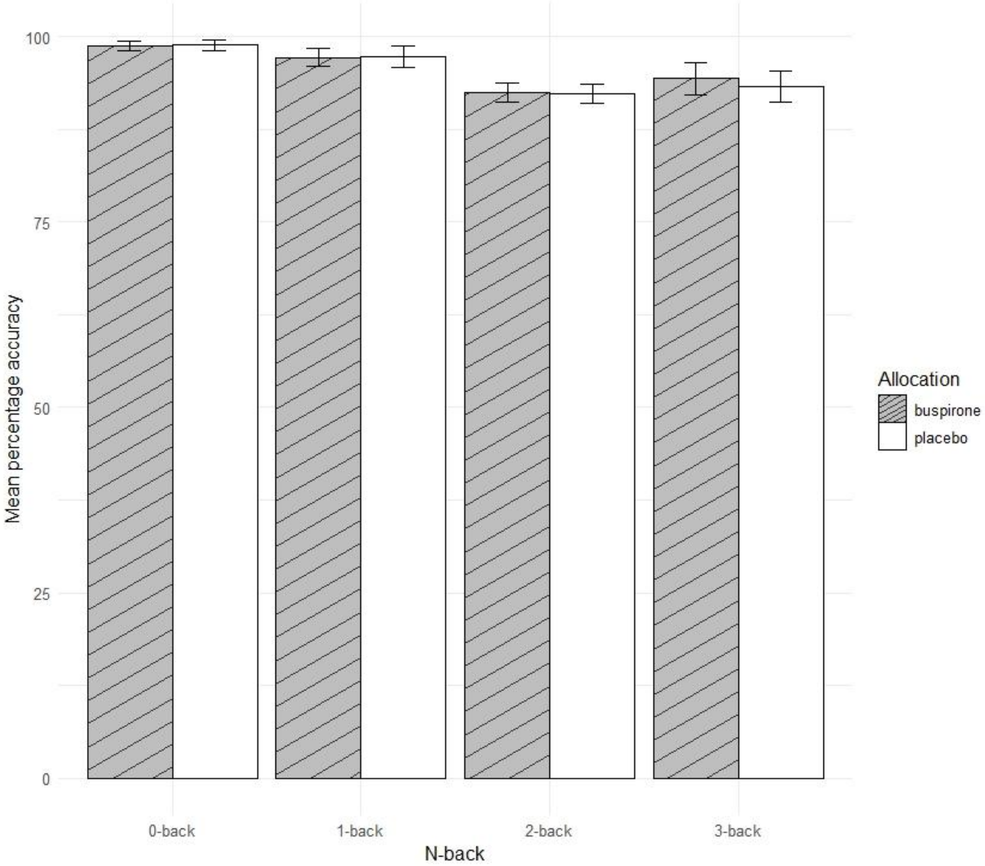


Figure 5. Mean percentage accuracy for each N-back category in the N-back task. Error bars = 95% C.I.

Sensitivity analysis: side effect as a covariate

The analyses were repeated with the inclusion of the composite side effect score (one-hour post drug administration) as a covariate in order to investigate whether any of the observed effects were driven by the different side effects experienced by the two groups. Despite a group difference in side effect scores, no statistically significant main effect of side effect was found in subsequent sensitivity analysis. To explore the effect further Pearson's correlation coefficients were calculated for outcome measures and the composite side effect score and mood score separately. A significant negative correlation between composite mood and side effect scores at 1-hour post intervention was observed (r = - 0.44, p = 0.0096) (Appendix: Chapter 2: table 3). A significant correlation between timepoint and a subjective energetic feeling was observed across all participants (r = - 0.21, p = 0.022) (Appendix: Chapter 2: table 4).

Facial Expression Recognition Test (FERT) – Accuracy

The significant group by emotion interaction on accuracy of facial expression recognition in the FERT remained significant when including side effect score as a covariate (F(6, 315.1) = 2.68, p = 0.015, η^2_p = 0.05). There was a positive correlation between accuracy for happy faces and the composite mood score, r(36) = 0.36, p = 0.026. No other correlations were significant.

Facial Expression Recognition Test (FERT) – Misclassifications

When including side effect score as a covariate the significant interactions remained between group and emotion for broad misclassifications [F(6, 367) = 1.60, p = 0.15, η^2_p = 0.03], between group and emotion of specific misclassification (F(41, 2337) = 1.45, p = 0.034, η^2_p = 0.025) and between group and valance of misclassification interaction [F(7, 455) = 2.83, p = 0.007, η^2_p = 0.04]. A positive correlation arose only for percentage of anger misclassifications and composite side effect score, r(55) = 0.27, p = 0.046. Examining specific misclassification, the number of anger to sad misclassifications negatively correlated with mood scores (r(53) = -0.33, p = 0.013) and positively correlated with side effect scores (r(57) = 0.30, p = 0.02). Misclassification of sad to anger also positively correlated with side effects core at time of testing (r(57) = 0.36, p = 0.0049). Misclassification of happy to fear positively correlated with mood scores as well (r(53) = -0.45, p < 0.001). No significant correlations arose between valence and composite scores.

AVLT

The significant interaction of group and block remained in the AVLT when side effect score was included as a covariate (F(6, 348) = 2.60, p = 0.018 η^2_p = 0.042). No significant correlation arose between number of words successfully recalled at each trial stage and the composite mood or side effect score.

Discussion

Summary of results

This chapter broadens the evidence for the effects of acute 5-HT_{1A} receptor agonism in cognition and emotional processing. Specifically, in a healthy volunteer sample, we found that buspirone reduced sensitivity to disgust emotions, increased sensitivity to sad emotions and increased misclassifications of negative emotions to other negative emotions. We found no effect of buspirone on working memory performance but a subtle impairment in verbal memory.

Emotional processing

Our findings demonstrate that acute buspirone administration increases negative to negative emotion misclassifications compared to placebo. This could indicate that buspirone induces a subtle negative bias in emotional processing perhaps through the reduction of serotonergic transmission that follows acute activation of 5-HT_{1A} autoreceptors. In support of this, it has been reported that a reduction in serotonergic transmission, through depletion of the 5-HT precursor tryptophan, induces a negative affective bias in healthy participants and remitted depressed patients (Hayward et al. 2005; van der Veen et al. 2007). Consistent with this acute tryptophan depletion (ATD) attenuated a positive bias in an affective word task (Roiser et al. 2008) and an increase in 5-HT transporter binding potential has been linked to a negative affective bias in healthy volunteers (Armand et al. 2022). Broadly it seems a reduction in serotonergic transmission leads to a negative affective bias behaviourally. However, it is noteworthy that several studies have observed no effect of ATD on performance, albeit with incidental, gender identification tasks rather than explicit emotional recognition tasks as used here (Daly et al. 2010; Fusar-Poli et al. 2007). A common finding of all studies though is that of neural responses to emotional stimuli with reduced serotonergic transmission. These observations have been with accompanying behavioural changes in emotional-valanced verbal stimuli (Roiser et al. 2008) and a facial emotion perception task (van der Veen et al. 2007) and without accompanying behavioural changes (Fusar-Poli et al. 2007; Daly et al. 2010). Studies have implicated similar regions in responses to emotionally-valanced tasks such as the cingulate cortex and insula (Roiser et al. 2008; Daly et al. 2010) as well as regions commonly associated with emotional processing such as the amygdala (van der Veen et al. 2007). Others identifying increased amygdala activity in emotionally valanced tasks under ATD conditions, also found a decrease in prefrontal-amygdala connectivity when viewing negative vs positive emotions, a so-called 'aversive amplification circuit' (Robinson et al. 2013). It is possible, therefore, that reduced serotonergic transmission from acute buspirone administration could lead to disinhibition of a prefrontal – amygdala aversive amplification circuit and increased sensitivity to negative emotions, as observed in results presented here.

However, ATD and buspirone are not equivalent in terms of pharmacological effects, which may mitigate the behavioural effects of a net reduction in serotonergic transmission brought about from buspirone-induced 5-HT$_{1A}$ autoreceptor activation. Namely buspirone is known to possess partial agonism activity at post-synaptic 5-HT$_{1A}$ heteroreceptors (Bantick et al. 2004), many of which are found in regions implicated in emotional processing (Puig and Gulledge 2011; Barnes and Sharp 1999). As such, the partial agonism of buspirone at 5-HT$_{1A}$ heteroreceptor may maintain 5-HT$_{1A}$-related inhibition of these regions, which would not be the case with the broad decrease in 5-HT neurotransmission produced by ATD. Speculatively acute buspirone may result in a partial disinhibition of the prefrontal-amygdala circuit, thereby producing a minor increase in sensitivity to negative emotions.

Our observation of a subtle negative bias following buspirone is in partial agreement with the only directly comparable study of 15 healthy volunteers (Bernasconi et al. 2015). This study found buspirone reduced sensitivity to fear when presented for 90msec only (Bernasconi et al. 2015). Here we find reduced sensitivity to another negative emotion, namely disgust, but also an increased sensitivity to sad emotions.

Several reasons could account for this discrepancy in results. Firstly the task used here presented emotional faces for 500msec, greater than 90msec presented in the task used by Bernasconi et al.; this would result in greater cognitive analysis of faces, employing cortical regions such as the prefrontal cortex (Wong et al. 2009). This study extends the work of Bernasconi et al. in indicating that the behavioural influence of buspirone, a 5-HT$_{1A}$ receptor agonist, can have mixed effects on differing emotions of the same valence, potentially through effects in cortical areas. The study presented in this chapter used a greater number of emotions and required specific labelling of emotions, as opposed to the emotion matching task of either fearful or happy faces which requires a binary response, used by Bernasconi et al.. As such, our design allowed effects of buspirone on other emotions e.g. disgust and sad, to be measured. However, the fact that no significance difference in accuracy for recognition of fear or sad faces was observed, could be due to the complex nature of the ETB emotion recognition task requiring greater cognitive resource in specific labelling of emotions. Finally, Bernasconi et al. recruited more males than females, compared to the participant group here which had a greater number of females. This may be important because females are thought to be more sensitive to negative emotions and emotion processing in general (Thompson and Voyer 2014), perhaps allowing more treatment effects to be detected in the present study.

Accuracy in emotional facial expression recognition has been shown to worsen in individuals diagnosed with depression (Surguladze et al. 2004). Hence changes in mood state induced by buspirone could alter accuracy of facial emotion recognition. Whilst buspirone elicited a decrease in subjective mood, this did not correlate with significant differences in accuracy emotion recognition or misclassification. It is also worth noting that with the ETB, depressed patients have shown an improvement in recognition of positive emotions, after a single dose of reboxetine, which had no impact on mood ratings (Harmer et al. 2009) i.e. performance on emotional tasks can be improved by pharmacological interventions prior to clinical enhancement in mood, which typically takes days or weeks to develop with conventional agents (Harmer et al. 2003).

Cognition

The results presented here indicate acute buspirone produced no significant effects on working memory but did elicit a small, deleterious effect on short term, verbal memory. This observation is consistent with some but not all previous buspirone studies. For example, a small, healthy male volunteer study observed that acute administration of buspirone 30mg negatively affected performance on a verbal memory task (Grasby et al. 1992). This study also reported a reduction in activity following buspirone administration in the retro-splenial cortex (a region linked to the hippocampus), the right prefrontal cortex and the parahippocampal gyrus during performance of the task (Grasby et al. 1992). Our results are also supported by behavioural studies which report a worsening of memory performance, following administration of other 5-HT$_{1A}$ receptor probes such as tandospirone (Yasuno et al. 2003) and ipsapirone (Riedel et al. 2002).

A potential pharmacological mechanism for our results could be found from animal models of cognition, which support the concept of differential effects of 5-HT$_{1A}$ agonism depending on receptor location. Specifically post-synaptic 5-HT$_{1A}$ agonism could impair cognition in humans (King, Marsden, and Fone 2008). Therefore, a probe such as buspirone with modest post-synaptic action, could impair cognition via its downstream inhibitory effect on GABA interneurons and glutamatergic pyramidal cells. This would occur in regions relevant to cognition, such as the hippocampus, where a reduction in glutamatergic transmission could lead to an overall impairment in cognitive function.

However, similar to results presented here, several studies have observed buspirone to have no deleterious effect on episodic memory (Barbee et al. 1991; Unrug-Neervoort, Van Luijtelaar, and Coenen 1992; Chamberlain et al. 2007). One commonality for all studies is that an aggregate of free recall was used as an outcome measure. Similarly, our analysis using an aggregate score found no difference. However, when separating by trial block (a comparison not performed in many of the aforementioned studies), a difference between groups emerged, indicating an impairment in immediate recall but at an earlier timepoint than is often tested.

Cognition may change prior to subjective mood, as rated through questionnaire scores, for several reasons. Firstly, the measurement may contribute to this observation. Namely the sensitivity of a percentage accuracy from behavioural cognitive testing may have less ‘noise’ compared to the analogue rating scale for mood, which would have higher within subject variability and relies on a single measurement. Secondly the nature of the construct differs, especially in terms of neural circuits. Cognition engages the prefrontal-hippocampal circuits that are immediately influenced by serotonergic transmission (Robbins and Arnsten 2009), however limbic-midline cortical system (e.g. amygdala, pregenual/ventromedial PFC) for emotional processing in general is slightly slower, involving integrating emotional information over a longer time course (Harmer, Goodwin, and Cowen 2009). Supported in this view is that the early ‘affective bias’ changes observed behaviourally by others often precedes a subjective feeling change (Harmer, Hill, et al. 2003; Harmer et al. 2009).

Limitations

Limitations of this work include the use of buspirone as a probe of 5-HT$_{1A}$ agonism, in that it has differing efficacy at pre-and post-synaptic 5-HT$_{1A}$ receptors. Namely, acute buspirone would be expected to reduce 5-HT release in the terminal fields of serotonergic neurones originating from the raphe nucleus, leading to a broad decrease in activation of 5-HT receptors in post-synaptic sites. However the partial agonism of post-synaptic 5-HT$_{1A}$ receptors could maintain the inhibitory influence on brain regions relevant to memory and emotion such as the hippocampus and enterohinal cortex as well as the insula, anterior cingulate, frontal cortex (Ito, Halldin, and Farde 1999).

The activity of buspirone's major metabolite l-(2-Pyrimidinyl)-pipcrazine (1-PP) also complicates interpretation. For example, 1-PP has been shown *in vivo* to inhibit somatodendritic and terminal α_2-adrenergic autoreceptors in the rat brain, leading to a disinhibition of noradrenergic system (Blier et al. 1991). Intriguingly a previous healthy volunteer study using a single dose of the noradrenaline-reuptake inhibitor, reboxetine, had no effect on AVLT but increased positive bias to positive emotional faces, results which are not replicated here (Harmer, Hill, et al. 2003). One potential explanation for this discrepancy is the noradrenergic actions of 1-PP after a single dose of buspirone are insufficient to induce a net positive shift in emotional processing.

Furthermore buspirone possess affinity for D_2 and D_3 receptors, albeit to a lesser degree than 5-HT_{1A} receptors (Loane and Politis 2012) and with a binding affinity at the dose used in the current study, that is unlikely to yield significant behavioural effects alone (Le Foll et al. 2016). However, this multimodal pharmacology of buspirone could contribute synergistically to widespread effects on cognition and emotional processing, making it challenging to attribute any effects (or lack thereof) to 5-HT_{1A} receptor agonism alone. This question could be addressed by more specific probes, such as the biased 5-HT_{1A} agonist NLX-101 (post-synaptic preference) or NLX-112 (pre-synaptic preference) (Newman-Tancredi et al. 2022; Smith et al. 2023).

As expected, buspirone produced characteristic side effects such as light-headedness and nausea. These did influence the significance of a few results, for instance for the FERT, accuracy and specific misclassifications. However, the majority of significant results within these analyses remained e.g. accuracy for disgust and misclassification of disgust to sad. This indicates side effect burden does not generally account for the results presented here.

This study's use of cortisol measurements provides a surrogate marker of 5-HT_{1A} post-synaptic target engagement. However, a hypothermic response was not observed; this is surprising as buspirone is known to possess significant presynaptic 5-HT_{1A} receptor activity. This could be due to temperature measurement issues e.g. inconsistency in measurement, thermometer imprecision. In future, alternative means of measurement could be used, such as tympanic thermometers. Alternatively, it could be a feature of the buspirone dose used, as administration of a higher dose buspirone (30mg) does produce a hypothermic response (Cowen, Anderson, and Grahame-Smith 1990; Young et al. 1993), as does acute challenge with other 5-HT$_{1A}$ receptor agonists such as tandospirone and gepirone (Yasuno et al. 2003; Anderson, Cowen, and Grahame-Smith 1990). A PET-fMRI study with a 5-HT$_{1A}$ agonist, could provide more direct evidence of target engagement.

Another drawback is the number and breadth of cognitive tests used (N-back and AVLT). The number of tests used was balanced against participant fatigue, although it is acknowledged this restriction in number of cognitive tests used limits the cognitive domains explored. In future, additional cognitive domains could be assessed such as executive function (e.g. Wisconsin card sorting task) or processing speed (e.g. Digit Symbol Substitution Test), using widely available tasks.

The use of healthy volunteers may limit the sensitivity of the measures to detect a drug effect, because task performance is already high in this group. As such, examination of clinical samples, such as those diagnosed with major depressive disorder, may give richer insights into the effects of 5-HT$_{1A}$ manipulation on cognition and emotional processing. Furthermore, the effects of buspirone may be different in groups in which there are pre-existing alterations in the monoaminergic system, such as those with mood disorder.

A final limitation would be the use of between subject comparison which, although improving generalizability of results and using relatively greater numbers than comparable studies it may be insufficient to detect subtle, individual-level effect that could manifest with a within-subject design (Bernasconi et al. 2015).

Chapter 3: The behavioural effects of acute buspirone on reward processing

Introduction

As outlined in chapter 1, 5-HT$_{1A}$ receptors are implicated in the pathophysiology of depression through several lines of evidence, including post-mortem, pharmacological and neuroimaging studies (Drevets et al. 1999; Savitz, Lucki, and Drevets 2009). The 5-HT$_{1A}$ receptor is widespread throughout the brain (Ito, Halldin, and Farde 1999) and a reduction in binding in the raphe and cortical regions has been observed in depression (Drevets et al. 2007). Furthermore, in a healthy volunteer PET study there was a positive correlation between 5-HT$_{1A}$ binding in the hippocampus and reward sensitivity in a probabilistic decision-making task (Faulkner et al. 2014). Additionally, 5-HT$_{1A}$ binding in the raphe nucleus has been shown to positively correlate with orbitofrontal cortex activation when viewing rewarding images (Hahn et al. 2009).

It is important to clarify the effects of 5-HT$_{1A}$ receptors on reward processes because manipulation of this receptor may be implicated in pharmacotherapy of depression, including anhedonia. Evidence for this includes 5-HT$_{1A}$ auto-receptor antagonism expediting the response to SSRIs (Portella et al. 2011), and 5-HT$_{1A}$ receptor agonism being involved the clinical effects of the multimodal antidepressant vortioxetine (D'Agostino, English, and Rey 2015), a medication that has shown efficacy for anhedonia (McIntyre, Loft, and Christensen 2021). There is increasing consensus that 5-HT$_{1A}$ receptors have a role to play in antidepressant treatments (Blier and Ward 2003; Celada, Bortolozzi, and Artigas 2013).

Converging evidence indicates that 5-HT$_{1A}$ receptor agonism improves reward behaviours (Hayes and Greenshaw 2011; Kinney, Griffith, and Hudzik 1998). Furthermore 5-HT$_{1A}$ receptors appear to play a role in the anti-depressant effect of the rapidly acting antidepressant ketamine in the forced swim test model of depression (Fukumoto et al. 2018). Specifically, blocking the 5-HT$_{1A}$ receptor attenuates the antidepressant effect of ketamine while cortical injection of a 5-HT$_{1A}$ agonist mimics the action of ketamine in this depression model (Fukumoto et al. 2018).

The aforementioned studies examining the role of 5-HT_{1A} receptors in reward are animal experimental studies. However, currently there are no reported human studies directly probing the 5-HT_{1A} receptor in reward processing or anhedonia.

In this chapter I explored the role of 5-HT_{1A} receptor agonism in reward processing in humans by administering a single dose buspirone 20mg to healthy volunteers in a between subject, placebo-controlled study and performed tasks to measure differing stages of reward processing. These included as subjective rating of the pleasurableness of four different tastes, an effort-discounting decision-making task and a probabilistic instrumental learning task.

At this juncture it is worthwhile considering the effects of buspirone on serotonergic transmission. 5-HT_{1A} receptors are inhibitory, therefore the pre-synaptic 5-HT_{1A} auto-receptors that reside at pre-synaptic somato-dendritic sites on serotonin neurons, originating from the raphe nucleus, would result in a reduction in 5-HT release from at terminal sites. However, the effect of 5-HT_{1A} post-synaptic receptors depend on the neuron, for example if inhibiting the inhibitory GABA interneuron may lead to disinhibition of downstream pyramidal neurons (Becker et al. 2016).

Buspirone has a high affinity for pre-synaptic 5-HT_{1A} and high intrinsic activity (i.e. not only binds readily to presynaptic sites, once it does it has an inhibitory effect similar to endogenous 5-HT). As this happens via $G_{i/o}$ *protein-coupled inwardly rectifying K^+ channels* (GIRK), this hyperpolarising effect is rapid, leading to a rapid reduction of up 50% of 5HT at downstream sites (Gobert et al. 1999). Acting on However, buspirone has lower affinity for post-synaptic sites, supported by PET occupancy data (Rabiner et al. 2000; Passchier et al. 2001). Additionally, at post synaptic 5-HT_{1A} sites buspirone has an intrinsic activity of < 40% of endogenous serotonin (Yocca 1990), acting as a partial agonist, giving contextual effects dependent on surrounding 5-HT levels (e.g. at hypo-serotonergic states it acts as a functional agonist, in baseline or hyper-serotonergic states it acts as a functional antagonist). Speculatively, one could hypothesize acute buspirone would induce a hypo-serotonergic state, and where there is a large, cortical 5-HT_{1A} receptor reserve buspirone may induce a subtle functional disinhibition, such as the hippocampus, ventromedial PFC and anterior cingulate (Savli et al. 2012; Beliveau et al. 2020).

This chapter aimed to examine whether direct 5-HT_{1A} receptor agonism influenced consummation of primary, taste stimuli; motivation through willingness to exert effort for reward and finally reward and aversive learning, all key stages in reward processing (Husain 2018; Berridge, Robinson, and Aldridge 2009).

I hypothesized that acute 5-HT_{1A} receptor agonism would improve subjective pleasurableness of rewarding tastes, increase willingness to exert effort for reward and increase reward learning. Secondly, I predicted that minimal significant effects would be observed for aversive stimuli e.g. bitter tastes, increased effort or loss outcomes.

Methods

Recruitment & study design

Recruitment and study design were described in detail in chapter 2. To recap, 63 healthy volunteers were randomized to a single oral dose of either placebo or buspirone 20mg. Temperature and salivary cortisol measurements were taken at baseline and every 30 minutes following capsule administration. The effort expenditure task was performed at +60 mins, the taste task at +90 mins and probabilistic instrumental learning task (PILT) at +150 mins.

Reward tasks

Taste task

The taste task involved subjects receiving four separate tastes (sweet, sour, salty and bitter) administered separately on paper strips. Strips were impregnated with the taste chemical at concentrations deemed suprathreshold and comparable to those found in foods to ensure the taste could be identified.

The tastes were as follows:

Taste	Substance
sour	Citric acid
salty	Sodium chloride
sweet	Sucrose
bitter	Quinine

To control for individual differences in terms of liking of disliking individual tastes subjects were asked to rate the degree to which they would like a taste before tasting it (termed ‘anticipation’). This was performed on a Visual Analogue Scale (0 – 100; very unpleasant to very pleasant).

Subjects were told what taste they were about to receive. The paper strip was removed with tweezers from the storage container, given to the subject to place in the middle of their tongue, with their mouth closed, for 10-20 seconds. Subjects then disposed of the paper strip and rated the intensity of the taste to ensure it was detected (termed ‘intensity’) and then how pleasurable they found the taste (termed ‘pleasure’). Again, this was performed on a Visual Analogue Scale (0 – 100; representing barely detectable – most intense imaginable for ‘intensity’ and very unpleasant to very pleasant for ‘pleasure’).

After rating the intensity and pleasure of the taste subjects were invited to sip water to clear their palette before receiving the next taste and repeating the above procedure (i.e. rating anticipation then intensity and pleasure).

The decision to inform participants prior to taste administration was due to the concern ratings of pleasure and intensity could be confounded by novelty and the sensitivity to taste intensity. By removing this novelty, it was believed the pleasure and intensity rating would be more accurate. However, it is acknowledged this could also be a limitation as it introduces a bias (e.g. ‘I usually like sweet things so I will rate sweet higher’), however this potential limitation is mitigated by the pre-taste expected pleasure rating recorded. The decision to administer sweet, sour & salt was arbitrary (although always administered in the same order), however manufacturer advice was to administer bitter quinine last as this would influence subject taste sensitivity for the remaining three tastes.

Effort expenditure task

The effort expenditure task is an effort discounting task originally described by Bonelle et al (Bonnelle et al. 2015) and here used as per Attaallah et al. (Attaallah 2022).

Stimulus presentation was programmed in MATLAB (The MathWorks, Inc., USA). Force was recorded using a BioPac M46 and SS25LB hand dynamometer (BIOPAC Systems, Inc., USA).

At the beginning of the task, participants were presented with a force bar, with a yellow horizontal threshold mark near the top, in the centre or the screen. Participants were instructed to squeeze the dynamometer as hard as they could, with their dominant hand, in order the reach the yellow threshold mark. This was repeated a further two times. To encourage maximal effort exertion and unbeknownst to the participant, the task was programmed to never reach the yellow threshold regardless of the force exerted. The maximum force exerted over the three trials was deemed as the participant's maximum voluntary contraction (MVC) and subsequent trials were calibrated to this individual MVC accordingly.

Participants then underwent familiarisation trials with each of the five effort levels (graded as a percentage of their unique MVC: 16%, 32%, 48%, 64% & 80%). Participants undertook two familiarisation trials for each effort level before progressing to the decision phase of the task.

In the decision phase of the task, participants were presented with an offer comprising of a hypothetical number of apples (1, 4, 7, 10 & 13 – five levels of reward) in exchange for physical effort (one of five levels of effort previously familiarised) demonstrated as an effort bar on the screen. Participants could either accept or decline the offer by selecting Yes or No presented either side of the screen (achieved by pressing left or right arrows on a keyboard) (Fig. 1). Randomly Yes and No changed sides throughout the task.

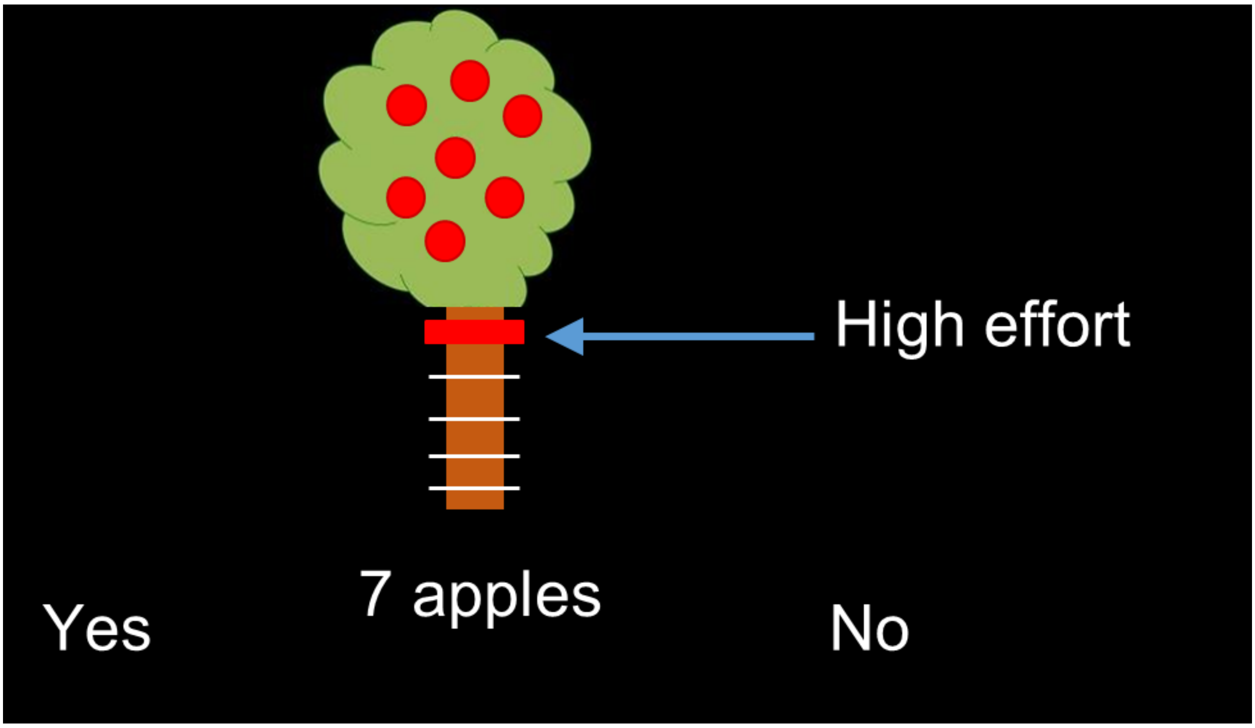


Figure 1: Example offer presented to participants in the decision phase of the effort expenditure task. The quantity of apples to be awarded for the offer are shown visually on the cartoon tree and in writing below. The effort required for the offer is represented by marking on the ‘stump’ of the cartoon tree (e.g. effort level 5 here). Decision to accept is presented as Yes or No option on the left and right of the screen (for subsequent trials this could switch randomly to alternative sides to mitigate a left/right bias).

Five levels of reward combing with five levels of effort resulted in 25 unique offers, which were presented in a pseudo random order. This was repeated a further four times giving 125 trials in total (5 trials of each 25 unique offers).

In the work phase of the task participants were informed that 10 trials from the previous 125 trials would be chosen at random for them to attempt (i.e. exert physical effort sufficient to ‘win’ the apples in the offer) (Fig. 2). Unbeknownst to the participant these were fixed for the following combinations of effort x stake: 1x1, 1x2, 1x3, 1x4, 1x5, 2x3, 2x4, 3x3, 4x2 (twice), 5x4. These were presented as accepted or declined irrespective of the previous responses in the decision phase of the task.

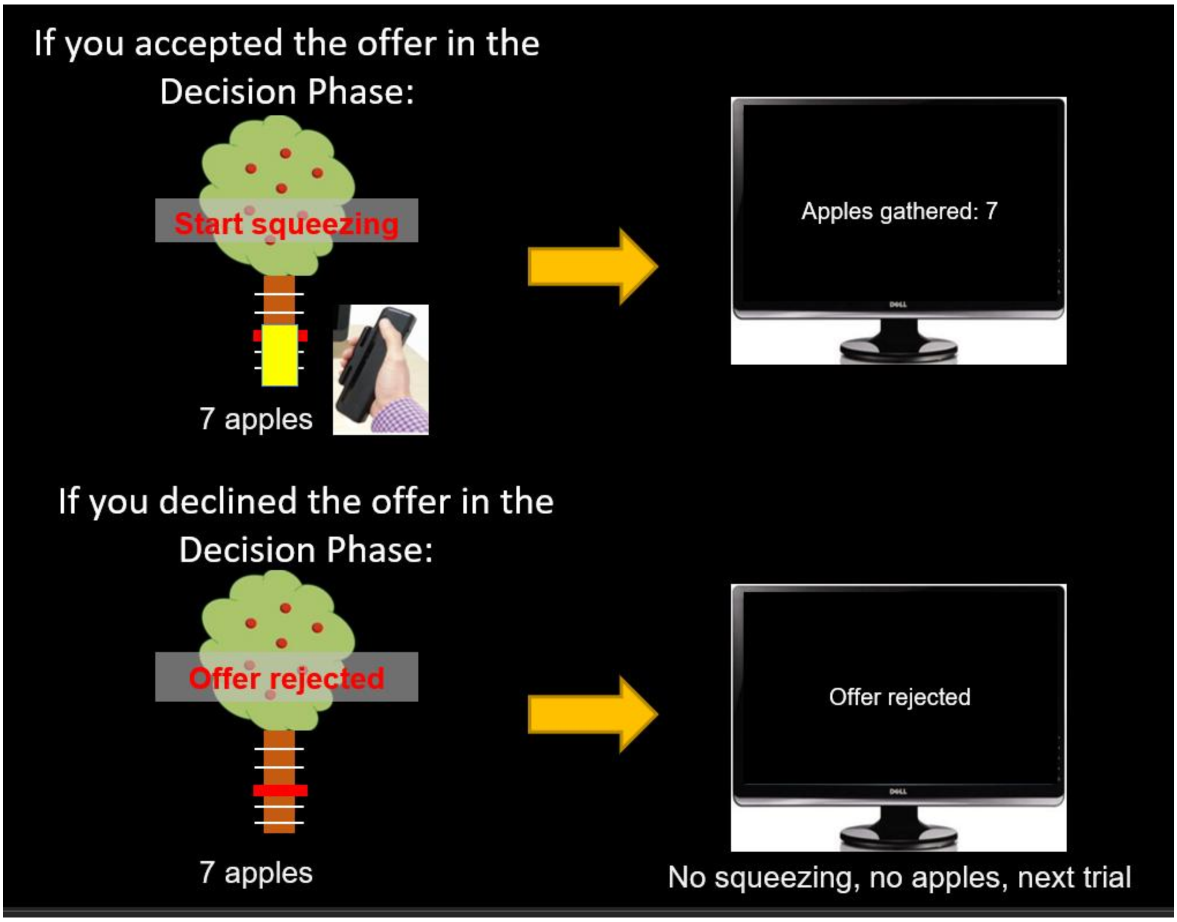


Figure 2: Example offer presented to participants in the work phase of the effort expenditure task. The quantity of apples to be awarded for the offer are shown visually on the cartoon tree and in writing below. The effort required for the offer is represented by marking on the 'stump' of the cartoon tree (e.g. effort level 5 here). If the task had randomly decided to make the offer the [participant was required to squeeze the dynamometer and then, if exerting sufficient grip strength to reach the target, was informed they had won the reward offer. If the offer was randomly decided to not to be offer not effort was required and the subject moved to the next trial offer.

Probabilistic Instrumental Learning Task (PILT)

The probabilistic instrumental learning task is a two-arm bandit task, originally described by Pessiglione et al. (Pessiglione et al. 2006), of interleaved win and loss trials. In win trials a pair of symbols are presented with implicit reciprocal probabilities (70% and 30%) of either winning 20p or winning nothing. In win trials the optimal choice is correctly selecting symbol which has a probability of winning 70% of the time. Similarly, in loss trials a pair of symbols are presented with implicit reciprocal probabilities (70% and 30%) of either losing 20p or losing nothing. In loss trials the optimal choice is correctly selecting symbol which has a probability of losing 30% of the time (Fig. 3). Participants had to learn to select the optimal symbol in each pair/trial.

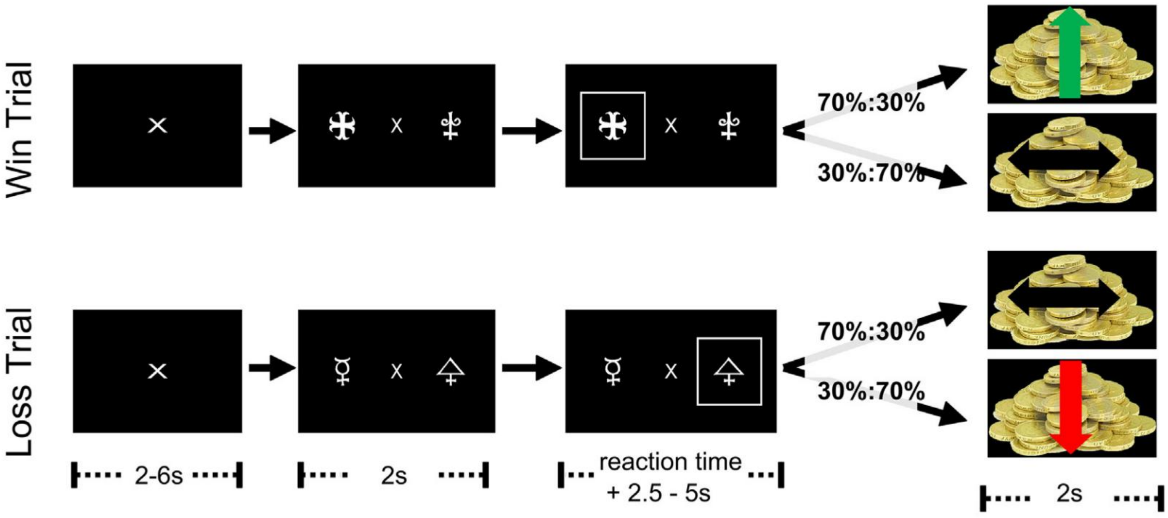


Figure 3: Probabilistic instrumental learning task. For each trial participants were presented with one of two a pair of symbols (2 seconds). Implicitly one pair was win (outcomes of either win or no change) and the other loss (outcomes of either loss or no change). Within each pair, one symbol was associated with the outcome 70% of the time (e.g. in win trials this was win) and 30% the remaining time. The other symbol in the pair was associated with reciprocal probabilities. Participants then made their selection by pressing a left or right arrow on a keyboard to select the symbol (2.5 – 5 seconds). They then received the outcome of their selection (2 seconds). Image taken from Halahakoon D.C. et al., Biological Psychiatry, 2024; 95:286–296.

Statistical analysis

Data cleaning

Taste task

Where participants could not register a taste (intensity = 0), this was removed from the specific taste dataset e.g. a participant could not taste a bitter taste, their data would be removed from bitter tastes analyses only but remain for salt, sweet & sour taste analyses). This occurred for two participants for pre-intervention intensity of bitter tastes and one participant for post-intervention bitter taste. All three participants were allocated to the buspirone group. To account for the potential effect of nausea as a side effect on pleasurableness, a rating of nausea was collected. An error in data collection resulted in mood and side effect scores not being collected for two participants (two female participants in buspirone group). The entire data from these two participants were removed when side effects and nausea were included in analysis.

Effort expenditure task

Several participants accepted all offers or had very quick reaction times across all trials, interpreted as not engaging with the task and were therefore removed from the dataset prior to unblinding. These included one female (placebo) and three males (one placebo, two buspirone). Similar to previous studies using this task (Saleh et al. 2021) at the trial level, trials with reaction time <0.4sec were deemed accidental and for each participant, trials with reaction times above three standard deviations above the participants mean reaction time were removed. This equated to 2.4% of all trials.

PILT

Trials which were particularly fast (< 0.2 sec) were removed on the presumption of the participant not engaging in the task for that trial. Twelve trials were removed owing to fast response times, across seven participants (2 subjects in buspirone & 5 subjects in placebo group), representing 0.1% of all trials. The range for proportion of optimal choices made across trials was 52.8% - 96.7%, indicating all participants performed better than chance. No participant's data was entirely removed from analysis.

Reward task analysis

Taste task

To investigate the effect of acute buspirone on the pleasurableness of each taste, linear regression models were created including pre-intervention pleasurableness, post-intervention intensity, post-intervention anticipation, post intervention nausea and allocation as predictor variables. Models tested include a mixture of interactions and no interaction between allocation and post-intervention measurements (e.g. anticipation & intensity) as well as inclusion or exclusion of post-intervention nausea. This was to account for allocation having an effect of post-intervention pleasurableness through these mechanism (e.g. buspirone led to more intense/sensitivity to taste, lowered the anticipation of sweet taste pleasurableness, nausea influencing pleasurableness of taste).

A preference for the simplest models that adequately explained each dataset was taken (to avoid 'over-fitting'), unless there was a significant improvement in fit of more complicated models (e.g. improved goodness of fit with higher R^2, lower RSE & lower AIC/BIC) with AIC/BIC measures given priority when deciding model selection for each unique taste. Models selected for each taste are stated below (with details of model fit in Appendix: Chapter 3, Table 1).

Bitter & Salt models

$$\begin{aligned}
\text{post-intervention pleasurableness} \sim\ & \beta_0 + \\
& \beta_1(\text{pre-intervention pleasurableness}_i) + \\
& \beta_2(\text{post-intervention intensity}) + \\
& \beta_3(\text{allocation}) + \\
& \beta_4(\text{post-intervention anticipation})
\end{aligned}$$

Sweet & Sour models

$$\begin{aligned}
\text{post-intervention pleasurableness}_{ij} \sim\ & \beta_0 + \\
& \beta_1(\text{pre-intervention pleasurableness}_{ij}) + \\
& \beta_2(\text{post-intervention intensity}) + \\
& \beta_3(\text{allocation}) + \\
& \beta_4(\text{post-intervention anticipation}) + \\
& \beta_5(\text{post-intervention nausea}) + \\
& \beta_6(\text{post-intervention intensity x allocation}) + \\
& \beta_7(\text{post-intervention anticipation x allocation}) + \\
& \beta_8(\text{post-intervention intensity x post-intervention} \\
& \qquad \text{anticipation}) + \\
& \beta_9(\text{post-intervention intensity x post-intervention} \\
& \qquad \text{anticipation x allocation})
\end{aligned}$$

Effort expenditure task

To compare overall offer acceptance, the total number and proportion of offers accepted was calculated as an outcome variable, in total and across each level of reward, effort and reward x effort level. An unpaired t-test was performed to compare overall mean proportion of offers accepted. The proportion of offers accepted within each effort level or reward levels was calculated for each participant and separate mixed effects linear regression were performed (i.e. one for effort, one for reward and one for effort & reward). An example of the regression model, specifically for effort, if provided below:

$$\begin{aligned}\text{Proportion of offers accepted)) } = \quad & \beta_0 + \\ & \beta_1(\text{effort}_{ij}) + \\ & \beta_2(\text{allocation}_{ij}) + \\ & \beta_3(\text{effort}_{ij} \times \text{allocation}_{ij}) + \\ & u_{0i}\end{aligned}$$

To explore the relationship between raw offer acceptance between groups at each unique effort x reward combination (e.g. a binary outcome of accept or decline), a logistic regression was also performed for the whole dataset with fixed effects of reward, effort and allocation group and reward and effort as within subject random factors as per below:

$$\begin{aligned}\text{logit}(P(\text{Accept}_{ij})) = \quad & \beta_0 + \\ & \beta_1(\text{reward}_{ij}) + \\ & \beta_2(\text{effort}_{ij}) + \\ & \beta_3(\text{allocation}_{ij}) + \\ & \beta_4(\text{reward}_{ij} \times \text{effort}_{ij}) + \\ & \beta_5(\text{reward}_{ij} \times \text{allocation}_{ij}) + \\ & \beta_6(\text{effort}_{ij} \times \text{allocation}_{ij}) +\end{aligned}$$

$$\beta_7(\text{reward}_{ij}\times\text{effort}_{ij}\times\text{allocation}_{ij}) +$$

$$u_{0i} +$$

$$u_{1i}(\text{reward}_{ij}\times\text{effort}_{ij}),$$

1. $\text{logit}(P(\text{Accept}_{ij}))$ is the log-odds of the probability that participant $_i$ in trial $_j$ responded "Yes".
2. β_0 is the fixed intercept.
3. β_1 to β_7 are the fixed effect coefficients.
4. u_{0i} is the random intercept for participant $_i$
5. u_{1i} is the random slope for the interaction between reward_{ij} and effort_{ij} for participant $_i$.
6. $_{ij}$ denotes the indices for individual participants $_i$ and trials $_j$

To visualise the differences in acceptance of an offer, using the full mixed effects logistic regression (which included random effects of subject, in order to account for individual differences), log odds of accepting an offer were calculated at the trial level for each participant. The mean average of log odds was calculated for each unique effort x reward combination for each allocation group.

To calculate individual probabilities of accepting a particular offer at the individual trial level for each participant, the mixed effects logistic regression was used to calculate the log odds of accepting an offer for each trial and then transformed to a probability of accepting an offer using the following formula:

$$P(\text{accept}) = 1 / (1 + e^{(-\text{log odds})})$$

To examine the effect of allocation on log-transformed reaction times, the mean and standard error of log-reaction times were calculated and a repeated measures ANOVA was performed with allocation, reward level and effort level as factors.

Maximum and mean (with standard error of mean) grip strength was also calculated from the final 10 trials within the task that requested effort and analysed using a repeated measures ANOVA test.

PILT

Each subject underwent an equivalent of three runs of the 30 trials of the win & loss condition. An average optimal choice for each trial for each participant was calculated (taking a value between 0/3 to 3/3). This was used to calculate a mean average for each group at each trial. Data was separated into win and loss conditions, then mean optimal choice in each trial (1-30) was compared between groups using a two-way ANOVA test with allocation, trial number and their interaction as fixed effects:

$$\begin{aligned}\text{Mean Optimal Choice}_{ij} \sim \quad & \beta_0 + \\ & \beta_1(\text{Allocation}_{ij}) + \\ & \beta_2(\text{Trial Number}_{ij}) + \\ & \beta_3(\text{Allocation}_{ij} \times \text{Trial Number}_{ij}) + \\ & \varepsilon_{ij}\end{aligned}$$

β_0 = intercept;
$\beta_{1\text{-}3}$ = main & interaction effects;
ε_{ij} = residual error

To examine an effect after learning, trials 11-30 (where optimal choice reaches asymptote) in each condition were also analysed separately as per above.

Results

Taste task

Pleasurableness

A linear regression revealed no significant main or interacting effect of allocation on post-intervention pleasurableness of sweet, sour or salt tastes ($p > 0.1$).

A main effect of post-intervention anticipation was found for pleasurableness of sour tastes ($\beta = 1.07$, $p = 0.032$), indicating the greater the anticipation of the sour taste being pleasant the more pleasurable it was found to be.

In analysis of the post-intervention pleasurableness of bitter tastes a main effect of allocation (β = 7.56, p = 0.039) was observed, indicating that the buspirone group found bitter tastes more aversive than the placebo group. Furthermore, small main effects of intensity (β = -0.21, p = 0.015) and anticipation (β = 0.40, p = 0.001) on post-intervention pleasurableness of bitter tastes were observed. These results indicate the more intense bitter was experienced the less pleasurable it was found to be, however the greater the anticipation of the bitter taste being pleasant the more pleasurable it was found to be.

Anticipation & Intensity

No significant main effect or interacting effect of allocation was found for post-intervention intensity or anticipation of any taste.

Nausea

A main effect of post-intervention nausea was found for sweet tastes (β = -0.52, p = 0.045), interpreted as the more nausea experienced the less pleasurable a sweet taste was experienced.

Effort expenditure task

Overall comparison of offer acceptance

No significant difference between groups in overall acceptance rate was identified [t(47) = -0.83, p = 0.41; buspirone = 0.62, placebo = 0.66].

Comparison at level of effort or reward

The separate mixed effects linear regression analyses demonstrated a significant effect of effort level (β = -8.8, p < 0.005) and reward level (β = 19.8, p < 0.005). The main effect of allocation, or two-way interaction between allocation and effort level or reward level, did not reach significance (p > 0.1). The mean of the proportion of offers accepted for each group was then calculated at each level of either effort or reward and can be seen in (Figure 1 & 2).

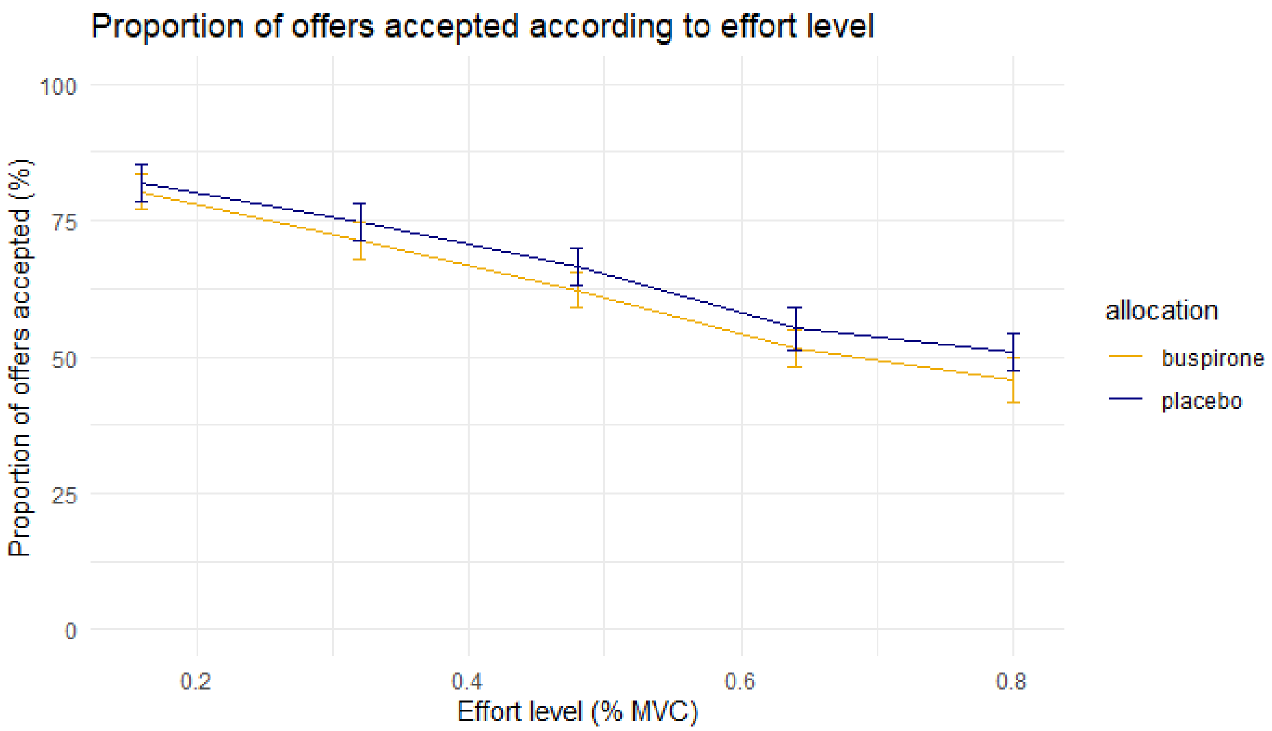

Proportion of offers accepted according to effort level

Figure 1: Mean proportion of offers accepted at each effort level between groups.
Error bars = +/- 1 sem


Proportion of offers accepted according to stake level

Figure 2: Mean proportion of offers accepted at each reward level between groups.
Error bars = +/- 1 sem

Comparison at level of effort and reward

The interaction effect of effort and reward was explored by dividing each offer into its unique reward x effort combination (25 levels in total derived from five levels of reward and five levels of effort). Mixed effects linear regression of the proportion of offers accepted within each group at each unique offer combination indicated no significant main or interaction effect of allocation ($p > 0.1$). However, upon data visualisation a pattern emerges, whereby at lower reward levels, the increasing effort reduces the proportion of offers accepted in both groups (Figure 3). This trend reduces however at the highest reward level, where most offers are accepted regardless of effort. Furthermore, at lower reward levels the proportion of offers accepted is lower in the buspirone group compared to the placebo group in the majority of offer combinations.

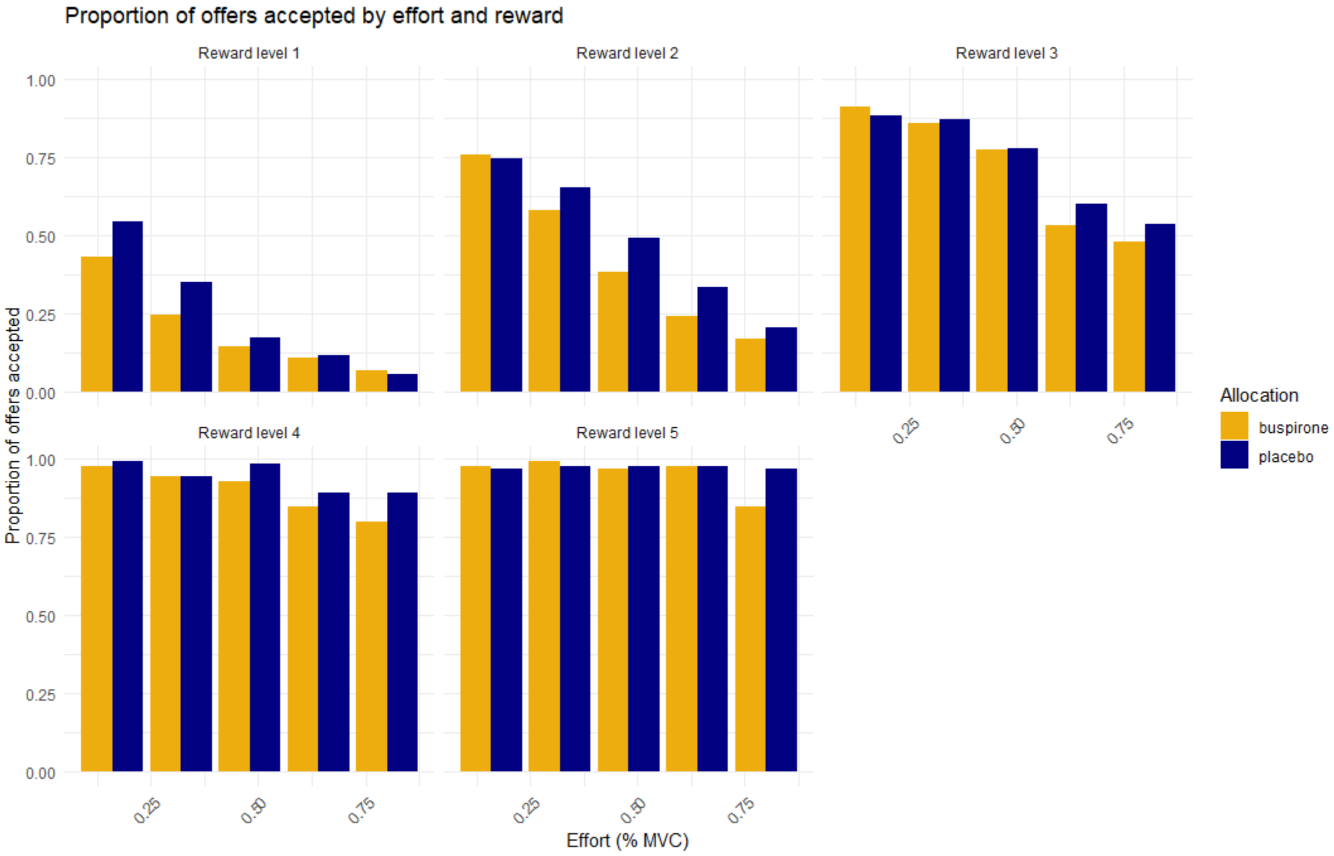


Figure 3: Proportion of offers accepted at each unique effort*reward level combination between groups.
Proportion derived from total offers accepted within group / total offers made within group, for each unique effort*reward level combination

The mixed-effects logistic regression indicated significant effects were observed for reward level ($\beta_1 = 3.11$, $p < 0.005$), effort level ($\beta_2 = -1.21$, $p < 0.005$) and a non-significant two-way interaction between effort and reward levels ($\beta_4 = 0.35$, $p = 0.062$). The main effect of allocation was not significant ($\beta_3 = 0.26$, $p = 0.62$), however a significant three-way interaction between reward level, effort level, and allocation was observed ($\beta_7 = 0.59$, $p = 0.021$).

To visualise the significant three-way interaction between reward level x effort level x allocation, the probability of accepting each unique effort x reward offer for each allocation group (derived from the mean & 95% C.I. of log odds of accepting an offer from the mixed effect logistic regression model) was calculated (Figure 4).

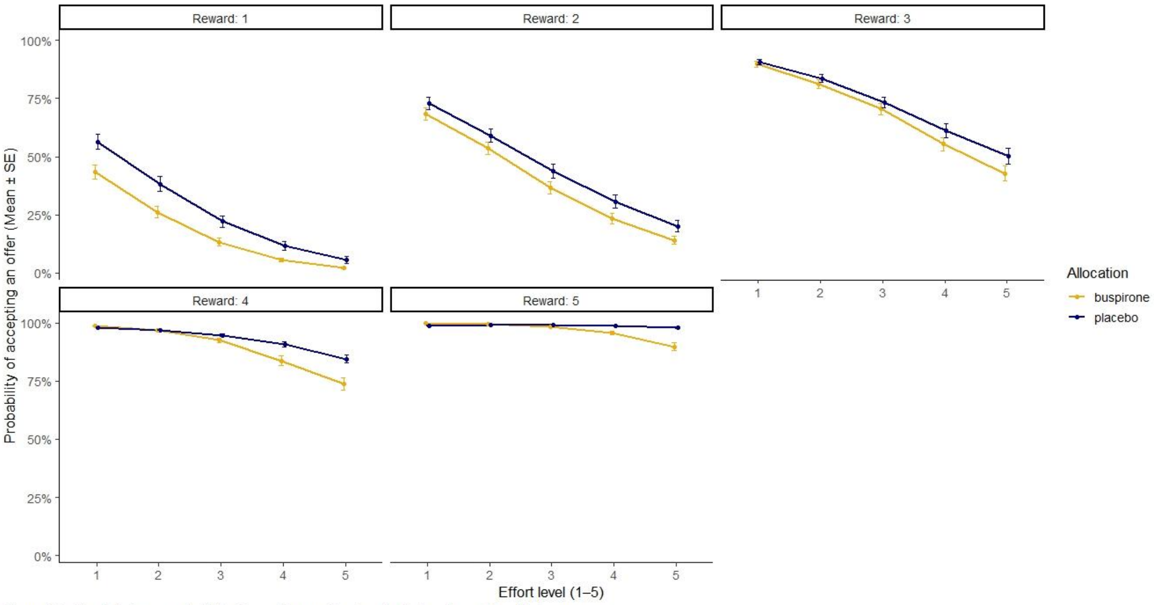


Figure 4. Scatter plot of mean probability of accepting an offer at each effort and reward level between groups.
Mean probability calculated using the estimated marginal means from the full mixed effects logistic regression model.
Error bars = S.E.M

This illustrates the general trend of decreased probability of offer acceptance as effort increases across all reward levels. This trend is fairly consistent for the buspirone group, indicating sensitivity to effort is maintained regardless of reward level. However, a reduction in the slope of acceptance rate for the placebo group indicates as reward level increases, sensitivity to effort decreases as offers appear to be accepted at a consistent rate across the effort range.

The largest group difference appears at lowest reward and effort combinations, where the buspirone group have lower probability of accepting an offer. In spite of the significant reward * effort * allocation interaction, pairwise contrasts using estimated marginal means, and corrected for multiple comparisons (Holm), revealed no significant differences emerged between allocation groups at any of the 25 unique effort * reward combinations.

To explore the validity of the model in predicting the probability of accepting an offer for each unique reward and effort combination within each group, the model was used to make predictions for the probability of accepting an offer and provided a good fit to observed group behaviour (Figure 5).

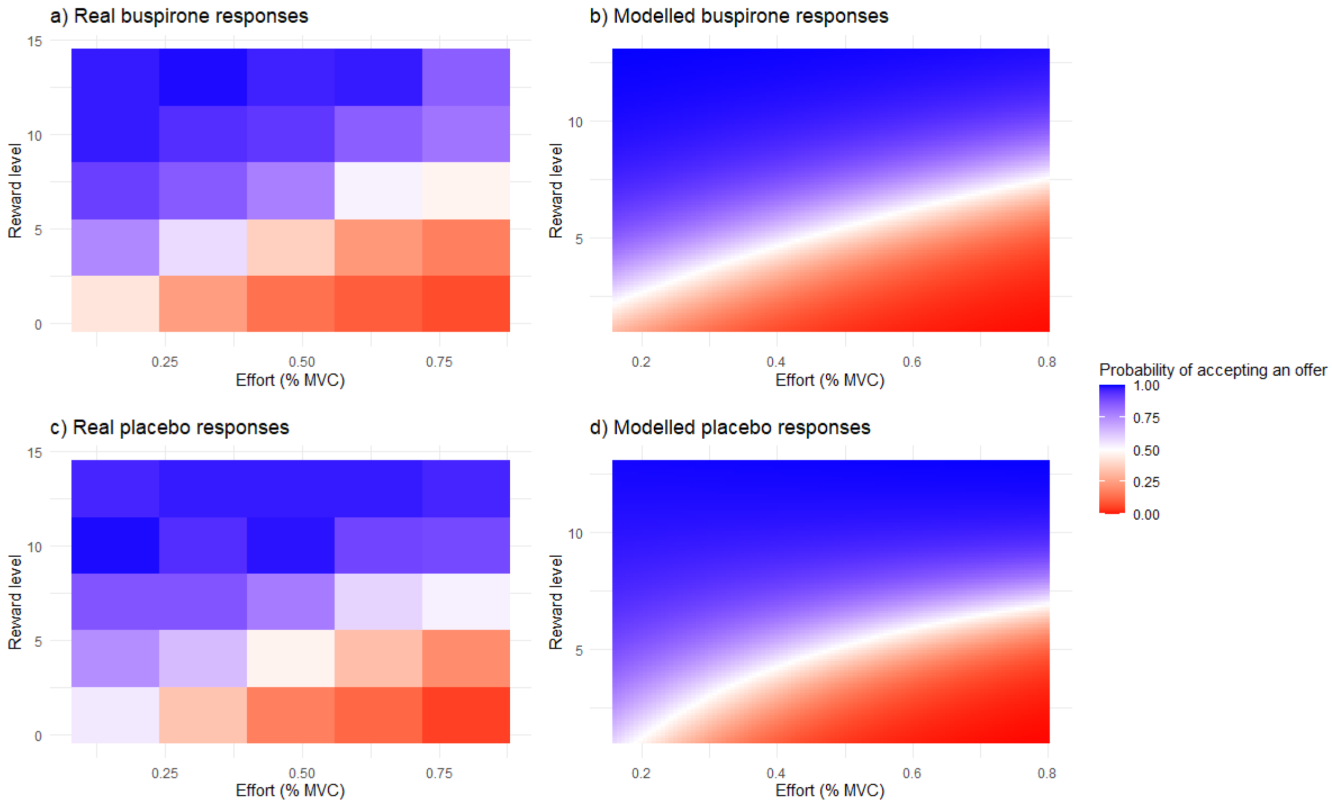


Figure 5: Heat maps of mean proportion of offers accepted in buspirone (a) and placebo (c) groups.
The modelled probability of accepting an offer, based on the mixed effect logistic regression model [equation (1)], for buspirone (b) and placebo (d)

Reaction times

A repeated measures ANOVA was performed with allocation, reward level and effort level as fixed effects for log-transformed reaction times. No significant main or interaction effect of allocation was observed ($p > 0.1$) (Fig. 6).

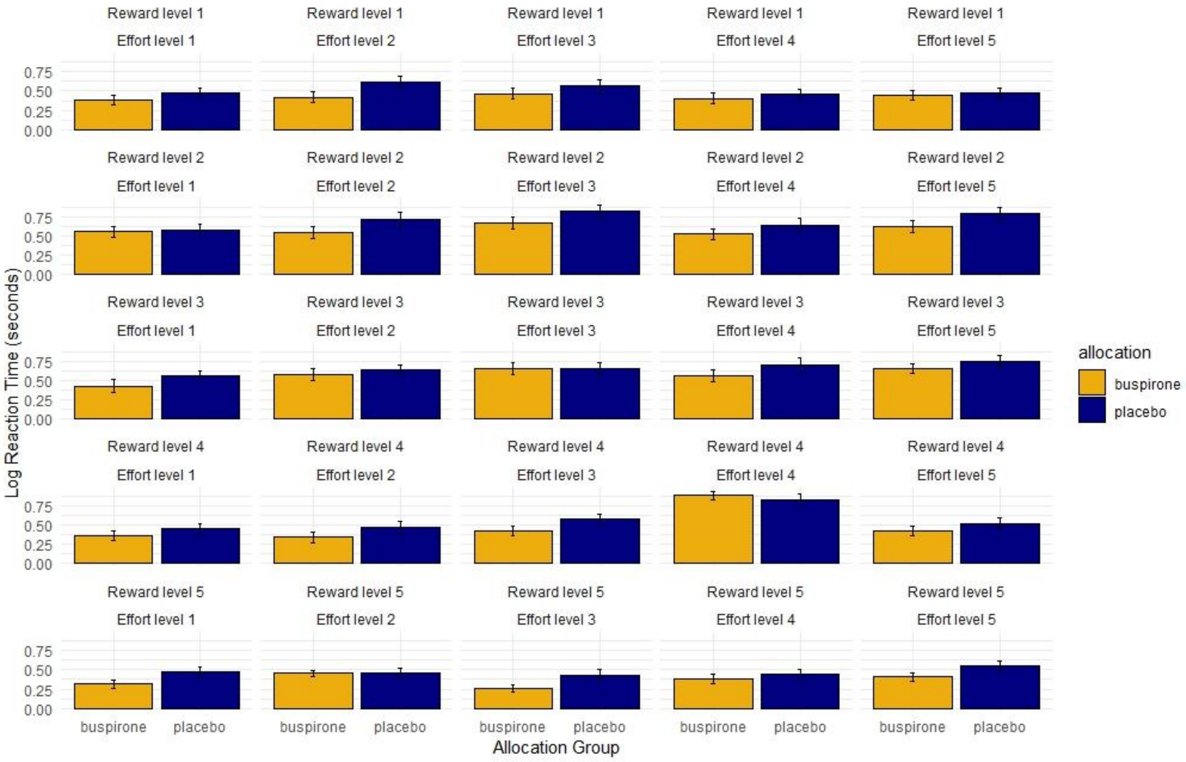


Figure 6. Average log reaction times (seconds) grouped by effort * reward combination. Error bars = +/- 1 S.E.M.

Maximum voluntary contraction strength

No significant difference in maximum force for offers was observed between groups [$t_{(374)}$ = -1.09, p = 0.28, 95% CI: -0.004 – 0.001]. A repeated measure ANOVA was performed to study the effects of effort and reward levels on maximum force exerted. As expected a significant main effect of effort on maximum force applied was observed [$F_{(1, 44)}$ = 4.79, p = 0.034]. A non-significant two-way interaction effect of reward level by allocation was also observed [$F_{(1, 44)}$ = 2.51, p = 0.073]. Post-hoc tests indicated no significant difference (p > 0.5) between allocation groups within each reward level.

Conclusions

A significant difference appears between allocation groups for unique combinations of reward and effort when modelled with a mixed effect logistic regression. Specifically, at higher reward levels the placebo group are less sensitive to effort requirements than the buspirone group when deciding to accept an offer, significantly so at the highest effort level, albeit by a small degree. In contrast, at lower reward levels, both buspirone and placebo groups seem to show similar sensitivity to effort e.g. the slope of acceptance line is similar in Fig. 4. It is noteworthy at the lowest and highest effort and reward level, the buspirone group is less sensitive to effort requirements than the placebo group. The effect of buspirone in maintaining effort sensitivity across reward levels, however seems very small. No significant difference in reaction times or maximal voluntary contraction between allocation groups.

PILT

Comparison of optimal choice

In the loss condition a significant difference in optimal choice was found between allocation groups [$F_{(1, 56)} = 9.08$, $p = 0.0039$, $\eta_p^2 = 0.14$] (Fig. 7). In corresponding analysis, no significant difference was observed in the win condition [$F_{(1, 56)} = 2.49$, $p = 0.12$] (Fig. 8). Visualisation of optimal choice indicates that for both win and loss condition the buspirone group show increased number of optimal choices compared to the placebo group, especially when it appears learning has plateaued after trial 10.

Analysis of mean optimal choice after trial 10 was performed and indicated a significant difference between groups exist in both the win [$F_{(1, 38)} = 11.23$, $p = 0.0018$, $\eta_p^2 = 0.23$] and loss [$F_{(1, 38)} = 10.28$, $p = 0.0027$, $\eta_p^2 = 0.21$] conditions.

Comparison of reaction time

No significant main effect of allocation or interaction effect of allocation & condition was observed for log-transformed reaction times ($p > 0.1$).

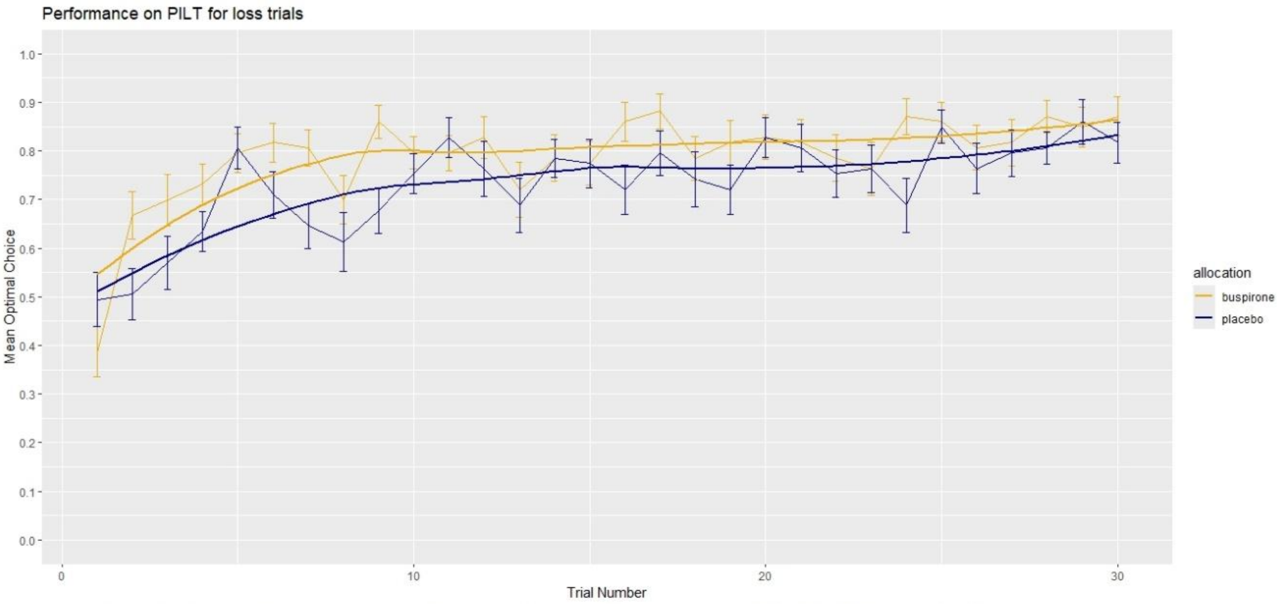


Figure 7: Mean optimal choice in loss trials. mean optimal choice was calculated by subjects choosing the symbol least likely to lose in a loss trial. The number of optimal choice trials was divided by total loss trials for each participant to give proportion of optimal choices at each trial (e.g. two runs therefore optimal choice can be made on both, one or neither trial giving a proportion of either 0, 0.5 or 1). The mean of proportion of optimal choices was then calculated for each group. Error bars = +/- 1 S.E.M. \n Line of best fit uses LOESS (Locally Estimated Scatterplot Smoothing) function in geom_smooth() in R.

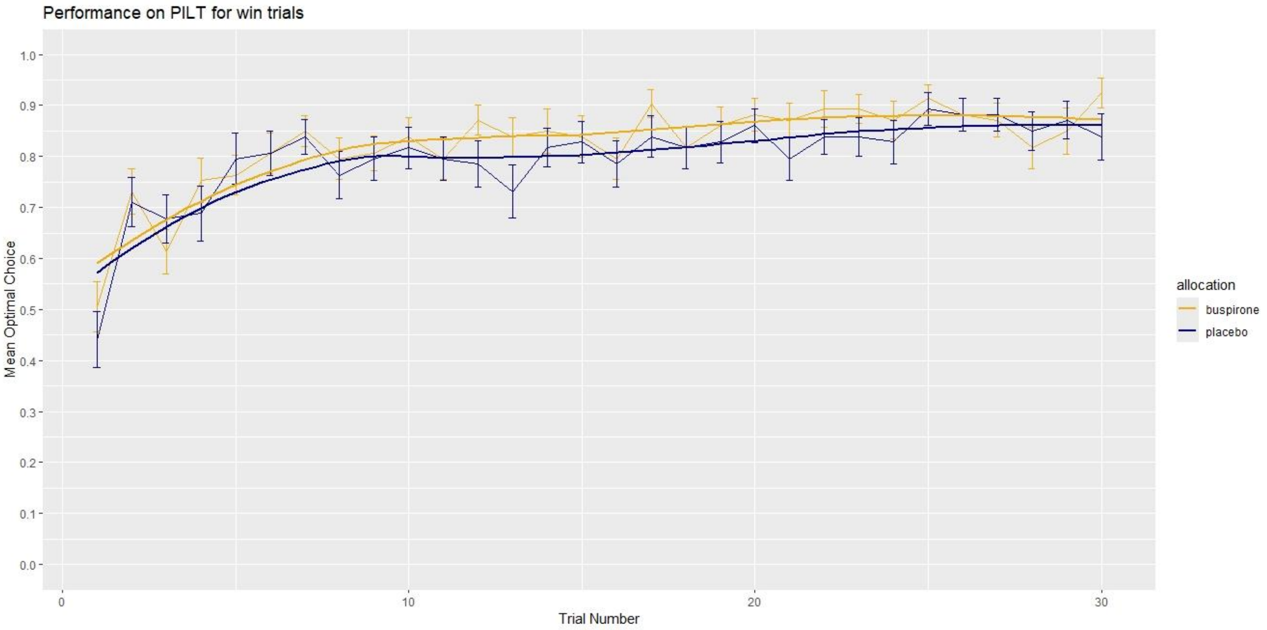


Figure 8: Mean optimal choice in win trials. mean optimal choice was calculated by subjects choosing the symbol least likely to lose in a loss trial. The number of optimal choice trials was divided by total loss trials for each participant to give proportion of optimal choices at each trial (e.g. two runs therefore optimal choice can be made on both, one or neither trial giving a proportion of either 0, 0.5 or 1). The mean of proportion of optimal choices was then calculated for each group. Error bars = +/- 1 S.E.M. \n Line of best fit uses LOESS (Locally Estimated Scatterplot Smoothing) function in geom_smooth() in R.

Computational analysis

A simple reinforcement Q-learning model, as used previously with the task (Pessiglione et al. 2006), was fitted to subjects choice data. The model used reciprocal updating in that not only is the subjects' understanding of the chosen symbol updated with every trial, but also the unchosen symbol e.g. 'this symbol did not win on this trial so the other symbol must be a winning symbol, I should choose that next time'. The equation to update the symbol value, *a*, was:

$$Q_{a(t+1)} = Q_{a(t)} + \propto (\rho R_{a(t)} - Q_{a(t)})$$

In the above equation, $Q_{a(t)}$ is the current value of shape '*a*' at trial '*t*'; $R_{a(t)}$ is the outcome that was observed for symbol '*a*' at trial '*t*' and $Q_{a(t+1)}$ is the new, updated value of symbol '*a*' for the next trial '*t*+1'. Finally, ∝ is the learning rate and ρ is the sensitivity to the outcome. The starting value of each symbol (e.g. $Q_{a(t)}$) for each block was set to zero. Separate learning and outcome sensitivities were set for win and loss trials as the model-free analysis indicated differences between the trial types.

A softmax equation was then used to calculate the choice probability for each trial. The Q values for each symbol ($Q_{a(t)} and\ Q_{b(t)}$) within each pair (win pair or loss pair) were combined for this equation:

$$P_{a(t)} = \frac{1}{1 + exp^{(Q_{a(t)} - Q_{b(t)})}}$$

To enable a wide and even spaced sample of potential parameter estimates, potential learning rates were sampled in a logit space with a 110 x 100 grid and outcome sensitivity sampled in a log space with a 100 x 100 grid. Learning rates and outcome sensitivities for each trial type for each subject were then averaged (across the three trial blocks they undertook) and compared between groups using a Welches t-test [mean parameter ~ allocation], using Holm-Bonferroni for family-wise error rate.

No main effect of allocation of interaction effect with trial type was observed for win outcome sensitivity or for win and loss learning rates ($p > 0.1$) (Fig. 9). However, a nominal difference emerged for loss sensitivity, with the buspirone group a higher sensitivity (rho = 25) for loss trials than placebo (rho = 10) ($t(60)=2.02$, $p_{unadj}=0.048$, $d=0.51$), although this effect did not survive correction for the Holm-Bonferroni correction for the four multiple comparisons (Holm-adjusted $p > 0.05$).

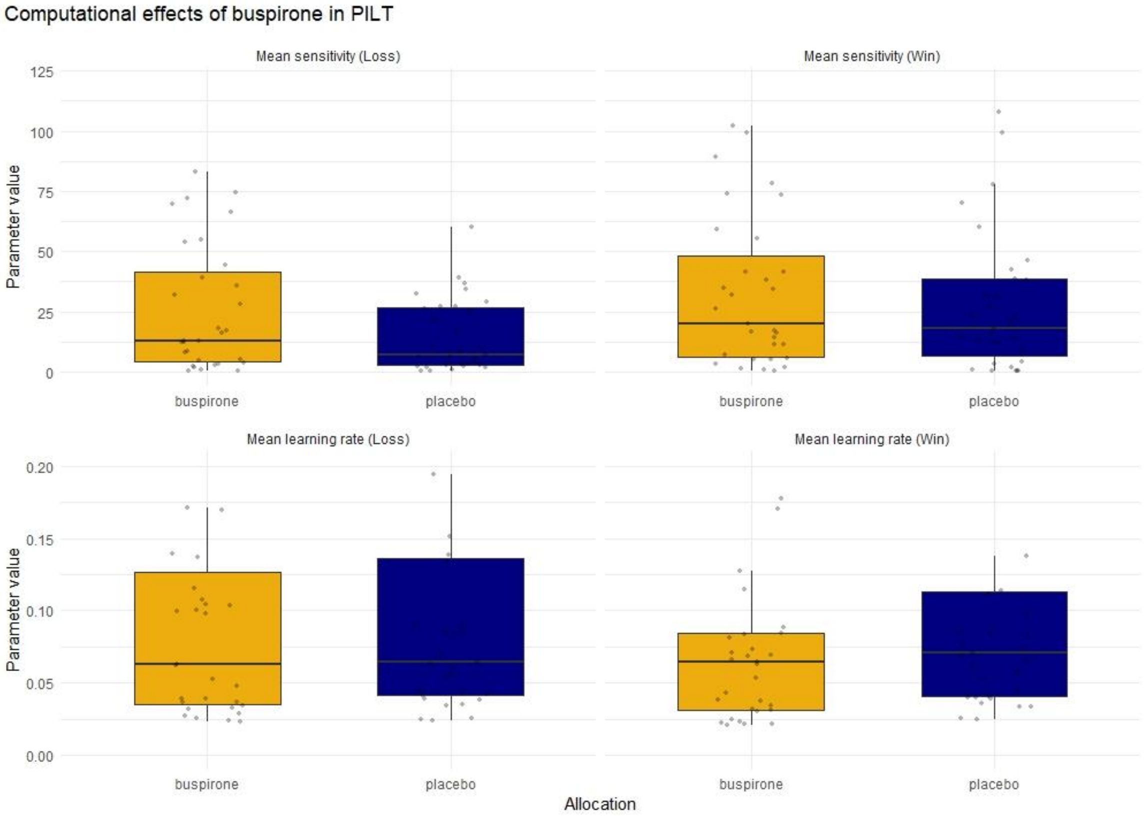


Figure 9: Boxplots of modelled learning rate and outcome sensitivity for win and loss trials between allocation groups.

Conclusion

Model-free analysis indicates buspirone appears to increase learning and sensitivity to loss as seen by significant differences in optimal choice across all trials and trials when learning has plateaued e.g. after trial 10. However, this is supported by model-based (computational) analysis which found a difference between buspirone and placebo for sensitivity to, loss. Buspirone appears to increase sensitivity to reward after learning has plateaued in model-free analysis. This is not supported by model-based analysis which found no difference between buspirone and placebo for either learning rate for or sensitivity to win outcomes.

Discussion

Acute buspirone appears to increase the aversiveness of bitter tastes, considering intensity and anticipation of the taste experience. Furthermore, acute buspirone maintains effort discounting in an effort-expenditure task. Finally, buspirone does not appear to alter learning about or sensitivity to reward but does increase sensitivity to loss. To the best of my knowledge, there are no previous human studies investigating the effect of buspirone in these models of reward.

General reduction in serotonergic transmission – Acute tryptophan depletion & other studies

The principle action of buspirone is full agonism of 5-HT$_{1A}$ autoreceptors, which acutely reduces 5-HT release from serotonergic neurones and thus decrease overall 5-HT receptor transmission, particularly at non-5-HT$_{1A}$ post-synaptic receptors. However, few studies use buspirone as a serotonergic probe. It is therefore of interest to compare the findings in this study with other methods of reducing overall 5-HT receptor activity in humans, such as those of acute tryptophan depletion (ATD), which would be expected to lower overall 5-HT neurotransmission by decreasing serotonin release from presynaptic terminals.

Primary reward & taste

A study of 25 healthy female volunteers found acute tryptophan depletion led to the rating of bitter tastes as less pleasurable, compared to the placebo group, consistent with results presented here. The same study found the ATD group experienced bitter and sour tastes as more intense (Smith et al. 2021), an observation not found in results presented here. One potential explanation for the discrepancy in results could relate to study design, specifically here a between group design is used compared to the within subject design used by Smith et al (Smith et al. 2021), which could be more sensitive to detecting sensory effects.

A study of 36 lean, healthy volunteers (BMI between 18.5 and 24.9) found acute tryptophan depletion, compared to placebo, led to a non-significant decrease in calories consumed through sweet foods and a reduction in first choice sweet foods (Pagoto et al. 2009). This was interpreted as a decreased preference or salience, of pleasurable sweet foods. A similar trend was observed in results presented here (i.e. buspirone reducing the pleasurableness of sweet tastes).

The changes to taste after ATD were believed to be due to a reduction in central 5-HT as opposed to changes in peripheral 5-HT (Smith et al. 2021), which seem to be unaffected by central changes in tryptophan (Geeraerts et al. 2011; Keszthelyi et al. 2012). This would support the influence of buspirone, and serotonergic manipulation more broadly, on taste being at the level of reward centres in the brain as opposed to peripheral, sensory neurones.

However, one theory of the influence of serotonin on taste involved 5-HT$_{1A}$ receptors but at peripheral sites. Heath et al. (2006) found that acute SSRI treatment induced an increase in sensitivity to bitter and sweet tastes specifically, and postulate that 5-HT$_{1A}$ receptors on taste cells could be the reason for this (Heath et al. 2006). Specifically peripheral presynaptic 5-HT$_{1A}$ receptors on taste cells provide feedback to increase signal transduction from taste cells (by increasing intracellular calcium release and subsequent ATP release) to gustatory afferent pathways (Heath et al. 2006). If true, one would expect an increase in intensity for bitter and sweet tastes, which was not observed here. Several reasons could explain this as Heath et al. used incremental taste concentrations, enabling accurate threshold detection and thus intensity scores. Here we used a single supra-threshold concentration and a subjective rating of intensity, which is likely less sensitive to intensity changes after intervention. However, in addition, Heath et al. increased 5-HT through paroxetine, a drug that would influence several 5-HT receptors specifically such as 5-HT$_2$ receptors as well as possessing weak anti-muscarinic actions which can lead to dry mouth and potentially influence taste. Furthermore, several premises of the proposed mechanism relied on *in vitro* evidence which may not directly translate into humans.

In summary evidence indicates reducing serotonergic transmission, through reduction in the serotonin precursor tryptophan, results in less pleasure being derived from taste stimuli, significantly so for aversive bitter tastes and non-significantly for rewarding, sweet tastes, congruent with results presented here.

Motivation

Results presented here indicate a reduction in serotonergic transmission could impair subjects' ability to change motivation in a relation to the incentive offered.

This is in contrast to two pre-clinical studies indicating that reduction in serotonin has no significant effect on effort discounting. The studies examined effort discounting using a T-maze task, which provided a choice of either a high reward option requiring the scaling of a barrier and an alternative, low reward option without a barrier (Izquierdo et al. 2012; Denk et al. 2005). After blocking serotonin synthesis with para-chlorophenyl-alanine methyl ester (pCPA), rats continued to pursue high reward options by climbing the barrier. Importantly one of the studies found animals given high pCPA doses had significantly lower 5-HT than controls but also were slower than controls, compared to low dose pCPA, which paradoxically had higher 5-HT and were quicker in completing the task (Izquierdo et al. 2012). These pre-clinical studies indicate that whilst lowering 5-HT did not influence effort discounting may have slowed decision making. The discrepancy with results found here could be related to common challenges with preclinical translation. These include potential species-related differences but also could be due to task differences, in that primary reward with actual outcome was used for the animal studies cited however financial and delayed rewards were used for the current study. Furthermore, the technique of lowering 5-HT in animals (pCPA) reduced 5-HT by up to 90% in the brain and testing took place after 3-4 days, compared to here where the degree of reduction in 5-HT release from serotonergic neurones is not known and testing as performed acutely post-intervention, where any compensatory changes in neurotransmission may not have occurred.

Few human studies exist involving reduction of serotonergic transmission and tasks involving effort discounting as a measure of motivation. However, several studies have interpreted changes in reaction times following ATD as changes in incentive motivation.

Several within-subject studies, utilising a novel cued-reinforcement reaction time task, found that reaction times became faster as the probability of reward increased, an effect that was abolished with ATD, suggesting reducing serotonergic transmission reduces motivational vigour for high probability rewards (Cools et al. 2005). A second study using the same task found tryptophan depletion induced a similar slowing of reaction times to high probability offers compared to tryptophan replete conditions (Roiser et al. 2006). A key caveat however to these studies is that they do not explicitly measure willingness to exert effort for reward but rather use reaction time as a surrogate for motor vigour as part of motivation in reward processing. With this caveat in mind these studies do identify an effect on reaction time, interpreted as a reduction in serotonergic transmission resulting in a reduction in motivation for high probability rewards.

This finding is broadly consistent with results presented here, namely that reducing serotonergic transmission maintains a sensitivity to effort that would otherwise diminish with increasingly rewarding outcomes. It is noteworthy that no reaction time difference was found in results presented here. This could be owing to the studies mentioned used a within-subjects approach which could have greater sensitivity to reaction time difference, as well as task difference, namely the aforementioned task had to discern between three shapes, was response time-dependent and with the outcome of winning a reward. In contrast, the task used here to measure motivation evaluated only one option at a time, deciding if an offer ‘is worth it’ to the individual and with outcome not dependent on speed of response. Therefore, response times here were possibly too great to detect group differences.

The influence of serotonergic modulation on motivation can be extended to aversive outcomes. Using a novel Go/No-Go task with rewarding and aversive outcomes in healthy volunteers, slower reaction times for punishment trials became faster after ATD, becoming similar to the fast reaction times observed for rewarding trials. This effect was believed not to be due to reduced sensitivity to aversive outcomes but rather acute tryptophan depletion weakening the link between behavioural inhibition and the prediction of aversive outcomes (Crockett, Clark, and Robbins 2009). One interpretation is that a reduction in serotonergic transmission could reduce motivational salience of aversive outcomes thus enabling faster reaction times as subjects are less inhibited by potentially aversive outcomes. This pattern is not observed here, namely buspirone group appear to maintain a sensitivity to effort throughout differing reward levels, unlike the placebo group who appear less sensitive to effort as reward increases. Comparison between studies should be met with caution however for several reasons. The Go/No-Go task was designed to specifically measure executive and aversive motor inhibition, with reaction time as an implicit measure of aversive salience. Results here are explicit in using effort as a discounting factor and measure of aversiveness, with no loss or omission of reward if reaction time was slow, therefore measuring a different decision-making construct and thus differing results. Furthermore, the Go/No-Go task used monetary omission or loss, as oppose to effort, as an aversive factor which are not directly comparable owing to potentially different degrees of aversion e.g. subject may experience monetary loss as worse than a mild increase in grip strength requirement.

In summary results of acute buspirone maintaining the aversive effect of effort is indirectly supported by some ATD studies in humans, and contributes novel evidence to the field of serotonin and motivation. Thus reduction in serotonergic transmission impacts on explicit effort-discounting decision making, an effect presumably mediated by neurones synapsing with serotonergic neurones originating from the raphe nucleus (via inhibition from somatodendritic 5-HT_{1A} receptors) such as the striatum, frontal cortex and cingulate cortex (Delcourte, Etievant, and Haddjeri 2021).

Learning

Acute buspirone does appear to improve learning to aversive (loss) or rewarding (win) outcomes in a probabilistic instrumental learning task, although computational analysis indicates no difference in sensitivity or learning to the outcomes.

The model-free findings are consistent with a smaller, healthy volunteer study examining the influence of acute tryptophan depletion on a two-arm bandit, gambling task varying the magnitude and probability of reward. This study found ATD did not influence probabilistic choice of healthy volunteers between a high-probability small reward and a low-probability high reward (Anderson, Richell, and Bradshaw 2003) . However, this study adds to this evidence in that it indicates reductions in serotonergic transmission extend to insensitivity to implicit probabilities (without variation in reward magnitude) as well as loss (rather than just reward omission).

Whilst data involving instrumental probabilistic learning is sparse much evidence exists examining the role of serotonin depletion and reversal learning in humans. One group used an observational reversal learning task and found that healthy volunteers made less prediction errors for punishment when lowering serotonin via ATD (Cools, Robinson, and Sahakian 2008). Specifically, under baseline conditions, an increase in prediction errors for punishment trials, but not for reward trials, was observed. However, upon acute tryptophan depletion, this difference was abolished with prediction error for both reward and punishment trials being similar. This was hypothesized to indicate normal levels of serotonergic transmission could provide a resilience to aversive outcomes or protective bias against anticipation of punishment, however upon lowering serotonin this bias is lost and subjects have an enhanced ability to anticipate punishment (Cools, Robinson, and Sahakian 2008).

These findings were replicated in a larger, between-subjects healthy volunteer study, and extended in that the increased sensitivity to prediction of punishment was not accompanied with any changes in mood (Robinson, Cools, and Sahakian 2012). There are many contrasts between these studies of reversal learning and the results of the probabilistic instrumental learning task presented here. One possibility could be due to the options in the reversal learning tasks, namely the subject had to decide whether an option was a probably a loss or a win, which comparatively may have greater salience than the loss or no loss options respectively used in trials here, which may not be sufficiently sensitive, to detect differences in behavioural prediction error. Furthermore, whilst hypothetical, the win and loss outcomes were possibly more salient in the reversal learning study (+/- $100) compared to incentives used in this study (+/- 20p), which may be insufficient to elicit a substantial difference in behaviour than is amenable to serotonergic manipulations.

In contrast to the findings presented here, some studies have found reducing serotonergic transmission via ATD in healthy volunteers was detrimental to reward learning, specifically through reducing sensitivity to reward. The study used a probabilistic 4-arm bandit task and found increased perseveration (or reduced flexibility) and reduced sensitivity to rewarding, but not aversive, stimuli in the tryptophan depletion group (Seymour et al 2012). These divergent findings could be due to differences in task effects. In Seymour et al. a four-arm bandit was used with reward and punishment being delivered together, with varying probability. This makes it challenging to separate the effects of either outcome on learning in that reward is perhaps more salient as it is always being compared to pain, rather than reward omission, which is more likely to reflect reality e.g. if you do not win the lottery having bought a ticket, you do not worry about getting an electric shock.

Specific 5-HT$_{1A}$ modulation

Acute buspirone may have comparable effects to acute tryptophan depletion (ATD) through its somato-dendritic 5-HT$_{1A}$ action. However, the two interventions are not directly comparable. ATD induces a transient and modest decrease in 5-HT, whilst buspirone may reduce 5-HT for a longer duration through DRN somato-dendritic 5-HT$_{1A}$ agonism. Buspirone and other 5-HT$_{1A}$ probes would have additional effects, such as maintaining post-synaptic 5-HT$_{1A}$ inhibition through partial agonism in spite of a decrease in the release of 5-HT in the brain.

Primary reward & taste

An early pre-clinical study indicates that buspirone can mitigate the experience of aversive tastes using a conditioned taste aversion task. Mice received daily saccharin drink, until day 8 when they received an aversive lithium chloride i.p. injection, subsequently conditioning them to suppress saccharin intake (Ervin, Soroko, and Cooper 1987). This conditioned aversion was reduced in mice pre-treated with 1-5mg/kg of buspirone. Importantly mice not receiving the aversive intervention (but a saline i.p. injection instead) did not increase saccharin intake, indicating the action of buspirone was in reducing aversive experience as opposed to enhancing rewarding experiences.

Motivation

Pre-clinical evidence indicates 5-HT$_{1A}$ agonism may enhance the motivation to exert effort. Using a response duration differentiation schedule, whereby rats had to learn to press a level for 1 to 1.3 sec to receive a food reward, one group demonstrated that 8-OH-DPAT and buspirone 10mg/kg i.p. increased the mean duration of responses (Kinney, Griffith, and Hudzik 1998). The authors hypothesized the effects of buspirone and 8-OH-DPAT in increasing the mean duration of response could have been due to post-synaptic 5-HT$_{1A}$ receptor agonism, which may account for the differing results from the global lowering of 5-HT in the previous studies using the T-maze task.

However, results from the modelled data at higher reward levels indicate the subjects receiving buspirone are less likely to accept an offer associated with higher effort requirement i.e. more aversion to effort. One possible reason is the effect noted by Kinney et al. was attributed to post-synaptic 5-HT$_{1A}$ action, whereas buspirone is thought to have primarily auto-receptor preference. Furthermore, the effect reported here is small and is not apparent from initial, grouped analysis and thus this conclusion should be treated with caution. Nevertheless, in the context of effort being aversive, the effort expenditure task gives partially consistent results with increased sensitivity to aversive tastes in the primary reward task.

Learning

A potential divergent effect on reward sensitivity, motivation and learning with pre- vs. post-synaptic 5-HT$_{1A}$ agonism is highlighted with pre-clinical studies. Increasing concentrations of the prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT produces a biphasic response in fixed-threshold electrical intracranial self-stimulation of the lateral hypothalamus in rats, a measure of consummatory reward & motivation (Montgomery, Rose, and Herberg 1991). In this animal study, low dose 8-OH-DPAT was believed to have a pre-synaptic 5-HT$_{1A}$ preference (leading to reduced serotonergic transmission) and self-stimulation increased (interpreted as being more motivated or sensitive to reward). However, at higher 8-OH-DPAT doses, when post-synaptic 5-HT$_{1A}$ receptors are believed to be engaged, with subsequent inhibition of dopaminergic reward pathways, a reduction in self-stimulation frequency was observed (Montgomery, Rose, and Herberg 1991). This pattern was also observed when buspirone was administered to rats in the same task, an effect potentially from the post-synaptic 5-HT$_{1A}$ agonism of buspirone. Thus, pre-synaptic 5-HT$_{1A}$ agonism increases sensitivity to reward & motivation and post-synaptic 5-HT$_{1A}$ agonism may have the opposite effect.

In support of this hypothesis, Harrison et al. found similar results, specifically a reduction in the electrical current required for a rewarding effect in a brain stimulation paradigm in rats for low dose, systemic 8-OH-DPAT (Harrison and Markou 2001). A comparable reduction was seen when 8-OH-DPAT was directly injected in to the median raphe nucleus, which implicates the pre-synaptic 5-HT$_{1A}$ autoreceptors in the reward enhancing effect of low dose, systemic 8-OH-DPAT administration e.g. reducing serotonergic transmission (Harrison and Markou 2001). Furthermore, higher dose of 8-OH-DPAT exhibited a reward sensitivity-reducing effect in the same brain stimulation task (Harrison and Markou 2001).

Results here contrast with these findings, namely buspirone did not lead to a significant increase in outcome sensitivity. A possible reason for this is differences in species and task, specifically direct brain stimulation of the hypothalamus in animals not being directly comparable to instrumental learning tasks in humans, which uses financial incentive, a degree of uncertainty of outcome and involves competing aversive learning alongside rewarding trials. These additional factors may limit any effect of 5-HT$_{1A}$ receptor agonism on reward learning, as seen in the aforementioned pre-clinical studies.

Evidence from an animal study using an operant reward learning task involving a food reward found a mild detrimental effect of buspirone on acquisition learning but no effect on extinction (Frick et al. 2015). This is in contrast to results here whereby no difference in reward learning rate between buspirone and control groups was observed. Again, this could be explained by species differences but also task differences. Whilst operant conditioning tasks are similar to instrumental learning tasks, the latter is more complex involving implicit probability, symbolic cues and outcomes, which may have more cognitive demands and thus conceal any effects of 5-HT$_{1A}$ receptor agonism.

One healthy volunteer PET study examined the availability of 5-HT$_{1A}$ and performance on a decision-making task involving probability of reward and loss (Faulkner et al. 2014). It found subjects' sensitivity to probability (regardless of trial valence) positively correlated with availability of hippocampal, post-synaptic 5-HT$_{1A}$ receptors. One interpretation of this could be that with increased inhibition of hippocampal neurones, through post-synaptic 5-HT$_{1A}$ binding, greater sensitivity to probability occurs. This was not observed in the present study. This could be because nature of the studies, in that Faulkner et al. (2014) was observational in nature, so caution should be taken in directly linking the correlation found in that study with results of the interventional study here, as subjects presumably were tested with baseline levels of 5-HT and constitutive activity of the many 5-HT receptors. This would not be the case in the current study where baseline levels of 5-HT and constitutive activity of 5-HT receptors would be reduced from 5-HT$_{1A}$ auto-receptor activation whilst post-synaptic 5-HT$_{1A}$ inhibition would be maintained at a constitutive level.

Limitations

Duration of intervention

An acute buspirone dose would principally activate somato-dendritic 5-HT_{1A} receptors, leading to a reduction 5-HT release in terminal fields. It is possible that a longer course of buspirone may lead to down regulation of somato-dendritic 5-HT_{1A} receptors specifically (Sim-Selley et al. 2000), with the post-synaptic 5-HT_{1A} agonist action of buspirone predominating. Thus, repeated administration of buspirone would enable a hypothetical comparison of the influence of pre- vs post-synaptic 5-HT_{1A} agonism on reward processing using an identical compound.

Lack impulsivity testing for individual differences

One key feature of serotonergic manipulations is that of impulsivity. Previously mentioned studies indicate individual differences could be key to the motivational effect of modulating serotonergic transmission. The original study found the slowing of response significantly correlated with high impulsivity (Cools et al. 2005). The study presented here has not measured impulsivity and therefore is unable to examine where impulsivity may moderate the relationship between a reduction in 5-HT signalling and decision making.

Lack of genetic testing

It has been shown that genotype can moderate the influence of 5-HT reduction and motivation. Namely in a homozygote for the short allele in the serotonin-transporter-linked promoter region (5-HTTLPR), a group generally considered at risk of depression, demonstrated slower reaction times for reward (Roiser et al. 2006). Intriguingly the opposite pattern emerged for individual homozygous for the long allele at (5-HTTLPR) i.e. ATD induced faster reaction times as probability of reward increased. The present study did not undertake and was not powered for, genetic testing, therefore it is unclear if specific genotypes would mediate the effects of buspirone on any stage of reward processing, such as motivation.

Non-serotonergic effects of buspirone

Furthermore, buspirone possesses antagonism of D_2-like auto-receptors (Ford 2014), which when buspirone is given acutely leads to an increase in dopamine release from in the striatum (Algeri et al. 1988), with only mild post-synaptic dopamine antagonism (Loane and Politis 2012). Thus the increase in dopamine release in striatal regions from D_2-like autoreceptor antagonism may mitigate the impact of reduced reward sensitivity seen with reduction in global 5-HT brought about by ATD seen by others (Seymour et al. 2012). Broadly the aforementioned reward enhancing effect of pre-synaptic 5-HT_{1A} agonism in the raphe nucleus found in animals with intra-cranial self-stimulation, is believed to be due to disinhibition of dopaminergic reward pathways. However biochemical evidence from rodents indicates that relatively higher doses of systemic (subcutaneous) 8-OH-DPAT injection, presumed to activate post-synaptic receptors, elicit an increase in dopamine release in the prefrontal cortex, but not the striatum (Rollema et al. 2000). This dose-dependent differential effect was broadened with the finding that the 5-HT_{1A} agonist BAY x 3702 may both increase and decrease prefrontal cortex dopamine release (Diaz-Mataix, Artigas, and Celada 2006). The former observed at lower doses and thought to be due to 5-HT_{1A} activation on pyramidal glutamatergic neurons projecting to the ventral tegmental area and the latter owing to 5-HT_{1A} activation on inhibitory GABA interneurons (Alex and Pehek 2007).

Healthy volunteer studies have used pramipexole, a D_2/D_3 agonist, and sulpiride, a D_2 antagonist, to investigate the subjective rating and neural correlates of rewarding and aversive tastes (McCabe et al. 2011; McCabe et al. 2012). Except for a significant change in the aversive bitter taste here, these studies found no changes in subjective ratings of taste similar to results here. Speculatively this may be due to reduction in dopaminergic transmission through either pramipexole action at D_3-like autoreceptors or the antagonism of post-synaptic D_2/D_3 receptors by sulpiride. Whilst buspirone may have some post-synaptic D_2 blockade it also mildly antagonises D_2-like autoreceptors increasing dopamine release, which may enable for the significant worsening of, or possibly sensitivity to, and an aversive bitter taste.

It is clear that relationship between the buspirone and the dopamine system is complex. It is possible the influence of buspirone on increasing dopaminergic signalling in cortical regions via the 5-HT_{1A} receptor may be complemented by the aforementioned increase in striatal dopamine from D_2-like auto-receptor antagonism of buspirone.

Computational models

Initial analysis indicates an effect of buspirone in the probabilistic instrumental learning task for reward and loss trials, however further computational analysis indicates no significant difference between groups for outcome sensitivity or learning rate for reward or learning rate for loss. The model chosen replicates what parameters others have found valuable in explaining differences the behavioural responses of depressed patients, namely outcome sensitivity and learning (Huys et al. 2013). Furthermore, others have commented that additional parameters, such as choice stochasticity (i.e. the degree to which a subject may use information gained to make a decision), could be redundant when including outcome sensitivity in a model for depressed patients (Browning, Paulus, and Huys 2022). In spite of the rationale for this approach, an exploration of various other models that include non-reciprocal updating or inclusion of other parameters such as choice stochasticity which may be sensitive to 5-HT_{1A} manipulation, or decay in values during reward learning, which has been shown to be influence by pramipexole (Halahakoon et al. 2024).

Conclusion

This study demonstrates the diverse role of buspirone on various stages of reward processing. It seems to reduce the subjective experience of an aversive taste, without influencing other tastes, and maintain a sensitivity to effort when deciding whether to accept a reward in an effort-discounting task. Finally, whilst it finds an increase in sensitivity to reward and loss learning in model-free analysis, this is not replicated in a simple reinforcement model. Whilst buspirone possesses a complex pharmacology with mild affinity for dopamine receptors and partial agonism of post-synaptic 5-HT_{1A} receptors its main mechanism of action is activation of somato-dendritic 5-HT_{1A} receptors leading to reduction in 5-HT at sites proximal to midbrain serotonergic neurones, such as the striatum and frontal cortex. Therefore, a speculative but plausible hypothesis is a reduction in 5-HT in these regions may increase aversion to primary stimuli and effort in aversive learning.

Chapter 4: The behavioural effects of sub-chronic citalopram on reward processing

Introduction

Selective Serotonin Reuptake Inhibitors (SSRIs), particularly sertraline and citalopram, remain widely prescribed (Lalji, McGrogan, and Bailey 2021), in part related to their safety profile, which has enabled their frequent use in experimental and translational studies. This has enabled the robust pharmacological and neuropsychological characterisation of SSRI treatment, and allowed identification of the challenges involved when SSRIs are used to treat clinical depression (Cowen 2024). This can include poor efficacy for symptoms such as amotivation and anhedonia and possible inducement of emotional blunting (Price, Cole, and Goodwin 2009; Masdrakis, Markianos, and Baldwin 2023).

Whilst the efficacy of SSRIs in treating depression is clear (Cipriani et al. 2018) it should be tempered with the knowledge that only around 45% individuals experiencing depression will likely respond fully to either the first or second antidepressant they trial, both of which would typically involve some serotonergic action (McIntyre et al. 2023). This warrants a detailed evaluation of the ways in which SSRIs fail to ameliorate specific depressive symptoms and the associated neural correlates, enabling a finer characterisation of the limitations in their actions. Such an approach is consistent with the Research Domain Criteria, a framework developed in the late 2000s conceptualising mental disorder along behavioural and psychological axes and their corresponding neural circuitry (Insel et al. 2010). This is with the expectation that results can inform future research into pharmacotherapies that can demonstrate domain-specific efficacy and thereby be used in a more focussed way in treatment programmes. This approach was taken here in evaluating anhedonia through study of reward processing, examined as per Berridge et al. (2009) as liking (e.g. consummatory), wanting (e.g. motivation) and learning about reward and punishment (Berridge, Robinson, and Aldridge 2009).

These processes were measured using a taste task, an effort grip task and a probabilistic learning task, as described in chapter 3.

Although using different tasks, evidence to date indicates repeated citalopram may not influence subjective ratings of various tastes (McCabe et al. 2010), but can increase willingness to exert effort for reward (Meyniel et al. 2016) and improve aversive learning but worsens reward learning (Michely et al. 2022).

Chapter 2 & 3 examine 5-HT$_{1A}$ receptor, having been implicated in depression. A key effect of 5HT$_{1A}$ agonism is an acute reduction 5-HT levels. A longer course of buspirone would lead to early desensitisation of pre-synaptic receptors (Albert and Vahid-Ansari 2019) however persistent signalling on 5-HT$_{1A}$ receptors, which are known to desensitise more slowly, if at all (Altieri et al. 2013). Furthermore, at longer time courses the specific post-synaptic 5-HT$_{1A}$ partial agonism would switch from a tenuous functional agonism to a functional antagonism, specifically at 5-HT$_{1A}$ receptors, whilst 5-HT levels return to baseline from normalisation of raphe serotonergic neurones.

In order to enable a directional examination, the impact of 5-HT on reward levels, I wished to increase 5-HT, at an early stage, where it may be increasing just beyond baseline levels. Therefore, an SSRI mechanism wold enable this (Smith et al. 2000), citalopram chosen as it's a relatively uni-modal SSRI. Furthermore, it would enable a gradual, persistent tonic increases in 5-HT levels that would ensure the increases in 5-HT are maintained and any homeostatic responses of other neurotransmitter systems to occur, including the potential for any downstream neuroplastic effects to occur, if any as seen in animals (Alboni et al. 2010).

The choice of this intervention enables serotonergic modulation across two complementary mechanisms and timescales: immediate, receptor-specific effects (buspirone) versus early adaptive transporter-driven effects (citalopram).

The aim was to examine the effects of a 7-day course of SSRI treatment, and the ensuing generalised increase in brain serotonin levels, on each of these tasks to gain insights in to the consequences for each of the aforementioned stages of reward processing. I hypothesized that a 7-day course of citalopram would:

1. produce no significant difference in optimal choice reward learning (i.e. reward selection) in the citalopram group compared to the placebo group.
2. produce a significant increase in optimal choice aversive learning (i.e. loss avoidance) in the citalopram group compared to the placebo group.
3. produce a reduced rating of subjective pleasurableness of rewarding taste, but not of aversive taste, in the citalopram group compared to the placebo group.
4. produce a higher willingness in the citalopram group to expend effort for varying degrees of reward compared to the placebo group

Methods

Recruitment, screening and randomisation

Forty-three healthy volunteers aged 18-65yrs old were recruited through university, local and social media advertising. Interested individual were screened by telephone to assess eligibility for the study (e.g. do not meet exclusion criteria as detailed in Appendix: Chapter 4, Table1), which included no contraindications to citalopram, no previous mental health disorder or previous use of study tasks within the last year. Eligible individuals were invited to attend a second, in-person screening session where various eligibility criteria were screened for, these included urine pregnancy and drug test, vital signs, BMI and a Structured Clinical Interview for DSM-5 to screen for current or past mental health disorders.

Eligible participants were then randomised, using an online, block randomisation tool, to a 7-day course of either lactose placebo or citalopram 20mg once daily. Randomisation occurred on the day of screening and was stratified by gender.

Citalopram peaks in plasma concentration by 4 hours, has a half-life of 1.5 days and reaches steady state by 1 week (Rivopharm 2024). In terms of pharmacodynamics, citalopram possesses low affinity for other neurotransmitter systems or serotonergic receptors (Rivopharm 2024). Repeated dosing of citalopram 20mg achieves 60-80% SERT occupancy, at which point the dose-occupancy relationship plateaus (Sørensen, Ruhé, and Munkholm 2022). After 7 days of daily dosing citalopram maintains a 60% occupancy of SERT by 6-hrs post-dose (Klein et al. 2007). Of note SERT occupancy declines at a slower rate than citalopram concentration (Sørensen, Ruhé, and Munkholm 2022). This indicates that by 7-days citalopram plasma concentration and SERT occupancy would reach steady state, and after the final morning dose SERT occupancy would remain at time of testing that day.

Study design

After randomisation, participants completed several questionnaires and a taste task (described in detail in chapter 3). Participants were then given the study medication to take home.

Participants were contacted on days 2-3 and 4-5 to discuss their wellbeing whilst taking the study medication and provided an opportunity for them to raise any concerns or questions to support full engagement with the study. After the 7th day of taking study medication, participants attended for a final, testing session visit.

Participants then completed the taste task and effort-expenditure task (described in detail in chapter 3). They then read instructions for a probabilistic instrumental learning task (described in chapter 3) and undertook a practice session for the task before entering the MRI scanner.

Participants then undertook the task whilst undergoing an fMRI scan. The task involved two runs of the task described in chapter 3.

Behavioural data from a previous study measuring reward learning after a 7-day course of SSRI (Michely 2022) found the reduction in reward learning in the SSRI group was significantly different from placebo (t_{64} =2.7, p=0.008). This results in an effect size of 0.675. Using 90% power and two-tailed alpha of 0.5 the resultant minimum sample size was 15 in each group. It was decided a sample size of 40 (20 per group) would provide a robust test of our hypotheses. We replaced participant attrition with additional participants.

Questionnaire measures

Prior to commencing the study medication, randomised participants completed the Beck Depression Inventory (BDI) to measure general depressive symptomatology, Snaith-Hamilton Pleasure Scale (SHAPS) to measure of anhedonia, the Temporal Experience of Pleasure scale (TEPS), to distinguish separate anticipatory from consummatory aspects of reward, the Apathy Motivation Index (AMI), to identify symptoms of apathy and motivation, the positive valence system scale-21, a scale suggested to capture the various aspects of reward processing and finally the Spielberger State-Trait Anxiety Inventory (STAI).

During the testing session on day 7, participants repeated the TEPS & SHAPS as well as completing the Oxford Depression Questionnaire to ascertain any emotional blunting related to SSRI use.

Reward tasks

Tasks used to measure reward processing were described in detail in chapter 3. To recap, the taste task consisted of tasting four tastes (sweet, sour, salty and bitter) and first rating how much pleasure was anticipated from the taste, then after tasting how intense and pleasurable the taste actually was.

The effort expenditure task entailed deciding whether or not to accept an offer of reward for a certain requirement of physical effort through gripping a hand-held dynamometer. Five levels of reward and five levels of effort required were combined to result in 25 unique reward x effort combinations. In a pseudo-randomised order, participants were offered the 25 unique offer combination in five blocks. Ten trials at the end of the task required the participant to exert hand grip effort for a reward, depending on whether they had decided to accept an offer previously.

The probabilistic instrumental learning task involved sequential presentation of pairs of symbols, one pair associated with winning money or no change as outcomes and the other pair associated with losing money or no change as outcomes. Within each pair one symbol was associated with the outcome more often than the other symbol (70% vs 30%). The participant underwent two runs of the task, each run used a different set of symbols.

Statistical analysis

Data checking

Taste task

Two participants in the placebo allocation group could not register a taste (intensity = 0) for the bitter taste (either rating at pre- and post-intervention or both). Data for bitter taste from these two participants was removed from the bitter taste dataset before group unblinding.

Effort expenditure task

Several participants accepted all offers or had very quick reaction times across all trials, interpreted as not engaging with the task and therefore removed from the dataset before unblinding. Similar to previous studies using this task (Saleh et al. 2021) at the trial level, trials with reaction time < 0.4 secs were deemed accidental and for each participant, trials with reaction times above three standard deviations above the participants mean reaction time were removed. This resulted in 78 trials being removed (1.4% all trials). Two participants (both in citalopram group) were removed owing to accepting all trials in the task, therefore deemed not to have engaged in the task.

PILT

Trials which were particularly fast (< 0.2 secs) were removed on the presumption of the participant not engaging in the task for that trial. In total 114 trials (2.5%) were below 0.2 seconds in response time and removed from the dataset. One participant (in placebo group) was removed owing to incorrect button presses during the task, leading to an excess number of missed/non-registered trials.

Additional exclusion

One participant (citalopram group) was removed from all final analysis after it emerged they had not disclosed information that would have rendered them ineligible for the study.

Reward task analysis

Analyses were similar to those described in chapter 3. They will be briefly recapped below.

Taste task

Linear regression models were created including pre-intervention pleasurableness, post-intervention intensity, post-intervention anticipation and allocation as predictor variables.

A preference for the simplest models that adequately explained each dataset was taken, unless there was a significant improvement in fit of more complicated models (e.g. improved goodness of fit with higher R^2, lower RSE & lower AIC/BIC).

Similar approaches were taken in selecting the best fitting models for post-intervention anticipation and intensity of taste.

Effort expenditure task

To compare overall offer acceptance, the total number and proportion of offers accepted, in total and across each level of reward, effort and reward x effort level, was calculated as an outcome variable. An unpaired t test was performed to compare overall mean proportion of offers accepted. The proportion of offers accepted within each effort level or reward levels was calculated for each participant and separate mixed effects linear regression were performed (i.e. one for effort and one for reward).

To explore the relationship between raw offer acceptance (accepting an offer was analysed as a binary yes or no) between groups at each unique effort x reward combination, a logistic regression was performed for the whole dataset with fixed effects of reward, effort and allocation group and reward and effort as within subject random factors.

To calculate individual probabilities of accepting a particular offer at the individual trial level for each participant, the mixed effects logistic regression was used to calculate the log odds of accepting an offer for each trial and then transformed to a probability of accepting an offer using the following formula:

$$\mathrm{P(accept)} = 1 \,/\, (1 + e^{(-\,\mathrm{log\ odds})})$$

To examine the effect of allocation on reaction time, reaction times were log transformed and compared using a t-test. A mixed-effects linear regression was performed with allocation, reward level and effort level as factors.

Mean grip strength, grouped by allocation, effort & reward level, was also calculated from the final 10 trials within the task that requested effort and analysed using a linear regression.

PILT

Each subject underwent an equivalent of two runs of the 30 trials of the win & loss condition. An average optimal choice for each trial for each participant was calculated (taking a value of either 0/2, 1/2 or 2/2). This was used to calculate the proportion of participants in each group selecting the optimal choice at each trial. Data was separated into win and loss conditions, then mean optimal choice in each trial (1-30) was compared between groups using a two-way ANOVA test (with allocation and trial number as fixed effects). Reaction times were log transformed to enable normal distribution for subsequent repeated measure ANOVA testing.

Results

Demographics and pre-intervention questionnaire results

No significant differences in psychometric questionnaires between groups was observed, however a significant difference in age occurred, specifically the citalopram group were younger than the placebo group (Table 1). No significant difference between groups existed for ethnicity, income or education level. Psychometric questionnaire revealed no significant differences in symptoms related to reward processing and anhedonia. However, a non-significant difference appeared in the Beck Depression Inventory rating between groups, with the citalopram group reporting a higher score i.e. more depressive symptomatology, than the control group, albeit still not above a clinical threshold.

Table 1. Baseline Questionnaire results

	Citalopram *n* = 21	Placebo *n* = 21
Age	32.3 (14.1)	44.2 (17.6)
Male/Female	8/13	7/14
BDI	2.4 (0.5)	1.2 (0.3)
SHAPS	20.0 (0.9)	19.9 (0.7)
AMI: Emotional sensitivity	17.5 (0.5)	17.5 (0.8)
AMI: Social motivation	15.5 (1.0)	14.7 (0.8)
AMI: Behavioural activation	17.7 (0.9)	16.6 (0.7)
AMI: total	50.7 (1.9)	48.7 (1.6)
PVSS-21	157.5 (4.7)	156.9 (4.1)

BDI: Beck Depression Inventory; SHAPS: Snaith-Hamilton Pleasure Scale; AMI: Apathy Motivation index; PVSS-21: Positive Valence Sub-Scale. Values are mean values with S.E.M in parentheses. Age is in years with S.D. in parentheses.

Post intervention questionnaire results

The Oxford Depression Questionnaire indicates the citalopram group had higher (i.e. worse) scores for general reduction in emotions, positive emotions and antidepressant associated emotional blunting (which is the total of reduction in positive emotions and not caring, which validation indicates may be closely related to antidepressant-associated emotional blunting) (Table 2).

No significant difference emerged between groups in anxiety symptomatology, either pre- or post-intervention, similarly within groups between time points. Likewise, no difference was observed for anhedonic symptoms (Table 3).

Table 2. Oxford Depression Questionnaire results

	citalopram	placebo	*t value*	*df*	*p*
Total	31.5 (2.5)	24.9 (1.2)	2.40	28	0.023
General reduction in emotions	9.5 (0.95)	7.1 (0.6)	2.21	31	0.034
Reduction in positive emotions	7.8 (0.6)	5.5 (0.2)	3.32	24	0.003
Emotional detachment from others	6.8 (0.7)	6.1 (0.5)	0.79	33	0.437
Not caring	7.5 (0.6)	6.2 (0.3)	1.87	31	0.070
Antidepressant as cause	8.5 (0.9)	6.7 (0.6)	1.62	32	0.114
Antidepressant-associated emotional blunting	15.2 (1.1)	11.7 (0.5)	2.96	28	0.006

Values are mean values with S.E.M in parentheses

Table 3. TEPS & STAI questionnaire results

	pre		post	
	placebo	citalopram	placebo	citalopram
STAI (state)	28.9 (0.9)	29.4 (1.4)	28.0 (0.9)	29.7 (1.6)
STAI (trait)	30.6 (1.0)	31.4 (1.5)	30.7 (1.0)	31.0 (1. 7)
TEPS (total)	80.6 (1.1)	83.3 (1.9)	86.6 (1.6)	89.9 (2.0)
TEPS (ant)	40.2 (1.1)	42.1 (1.3)	45.6 (1.4)	47.6 (1.4)
TEPS (cons)	40.2 (0.6)	41.0 (0.96)	41.1 (0.8)	42.3 (0.8)

TEPS = Temporal Evaluation of Please Scale; TEPS (ant) = anticipatory subscale; TEPS (cons) = consummatory subscale; TEPS (tot) = total of ant + cons subscales scores. STAI = State Trait Anxiety Inventory. Value represent mean and S.E.M values in parentheses

Reward processing

Taste task

Pleasurableness

A non-significant main effect of allocation was observed for the post-intervention pleasurableness of the sour taste (β = 5.746, p = 0.098), indicating the citalopram group found the sour taste less pleasurable than the placebo group. No significant main effect of allocation was observed for any of the remaining tastes (p > 0.05) (Fig. 1). A non-significant, three-way interaction effect of allocation x intensity x anticipation was observed for bitter tastes (β = -0.009, p = 0.099). This indicates that the placebo group have slightly lower post-intervention pleasure than the citalopram group when taking in to account post-intervention intensity and anticipation. This effect is weak and significance marginal however so this result should be taken with caution.

Anticipation

A non-significant main effect of allocation was observed for the post-intervention anticipation of the sweet taste (β = 6.083, p = 0.065), indicating the citalopram group anticipated the sweet taste to be less pleasurable compared to the placebo group.

Intensity

A non-significant main effect of allocation was observed for the post-intervention intensity of the salt taste (β = -10.309, p = 0.054), indicating the citalopram group found the salt taste more intense than the placebo group.

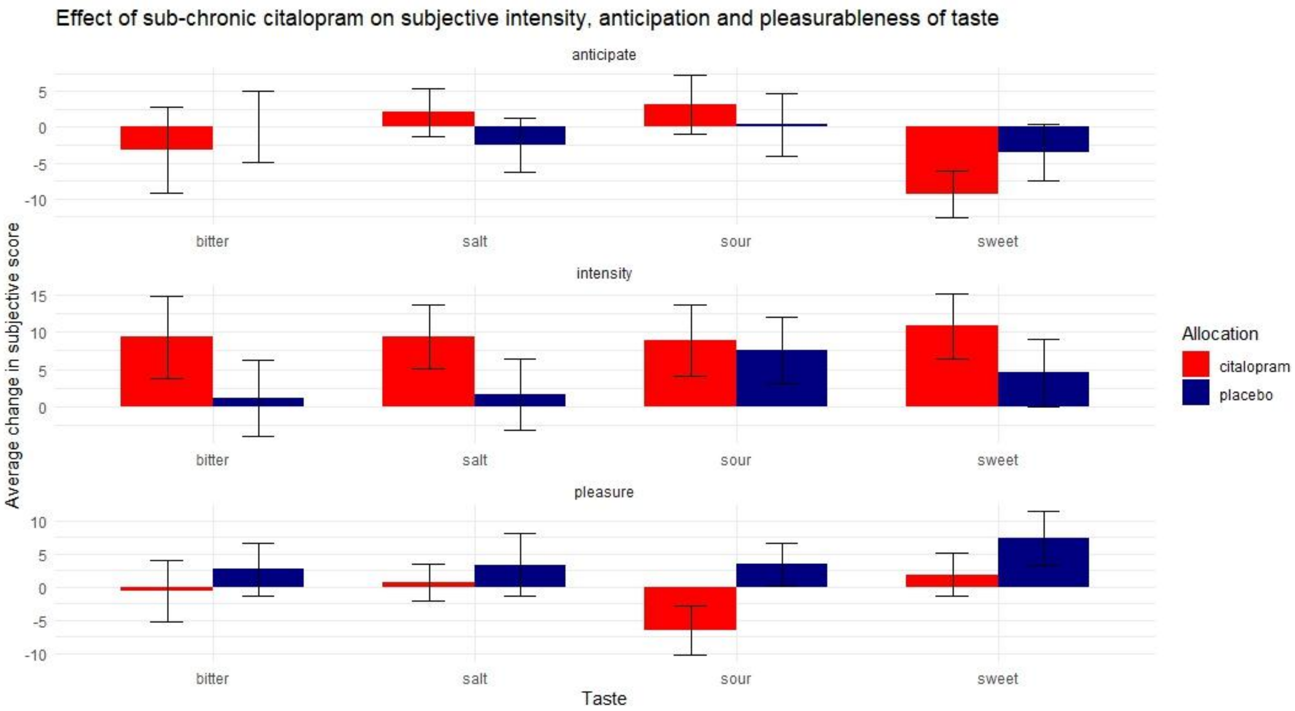


Figure 1: Barchart of the mean change in rating from baseline for each stage (anticipation, intensity & pleasurableness) for each taste (bitter, salt, sour & sweet) between groups. Error bars = 95% CI

Effort expenditure task

Overall comparison of offer acceptance

No significant difference between groups in overall acceptance rate was identified [$t_{(38)}$ = -0.50, p > 0.5; citalopram = 0.67, placebo = 0.69].

Comparison at level of effort or reward

The mixed effects linear regression for effort only indicates no significant main effect of allocation, or interacting effect between allocation and effort level, on proportion of offers accepted (Fig. 2). The mixed effects linear regression for reward level indicates non-significant main effect of allocation on offer acceptance (β = 0.13, p = 0.074) and significant interaction effect between allocation * reward level (β = -0.036, p = 0.042) (Fig. 3).

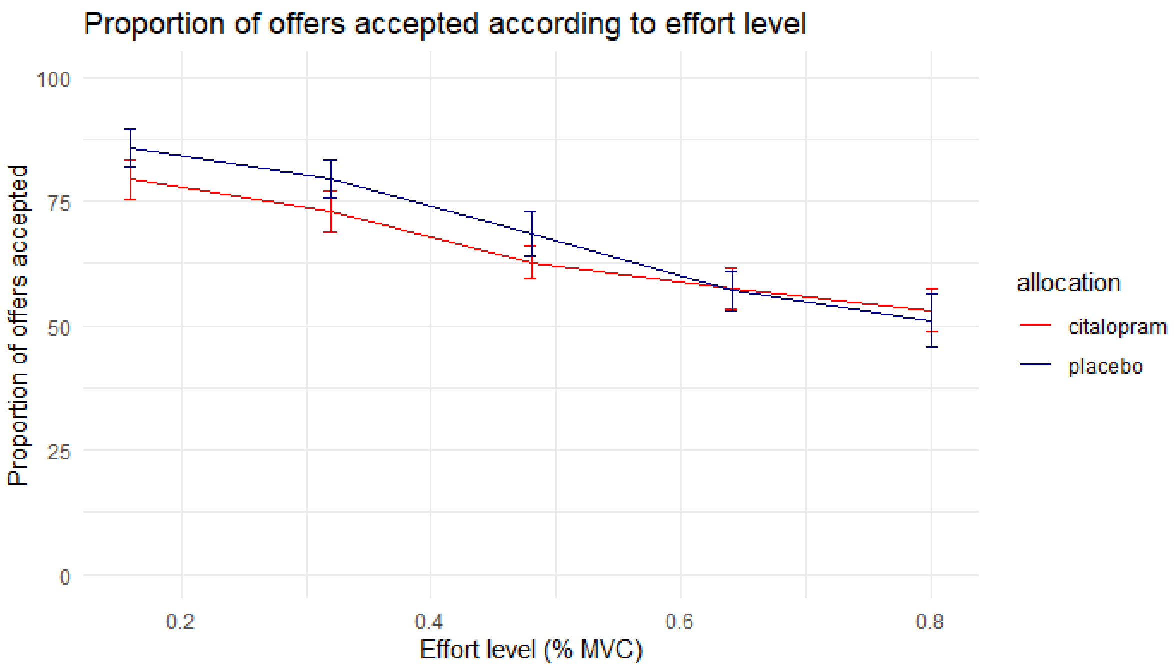

Proportion of offers accepted according to effort level

Figure 2: Mean proportion of offers accepted at each effort level.
Error bars = +/- 1 sem


Proportion of offers accepted according to reward level

Figure 3: Mean proportion of offers accepted at each rewadr level between groups.
Error bars = +/- 1 sem

Comparison of level of effort and reward

The interaction effect of effort and reward was explored by dividing each offer into its unique reward x effort combination (25 levels in total derived from five levels of reward and five levels of effort). The proportion of offers accepted within each group at each unique offer combination was calculated. The mixed effects linear regression indicated a main effect of allocation ($\beta = 0.28$, $p = 0.006$; Cohen's d = -0.06, 95% C.I.: -0.19 to 0.06) and interaction effect of reward * allocation ($\beta = -0.059$, $p = 0.02$). However, estimated marginal means did not identify a significant group difference at any separate reward level, which may be due insufficient power/small effect sizes /high variability within reward levels. However, there was a trend of the citalopram accepting less offers proportional at reward levels 1-3 but more offers at reward levels 4-5 (Fig. 4).

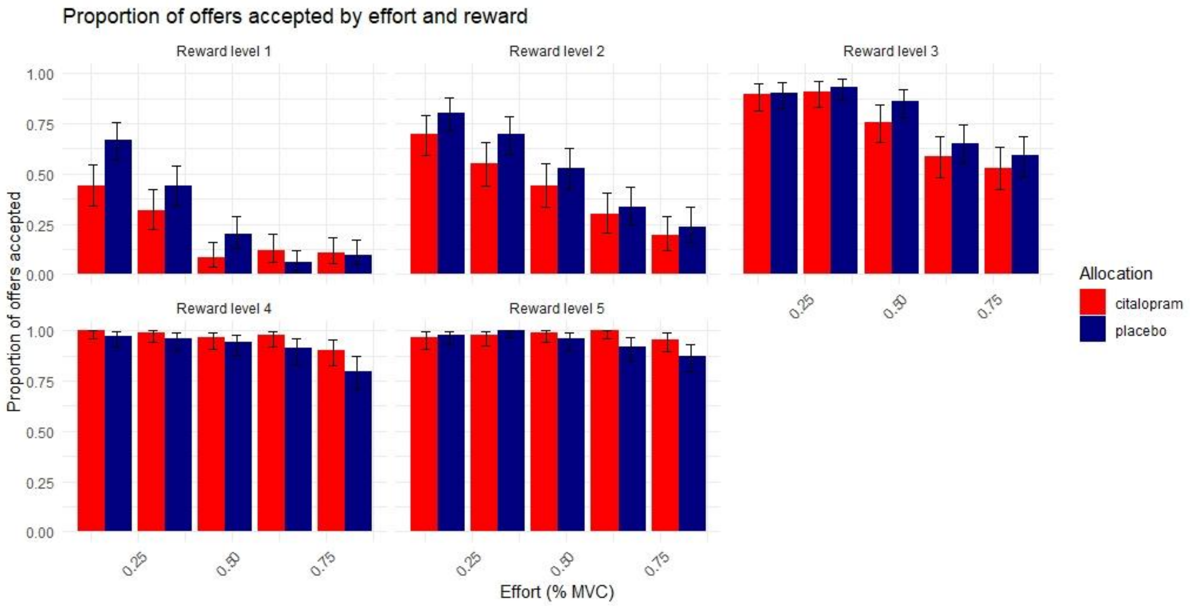


Figure 4: Proportion of offers accepted at each unique effort*reward level combination between groups.
Proportion derived from total offers accepted within group / total offers made within group, for each unique effort*reward level combination

The mixed-effects logistic regression indicated significant effects were observed for reward level ($\beta_1 = 4.19$, $p < 0.005$), effort level ($\beta_2 = -1.12$, $p < 0.001$) and a significant two-way interaction between effort and reward levels ($\beta_4 = 0.79$, $p = 0.0248$). This indicates that participants weigh up both reward and effort level when deciding to accept an offer, or put another way they are less likely to accept a high reward offer when the effort level is also high compared to when effort required is low. The main effect of allocation was not significant ($\beta_3 = 0.56$, $p > 0.1$). Somewhat consistent with the mixed linear regression result, a non-significant two-way interaction trend was observed between reward level and allocation ($\beta_5 = -1.01$, $p = 0.07$), indicating that the effect of increasing reward value on accepting an offer may be increased in the citalopram group compared to the placebo group (Fig. 4). The non-significant three-way interaction between allocation, effort and reward level ($\beta_7 = 0.23$, $p = 0.54$) suggests the interaction between reward value and allocation on offer acceptance does not vary across effort levels.

To visualise the trend-level interaction between reward level and allocation the probability of accepting an offer was derived from the mean log odds of accepting an offer from the logistic regression model (Fig. 5). It indicates at lower reward levels the probability of accepting an offer is lower in the citalopram group compared to the placebo group.

To investigate the non-significant two-way interaction between reward value and allocation further, estimated marginal means from the mixed-effects logistic regression was calculated for each level of reward (whilst averaging over effort levels). For each reward level a pair-wise contrast comparing citalopram with placebo on the log-odds scale was performed across the five reward levels; correcting for multiple comparisons using the Holms method. Citalopram reduced the odds of accepting an offer at reward level 1 by about 86 % relative to placebo (OR = 0.14, 95 % CI [0.02–0.87], $p_{adj} = 0.087$). In the remaining reward levels (2-5), the odds ratio of accepting an offer between allocation groups became progressively similar, with no significant difference between them.

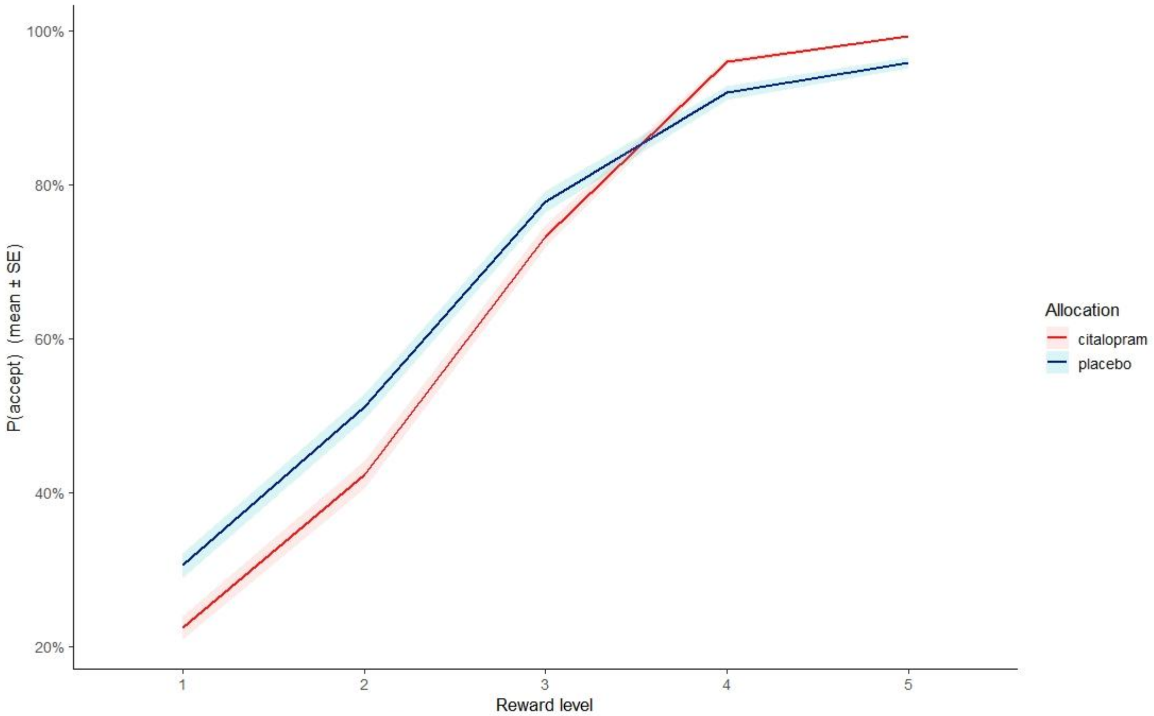


Figure 5. Scatter plot of mean probability of accepting an offer at each reward level between groups.
Mean probability calculated using the estimated marginal means from the full mixed effects logistic regression model.
Error bars = +/- 1 S.E.M

Reaction times

No significant difference in log transformed reaction times was observed between groups ($t_{(36)}$ = 0.169, p > 0.5, 95% CI = -0.169 – 0.200, citalopram = 0.624, placebo = 0.609).

A mixed-effects linear regression of log transformed reaction times indicated a significant effect of reward level (β = -0.047, p = 0.0026), indicating with increasing reward, reaction times became faster. Main effects of allocation and effort level on log transformed reaction times were non-significant (p > 0.5). Two- and three- way interaction effects of reward and/or effort with allocation were non-significant (p > 0.5). A small, non-significant two-way interaction between effort and reward level was observed however (β = 0.0089, p = 0.056), indicating the effect of reward level on reaction time was influenced by effort level required (Fig. 6).

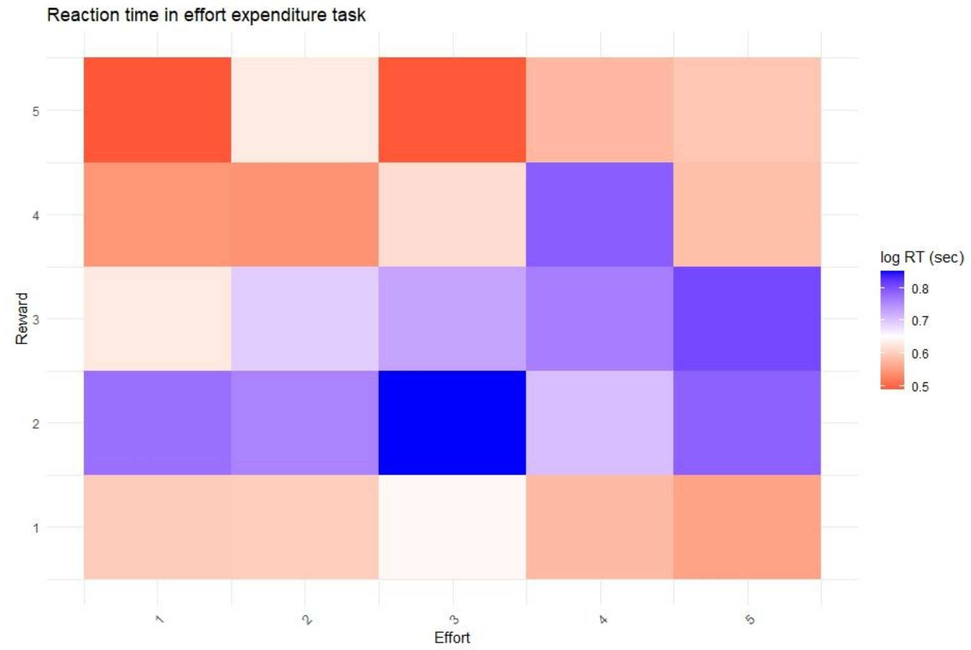


Figure 6: Heatmap of log-transformed mean of reaction times of all participants, group by effort and reward level

Maximum voluntary contraction strength

Linear regression indicated a small but significant main effect of reward ($\beta = 0.005$, $p = 0.0056$) and effort level ($\beta = 0.009$, $p = 0.0043$) on force applied in the offers which required effort. No significant main, or interaction, effect of allocation was observed ($p > 0.5$).

Summary

Sub-chronic citalopram appears to induce a small, non-significant reduction in willingness to accept offers at the lowest levels of reward. Sub-chronic citalopram does not seem to influence maximum force when applying grip strength for reward or reaction time across offers, indicating no alteration of motor invigoration rewarding stimuli was observed.

PILT

Comparison of optimal choice

A significant main effect of allocation on optimal choice was found between in loss trials [$F_{(1, 56)} = 6.54$, $p = 0.013$] and win trials [$F_{(1, 56)} = 7.87$, $p = 0.0069$].

Estimated marginal means were calculated to further investigate these results. This indicated in the loss condition the citalopram group made more optimal choices than the placebo group (($t_{(115)} = 2.58$, $p = 0.011$; citalopram = 0.75, S.E. = 0.028; placebo = 0.70, S.E. = 0.014) (Fig. 9). However, in the win condition the placebo group made more optimal choices than the citalopram group ($t_{(115)}$ = -2.71, p = 0.077; citalopram = 0.78, S.E. = 0.014; placebo = 0.83, S.E. = 0.014) (Fig. 9).

Analysis of mean optimal choice after trial 10 was performed and indicated a significant difference between groups exists in both the win [$F_{(1, 38)}$ = 9.34, p = 0.0041] and loss [$F_{(1, 38)}$ = 5.825, p = 0.021] conditions.

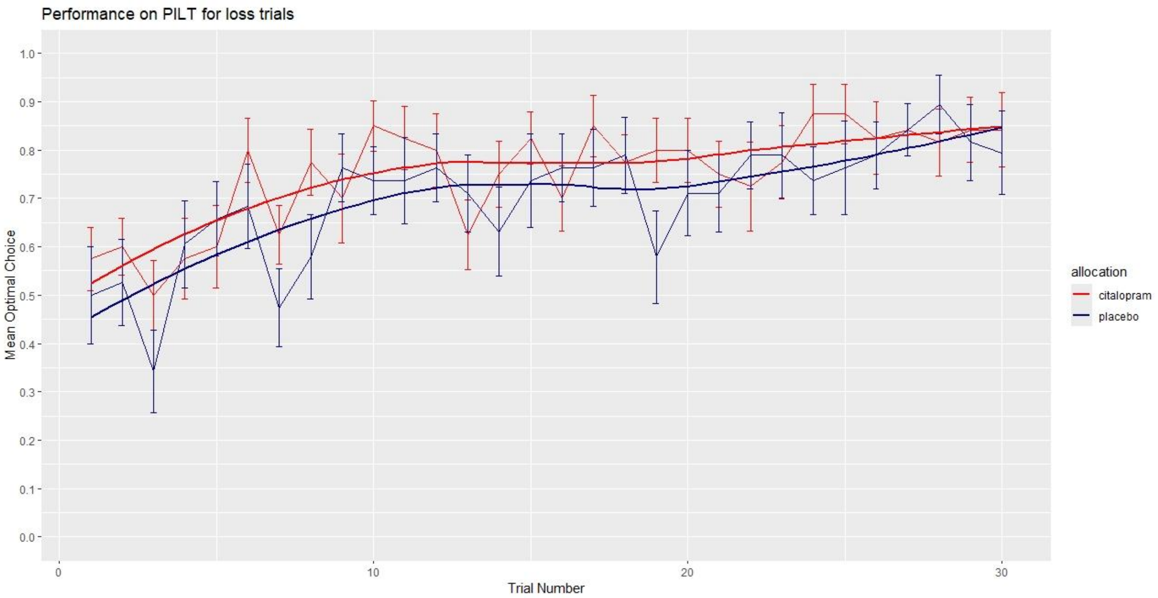


Figure 7: Mean optimal choice in loss trials. mean optimal choice was calculated by subjects chosing the symbol least likely to lose in a loss trial. The number of optimal choice trials was divided by total loss trials for each participant to give proportion of optimal choices at each trial (e.g. two runs therefore optimal choice can be made on both, one or neither trial giving a proportion of either 0, 0.5 or 1). The mean of porportion of optimal choices was then calculated for each group. Error bars = +/- 1 S.E.M.
Line of best fit uses LOESS (Locally Estimated Scatterplot Smoothing) function in geom_smooth() in R

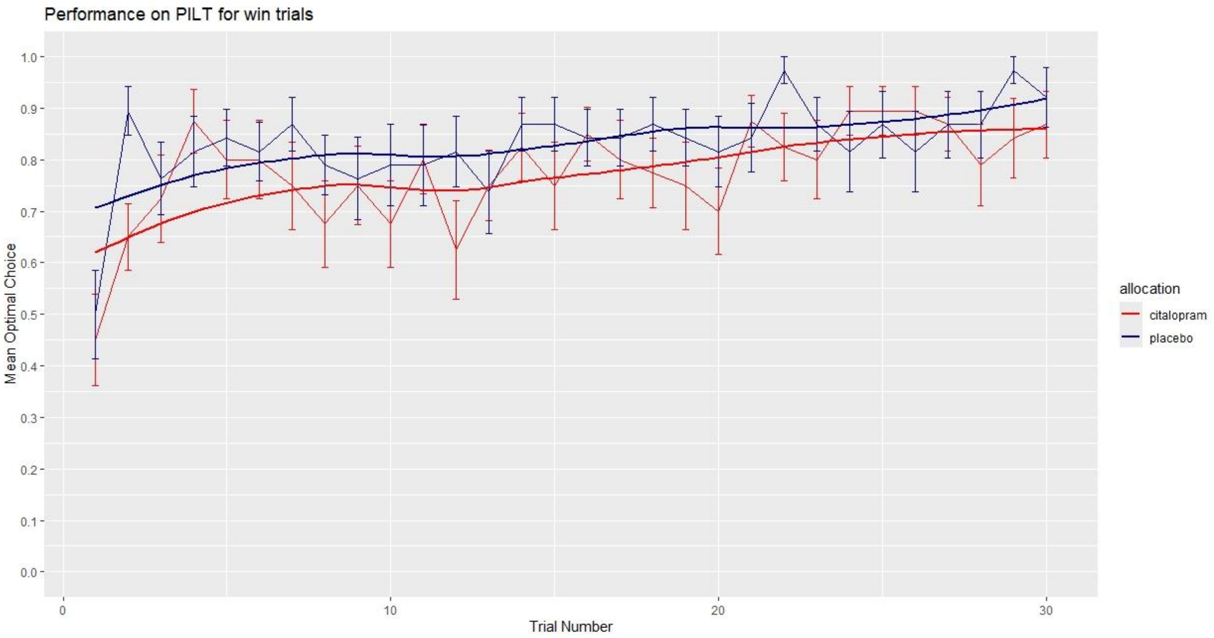


Figure 8: Mean optimal choice in win trials. mean optimal choice was calculated by subjects chosing the symbol most likely to win in a win trial. The number of optimal choice trials was divided by total win trials for each participant to give proportion of optimal choices at each trial (e.g. two runs therefore optimal choice can be made on both, one or neither trial giving a proportion of either 0, 0.5 or 1). The mean of porportion of optimal choices was then calculated for each group. Error bars = +/- 1 S.E.M.

Line of best fit uses LOESS (Locally Estimated Scatterplot Smoothing) function in geom_smooth() in R

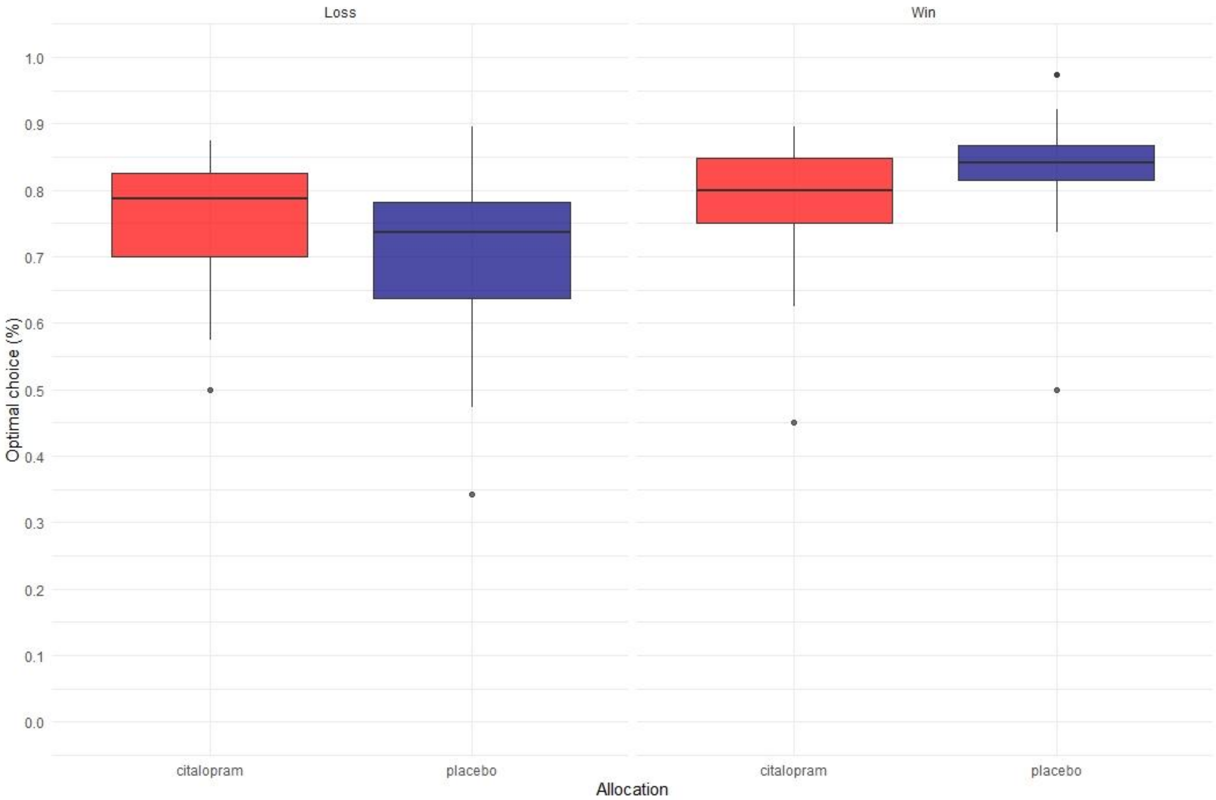


Figure 9. Proportion of subjects making the optimal choice on loss and win trials.
Each point is a participant's mean proportion across 120 trials; boxes show the group distribution.

Comparison of reaction time

A repeated measure ANOVA indicated a significant effect of trial number ($F_{(14, 21)} = 2.28$, $p = 0.043$), indicating participants reacted faster as the task progressed. No main effect of allocation or trial type on reaction time was observed however ($p > 0.1$).

Conclusion

Sub-chronic citalopram increases sensitivity to loss but diminishes sensitivity to win trials

Summary

No statistically significant differences between allocation groups were observed for pleasure, anticipation or intensity in the taste task, indicating sub chronic citalopram does not influence consummatory aspects of rewarding or aversive primary stimuli when measured subjectively. However non-significant effects of citalopram were observed in some instances, including a reduced anticipation of pleasure for a rewarding sweet taste and a reduced pleasure of an aversive sour taste.

No significant difference arose in the effort expenditure task between groups. However, a small, non-significant two-way interaction effect on accepting an offer appeared between reward and allocation. Cautiously, one could hypothesize citalopram reduces sensitivity to low reward in this effort-based task.

In contrast from the instrumental learning task one could hypothesize sub-chronic citalopram increases sensitivity to loss but reduces sensitivity to reward in a reward learning task.

Discussion

Primary taste reward

The few studies that have examined the effect of SSRI treatment on taste as a primary rewarding or aversive stimulus, mainly observe minimal effect of SSRI on the experience of taste. Similarly, results presented here indicate sub-chronic citalopram has no significant effect on subjective experience of pleasurable or aversive taste stimuli.

Increasing 5-HT increases aversiveness of rewarding and aversive tastes in animals

Animal studies using fenfluramine, an agent that increases serotonin levels, indicate divergent effects on rewarding and aversive tastes. One study of rats, measuring separate aversive and hedonic behaviours, found only the aversive responses to quinine were increased with higher doses of *dl*-fenfluramine and only aversive responses (not hedonic responses) to sucrose were increased at the highest *dl*-fenfluramine dose (Barnfield et al. 1994). Conversely a separate study found *d*-fenfluramine, an agent more specific for the serotonin system, reduced hedonic responses to sucrose but had no significant effect on aversive behaviours to quinine solution (Gray and Cooper 1996). Differences were attributed to contrasting methodology, as Gray et al. used a weaker solution of aversive quinine, potentially below a threshold for eliciting aversive behaviours (Gray and Cooper 1996). Secondly Gray et al. (1996) used voluntary exposure to solution, enabling animals to voluntarily avoid or pursue tastes and thus limit the resultant aversive and hedonic behaviours respectively (Gray and Cooper 1996). Importantly Gray et al. (1996) did observe a reduction in consumption of both sucrose and quinine solutions (Gray and Cooper 1996), which is consistent with increasing the aversive experience of both solutions observed by Barnfield et al (Barnfield et al. 1994).

SSRI do not alter subjective liking or wanting of tastes

A healthy volunteer study using a 7-day course of citalopram found no change in subjective liking or wanting of either a rewarding chocolate taste or an aversive strawberry taste (McCabe et al. 2010), consistent with results presented here.

However, one within-subject healthy volunteer study found that acute SSRI administration increased sensitivity to both sweet and bitter tastes (Heath et al. 2006).

Motivation

No consistent significant differences arose between allocation groups in willingness to exert grip effort for reward. However, at lower reward levels citalopram appeared to decrease willingness to exert effort, which reversed at higher reward levels, which could support findings in the literature, albeit in different species and contexts.

Pre-clinical evidence indicates SSRI decrease willingness to exert effort. This evidence arises from a study using a lever-pressing operant behaviour task in rats that found varying concentrations of a single dose of escitalopram could not reverse the deleterious effects of tetrabenazine (a vesicular monoamine transport inhibitor that has been shown to reduce willingness to exert effort in such tasks) on willingness to exert effort for reward (Yohn et al. 2016). Using the same lever pressing task, as well as an additional wheel running task, another study found fluoxetine also reduced willingness to exert effort (Presby et al. 2021).

On the premise of shared over-lapping mechanisms for apathy and anhedonia (Husain 2018), epidemiological data is consistent with this SSRI-related amotivation, albeit in clinical populations and self-report (Fava et al. 2006; Bolling and Kohlenberg 2004; Wongpakaran et al. 2007). A postulated biological basis for this view is based upon case reports of SSRI inducing a frontal lobe syndrome of apathy and amotivation with a reduction in frontal lobe blood flow, which could either be through direct modulation of frontal lobe activity by the serotonergic system or via an intermediary, such as the dopaminergic system (Barnhart, Makela, and Latocha 2004). Further evidence for its contribution is its dose-dependent nature and reversibility with SSRI cessation (Barnhart, Makela, and Latocha 2004; Garland and Baerg 2001).

Chronic SSRI increases willingness to exert effort in humans

A placebo-controlled, healthy volunteer study administering an 8-week course of citalopram and using handgrip force as effort, found the intervention group received more money overall compared to the placebo group (Meyniel et al. 2016). The authors interpreted this as an 8-week course of citalopram increasing the willingness to exert effort for reward, with subsequent computational analysis indicating this was due to reduction in effort cost as opposed to increase in value of the monetary reward (Meyniel et al. 2016). Of note, this study observed no significant difference between groups in reward amount received at approximately 3 days or 60 days from citalopram initiation (a group difference was observed in testing between days 12-15 however). This is important in placing results in context of duration of treatment, as this can point to specific pharmacological mechanistic effects that could be responsible for behaviour observed but also for translational perspectives e.g. patients are often on medication long-term, therefore it would be important to establish whether effects on reward processes persist during longer-term treatment.

The absence of consistent effect in the present study may be due to the duration of SSRI being insufficient to influence the behavioural indicators of effort cost, as seen in healthy volunteers previously (Meyniel et al. 2016). Finally, with the observation that not all MDD patients prescribed SSRI develop apathy, some have posited that the influence of SSRI on developing apathy and amotivation could be mediated by genotype (Barnhart, Makela, and Latocha 2004). This is supported by the observation that after acute tryptophan depletion individuals possessing the short allele polymorphism in the promoter region of the 5-HT transporter gene, conferring vulnerability to depression and SSRI-resistance, demonstrate a reduction in reaction speed (e.g. motivation) in a Cued-reinforcement Reaction Time Task compared to individuals homozygous for the long allele (Roiser et al. 2006). One might surmise therefore that in the subjects used here, a mixture of genotypes was present, therefore any overall effect of serotonergic manipulation on motivation responses and decisions may not be apparent.

Learning

SSRI reduce reward learning but increase aversive learning

Results presented here are consistent with the computational results from a similar study using a 7-day course of citalopram, that found a reduction in reward learning but enhancement in aversive learning (Michely et al. 2022). More specifically in the latter study, SSRIs enhanced the impact of failure, but not success, on willingness to gamble, with citalopram increasing the avoidance of gambles after a loss/failure, compared to placebo.

The similarities in results could be explained by the similar duration of treatment as well as the broad nature of each task, for example choices had an implicit but different probability of winning. However, there were some important differences to highlight between the studies. Firstly Michely et al. (2022) used three options (high, low & even), in which participants were given one of the options and then had to weigh up accepting the deck offered or accept a 'non-gamble' option of 50:50 probabilities. In contrast here the same two options were presented together (high vs low). The task presented here is simpler therefore the direct comparison of implicit high vs low probabilities, allow learning and sensitivity to be examined without the further cognitive burden that accompanies having to remember the probability contingencies of a third option. Secondly, the task used by Michely et al. (2022) explicitly introduces the concept of gambling, which could be influenced by other psychological processes such as impulsivity, as opposed to just learning and sensitivity. Finally, the different outcome measure used here influences direct comparison of results (outcome measure here is choosing the optimal choice yes/no; as opposed to willing to gamble yes/no, which could align more with impulsivity). Therefore, the result presented here contribute to our understanding by supporting the finding of increased aversive learning and reduced reward learning and extends it by removing the possible confounds of impulsivity and willingness to gamble.

A second study using a 14-day duration of citalopram, found no difference in learning about reward or effort (labelled as aversive) in healthy volunteers (Scholl et al. 2017). This was attributed to healthy volunteers having an optimal ceiling of learning that would not be improved further with medication. It therefore lowered the 'ceiling' with uncertainty (e.g. reward would be real or fake; whether high effort or low effort would be required for the reward – namely effort discounting), which it viewed as interference for learning and attention for the task. It found SSRI improved resilience of reward learning to the interference of uncertainty, compared to placebo. Several reasons could contribute to the discrepancy with our results of SSRI worsening reward learning.

Firstly, the task uses a differing aversive factor, namely effort (vs monetary loss, used here), which involves the sub-component of motivation and wanting within reward processing, introducing effort-discounting, as opposed to simply learning and sensitivity. Secondly, the aversive outcomes were paired to rewarding outcomes within the trials, as opposed to here having loss separate from win (each loss trial having either no loss or loss, similarly each win trial having either win or no win). The coupling of effort and reward could confound conclusions drawn about learning alone.

Another study used a 21-day course of citalopram in a probabilistic reversal learning task involving an acquisition phase and a single reversal phase, and found no significant differences between groups in standard outcome measures such as errors (akin to non-optimal choices here) and probability of switching choices in either phase of the task (Langley et al. 2023). However, computational analysis revealed the escitalopram group demonstrated reduced sensitivity to outcomes (both win & loss), without affecting learning rates or stimulus stickiness/perseveration.

One possible explanation for null results from initial analysis by Langley et al. (2023) differing from the potential reduction in reward but increase in loss sensitivity seen here, could be due to the nature of the tasks. The PILT used here uses static probability contingencies, suited to studying aspects of reward processing such as sensitivity and learning rates. The reversal learning task used by Langley et al. (2023), would be cognitively more demanding, such as flexibility in learning under uncertainty or introduce other behaviours such as perseveration. Similar to the challenges in directly comparing the present results to Scholl et al. (2017) this design may confound any effect of SSRI on learning and sensitivity. However, the same group used an identical task to that used here, again with a 21-day course of escitalopram and through Bayesian modelling found reduced learning from punishment, opposite to what was observed in the present study (Langley et al. 2024).

A key difference in both Scholl et al. (2017) and Langley et al. (2023) studies is the duration of treatment. It maybe that the required molecular, synaptic, network changes required to elicit behavioural change are not yet manifest to a sufficient degree to influence behavioural changes after 7-days of SSRI (Page et al. 2024). This highlights a further theme in studies involving SSRI medications, namely the frequently contrasting effects on neuropsychological processes. However, this may well reflect the heterogeneity of studies, such as the use of varying durations of administrations, with differing tasks that measure contrasting cognitive processes (e.g. reversal learning, probability learning & discounting, instrumental or passive engagement, differing aversive stimuli as effort, electric shock or monetary loss), and differing computational models/analysis revealing differing latent processes e.g. resistant to interference, more sensitive to outcomes, increased learning from punishment but not reward.

Although results presented here are consistent with a previous study (Michely et al. 2022), they are not in agreement with a recent study using the same PILT task that increasing brain 5HT release via alternative pharmacological means, namely fenfluramine (Colwell et al. 2024). In this study, sub-chronic treatment with fenfluramine (8 days) decreased sensitivity to loss in the PILT task.

In the light of these contradictions it is helpful to examine the effects of tryptophan depletion on sensitivity to loss because this dietary manipulation should produce the opposite on effect on brain 5-HT levels to that seen with 7-days' SSRI treatment (i.e., a decrease in synaptic 5-HT levels rather than an increase). Again, there is difficulty because of the number of different experimental models used in the depletion studies. However, in a review, Faulkner & Deakin (2014), concluded that acute tryptophan depletion generally diminished the ability of punishment to influence or restrain behaviour (Faulkner and Deakin 2014) - that is, the opposite to the present finding where SSRI treatment increased aversive learning.

The effect of tryptophan depletion is therefore similar to that reported by Colwell et al (2024) with fenfluramine, namely a reduction in optimal choices in loss trials. This observation could be consistent with a view that fenfluramine while transiently increasing synaptic 5-HT, can subsequently deplete serotonin reserves (Choi et al. 2002; Fattaccini, Gozlan, and Hamon 1991).

Another possible explanation for these divergent results could be related to the mechanism of action of fenfluramine. Fenfluramine increases synaptic 5-HT by disrupting the storage of 5-HT in presynaptic neurons and reversing the direction of action of the serotonin transporter. This results in a large, action-potential independent increases in extracellular 5-HT which are much greater than that seen with SSRIs and may dysregulate the serotonin system.

Finally, there is evidence that most of fenfluramine's behavioural effects in animals and humans are mediated by the 5-HT_{2C} receptor (Vickers et al. 1999; Vickers, Dourish, and Kennett 2001; Goodall et al. 1993), perhaps due to the 5-HT_{2C} agonist properties of its metabolite, norfenfluramine. This could provide fenfluramine with a more selective action on 5-HT neurons than SSRIs. It is also consistent with animal experimental work indicating that direct 5-HT_{2C} receptor agonism in animals reduces sensitivity to punishment (Phillips et al. 2018).

Subjective ratings of mood and pleasure

Results of the Oxford Depression Questionnaire (ODQ) indicated citalopram worsens subjective experience of general and positive emotions, particularly emotional blunting thought attributable to antidepressants, in healthy volunteers. This is consistent with qualitative results from MDD patients prescribed SSRI medication (Price, Cole, and Goodwin 2009). Intriguingly the significant group differences in ODQ responses did not significantly correlate with the group differences in reward or loss learning. Speculatively this could indicate that differences in reward learning from a 7-day course of citalopram may not arise from emotional blunting.

Interestingly, there were no significant increases in state anxiety in the citalopram group, as SSRIs are commonly thought of as anxiogenic early in treatment. Also, there were no subjective changes in the TEPS anhedonia questionnaire, indicating that subjectively SSRI did not lead to consummatory or anticipatory anhedonic symptoms. The latter is important as it could indicate the behavioural results seen in the reward learning task would not yet have translated into subjective experiences of anhedonia. Speculatively, this could either be due to the behavioural effects not being of significant size to influence conscious experience of pleasure or perhaps more time-related, in that longer periods of behavioural change are required before they declare themselves subjectively in the form of questionnaire responses.

Limitations

There are several limitations to the present study that warrant caution when interpreting the results presented here.

No citalopram levels were recorded during the study which introduces much variability with the intervention effects. This could be either through non-compliance or individual differences in pharmacokinetics of SSRIs. If citalopram levels were available, we could infer presumed drug effects with behavioural effects with greater confidence through correlation or regression analysis.

In spite of randomisation, baseline differences in age (e.g. the placebo group were older) and BDI scores (a trend-significance of the citalopram group having higher BDI scores) was observed. A narrative review presents evidence that older age is associated with increased loss sensitivity & learning and that this could be related to depletion, or possibly dysregulation, of serotonergic and dopaminergic function with increasing age (Eppinger, Hämmerer, and Li 2011). The implications for the present study could be that not only are there behavioural differences between groups (e.g. the placebo group are more sensitive and learn more from losses in the PILT), sensitivity to an SSRI intervention may also be different. One could conjecture that, if at baseline the placebo group are more sensitive to loss than the SSRI group, the group effect of SSRI increasing loss sensitivity and learning would be attenuated.

Similarly, the SSRI group subjectively felt lower in mood at baseline, as defined by a higher BDI score. Whilst this may not represent clinical MDD, it can be informative in that a meta-analysis of reward processing in MDD, compared to healthy controls, demonstrated reduced reward valuation, grip force strength and reinforcement learning for rewards (of note loss was not investigated) (Halahakoon et al. 2020). Speculatively this could mean at baseline in the current study, the SSRI group would have reduced reward valuation, reward learning and willingness to exert effort. The implications for the current study could be that the effect of citalopram in reward trials could be due to baseline differences in the group, whereas the null findings in the effort grip task could represent an effect of SSRI in normalising' willingness to exert effort. This interpretation is highly speculative however, and uses extrapolation from medicated, clinical samples to healthy volunteers, and should be met with caution. The limitation of baseline differences could be mitigated by a baseline behavioural assessment to enable a within-subjects comparison.

A limitation concerning the PILT data is that it was completed whilst undergoing an fMRI scan. One could postulate that responses whilst relaxed sat in a chair in normally lit room and alone may not be the same as responses in an fMRI scanner (e.g. supine, dark but noisy room, trying not to move whilst being watched). Indeed evidence exists indicating behavioural results have not been replicated whilst undergoing an fMRI scan, albeit using a different task to that used here (a working memory task under threat of shock) (Garibbo, Aylward, and Robinson 2019). It has also been suggested that the use of fMRI may also lead to a selection bias in samples (i.e. those less anxious regarding fMRI may enrol, unlike a behavioural only study), which may influence the generalizability of results (Charpentier et al. 2021). Consistent with this is the observation here of trait anxiety scores found here being similar to those found by others who've measured trait anxiety scores of subjects for fMRI studies. Importantly, these are lower than trait anxiety scores for subjects enrolled in behavioural studies (Charpentier et al. 2021).

The 7-day duration of intervention in the present study may limit mechanistic insights drawn. Animal studies indicate 7-day SSRI may be insufficient to induce neuroplasticity, either through not increasing 5HT levels sufficiently or for adequate duration for the desired network or downstream molecular effects to occur. However, meta-analysis of animal micro-dialysis data indicates this may be region specific, for example 5-HT is reduced in the frontal cortex but increased in other regions such as the ventral striatum, ventral tegmental area, prefrontal cortex and hypothalamus after 3 days of SSRI (Fritze, Spanagel, and Noori 2017). However, reinforcing the importance of downstream neuroplastic effects, the key neuroplastic effects may not mirror the increase in short term 5-HT. For example, in rats it was not until 21 days of SSRI treatment that increases in BDNF were observed in the frontal cortex (Nibuya, Morinobu, and Duman 1995) or hippocampus, habenular or thalamic nuclei (de Foubert et al. 2004).

The taste task and learning task have shown to be sensitive to pharmacological intervention in healthy volunteers previously, however these have been with dopaminergic agents (Halahakoon et al. 2024; Kaltenboeck et al. 2022). It may be that tasks are not sensitive to the effects of a 7-day course of SSRI or that they rely on non-serotonergic effects. Secondly, the effort grip task and its analysis used here, whilst similar, is not identical to previous tasks, outcome measures or analysis evaluating the effect of SSRI on effort grip i.e. motor invigoration (Meyniel et al. 2016). The task used here was developed with healthy volunteers (Bonnelle et al. 2016) and principally been used to examine clinical samples (Saleh et al. 2021; Le Heron et al. 2018) and is used here for the first time in an experimental medicine context, therefore it could be possible that the effort grip task as used here is not sensitive to drug interventions in healthy populations, particularly serotonergic manipulations. Furthermore, the task used here measures the cost-benefit decision making stage, distinct from motor invigoration measured by others (Husain and Roiser 2018; Meyniel et al. 2016). Although inter-dependent, it cannot be assumed that serotonergic effects observed in one stage (i.e. motor invigoration) are matched by to effects in earlier stages of processing (i.e. cost benefit decision making).

Summary & conclusions

This chapter has demonstrated a 7-day course of citalopram can improve learning from punishment but worsen learning from reward outcomes, consistent with a recent SSRI study (Michely et al. 2022). It is partially consistent with the absence of early effect of SSRIs on willingness to exert effort for reward, as observed by others (Meyniel et al. 2016). Finally, it is consistent with the absence of effect on subjective ratings of taste stimuli (McCabe et al. 2010). It also suggests that short-term treatment with SSRIs in healthy participants can result in a degree of subjective emotional blunting.

Chapter 5: The effects of sub-chronic citalopram on the neural correlates of reward processing

Introduction

Major Depressive Disorder (MDD) is associated with reduced activation in several fronto-striatal regions during all stages of reward processing (Pizzagalli et al. 2009; Zhang et al. 2013; Borsini et al. 2020), such as the dorsal striatum (caudate & putamen), the ventral striatum (nucleus accumbens) and the pregenual Anterior Cingulate Cortex (pgACC) during tasks investigating reward consumption (Forbes et al. 2006; Pizzagalli et al. 2009), anticipation (Pizzagalli et al. 2009; Zhang et al. 2013; Borsini et al. 2020) and learning (Kumar et al. 2018; Kumar et al. 2008; Gradin et al. 2011; Rupprechter et al. 2020).

A key premise of using neural correlates of reward processing to study anhedonia is being able to link the neural correlates to the subjective experience of anhedonia. Some evidence for this is provided through reward learning, including a focus on prediction errors (i.e. outcome being better (positive) or worse (negative) than expected). MDD patients demonstrated reduced striatal & midbrain signalling during prediction errors in an instrumental learning task compared to healthy volunteers. Importantly, in the medicated depressed group the greater the reduction in midbrain, bilateral caudate and nucleus accumbens signal, the greater the anhedonia severity (Gradin et al. 2011). A second study, using an instrumental learning task involving monetary gains & losses, compared unmedicated depressed patients and healthy volunteers and found no difference in behavioural performance between groups or in prediction error signals in the ventral striatum or insula (Rothkirch et al. 2017). However, a reduction in medial orbitofrontal cortex signal during reward prediction error was observed in patients, which correlated with anhedonia severity, as measured by the SHAPS self-report scale (as did the reduction in ventral striatum activity in patients only) (Rothkirch et al. 2017). Therefore, in the MDD population, one could conclude greater anhedonia symptoms correlate with a greater reduction in midbrain, striatum (dorsa and ventral) and orbito-frontal activation during reward learning tasks.

As described earlier, serotonergic medication appears to not improve, and potentially worsen, the subjective experience of anhedonia (Price 2009). It could be hypothesized that serotonergic medications, specifically SSRI, could therefore lead to either no change or hypoactivation in the aforementioned reward-related brain regions in MDD. Indeed, this possibility is extended to healthy volunteers where it was observed chronic SSRI administration led to reduced activity in reward related regions during receipt of various types of rewarding outcomes (Abler et al. 2011; Macoveanu et al. 2014; McCabe et al. 2010). In contrast, following a 14-day course of citalopram, an increase in activity in certain regions during reward prediction errors was noted (e.g. striatum, mid-cingulate cortex, parietal cortex and vmPFC) (Scholl et al. 2017).

Recently it has been reported that after a 7-day course of citalopram, behavioural differences in reward and aversive processing emerge in healthy volunteers (Michely et al. 2022). However, this study did not measure neural correlates of these findings (Michely et al. 2022) and the aforementioned studies used different stimuli (for instance primary rewards, such as food and erotic videos, rather than secondary monetary rewards) or different durations of treatment (Macoveanu et al. 2014; Scholl et al. 2017) making direct comparisons challenging.

To probe the neural correlates of reward processing in the present chapter, the probabilistic reward learning task, described in Chapter 3, was used. Tasks such as this have been shown to activate regions of interest in the brain dependent on the stage of reward and aversive processing in healthy volunteers. Grey literature evidence has compared baseline differences between MDD and healthy volunteers, using an identical task to that used here (Pickenhan 2021). Whole brain level analysis revealed MDD patients, compared to healthy volunteers, showed reduced neural activation during positive outcomes but increased neural activation during negative outcomes in certain regions (Pickenhan 2021). This finding was corroborated with Region of Interest analyses, specifically depressed patients exhibited increased neural activation during either loss or no win (in win trials) outcomes in bilateral dorsal striatum, right nucleus accumbens and insular cortex but reduced activation in these areas during positive outcomes such as win or no loss (in loss trials) (Pickenhan 2021). It did not find significant correlations between parameter estimates and anhedonic ratings (as defined by SHAPS or PANAS scales).

The monetary incentive delay task (MID) is an established task that can measure neural responses to both anticipation and receipt of win or loss outcomes. A meta-analysis of healthy volunteer, whole brain fMRI studies revealed the striatum (dorsal & ventral) as well as the anterior cingulate and anterior insular cortex are activated during anticipation of either win or loss (Wilson et al. 2018). Furthermore, the left inferior frontal gyrus operculum (MNI: -44, 38, -10) was found to deactivate during anticipation, more so for loss than reward (Wilson et al. 2018). A separate meta-analysis specifically examining loss in the MID task found that during loss anticipation the anterior/median cingulate gyri, bilateral insula, nucleus accumbens and caudate/putamen are activated (Dugré et al. 2018). The same meta-analysis found that during loss receipt the bilateral putamen, right insula and anterior cingulate gyri were activated (Dugré et al. 2018). A further meta-analysis of MID data in healthy volunteers using an alternative, Activation Likelihood Estimation (ALE) analysis approach, found that the bilateral thalami, amygdala and striatal regions were activated during reward anticipation, with a similar activation pattern for loss anticipation (Oldham et al. 2018). During reward outcomes, significant, albeit lower, ALE values were found for the bilateral ventral striatum, bilateral OFC/vmPFC and small regions incorporating the right posterior cingulate cortex and bilateral amygdala (Oldham et al. 2018). Using a similar but more extensive approach to that of Oldham et al. (Oldham et al. 2018) a further meta-analysis of reward anticipation and outcome, supports and extends the aforementioned evidence, namely reward anticipation activates ventral striatum and insula as well as the mid-cingulate cortex and rewarding outcomes activates the posterior and anterior cingulate cortices (Jauhar et al. 2021).

In summary the following regions could be expected to be activated during the following stages of the PILT task used:

1. Win & loss anticipation: dorsal & ventral striatum (putamen/caudate & nucleus accumbens respectively), the anterior & mid-cingulate cortex and the anterior insular cortex are activated (Wilson et al. 2018; Jauhar et al. 2021; Oldham et al. 2018). The left inferior frontal gyrus (MNI coordinates: x = -44, y = 38, z = -10) has also been thought to activate during loss anticipation (Wilson et al. 2018).
2. Win outcome: bilateral ventral striatum (nucleus accumbens), bilateral orbito-frontal cortex/ventro-Medial Prefrontal Cortex, small regions incorporating the right posterior cingulate cortex and bilateral amygdala (Wilson et al. 2018) as well as the anterior cingulate cortex (Jauhar et al. 2021).
3. Loss outcome: increased activation observed in bilateral ventral striatum (nucleus accumbens) (Pickenhan 2021) as well as bilateral putamen, the anterior cingulate cortex and right insula (Dugré et al. 2018).

Recently a PET/fMRI healthy volunteer study, using the MID found an increase in 5-HT synthesis in the ventral striatum and insula during both win and loss outcomes. Importantly 5-HT synthesis in the nucleus accumbens negatively correlated with its activation during reward outcome whilst 5-HT synthesis in the insula negatively correlated with its activation during reward anticipation (Hahn et al. 2024). No significant difference arose between loss and win trials for 5HT synthesis or activation (Hahn et al. 2024). These findings could be interpreted as 5-HT having a modulatory role during these stages, attenuating insula and nucleus accumbens response to anticipation and receipt of reward respectively, speculatively through serotonergic inhibition.

Therefore, an aim of this experiment was to explore the influence of a 7-day course of citalopram, which has been shown to influence reward and aversive processing behaviourally, on the neural correlates of a monetary probabilistic reward learning task in healthy volunteers. We hypothesised that a 7-day course of citalopram would reduce activation in reward related areas, such as striatum, during the anticipation and receipt of rewarding outcomes (e.g. win). However in line with behavioural data presented here and elsewhere (Michely et al. 2022) that suggests 7-day SSRI treatment increases sensitivity to aversive outcomes, we hypothesised an increase in activation of regions related to aversive processing , such as the insula and anterior cingulate cortex, during the anticipation and receipt of aversive outcomes (e.g. loss).

Methods

Study participants & design

This chapter reports data from the same study as reported in the previous chapter. It used the same study design and ethical approval, details of which will not be repeated here.

Owing the scanner malfunction after enrolment, two participants could not undergo fMRI scanning (but did undergo behavioural testing as presented in Chapter 4). As per Chapter 4, a further participants’ data was removed after it emerged they had not disclosed information that would have rendered them ineligible for the study.

Reward learning task

The PILT was described in Chapter 4. However, in contrast to Chapter 3 only two 'runs' of the PILT are used (as opposed to three).

Image acquisition

All MRI images were obtained using a 3T Siemens Prisma scanner with a 32-channel head coil at the Oxford centre for Human Brain Activity (OHBA). Foam padding and a head restraint were used to control head movement.

A T1 weighted structural scan with 1mm isotropic voxel resolution was first acquired. Further specifications included an echo time of 3.97msec, flip angle of 8 degrees, field of view of 192mm and repetition time of 1900msec.

A field map was taken to correct for distortions (echos at 4.92 and 7.38ms, TR = 482ms, flip angle = 46°).

Functional imaging consisted of 72 T2-weighted echoplanar imaging (EPI) slices (TR = 1500ms, TE = 25ms, flip angle = 70°, multiband accelerator factor 3, GRAPPA = 2, resolution = 2mm isotropic, -30 angulation). Participants underwent two runs of the PILT, each run lasting 13 minutes, (each run had different symbols) with the aforementioned setup were performed.

Reward learning task fMRI data analysis

All imaging data were analysed with FSL (www.fmrib.ox.ac.uk/fsl) version 6.0.7.15

Pre-processing

All raw DICOM (Digital Imaging and Communications in Medicine) files were converted to BIDS (Brain Imaging Data Structure) standardised NIfTI (Neuroimaging Informatics Technology Initiative) using heudiconv 1.0.1 (using heudiconv (heudiconv 1.0.1 (https://github.com/nipy/heudiconv)) format to facilitate analysis.

The structural anatomical scans were brain extracted using Synthstrip (Hoopes et al. 2022).

fMRI data were pre-processed and analysed using FEAT (FMRI Expert Analysis Tool). The following pre-statistics processing was applied to reduce unwanted variability in the data and improve validity of statistical analyses: Non-brain structures were removed using the FSL Brain Extraction Tool (BET) (Jenkins 2002) and then motion corrected using FMRIB's Linear Image Registration Tool (MCFLIRT) (Jenkinson et al., 2002a). Spatial smoothing was performed with a Gaussian kernel of FWHM 5mm to increase signal-to-noise ratio. High pass temporal filtering with a cut-off of 60 seconds to remove low-frequency drifts, and B0 unwarping using fieldmap rads and magnitude images for distortion correction and high-pass temporal filtering with a cut-off of 60 seconds to remove low-frequency drifts due to noise, for example from the MRI scanner.

Boundary Based Registration and high contrast functional images were used to register non-linearly functional images to corresponding structural images (Jenkinson et al., 2002; Jenkinson and Smith, 2001). Registration to high-resolution structural images was carried out using FLIRT (FMRIB's Linear Image Registration Tool) and further refined using FNIRT (FMRIB's Non-Linear Image Registration Tool) nonlinear registration. Data were normalized to the Montreal Neurological Institute (MNI) template

First level analysis

A custom three-column format convolved with a gamma hemodynamic response function and its temporal derivative was used to model the data.

Participants neural activity during run 1 and run 2 of the PILT was examined with six explanatory variables (EV). EVs modelled included win anticipation; loss anticipation; loss outcome, win outcome; no loss in loss trials & no win in win trials. Contrasts analysed included means for each condition versus baseline, as well as directional comparisons, as follows. The fifteen contrasts were defined based on the PILT's task-related events:

1. Win outcome versus implicit baseline (fixation cross)
2. Loss outcome versus implicit baseline (fixation cross)

3. Win anticipation versus implicit baseline (fixation cross)
4. Loss anticipation versus implicit baseline (fixation cross)
5. No win in win trials versus implicit baseline (fixation cross)
6. No loss in loss trials versus implicit baseline (fixation cross)
7. Win anticipation minus loss anticipation
8. Loss anticipation minus win anticipation
9. Win outcome minus no win outcome
10. No win outcome minus win outcome
11. Loss outcome minus no loss outcome
12. No loss outcome minus loss outcome
13. Win outcome minus loss outcome
14. Loss outcome minus win outcome

Motion outliers (from fsl_motion_outliers) and motion parameters (estimated by MCFLIRT Motion Correction by FMRIB's Linear Image Registration Tool) were added to the model as nuisance regressors. Absolute and relative motion values did not differ significantly between groups.

All participants were included in the main analysis.

Second level analysis / intermediate analysis

Since the PILT task comprised of two runs, an intermediate-level analysis using fixed effects analysis in FEAT was implemented to estimate each subject's mean response for each contrast.

Using Featquery (Woolrich et al. 2001), individual run results for each subject from the first level analysis were used to extract parameter estimates for each region of interest (RoI) for each contrast. Ten RoI were defined by the following structural masks from the Harvard-Oxford Structural Atlas library (Desikan et al. 2006): left & right caudate, left & right putamen, left & right nucleus accumbens, orbito-frontal cortex, fronto-medial cortex, insula and anterior cingulate cortex). RoI masks were thresholded at 50%. Parameter estimates were averaged to provide a mean activation for each subject

Third level analysis

Main effect of task

Group level analysis was performed using the FSL nonparametric permutation tool 'Randomise' (5000 permutations) (Jenkinson et al. 2012). Statistics were assessed using the threshold-free cluster enhancement method with family-wise error correction of 0.05 (or 0.95 threshold within randomise) (Smith and Nichols 2009).

The combined average of all participants was first calculated to identify the main effect of task on the whole brain for each contrast. This was repeated for the ten aforementioned regions of interests.

Main effect of group with anatomical masks

For the between subjects' analysis the GLM included two EVs: placebo and citalopram. Contrasts were citalopram > placebo and placebo > citalopram.

Regions of 20 voxels or more was a threshold arbitrarily set as representing a meaningful difference in activation between groups.

Results

Main effect of task

Whole brain analysis

Neural activation in response to each contrast was compared across all participants to determine the validity of the task in engaging brain regions previously associated with anticipation or receipt of loss and win outcomes. A whole brain analysis revealed significant activations in several clusters (Appendix: Chapter 5, Table 1), however results did not replicate activation in certain brain regions as per a previous study using the same task (Pickenhan 2021).

Win trials

Win anticipation (vs baseline**)** activated bilateral putamen, bilateral anterior insula cortex/orbito-frontal cortex, the cingulate cortex (posterior, mid- and anterior) & the supplementary motor cortex, which aligns with previous literature (Wilson et al. 2018).

Win outcome (vs baseline) activated the right insula cortex, right frontal pole and bilateral precentral gyrus/inferior frontal gyrus. Results did not replicate previous findings of win outcome activating OFC, vmPFC, posterior cingulate cortex or amygdala (Wilson et al. 2018). The win outcome vs no win outcome (i.e. positive prediction error) activated bilateral striatal regions & anterior cingulate gyrus.

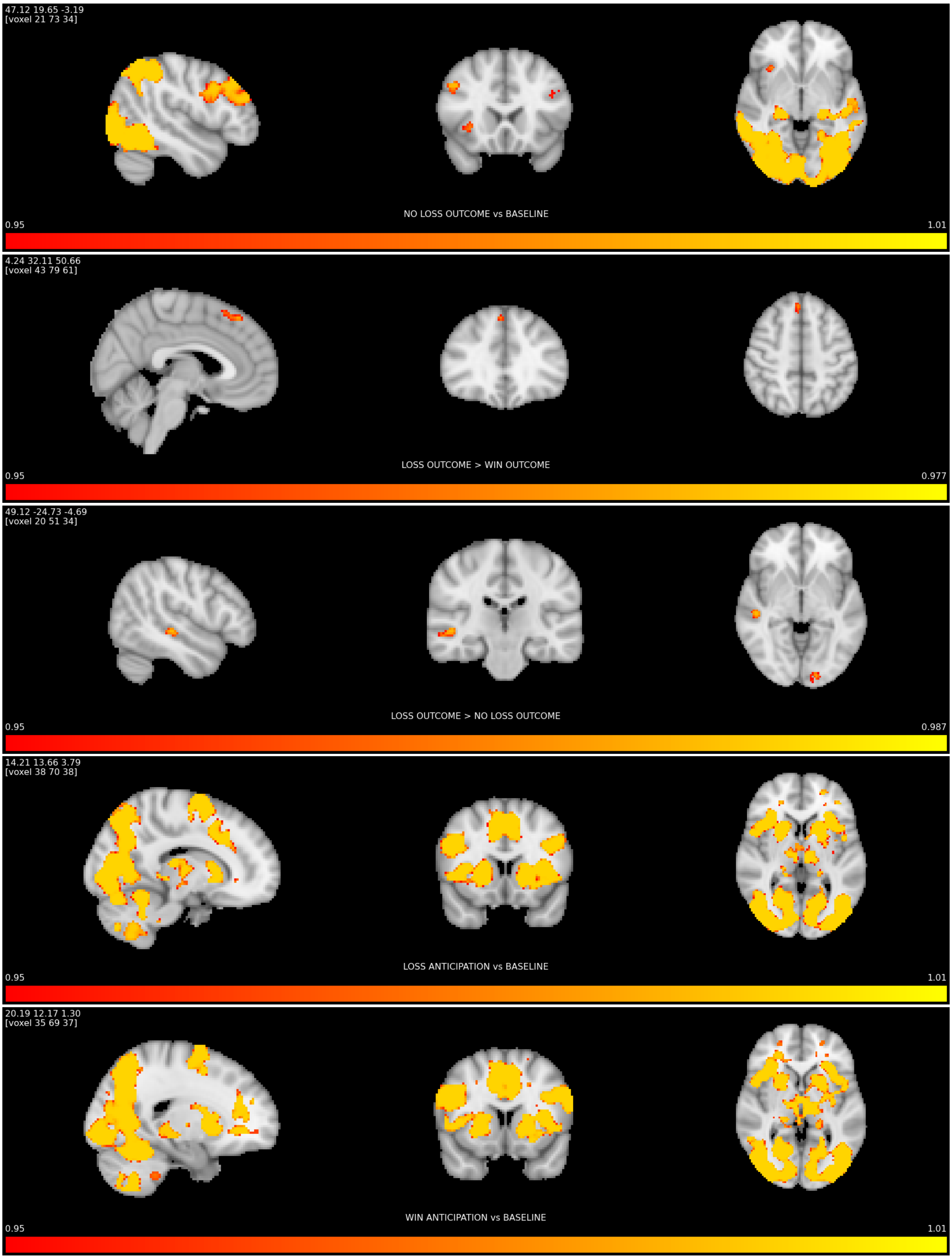
Loss trials

Loss outcome (vs baseline) activated the right insula cortex, similar to previous literature (Dugré et al. 2018) as well as the right middle frontal gyrus/right inferior frontal gyrus and right paracingulate gyrus. Of note, no striatal activation was observed as found in similar, previous studies (Dugré et al. 2018; Pickenhan 2021).

Consistent with previous literature, loss anticipation (vs baseline) activated bilateral caudate and putamen, left nucleus accumbens, bilateral insular cortex, bilateral inferior frontal gyrus and paracingulate/anterior cingulate gyrus (Wilson et al. 2018; Jauhar et al. 2021; Oldham et al. 2018).

Additional contrasts used in the current study, not explicitly reported in previous literature, include no loss outcome (vs baseline), an indicator of positive prediction error, which activated the right insular cortex and bilateral middle & inferior frontal gyrus. Also included were contrasts for loss outcome (vs win outcome), which activated a small region in the superior frontal gyrus, and loss outcome (vs no loss outcome), which activated a small superior/inferior temporal gyrus (posterior) region.

Contrasts not shown in Figure 1 include no win outcome (vs baseline); win anticipation vs loss anticipation; loss anticipation vs win anticipation and no loss outcome vs loss outcome as these contrasts did not appear to activate regions specifically implicated in reward and aversive processing, but rather appeared to activate regions thought of as being primarily involved in visual processing e.g. occipital cortex.



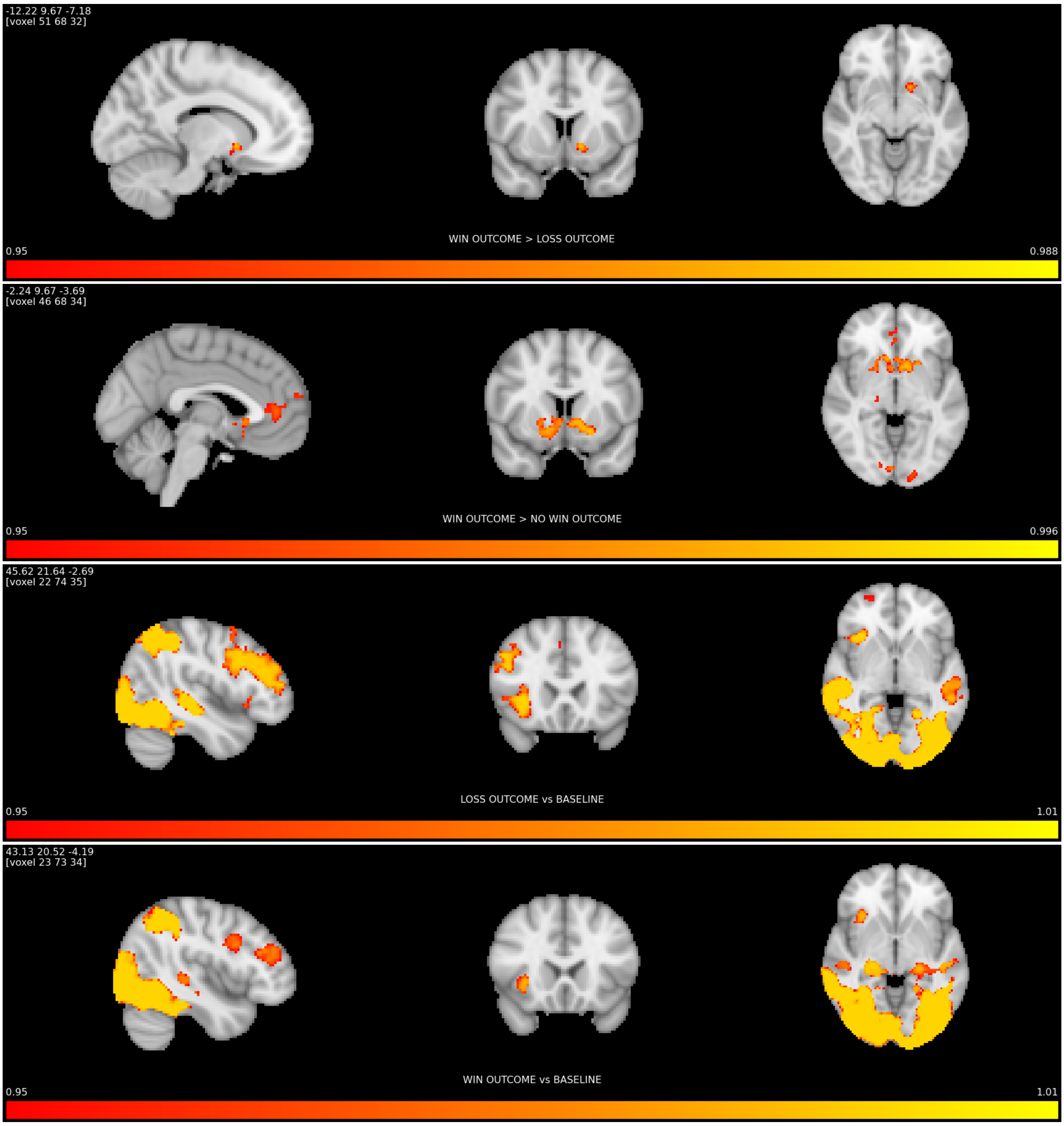


Figure 1: Summary overview of significant whole brain neural activation across all participants for each of the lower-level contrasts where significant activations occurred. Scans show sagittal, coronal, and horizontal planes (left to right) & thresholded to 0.95.

Region of interest analysis

A main effect of task analysis using regions of interest structural masks indicated several regions were activated consistent with previous literature (Appendix: Chapter 5, Table 2).

Win trials

During both anticipation and receipt of win outcome (vs baseline) bilateral insula and small regions encompassing the left nucleus accumbens and bilateral putamen were activated.

Whilst several regions were common amongst reward stages (e.g. outcome and anticipation), a differential pattern of activation, dependent on processing stage, emerged for a few regions.

During win anticipation, but not outcome, the left caudate and anterior cingulate cortex (ACC) were activated. Unlike in whole brain analysis, the putamen or insula regions were not activated. Of note, similar to the whole brain analysis, the ventral striatum was not activated during win anticipation (vs baseline).

Win outcome (vs baseline), but not win anticipation, activated the right nucleus accumbens, right caudate & right orbito-frontal cortex, which was not observed in the whole brain analysis or previous literature (Wilson et al. 2018).

Positive prediction error in win trials (win outcome vs no win outcome) activated the ACC, bilateral accumbens, caudate and putamen and the right orbito-frontal cortex, these are consistent with previous findings of striatal activation during positive prediction error (McClure, Berns, and Montague 2003; Pessiglione et al. 2006).

Loss trials

A differential pattern of activation emerged dependent on processing stage during loss processing. Specifically, during anticipation of loss (vs baseline) significant activation was observed in the bilateral insula, ACC, left accumbens, bilateral caudate & putamen and right orbito-frontal cortex. Loss outcome (vs baseline) activated bilateral insula, the ACC and bilateral orbito-frontal cortex (but unlike loss anticipation, not the left accumbens or bilateral caudate/putamen). The left orbitofrontal cortex was activated during loss outcome, but not in loss anticipation, too.

Prediction error appeared to differentially activate regions as well. In loss trials, positive prediction errors (i.e. no loss outcome vs loss outcome) activated bilateral nucleus accumbens, consistent with previous literature (Pessiglione et al. 2006). A negative prediction error for loss trials (e.g. loss outcome vs no loss outcome) activated bilateral caudate, unlike previous studies (Pickenhan 2021).

Main effect of allocation

Whole brain analysis

The whole brain analysis revealed no significant difference in BOLD activation between groups for any of the contrasts involving anticipation or outcome for either win or loss trials.

However, a trend-significant hyperactivation of a small cluster within the pre-central gyrus in the citalopram group was found for the win outcome relative to the no win outcome in the whole brain analysis (Figure 2).

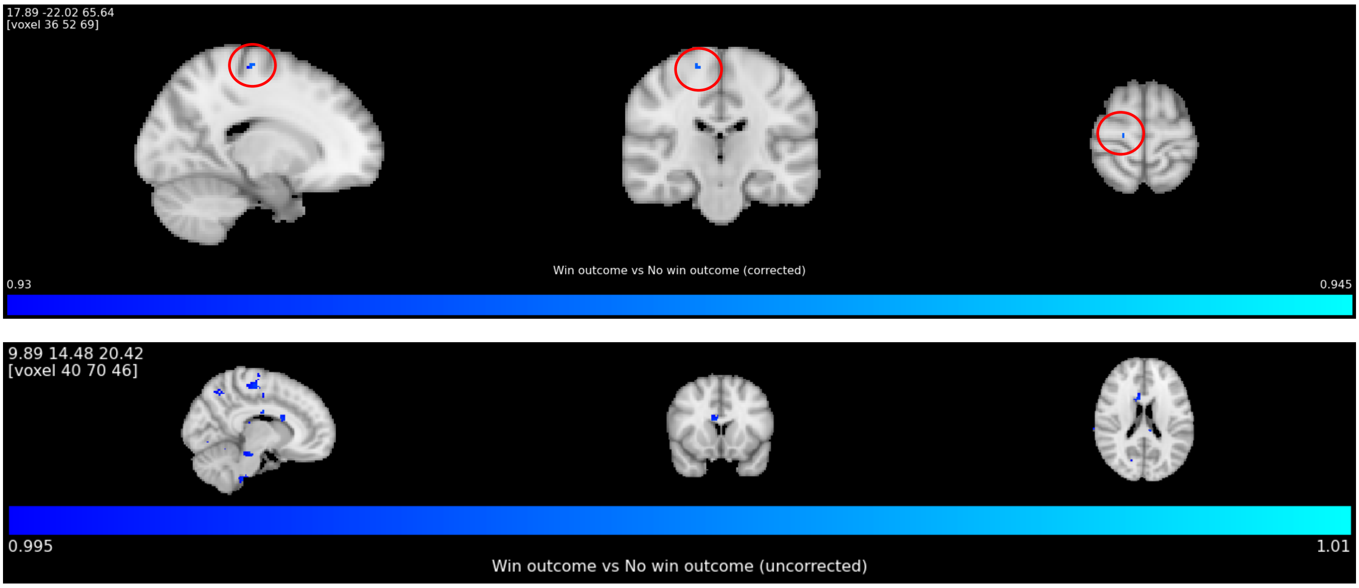


Figure 2: A small, non-significant cluster was identified as being hyper-activated in the citalopram group vs placebo group for win outcome vs no win outcome (i.e. positive prediction error) (t = 4.41, = 0.064, peak voxel location: x=18, y =-20, z= 66, cluster size = 6 voxels. Images show upper TFCE-corrected p = 0.07 image and lower p = 0.07 uncorrected image.

Structural region of interest analysis

The voxel-wise region of interest (RoI) analysis using structural masks defined through Harvard Oxford structural atlas, revealed significant differences in a number of small regions as outlined in table 3, including the left nucleus accumbens during no win outcome (Figure 3).

Table 3: Significant regions from structural region of interest analysis

Contrast	Brain region	Cluster size (voxels)	MNI maximum (x,y,z)	T value	p value	
No win > baseline	left accumbens	23	-10, 8, -10	2.72	0.027	placebo > citalopram

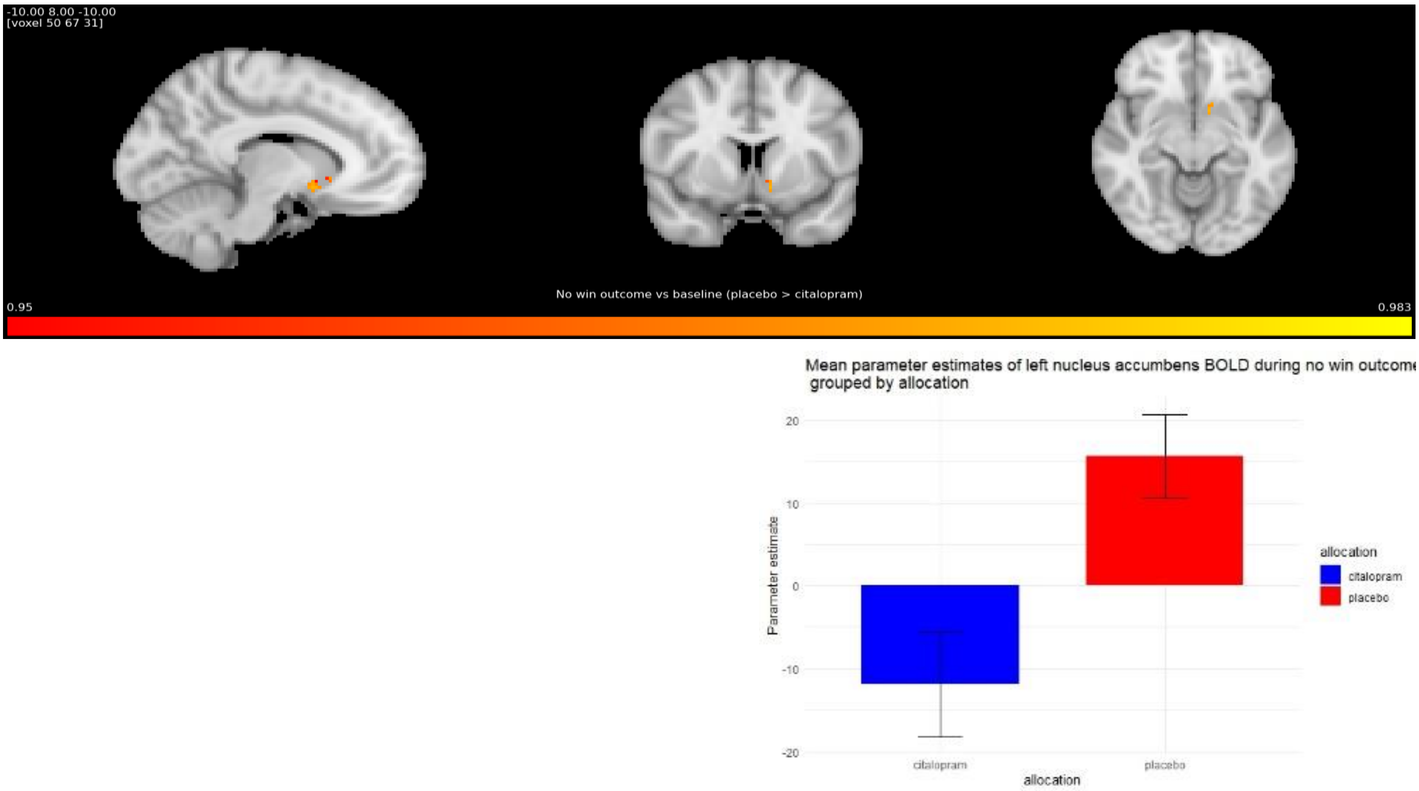


Figure 3. The red coloured cluster represents the significant change in BOLD activation between groups in the left nucleus accumbens RoI during the no win outcome vs baseline. Figure on the right shows extracted parameter estimates for significant cluster. Error bars are SEM.

FEAT structural region of Interest analysis

Parameter estimates were extracted from first level FEAT results for each contrast and prespecified RoI based on Harvard-Oxford structural masks. A mixed effects linear regression was performed for each RoI dataset (regressors were run, contrast & allocation) revealed a significant two-way interaction between allocation and run (F(1, 1026) = 4.94, p = 0.026) and a non-significant three-way interaction effect between contrast x allocation x run on parameter estimates for the orbito-frontal cortex ($F_{(13, 1026)}$ = 1.57, p = 0.088). Estimated marginal means (EMM) indicated that the citalopram group, compared to the placebo group, exhibited significant hypoactivation in the orbitofrontal cortex for loss outcome in the second run of the task ($t_{(1048)}$ = −1.98, p = 0.048) which was not the case for the first run of the task ($t_{(1048)}$ = 1.79, p = 0.073) (Figure 4). Furthermore, there is no significant effect of allocation if parameter estimates for orbitofrontal cortex during loss outcome are averaged across runs ($t_{(37)}$ = -0.11, p > 0.1).

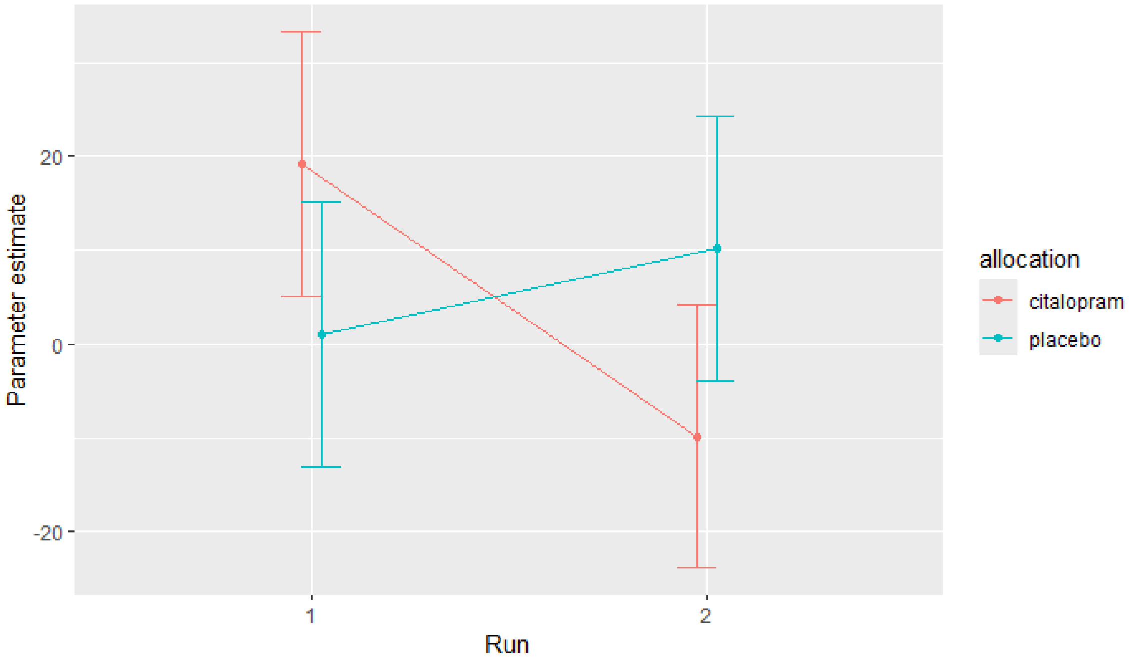


Figure 4: Orbitofrontal cortex FEAT parameter estimates to loss outcomes

Each subjects' parameter estimates were averaged across runs for each contrast * RoI combination. A mixed effect linear regression found a significant two-way interaction between contrast and allocation (F(13, 4750) = 3.17, p < 0.001), which seemed to be driven by a significant difference between no win outcome (vs baseline) (EMM ± SE = -9.413 ± 3.08, p = 0.0023) and no-win outcome (vs win outcome) (EMM ± SE = -7.064 ± 3.08, p = 0.0022). No main or other interaction effect of allocation was observed.

To investigate this further estimated marginal means between allocation groups for each contrast * RoI combination were calculated (Bonferroni corrected). This revealed a significant difference between allocation groups for the left accumbens in the no win outcome vs baseline (Estimated Marginal Mean ± SE = -22.74 ± 7.56, p = 0.0026).

Discussion

The instrumental learning task used here activated several brain regions implicated in different stages of reward and aversive processing. In the citalopram group a small cluster in the left nucleus accumbens showed significant hypoactivation during no-win outcome in the structural region of interest analysis (Table 3).   A non-significant hyperactivation during anticipation of loss was observed in the left caudate of the citalopram group too.

However, it is important to note that many regions implicated in reward and aversive processing were not activated by the task. This included no activation in the right nucleus accumbens during the anticipation of win or loss or bilateral nucleus accumbens activation during receipt of loss outcome.

No significant difference was found between allocation groups in the whole brain analysis. However, the citalopram group exhibited a non-significant increase in activation of a 6-voxel cluster in the pre-central gyrus (x=18, y =-20, z= 66) during win outcome vs no win outcome.

These results add to the wide array of fMRI results from using differing task and durations of SSRI.

SSRI reduce nucleus accumbens activity in no win outcome

The citalopram group exhibited hypoactivation in a small cluster in the left nucleus accumbens (e.g. ventral striatum) in response to a no-win outcome compared with baseline. Few studies have reported the neural correlates of win omission in instrumental tasks, least of all the influence of serotonergic modulation on it. This novel finding could indicate neural responses to reward omission is attenuated by SSRI or an increase in serotonergic transmission. Speculatively this could indicate reward omission has less salience after sub-chronic citalopram.

A female healthy volunteer study using a passive, Pavlovian task found that reward omission (juice vs water) activated the lateral habenula, insula, orbito-frontal cortex and the putamen (uncorrected) (Mollick et al. 2021). Consistent with this, the present study found activation of the insula (corrected) with reward omission (Appendix: Chapter 5, Table 2. RoI main effect of task), however not in the lateral habenula, which may be due to resolution constraints of 3T fMRI and the lateral habenula size. Some studies have found no significant activation of brain regions during reward omission in instrumental monetary reward tasks (Ernst et al. 2005). A healthy volunteer study using a similar task found a slightly delayed, reward magnitude-dependent, deactivation of the bilateral nucleus accumbens (NAcc) and left superior frontal gyrus after reward omission (Pedroni et al. 2011). The same study found a negative correlation between dorsal raphe nucleus (dRN) and NAcc activation in relation to magnitude of the reward omission i.e. as the value of reward omission increased, dRN activity increased whilst NAcc activity decreased, postulating that the dRN exerted an inhibitory effect on the NAcc during reward omission (Pedroni et al. 2011).

This hypothesis could be consistent with findings here, namely one could conjecture the baseline inhibitory influence of serotonergic neurons from the dRN on the NAcc is enhanced with a 7-day course of citalopram, possibly through downregulation of the 5-HT$_{1A}$ auto-receptor on serotonergic neurones but also increased synaptic serotonin availability through serotonin reuptake blockade. As noted, literature reporting the neural correlates of reward omission or negative prediction error in reward processing following serotonergic manipulation is sparse, therefore direct comparison with other studies is not possible.

Absence of task and group effects

The effect of the task used in the present investigation in activating certain brain regions contrast with many studies examining reward processing and SSRI medication in healthy volunteers. These may be due to several factors such as differences in task or intervention.

Differences in the main effect of task

Here anticipation of win and loss activated the anterior & posterior cingulate gyrus, thalamus, insular, orbito-frontal cortex, putamen & caudate. These results are broadly consistent with meta-analyses of healthy volunteer studies using the Monetary Incentive Delay task (MID). These include activation of the striatum, insular and anterior cingulate during anticipation of win and loss outcomes (Wilson et al. 2018). However, some studies have specifically identified the ventral striatum (e.g. nucleus accumbens) as being activated during reward anticipation in healthy volunteers (Jauhar et al. 2021; Oldham et al. 2018; Liu et al. 2011) – a finding partially replicated here with left nucleus accumbens being activated during win anticipation vs baseline in the main effect of task RoI analysis. That only left, and not bilateral ventral striatal activity, was identified could be attributable to insufficient sample size in the present study. It may also be attributable to the main effect of task analysis here including subjects who have received citalopram, which may influence detection of any main task effects e.g. SSRI participants may exhibit reduced activation within certain brain regions, therefore regions for the whole sample would not appear as significant in main effect of task analysis. It is possible the inclusion of citalopram is of little significance however, as no group differences arose in for the win anticipation contrast in any region of interest analysis.

The present study found receipt of win and loss outcomes activated regions in the temporal and frontal cortices as well as the insular and orbito-frontal cortex regions, however not striatal regions, as others have found (Oldham et al. 2018; Liu et al. 2011). However, the win outcome vs no win outcome contrast in the current study did activate striatal regions and anterior cingulate cortex. One possible explanation for win outcome vs baseline not eliciting nucleus accumbens activation is the magnitude of financial reward (e.g. 20p) is insufficient to elicit striatal activation, or during the task, activation of the striatum represents a comparative value of reward (as seen in win outcome vs no win outcome) rather than absolute value of reward (as seen in win outcome vs baseline).

The most comparable interventional study used a similar 7-day course of citalopram in healthy volunteers and used a paradigm in which participants were presented with picture cues indicating what an upcoming taste could be, and on some occasions delivering the cued taste e.g. rewarding chocolate or aversive strawberry, comparable to receipt of monetary outcome here (McCabe et al. 2010). The task used in the current study activated similar regions to those activated by the task used in this previous study, however a few key differences did arise which may influence the ability to detect results of a 7-day course of citalopram. These include the unpleasant taste in mouth (akin to the negative (loss) outcome in the present study) activated the caudate and ventral striatum, which was not found in the current study.

McCabe et al. (2010) found the citalopram group exhibited hypoactivation in the ventral striatum when receiving a pleasant chocolate taste (akin to win outcome here) and hypoactivation of the ventral striatum, mid-OFC and vmPFC/medial OFC when viewing a picture of chocolate (akin to win anticipation here). Although a win outcome activated the ventral striatum in the present study, no group differences emerged.

Similarly, when viewing an unpleasant picture of mouldy strawberries (akin to loss anticipation) hypoactivation of the insula was observed for the citalopram group and receiving an unpleasant taste (akin to loss outcome) elicited a hypoactivation of the insula and lateral OFC in the citalopram group (McCabe et al. 2010). However, no similar group differences were identified here, rather the citalopram group exhibited a single voxel hyperactivation in the left caudate during loss anticipation (vs win anticipation) (Table 3).

One possible explanation is that the influence of sub-chronic citalopram on aversive anticipation and outcome is modality dependent. For example, primary (taste) stimuli may have greater aversive salience, compared to secondary (monetary) stimuli, on neural activation in the insula, to a sufficient degree to detect a group difference (Sescousse et al. 2013). This is not the case for secondary or monetary loss, or tasks involving an instrumental learning of probabilistic cue-outcome associations and prediction errors, which activate the ventral striatum more reliably than tasks involving passive delivery of food (Sescousse et al. 2013).

A healthy volunteer, placebo-controlled study using a 2-week course of citalopram, used an effort reward paradigm to examine prediction errors of reward (financial) and aversive (effort) outcomes (Scholl et al. 2017). The study generated prediction errors through a Bayesian learning model and then measured activity in specific regions during these prediction errors. During reward prediction errors the citalopram group demonstrated significant increases in activation in the ventral striatum, mid-cingulate cortex, vmPFC, posterior and parietal cortex, amongst others (posterior cingulate cortex, middle temporal cortex and inferior frontal cortex). During effort prediction errors the citalopram group also demonstrated a significant increase in activation of the dorsal anterior cingulate cortex. Other regions activated, but not significant were the posterior parietal cortex, anterior insula and anterior prefrontal cortex.

Results presented here use a model-free definition of prediction errors. This is win vs no win and no loss vs no loss for positive prediction errors and no-win vs win and loss vs loss for negative prediction errors. The main effect of task for positive prediction errors (only for win vs no win contrast) activated ventral and dorsal striatal regions as well as anterior cingulate cortex regions. Likewise, negative prediction error (only for loss vs no loss contrast) activated superior temporal gyrus only. When examining group differences the citalopram group demonstrated a non-significant increase in activation of a cluster in the pre-central gyrus (MNI152 coordinates: x = 18, y = -20, z = 66) that extended into the posterior and anterior cingulate cortex for the win vs no-win outcome (positive prediction error) contrast. Similarly, the citalopram group demonstrated hypoactivation for the no-win vs win outcome contrast in an identical cluster (the pre-central gyrus (peak MNI152 coordinates: x = 18, y = -20, z = 66) to win vs no-win outcome contrast. This is broadly consistent with Scholl et al. (2017) in that they found with increasing effort (or increasing negative prediction error), a reduction in dACC activity was observed. Here we find during a model-free negative prediction error in win trials (i.e. no-win vs win outcome) a non-significant decrease in ACC/PCC activity in citalopram group relative to placebo group.

However, many of the results from Scholl et al. (2017) are not replicated here. There are several reasons why this could have occurred. Whilst the studies are similar in terms of using monetary reward and being instrumental in nature, the paradigm used by Scholl et al. (2017) possibly required higher cognitive demands in computing whether the reward was real or hypothetical, as well as a degree of effort discounting, as reward and effort (i.e. aversive stimuli) were paired together in the study by Scholl et a. (2017). It is possible such a complex paradigm required incorporation of more information, therefore engagement of more brain regions and to a greater extent, compared to the PILT as used here. This could explain why more regions demonstrated significant group differences in the Scholl et al. (2017) study compared to results presented here. Further, related to task design, Scholl et al. (2017) used effort as an aversive outcome, whereas here monetary loss is used instead. It may be that effort expenditure is more salient than monetary loss, therefore eliciting greater neural activation, thus providing a greater range of neural activation that is amenable to SSRI treatment. Another potential explanation could be that the neural correlates of effort expenditure in healthy volunteers rely upon a neural circuit that is influenced by SSRI action, whereas the probabilistic instrumental learning task used here does not engage such circuits. Differences in defining prediction could also account for the lack of findings here compared to Scholl et al. (2017), who used a potentially more accurate and nuanced measure of prediction errors than used here e.g. model-free contrast of win vs no-win outcome. Finally, it may also be possible that a 14-day duration of treatment is sufficient to enable neuroplastic effects to influence neural activity during reward processing, which may not manifest after a 7-day course of SSRI, as used here.

A within-subject healthy male volunteer study using a 7-day course of the SSRI paroxetine observed reduced BOLD response in the ventral striatum, anterior cingulate cortex and midbrain, when viewing erotic video clips compared to placebo (Abler et al. 2011). No group differences were found in these regions from whole brain analyses presented here. Similar to comparisons with McCabe et al (2010), this may be due to Abler et al. (2011) using stimuli that are primary in nature are more susceptible to changes in serotonergic transmission, compared to monetary stimuli, that are secondary in nature, and more resilient to serotonergic manipulation.

Furthermore, the task involved male only subjects which may have led to greater activation in more regions, compared a mixed-sex sample as used here, as it has been reported males exhibit stronger functional connectivity to reward areas when viewing erotic images (Lee et al. 2015). Finally, paroxetine is an SSRI with some additional pharmacological actions, , such as modest noradrenaline reuptake inhibition, which has been demonstrated to reduce activation in regions involved in reward processing (Graf et al. 2018).

Finally, a healthy volunteer study using a similar task as used here, but after a 21-day course of escitalopram, found reduced activation of the intraparietal sulcus (28, -66, 32) during reward trials (Langley et al. 2024). One potential explanation for not replicating this finding could be the duration of intervention, with a 7-day course of citalopram insufficient to lead to neuroplastic changes in regions relevant for reward processing.

Limitations

A major drawback of these findings is the absence of significant task effects in regions that are commonly activated by rewarding and aversive tasks. The task required two 60-trial runs of each participant and during extraction of parameter estimates its noted that the values from each run from most subjects were numerically far apart, making it difficult to determine the accuracy of responses. With additional runs of the task it may have been better placed to calculate an average parameter estimate for each subject, giving a more accurate effect of the effect of a 7-day course of citalopram on neural correlates of reward processing. This may also have enabled the first ‘run’ to be used as a practice run as the novelty of completing the task in an fMRI scanner may influence neural response (which could not be equivalent to a 10-trial practise task outside of the scanner).

Another limitation is potentially the study using a between-group design. Whilst between group designs have been sufficient to detect group differences when comparing clinical and healthy volunteer samples or other interventional studies, the effect size of serotonergic modulation on reward processing tasks used here may be considerably less and therefore a study design that reduces between subject variability and be sensitive to smaller neural effects sizes may be more suitable.

The 7-day course of citalopram was selected as a diverse reward processing tasks & analyses have found behavioural effects using 7-, 14- and 21-day courses of citalopram (Michely et al. 2022; Scholl et al. 2017; Langley et al. 2023). However corresponding neural effects have not been identified here. It is possible that the neural effects of serotonergic modulation on reward processing may require longer durations of treatment, possibly to facilitate necessary neuroplastic changes to occur in the regions of interest or because they have indirect effects on the dopaminergic reward system that require longer time than 7-days for the brain neurotransmitter systems to reach a new homeostatic state. Therefore, performing fMRI scans at longer time points such as 14- or 21-days may be valuable to ascertaining to effect of SSRI on reward processing. However, as mentioned previously, neural effects of 7-day citalopram course on instrumental reward learning have yet to be studied in healthy volunteers, therefore the study does add to the field of reward processing and serotonin.

A further limitation of the study is that the blood levels of citalopram were not measured. Not only would this enable an assessment of participant compliance with the study protocol but also mitigate any biological variability between individual in terms of pharmacokinetics, such as citalopram absorption, distribution throughout the brain and body and its metabolism.

A 7 day course of SSRI has been shown to reduce resting state cerebral blood flow, of note in regions related to reward processing such as striatum and anterior cingulate cortex (Viviani et al. 2012). Potentially any increase in activation from the anticipation of an aversive outcome could be ameliorated by the reduction in cerebral blood flow brought about by SSRI. This could be addressed in future studies by incorporating additional analysis, such as Arterial Spin Labelling, to account for any drug-induced vascular changes.

Conclusion

In summary this study found a 7-day course of citalopram reduced left nucleus accumbens activity during win omission in a probabilistic instrumental learning task. Speculatively this could indicate a 7-day course of citalopram could attenuate the neural substrates of loss (or reward omission) during reward whilst having little influence on rewarding outcomes. However, the contribution of these results to the field are caveated with paucity of task effects on regions associated with reward and aversive processing.

Thesis discussion

Thesis summary

This thesis has examined the role of 5-HT$_{1A}$ receptor agonism in cognition, emotional and reward processing and the role of serotonin, through a 7-day course of citalopram, in reward processing. It has examined these effects using behavioural and neuroimaging techniques in healthy volunteer samples.

In **Chapter 2** I reported the effects of a single dose of buspirone 20mg on established cognitive and emotional processing tasks. Buspirone had no significant effect in the N-back task but did have a significant group by block effect in the AVLT and specifically at block 3, with the buspirone group performing worse than placebo. Regarding emotional processing, buspirone had no significant effects on emotional word recall (EREC) or the recognition of emotive words (ECAT) but did have a significant effect on accuracy of identifying certain emotions from faces (FERT). Specifically, the buspirone group were less accurate for disgust and more accurate for sad faces. Furthermore, when considering misclassification of emotion, which could be driven by the buspirone group misclassifying the anger emotion more than the placebo group. Buspirone made significantly more negative to negative emotion misclassifications than the placebo group as well.

In **chapter 3** I similarly report the effects of a single dose of buspirone on three behavioural reward tasks with the aim to capture several stages of reward processing. These include the subjective rating of four tastes to measure consummatory pleasure of primary stimuli; an effort-grip task to measure cost-benefit decision making and appetitive behaviour and finally a simple reinforcement learning task to measure reward learning. The buspirone group found bitter tastes more unpleasant than the placebo group. A significant allocation by reward by effort level was observed, it seemed to reflect a general trend of buspirone maintaining the disincentivising effect of effort throughout all reward levels, whereas the placebo group appeared less influenced by effort at higher reward levels. Finally, in the reward learning task buspirone significantly increased optimal choice selection in loss trials but not win trials throughout the task. Additionally, buspirone significantly improved optimal choices when learning had reached asymptote (taken as trials 10 to 30) for both win and loss trials.

In **chapter 4**, using the same tasks as chapter 3, a 7-day course citalopram resulted in no significant effects in the taste task or the effort-grip task. In the reward learning task, which unlike chapter 3 was performed in an fMRI environment, the citalopram group made more optimal choices in the loss trials but less optimal choices in win trials throughout the task. Additionally, the citalopram group reported greater emotional blunting at day 7 in self-report questionnaires.

Chapter 5 presented the neural correlates of the reward learning task, which included the neural correlates for anticipation and receipt of win and loss outcomes. The citalopram group demonstrated a small cluster of hypoactivation located in the left nucleus accumbens during win omission vs neutral contrast.

Summary of main conclusions

Emotional processing and cognition

Acute administration of the 5-HT$_{1A}$ receptor partial agonist, buspirone, increases sensitivity to sad emotions whilst decreasing sensitivity to disgust. Buspirone also increases misclassification of negative emotions to other negative emotions. This is partially consistent with a previous study finding reduced accuracy of negatively valanced faces (fear) after acute buspirone (Bernasconi et al. 2015). While my study did not find an effect of buspirone on fearful faces specifically, it used a wider repertoire of emotional faces, enabling the divergent responses to sad and disgust emotions to be identified. This observation is a novel contribution to the field, and implicates 5-HT$_{1A}$ receptor agonism as potentially valuable in reducing sensitivity to disgust, which may be relevant to disorders including obsessive-compulsive disorders (Bhikram, Abi-Jaoude, and Sandor 2017). However, in increasing sensitivity to sad emotions, 5-HT$_{1A}$ receptor agonism, at least acutely, is potentially unhelpful in disorders such as depression.

Although results presented here have been discussed in the context of the potentially similar intervention of acute tryptophan depletion (ATD), caution should be used when comparing it directly to the effects of acute buspirone. The effects of ATD on emotional processing are somewhat variable. Decreased fear and happy facial processing has been reported, phenomena not seen here. Speculatively this may be due to e acute buspirone administration, though it can decrease much 5-HT neuronal activity similar to ATD, it also possesses partial agonist activity of buspirone at post synaptic 5HT$_{1A}$ receptors, which may not be observed in ATD.

The findings of this thesis also support previous findings that acute buspirone had minimal effect on cognition, specifically the N-back memory task. However, a small but significant deleterious effect in the AVLT, specifically on block 3 of word recall, was observed. The modest effect of acute buspirone on cognition is somewhat consistent with previous evidence (Chamberlain et al. 2007), but extends this by including males and females, important for generalisations of these findings.

Reward processing

Liking

Buspirone appears to make an aversive, bitter taste subjectively worse whilst having no significant effect on pleasant sweet tastes, this is consistent with the effect of ATD in another healthy volunteer study (Smith et al. 2021), indicating that the effect of buspirone observed here may be attributable to reduced 5-HT availability, presumably through activation of 5HT$_{1A}$ auto-receptors.

A 7-day course of SSRI, did not influence the subjective rating of either pleasant or aversive tastes, similar to previous studies (McCabe et al. 2010). Using supra-threshold taste concentration in a non-fMRI context these results could indicate a ceiling effect of serotonin levels, and its downstream molecular effects, on consummation of primary taste rewards may exist, whereby only reductions can influence subjective experiences, albeit valence specific (e.g. aversive).

Wanting

Buspirone seems to reduce willingness to accept high reward:high effort offers and maintain a sensitivity to effort as reward increases (e.g. maintain effort sensitivity in effort discounting) but has minimal influence on appetitive behaviour as seen through the lack of group differences of force grip effort during trials. This novel observation is somewhat inconsistent with pre-clinical evidence with specific 5-HT$_{1A}$ receptor agonism, which broadly observes a reduction in appetitive behaviours. Intriguingly sub-chronic SSRI treatment does not seem to influence cost-benefit decisions or appetitive behaviour in healthy volunteers as found in the task used here. As discussed, this is in contrast to evidence from Meyniel et al (2016) which found SSRI increased monetary gain in an effort-grip task (Meyniel et al. 2016). This divergence in results could be due to the present study measuring only effort-cost decisions, whereas the outcome measure used by Meyniel et al. (2016) incorporated both cost-benefit decision making as well as appetitive behaviour. Results here could reflect a similar hypothesis for consummation being applicable to effort, specifically a narrow ceiling effect of serotonin levels on cost-benefit decision exists limiting behavioural effects of the 7-day course of citalopram as used here. It could be that only reductions in 5-HT are able to elicit behavioural changes in cost-benefit decision making.

Learning

Buspirone and citalopram both appeared to increase sensitivity to loss, as defined by increases in optimal choices in loss trials. Whilst this is consistent with previous literature for citalopram (Michely et al. 2022) such an effect has not been reported previously for a 5-HT$_{1A}$ receptor partial agonists. Such a finding is not consistent with the effects of ATD. It is possible therefore that the effects of buspirone on loss sensitivity could be attributable to its post-synaptic 5-HT$_{1A}$ receptor effects. The increase in saliva cortisol, described in Chapter 2, indicated acute buspirone 20mg seemed to activate postsynaptic 5-HT$_{1A}$ receptors.

Similarly, the finding that SSRIs can impair reward learning is consistent with previous evidence using SSRI medication. However, buspirone improves optimal choices in reward contrasts with this. This effect may be linked to the possibly separate effects of buspirone of decreasing 5-HT transmission through serotonergic neurones whilst also engaging specific post-synaptic receptors. Supporting this view is that broadly ATD is reported to have little effect on reward learning.

A novel finding is the counterintuitive sparse neural effects of citalopram during reward or loss trials, despite the behavioural effects. However, citalopram reduces left ventral striatum activation in win omission. This is somewhat consistent with the behavioural data in that the ventral striatum is understood to encode prediction error in reward processing i.e. increase activation in either unexpected win or win omission. As such that the SSRI group demonstrate reduced ventral striatum activation during win omission could indicate a blunting of reward prediction errors following treatment.

Self-reported effects

No significant group differences in anhedonia self-report scores were observed, however the SSRI group did demonstrate an increase in emotional blunting, consistent with some clinical evidence (Price, Cole, and Goodwin 2009).

Strengths & limitations

Limitations

Many limitations have already been discussed in previous experimental chapters; these will briefly be revisited here alongside some broader considerations.

Pharmacological effects of probes used

Much of the posited mechanisms of action of the two interventions used are theoretical and inferred from animal studies, without direct measurement we cannot be sure of the postulated effects. These include the levels of extracellular 5-HT in different regions of the brain during testing and the direct or indirect non-serotonergic effects of buspirone and SSRIs. Furthermore, the presumed final common pathway of antidepressants, namely the neuroplastic & molecular effects, such as altered neuronal architecture or increases in BDNF, were not measured. Therefore, conclusions regarding any effects from mechanisms of action and subsequent effects on brain circuits should be treated with caution. This could be explored with assays of blood samples for plasma BDNF concentration, or of measurement of synaptic 5-HT levels using PET imaging

Pharmacokinetics

Similar to clinical practice, variation in how the body handles a medication can vary e.g. metabolism, clearance, leading to variation in exposure of the brain to the medication in question. To a certain extent in the buspirone study this was mitigated with measurement of temperature and cortisol to infer the degree of pre- & post-5-HT$_{1A}$ receptor agonism respectively. However, compliance with taking the medication as requested in the citalopram study was assumed. To resolve this, plasma citalopram concentrations could be measured. Similar criticisms could arise for individual differences in genotype, specifically serotonin transporter-linked promoter region (5-HTTLPR) polymorphism, which could influence the effect of an SSRI on 5-HT levels or the behavioural and neural responses in the tasks used, with potential confounding of any group effects therein. This limitation could be addressed by genetic testing for this specific polymorphism.

Nature of the tasks used

The effort grip task used here, specifically measurement of cost-benefit decision making, was developed in clinical samples but has not yet been used in interventional studies of healthy volunteers. It may be that increasing range of effort or reward is required to elicit effects of pharmacological interventions in the healthy population.

Similarly, subjective taste has proven inconsistent in revealing group difference of pharmacological interventions. Sub-chronic dopaminergic agents have elicited a difference in taste tasks, however serotonergic agents less so. Gustatory experience is a complex process and liable to several confounds such as smoking, recent food intake and caffeine intake. While smoking status was established and subjects requested not to consume food, nicotine or caffeine close to testing, compliance could not be guaranteed and therefore may influence results. It is expected that randomisation would help mitigate such effects.

Healthy volunteer samples

Physiological reserves of neurotransmitters and homeostatic responses of neural circuits to interventions may mitigate any effects of said interventions in the tasks used. It may be that alternative measurements in the tasks must be taken to find effects, such as those by Scholl et al. (2017) who found 14-day course of SSRI led to learning being more robust to interference as opposed to direct improvement/decrease per se (Scholl et al. 2017).

Self-report prior to testing indicated no anhedonia was present for either study sample at baseline. No significant differences in self-report anhedonia emerged after a 7-day course of citalopram. These studies could be criticised for low expected levels of anhedonia in healthy participant samples leading to floor effects. However, SSRIs are believed to not improve, or perhaps worsen, hedonic experience and reward processing, so this criticism may not be fully applicable to the citalopram study. However, a key premise of this thesis is distilling reward processing and thus anhedonia into its constituent stages, and that even if the final common outcome of anhedonia self-report does not change, this would not necessarily preclude changes in its constituent underlying behavioural or neural processes, such as an increase in sensitivity to reward. Such an issue may be addressed by performing similar studies in clinical, anhedonic populations, where any changes in the behavioural stages of reward processing could have meaningful subjective effects for a subject's anhedonic experience. Extending this theme of generalisability, it should be recognised too that tasks were somewhat artificial in nature, lacking 'real world' consequences and were performed in a testing room or fMRI scanner on a university site. This limits their ecological validity in respect to everyday decision making and functioning. This could be addressed using portable behavioural tasks, that could be performed at home or with a wider range of stimuli, closer to everyday, salient decisions e.g. speaking with friends; drinking coffee; saving money when shopping.

Study design

Another limitation of the study is the between-group design, which is statistically less powerful than a within-subject design, which may also reduce the impact of between subject variability arising from individual differences. However, it should also be acknowledged that between-group testing does mitigate practice effects, which may be significant as the intervention may differentially influence learning from tasks as opposed to the construct of the task itself.

Strengths

The tasks described in this thesis measure primary and secondary reward explicitly and implicitly, thereby capturing a broader range of hedonic experiences for reward processing. Furthermore, assessing several stages of reward processing and punishment processing with one intervention, enabling parallels to be drawn and effects easier to isolate, as stages are all interlinked to a certain extent and as such, distal effects could indirectly be due to proximal changes in the reward processing pathway.

Another strength of this thesis is its use of licensed pharmacotherapies used in day to day clinical practice, that in an experimental medicine context have been relatively well characterised. In adding both behavioural, and in the case of citalopram, neuroimaging findings, this thesis enables a richer understanding of why they may, or may not, help the common and often difficult to treat symptom of anhedonia.

A motivation for this thesis is what the results could mean for the patient sat in clinic or in hospital who is experiencing anhedonia. The behavioural results for chapter 4 indicated a 7-day course of citalopram may have minimal effect on consummatory and cost:benefit decision making, albeit in healthy volunteers, which supports the observations made by others that SSRI treatment, of various durations, do not improve self-reported anhedonia in depressed individuals (Cao et al. 2019).

Furthermore, the finding of changes in optimal choices for win and loss trials, alongside the changes in subjective emotional blunting, are consistent with observational evidence that SSRI may induce an emotional blunting or apathy (Price, Cole, and Goodwin 2009; Masdrakis, Markianos, and Baldwin 2023). In this context, results presented in chapter 4 may contribute to the discussion between clinicians and patients in terms of expectations of effects for anhedonia and motivation in the first week of SSRI treatment.

The acute effects of buspirone, which may be due to pre- & post-synaptic 5-HT$_{1A}$ receptor activation, show an increased sensitivity to subjective pleasure of primary aversive stimuli and increased optimal choices in aversive learning. This may aid clinical discussion around potential increases in sensitivity to negative events in the early stages of drugs which agonise the 5-HT$_{1A}$ receptor, either directly such as vortioxetine or indirectly such as SSRI. Although caution should be taken with this view as although 5-HT$_{1A}$ receptor agonism could be common action of many serotonergic drugs it would not be the only action, with other 'non-5HT$_{1A}$ receptor' effects contributing to the early effects of drugs possessing either direct or indirect effects on the 5-HT$_{1A}$ receptor. One avenue of exploration that could aid the clinical relevance of 5-HT$_{1A}$ receptor agonism for anhedonia would be a longer period of buspirone administration. This could facilitate downregulation of pre-synaptic 5-HT$_{1A}$ receptors, enabling disinhibition of serotonergic neurones, whilst maintaining a degree of post-synaptic 5-HT$_{1A}$ partial agonism.

Future considerations

In addition to the aforementioned work in this chapter, further work could be completed to add to our understanding of reward processing and pharmacotherapies to target it. This could include incorporation of other factors in the tasks used here such as a theoretical delay in outcome, alongside further computational analysis of cost-benefit decision making and reward learning models. This would enable discounting of effort, probability and delay to be measured in a single battery of tests.

Validation of the taste task and effort grip task as sensitive to changes in anhedonia in clinical samples should be performed. Furthermore, inclusion of a wider range of effort and reward magnitudes to improve the potential sensitivity of the effort grip task to enable changes in cost-benefit decision making from pharmacotherapies in healthy volunteers to be detected.

Measurement of cognitive effort, as opposed to physical effort, should also be investigated in exploring the effect of changes in serotonin transmission on reward processing, as cognitive effort discounting may have greater ecological validity to individuals experiencing anhedonia.

One of the major difficulties in predicting and understanding the psychotropic effects of buspirone is its actions at both pre-and post-synaptic $5HT_{1A}$ receptors. Over the last decade a novel concept of biased agonism has emerged in drug action, in that different compounds may be able to trigger differing intracellular pathways via the same receptor. Such a compound for the $5\text{-}HT_{1A}$ receptor is NLX-101, which acts selectively at post-synaptic $5\text{-}HT_{1A}$ receptors (Newman-Tancredi et al. 2022), and both acute and repeated dosing has shown positive effects in animal models of depression (Assie et al. 2010; Jastrzebska-Wiesek et al. 2018) and anhedonia (Depoortere et al. 2019) as well as repeated doses having neuroplastic effects (Aguiar et al. 2020). Testing agents such as NLX-101 in the experimental models described here could be important avenue to explore in developing new therapeutics for anhedonia

Whilst medications probing different neurotransmitter systems have been used already in some of these tasks (e.g. pramipexole on taste and PILT paradigms) there is scope to revisit these compounds using additional tasks, for example, the effort grip task or a novel cognitive effort task. These tasks could also be helpful to assess compounds in development e.g. kappa opioid receptor antagonists or KCNQ channel modulators. This approach could provide new insights in potential non-monoaminergic treatments for anhedonia in the future.

This thesis contributes novel evidence to the field by implicating components of the serotonin system in aspects of emotional and reward processing, thus potentially low mood and anhedonia.

However, at the same time it cannot convincingly dissuade us from the idea that serotonin is ‘implicated in virtually everything, but responsible for nothing’ (Jacobs and Fornal 1995).

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