# DISSOCIATING THE ROLE OF PREFRONTAL NORADRENALINE SIGNALING IN COST-BENEFIT DECISION MAKING AND IMPULSIVE ACTION

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## Abstract

Win-paired stimuli can promote risk taking on experimental gambling paradigms in both rats and humans. We previously demonstrated that atomoxetine, a noradrenaline reuptake inhibitor, and guanfacine, a selective α2A adrenergic receptor agonist, reduced risky choice on the cued rat gambling task (crGT), a rodent decision making task in which wins are accompanied by salient audiovisual cues. Both compounds also decreased impulsive premature responding. However, the central mechanisms behind noradrenergic regulation of cue-guided risk taking and impulsivity have not yet been elucidated. Areas of the prefrontal cortex such as the lateral orbitofrontal cortex (lOFC) and prelimbic (PrL) subregion of the medial prefrontal cortex receive dense noradrenergic innervation and are highly implicated in risk assessment, action selection and impulse control. I therefore probed the prefrontal substrates of noradrenaline's influence over gambling-like behaviour by infusing atomoxetine and guanfacine directly into either the lOFC or PrL prior to task performance. Atomoxetine infused into the lOFC improved decision making score in male rats but did not alter decision making in females. Atomoxetine also improved impulse control when infused into the PrL, yet only in risk preferring animals. Contrastingly, intra-PrL guanfacine exacerbated motor impulsivity in all subjects. Our data reveal a double dissociation such that lOFC noradrenaline importantly guides decision making, at least in males, while PrL noradrenaline signaling regulates motor impulsivity. We also show that noradrenergic manipulations differentially influence behaviour depending on baseline risk preference, suggesting that the noradrenaline system may function differently in subjects that are susceptible to the risk-promoting lure of win cues.

## Lay Summary

Adding casino-like win-paired sound and light cues to laboratory gambling tasks promotes risk taking. Drugs that alter noradrenaline signaling improve decision making and impulse control on the cued rat gambling task (crGT), a rodent decision making task where audiovisual cues accompany wins. I sought to determine the mechanisms behind how noradrenaline guides crGT performance by infusing small amounts of noradrenergic drugs directly into prefrontal brain regions before rats gamble. I found that atomoxetine, a reuptake blocker, improved decision making in male rats when infused into the lateral orbitofrontal cortex (lOFC). Atomoxetine reduced impulsivity in risk preferring rats when infused into the prelimbic cortex (PrL), while intra-PrL guanfacine, an α2 noradrenaline receptor agonist, increased impulsivity. These findings help define the mechanisms that underly cue-invigorated risk taking, highlighting the lOFC and PrL as mediators of decision making and impulsivity, respectively, and how noradrenaline's effects may depend on sex and risk preference.

## Preface

This thesis is based on original, unpublished data collected by the author, C. Chernoff, to fulfill the requirements for a Master of Science in Neuroscience at the University of British Columbia. My supervisor, Dr. Catharine Winstanley, and Dr. Stan Floresco oversaw and guided the experimental planning, data collection, and data interpretation for these experiments. The writing, data analysis, and figure generation for the thesis were completed by the author.

The author performed stereotaxic surgeries, intracranial microinfusions, histology, and behavioural training and testing. Fabrication of the microinfusion hardware and preparation of the drugs were conducted by the author. Jackson Schumacher performed stereotaxic surgeries and microinfusions with the author, and provided guidance on animal husbandry, hardware manufacturing, and data analysis. Shrishti Ramaiah assisted with acute microinfusions and behavioural training. Dimitrios Avramidis and Shrishti Ramaiah assisted with the histology. Dr. Tristan Hynes provided guidance on statistical analysis and data interpretation.

All procedures were in accordance with the Canadian Council on Animal Care and the University of British Columbia Animal Care Committee (ACC) under ACC protocol number A21-0012.

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## List of Abbreviations

| | |
|---|---|
| ADHD | Attention deficit hyperactive disorder |
| ANOVA | Analysis of variance |
| A-O | Action-outcome |
| AP | Anteroposterior |
| ATX | Atomoxetine |
| CNO | Clozapine-N-oxide |
| crGT | Cued rat gambling task |
| DAT | Dopamine transporter |
| DREADD | Designer receptor exclusively activated by designer drugs |
| DV | Dorsoventral |
| GD | Gambling disorder |
| GFC | Guanfacine |
| HI | High impulsive |
| IGT | Iowa Gambling Task |
| IL | Infralimbic cortex |
| LC | Locus coeruleus |
| LI | Low impulsive |
| lOFC | Lateral orbitofrontal cortex |
| ML | Mediolateral |
| mPFC | Medial prefrontal cortex |
| NET | Noradrenaline transporter |
| OFC | Orbitofrontal cortex |

| | |
|---|---|
| OPT | Optimal performing |
| PFC | Prefrontal cortex |
| PrL | Prelimbic cortex |
| rGT | Rat gambling task |
| RP | Risk preferring |
| SEM | Standard error of the mean |
| TH | Tyrosine hydroxylase |
| VEH | Vehicle |
| VTA | Ventral tegmental area |

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# Chapter 1: Introduction

## 1.1 Gambling Disorder

Gambling disorder (GD) imposes an immense financial burden at the level of both the individual and society, inflicting annual costs of around $5 billion in the U.S. alone (Gerstein et al. 1999). GD is further associated with significant emotional and psychological afflictions, and is often comorbid with other psychiatric conditions such as major depressive disorder, anxiety disorders and substance use disorder (Karlsson & Håkansson, 2018). Considering the high prevalence of psychiatric comorbidities in GD patients, excessive gambling and risk taking may exemplify coping strategies, whereby gameplay may provide an "escape" from negative internal states (Kruger et al., 2020; Quigley et al., 2015). There are also notable gender differences in the prevalence and manifestation of problematic gambling, as men are generally at a greater risk of developing GD than women (Merkouris et al., 2016), and men and women differ in the factors that contribute to GD severity (Jiménez-Murcia et al., 2020). Women also tend to progress from casual to problematic gambling more rapidly than men, a phenomenon termed 'telescoping' (Grant et al., 2012). However, the biobehavioural and environmental elements that facilitate the transition from recreational to pathological gambling, and how these may differ between males and females, are not fully understood.

### 1.1.1 Win-paired cues

Risk taking is hallmark characteristic of GD. Studies in both humans and rodents demonstrate that adding win-paired audiovisual cues to experimental gambling paradigms increases the proportion of subjects that develop risky decision making strategies (Barrus &

Winstanley, 2016; Cherkasova et al., 2018; Spetch et al., 2020). As such, the salient lights and sounds of a modern gambling environment are not as innocuous as once believed and may promote detrimental risk taking behaviour. Gambling-related cues can trigger the urge to gamble in both casual and pathological gamblers (Park et al., 2015; Potenza et al., 2003), and those with GD exhibit heightened reactivity and attentional biases to such cues (McGrath et al., 2018; van Holst et al., 2012). These phenomena parallel those described by the incentive salience theory of addiction, which posits that drug-paired cues can take on motivational value and subsequently trigger drug cravings in individuals with substance use disorder (T. E. Robinson & Berridge, 1993). This may suggest a shared underlying mechanism that might contribute to the high comorbidity between GD and substance abuse (Olney et al., 2018; Winstanley & Hynes, 2021). Gambling-related stimuli can also enhance physiological arousal which is itself a significant reinforcer in the initiation and maintenance of problematic gambling behaviour (Baudinet & Blaszczynski, 2013; Brown, 1986). Importantly, pathological gamblers experience larger cue-induced increases in arousal compared to controls or recreational gamblers (Meyer et al., 2004; Starcke et al., 2018). Some theorize that heightened reactivity to gambling cues may mediate the transition into maladaptive, trance-like states referred to as “the zone” or “dark flow” (Tricker et al., 2016). In such states, individuals become completely absorbed in the game, disregarding non game related stimuli such as passing time, general casino commotion, and personal life stressors (Dixon et al., 2019; Schull, 2005). This mode of total fixation is problematic as it can lead to greater financial losses and more time spent gambling, and levels of maladaptive game immersion positively relate to GD severity (Murch & Clark, 2021; Rogier et al., 2021). Recent evidence suggests that shifts in flow-like states of arousal and attention may involve activation of the locus coeruleus noradrenaline system (Lu et al., 2023), the main regulator of central arousal

and attention (Bouret & Sara, 2004; Robbins, 1984). This is consistent with work indicating disruptions in noradrenergic system function in problematic gamblers compared to controls (Meyer et al., 2004; Pallanti et al., 2010; Roy et al., 1988). The neurobiology behind how win cues promote pathological risk taking is, however, not fully elucidated, and is important to disseminate if we are to aptly understand, prevent, and treat disorders of decision making.

## 1.2 Noradrenaline and behaviour

Experimental investigations of the neurochemical basis of cue-invigorated risk taking have predominantly focused on dopaminergic processes (Hynes et al., 2020; Mortazavi et al., 2023; Winstanley & Hynes, 2021). Given that win cues have such a prominent influence on attention, arousal, and states of consciousness (Brown, 1986; Tricker et al., 2016), noradrenaline is an integral neurotransmitter to consider when probing the neural mechanisms behind the behavioural consequence of cues.

### 1.2.1 Adaptive gain theory of locus coeruleus noradrenaline

The noradrenergic locus coeruleus (LC) in the brainstem fires in distinct patterns, allowing noradrenaline to differentially guide reward motivated behaviours depending on its manner of release. As described by the adaptive gain theory, noradrenergic cells of the LC exhibit phasic responses to salient and motivationally relevant stimuli (Aston-Jones et al., 1994; Bouret & Richmond, 2015; Bouret & Sara, 2004). These phasic LC bursts promote fixed attention and persistent ‘exploitation’ of the current, profitable behavioural strategy (Aston-Jones & Cohen, 2005). As the utility of an option wanes, LC activity shifts to high tonic levels, during which phasic LC responses are diminished and prior behavioural strategies are disengaged,

encouraging ‘exploration’ of other potentially lucrative options (Aston-Jones & Cohen, 2005; Usher et al., 1999). As such, LC noradrenaline can optimize decision making by tracking behavioural efficacy and precipitating appropriate changes in strategy. The main principles of the adaptive gain theory are echoed and expanded by Sales et al.’s work mathematically fitting LC activity to an active inference behavioural model (2019). In short, this model supports the hypothesis that phasic and tonic LC firing work in concert to promote flexible updating of internal action-outcome and task structure representations when ‘state-action prediction errors’ occur, ie. when recent observations necessitate a substantial change in an individual’s overall cognitive model of the world/task (Sales et al., 2019). This active-inference model is remarkably consistent with multiple empirical observations of *in vivo* task-related LC cell activity (Aston-Jones et al., 1994; Bouret & Richmond, 2015; Dayan & Yu, 2006). Importantly, the authors also suggest the prefrontal cortex as the critical site at which LC noradrenergic signals may converge to refine internal representations and optimize decision making (Sales et al., 2019).

### 1.2.2 Alpha-2 adrenergic receptors and prefrontal function

In addition to large-scale patterns of noradrenaline release, certain adrenergic receptor subtypes have been implicated in the beneficial effects of noradrenaline on attention and cognitive performance. The α2 adrenergic receptor, particularly at postsynaptic sites, has been purported to be a main regulator of prefrontal cortex (PFC) function (Arnsten et al., 1996). Catecholaminergic denervation impairs performance on a PFC-dependent working memory task, which can be rescued by α2 agonists (Arnsten et al., 1988; Cai et al., 1993). Improvements in working memory following α2 agonism are also more pronounced in denervated animals compared to intact subjects (Arnsten & Goldman-Rakic, 1985), further suggesting that the

benefits of α2 receptors on certain prefrontal functions are orchestrated postsynaptically. The α2A receptor subtype, compared to the α2B and α2C subtypes, is pervasively expressed in the LC and prefrontal cortex (Aoki et al., 1994), suggesting it as a potential key player in executive functions that are regulated by noradrenaline. Selective mutations of the α2A receptor negate the benefits of guanfacine, an α2A agonist, on working memory (Franowicz et al., 2002), further supporting that action at α2A receptor subtypes drives guanfacine-mediated improvements in certain cognitive functions. Interestingly, guanfacine bidirectionally altered performance on various attentional tasks. Guanfacine impaired accuracy on variations of the five choice serial reaction time task which require 'scanning attention' (Milstein et al., 2007), yet benefitted performance on tasks requiring narrow attentional focus and resistance to distractors (Arnsten & Contant, 1992; O'Neill et al., 2000). These disparate findings indicate that the benefits of α2A agonism critically depend on the cognitive demands of the task. While there is plenty of support for the role of noradrenaline signaling, and specifically α2A adrenergic receptors, in PFC-dependent functions such as working memory and attention, studies investigating noradrenergic mechanisms underlying prefrontal regulation of cost-benefit decision making or impulse control are currently lacking.

### 1.2.3 Noradrenaline and the cued rat gambling task

We previously demonstrated that pharmacological manipulations of noradrenergic signaling can significantly alter behaviour on the cued rat gambling task (crGT), a preclinical cost-benefit decision making paradigm in which risk-promoting audiovisual cues accompany wins, reminiscent of the human gambling environment (Barrus & Winstanley, 2016). During the crGT, rats maximize sugar pellet wins by choosing between options of varying probability and

magnitude of wins and time out punishments. The optimal strategy on the crGT is to select low-risk, low-reward options that amount to larger overall winnings throughout a 30-minute session due to smaller, less frequent time-out punishments. When injected systemically, atomoxetine, a selective noradrenaline transporter (NET) blocker, and guanfacine both independently improved decision making on the crGT by reducing preference for risky, highly-cued options and promoting choice of safer options that deliver smaller, more likely wins (Chernoff et al., 2021). Both compounds also reduced premature impulsive responses, consistent with the body of work describing the potent anti-impulsivity effects of atomoxetine and guanfacine in both clinical and preclinical settings (Chamberlain et al., 2006; Fernando et al., 2012; Nishitomi et al., 2018; E. S. J. Robinson et al., 2008). The ability of these noradrenergic medications to improve decision making on the crGT was in complete contrast to findings in the uncued version of the task, whereby atomoxetine did not influence, or even slightly impaired, rGT performance (Baarendse et al., 2014; Silveira et al., 2016). Such opposing results indicate that salient cues may uniquely engage the noradrenaline system, allowing noradrenergic manipulations to selectively benefit decision making processes in the presence of risk-promoting win-paired cues. These findings highlight the importance of noradrenaline in cue-guided risk taking and impulsivity, yet the neural mechanisms behind these behavioural effects are not yet identified.

### 1.3 Prefrontal regulation of decision making and impulsivity

The majority of cortical noradrenaline is supplied by the LC, with various prefrontal regions receiving direct, non-collateralized noradrenergic projections from LC neurons (Chandler et al., 2013, 2014). Accordingly, the central noradrenaline system is organized to allow for independent regulation of frontal brain regions that orchestrate distinct cognitive and

behavioural functions. Subregions of the prefrontal cortex, such as the orbitofrontal cortex (OFC) and medial prefrontal cortex (mPFC), are densely innervated by noradrenergic LC terminals (Chandler et al., 2013, 2014) and are critically involved in higher order processes underlying decision making, probabilistic reward learning, and top-down behavioural control.

### 1.3.1 Orbitofrontal cortex and decision making

OFC dysfunction is observed in multiple psychiatric disorders associated with impaired decision making and impulse control, such as GD, attention deficit hyperactive disorder (ADHD), and substance use disorder (Dom et al., 2005; Verdejo-Garcia et al., 2015; Yang et al., 2019). It is well-established that the OFC is integral for forming, updating, and using cue- and action-outcome associations to guide optimal decision making, with evidence coming from both clinical and preclinical studies (reviewed in Bechara et al., 2000; Izquierdo, 2017; Schoenbaum et al., 2009). Some suggest that the OFC may generate a predictive 'value signal', based on experience with task contingencies, to help guide optimal decisions (Balleine et al., 2011). Consistent with this view, the activity of OFC neurons directly tracks the value of an option in a risk-based 2-choice task as the utility of each option changes across a session (Hong et al., 2019). An emerging perspective is that the OFC may, instead of strictly assessing predicted value, establish a more wholistic representation of task or world structure allowing the OFC to orchestrate appropriate actions from a comprehensive internal model of the environment (Zhou et al., 2021). Recent work reveals that noradrenergic inputs to the OFC are necessary for rats to update action-outcome (A-O) representations in the face of changing contingencies and adjust subsequent decisions accordingly (Cerpa et al., 2022). However, intra-OFC dopamine was not required for the same flexible regulation of behaviour (Cerpa et al., 2022), indicating the

particular importance of noradrenaline signaling in OFC-dependent decision making processes. Others suggest that, more specifically, *tonic* noradrenaline in the OFC is the neurochemical mediator of flexible behaviour (Sadacca et al., 2017), marrying the principals of the adaptive gain theory with our understanding of the OFC as a hub for cue-outcome and A-O representations. How OFC noradrenaline relates to cost-benefit decision making in the presence of win cues, however, remains to be tested.

### 1.3.2 Medial PFC: action selection and impulse control

Within a given decision making task, mPFC involvement appears to be dissociable from that of the OFC. In a 2-choice task, neurons in the mPFC did not track the shifting utility of an option across a session, as OFC cells did, but reliably responded to small immediate rewards (Hong et al., 2019). As such, the mPFC may function to signal immediate gain, irrespective of overall value, which could guide behaviour on a trial-by-trial basis. Consistently, optogenetic inhibition of the PrL mPFC during pre-choice epochs and during presentation of losses or wins impaired the ability of recent outcomes to guide advantageous decisions on a probabilistic discounting task (Bercovici et al., 2023). These findings support that the PrL is important for using recent A-O observations to inform subsequent action selection in the face of changing reward probabilities. Interestingly, noradrenergic inputs to the PrL mPFC were not required for rats to properly adjust their behaviour in an A-O reversal task, while noradrenergic afferents in the OFC were (Cerpa et al., 2022). This functional dissociation indicates that the influence of noradrenaline on prefrontally-governed behaviours critically depends on the site of action.

The above evidence generally implicates the OFC as a prefrontal center for building and adapting internal representations of the world/task, while the mPFC may be more involved in

using recent observations to mold subsequent actions. In addition to directing action selection, the mPFC is also a marked regulator of response inhibition. Inactivation of the mPFC impairs the ability of subjects to wait for a target stimulus before responding (Feja & Koch, 2014; Hardung et al., 2017; Narayanan et al., 2006), and the activity of neurons in the PrL mPFC track and predict impulsive premature responding in various experimental paradigms (Hardung et al., 2017; Moschak & Carelli, 2021; Narayanan et al., 2006). Extensive training on a response inhibition task even altered the intrinsic excitability of PrL neurons, which was inversely related to task performance (Hayton et al., 2011). It is well-established in the literature that noradrenaline is a significant neurochemical mediator of impulse control and motor impulsivity (Bari et al., 2009; Chamberlain & Sahakian, 2007; Robinson et al., 2008). Bari et al. (2011) indicated prefrontal regions such as the OFC and dorsal PrL as critical sites for noradrenergic control over stopping impulsivity using the stop signal task. How noradrenaline contributes to risk taking and motor impulsivity on the crGT, and the potentially distinct prefrontal substrates underlying noradrenergic regulation of such behaviours, have not yet been disseminated.

### 1.4 Summary of research objectives

I therefore sought to determine the prefrontal regions at which atomoxetine and guanfacine may act to improve performance on the crGT (Chernoff et al., 2021). I did so by infusing either noradrenergic drug into the lOFC or PrL of rats trained on the crGT, prior to performance of the task. I used rats of both sexes in all of experiments to explore any potential sex differences in noradrenergic modulation of gambling-like behaviours, given the gender differences in GD prevalence, progression and presentation (Grant et al., 2012; Jiménez-Murcia et al., 2020; Merkouris et al., 2016), as well as sex differences in the sensitivity and function of

the noradrenergic system (Bangasser et al., 2019; Luque et al., 1992; Mei et al., 2021). Given the differential roles of the OFC and PrL in flexible A-O assessment and response inhibition, respectively, I hypothesized that noradrenergic lOFC manipulations would selectively guide risk taking while intra-PrL drug infusions would affect motor impulsivity. Specifically, I expected that increasing noradrenergic tone with intra-lOFC atomoxetine will improve decision making, shifting preference away from disadvantageous high-risk, high-reward options on the crGT. I further predicted that both atomoxetine and guanfacine, when infused directly into the PrL, will reduce impulsive premature responses. These experiments will help elucidate the underlying neural mechanisms behind noradrenergic regulation of gambling-like behaviour. It is critical to understand how and where noradrenergic compounds may act in the brain to improve decision making and impulse control in an environment that is inundated with risk-promoting cues. Such mechanistic insight could facilitate the safe and effective transition of FDA-approved compounds like atomoxetine and guanfacine into clinical settings as potential pharmacotherapies for GD and related psychopathologies.

# Chapter 2: Local pharmacology experiments

## 2.1 Background and rationale

The thrilling lights and buzzers of a modern casino may have a greater impact on the development of problematic gambling behaviour than once thought. When light and sound cues are paired with wins in experimental gambling tasks, a larger proportion of both rodent and human subjects adopt risky decision making strategies (Barrus & Winstanley, 2016; Cherkasova et al., 2018; Spetch et al., 2020). The neurobiology behind cue-exacerbated risk preference, however, has not been wholly elucidated. Gambling-related cues enhance arousal (Dixon et al., 2014) and can trigger cravings to gamble (Park et al., 2015; Potenza et al., 2003). Those with GD demonstrate heightened attentional biases to such cues (McGrath et al., 2018; van Holst et al., 2012). This exaggerated cue-reactivity may contribute to the propensity toward maladaptive states of altered consciousness and attention (Tricker et al., 2016), such as 'the zone' or 'dark flow', wherein individuals with GD exhibit trance-like game immersion and become insensitive to non-game related stimuli, such as passing time and personal life stressors (Dixon et al., 2019; Schull, 2005). Noradrenaline, as the main regulator of attention and arousal in the central nervous system, is therefore integral to consider when examining the mechanisms behind the risk promoting effects of win-paired stimuli.

We previously showed that systemic administration of a noradrenaline transporter (NET) blocker, atomoxetine, and an α2 adrenergic receptor agonist, guanfacine, improved decision making on the crGT by shifting preference away from highly cued, high-risk high-reward options toward safer options that yield more consistent smaller wins (Chernoff et al., 2021). This is in stark contrast to data from the uncued version of the rat gambling task in which atomoxetine

had no effect or slightly reduced optimal choice (Baarendse et al., 2014; Silveira et al., 2016). Noradrenaline may therefore uniquely modulate decisions that are made under the influence of risk-promoting win cues. Both atomoxetine and guanfacine were also able to reduce impulsive responses made prematurely on the crGT (Chernoff et al., 2021). Noradrenaline action in the prefrontal cortex, particularly at postsynaptic α2 receptors, underlies the beneficial effects of noradrenergic compounds on working memory and attention (Arnsten et al., 1988; Arnsten et al., 1996; Cai et al., 1993). Whether noradrenaline likewise acts in the frontal cortices to alter decision making and impulse control on the cued rGT has yet to be tested.

Notably, areas of the prefrontal cortex such as the OFC and mPFC receive discrete and direct noradrenergic projections from the locus coeruleus (Agster et al., 2013; Chandler et al., 2013, 2014). These frontal areas are also functionally heterogeneous. The OFC is important for establishing, updating, and using cue- and action-outcome associations to guide optimal decision making (reviewed in Bechara et al., 2000; Izquierdo, 2017; Schoenbaum et al., 2009). Recent evidence indicates that noradrenaline in the OFC, but not mPFC, is necessary for appropriately updating action-outcome representations to guide subsequent decisions (Cerpa et al., 2022), specifically implicating noradrenaline in OFC-dependent value assessment. Whereas OFC neurons tracked the utility of an option as it changed across a session, mPFC neuron activity instead consistently responded to small immediate rewards (Hong et al., 2019).

The PrL cortex of the mPFC is strongly implicated in response inhibition and action selection (Feja & Koch, 2014; Narayanan et al., 2006), with multiple studies demonstrating a significant relationship between PrL neuron activity and motor impulsivity (Hardung et al., 2017; Hayton et al., 2011; Moschak & Carelli, 2021; Narayanan et al., 2006; Narayanan & Laubach, 2006). Noradrenaline itself is known to be a potent regulator of impulsive action (Bari et al.,

2009; Chamberlain & Sahakian, 2007; E. S. J. Robinson et al., 2008), with the anti-impulsivity properties of noradrenergic drugs like atomoxetine well-established in the literature (Bari et al., 2009; Economidou et al., 2012; E. S. J. Robinson et al., 2008), and further corroborated by rGT data from our laboratory (Chernoff et al., 2021; Silveira et al., 2016). Yet, it is unknown whether noradrenaline modulates impulse control on the crGT through direct action in frontal areas such as the PrL.

Given our previous findings that noradrenergic manipulations significantly influence cue-guided gambling-like behaviour in rats, I aimed to discern the prefrontal substrates which may orchestrate noradrenaline's contributions to risk taking and motor impulsivity in the presence of win-paired cues. Here, I pharmacologically manipulated local noradrenaline signaling in the lateral OFC or PrL as rats performed the crGT. Considering the dissociable roles of the OFC and PrL in risk assessment and impulse control, I predicted that pharmacologically enhancing noradrenaline signaling in the OFC would promote safer decision making, while intra-PrL noradrenergic manipulations would reduce impulsive premature responses.

## 2.2 Methods

### 2.2.1 Subjects

In total, 32 male and 33 female Long Evans rats (Charles River Laboratories, St. Constant, QC), completed the behavioural experiments, divided into four cohorts based on sex and targeted brain region (16 male-lOFC, 16 female-lOFC, 16 male-PrL and 17 female-PrL). Rats were pair- or trio-housed with same-sex cagemates in a climate-controlled colony room on a reverse 12-hour light-dark cycle (lights off at 08:00am; temperature 21°C). At least one week prior to the start of behavioral training, animals were food restricted to ~85% of their free feeding weight and maintained at ~15 grams of standard rat chow per day for males and ~11 grams per day for females. Water was available *ad libitum* in the homecage. For days on which behavioural testing or training occurred, rats were fed directly following the behavioural session. All housing conditions and testing procedures were in accordance with the guidelines of the Canadian Council of Animal Care, and all protocols were approved by the Animal Care Committee of the University of British Columbia, Vancouver.

### 2.2.2 Apparatus

Behavioral testing was conducted in 32 identical five-hole operant chambers (30.5 × 24 × 21 cm; Med Associates, St. Albans, VT, USA), each enclosed in a ventilated sound-attenuating cabinet (Med Associates, St. Albans, VT, USA). Boxes were equipped with fans for air circulation and extrinsic noise cancellation. Along the curved wall of the chamber was an array of five nose-poke holes, each equipped with an infrared detector and a yellow LED stimulus light. On the

opposite wall, a food tray was positioned to deliver dustless sugar-coated food pellets (45 mg, Formula P, Bio-Serv, Frenchtown, NJ, USA). The chamber was illuminated by a white house light attached to the roof. Apparatus control and data collection were conducted using code written by CAW in MEDPC (Med Associates) running on standard IBM-compatible computers.

### 2.2.3 Cued rat gambling task (crGT)

As described previously, behavioural training began with two daily 30-min chamber habituation sessions followed by operant nose-poke training and seven days of forced choice crGT sessions, during which rats are exposed to the contingencies of each task option (Barrus & Winstanley, 2016; Ferland et al., 2019; Zeeb et al., 2009). Rats then went on to perform the full, free choice version of the crGT. In short, a nose-poke into the illuminated food tray initiated a 30-min crGT session during which rats sampled from four of the response openings (the middle hole of the 5-hole array is not used in the crGT). Each option was associated with a unique magnitude and probability of both a sugar pellet reward and time-out punishment (Figure 1). During the time out punishments, the house light turned on and subjects were unable to initiate a new trial. The optimal strategy on the crGT is to favor the options that result in a smaller per-trial reward coupled with shorter time-out punishments (P1 and P2). These ‘safe’ options earned the most reward throughout the task due to more consistent wins, less frequent punishment, and shorter time-out penalties compared to the ‘risky’ options (P3 and P4) that delivered larger, uncertain rewards and longer, more frequent punishments. In the crGT, audiovisual cues were concurrently presented with sugar pellet rewards on winning trials. The cues increased in complexity with the magnitude of the reward, similar to the human gambling experience (Barrus & Winstanley, 2016).

Much like in the five-choice serial reaction time task, premature responses were used as a measure of motor impulsivity, and were defined as nose poke responses made at the aperture array during the 5 second period between trial initiation and illumination of the stimulus lights (ie. intertrial interval, ITI). A premature response resulted in a time-out punishment of 5 seconds. An omission was recorded if the rat failed to respond at one of the four nosepoke options within 10 seconds of their illumination.

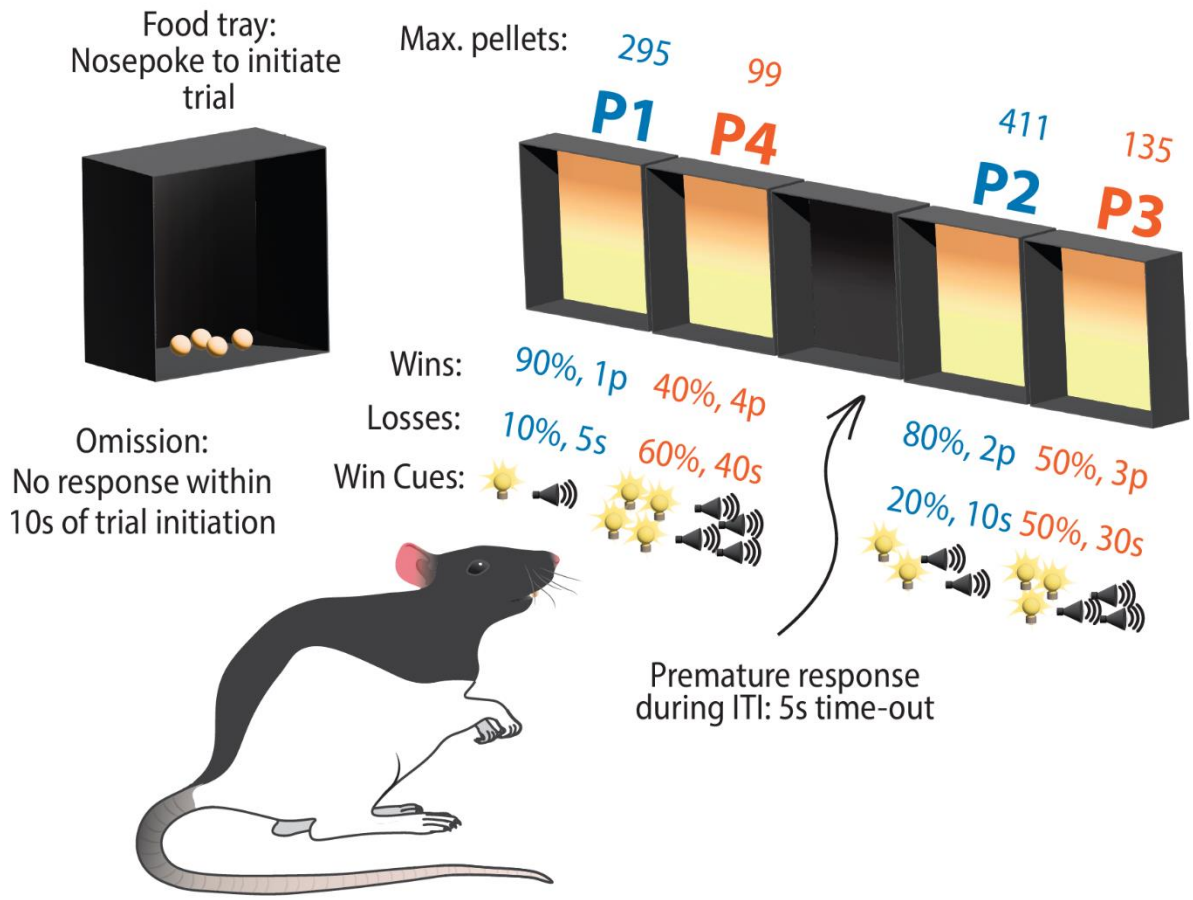


**Figure 1 Cued rat gambling task (crGT) schematic.**
Rats sample from four nosepoke holes associated with varying probabilities and magnitudes of sugar pellet wins and time-out punishments. A 30-minute trial is initiated by a nosepoke in the food tray (left side of the diagram), following which the four options are illuminated and the rat can make a choice. On winning trials, sugar pellet rewards are accompanied by audiovisual cues that scale in complexity with win magnitude, such that the riskiest options are associated with the most complex cues (see Barrus & Winstanley, 2016 for cue details)

Rats were trained on the crGT 5–7 days a week until their performance on all behavioural measures (described below) were deemed statistically stable over the last five consecutive sessions, meaning a repeated measures ANOVA revealed no significant interactions or main effect of session. In the current study, statistically stable behaviour was achieved after 32-43 free choice crGT sessions, depending on the cohort.

### 2.2.4 Surgery

After stable baseline crGT performance was attained, animals underwent stereotaxic surgery under isoflurane anesthesia (5% induction; 2% maintenance) to implant beveled 23-gauge stainless steel guide cannulae bilaterally into either the lOFC (n = 16 males, 16 females; lOFC: AP = +3.5 mm from bregma, ML = ±2.6 mm from midline, DV = −2.9 mm from dura) or PrL (n = 16 males, 17 females; PrL: AP = +3.0 mm from bregma, ML = ±0.7 mm from midline, DV = −2.8 mm from dura). Guide cannulae were secured to the skull through the aid of 4 stainless steel screws and a dental acrylic headcap. Sterile 30-gauge obdurators flush with the end of the cannulae were inserted and replaced as necessary throughout the duration of the experiment. Appropriate surgical post-care procedures were followed and animals were given at least 1 week of post-operative recovery in the homecage, with *ad libitum* food supply, before resuming any behavioural procedures.

### 2.2.5 Microinfusion procedure

Following post-surgical recovery, animals were reintroduced to the task with 5-8 free choice crGT sessions, to re-establish statistically stable performance. Animals were then habituated to the microinfusion process with a mock infusion, during which sterilized 30-gauge

injectors were inserted into the guide cannula and left in place for two minutes. No drug was infused. Animals were then left in the operant box for ten minutes following the mock infusion, prior to performing a crGT session.

Two days after the mock infusion, rats began a series of acute drug challenges with atomoxetine (ATX: 1.5 μg/side, 5.0 μg/ side, saline vehicle) and guanfacine (GFC: 0.005 μg/side, 3 μg/side, saline vehicle). Doses for intracerebral microinfusions were determined based on previous behavioural experiments (Bari et al., 2011; Economidou et al., 2012; Pardey et al., 2013). Atomoxetine hydrochloride and guanfacine hydrochloride were purchased from Sigma-Aldrich (Oakville, Canada). Drug doses were calculated as the salt and dissolved in sterile 0.9% saline. Atomoxetine was infused as a 11mM or 39mM solution for low and high doses, respectively, while low and high doses of guanfacine were administered using 41μM and 24mM solutions, respectively. Each rat received a total of six infusions: low dose, high dose, and vehicle for both atomoxetine and guanfacine. Each drug was microinfused following a balanced Latin square design (doses: ABC, BCA, CAB; Cardinal and Aitken 2013). Every subject first received either atomoxetine or guanfacine, with drug order counterbalanced across subjects, followed by at least one week of washout prior to beginning a second Latin square for the other compound to mitigate potential carryover effects.

Drug administration followed a 3-day cycle, starting with a baseline, drug-free crGT session. The following day, animals were dosed and behaviourally tested. Bilateral microinfusions of 0.5 μL per hemisphere were administered at a rate of 0.5 μL/min (for a total infusion time of 1 min) via 30-gauge injector tips that extended 0.8 mm beyond the guide cannulae. Injectors were left in place for an additional minute to allow for diffusion. Following the diffusion period, injectors were removed, sterile obdurators replaced, and animals were

placed in the operant chambers for 10 min prior to performing the crGT (Bari et al., 2011; Economidou et al., 2012; Yates et al., 2016). Animals were not tested nor dosed on the third day of the Latin square schedule.

### 2.2.6 Histology

Following completion of all behavioral testing, animals were anesthetized with isoflurane and euthanized by acute carbon dioxide exposure. Brains were immediately extracted and fixed in 4% phosphate buffered formaldehyde for 24-48 hours before being transferred to a cryoprotective 30% sucrose solution. They were then frozen and sliced into 40-µm coronal sections. Frontal brain sections were stained with cresyl violet for visualization on the Zeiss Axioscan 7 slide scanner (Zeiss, Oberkochen, Germany). Brightfield images were captured at 10x magnification to confirm cannula placement, and the projected locations of the injector tips protruding from the guide cannulae were mapped onto standard sections from the Rat Brain Atlas (Paxinos & Watson, 1998). Rats were excluded from the analyses if their cannulae were misplaced or did not accurately target the brain region of interest as defined by the Rat Brain Atlas (Paxinos & Watson, 1998).

### 2.2.7 Behavioural measures and data analysis

All statistical analyses were completed using SPSS Statistics 27.0 software (IBM, Chicago, IL, USA). As per previous reports, the following crGT variables were analyzed: percentage choice of each option (number of times option was chosen/total number of choices × 100), decision making score (calculated using precent choice variables ie. score = [(P1 + P2) − (P3 + P4)]), percentage of premature responses (number of premature responses/total number of

trials initiated × 100), sum of omitted responses, sum of trials completed, and average latencies to choose an option and collect reward. Variables that were expressed as a percentage were subjected to an arcsine transformation prior to statistical analysis to limit the effect of an artificially imposed ceiling (i.e., 100%). The last five post-surgery crGT sessions that were statistically stable (ie. a repeated-measures ANOVA in which neither the main effect of session or the session × choice interaction were not significant) were used to determine baseline performance. Animals with mean positive (ie. ≥ 0) baseline decision making scores were designated as "optimal performing" (OPT) subjects, whereas rats with negative risk scores at baseline were classified as "risk-preferring" (RP). If a two-way repeated measures ANOVA with dose (three levels: vehicle, low dose and high dose) and choice (four levels: P1, P2, P3, and P4) as within-subjects factors came out with a significant interaction or main effect of choice, individual options were subject to separate repeated measures analysis. All variables were analyzed using a repeated measures ANOVA with dose as a within-subjects factor, and both sex and risk preference as between-subjects factors. If sphericity was violated as determined by Mauchley's test of sphericity, a Huynh–Feldt correction was applied.

Results were considered statistically significant if p-values were less than or equal to $\alpha = 0.05$. Any main effects or interactions of significance resulting from the repeated measures ANOVA were further analyzed via one-tailed paired samples t-tests with a Bonferroni correction applied for the number of comparisons made, given the directionality predicted based on the graphical ANOVA output. Any p-values > .05 but < .08 were reported as a statistical trend in the interest of transparency. All data were plotted as mean ± SEM. For within-subjects analyses, error bars were corrected as described previously to prevent overestimation of standard error and to more accurately depict within-subjects variation (Betts et al., 2021).

## 2.3 Results

### 2.3.1 Histology

Cresyl violet staining was used to confirm cannula placements in either the lOFC or PrL cortex, as defined by Paxinos & Watson (1998). Acceptable projected infusion sites were plotted onto coronal sections adapted from the Rat Brain Atlas (Figure 2A; Paxinos & Watson, 1998). Representative micrographs of proper cannula placements are provided in Figure 2B. One lOFC male and two PrL females were excluded from subsequent analyses due to misplaced cannulae that were ventral to the respective targets. One lOFC male, two PrL males, and two PrL females were excluded for analyses due to obstructed, bent, or damaged cannulae that precluded completion of all six microinfusions. One lOFC male died during surgery, and two lOFC females were euthanized prior to undergoing microinfusions due to poor health. The total number of rats in each group that were included in analyses were as follows: 13 male-lOFC, 14 female-lOFC, 14 male-PrL and 13 female-PrL.

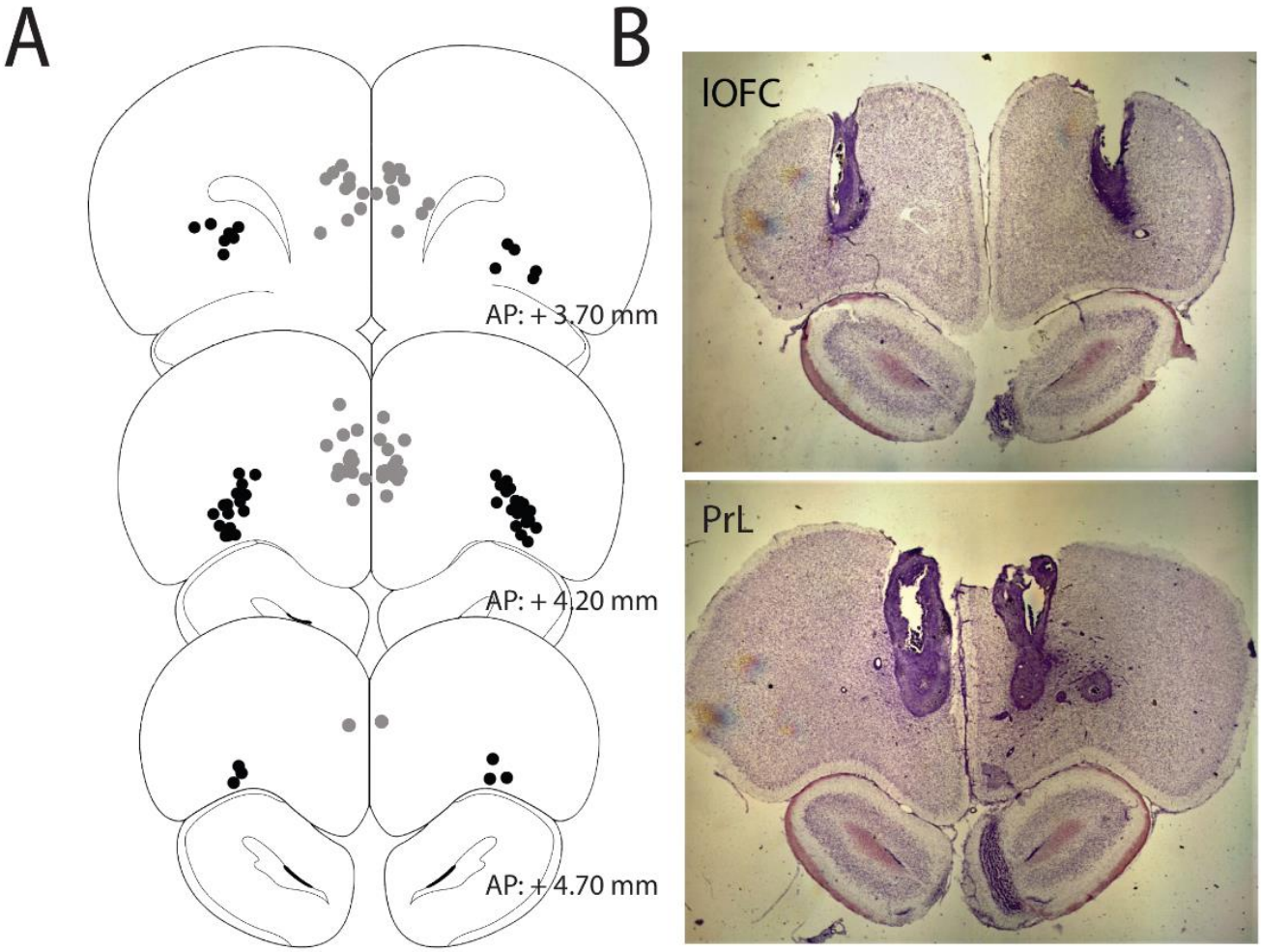


**Figure 2 Accepted cannula placements.**
A) Black circles indicate lOFC placements while grey circles correspond to PrL cannula placements. Subjects with inaccurate cannula placements (not shown) were excluded from the analyses. B) Representative Cresyl stained coronal sections for lOFC and PrL cannulae, respectively.

### 2.3.2 Baseline behaviour

Baseline performance was established from the last five statistically stable post-surgery crGT sessions. Decision making scores of male and female rats in the lOFC cohort did not significantly differ (Figure 2.3 A; sex: $F_{1,23} = 1.882$, $p = 0.083$), yet PrL males demonstrated

more optimal decision making scores at baseline than PrL females (Figure 2.3 B; sex: $F_{1,24}$ = 4.597, p = 0.042). As expected, there was a significant between-subjects effect of risk preference on score in both cohorts (lOFC: $F_{1,23}$ = 82.945, p < 0.001; PrL: $F_{1,24}$ = 55.402, p < 0.001). Individual choice options were analysed using a two-way ANOVA with choice and session as within-subjects factors. No significant interactions with choice were found for either lOFC or PrL animals (all Fs < 3.210, ps > 0.085), and as such subsequent analysis of individual options were not justified.

Regarding impulsive premature responses, risky females in the lOFC cohort made more impulsive premature responses than their optimal performing counterparts at baseline (Figure 3 C; females- risk preference: $F_{1,12}$ = 4.873, p = 0.047), yet impulsivity did not vary with risk preference in male lOFC rats (Figure 3 D; sex x risk preference: $F_{1,23}$ = 6.787, p = 0.016; males- risk preference: $F_{1,11}$ = 2.261, p = 0.161). Risk preferring rats in the PrL cohort, irrespective of sex, exhibited higher motor impulsivity (Figure 2.3 E; risk preference: $F_{1,24}$ = 4.420, p = 0.046). Further, females took longer to both make a choice and collect food reward than males, yet only in the PrL cohort (PrL- collect latency: $F_{1,24}$ = 7.865, p = 0.010; choice latency: $F_{1,24}$ = 5.132, p = 0.033; lOFC- all Fs < 3.129, p = 0.090). lOFC females completed more trials than lOFC males (sex: $F_{1,23}$ = 5.812, p = 0.024) while PrL males completed more crGT trials than PrL females (sex: $F_{1,24}$ = 15.755, p < 0.001), consistent with the directionality of observed differences in baseline decision making scores in both cohorts. There were no significant differences in omitted trials in either group (all Fs < 2.355, ps > 0.139).

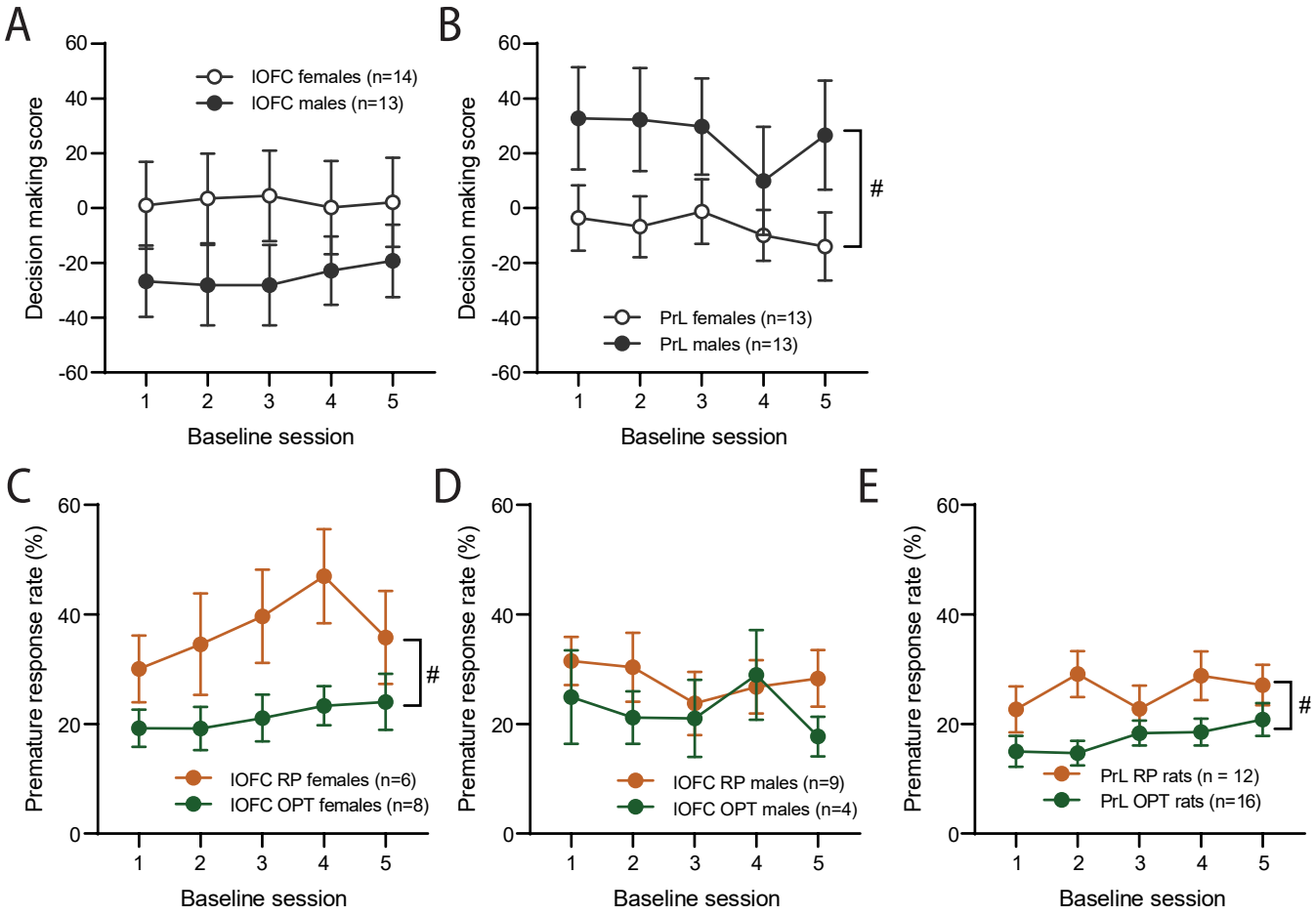


**Figure 3 Baseline decision making and impulsivity**
A) Decision making score did not significantly differ between sexes in the lOFC cohort, yet B) PrL males demonstrated higher decision making scores than PrL females at baseline. Regarding baseline motor impulsivity, C) risk preferring (RP) females in the lOFC cohort made more premature responses than optimal performing (OPT) females, yet D) RP and OPT males in the lOFC cohort did not differ in premature responding. E) RP rats in the PrL cohort, irrespective of sex, made more prematures responses than OPT PrL rats. Data are mean ± SEM. # $p < 0.05$ between subjects effect.

### 2.3.3 Effects of drug infusions into the lOFC

#### 2.3.3.1 Atomoxetine

*Decision making:* Atomoxetine had differential effects in males vs females, as indicated by a significant dose x sex interaction ($F_{2,46} = 6.392$, $p = 0.005$). Subsequent analyses revealed that intra-lOFC atomoxetine increased decision making score in male rats only (Figure 4 A;

females- dose; $F_{2,24} = 2.392$, $p = 0.113$; males- dose: $F_{2,22} = 4.109$, $p = 0.030$; 1.5µg vs VEH: $t_{12} = -1.879$, $p = 0.042$; 5.0µg vs VEH: $t_{12} = -1.823$, $p = 0.047$). It appeared as if males had lower decision making scores than females, although the between subjects sex difference did not reach statistical significance (sex: $F_{1,23} = 0.248$, $p = 0.623$). To explore the possibility that the sex-specific effect of lOFC atomoxetine on score may have been partially driven by the high risk preference demonstrated by males, baseline risk score was covaried in the above repeated measures ANOVA. The dose x sex interaction ($F_{2,44} = 6.462$, $p = 0.003$) and main effect of dose in males remained significant when controlling for baseline score (males: $F_{2,20} = 3.609$, $p = 0.046$; females: $F_{2,22} = 1.802$, $p = 0.188$), validating that the selective effect in males was not driven by higher baseline risk preference. An omnibus analysis revealed that atomoxetine influenced choice of individual options in males only (choice x dose x sex: $F_{6,138} = 4.872$, $p = 0.003$; choice x dose- males: $F_{6,66} = 3.014$, $p = 0.029$; females: $F_{6,72} = 1.185$, $p = 0.324$). As such, the individual choice options were further analyzed for males only. The lower dose of atomoxetine increased choice of the best option, P2, in optimal decision-makers (Figure 4 B; dose x risk preference: $F_{2,22} = 4.228$, $p = 0.028$ ; OPT rats- 1.5 µg vs VEH: $t_3 = -2.539$, $p = 0.042$; all other Fs < 0.921, ps > 0.212), and reduced choice of the risky option P3 in all rats, albeit only at the level of a statistical trend (Figure 2.4 B; dose: $F_{2,22} = 2.975$, $p = 0.072$). No changes were observed in choice of the safe option P1 nor the riskiest option P4 (all Fs < 2.622, ps > 0.095).

*Impulsivity:* Repeated measures analyses reveal only a trend-level main effect of intra-lOFC atomoxetine on the number of impulsive premature responses made (Figure 4 C; dose: $F_{2,46} = 2.675$, $p = 0.080$). Visual inspection of the data indicated that females may be more susceptible to the subthreshold anti-impulsivity effect of atomoxetine infused into the lOFC, yet

the ANOVA yielded no significant interactions with sex (all Fs < 0.930, ps > 0.402). There was, however, a significant between-subjects effect of sex whereby males exhibited greater motor impulsivity than females (Figure 4 C; sex: $F_{1,23} = 9.253$, $p = 0.006$). No other significant main effects or interactions were reported (all Fs < 1.829, ps > 0.189).

*Other behavioural variables:*

Intra-lOFC atomoxetine did not significantly influence latency to collect food pellet rewards in either sex, despite a significant dose x sex interaction (dose x sex: $F_{2,46} = 3.909$, $p = 0.037$; dose- males: $F_{2,22} = 2.344$, $p = 0.119$; females: $F_{2,24} = 1.748$, $p = 0.203$). No significant effects or interactions were noted for trials completed, omissions or choice latency (all Fs < 2.310, ps > 0.142).

#### 2.3.3.2 Guanfacine

*Decision making:* Intra-lOFC guanfacine did not significantly impact decision making score (Figures 4 D; all main effects and interactions: all Fs < 0.791, ps > 0.460), nor did it influence choice of individual options (all Fs < 1.546, ps > 0.183).

*Impulsivity:* There was no main effect of, or interactions with, dose of intra-lOFC guanfacine on premature responses (Figure 4 E; all Fs < 2.353, ps > 0.107). There was, however, a between-subjects effect of sex wherein males made more impulsive premature responses than females (Figure 4 E: sex: $F_{1,22} = 6.424$, $p = 0.019$).

*Other behavioural variables:* No significant main effects or interactions were detected for trials completed, omissions, and latencies to choose and option or collect reward (all Fs < 1.176, ps > 0.318).

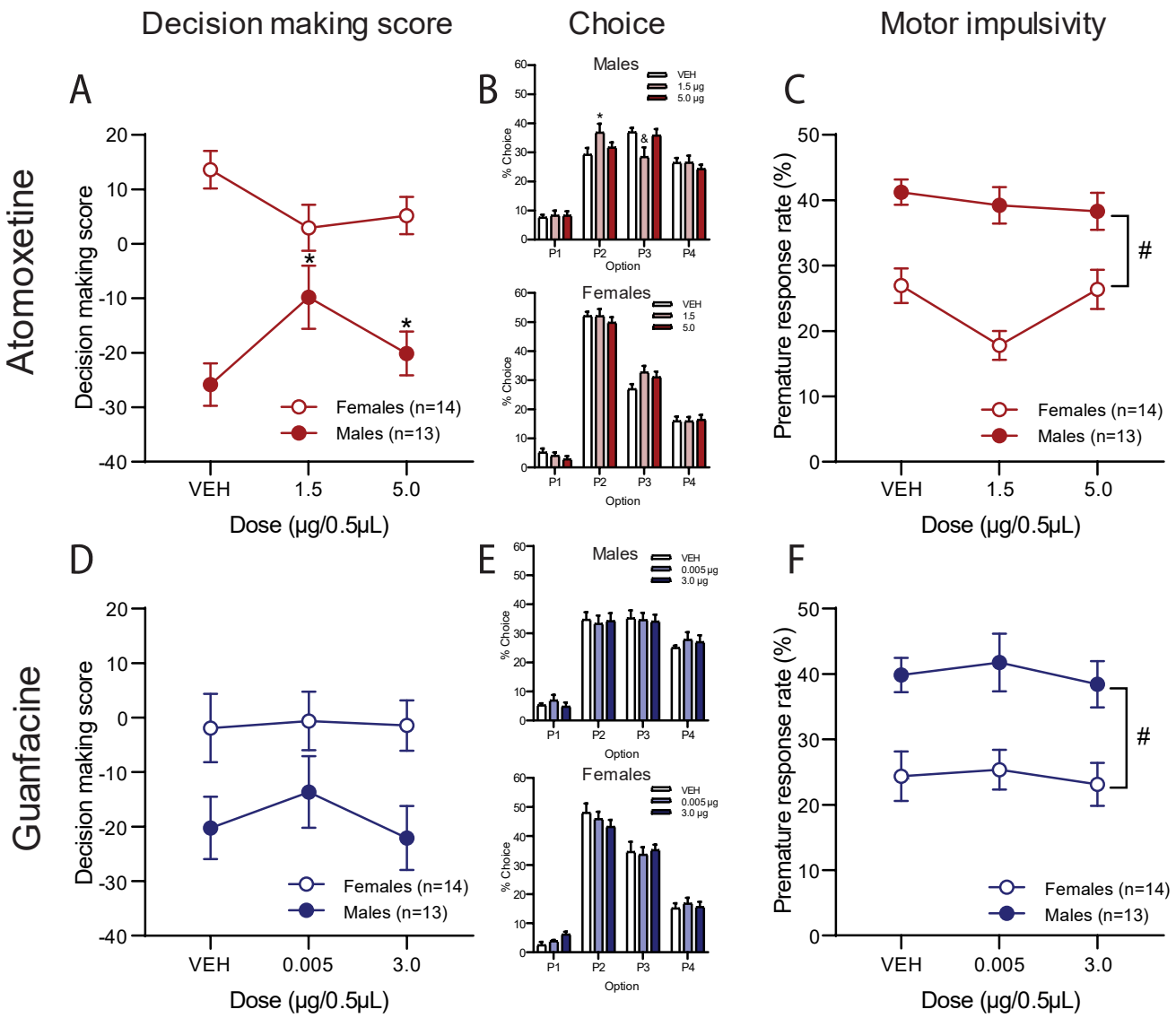


**Figure 4 Behavioural effects of intra-lOFC infusions.**

Intra-lOFC atomoxetine improved decision making in male rats. A) Both doses of atomoxetine improved decision making score in male rats when infused into the lOFC, yet intra-lOFC atomoxetine was unable to significantly influence score in females. B) In males, the low dose of atomoxetine enhanced preference for the most advantageous option P2, driven by a significant increase in P2 choice in OPT males, and produced a trend-level decrement in choice of the risky option P3. No significant changes in choice were observed for females. C) Intra-lOFC atomoxetine did not precipitate significant changes in impulsive premature responses in either sex, yet males demonstrated higher impulsivity than females overall. D) Guanfacine did not influence decision making score nor B) premature responding when infused into the lOFC, yet males demonstrated higher premature responding than females. * $p < 0.05$ compared to VEH. & $p < 0.08$ main effect of dose. # $p < 0.05$ between-subjects effect of sex. Data are presented as mean ± within-subjects corrected SEM.

### 2.3.4 Effects of drug infusions into the PrL

#### 2.3.4.1 Atomoxetine

*Decision making:* Atomoxetine infused into the PrL did not significantly influence decision making score (Figure 5 A; all Fs < 2.466, ps > 0.098). Our analyses also did not reveal any significant interactions between dose and choice (all Fs < 2.205, ps > 0.081), and therefore individual options were not separately analyzed.

*Impulsivity:* Atomoxetine significantly reduced impulsive premature responses when microinfused into the PrL, but this effect was only observed in risk preferring animals following the high dose (Figure 5 B; dose x risk preference: $F_{2,44} = 5.760$, $p = 0.006$; RP rats- dose: $F_{2,20} = 4.635$, $p = 0.022$; 5.0µg vs VEH: $t_{11} = 2.545$, $p = 0.014$; OPT rats- dose: $F_{2,24} = 1.525$, $p = 0.238$). In the interest of transparency, it appeared that risk preferring rats were more impulsive than optimal performers following vehicle infusions, suggesting that this selective effect on premature responding in risky rats may have been driven by high baseline impulsivity. Rats were therefore categorized as either high impulsive (HI) or low impulsive (LI) based on a median split for average baseline premature responding, yet baseline impulsivity level did not interact with atomoxetine dose to alter premature responding (dose x baseline impulsivity: $F_{2,38} = 0.322$, $p = 0.727$). When baseline risk score was covaried in the initial repeated measures analysis, intra-PrL atomoxetine no longer impacted premature responding (dose- RP rats: $F_{2,18} = 1.640$, $p = 0.222$; OPT rats: $F_{2,22} = 0.086$, $p = 0.918$), further confirming that the observed selective reduction in impulsivity was related to high baseline risk preference.

*Other behavioural variables:* Repeated measures ANOVAs revealed no significant main effects of, or interactions with, atomoxetine dose for trials completed, omissions, reward collection latency, or choice latency (all Fs < 2.179, ps > 0.136).

#### 2.3.4.2 Guanfacine

*Decision making:* Guanfacine did not significantly change score when administered into the PrL, even though there was a significant dose x sex x risk preference interaction (Figure 5 C; dose x sex x risk preference: $F_{2,46} = 3.728$, $p = 0.032$; dose- OPT females: $F_{2,10} = 0.392$, $p = 0.686$; RP females: $F_{2,12} = 0.258$, $p = 0.777$; OPT males: $F_{2,16} = 0.280$, $p = 0.672$; RP males: $F_{2,8} = 3.227$, $p = 0.146$). An omnibus analysis revealed a significant choice x dose x sex x risk preference interaction ($F_{6,138} = 2.612$, $p = 0.020$), yet further analyses of individual options revealed that guanfacine was unable to significantly influence choice in any of four options when compared to vehicle infusions (all Fs < 3.022, ps > 0.110).

*Impulsivity:* Both doses of intra-PrL guanfacine significantly increased the rate of impulsive premature responding in all rats compared to vehicle (Figure 5 D; dose: $F = 4.436$, $p = 0.017$; 0.005µg vs VEH: $t_{26} = 2.415$, $p = 0.012$; 3.0µg vs VEH: $t_{26} = 2.344$, $p = 0.013$).

*Other behavioural variables:* No main effects or interactions were noted for omitted trials, choice latency, reward collection latency, or trials completed (all Fs < 2.098, ps > 0.161).

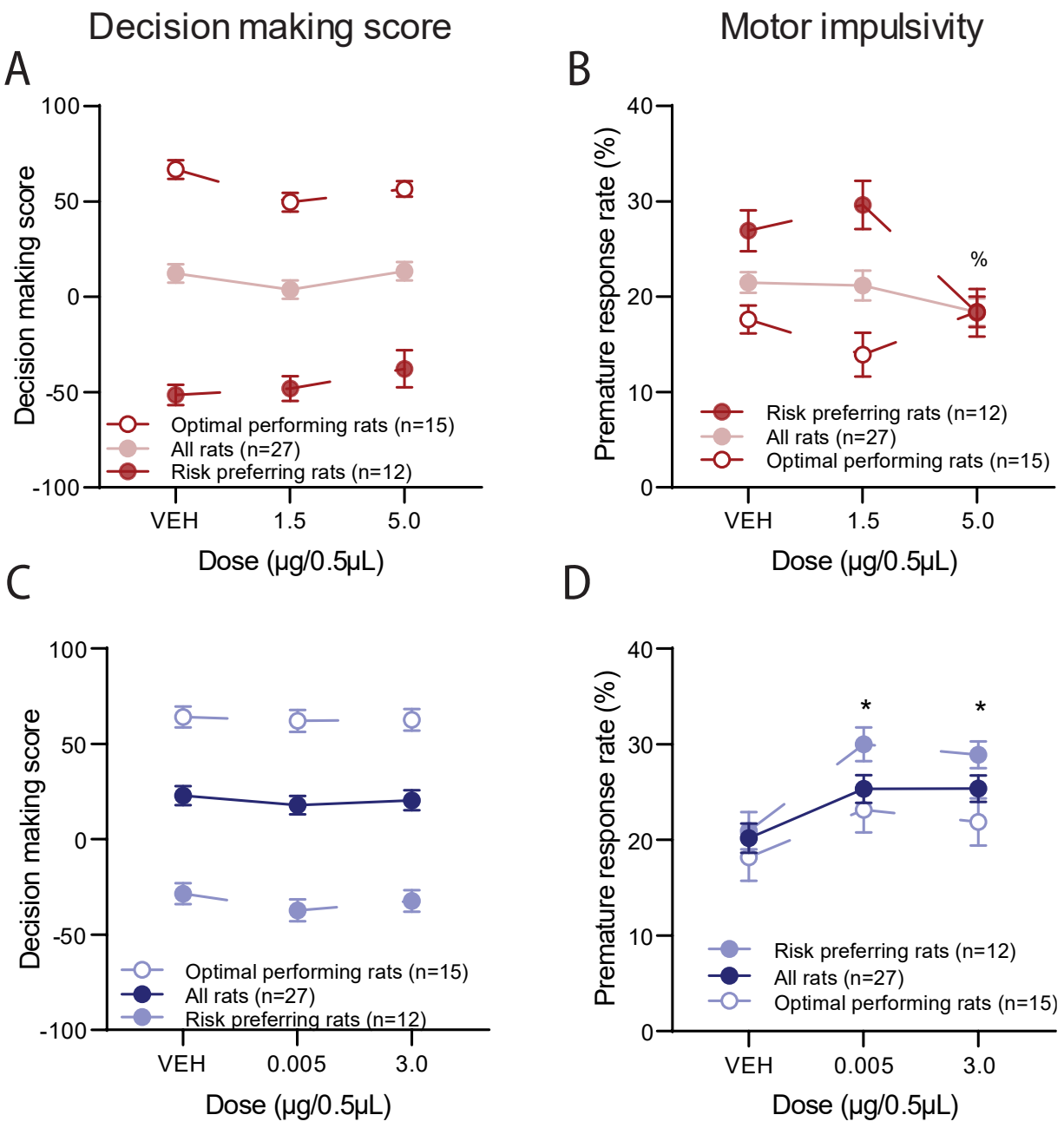


**Figure 5 Behavioural effects of intra-PrL infusions.**
A) Atomoxetine infused into the PrL did not alter decision making score, yet B) the highest dose of intra-PrL atomoxetine selective reduced premature responding in RP rats. C) Guanfacine also did not alter decision making score when infused into the PrL. D) Intra-PrL guanfacine increased premature responses in all rats at both doses. % $p < 0.05$ compared to VEH in RP group only. * $p < 0.05$ compared to VEH. Data are presented as mean ± within-subjects corrected SEM.

## 2.4 Discussion

Using local pharmacological manipulations, I revealed the previously undefined role of prefrontal noradrenaline signaling in cue-guided risk taking and impulsivity. These data present a double dissociation, such that noradrenergic action in the lOFC influenced decision making in a sex-specific manner, while PrL noradrenaline signaling was a more potent regulator of impulsive action. Specifically, I show that the selective NET inhibitor atomoxetine improved decision making in male rats when microinfused into the lOFC, increasing choice of the most fruitful option P2 in optimal performers, while decision making in females was relatively immune to the beneficial effects of intra-lOFC NET blockade. Atomoxetine in the lOFC did not influence premature responding, a measure of motor impulsivity, and guanfacine, an α2A receptor agonist, had no behavioural effects when infused into the lOFC. Contrastingly, when administered into the PrL neither noradrenergic compound altered decision making, yet the drugs produced divergent effects on impulse control. A high dose of intra-PrL atomoxetine selectively improved impulse control in risk preferring rats, while both doses of intra-PrL guanfacine conversely enhanced impulsive premature responding in all rats.

Here I replicate our past finding that atomoxetine improves score on the crGT, with the effect size being nearly 1.5 times greater following intra-lOFC administration in males than that previously observed following i.p. atomoxetine in all rats (Chernoff et al., 2021). This suggests the lOFC as a main locus at which atomoxetine acts in the male brain to improve cue-guided decision making. The adaptive gain theory could provide important insight into a potential mechanism behind the ability of local NET blockade to produce these results. The theory posits that burst-like phasic noradrenaline release from the LC, which can be evoked by salient and motivationally relevant stimuli (Aston-Jones et al., 1994; Bouret & Richmond, 2015; Bouret &

Sara, 2004), promotes fixed attention and persistent engagement in the current behavioural strategy (Aston-Jones & Cohen, 2005). Conversely, as a behaviour becomes less profitable, tonic LC noradrenaline release increases, which attenuates stimulus-triggered phasic responses and encourages exploration of other potentially beneficial options (Aston-Jones & Cohen, 2005; Usher et al., 1999). As such, artificially enhancing synaptic noradrenergic tone with a NET blocker like atomoxetine could conceivably mimic the synaptic environment induced by heightened tonic LC activity, blunting the behavioural impact of cue-evoked phasic bursts and shifting behaviour toward sampling from more advantageous choices on the crGT. This interpretation is consistent with a more recent theory proposing that, specifically, tonic noradrenaline within the OFC is important for managing internal representations of task contingencies and precipitating appropriate changes in behavioural strategies (Sadacca et al., 2017).

Local atomoxetine may also improve crGT performance by increasing the signal-to-noise ratio in the lOFC. The noradrenaline system tunes cortical information processing by enhancing neuronal responses to relevant stimuli (signal) and diminishing responsivity to erroneous or distracting stimuli (noise) (Berridge & Waterhouse, 2003; Gamo et al., 2010; Hasselmo et al., 1997; Salgado et al., 2012). Given that the lOFC responds to and encodes punishment (O'Doherty et al., 2001; Turner et al., 2021), increasing synaptic noradrenaline levels in the lOFC could potentially enhance the resultant 'punishment' signal following losses on the crGT and increase behavioural sensitivity to time-out punishments, driving preference away from the risky options that deliver longer and more frequent time-outs. This interpretation is in line with recent work fitting a reinforcement learning model to rGT data suggesting that a relative

insensitivity to punishment, instead of alterations in reward learning, drives risk taking in the presence of win cues (Langdon et al., 2019).

A higher signal-to-noise ratio could conversely promote safer decisions by boosting orbitofrontal ‘value’ signals and/or regulating internal representations of task structure (Padoa-Schioppa & Conen, 2017; Zhou et al., 2021) such that subjects increasingly favor optimal options that yield greater overall gain across a session. In further support of this hypothesis, value signals in the OFC selectively reflect the value of an attended target (Y. Xie et al., 2018), and noradrenergic signaling importantly guides attentional allocation. Higher noradrenergic tone promotes broader, ‘scanning’ attention (Aston-Jones & Cohen, 2005; Milstein et al., 2010; Valentino & Van Bockstaele, 2008), and as such, intra-lOFC atomoxetine could encourage subjects to attend to all crGT options more equally throughout the session. Therefore, value signals for each option would be more accurately represented in the decision making landscape, which could promote preference for safe crGT options that are, objectively, more profitable.

However, the inability of intra-lOFC atomoxetine to alter decision making in females is in stark contrast to our previous findings that systemic atomoxetine improves choice score on the crGT in all subjects, irrespective of sex (Chernoff et al., 2021). This may speak to possible sex differences in lOFC contribution to the neural processes underlying risk taking. Human data indicate potential sex differences in decision-related lOFC recruitment, whereby men demonstrated significant lOFC activation during performance of the Iowa Gambling Task (IGT), the human decision making paradigm from which the rGT was adapted, while women did not (Bolla et al., 2004). Men were also quicker to adopt the optimal IGT strategy in this study. Together, these findings could suggest that differences in frontal activation patterns, including recruitment of the lateral OFC, may relate to more efficient IGT performance in men and,

importantly, that the lOFC is less involved in such decision making processes in women. A study in rodents also found significant sex differences in on-task lOFC activation in rats during a probabilistic decision making task, yet the directionality of this sex difference was not indicated (van Hasselt et al., 2012). If the lOFC is in fact less critical to cost-benefit decision making in females compared to males, then this may explain why the intra-lOFC pharmacological manipulations did not influence any decision making variables in females yet significantly improved score in male rats. Underlying differences in risk preference between males and females could also drive this sex-specific benefit of intra-lOFC atomoxetine on risk score. While it appears that males in the current lOFC cohort generally had much lower decision making scores than females, this was not statistically significant. Further, when baseline score was covaried in the analyses, intra-lOFC atomoxetine still significantly and selectively improved score in male rats. As such, the statistics do not support the conclusion that baseline differences in decision making drove the ability of atomoxetine to reduce risk preference in males only.

While the data implicate the lOFC as an important mediator of atomoxetine's benefits on decision making, at least in males, I find that intra-lOFC guanfacine was unable to sway risk preference on the crGT. This is inconsistent with our past findings, whereby systemically administered guanfacine improved decision making (Chernoff et al., 2021), suggesting that guanfacine does not reduce risk preference through action in the lOFC. In our previous experiment, however, precise dosing and behavioural phenotypes (i.e. intermediate baseline choice profiles) were required for guanfacine to improve score (Chernoff et al., 2021). Given the limited number of infusions possible with the current experimental design, it could be that I did not administer a dose of guanfacine that was selective enough to improve decision making when

infused into the lOFC. Future experiments using a larger range of doses are needed to ascertain whether there is an optimal dose for intra-lOFC guanfacine to benefit crGT behaviour.

The disparity between our findings here and those from prior systemic experiments could also be due to the differential route of administration. When injected intraperitoneally, guanfacine acts through autoreceptors to inhibit LC cell firing and reduce noradrenaline release (Callado & Stamford, 1999; Engberg & Eriksson, 1991), an effect which can be replicated by direct infusion of guanfacine onto LC cell bodies (Okada et al., 2019). Conversely, when administered directly into the prefrontal cortex, guanfacine does not alter synaptic catecholamine levels (Okada et al., 2019). Multiple experiments testing working memory, attention, and impulsive choice indicate that the cognitive benefits of guanfacine are not driven by autoreceptor-mediated mechanisms, but instead by action at postsynaptic α2A receptors in the prefrontal cortex (A. Arnsten et al., 1988; Nishitomi et al., 2018; Ramos & Arnsten, 2007; Wang et al., 2007). Our current results could suggest that prefrontal α2A receptor activation may not be as critical for neural processes underlying cost-benefit decision making as it is for other prefrontal functions. However, given the substantial body of evidence indicating the particular importance of postsynaptic α2A receptors in prefrontal function (A. Arnsten et al., 1988; A. F. T. Arnsten & Jin, 2012; Nishitomi et al., 2018), it is unlikely that somatodendritic autoreceptor mechanisms drive the benefits of guanfacine on the crGT. Enhancing synaptic concentrations of noradrenaline in the prefrontal cortex with atomoxetine may increasingly activate lower affinity α1 receptors in the cortex in addition to α2 receptors. Alpha-1 receptors are implicated in selective attention, multiple forms of memory, behavioural stress responses, and general behavioural activation (Birnbaum et al., 1999; Doze et al., 2011; Nalepa et al., 2013; Puumala et al., 1997; Stone et al., 1999), yet it is unknown how α1 receptors contribute to cue-induced risk

taking. Future behavioural pharmacology studies should consider the potential role of other adrenergic receptor subtypes, such as α1 receptors, in the noradrenergic contributions to risky decision making.

Whereas intra-lOFC infusions of atomoxetine led to changes in decision making score but not motor impulsivity, intra-PrL infusions of both noradrenergic drugs altered motor impulsivity but not decision making score. This functional double dissociation is consistent with anatomical studies revealing separate, direct projections from the LC to various prefrontal regions including the lOFC and PrL (Chandler et al., 2013, 2014; Robertson et al., 2013) which could allow for noradrenaline to exert distinct effects on prefrontally-governed behaviours. The dissociable consequences of intra-lOFC and intra-PrL noradrenergic manipulations are also in line with the separable roles of these frontal regions in action-outcome evaluation and impulse control, respectively (Izquierdo, 2017; Moschak & Carelli, 2021).

Interestingly, while both atomoxetine and guanfacine reduced motor impulsivity on the crGT and other behavioural paradigms when administered systemically (Chernoff et al., 2021; E. S. J. Robinson et al., 2008), I observed opposing effects on impulsivity following local microinfusion of either noradrenergic compound into the PrL. The high dose of atomoxetine reduced impulsivity selectively in risk preferring rats, yet both doses of intra-PrL guanfacine increased the number of impulsive premature responses made by all subjects. These current findings indicate that the anti-impulsivity benefits conferred by systemic α2A agonism are not driven by direct activation of PrL α2A receptors, and that guanfacine acting in the PrL might actually oppose such α2-mediated benefits. Given that the α2A receptor is $G_i$-coupled and generally inhibits prefrontal neuronal transmission (Ji et al., 2008), local guanfacine acting in the PrL might therefore enhance motor impulsivity largely through its inhibitory influence on

prelimbic principal neurons. Reversible inactivation of the PrL impaired the ability of animals to wait for a target stimulus (Narayanan et al., 2006), increasing premature responding similar to the effects of intra-PrL guanfacine reported here. Additionally, PrL lesions or inactivation also lead to non-specific behavioural activation (Brito & Brito, 1990; Jonkman et al., 2009), suggesting that the PrL might play a more general role in orchestrating behavioural inhibition. However, I did not observe any general signs of increased motor output following intra-PrL infusions, as response latencies, omissions, and trials completed were all unaffected. Guanfacine's actions therefore appear more nuanced than expected from a simple global inhibition of PrL activity.

Rather than just being mediated through one area, the ability to withhold an inappropriate response may rely on the relative balance in activity between PrL and the more ventral infralimbic (IL) region of the mPFC (Hardung et al., 2017). In a reaction time task requiring rats to release a lever upon delivery of an auditory cue, optogenetic inhibition of the PrL increased premature releases while inhibition of the IL had the opposite effect (Hardung et al., 2017). Accordingly, the inhibitory influence of local guanfacine on PrL neurons may prevent PrL involvement in action selection, biasing behavioural output toward IL-dependent facilitation of responding and impairing impulse control on the crGT. It is important to note that inactivations of either the IL or PrL did not influence premature responding rates on the uncued rGT (Zeeb et al., 2015), suggesting that the pro-impulsivity effect observed following intra-PrL guanfacine may depend on the presence of win-paired cues, or result in a more selective inhibition of "stop" circuits within the PrL (Hu et al., 2019) instead of inhibiting overall PrL activity. These hypotheses remain to be experimentally tested.

The selective impact of intra-PrL atomoxetine on premature responding in risk preferring rats could indicate potential differences in noradrenergic regulation of behaviour between subjects that are vulnerable versus resistant to developing risky choice profiles in the presence of win-paired cues. While, to our knowledge, there is currently no work defining underlying alterations in the noradrenaline system between risky and safe decision makers, prefrontal NET availability correlated with trait impulsivity scores in human subjects (Hesse et al., 2017). Atomoxetine also reduced both premature responding and stress-induced compulsive water drinking in highly impulsive rats only, leaving behaviour unaltered in low impulsive rats (Ansquer et al., 2014). These findings suggest there may be biobehavioural differences in noradrenaline system function between high and low impulsive individuals. Previous work from our lab indicates that motor impulsivity and decision making are not wholly independent behavioural constructs, as premature response rates negatively correlate with decision making scores on the uncued rGT (Barrus et al., 2015). Individual differences in impulsivity account for ~7% of the variability in choice score on the crGT (Hynes et al., 2021). Considering this relationship between risk preference and impulsivity, risk preferring rats may exhibit some of the aforementioned alterations in noradrenaline function characteristic of highly impulsive individuals, which could help explain why risk preferring animals were uniquely sensitive to intra-PrL atomoxetine over their optimal performing counterparts. Our statistical analyses demonstrated that baseline impulsivity was not a contributing factor to the selective effect of PrL atomoxetine on premature responding, and covariate analyses further confirm that this effect was indeed driven by risk preference. As such, the ability of intra-PrL NET blockade to selectively improve impulse control in risk preferring rats suggests that noradrenaline function, or more

specifically, NET function, in the PrL differs between risk preferring and optimal performing rats, rendering risky rats more susceptible to the behavioural benefits of NET inhibition.

While atomoxetine and guanfacine demonstrate incredibly selective affinities at their respective noradrenergic targets, NET and α2A adrenergic receptors (Devedjian et al., 1994; Gehlert et al., 1995; Uhlén & Wikberg, 1991), we cannot discount potential effects on the dopamine system. Given the relative dearth of dopamine transporter (DAT) in the prefrontal cortex, NET plays an important role in prefrontal dopamine clearance (Yamamoto & Novotney, 1998). As such, atomoxetine can increase synaptic dopamine at target prefrontal regions while leaving dopamine levels in DAT-rich areas, such as the striatum, unaffected (Bymaster et al., 2002). Dopamine can also be co-released by noradrenergic terminals in the PFC (Devoto et al., 2005), and α2 adrenergic receptors are expressed on non-noradrenergic neurons in the PFC, including dopamine neurons (Castelli et al., 2016). Accordingly, noradrenaline can modulate prefrontal dopamine release (Shinohara et al., 2020), making it difficult to completely disentangle the contributions of either catecholamine to prefrontal function. However, early studies investigating NET-mediated dopamine clearance predominantly assess reuptake in the mPFC. More recent evidence challenges the fact that dopamine clearance mechanisms are identical across subregions of the frontal cortex, demonstrating that DAT-mediated dopamine uptake was nearly twice the magnitude in the OFC than in the mPFC (Yates et al., 2016). This could imply that atomoxetine-induced increases in dopamine may be less of a concern for our lOFC pharmacology data. In efforts to circumvent potential dopaminergic confounds, I administered a notably low dose of guanfacine (0.005 µg). Doses of this magnitude are purported to more selectively target the noradrenergic system (Bari et al., 2011), as lower concentrations of guanfacine should more readily bind to the denser population of α2A receptors on noradrenergic

terminals versus the more sparsely expressed α2A receptors on other types of prefrontal neurons (Castelli et al., 2016). I observed the same behavioural effect of intra-PrL guanfacine at both the low and high dose (0.005µg and 3.0µg), suggesting that, even if the higher dose had off-target effects on dopamine release, this was not behaviourally relevant with respect to impulsivity on the crGT.

Here I have illustrated a double dissociation of prefrontal noradrenergic contributions to risk taking and impulsivity across the lOFC and PrL respectively. I also highlight a novel sex difference, such that noradrenergic tone in the lOFC critically guides cost benefit decision making in males but not females. Our data indicate potential differences in noradrenaline function between rats that develop risky versus optimal decision making profiles in the presence of risk-promoting win cues, such that only rats with high risk preference at baseline benefitted from the anti-impulsivity effects of intra-PrL NET blockade. These data deepen our understanding of the neural mechanisms behind noradrenergic regulation of risky and impulsive behaviours, providing important insight into the means by which noradrenergic medications such as atomoxetine and guanfacine could ameliorate maladaptive behaviours in gambling disorder and related psychopathologies.

# Chapter 3: General discussion

## 3.1 Summary of findings

### 3.1.1 Double dissociation across prefrontal brain regions

Here I present novel evidence for the important role of prefrontal noradrenaline signaling in cue-exacerbated risk taking and impulsivity. Critically, these experiments dissociated the prefrontal loci at which noradrenergic compounds act to guide distinct behaviours. Enhancing noradrenergic tone within the lOFC, via local administration of the NET blocker atomoxetine, improved decision making by increasing preference for the most lucrative low-risk low-reward option. However, the benefits of intra-lOFC atomoxetine were only observed in male rats. In contrast, intra-PrL noradrenergic manipulations selectively influenced motor impulsivity, yet did not alter decision making. Atomoxetine infused into the PrL reduced premature responding in risk preferring rats only, yet intra-PrL infusions of guanfacine, an α2A receptor agonist, increased impulsive actions irrespective of baseline risk preference. The behavioural pharmacology results are summarized in Table 3.1. Our findings indicate that noradrenaline signaling in the lOFC is a main contributor to cost-benefit decision making, while the PrL mPFC is a site at which noradrenaline acts to regulate impulse control. Thus, these data provide novel mechanistic insight into the prefrontal regions that drive the ability of noradrenergic compounds to alter decision making and various measures of impulsivity (Bari et al., 2009; Chernoff et al., 2021; Fernando et al., 2012; Nishitomi et al., 2018; E. S. J. Robinson et al., 2008). Furthermore, the current data are consistent with the separable involvement of the OFC and mPFC in various cognitive processes within a given behavioural paradigm (Cerpa et al., 2022; Hong et al., 2019; St. Onge & Floresco, 2010; Woon et al., 2020). The double dissociation revealed by our present

data suggests that noradrenaline signaling underlies, or at least significantly contributes to, the dissociable roles of such prefrontal regions in gambling-like behaviours.

| | | Lateral orbitofrontal cortex | | Prelimbic cortex | |
|---|---|---|---|---|---|
| | | Decision making | Motor impulsivity | Decision making | Motor impulsivity |
| Atomoxetine (NET blocker) | Low: 1.5 µg/side | ↑ score in males | ____ | ____ | ____ |
| | High: 5.0 µg/side | ↑ score in males | ____ | ____ | ↓ prematures in RP rats |
| Guanfacine (α2A agonist) | Low: 0.005 µg/side | ____ | ____ | ____ | ↑ prematures in all rats |
| | High: 3.0 µg/side | ____ | ____ | ____ | ↑ prematures in all rats |

**Table 3.1 Summary of behavioural pharmacology findings.**
All results presented reached statistical significance of $p < 0.05$ compared to vehicle. A dash indicates no significant effect. "Prematures" refers to impulsive premature responses.

### 3.1.2 Sex differences in OFC noradrenaline

The current understanding of orbitofrontal contributions to decision making and flexible risk assessment has come from experiments performed almost exclusively in male subjects. The present findings suggest, however, that the behavioural consequence of OFC neuromodulation may differ between males and females. The current experiment revealed a sex difference whereby OFC NET blockade reduced risk taking in male rats only. The null effect in females is unlikely to be due to circulating ovarian hormones, as decision making on the crGT is not influenced by estrous stage (Hynes et al., 2020). This suggests that we are observing an organizational sex difference such that noradrenergic signaling in the OFC is more important for cost-benefit decision making in males than in females. These findings exemplify the first preclinical evidence, to our knowledge, describing this functional difference. What

organizational and physiological alterations may drive differential OFC involvement in cost-benefit decision making between males and females, however, remain to be disseminated. Nonetheless, the current experiment provides strong evidence that female subjects are integral to include in studies probing the role of the OFC in complex behaviour, and indicate that the field needs to know more about potential sex differences in noradrenergic regulation of behaviour.

### 3.1.3 Noradrenaline function: Interaction with baseline risk preference

The current data also reveal a potential biobehavioural difference between individuals that are vulnerable vs. resistant to developing risk preferring phenotypes in the presence of reward-paired cues. Intra-PrL atomoxetine reduced impulsivity selectively in rats with risky choice profiles at baseline. This could suggest an underlying difference in PrL mPFC and/or noradrenaline system function between individuals that eventually become risk preferring or optimal performing on the crGT. Compared to that of optimal performers, the mPFC of risk preferring rats may be relatively hypo-noradrenergic, which could allow the latter group to uniquely benefit from pharmacological enhancements of synaptic noradrenaline. This hypothesis, however, has yet to be experimentally tested. Additionally, our findings could illustrate functional adaptations that occur in vulnerable individuals during cue-guided learning, which could contribute to the development of a risky phenotype. Whether such differences in the function of PrL noradrenaline in risky and safe individuals are pre-existing or a consequence of learning in a heavily cued environment remains a question. Both rodent and human studies have demonstrated differences in NET availability (Hesse et al., 2017) or behavioural sensitivity to NET blockade (Ansquer et al., 2014; Zlebnik & Carroll, 2015) between individuals with high and low baseline levels of impulsivity. In the current experiment baseline impulsivity did not

drive the selective anti-impulsivity effect of PrL atomoxetine in risk preferring animals, and the statistics suggest that the effect was indeed driven by risk preference. As such our data present novel evidence for differences in prefrontal noradrenergic function between risk preferring and optimal performing individuals.

## 3.2 Toward a unified theory of noradrenergic regulation of decision making

Our data not only show a novel double dissociation of noradrenaline function across frontal brain regions but also corroborate and extend existing theories on the neurochemical bases of decision making. Below I combine theories of noradrenergic regulation of behaviour with those of OFC function to propose a theoretical mechanism for how prefrontal noradrenaline may guide cost-benefit decision making.

### 3.2.1 Noradrenergic tone and adaptive decision making

The influential adaptive gain theory describes the role of LC noradrenaline in flexible behaviour (Aston-Jones & Cohen, 2005). The theory posits that enhanced noradrenergic tone can facilitate adaptive switches in behavioural strategies when the current strategy proves to be disadvantageous, as high LC tone can blunt the physiological and neurochemical impact of phasic noradrenaline bursts. These phasic bursts, importantly, can be triggered by salient and motivationally relevant stimuli (Aston-Jones et al., 1994; Aston-Jones & Bloom, 1981; Bouret & Richmond, 2015). Our current data fit nicely within the adaptive gain framework. The risk-promoting cues of the crGT presumably provoke phasic LC firing due to their salience, relation to reward, and task relevance. Enhancing prefrontal noradrenergic tone with a reuptake blocker like atomoxetine could therefore lessen the impact of cue-induced phasic LC firing and

encourage rats to shift preference away from highly-cued risky options. This is consistent with the observed improvements in score and choice profiles following intra-lOFC atomoxetine in males. Sales et al. (2019) expand upon the tenets of the adaptive gain theory, using an active-inference mathematical model to further implicate LC dynamics in flexibly updating behaviour and internal task representations. The authors show that ramping LC noradrenaline release may be particularly important in updating behavioural strategies when recent observations significantly violate one's internal model of the world or task, termed a 'state-action prediction error'.

### 3.2.2 Orbitofrontal punishment signals and noradrenaline

Considering noradrenaline's ability to increase signal-to-noise ratio in the cortex (Berridge & Waterhouse, 2003; Gamo et al., 2010; Hasselmo et al., 1997), and the important role of the OFC in tracking, encoding, and responding to punishment (O'Doherty et al., 2001; Orsini et al., 2015; Turner et al., 2021; C. Xie et al., 2021), our current data may exemplify a union between adaptive gain/active-inference processes and OFC punishment encoding. Enhancing frontal noradrenaline with atomoxetine may boost on-task 'punishment signals' within the OFC, rendering every time-out punishment subjectively more severe than expected based on the internal task representation developed during drug-free training on the crGT. As such, this would exemplify a state-action prediction error, as the previously learnt model of the task was significantly violated. In the face of such a prediction error, the pharmacologically enhanced noradrenergic tone would also facilitate behavioural switches away from high-risk high-reward options toward safer crGT choices. Therefore, intra-lOFC atomoxetine may improve decision making by simultaneously exacerbating punishment-related prediction errors and promoting

adaptive shifts in decision making strategy. These speculations are consistent with theories derived from experiments done in male subjects (Aston-Jones & Cohen, 2005; Sales et al., 2019), and fit well with our male lOFC data. However, the mechanisms underlying noradrenergic regulation of decision making in females are less understood, and the above theories do not adequately account for why intra-lOFC atomoxetine does not improve decision making in females. This highlights the need for future studies to extend or adapt existing theories to fit female behaviour, as well as further investigations on sex differences in the neural substrates of risk taking.

### 3.3 Future directions

The double dissociation revealed by the current data prompts future inquiries into the mechanisms behind noradrenaline's behavioural effects. One logical future experiment would be to investigate the necessity and sufficiency of endogenous noradrenaline release in the development of cue-invigorated risk taking. This could be attained by bidirectionally altering the activity of LC noradrenaline neurons while rats learn the crGT using inhibitory (hM4-Di) or excitatory (hM3-Dq) designer receptors exclusively activated by designer drugs (DREADDs). With a transgenic TH::Cre rat line coupled with Cre-dependent viral vectors, DREADD expression of can be restricted to catecholaminergic neurons in the LC. Injection of the DREADD ligand, clozapine-N-oxide (CNO), prior to acquisition sessions of the crGT would lead to the suppression or enhancement of noradrenaline release from the LC, depending on the DREADD that is expressed. Consistent with the adaptive gain theory and our current findings, I predict that chemogenetically blunting the excitability of LC neurons could improve decision making on the crGT by reducing the physiological and behavioural impact of cue-elicited phasic

noradrenaline release. Conversely, enhancing the excitability of LC noradrenaline neurons may exacerbate task-evoked LC firing and promote preference for risky options that are associated with the most salient win cues. Interestingly, there is some evidence that cell-type specific chemogenetic manipulations of noradrenaline neurons may not lead to the same physiological changes as they do in other types of neurons. DREADD-mediated excitation leads to increased tonic firing rate in LC noradrenaline neurons (Vazey & Aston-Jones, 2014). However, chemogenetic activation of ventral tegmental area (VTA) dopamine neurons enhances phasic firing without altering measures of tonic release (Mahler et al., 2019; unpublished data). Inhibiting neuronal firing with an hM4-Di DREADD also has disparate effects on action potential-mediated vs spontaneous neurotransmitter release, depending on whether CNO was infused at the soma or axon terminals (Ferguson et al., 2011; Stachniak et al., 2014). However, it is currently unknown how systemic chemogenetic inhibition of LC noradrenaline neurons influences the balance between phasic and tonic firing. Given the notable differences in the behavioural correlates of phasic and tonic LC firing (Aston-Jones & Cohen, 2005), future studies should also use fibre photometry or electrophysiology to confirm the effects of DREADD manipulations on LC neuron activation and firing pattern.

To gain temporal control over LC manipulations, future work should consider employing optogenetics during crGT performance. With this approach, LC inhibition can be restricted to relevant crGT time points, such as at cue/reward presentation, throughout time-out punishments, or during the pre-choice epoch. This would interrogate the role of LC noradrenaline in various cognitive processes engaged during a crGT session. Additionally, laser illumination of an excitatory opsin could be tailored to mimic frequencies of physiological phasic or tonic LC firing. This could allow us to dissociate the functional roles of phasic vs tonic noradrenaline

release in a complex decision making task. Optogenetics would also allow for projection-specific manipulations to probe the functional role of various prefrontal LC projections. These experiments could corroborate our current findings and explore LC contributions to the dissociable behavioural roles of lOFC and PrL noradrenaline signaling in the crGT.

Additionally, future inquiries of noradrenergic contributions to gambling-like behaviour should use risk preference and baseline impulsivity as covariates or between-subjects factors in all analyses, considering the reports from our lab and others that the behavioural effects of noradrenergic manipulations may vary as a function of baseline risk preference or impulsivity (Ansquer et al., 2014). Further, given the sex differences revealed by the current data, it is critical for subsequent behavioural work to use both male and female subjects.

### 3.4 Significance and conclusions

The work presented here doubly dissociate noradrenaline function across prefrontal subregions such that noradrenergic tone in the lOFC is important for guiding cost-benefit decision making in males while PrL noradrenaline signaling regulates motor impulsivity. The data further demonstrated a functional sex difference that renders decision making in females insensitive to the benefits of intra-OFC atomoxetine that were observed in males. These experiments also showed that noradrenergic manipulations differentially alter behaviour depending on baseline risk preference, whereby only risk preferring animals benefited from the anti-impulsivity effects of intra-PrL NET blockade while all rats, irrespective of risk preference, made more premature responding after intra-PrL guanfacine treatment. This suggests that the noradrenaline system responds differently to pharmacological perturbations in individuals that are vulnerable versus protected from developing maladaptive decision making strategies in the

presence of risk-promoting win-paired stimuli. Together, these data provide novel insights into the prefrontal substrates that independently orchestrate noradrenergic influences over impulsivity and decision making. This importantly adds to our understanding of the mechanisms through which noradrenergic drugs can improve maladaptive behaviours common to psychiatric conditions like gambling disorder, impulse control disorders, and substance use disorder. They also highlight that the therapeutic mechanisms and efficacy of such medications may vary based on an individual's biological sex and natural levels of risk preference, which could have important implications for the development of novel pharmacotherapies.

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