Neurobiological and behavioural studies of individual variation in cue-evoked motivation across rodents and humans

Morgane Colom
BSc. MSc.

A thesis submitted for the degree of
Doctor of Philosophy

January 2023

Supervised by Dr Bryan F. Singer, Dr Eleanor J. Dommett,
Dr Claire L. Rostron, Dr Christopher J. Heath.

The Open University
Faculty of Science, Technology, Engineering and Mathematics
School of Life, Health and Chemical Sciences
United Kingdom

Declaration

I declare that the work presented in this thesis is my own original work and does not contain any material submitted for any other qualification at the Open University, or any other University. Contributions made by other researchers are fully acknowledged in relevant parts of the thesis.

Abstract

For a subset of individuals known as sign-trackers, discrete pavlovian cues associated with rewarding stimuli can acquire incentive properties in their own right. When they do so, cues have the ability to exert control over behaviour, which appears to contribute to specific sets of symptoms within neuropsychiatric conditions such as substance use disorder or post-traumatic stress disorder.

Characterising the neurobiological mechanisms mediating variation in cue responsivity is essential to better understand differences in the susceptibility to such disorders. Converging evidence points towards the involvement of dopaminergic neurotransmission in the nucleus accumbens core in the development of sign-tracking; yet, whether this phenotype is associated with specific accumbal postsynaptic properties is unknown. The first section of this thesis investigated the morphology of dendritic spines, presynaptic and postsynaptic markers of activity in the nucleus accumbens core of male and female rats following a pavlovian conditioned approach procedure. Results suggest that individuals who attributed the most incentive salience to a food cue displayed unique dendritic spine organisation; such observations were modulated by the presence or absence of reward. The influence of the oestrous cycle and the altering of sign-tracking by propranolol were also examined.

Developing transitional tools to detect sign-tracking in humans might provide a valuable means to identify profiles conferring vulnerability to maladaptive behaviours. The second section of this thesis describes responses of male and female participants in a computerised image-based pavlovian procedure, a virtual room-based pavlovian environment, and a 'real-life' pavlovian procedure in which participants physically interacted with the apparatus. The second and third approaches enabled the development of distinct conditioned phenotypes. Because rodent sign-trackers are more impulsive, the relationship between phenotypes and impulsivity was also considered.

This thesis provides further insight into the neural underpinnings of motivated behaviours and offers guidance for future translational investigation.

Acknowledgements

If I had to summarise my PhD experience, I would say that I spent a good portion of my time in the laboratory observing rats enthusiastically nibbling levers. Human participants, sadly, were not willing to do the same when offered the opportunity, which shows how dull humans are.

Some say that the environment in which the PhD is undertaken is more important than the topic itself. After hearing numerous testimonies of absent or even hostile supervisors, I consider myself extremely fortunate to have had such great PIs. First and foremost, I want to thank them all for proposing the project and for choosing me. My sincerest gratitude goes to Bryan, Ellie, Claire and Chris for their unwavering patience, their great mentorship, their guidance, and constant reassurance. It is almost as if the four supervisors tacitly understood that my insecurities would benefit from frequent and explicit praise, and they willingly did so through each step of the process, thus pushing me forward. They all provided prompt and valuable feedback whenever the situation required, and complemented each other perfectly considering this multi-technique, multi-species, multi-approach project. Despite negligeable downsides of having four supervisors – for example, when everyone reviews writing at the same time – it is an undeniable advantage to be able to draw from such a vast well of knowledge.

I am indebted to Agata and Iwona for taking care of my ratties, for showing me how to tickle them (for their own well-being of course), and teaching me many other techniques. This project would not have been possible without their work. Above all that, thank you for always being so sweet. Thank you to the other lab members for the trainings and the endless assistance: Igor, Karen, Brett and George. I also want to acknowledge Alexandra Georgescu from KCL for writing the scripts used to analyse the human experiments; Gwyneth Morgan for creating and amending the virtual pavlovian environment; David Pound for designing and building the human pavlovian apparatus. In addition, I must thank the University of Sussex for letting me use their microscopes when technical issues arose at the Open University.

Because of a set of circumstances leading to a shortage of fellow behavioural neuroscientists – even of fellow postgraduates, full stop – in my department, I was at risk of being extremely lonely during this PhD. However, through a fortunate chain of events, I was quickly adopted by geologists, astrophysicists, chemists and other space engineers from the Physics department who raised me as their own. In hindsight, I discussed more about asteroid lithologies and multiple star systems than about my own field; what more could a curious

mind ask for! Many thanks are owed to Vincent (who had the power to make ‘no smoking’ signs magically appear everywhere on campus), Oleg, Giulia, Oliver, Megan, Anton, Tara, Feargus, Josh, Tom, Chiaki, Pete, Lewis and all the others.

A few long-lasting friends outside of the Open University have indirectly participated in making this PhD more comfortable. Constance, of course, is the first that comes to mind. It is almost unbelievable that we ended up going through the same process for the past thirteen years, and now sharing the same doctoral experience from different territories, both exiled from home, in strange lands speaking strange languages. I cherish this shared experience and the mutual understanding that comes with it. I want to give thanks to Lucie, who also had the idea of undertaking her PhD in England and who was always eager to show me the best climbing spots in London. Thank you to the Ascenders, Rich and Francis, for the much-needed rock climbing breaks (because after all, who needs skin on their fingers?). I should probably also thank the 75 m² allotment plot that kept Ross and me – and not Ross and I – sane during the 2020 lockdown.

I am very fortunate to have a loving and supporting family. My parents fought against my lack of self-confidence with all their might since I was able to speak, and always refused to believe that the (undeniably fortuitous) opportunities I was given were due to anything else than talent. An immense thank you to them for their unceasing support, and to Mélie who will definitely not be undertaking a PhD after witnessing her sister’s descent into madness – but who nonetheless patiently listened to my numerous (and unsuccessful) attempts to explain the concept of sign-tracking in French. I also want to extend a thought to my grandparents and other family members who never understood a word of my project but were nonetheless unquestioningly proud – I will soon be a ‘docteur des souris’ (mouse doctor) indeed! Thank you also to my second family, Hazel, Andy and Mae, for providing a warm and welcome refuge in the wilderness when I was overdosing on screens.

And finally, an immense thank is due to my partner Ross, to whom I cannot show enough gratitude for handling my convoluted mind with such patience and virtuosity. You deserve another PhD for this achievement. Taking care of your impostor syndrome helped me reconsider and temper my own, which is no mean feat. This all experience would have been vastly different without the support we gave each other and the reciprocal sharing of knowledge.

Contents

List of figures

List of tables

Abbreviations

1DC	Validated at least one discrimination criterion
ADHD	Attention deficit/hyperactivity disorder
AST	Alcohol screening tool
AUC	Area under the curve
AUDIT	Alcohol screening tool
BIS-11	Barratt Impulsiveness Scale
BLA	Basolateral amygdala
BORIS	Behavioural Observation Research Interactive Software
CR	Conditioned response
CS	Conditioned stimulus
DA	Dopamine
DAST	Drug abuse screening test
DAT	Dopamine transporter
DDT	Delay discounting task
EEG	Electroencephalogram
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GNG	Go-NoGo
GT	Goal-tracker / goal-tracking
IF	Indifference point
I.P.	Intra-peritoneal
IGT	Iowa Gambling Task
ITI	Inter-trial-interval
LTP	Long-term potentiation
MDD	Major depressive disorder
MSNs	Medium spiny neurons
NAc	Nucleus accumbens
NMDA	N-methyl-D-aspartate
NoDC	Did not validate any discrimination criterion
NORET	Chap. IV: Control group without retrieval session
NORET10	Chap. IV: Control group without retrieval administered propranolol at 10 mg/kg

NORET20	Chap. IV: Control group without retrieval administered propranolol at 20 mg/kg
ONS	Office for National Statistics
PB	Phosphate buffer
PCA	Pavlovian conditioned approach
PFA	Paraformaldehyde
P	Non-rewarded paired group from Chapter III, Exp. 1
P30	Paired animals from Chapter III, Exp. 1 perfused 30 min after the test
P360	Paired animals from Chapter III, Exp. 1 perfused 360 min after the test
PFC	Prefrontal cortex
PIT	Pavlovian-to-instrumental transfer
PROP	Propranolol
PSD	Postsynaptic density
PTSD	Post-traumatic stress disorder
R	Rewarded paired group from Chapter III, Exp. 2
R30	Rewarded paired animals from Chapter III, Exp. 2 perfused 30 min after the test
R360	Rewarded paired animals from Chapter III Exp. 2 perfused 360min after the test
RET	Chapter IV: Group with retrieval sessions
RET10	Chapter IV: Retrieval group administered propranolol at a dose of 10 mg/kg
RET20	Chapter IV: Retrieval group administered propranolol at a dose of 20 mg/kg
RPE	Reward prediction error
SAL	Saline
SN	Substantia nigra pars compacta
ST	Sign-tracker / sign-tracking
SUD	Substance use disorder
U360	Unpaired control group of Chapter III, Exp. 1 perfused 360 min after the test
UNODC	United Nations Office on Drugs and Crime
UR	Unconditioned response
US	Unconditioned stimulus
VP	Ventral pallidum
VTA	Ventral tegmental area

Conference items

Poster presentations

2020 **Federation of European Neuroscience Societies Virtual Forum** (virtual). Evaluation of postsynaptic structural differences between sign- and goal-tracking rats (#3391).

2021 **Society for Neuroscience conference** (virtual). Variation in cue evoked motivation and dendritic spine dynamics in the nucleus accumbens (#P672.05).

2021 **British Neuroscience Association Festival of Neuroscience** (virtual). Postsynaptic structural organisation in sign- and goal-tracking rats (#TP001277).

Chapter I
General Introduction

Environmental stimuli

The environment organisms are exposed to differs vastly depending on the species. Skates are electrosensitive and use bioelectric fields emitted by other animals to locate prey and detect predators (Kalmijn, 1971). Common moles have an acute and stereoscopic sense of smell and are sensitive to vibrations, granting them the ability to reconstruct their surroundings in the absence of light (Catania, 2013). The vision of diurnal birds of prey, far superior to that of humans, allows them to perceive small details from great distance (González-Martín-Moro *et al.*, 2017). Animals are therefore surrounded by a range of drastically different environmental stimuli – but regardless of their nature, these stimuli can provide valuable information to the organism and when they do so, they take on the role of 'cues'. Cues can be discrete and localisable (*e.g.*, object, smell, sound), or contextual (*e.g.*, a static place); in a natural environment – as opposed to a laboratory – they are often a combination of these aspects (*e.g.*, a static but discrete tree). To be considered as cues, stimuli need to be biologically relevant to the animal by signalling the availability or the location of significant resources such as food, water, or mates, or indicating the presence of a threat. Being able to correctly identify and respond appropriately to environmental cues, for example approaching a 'reward' or fleeing a predator, is evolutionary beneficial and critical for survival.

Cue-based reward learning

Specific patterns of behavioural responses are acquired through prior experience. Cue-based associative learning can take many forms, but pavlovian (or classical) learning and instrumental (or operant) learning are the most significant as they allow animals to connect environmental elements – for the former – or actions – for the latter – to outcomes inherently significant for the individual, thereby developing adaptive and flexible decision-

making ensuring survival (Fanselow, 2018; Skinner, 1984). A conventional pavlovian procedure involves an appetitive or aversive stimulus (unconditioned stimulus; US) provoking an unconditioned (unlearnt) and reflexive response (UR) in the organism and an originally neutral stimulus temporally and/or spatially associated with the US which, after repeated pairings, becomes a predictive and conditioned stimulus (CS) capable of eliciting the same response than the US (learnt conditioned response; CR).

The mechanism by which rewards and associated cues influence behaviour has been subject to debates over the years and evolved in parallel with techniques and behavioural concepts. In his initially hedonistic Law of Effect, Thorndike hypothesised that responses followed by pleasant effects would be more likely to reoccur (Thorndike, 1898). Ignoring affective states in favour of a more measurable approach, learnt responses evoked by predictive cues were subsequently described as simple rigid and inflexible reflexes strengthened by rewarded outcomes (Carr and Watson, 1908; Moss and Thorndike, 1934; Pavlov, 1927). However, conditioned responses are often complex, and the same stimulus can induce various sequences of action depending on external but also internal conditions, which is critical to adapt to an evolving environment. In the beginning of the 20th century another very influential hypothesis was introduced to explain what guided and energised conditioned behaviour, called the 'drive reduction' theory (Hull, 1943; Richter, 1927). Proponents of this theory proposed that behaviour was stimulated into action by the need to maintain homeostasis through alleviation of aversive states such as hunger or thirst. According to this theory, a stimulus-response habit is reinforced when the response produced leads to the diminution of a 'negative' state – for example, a response which gains food diminishes the state of hunger. However, between the 50s' and the 70's, experiments started to point to the shortcomings of this hypothesis. For example, rats learnt and eagerly produced instrumental responses to stimulate rewarding regions of the brain (Olds and Milner, 1954) and indirect brain stimulations in the reward system triggered food and water consumption

(Valenstein *et al.*, 1971) in the absence of any specific drive, hence demonstrating that motivations and rewards could be sought after and not aversive. This suggested that drive reduction was not sufficient to explain how behaviours are reinforced.

Instead, researchers progressively developed the notion of an *incentive* stimulus capable of reinforcing responses and invigorating reward-seeking behaviours through its appetitive and motivational properties. Reverting to a hedonistic view of the reward perceived as such because of the pleasure felt when consumed, researchers then hypothesised that stimuli predictive of the reinforcer did not only induce an expectation of the latter but were also imbued with the same rewarding properties because of their pavlovian association, therefore being perceived as gratifying as well (Bolles 1972; Bindra, 1978). To further explain the ability of reward stimuli to control behaviour, Frederick Toates later suggested that the value of incentive stimuli was also modulated by internal states in such a way that specific rewards instigated motivations relevant to physiological needs in order to act as magnets and reach homeostasis (Toates, 1986). For example, in a state of thirst, the hedonic and incentive value of water and related stimuli would be specifically enhanced so as to be more attractive and guide animals towards it for survival. It is important to note that in this model, the concepts of pleasure (liking) and motivation (wanting) were still united – which is reasonable given that the conscious experience of pleasure is tightly linked to that of desire in humans.

However, as illustrated in Figure I.1, this incentive salience theory has since been adjusted by separating both notions and by distinguishing subjective, perceived experience (liking and wanting) from unconscious, physiological processes of rewards (‘liking’ and ‘wanting’; Berridge, 2007; Berridge and Robinson, 2003) – although not all researchers concur with using subjective words to refer to neurobiological processes that cannot be directly linked to specific desires (Robbins and Everitt, 2007). Using both behavioural and neurobiological evidence (as described in a subsequent section), Kent Berridge, Terry

Robinson and their team demonstrated that 'liking' and 'wanting' were in fact dissociable: in both human and non-human animals, physiological reactions of pleasure were not impacted by the manipulation of the neural circuits that otherwise disrupted the pursuit of reward, and vice versa (Berridge *et al*., 1989; Berridge and Robinson, 1998; Berridge and Valenstein, 1991; Reynolds and Berridge, 2002; Treit and Berridge, 1990; Wyvell and Berridge, 2000). Moreover, subjective ratings of pleasure were also found to be independent from subjective reports of desire (Brauer and De Wit, 1997; Leyton, 2010; Sienkiewicz-Jarosz *et al.*, 2013).

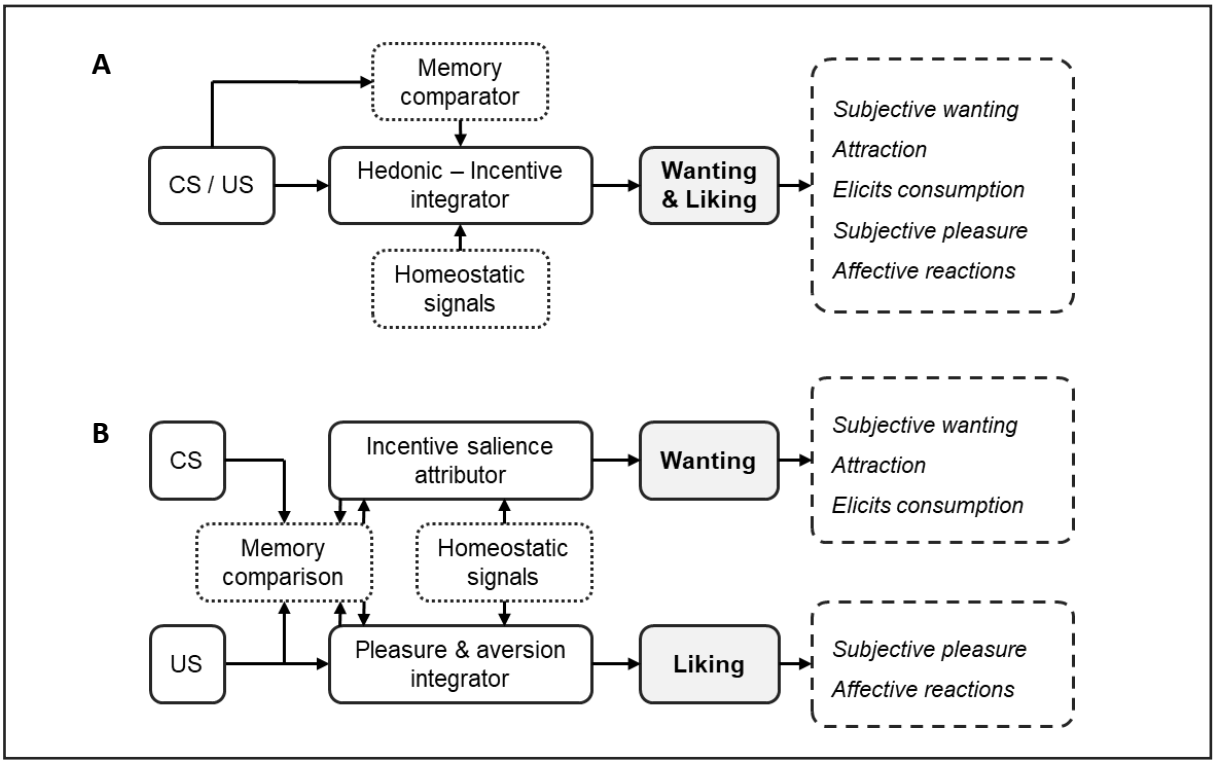


Figure I.1. Models of incentive motivation. (A) The concepts of pleasure (liking) and desire (wanting) are combined. Based on Toates (1986). (B) Incentive salience model from Robinson and Berridge (1993) in which liking and wanting are dissociated. *Image modified from Berridge (2000).*

Another example of this dissociation can be found in the scope of substance misuse, wherein individuals sometimes crave drugs despite experiencing no pleasure in their consumption and being aware of detrimental consequences (Robinson and Berridge, 1993). Motivation – and not pleasure – is therefore the central element of the incentive salience theory (Berridge

and Robinson, 1998). The incentive stimulus has three primary properties: it is attractive, and therefore elicits *conditioned approach* towards it; desirable, and can thus act as a *conditioned reinforcer* capable of maintaining behaviour; and can induce a *conditioned motivational* state energising behaviour (Cardinal *et al.*, 2002; Everitt *et al.*, 2001). Such incentive properties, typically possessed by valuable resources ('rewards'), can then be transferred to conditioned stimuli predicting them (CSs), thereby granting these incentive cues the ability to motivate reward seeking (Berridge *et al.*, 2009).

Neural mechanisms of reward learning

Appetitive learning is crucial for survival as it prompts organisms to actively seek out valuable resources, and much research has investigated the neural processes underlying the formation and persistence of learnt associations over the years. Dopamine (DA), a catecholamine neurotransmitter synthetised by most animals, plants and microorganisms (Roshchina, 2010), has quickly become the main focus – although it is not the only one involved (Ikemoto, 2010). In mammals, dopaminergic neurons primarily arise from the ventral tegmental area (VTA) and the substantia nigra pars compacta (SN) and are distributed along the mesolimbic pathway, the mesocortical pathway and the nigrostriatal pathway, in which dopamine binds to D1-type or D2-type receptors in subcortical and fronto-cortical areas (Watabe-Uchida *et al.*, 2012). Within the mesolimbic pathway, VTA dopaminergic neurons project to the ventral striatum (including the nucleus accumbens; NAc), the dorsal striatum, the ventral pallidum and the hippocampus; and neurons arising from this area also project to the prefrontal cortex (PFC) in the mesocortical pathway (Figure I.2, A and B; Ikemoto, 2007; Watabe-Uchida *et al.*, 2012). In return, the VTA receives GABAergic inputs (neurons releasing inhibitory γ-aminobutyric acid neurotransmitters) from the NAc and the ventral pallidum, as well as glutamatergic afferences (neurons

releasing excitatory glutamic acid neurotransmitters) from the hippocampus and the PFC, all of which regulate dopaminergic signalling (Figure I.2, C; Carr and Sesack, 2000).

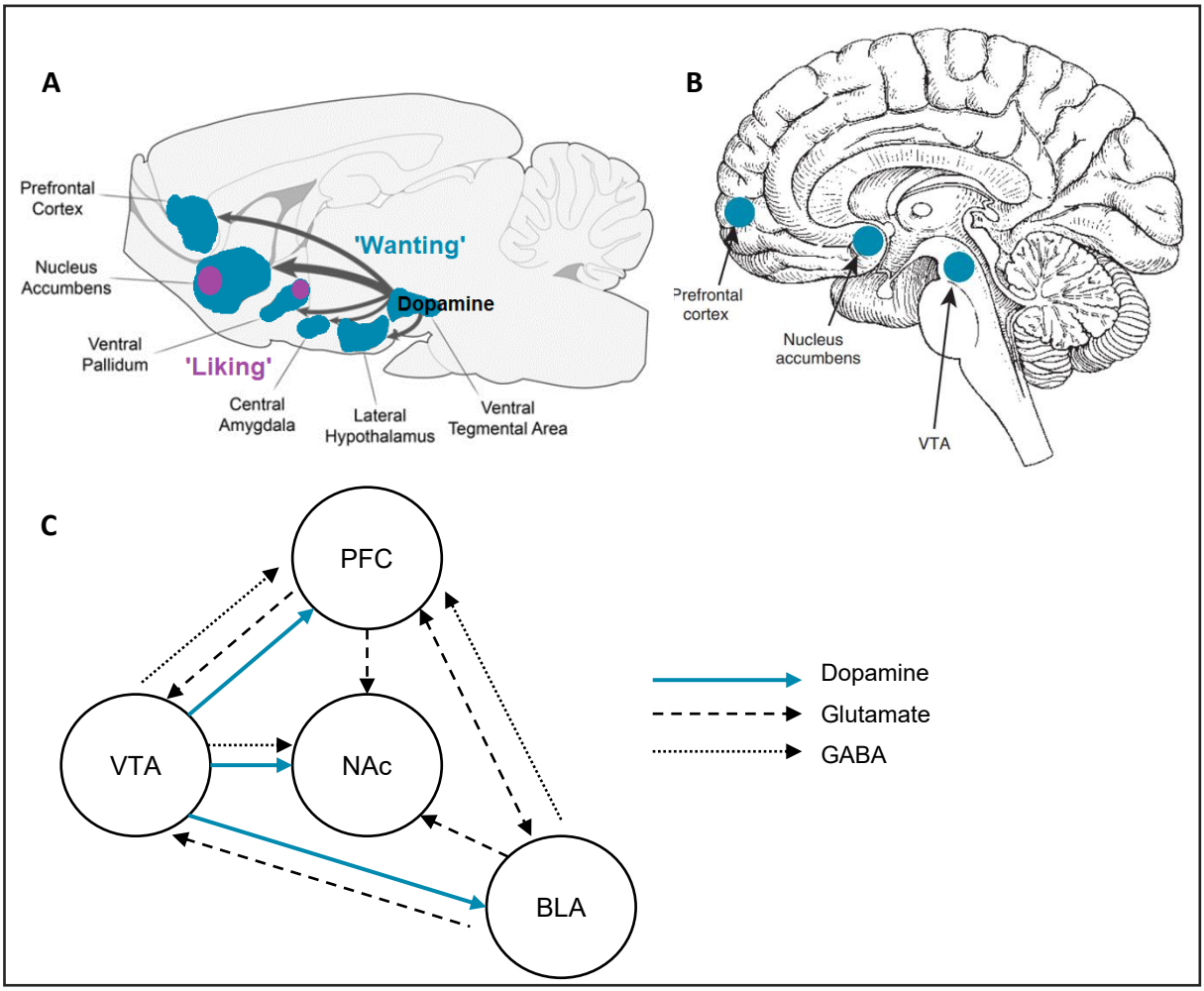


Figure I.2. Representations of the motive circuit. (A) Sagittal view of the mesocorticolimbic pathway in the rat. Dopaminergic neurons arising from the VTA and projecting to target areas are involved in 'wanting' (blue). 'Liking' (purple) is localised in hedonic hotspots. (B) Sagittal view of the mesocorticolimbic pathway in a human brain. (C) Schematic illustration of the connections within the motive circuit. VTA: Ventral tegmental area. NAc: Nucleus accumbens. PFC: Prefrontal cortex. VP: Ventral pallidum. BLA: Basolateral amygdala. *Images modified respectively from Robinson et al., 2016; Wand, 2008; Baker et al., 2002.*

One of the first experiments revealing the function of dopamine in reward processing did so by implanting electrodes along what would subsequently be identified as the mesolimbic pathway and offering rats to press a lever to obtain a stimulation in these areas (Olds and Milner, 1951), action which animals produced eagerly to the point where they would press the lever to activate these regions whilst neglecting available food (Olds, 1956). This suggested that positive reinforcement of behaviour was supported by 'pleasure centres'

in the brain. For decades dopamine was considered the neurotransmitter underlying the experience of pleasure, which was itself thought as the key mechanism reinforcing motivated behaviour and conferring rewarding properties to natural rewards and drugs (De Wit & Wise, 1977; Wise, 1982; Wise *et al.*, 1978). Another crucial suggested role of dopamine was to promote the acquisition of stimulus-reward associations by acting as a teaching signal. Wolfram Schultz's experiments indeed revealed that in instrumental or pavlovian learning tasks, mesolimbic dopaminergic neurons fired in phasic bursts in response to unexpected rewards and, more importantly, to discrepancies between a reward-predicting cue and the subsequent outcome, phenomenon commonly referred to as reward-prediction error or RPE (Montague *et al.*, 1996, 1996; Schultz *et al.*, 1993; Schultz, 2022). More specifically, the signal initially triggered by unexpected rewards transferred, over time, to the occurrence of the stimulus predicting it, and the magnitude of DA firing reflected changes in the value of the reward so that negative RPEs – the omission or delay of an expected reward – were accompanied by a decrease in dopamine spiking (Hollerman and Schultz, 1998; Schultz *et al.*, 1997). This mechanism has been proposed to support the update of existing associations as well as new learning. Other teams have since then replicated and extended these results to other associative learning settings, including pavlovian conditioning, using various techniques (Chang *et al.*, 2016; Bayer and Glimcher, 2005; Hart *et al.*, 2014; Saddoris *et al.*, 2015; Steinberg *et al.*, 2013). Recent work suggest that dopamine may also be involved in encoding and updating goal-directed predictive representations, and that dopamine neurons responded to unexpected or salient events that are neither appetitive nor aversive; these elements may be used prior to and alongside RPEs to adjust predictive relationships and stimulate new learning (Akam and Walton, 2021).

However, mesolimbic dopaminergic neurons do not only respond to error prediction signals in the scope of cue-based reward learning but have been suggested to also be involved in attributing incentive properties to rewards and associated cues (Berridge and Robinson,

1998; Berridge *et al.*, 2009). As mentioned in a prior section, contrary to the predominant concept of reward in the end of the 20th century stating that motivation and pleasure were one and the same, neurobiological evidence further supported the hypothesis that 'liking' and 'wanting' were distinct components. Disruption of dopaminergic signalling by lesions or administration of antagonists do not impact physiological hedonic reactions in rats but do alter the expression of reward-related behaviours (Berridge, 2007; Treit and Berridge, 1990), whereas stimulating dopamine release in the mesolimbic pathway (*e.g.*, NAc, ventral pallidum) or increasing dopamine availability specifically enhances the incentive impact, thereby prompting reward-seeking and increasing cue-induced instrumental learning (Berridge and Valenstein, 1991; Pecina *et al.*, 2003; Tindell *et al.*, 2005; Wyvell and Berridge, 2000). In humans, reduction or blockade of dopamine signalling affect subjective ratings of 'wanting', but not 'liking', food and drugs (Leyton *et al.*, 2005). Contrary to the widely distributed and easily activated motivational system, 'liking' originates from localised hedonic hotspots containing clusters of neurons in the insula, the ventral pallidum, the orbitofrontal cortex and the NAc shell, which are mainly activated by opioid signalling (Berridge and Kringelbach, 2015). Other authors have suggested an approaching – but in some aspects, distinct – interpretation of dopamine function in reinforcement. According to this alternative model, mesolimbic dopamine is implicated in habit learning, which can be supported, for example, by the fact that conditioned responses persist after reward devaluation if dopaminergic signalling is stimulated, and is particularly relevant to the scope of addiction research (Berridge, 2007; Nelson and Killcross, 2006; Robbins and Everitt, 2007). However, the pavlovian conditioned responses investigated in this work do not entirely correspond to simple S-R habits in that they do not appear to involve the dorsal striatum (see subsequent section: Fraser and Janak, 2017) and have been shown to adjust flexibly in some conditions (see subsequent section: Chang and Smith, 2016; Robinson and

Berridge, 2013). The hypotheses of this thesis were thus designed within the incentive salience framework.

If dopamine neurons within the motivational circuit can assign incentive value to all valuable stimuli – rewards and predictors alike, they are also involved in turning reward-associated *cues* into attractive, desirable stimuli capable of invigorating behaviour (Blaiss and Janak, 2009; Cardinal *et al.*, 2002; Dickinson *et al.*, 2000; Parkinson *et al.*, 1999). It seems that when pavlovian cues are attributed with incentive/motivational properties by the mesolimbic dopaminergic system, they can exert control over behaviour; however, strikingly, they only do so in a subset of individuals.

Individual variation in reward-cue processing

Individual differences in the responsivity to reward-associated cues were not described in the first experiments undertaken by Pavlov as dogs were restrained, thus preventing the expression and detection of variable CRs – although a few years later, Pavlov did write about animals contacting the stimulus and licking it when it was within reach (Pavlov, 1927; Pavlov, 1934). The first researcher to properly report variations elicited by pavlovian cues in unrestrained animals described a '*striking'* divergence in behaviours with some individuals approaching the CS and others constantly fixating the reward location (Zener, 1937). During the few decades following these initial observations, more experiments revealed similar cue-directed behaviours in both instrumental, pavlovian, and hybrid paradigms, as animals displayed species-specific consummatory behaviours towards reward-paired stimuli – pecking, rooting, rubbing, gnawing – instead of only producing the reinforced learnt response (Breland and Breland, 1961; Browns and Jenkins, 1968; Skinner, 1948; Williams and Williams, 1969). To explain these unexpected and surprising 'misbehaviours' researchers put forward several hypotheses, the most renowned being that

animals developed superstitious behaviours due to accidental unconditioned reinforcement (Breland and Breland, 1961; Browns and Jenkins, 1968; Skinner, 1948; Williams and Williams, 1969). In other words, individuals believed that these irrelevant responses produced during the interval between the CS and the US were in fact associated with the availability of the reward. However, this theory has been contested by procedures demonstrating that animals still approached and contacted the pavlovian cue when this action resulted in subsequent omission of the reward (Killeen, 2003; Lajoie and Bindra, 1976; Schwartz and Williams, 1972; Timberlake and Lucas, 1985). Later, students of Skinner introduced the notion of 'instinctive drift' to rationalise why animals behaved in a manner which appeared to defy the Law of Effect since it sometimes delayed – or cancelled – the delivery of the reward. They proposed that organisms had an instinctive tendency to drift back towards their natural reflexes – taken, in this situation, from their specific food-getting repertoire (Breland and Breland, 1961). Because of the consummatory-like nature of behaviours expressed towards the conditioned cue, the latter was also suggested to act as a substitute for the reward (Timberlake and Lucas, 1985). For the most part, and despite some calls to include such 'anomalous' data and to revise current paradigms to better accommodate and interpret them (Malone, 1975; Staddon and Simmelhag, 1971), these responses were considered aberrant and were not systematically studied until the 70s'. Hearst and Jenkins were the first to use the now conventional name of 'sign-tracking' to designate this particular behaviour in reference to the signalling role of the predictive cue (Hearst and Jenkins, 1974), and 'goal-tracking' was subsequently introduced to label the behaviour of individuals preferentially approaching the location of the reward (Boakes, 1977).

More recent experiments have allowed to methodically characterise individual variation in the response to pavlovian cues and have demonstrated that these disparities were attributed to differences in incentive/motivational salience assignation to CSs, thereby leading to different levels of engagement with the CS and the US (Flagel *et al.*, 2009). Using

a rodent pavlovian conditioned approach (PCA) paradigm consisting of repeated pairings of a lever (CS) with the delivery of food (US) in a magazine, researchers revealed the emergence of three phenotypes: as rats learn that the CS is predictive of the US availability, sign-trackers (STs) develop a *conditioned approach* response towards the lever and interact with it, only visiting the food-cup to eat the reward after retraction of the lever; whereas goal-trackers (GTs) acknowledge the cue but directly approach the reward location (Figure I.3). As mentioned above, interactions with discrete pavlovian cues closely resemble pattern of behaviours animals produce towards the US – pigeons peck, rats nibble, cuttlefish attack (Cole and Adamo, 2005; Flagel *et al.*, 2009; Hearst and Jenkins, 1974). Other animals display an intermediate behaviour and oscillate between both strategies depending on the trial. Importantly, sign- and goal-trackers learn their respective conditioned response at the same pace, indicating that the CS is equivalently predictive in both phenotypes (Robinson and Flagel, 2009), and both phenotypes seem to possess similar learning capabilities in other behavioural tasks (Morrow *et al.*, 2011; Robinson and Flagel, 2009; Saunders and Robinson, 2010). Further illustrating the dissociation between predictive and incentive values of reward stimuli, studies have shown that the motivational valence could be manipulated and reversed

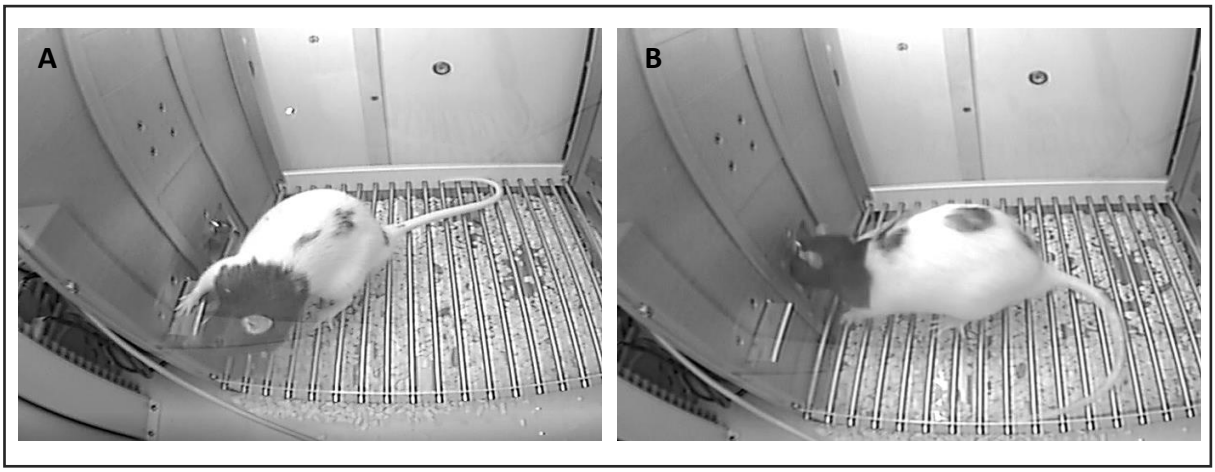


Figure I.3. Sign- and goal-tracking conditioned responses. (A) Sign-trackers approach and interact with the predictive cue during its presentation. (B) When the predictive lever is extended, goal-trackers wait for the reward in front of the food-cup.

(from repulsion to attraction) without affecting the learnt association between CS and US (Robinson and Berridge, 2013; Tindell *et al.*, 2005). Beyond conditioned approach, research has also shown variations in the extent to which reward cues act as *conditioned reinforcers*, in such a way that sign-trackers readily learn an instrumental response to trigger the presentation of the pavlovian cue in the absence of reward (Di Ciano and Everitt, 2004; Lomanowska *et al.*, 2011; Meyer *et al.*, 2012a; Robinson and Flagel, 2009). Reinforcing properties of reward cues are so powerful that they resist extinction procedures and are maintained when the value of the reward is subsequently reduced (Davis and Smith, 1976; Di Ciano and Everitt, 2005; Panlilio *et al*., 2005). Finally, CSs are more effective in spurring reward-seeking behaviours in sign-trackers, thus proving greater *conditioned motivators* (Barker *et al.*, 2012; Saunders *et al.*, 2013; Yager and Robinson, 2010). All the aforementioned elements strongly suggest that individual differences observed in the topography of conditioned responses reflect variations in the propensity to attribute incentive properties to pavlovian cues (Flagel *et al.,* 2009; Flagel *et al*., 2007; Meyer *et al*., 2012a; Robinson and Flagel, 2009; Robinson *et al.*, 2014). Critically, such variations in CRs might be conditional on sensory features of the CS: discrete, localisable and manipulable cues (*e.g.*, a lever) appear to have the ability to act as incentive stimuli, whereas auditory cues (*e.g.*, a tone) only produce goal-tracking-like responses and have equivalent conditioned reinforcing properties for all individuals (Flagel *et al.*, 2009; Meyer *et al.*, 2014; Singer *et al.*, 2016a), possibly contributing to adaptive flexible responding.

Observable behavioural variations in the tendency to attribute reward cues with motivational value are mediated by distinct and dissociable neural mechanisms. Various neurotransmitter systems allow to modulate sign-tracking behaviour including glutamatergic, cannabinoid, opioid, adrenergic and cholinergic systems (Bacharach *et al.*, 2018; Chow and Beckman, 2018; DiFeliceantonio and Berridge, 2012; Pasquariello *et al.*, 2018; Pitchers *et al.*, 2017a), but dopaminergic neurotransmission is thought to be

particularly involved in encoding the incentive component of reward-related stimuli, especially in the core subregion of the NAc. The NAc, part of the ventral striatum (Figure I.2), is divided into two functionally discrete areas: an inner core surrounded by an outer shell. Both are mainly composed of GABAergic medium spiny neurons (MSNs) predominantly expressing either D1-type receptors, forming the 'direct' pathway, or D2-type receptors, forming the 'indirect' pathway, although a few MSNs in the shell contain both receptors (Kravitz *et al.*, 2012; Meredith *et al.*, 2008; Salgado and Kaplitt, 2015). The NAc has been implicated in the acquisition (Dalley *et al.*, 2002; Di Ciano *et al.*, 2001, Parkinson *et al.*, 2002) and the expression (Di Ciano *et al.*, 2001; Parkinson *et al.*, 2002) of pavlovian learning. In a pavlovian procedure, the presentation of the reward cue induces a greater increase of dopamine release in the NAc core of sign-trackers compared to goal-trackers (Aitken *et al.*, 2016; Flagel *et al.*, 2011a; Singer *et al.*, 2016a), and the 'transfer' of phasic dopamine response from the time of the reward to the time of the predictive cue throughout learning noticed by Schultz (Schultz *et al.*, 1997) only occurs in these individuals; for goal-trackers, DA is still released after both the CS and the US (Flagel *et al.*, 2011b; Singer *et al.*, 2016a). What is more, disrupting said transfer of phasic dopamine impairs the development of sign-tracking behaviour whilst leaving goal-tracking intact (Parker *et al.*, 2010). However, manipulating the length of time between the presentation of the predictive cue and the delivery of the reward affects the animal's response towards the CS and the US, as well as the associated DA release: shorter intervals appear to decrease the CS interaction and to reinstate the RPE-like dopaminergic response (Lee *et al.*, 2018). Sign-trackers have also been shown to have higher surface expression of dopamine transporter (DAT) – and therefore a faster clearance and regulation of dopamine – than goal-trackers in the ventral striatum (Singer *et al.*, 2016b). The deactivation of DAT prevents the acquisition of an instrumental response in a pavlovian-to-instrumental transfer (PIT) procedure without impacting the initial pavlovian learning, suggesting the specific involvement of DAT in

incentive salience attribution (Savchenko *et al.*, 2022). Studies also suggest that D2-type receptors selectively contribute to incentive salience in that the blockade of D1 receptors prevents the acquisition of pavlovian associations in both phenotypes but antagonism of D2 receptors does not affect goal-trackers (Roughley and Killcross, 2019); and optogenetic activation or inhibition of D2 receptors in the NAc modulate motivational drive (Soares-Cunha *et al.*, 2016).

Due to their distinct profiles of dopaminergic neurotransmission, sign- and goal-trackers are differentially affected by dopamine manipulations. Systemic dopamine antagonist injections before or after pavlovian training have demonstrated that dopaminergic signalling was necessary for the specific acquisition and performance of sign-tracking approach (Danna and Elmer, 2010; Flagel *et al.*, 2010; Flagel *et al.*, 2011b; Saunders and Robinson, 2012). At higher doses the expression of goal-tracking was sometimes reduced as well, albeit to a lesser extent (Danna and Elmer, 2010; Flagel *et al.*, 2010; Flagel *et al.*, 2011b). This fact, along with the involvement of D1-type receptors in goal-tracking, indicates that dopamine signalling is still necessary for the development of this phenotype, and that RPE and incentive salience theories are not necessarily at odds; instead, different types of dopamine signals might support distinct functions (Akam and Walton, 2021; Chow *et al.*, 2016; Berke, 2018). Dopaminergic activity in the NAc core needs to be intact to learn a sign-tracking, but not goal-tracking, conditioned response, suggesting that dopamine activity in this area does not underlie associative learning or RPE, but motivational processes (Blaiss and Janak, 2009; Fraser and Janak, 2017; Saunders and Robinson, 2012). A study employing the opposite technique and stimulating dopamine signalling during pavlovian training using amphetamine injections found that increasing dopamine in the NAc core enhanced sign-trackers' approach towards the reward cue whilst leaving goal-tracking unaffected (Singer *et al.*, 2016b). It should be noted that when amphetamine is delivered intraperitoneally either two weeks before or after training, experimenters observe a *decrease*

of sign-tracking behaviour and an increase in goal-tracking (Holden and Peoples, 2010; Schuweiler *et al*., 2018; Simon *et al*., 2009). Further demonstrating the role of dopaminergic transmission in the NAc core, the optogenetic stimulation of VTA neurons projecting to the NAc core concomitant with the presentation of reward cues proved sufficient to turn these cues into incentive stimuli (Saunders *et al.*, 2018).

Sign-tracking has been observed in many vertebrate species including rats and mice (Dickson *et al.*, 2015; Flagel *et al.*, 2009; Tomie *et al*., 2012), monkeys (Bullock and Myers, 2009; Gamzu and Schwam, 1974), humans (Colaizzi *et al.*, 2022; Garofalo and di Pellegrino, 2015; Pithers, 1985; Wilcove and Miller, 1974), raccoons (Breland and Breland, 1961) dogs (Jenkins *et al.*, 1978; Zener, 1937), horses (Miyashita *et al.*, 1999), birds (Burns and Domjan, 1996; Hearst and Jenkins, 1974; Mauldin, 1981; Radevski and Rice, 2022), turtles (Yeh and Powers, 2005) and fish (Nilsson *et al.*, 2008; Scobie, 1977; Waxman and McCleave, 1978), but also in invertebrates such as cephalopods (Cole and Adamo, 2005; Purdy *et al.*, 1999). Attributing pavlovian cues with incentive salience thus seems to be an adaptive behavioural strategy which emerged ubiquitously regardless of the environment organisms are subjected to. Being attracted to stimuli signalling the presence of valuable resources during hunting or foraging might provide an advantage if it brings animals in proximity of said resources – potentially faster if animals are particularly sensitive to cues as is the case for sign-trackers. However, whilst this mechanism may have evolutionary significance, it can also be maladaptive.

Reward cues can promote maladaptive behaviours

Environmental stimuli can influence behaviour in adaptive ways but when irregularities arise in this associative process, such as an excessive attribution of incentive salience, they also have the power to promote maladaptive patterns of behaviours – which is

exacerbated by studies indicating that sign-trackers exhibit behavioural traits and neurobiological characteristics implicated in impulse control disorders. Rats with a propensity to attribute incentive salience to reward cues are more impulsive and make more premature responses than goal-tracking individuals (Flagel *et al.*, 2010; Lovic *et al.*, 2011; Tomie *et al.*, 1998), make suboptimal decision-making on rodent gambling tasks (Swintosky *et al.*, 2021), lack behavioural flexibility and their cue-directed behaviour perseveres instead of being extinguished when the CS is no longer reinforced in a pavlovian setting (Ahrens *et al.*, 2016; Beckman and Chow, 2015; Fitzpatrick *et al.*, 2019a; Gillis and Morrison, 2019; Nasser *et al.*, 2015). Furthermore, relative to goal-trackers, sign-trackers have poor attentional control and have difficulty regulating and maintaining attention over time especially in the presence of distractors (Koshy Cherian *et al.*, 2017; Paolone *et al.*, 2013; Pitchers *et al.*, 2017a; Sarter and Phillips, 2018). This 'bottom-up' attentional bias towards reward-related cues characterising sign-trackers and its associated salience-driven control over behaviour is thought to originate from a reduced level of cholinergic modulation in the prefrontal cortex; in contrast, the intact cholinergic system of goal-trackers might allow them to produce 'top-down' and goal-directed responses (Koshy Cherian *et al.*, 2017; Phillips and Sarter, 2020; Pitchers *et al.*, 2017a). Variations in responses to pavlovian cues and underlying neural processes are therefore believed to contribute to individual vulnerability to several disorders for which cues have significance and in which the aforementioned traits are also abnormal, including addiction, post-traumatic stress disorder (PTSD) and attention deficit/hyperactivity disorder (ADHD), but also schizophrenia and depression.

Substance use disorder (SUD) is a debilitating condition characterised by frequent relapses even after a prolonged period of non-reinforced and deliberate abstinence. For years the notion of 'drive reduction' appeared to fit with observable comportments of substance abusers in that it provided a reasoning as to why excessive and harmful drug-taking persisted despite known detrimental consequences – individuals suffering from this condition simply

needed to alleviate aversive states of withdrawal (Koob and Le Moal, 2001; Solomon and Corbit, 1974). The incentive salience theory and the rupture between 'wanting' and 'liking' offered an alternative explanation, and research now suggests that incentive cues might instead generate motivational states invigorating drug-seeking behaviours (Robinson and Berridge, 1993; Milton and Everitt, 2010; Stewart *et al.*, 1984). Indeed, although numerous factors influence the propensity to develop addiction including the type of drug, the pattern of use and social factors, the reinstatement of compulsive drug use is often triggered by re-exposure to stimuli previously associated with the substance (Everitt, 1997; Franken *et al*., 2004; Janes *et al.*, 2010; Papachristou *et al.*, 2014; Witteman *et al.*, 2015). According to the incentive-sensitisation theory of addiction (Robinson and Berridge, 1993), abuse patterns of substance use occur when the mesolimbic dopaminergic circuit responsible for 'wanting' and motivation becomes sensitised by repeated use of addictive substances, hence resulting in an excessive DA-mediated attribution of incentive salience to substance-related cues and to a persistent hyperresponsivity to said stimuli afterwards. Drugs of abuse highjack and dysregulate adaptive motivational systems processing naturally occurring rewards (Di Chiara *et al*., 1998; Koob and Volkow, 2016; Pitchers *et al.*, 2013; Robinson and Berridge, 1993), potentially acting as by-product 'supernormal stimuli' (Barrett, 2010; Tinbergen, 1951). Studies in rats, human and non-human primates demonstrated that exposure to psychostimulants triggered enhanced dopamine release and dopaminergic sensitisation in the ventral striatum (Boileau *et al.*, 2006; Bradberry *et al.*, 2000; Drevets *et al.*, 2001; Henry and White, 1995; Robinson *et al.*, 1988; Singer *et al.*, 2017), and that dopamine release in this area was associated with the subjective experience of wanting the drug (Leyton *et al.*, 2002) in a way which reflected subjective reports of euphoria (Laruelle *et al.*, 1995; Volkow *et al.*, 1999). Moreover, therapies enhancing dopamine signalling used in Parkinson's disease have been shown to strikingly escalate the propensity to gamble and sexual desire – but not reports of liking – in correlation with the increase in ventral striatal activity

(Averbeck *et al.*, 2014; Weintraub *et al*., 2010). Markedly, despite the prevalent use of recreational drugs or other substances in the population, only a subset of individuals – approximately 20% – lose control over their intake and develop compulsive drug-seeking (Degenhardt and Hall, 2012), a proportion found in non-human animals as well (Belin and Everitt, 2008; Deroche-Gamonet *et al.*, 2004; Pelloux *et al.*, 2007). As with any experience, the development of addiction always occurs in a complex environment composed of multiple discrete and contextual stimuli which have the potential to become pavlovian cues predictive of the drug-taking and, if attributed with incentive salience, to gain motivational control over behaviour in susceptible individuals. Sign-trackers have been shown to prefer psychostimulants over natural rewards when presented with the choice (Tunstall and Kearns, 2015) and exhibited a greater drug sensitisation (Flagel *et al.*, 2008). For sign-trackers, drug-associated incentive CSs induce *conditioned approach* and engagement with related objects (Cunningham and Patel, 2007; Krank *et al.*, 2008; Villaruel and Chaudhri, 2016; Meyer *et al.*, 2012a; Flagel *et al.*, 2009). Drug cues imbued with incentive salience act as *conditioned reinforcers* and therefore promote cue-induced reinstatement of drug-seeking behaviours after extinction to a greater extent in sign-trackers compared to goal-trackers (Peters and De Vries, 2014; Saunders and Robinson, 2010; Yager and Robinson, 2013; but see Kawa *et al.*, 2016 who used an immediate-access schedule of reinforcement and did not observe differences between sign- and goal-trackers). Drug-associated cues can also elicit a *conditioned motivational* state invigorating the rate of self-administration in hyperresponsive individuals (LeBlanc *et al.*, 2012) which has been compared to craving. Drug cues have also been shown to induce stronger emotional responses in sign-trackers, who produce a greater amount of 50-kHz vocalisations when exposed to the element previously paired with cocaine (Meyer *et al.*, 2012b). It is worth noting that whereas approach behaviour towards drug cues increases dopamine in the PFC without impacting acetylcholine in sign-trackers, discrete cues specifically and only enhance cholinergic

signalling in the PFC of goal-trackers (Pitchers *et al.*, 2017b). Moreover, whilst sign-trackers are hypersensitive to discrete localisable cues, contextual cues preferentially exert motivational control over goal-trackers and have the power to elicit drug reinstatement which can be prevented by disrupting dopaminergic transmission in the nucleus accumbens core (Saunders *et al.*, 2014). Relapses might therefore occur through distinct mechanisms depending on individual variation in neurochemistry. Consistent with sign-tracking endophenotype, beyond this apparent difficulty to resist addiction-related cues, human substance use disorder is also associated with impulse control and attentional deficits (Dalley *et al.*, 2011; Jentsch *et al.*, 2014; Jentsch and Taylor, 1999), and a lack of behavioural flexibility illustrated by the persistence of drug-taking despite adverse consequences and the absence of reinforcement (Belin and Everitt, 2008; Deroche-Gamonet *et al*., 2004; Pelloux *et al*., 2007). Together, these elements suggest that the way different individuals respond to discrete and contextual stimuli associated with rewards may have distinct implications for understanding factors contributing to the vulnerability to develop and maintain substance use disorder through relapse. Interestingly, the same mechanisms – individual reactivity to distinct cues, attentional biases, lack of self-regulation and inhibitory control – might have the ability to elicit motivational states leading to problematic usages of the internet or social media because of the multitude of associated auditory and visual cues individuals are subjected to whilst using related devices (Moretta *et al.*, 2022).

Another condition sharing commonalities with sign-tracking is ADHD, an impulse control disorder impairing daily functioning which can feature diminished sustained attention (Barkley, 1997; Bezdjian *et al.*, 2009) particularly when distractors are most salient (Friedman-Hill *et al.*, 2010) and/or impulsivity manifested by a preference for small and immediate – over larger but delayed – rewards (Scheres *et al.*, 2010; Sonuga-Barke *et al.*, 2008). What is more, it is possible that altered motivational processing might be involved in some symptoms of this condition, including the aversion to delayed gratification (Hinshaw,

2018; Luman *et al.*, 2010; Volkow *et al.*, 2011a). Literature suggests that ADHD is characterised by an alteration of mesolimbic dopaminergic signalling, including a hyporesponsiveness of the ventral striatum (Plichta and Scheres, 2014) and a reduced density of D2 receptors in the nucleus accumbens in patients self-reporting a lower motivation (Volkow *et al.*, 2011a). In particular, Furakawa and colleagues found dampened dopaminergic responses in the ventral striatum in response to reward-associated stimuli in individuals with ADHD – in other words, a defective transfer of dopamine release from the reward to the cue (Furukawa *et al.*, 2014). Studies investigating striatal DAT in humans have yielded mixed results, with some experiments revealing a higher density of DAT in individuals with ADHD (Dougherty *et al.*, 1999; Fusar-Poli *et al.*, 2012; Madras *et al.*, 2002) and others showing the opposite (Volkow *et al.*, 2009). Such heterogeneity may be attributable to prior exposure to psychostimulant medication (Fusar-Poli *et al.*, 2012). Similar to sign-trackers, alterations in the cholinergic system might also contribute to the symptomatology of ADHD as suggested by the increased tendency to smoke nicotine in individuals with ADHD (Pomerleau *et al.*, 1995), its apparent lessening of symptoms leading to the development of treatments using the nicotinic cholinergic system to treat ADHD (Levin *et al.*, 1996; Potter *et al.*, 2014) and the potential link between polymorphism in the choline transporter gene and ADHD (English *et al.*, 2009).

Whilst aberrant incentive salience in sign-trackers has mostly been demonstrated for appetitive stimuli, pavlovian cues also have the potential to elicit excessive and/or overgeneralised fear states in PTSD or phobias if they were associated with the initial traumatic event, and individuals for which these cues have motivational – and therefore, emotional – value might manifest stronger responses. In line with this hypothesis, studies have shown that sign-trackers had greater fear responses to cues paired with foot-shocks and that such fear behaviours were persistent and increasing over time as is observed in PTSD (Morrow *et al.*, 2011; Morrow *et al.*, 2015). This suggests that this phenotype might underlie

vulnerability to this disorder. A feature commonly observed in PTSD which is also a main symptom of major depressive disorder (MDD) is anhedonia. Anhedonia, named so due to the apparent lack of pleasure or interest displayed and reported by patients, and the decrease in reward-related behaviours in rodent models, is now believed to sometimes reflect a deficit in incentive motivation processes (Treadway and Zald, 2013; Whitton *et al.*, 2015). Research has indeed shown that when tested in real-time, patients and controls did not differ in their responses of 'liking' to a variety of tastes (Berlin *et al.*, 1998; Dichter *et al.*, 2010; Scinska *et al.*, 2004), indicating that they were still able to enjoy experiences. Recent reports appeared to have corroborated this hypothesis by establishing that stress-induced 'anhedonia' was accompanied by a specific impairment of sign-tracking – therefore, motivational – behaviour, and that this was accompanied by reduced levels of dopamine release in the nucleus accumbens (Fitzpatrick *et al.*, 2019b). Functional abnormalities in the nucleus accumbens – and dysregulation in the dopaminergic transmission arising from the VTA – appear to be involved in 'anhedonia' (Nestler and Carlezon, 2006) as it is hypoactivated by pictures of rewarding stimuli and conditioned stimuli (Keedwell *et al.*, 2005; Kumar, 2008; McCabe *et al.*, 2009 – but see Knutson *et al.*, 2008) in patients suffering from MDD. Researchers also demonstrated that deep brain stimulation of the nucleus accumbens improved symptoms of anhedonia (Schlaepfer *et al.*, 2008). It is possible that alterations in dopaminergic transmission in this area result in an impaired incentive processing of rewarding experiences, thus decreasing the value of appetitive associations, and promoting 'anhedonia'.

Schizophrenia is another disorder featuring 'anhedonia' in which experiments have yet shown that hedonic responses were not impacted in patients compared to controls (Strauss and Gold, 2012), thereby suggesting a specific blunting of incentive reward processing (Treadway and Zald, 2013). Concurrently, and similar to PTSD, psychoses found in this disorder are thought to arise from an aberrant attribution of emotional/motivational

salience to environmental cues (Kapur, 2003) linked to an abnormal dopaminergic signalling in the striatum, which has been proposed to also be involved in the formation of delusions (Heinz and Schlagenhauf, 2010; Howes and Kapur, 2009). Additionally, patients suffering from schizophrenia present cognitive symptoms including impaired sustained attention worsened by the presence of distractors (Demeter *et al.*, 2013; Filbey *et al.*, 2008), which is thought to be caused by a dysfunction of the cholinergic system in the striatum amongst other areas (Gibbons and Dean, 2016; Sarter *et al.*, 2010). Acetylcholine might also contribute to the manifestation of psychoses (Rowntree and Nevin, 1950), but recent research on this matter is lacking.

In most of the aforementioned conditions, individuals share common neurobiological features and exhibit functional abnormalities in incentive salience processing leading to an excessive motivational/emotional responses to stimuli associated with appetitive and aversive experiences. Sign-trackers reflect aspects of this pathophysiology, suggesting that this phenotype might have translational clinical relevance for the acquisition and maintenance of externalising disorders; this is supported by a recent study investigating the link between high impulsivity, addiction-like behaviours, obsessive-compulsive disorder and subjective / physiological responses to reward cues in humans (Schettino *et al.*, 2022).

Sex differences

Neurobiological and symptomatic sexual dimorphisms have been observed in most of the conditions mentioned above. Although men report more drug- and alcohol-related issues (Agabio *et al.*, 2017; Erol and Karpyak, 2015) – mainly due to social and environmental factors (McHugh *et al.*, 2018) – and exhibit stronger neurobiological responses to stimulants and alcohol (Munro *et al.*, 2006; Urban *et al.*, 2010), human and non-human female animals seem more sensitive to substances as well as reward cues and more

likely to develop a disorder after continued use (Agabio *et al.*, 2017; Carroll *et al.*, 2004; Daiwile *et al.*, 2022; Stringfield *et al.*, 2019; Volkow *et al.*, 2011b). In children, boys are more likely to be diagnosed with ADHD than girls – although this disparity fades in adulthood – and the manifestation of symptoms appears to differ between both sexes (Arnett *et al.*, 2015; Hinshaw, 2018). Despite a lower probability to experience traumatic events, women are more likely than men to suffer from PTSD due to a combination of societal factors, genetic predispositions, and hormonal characteristics (Christiansen and Berke, 2020; Tolin and Foa, 2008). Experiments in rat models of PTSD revealed sex differences in the resilience to traumatic events and in how such experiences affected levels of glucocorticoid receptors, which are involved in stress processing (Albrechet-Souza *et al.*, 2020; Keller *et al.*, 2015; Pooley *et al.*, 2018). Schizophrenia affects men earlier in life and to a greater extent than women, and they appear to exhibit distinct sets of symptoms and responses to antipsychotics (Aleman *et al.*, 2003; Leger and Neill, 2016).

Few experiments have included both male and female rodents to study individual differences in incentive salience attribution to reward cues, and those that did found heterogeneous results such as females exhibiting more sign-tracking tendencies than males (Fuentes *et al.*, 2018; King *et al.*, 2016; Madayag *et al.*, 2017), more goal-tracking tendencies (Hughson *et al.*, 2019), or both sexes showing similar conditioned responses (Dickson *et al.*, 2015; Kucinski *et al.*, 2018; Pitchers *et al.*, 2015). Although the literature incorporating male and female individuals is slowly growing, sex must remain a critical variable to consider when studying biological sciences to prevent painting an incomplete picture – which is why both male and female subjects were included in all following experiments.

Aims and summary of experiments

Due to the link between individual differences in the propensity to attribute reward-associated stimuli with incentive salience and the susceptibility to develop and maintain neuropsychiatric conditions, studying sign-tracking might provide a valuable translational tool to predict and screen for individual vulnerability to psychopathologies, enrich transdiagnostic tools, and elaborate personalised interventions and treatments. This thesis presents two distinct aims and is therefore divided into two sections.

Section One

As sign-tracking appears to be a core aberrant behaviour found in several neuropsychiatric disorders, characterising the neurobiological basis of this phenotype is essential to determine which mechanisms mediate the ability of pavlovian cues to drive behaviour and understand why some individuals are more vulnerable than others. Furthermore, such experiments can improve understanding of natural variations in the neurobiology of regular reward seeking as well. It is evident from the literature than dopaminergic transmission in the NAc core is strongly involved in sign-tracking. However, whilst there are extensive descriptions of variations in dopamine-related neurochemistry (*e.g.*, pattern of release, DAT) and in the involvement of specific dopamine receptors, nothing is known about whether individual differences in cue responsivity are associated with specific postsynaptic characteristics in this area despite the modulatory impact that dopaminergic signalling has on the structure of dendritic spines (Yao *et al.*, 2008), on which dopaminergic neurons typically connect. Therefore, the aim of **Section One** was to investigate how postsynaptic changes correspond with variations in the modulation of cue-motivated behaviours in rats.

The current Chapter (**Chapter I**) aimed to provide general background on the following sections.

Chapter II (in Section One) delves into postsynaptic literature in more details and introduces relevant knowledge for this section. In **Chapter III**, the morphology of dendritic spines of the NAc core are first examined in sign-tracking and goal-tracking rats of both sexes using a pavlovian conditioned approach procedure and Golgi staining. Presynaptic and postsynaptic activity inferred through the use of immunostained markers are explored as well. Secondly, results are compared to a control group that did not undertake associative training. Thirdly, a secondary analysis of existing data examining the relationship between the pavlovian training performances and the oestrous cycle is presented. The initial aim of **Chapter IV** was to investigate the postsynaptic mechanisms associated with the modulation of the motivational value of reward cues by interference with its reconsolidation, however technical issues halted the experiment. Chapter IV thus only describes behavioural manipulations. **Chapter V** reviews findings of the Section One.

Section Two

Rodent procedures such as PCA training allow to reliably recognise and characterise variations in appetitive responses to reward-related stimuli. Yet few studies have attempted to develop similar paradigms in humans, and the search for an optimal and standardised procedure is therefore still ongoing. Being able to formally identify sign-trackers in humans might offer a mean to detect behavioural markers of risk profiles to impulse control disorders. The objective of **Section Two** was to design and compare three distinct pavlovian settings to capture sign- and goal-tracking phenotypes in men and women, and relate potential cue-induced motivated behaviours to variations in impulsivity and addiction-related conducts – as has been observed in rodents.

Chapter VI (in Section Two) introduces notions and literature relevant to understanding the goals and techniques of this section. In **Chapter VII** we discuss the use of a computerised pavlovian task and a webcam-based eye-tracker to attempt to categorise

cue-related phenotypes. Impulsivity is measured using self-report and behavioural tasks. **Chapter VIII** considers the use of a virtual pavlovian environment in which participants can navigate in a computerised room to differentiate individual variation in appetitive behaviours. In addition to estimating self-report trait impulsivity, addictive-like behaviours towards drugs and alcohol are assessed as well. **Chapter IX** describes a pavlovian behavioural procedure mimicking the paradigm used in rodents, in which participants can interact with real elements. As with Chapter VII, variations in cue-induced behaviours are subsequently related with self-report trait impulsivity and impulsivity computerised tasks. In **Chapter X**, findings of the Section Two are summarised and discussed.

Finally, **Chapter XI** synthesises the work undertaken in this thesis.

Chapter II
A neurobiological and behavioural analysis of sign-tracking in rats: Introduction and methods

Introduction

Most previous research has focussed on networks and presynaptic regulation underlying sign-tracking behaviour and has demonstrated that dopaminergic neurotransmission in the NAc core is closely involved in the degree to which pavlovian cues become incentive stimuli (Flagel *et al.*, 2011a; Saunders *et al.*, 2012; Singer *et al.*, 2016a; Yager *et al.*, 2015); however, a crucial part of the process remains unknown. As shall be described in Chapter III, inputs from dopaminergic and glutamatergic neurons synapse onto medium spiny neurons in the NAc through dendritic spines whose dynamic structural plasticity is thought to support associative learning (Geinisman *et al.*, 2001; Gipson *et al.*, 2013; Leuner *et al.*, 2003; Matsuzaki *et al.*, 2004; Singer *et al.*, 2009 and 2016). Given that sign- and goal-tracking phenotypes depend on distinct dopaminergic characteristics, it is reasonable to question whether some individuals might be more motivated by reward cues than others because of variations in postsynaptic excitability or because of sex differences – which is what Chapter III aims to unravel. If this hypothesis is substantiated, it might provide a mechanism by which the motivational value of pavlovian cues can be modulated, which is the focus of a preliminary investigation in Chapter IV.

General methods

Oestrous cycle monitoring

Becker and colleagues highlighted the importance of considering the ovarian cycle when working with female rats as behaviour and physiology fluctuate across the cycle (Becker *et al.*, 2005; Steiner *et al.*, 1981), and this requires reliable recognition of the animal's oestrous stage. When in heat, female rats display a specific behaviour involving increased locomotor activity, darting, hopping, freezing, quivering of the ears and lordosis (Long and Evans, 1922), inciting the male to engage in mating. This 'oestrous dance' can be

used as a consistent and non-invasive method to identify the proestrus stage, as a gentle squeeze on the abdomen near the hind legs is sufficient to trigger it (Stramek *et al.*, 2019). In order to predict female's cycles and perform histology during the stage closest to male physiology (*i.e.*, diestrus; see Chapter III, experiment 3), we monitored the 'oestrous dance' daily throughout the 6 weeks during which animals were kept in their home cages before experimentation as well as during training weeks. Because stages evolve in a matter of hours, the lighting schedule was kept regular and undisturbed, behavioural observations were conducted at the same time of day whenever possible, and always in the dark phase during which these nocturnal animals are most active. Additionally, male rats were housed in the vicinity to encourage a natural and regular cycle. The cycle was considered established and regular after two weeks of consistency. Oestrous stage was confirmed by vaginal lavage immediately before perfusion at the end of the experiment. A clean 1mL pipette containing distilled water was gently inserted into the vagina

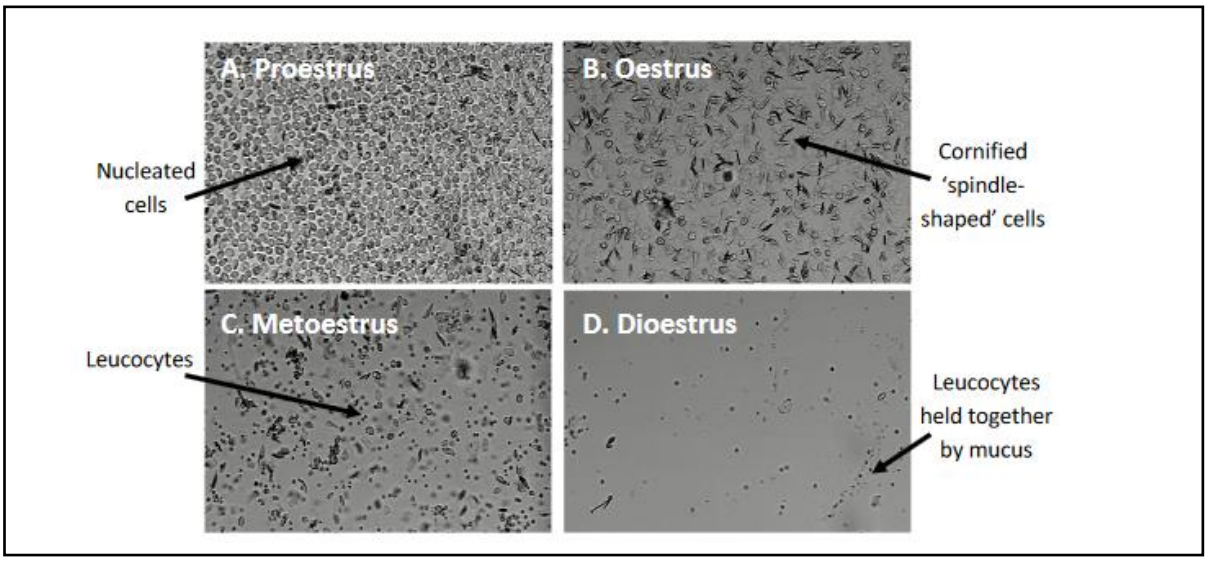


Figure II.1. Representative image of each stage of the oestrous cycle in rats. Magnification x100. *Image utilised with the permission of Stramek et al., 2019.*

of the anaesthetised rat and the smear was visually examined under a microscope using x10 magnification. Proestrus was characterised by very numerous round and fat cells, as opposed

to very few cells of any type in diestrus, or misshapen, needle-shaped cells or leucocytes observed in other stages (Figure II.1; Becker *et al.*, 2005; Stramek *et al.*, 2019).

Apparatus

Behavioural procedures were conducted using modular test chambers (Med Associates) located in sound-attenuating compartments. Chambers (29.53 x 23.5 x 27.31 cm) were composed of a stainless-steel grid floor, a food magazine in the centre of a wall 3 cm to the floor and a house light which remained illuminated throughout all sessions (Figure II.2). Chambers were fitted with infra-red cameras to observe animals during sessions. A retractable and illuminated lever was located 6cm to the floor, and its position was counterbalanced from the left- to the right-hand side of the food magazine between rats. The food dispenser released one food pellet per trial (unflavoured, AIN-76A Rodent Tablet 45mg, TestDiet). Sensors recorded head entries into the food cup and lever deflections. Med Associates software was used to record and quantify these data. Test chambers were cleaned with 70% ethanol before and after each rat, and a tray filled with corncob bedding under the grid floor was changed for each animal. Chambers were specifically attributed to male or female rats in order to avoid odour contamination. When exceptions occurred, chambers were thoroughly cleaned.

Figure II.2. Operant chambers. Food pellets (US) are delivered in the food magazine, and the retractable lever (CS+) is located on the left or right of the magazine.

Pavlovian conditioned approach (PCA)

Food habituation and pre-training. On the first day (D1), a handful of food pellets was placed in homecages in order for the rats to be exposed to the reward. The following day (D2), animals were pre-trained to familiarise themselves with the behavioural chamber. The box remained in the dark for 5 minutes, after which 25 pellets were delivered in a random variable 30-seconds ITI (0-45 seconds range).

PCA training. From day 3 to the end of the procedure, rats were systematically weighed before each daily session to monitor their welfare. For 5 consecutive days (D3-D7), rats were trained in a paired pavlovian setting during which twenty-five 8-seconds extensions and illuminations of the lever (CS+) were immediately followed by the delivery of one food pellet into the food cup (US). Pairings occurred at random variable 90-seconds intervals (30-150 seconds range). A separate cohort was subjected to the same number of lever presentations and food pellet deliveries, however these were not temporally associated (unpaired). These animals therefore acted as non-conditioned controls and allowed us to ensure that the behaviour observed in paired animals was specifically due to the association between the CS+ and the US and that there was no bias in our procedure preventing us from measuring sign- and goal-tracking CRs (Robinson and Flagel, 2009).

Sign- and goal-tracking classification

Animals were categorised into three phenotypes depending on their behaviour during the two last days of pavlovian training, once conditioned responses were established: sign-trackers (STs), goal-trackers (GTs) and intermediate rats. On days 4 and 5 we measured the probability of coming into contact with the lever and with the food magazine during the CS+ extension; the response bias to contact the lever and the food magazine during the CS+ period; and the average latency to first contact the lever and the food magazine during CS+ presentation. These elements were averaged for these two last sessions and used to calculate a global PCA index score ranging between -1.0 – the animal produces a GT conditioned

response on every trial and possesses a strong GT bias – and +1.0 – the animal produces a ST conditioned response on every trial and possesses a strong ST bias (formulae from Meyer *et al.*, 2012a). In order to ensure a sufficient number of animals would be attributed to the 'sign-tracking' and 'goal-tracking' groups, rats with PCA index score ranging from -1.0 to -0.4 were classified as GTs; rats with PCA index score ranging from -0.39 to 0.39 were classified as intermediates; and rats with a PCA index ranging from +0.4 to +1.0 were classified as STs. Only sign- and goal-trackers were analysed in order to compare the neurobiology of more extreme behaviours.

Histology

Tissue preparation. Rats were deeply anaesthetised with isoflurane 5% (Zoetis, US) followed by an intraperitoneal overdose injection of pentobarbitone sodium (0.6-0.8 ml/kg; Animalcare, UK). Animals were then perfused intracardially first with 0.1M phosphate-buffered saline (PBS; pH=7.4) and heparin, then with 3% paraformaldehyde (PFA) and 0.5% glutaraldehyde in 0.1M phosphate buffer (PB; pH=7.4). Fixed brains were collected and stored in 2.5% glutaraldehyde in 0.1M PB at +4°C, then sliced and processed as described in the two staining procedures hereunder.

Golgi-cox staining. Coronal sections of 100 µm were sliced through the nucleus accumbens core using a vibratome (Leica VT1000) and stored in 2.5% glutaraldehyde in 0.1M PB at +4°C. Three to six sections were chosen per animal. The following day, slices were rinsed in 0.1M PB, then processed in 1% osmium tetroxide and 0.2M PB for 40 minutes. They were subsequently left overnight at room temperature in 3.5% potassium dichromate in distilled water. On day 3, slices were mounted onto glass slides and immersed in 1.5% silver nitrate for up to 4 hours, until first signs of neuron staining could be observed under the microscope. The reaction was then stopped with glycerol, slides were enclosed with cover glasses and left overnight at +4°C. On the final day, slides were dehydrated in

ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted onto glass slides using DPX.

Immunohistochemistry. Two antibodies were investigated. Concurrently to slicing sections for the Golgi-cox staining, coronal sections of 50 µm were taken through the nucleus accumbens and stored in cryoprotectant storage solution (sucrose and ethylene glycol in PB 0.1M) at -20°C until staining. On the first day, two to five slices per animal and per antibody (homer1 and synaptophysin, as detailed below) were chosen and were left to warm at room temperature. Sections were then rinsed with 0.1M PB and placed in 1% sodium borohydride for 30 minutes. To block peroxidase, slices were transferred for 5 minutes in 10% methanol and 3% hydrogen peroxide in 0.1M PB. After both previous steps, slices were rinsed with 0.1M PB to remove effervescence. Endogenous biotin then was blocked for 15 minutes using 5% skimmed milk in 0.1M PB, after which slices were rinsed with 0.1M PB. Sections were subsequently moved to a blocking buffer (0.5% Bovine Serum Albumin and 0.3% Triton X-100 in 0.1M PB) for 30 minutes, and then incubated either in 1:100 polyclonal rabbit anti-homer1 antibody (Synaptic Systems, Germany), or in 1:1000 monoclonal mouse anti-synaptophysin antibody (Synaptic Systems, Germany) for 36h at +4°C on a gentle shaker. Two days after, slices were rinsed in 0.1M PB and incubated for two hours in either 1:200 donkey anti-rabbit antibody (Jackson ImmunoResearch, USA) or 1:200 donkey anti-mouse antibody (Jackson ImmunoResearch, USA), then rinsed with 0.1M PB, before being transferred in ABC peroxidase (two drops of solution A and B for every 10 ml of 0.1% Bovine Serum Albumin in 0.1M PB) for 30 minutes. Next, DAB in 30% hydrogen peroxide and 0.1M PB was squirted on the sections until the staining was satisfactorily developed, sections were rinsed with 0.1M PB and left to dry on glass slides for two days. Finally, slices were dehydrated in ascending concentrations of ethanol (30%, 50%, 70%, 90%, 100%), cleared in xylene and mounted onto gelatine slides with DPX.

Anatomical measures

Dendritic spine morphology. Stained brain slices were analysed using a light microscope (Nikon Eclipse 80i upright) and Neurolucida neuron tracing software (MBF Bioscience, Williston, VT, USA). The nucleus accumbens core was determined as the zone ≤ 250 µm around the anterior commissure. From image stacks taken through the z-axis, dendrites of interest were manually outlined whilst delineating the thickness, and dendritic spines were individually traced by adjusting the size of the head and attaching it to the dendrite (Figure III.1-C, Chapter III). In the interests of consistency, only proximal dendrites (Singer *et al.*, 2016c) from at least three different neurons and longer than 30 µm were reconstructed. Data were subsequently imported into Neurolucida Explorer (MBF Bioscience, Williston, VT, USA) for quantitative analysis. From these data we determined spine density, spine length, and spine diameter. All parameters were kept constant throughout images.

Pre- and postsynaptic markers. Stained brain slices were processed using 100x magnification on a light microscope (Olympus BX53) and iVision-Mac (Biovision) in order to take image stacks through the z-axis at 10 random sites within the nucleus accumbens core (determined as the zone ≤ 250 µm around the anterior commissure). ImageJ (Schneider *et al.*, 2012) was then used to merge the image stacks, subtract the background, create a threshold, delimitate merged puncta and display puncta ranging from 10 to 200 µm to exclude potentially remaining artefacts. The 'analyse particles' function allowed us to calculate the number, the average size and the density of puncta, as well as the percentage area covered by the staining (Figure II.3). All parameters were kept constant throughout images.

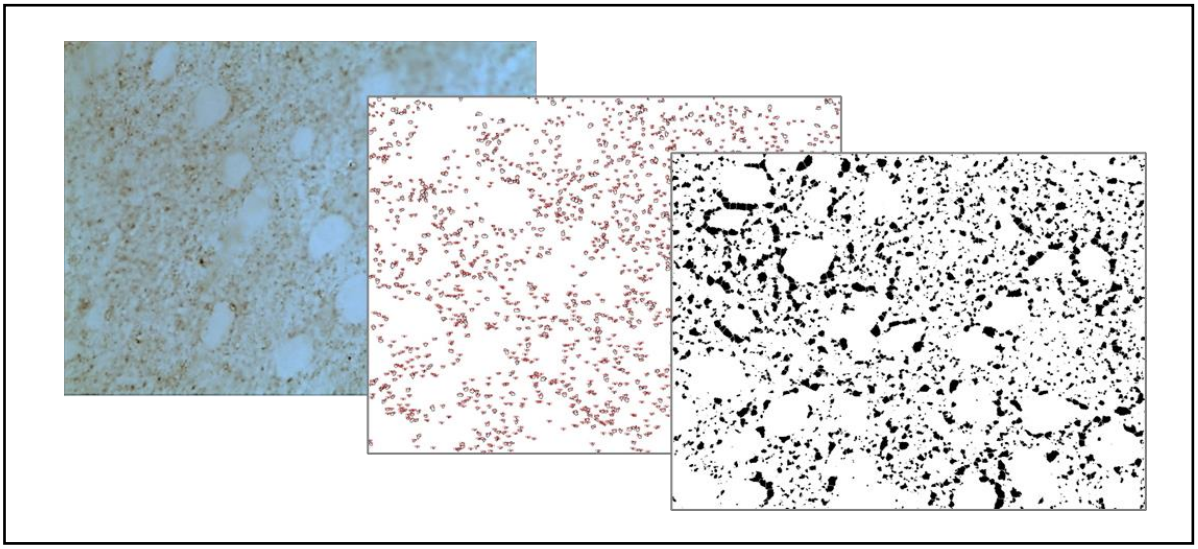


Figure II.3. Example of synaptophysin stained tissue in the NAc core. Images are merged through the z-axis and thresholded, puncta exceeding 200 µm are excluded, and the remaining puncta are quantified.

Statistical analysis

GraphPad Prism (versions 8 and 9; GraphPad Software Inc.; San Diego, CA, USA) was used for ANOVAs, correlations, independent group comparisons, and to identify outliers. Chi-square analyses were conducted on SPSS (versions 27 and 28; IBM Corp.; Chicago, IL, USA). All group comparison results are presented as mean + SEM. Statistical significance was set at 0.05. Measures were all checked for normality using the Shapiro-Wilk test, and non-parametric tests were used when appropriate. Phenotypic repartition between males and females was compared using the Chi-square test of independence. Repeated measures such as behaviour during PCA training sessions were analysed using two-way RM ANOVA. Relevant significant interactions and main effects were followed by post-hoc Šídák multiple comparison's correction tests in order to assess whether sex differences were present at the end of the training or whether they were removed by learning. Post-hoc Dunnett tests were also performed to compare the evolution of behaviour throughout sessions. Comparison of the neurobiology between paired and unpaired rats, P30 and P360 groups, propranolol and saline in RET vs. NORET groups were analysed using two-way ANOVA. Significant ANOVAs were followed by Šídák tests to dissect the effect

of sex, or Tukey tests to examine the effects of phenotype, treatment or retrieval. The effect of the oestrous cycle on behaviour was also assessed with two-way ANOVAs because animals in each groups were different depending on the PCA session – repeated measures were absent. When possible, violations of statistical assumption were delt with using log10 transformations. Comparisons between two independent groups such as interactions with the lever during the last test session between NR and R groups or the effect of propranolol on the PCA score during the test session were investigated using the parametric independent *t*-test, or the non-parametric Mann-Whitney test. All correlations were analysed using Pearson's correlations when the dataset met normality assumptions, or Spearman's correlations when it did not. A Bonferroni correction was applied after multiple correlations to reduce the risk of type I error. The ROUT method was applied to all groups to identify outliers with the false discovery rate set at 1%. All figures were produced on GraphPad Prism.

Chapter III
Synaptic plasticity in the nucleus accumbens core: Effect of sex, reward and oestrous cycle

Experiment 1. Conditioned behaviour in male and female rats: effect on synaptic plasticity

Introduction

Learnt behaviours are stabilised into memory traces through consolidation, a process often involving synaptic plasticity and long-term potentiation (LTP). LTP is a mechanism leading to a long-lasting increase in the efficiency of synaptic transmission (Muller *et al.*, 2000). Excitatory synapses are mainly found on dendritic spines, small protrusions located on dendrites of several neuron populations including MSNs, pyramidal cells and Purkinje cells on which they were first described in 1888 by Santiago Ramón y Cajal (Cajal, 1888). As early as 1949, Donald Hebb postulated that the repetitive activation of a presynaptic neuron with a postsynaptic neuron led to a growth of '*synaptic knobs*', thus to an increase in synaptic space area between both neurons, and ultimately a strengthening of their association and facilitation of their communication (Hebb, 1949). Research has since demonstrated that LTP and behavioural learning both result in structural changes and the formation of synapses on dendritic spines (Lamprecht and LeDoux, 2004; Matsuzaki *et al.*, 2004; Medvedev *et al.*, 2014). Dendritic spines are highly dynamic; they can be formed, retract, extend or expand in response to external (*e.g.*, environmental) and internal (*e.g.*, neuronal) activity (Arellano *et al*., 2007; Butz *et al.*, 2009; Jung and Herms, 2014). This structural plasticity is dependent upon protein synthesis, necessary to turn immature thin spines into mature, more developed synapses, and stimulate the formation of new spines (Bramham, 2008; Sala and Segal, 2014). Because most synapses are located on dendritic spines, thereby making the latter predominant targets of activity-dependent neuroplasticity, both appetitive and aversive associative conditioning are associated with structural reorganisation (Geinisman *et al.*,

2001; Leuner *et al.*, 2003) including in the NAc core (Gipson *et al.*, 2013; Singer *et al.*, 2009) which has wider dendritic trees and more spine density than the shell (Meredith *et al.*, 1995). Moreover, environmental stimuli paired with rewards can also induce morphological reorganisations of dendritic spines in the NAc (Singer *et al.*, 2016c).

The role of dopamine in the consolidation of pavlovian learning (Dalley *et al.*, 2005) and in the attribution of incentive salience to reward cues (Flagel *et al.*, 2011a; Saunders *et al.*, 2012; Singer *et al.*, 2016a) is well established. Other studies have shown that dopamine was also involved in modulating structural plasticity (Yao *et al.*, 2008). Indeed, artificially increasing dopamine levels through *in vivo* amphetamine injections (Robinson and Kolb, 1999; Singer *et al.*, 2009) or *in vitro* activation of dopamine receptors (Fasano *et al.*, 2013) increased spine density, branching and length in MSNs, whereas dopamine inhibition using animal models of Parkinson's Disease (Villalba *et al.*, 2009), chemical dopamine depletion (Meredith *et al.*, 1995; Wang and Deutch, 2008) or *in vitro* blockade of dopamine receptors (Fasano *et al.*, 2013) led to a decrease in spine length and density. Additionally, cue-induced reinstatement of drug-seeking has been shown to result in synaptic alterations in the NAc core (Gipson *et al.*, 2013). Considering the aforementioned elements together with differences in dopaminergic signalling observed between sign- and goal-trackers, it is conceivable that changes in dendritic spines within this area could reflect the incentive value of reward cues instead of the simple predictive aspect.

Experiment 1 examined baseline and cue-induced postsynaptic structural characteristics, presynaptic and postsynaptic activity associated with variations in cue-induced motivation in male and female rats in the core of the NAc (Figure III.1.1). This was first achieved using Golgi-cox staining to visualise and reconstruct dendritic spines after rats undertook a pavlovian procedure to discriminate sign- and goal-trackers learning biases (Flagel *et al.*, 2009). Synaptophysin, a protein involved in the regulation of synaptic vesicles, and Homer1, a core component of the postsynaptic density (PSD) that participates in the

growth of dendritic spines, were used as indicators of pre- and postsynaptic activity, respectively. In this experiment, the specific influence of the predictive cue on spine plasticity was observed by presenting the animals with said cue in the absence of reward in an ultimate session. Behavioural performances and the effect of sex on behaviour and synaptic plasticity were also considered.

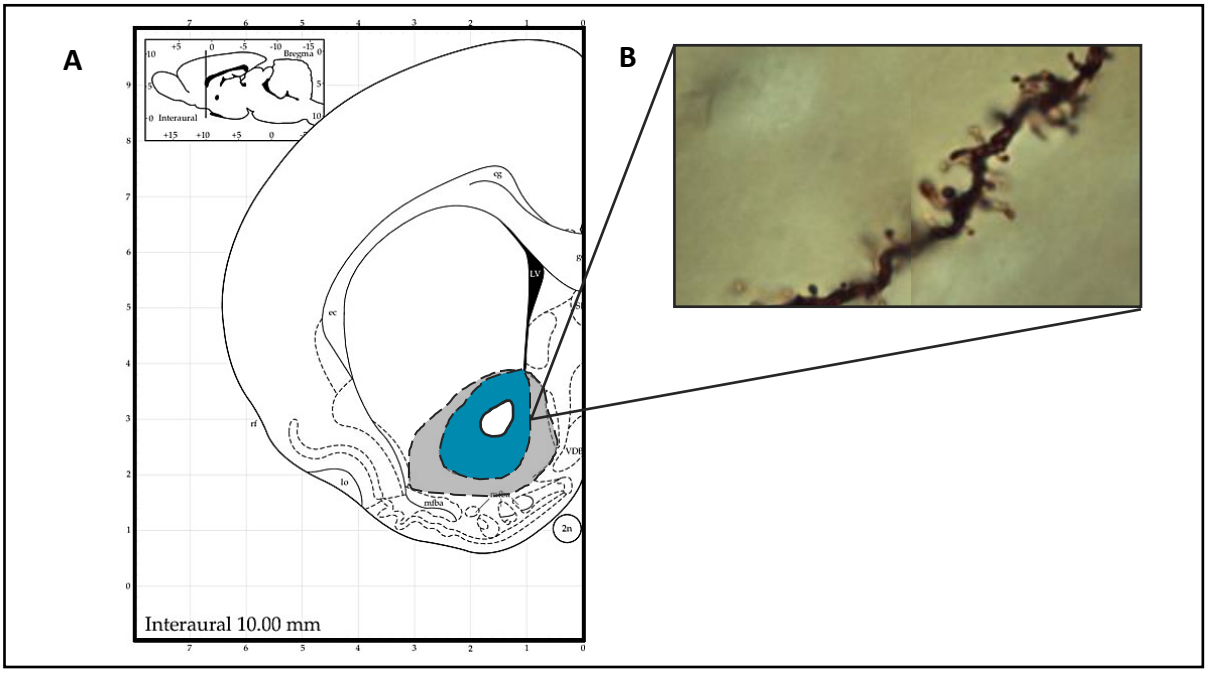


Figure III.1.1. Dendritic spines in the NAc core. (A) Coronal view of the NAc core in blue, and NAc shell in grey, in the ventral striatum. *Image modified from the Atlas of Paxinos and Watson, 2006*. (B) Photograph of a NAc core dendrite analysed in the present thesis.

Materials and methods

Animals

Male (n=32) and female (n=27) Lister Hooded rats (outbred; Charles River, Kent, UK) aged from four to six weeks upon arrival (males 88-157g, average 122g; females 72-112g, average 93g) were housed in same-sex groups of 3 rats. The housing room was set on a reverse light and dark cycle of 14h-light and 10h-dark (dark at 08:00). All procedures were performed during the dark phase, whilst these nocturnal animals are naturally active. Ventilated cabinets containing the homecages were maintained at 21-23°C, and water and

food were available *ad libitum*. Animals were gently handled regularly for six weeks prior to experiments to reduce the stress of manipulation. They were tested when they reached adulthood, between 11 and 14 weeks of age (males 337-513g, average 405g; females 214-369g, average 245g). All procedures were approved by Institutional Ethical Review Committee at the Open University (The Animal Welfare and Ethics Research Board; PPL numbers 70/7995 and PABC1F4D1) and were carried out in accordance with the Animals [Scientific Procedures] Act (1986) and EU Directive 86/609/EEC.

Non-rewarded cue re-exposure

After habituation and pre-training (D1 and D2), animals undertook pavlovian training or unpaired training (D3 to D7). During a final test day (D8), which occurred from 2 to 4 days after training in order to target the diestrus cycle of each individual female, animals from the paired group (females n = 24, males n = 21) and the unpaired group (females n = 6, males n = 6) were re-exposed to the discrete cue in the absence of reward (Figure III.1.2). After a contextual extinction of 5 minutes in the chamber to minimise contextual cues, the lever was extended 10 times for 4 seconds in a random variable 90-seconds ITI (30-150 seconds range). No food pellets were delivered following the lever retraction.

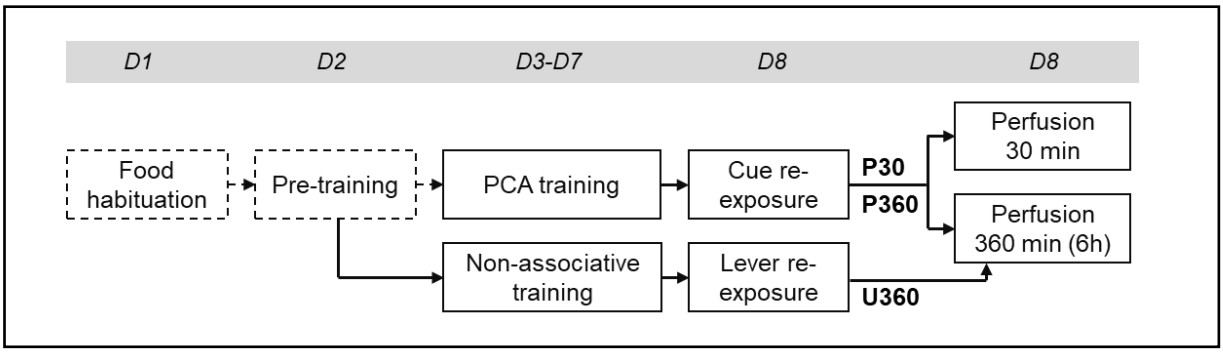


Figure III.1.2. Outline of the procedure used in Chapter III, Experiment 1.

Histology

In order to separate baseline spine plasticity from synaptic changes evoked by the predictive cue, a subset of paired animals (**P30**: females n=14, males=11) was anaesthetised and perfused 30 minutes after the end of the re-exposure session (D8). The remaining paired animals (**P360**: females n=10, males n=10) and all unpaired rats (**U360**: females n=6, males n=6) were returned to homecages after the re-exposure session and left undisturbed for 360 minutes (6 hours) before being perfused to observe synaptic plasticity induced by the behavioural procedure. Brains were subsequently processed as described in the Histology section of Chapter II.

Results

PCA training: behavioural phenotypes and the effects of sex and pairing

Paired animals were first trained in a pavlovian conditioning and their behavioural bias towards the predictive cue and the food magazine was monitored. Male and female rats showed similar individual variation in the PCA training (Figure III.1.3-A; $\chi^2 = 0.027$, df = 2, p = 0.987). A high majority was classified as sign-trackers (77.18% on average), whereas only an average of 8.92% of rats (2 females and 2 males) were categorised as goal-trackers. 13.39% of rats displayed no preference for the lever or for the food magazine ('intermediate'); these animals were not included in our analyses as we sought to compare the neurobiology of more radical phenotypes. In accordance with the aforementioned classification, the distribution of PCA scores across the five conditioning sessions shows a shift from the central range of the score (-0.5 and 0.5) to the highest range for both males and females and illustrates the formation of one main sign-tracking population (Figure III.1.3-B). It is interesting to note that the final propensity emerges earlier in males, for which the sign-tracking tendency can already be observed on day 2. When examining the PCA index score (Figure III.1.3-C), no sex difference was found in sign-trackers ($F_{1, 43} = 2.318$, p

= 0.1352). Unpaired rats diverged in the middle of the training (main effect of sex, $F_{1, 9}$ = 8.204, p = 0.0187; session 3, p = 0.0100), but male and female performed in a comparable manner at the end of the procedure.

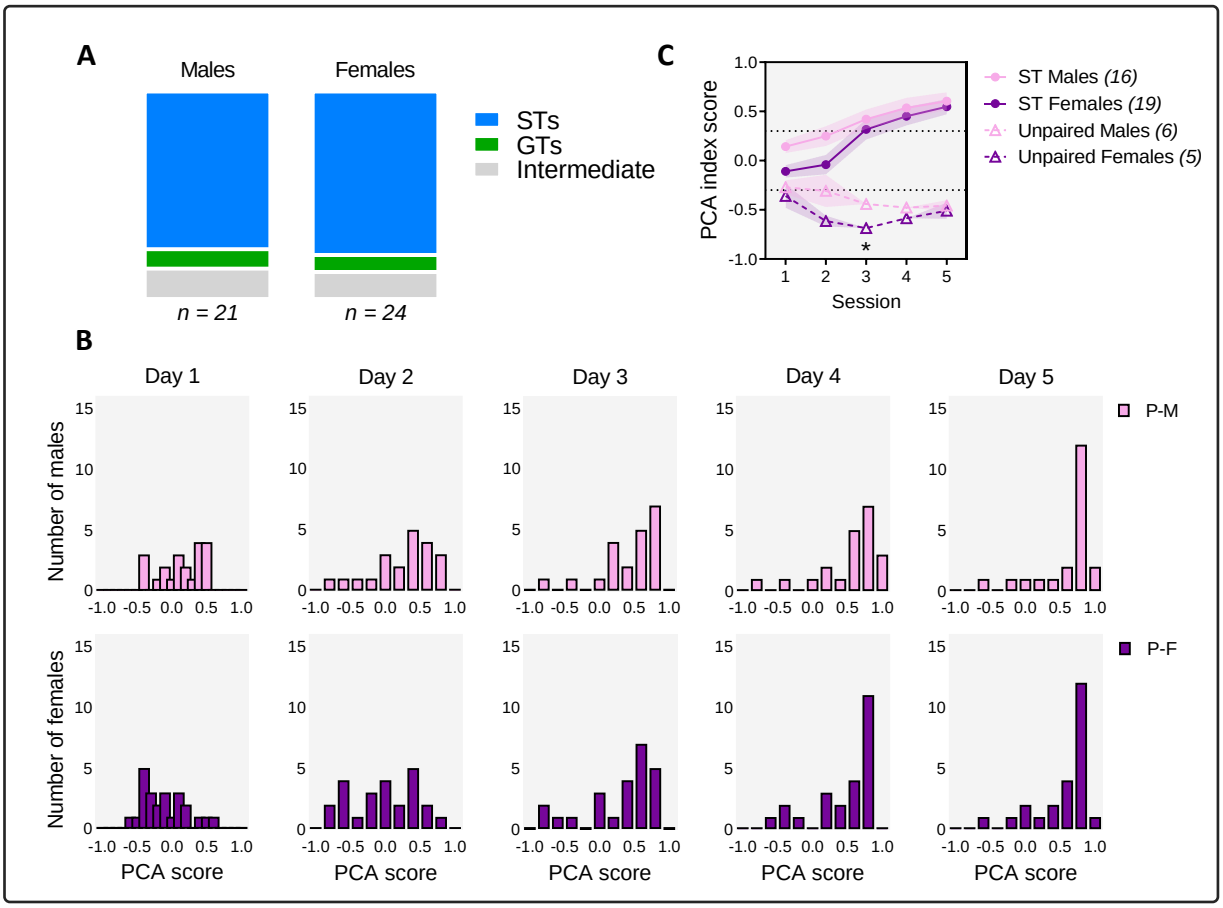


Figure III.1.3. Pavlovian phenotypic repartition of Experiment 1 and evolution across training. (A) Repartition of sign-trackers, goal-trackers and intermediate male and female rats from the paired group. (B) Distribution of the PCA score for each training session in male (P-M) and female (P-F) paired rats. (C) Evolution of the PCA index score across the five sessions of PCA training in male and female sign-trackers.

STs and GTs developed very distinct conditioned responses (Figure III.1.4-A). STs contacted the lever increasingly and faster across conditioning sessions, and quickly discontinued their interaction with the food-cup. Conversely, GTs interacted more with the food magazine from the second session and did not manipulate the lever as much as STs. It is worth noting that on the fifth session, GTs contacted the lever faster than during previous sessions and accordingly increased their probability to contact the lever. As opposed to these

two phenotypes, unpaired animals who were shown dissociated lever and food presentations did not learn and develop a specific CR across sessions, as evidenced by the stable interaction with the lever and the food magazine (Figure III.1.4-A). Figure III.1.4-B shows the evolution

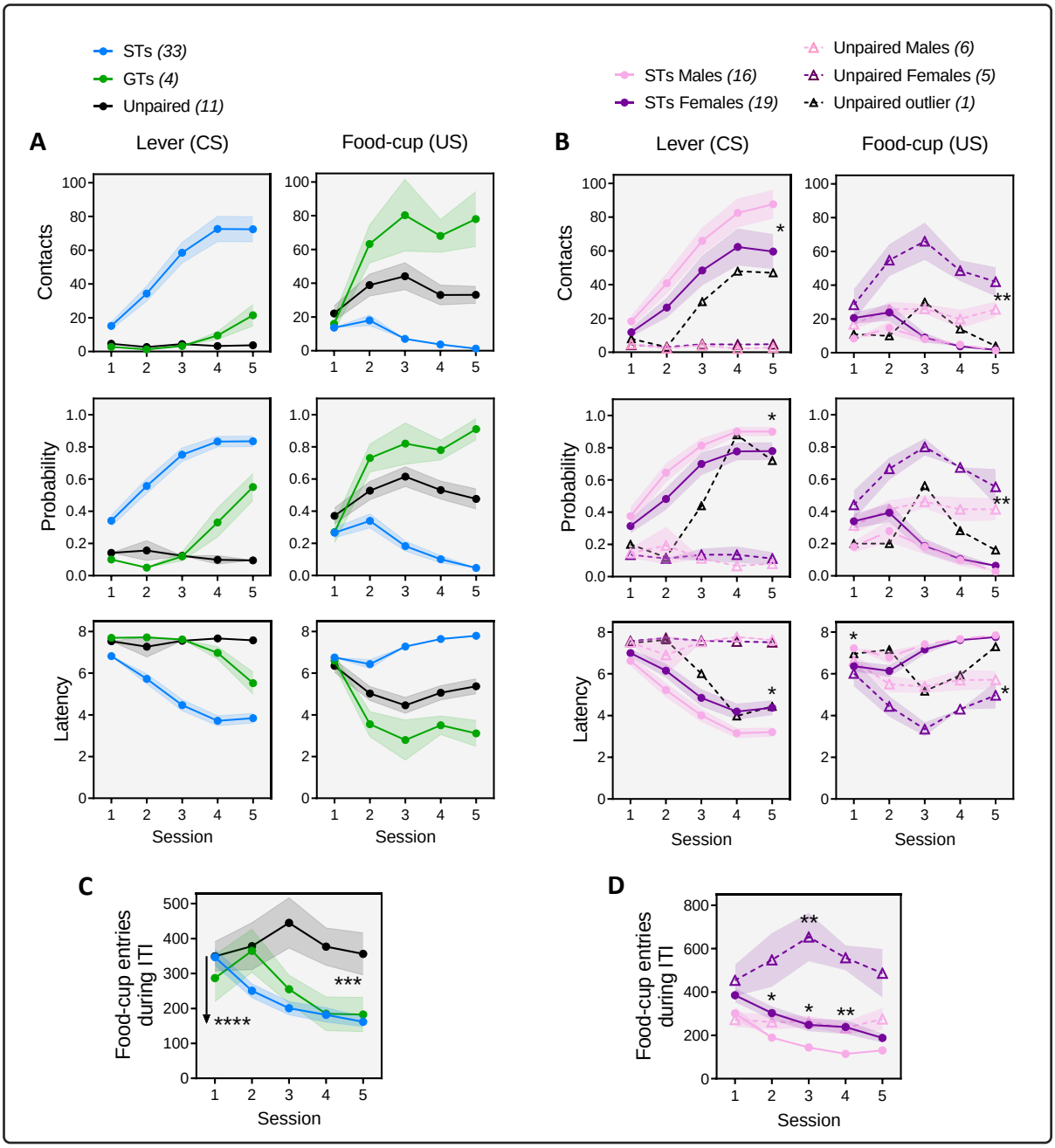


Figure III.1.4. Interaction with the CS+ and the US during training. (A) Comparison between sign-trackers, goal-trackers and unpaired rats. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation. Males and females combined. (B) Sex comparison between male and female sign-trackers, then between male and female unpaired rats, and contrast with a female unpaired outlier. Effect of sex. (C) Food-cup entries during the inter-trial interval of sign-trackers, goal-trackers and unpaired rats of both sexes. Comparison between phenotypes and evolution across sessions. (D) Sex comparison of food-cup entries during the inter-trial interval between male and female sign-trackers, then between male and female unpaired rats. Effect of sex for each session. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$).

of conditioned responses across between male and female rats. Two-way repeated measure ANOVAs with sex and phenotype as between-subjects factors and session as a within-subject factor showed a main effect of sex in sign-trackers for the number of contacts with the lever ($F_{1, 33} = 4.176$, $p = 0.0490$), the latency to first approach the lever ($F_{1, 33} = 6.244$, $p = 0.0176$) and the food-cup ($F_{1, 33} = 4.959$, $p = 0.0329$), and the probability to contact the lever ($F_{1, 33} = 4.284$, $p = 0.0464$). An interaction sex x session was also found for food-cup contacts ($F_{4, 132} = 3.290$, $p = 0.0132$). Contrary to the sex difference observed in food-cup latency which was only present at the beginning of the training (Šídák session 1, $p = 0.0282$), the differences in lever probability (Šídák session 5, $p = 0.0139$) and lever latency (Šídák session 5, $p = 0.0380$) were found at the end of the training. Unpaired males and females displayed similar lever-directed behaviours (all $F_{1,9} < 0.8198$, all $p > 0.3888$); however, one female displayed a different profile of responses resembling those of conditioned female STs. This female was identified as an outlier using the ROUT method and removed from subsequent analyses. Two-way repeated measures ANOVA revealed an effect of sex in food-cup contacts ($F_{1, 9} = 49.75$, $p < 0.0001$), latency ($F_{1, 9} = 9.824$, $p = 0.0120$) and probability ($F_{1, 9} = 18.29$, $p = 0.0910$) of unpaired rats. Post-hoc tests showed that these differences were neither found at the beginning nor at the end of the training, but in intermediate sessions (food-cup contacts: Šídák session 4, $p = 0.0340$; food-cup latency: Šídák session 3, $p = 0.0067$; food-cup probability: Šídák session 3, $p = 0.0044$).

Another element of the pavlovian training that can be studied is the number of visits to the food cup outside of lever presentation. This is sometimes considered as an indication of the animal's rate of learning in that it should decrease as the animal learns that food is delivered in the magazine only after lever retraction, and it can also suggest general activity level. Figure III.1.4-C illustrates the number of visits to the food-cup between sign-trackers, goal-trackers and unpaired rats across training sessions. A two-way repeated measures ANOVA with phenotype as a between-subjects factor and session as a within-subject factor

revealed a main effect of the phenotype (Figure III.1.4-C: $F_{2, 48} = 8.127$, $p = 0.0009$) as well as an interaction phenotype x session ($F_{8, 192} = 4.974$, $p < 0.0001$). When comparing the last training session to the first, a Dunnett's multiple comparison test showed that sign-trackers visited the food magazine significantly less as the association was learnt ($p < 0.0001$). A similar decreasing tendency can be observed in goal-trackers but is not significant ($p = 0.5566$); this might be due to the low number of animals. Conversely, unpaired animals who did not develop any conditioned response interacted with the food magazine outside of lever presentation in a stable manner throughout sessions (session 1 vs. 5, $p = 0.9978$). A two-way repeated measures ANOVA with sex and phenotype as between-subjects factors and session as a within-subject factor found an effect of sex in sign-trackers (Figure III.1.4-D: $F_{1, 33} = 12.60$, $p = 0.0012$) with females visiting the food magazine more than males during conditioning sessions 2, 3 and 4 (Šídák: $p = 0.0127$, $p = 0.0177$, $p = 0.0064$). Similarly, unpaired females visited the food-cup significantly more than males in the middle of the training (effect of sex: $F_{1, 9} = 21.20$, $p = 0.0013$). All animals behaved in a comparable way during the last training session.

Cue re-exposure: effects of sex and pairing

Two to four days after the last training session, rats underwent a single session during which they were re-exposed to the lever in the absence of reward. It is worth noting that the re-exposure session is not directly comparable to training sessions because it was composed of 10 trials as opposed to 25 in order to prevent extinction, and the lever was extended for 4 seconds instead of 8 seconds. A significant positive relationship was found in paired animals between the last training session and the test session for the number of contacts with the lever (Figure III.1.5; Pearson $r = 0.5447$, $p = 0.0001$) and the latency to first contact the lever (Pearson $r = 0.5806$, $p < 0.0001$) – but not lever probability (Spearman $r = 0.2931$, $p = 0.0507$). These results survived Bonferroni correction for multiple correlations. Figure III.1.5

also indicates that no relationship was found in lever-directed behaviours between the last training session and the re-exposure session in unpaired rats (all Spearman r > 0.09770, all p > 0.4427). The correlation between CRs expressed during the re-exposure session and the last training session enabled us to use the former reliably to classify our animals into groups and perform our future analyses.

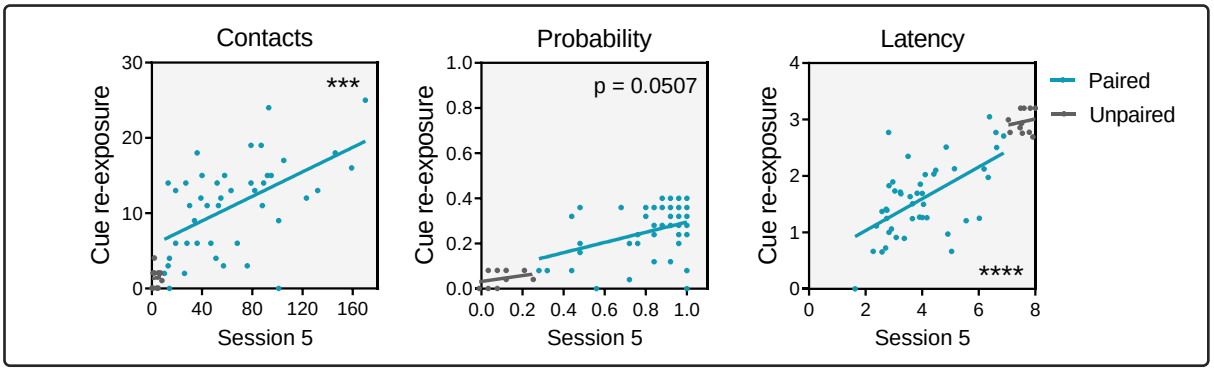


Figure III.1.5. Correlation between lever-directed conditioned responses of the last training session and the cue re-exposure. Number of contacts with the lever during CS presentation, probability to contact the lever during CS presentation, latency to first contact the lever during CS presentation. (*** p ≤ 0.001, **** p < 0.0001).

Spine morphology: effects of time, sex and pairing

Neurons from the nucleus accumbens core of paired and unpaired groups were stained using Golgi-cox staining. Dendritic spines were reconstructed and spine density, spine head diameter and spine length were quantified. One female STs from the P30 group was excluded from neurobiological analyses due to brain slices not being successfully fixed. The unpaired female outlier was excluded as well. A total of 259 (paired) and 65 (unpaired) first-order dendrites from at least three different neurons for each rat located in random locations in the nucleus accumbens core were reconstructed (4 to 7 per rat; average diameter = 0.97 µm; length ranging from 31.9 to 181.1 µm, average length = 75.35 µm). A two-way ANOVA comparing spine properties between P30 and P360 male and female rats shows that ST rats whose brains were fixed 30 minutes after cue re-exposure did not differ from rats perfused 360 minutes (6 hours) after re-exposure in their density of spines ($F_{1, 30}$ = 1. 093, p

= 0. 3041) or average diameter ($F_{1, 30}$ = 0.07029, p = 0.7927) in the nucleus accumbens (Figure III.1.6-A), however a difference was found in the length of spines ($F_{1, 30}$ = 5.256, p = 0.0291). No differences were observed in perfusion time between males and females (all $F_{1, 30}$ < 1.824, all p > 0.1870). GTs were not analysed as only one animal was allocated to the P30 group. Even though rats displayed similar spine properties regardless of the time they spent after the re-exposure session, only the P360 group is examined in subsequent sections as the conditions rats were exposed to and therefore resulting plasticity mechanisms were deemed to be too different.

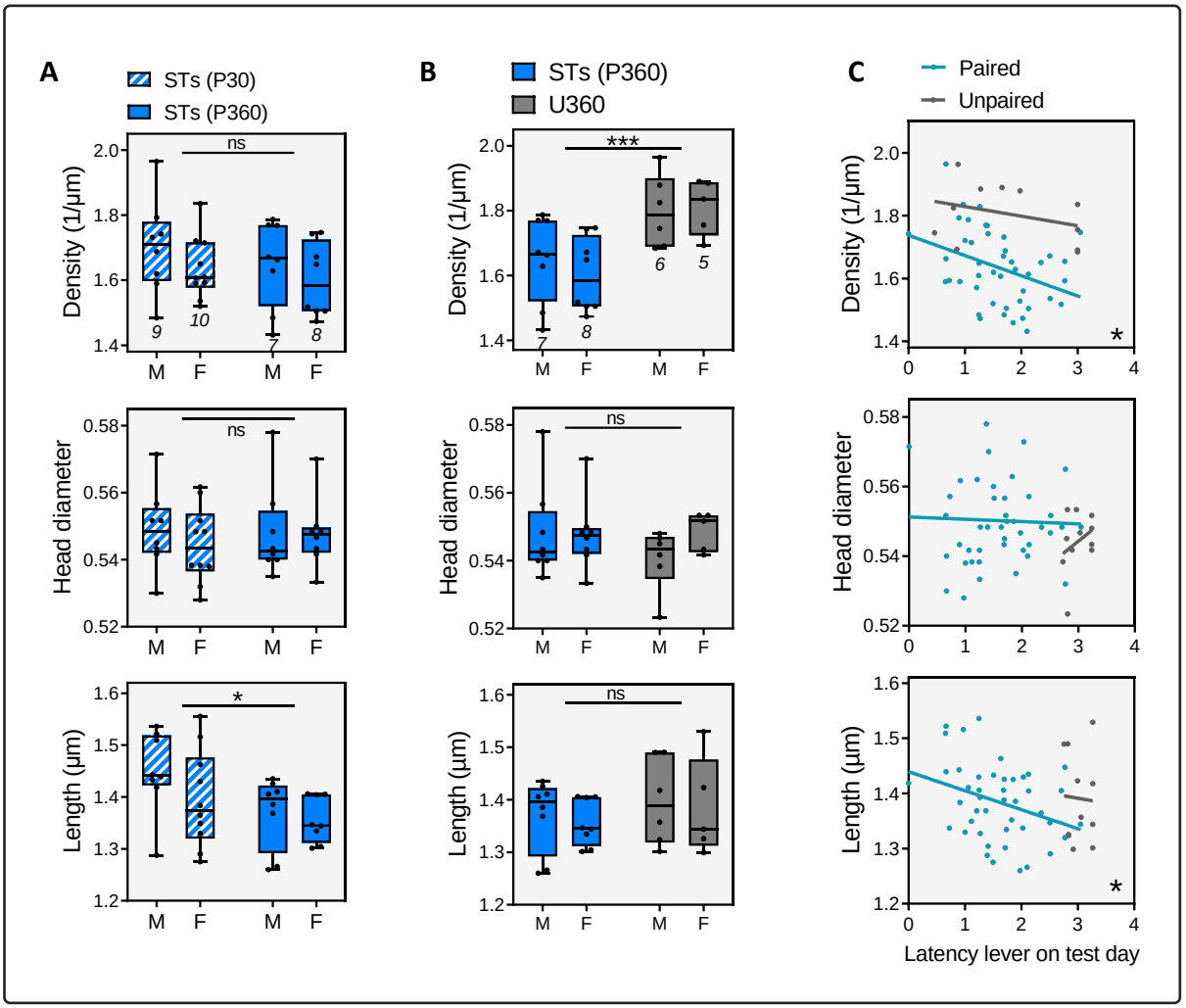


Figure III.1.6. Dendritic spine morphology in the NAc core. (A) Density of spines, average spine head diameter and average length of spines in male and female sign-trackers perfused 30 minutes and 360 minutes after cue re-exposure. (B) Comparison of the density of spines, average spine head diameter and average length of spines between male and female sign-trackers perfused 360 minutes after cue re-exposure, and male and female unpaired rats perfused 360 minutes after cue re-exposure. Effect of conditioning. (C) Correlation analysis between the latency to approach the CS during the re-exposure and the density, diameter and length of spines, in all paired rats and all unpaired rats. (* p ≤ 0.05, *** p ≤ 0.001).

Next, paired STs were compared to the unpaired group who did not undergo pavlovian conditioning (Figure III.1.6-B). A two-way ANOVA revealed an effect of the training ($F_{1, 23}$ = 15.67, p = 0.0006) wherein animals who learnt the association had a significantly smaller density of spines in the nucleus accumbens core. No difference was found between both groups in spine length ($F_{1, 23}$ = 1.009, p = 0.3256) or average spine head diameter ($F_{1, 23}$ = 0.5805, p = 0.4539), and no sex difference was observed (all $F_{1, 23}$ < 0.1510, all p > 0.3520). When combining all conditioned animals regardless of the time left before perfusion and the sex, rats who contacted the lever faster during the re-exposure session had a higher density of spines (Pearson r = -0.3596, p = 0.0165) and longer spines (Pearson r = -0.3325, p = 0.0275), but not larger heads (Pearson r = -0.03393, p = 0.8269), in the nucleus accumbens core (Figure III.1.6-C). The significantly higher density of spines was also found after Bonferroni correction for multiple correlations, however the difference in spine length did not survive the correction. No relationship was observed in unpaired rats who did not undertake conditioning between the rapidity to first contact the lever and spine density (Pearson r = -0.3288, p = 0.3236), diameter (Pearson r = 0.3142, p = 0.3467) and length (Figure III.1.6-C; Pearson r = -0.04309, p = 0.8999).

Plasticity mechanisms: effects of time, sex and pairing

To investigate the relationship between pavlovian bias and synaptic mechanisms within the nucleus accumbens core, synaptophysin and homer1 puncta were examined as a measure of pre- and post-synaptic activity, respectively. In addition to excluding the P30 female and the unpaired female, a P30 male and a P360 female were removed from the synaptophysin analysis due to brain slices being damaged during the staining procedure.

Synaptophysin: A two-way ANOVA revealed no effect of the time of perfusion in the number of puncta ($F_{1, 29}$ = 1.317, p = 0.2605), the puncta size ($F_{1, 29}$ = 0.04096, p = 0.8410), the percentage area covered by puncta ($F_{1, 29}$ = 1.202, p = 0.2819) or the density of

puncta ($F_{1, 29} = 1.178$, $p = 0.2867$). Within the P360 group (Figure III.1.7-B), no relationship was found between the speed at which animals contacted the lever during the re-exposure session synaptophysin staining in males (all Pearson $r > -0.4362$, all $p > 0.0952$) or females (all Pearson $r > -0.5793$, all $p > 0.1021$). The comparison of synaptophysin staining between STs and unpaired animals revealed sex and training differences (Figure III.1.7-C). More

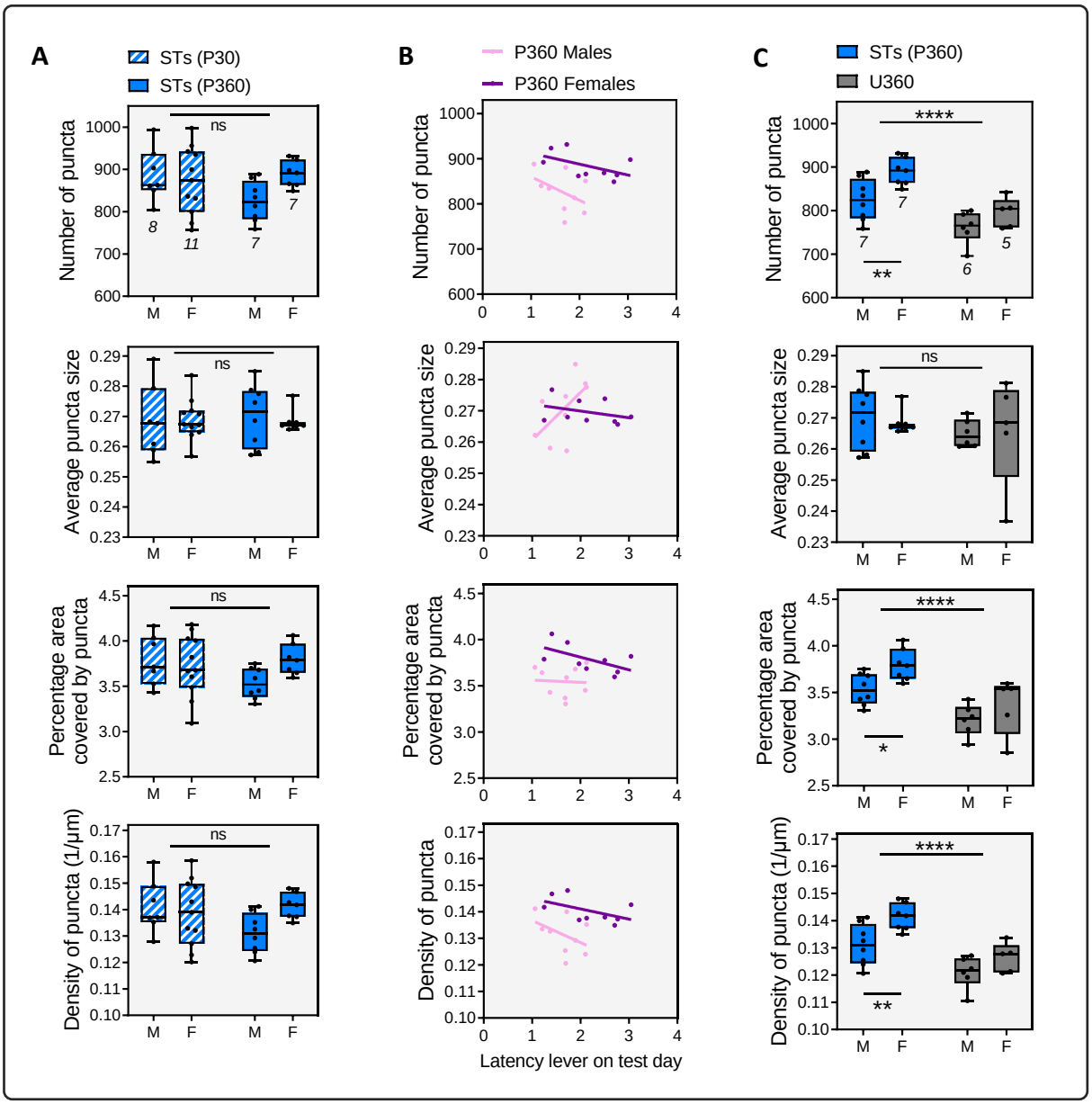


Figure III.1.7. Synaptophysin staining in the NAc core. (A) Number of synaptophysin puncta, average puncta size, percentage area covered by synaptophysin puncta, and density of puncta of male and female sign-trackers perfused 30 minutes after cue re-exposure and 360 minutes after cue re-exposure. (B) Relationship between the latency to first contact the CS during cue re-exposure and synaptophysin staining in male and female paired rats. (C) Comparison of synaptophysin staining between male and female sign-trackers perfused 360 minutes after cue re-exposure and male and female unpaired rats also perfused 360 minutes after cue re-exposure. Effect of sex and effect of conditioning. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

specifically, STs had significantly more synaptophysin puncta (effect of training: $F_{1, 22}$ = 25.96, p <0.0001), a greater density (effect of training, $F_{1, 22}$ = 25.95, p <0.0001) and larger area covered by puncta (effect of training, $F_{1, 22}$ = 22.57, p <0.0001) than unpaired animals. The size of synaptophysin puncta was similar between both groups (effect of training: $F_{1, 21}$ = 0.2893, p = 0.5963). Congruent with previous results, synaptophysin puncta of female STs were more numerous (effect of sex, $F_{1, 22}$ = 10.12, p = 0.0043; Šídák, p = 0.0079), covered a wider percentage area (effect of sex, $F_{1, 22}$ = 6.563, p = 0.0178; Šídák, p = 0.0408), and were denser (effect of sex, $F_{1, 22}$ = 10.13, p = 0.0043; Šídák, p = 0.0078) than those of male STs.

Homer1: Homer1 staining in the nucleus accumbens core of STs was comparable after both times of perfusion for the number of puncta (Figure III.1.8-A; $F_{1, 30}$ = 0.2002, p = 0.6577), the puncta size ($F_{1, 30}$ = 0.06002, p = 0.8081), the percentage area covered by puncta ($F_{1, 30}$ = 0.073, p = 0.3084), and the density of puncta ($F_{1, 30}$ = 0.2003, p = 0.6577). No relationship was found between animal's behaviour during the PCA task and Homer1 staining puncta (Figure III.1.8-B; all Pearson r < -0.5024, all p > 0.1389). A two-way ANOVA comparing homer1 staining between STs and unpaired rats who did not undergo training did not reveal any difference in the number ($F_{1, 23}$ = 1.610, p = 0.2172), the size ($F_{1, 23}$ = 1.469, p = 0.2390), the area covered by ($F_{1, 23}$ = 2.737, p = 0.1129) or the density of ($F_{, 23}$ = 1.610, p = 0.2172) puncta (Figure III.1.8-C).

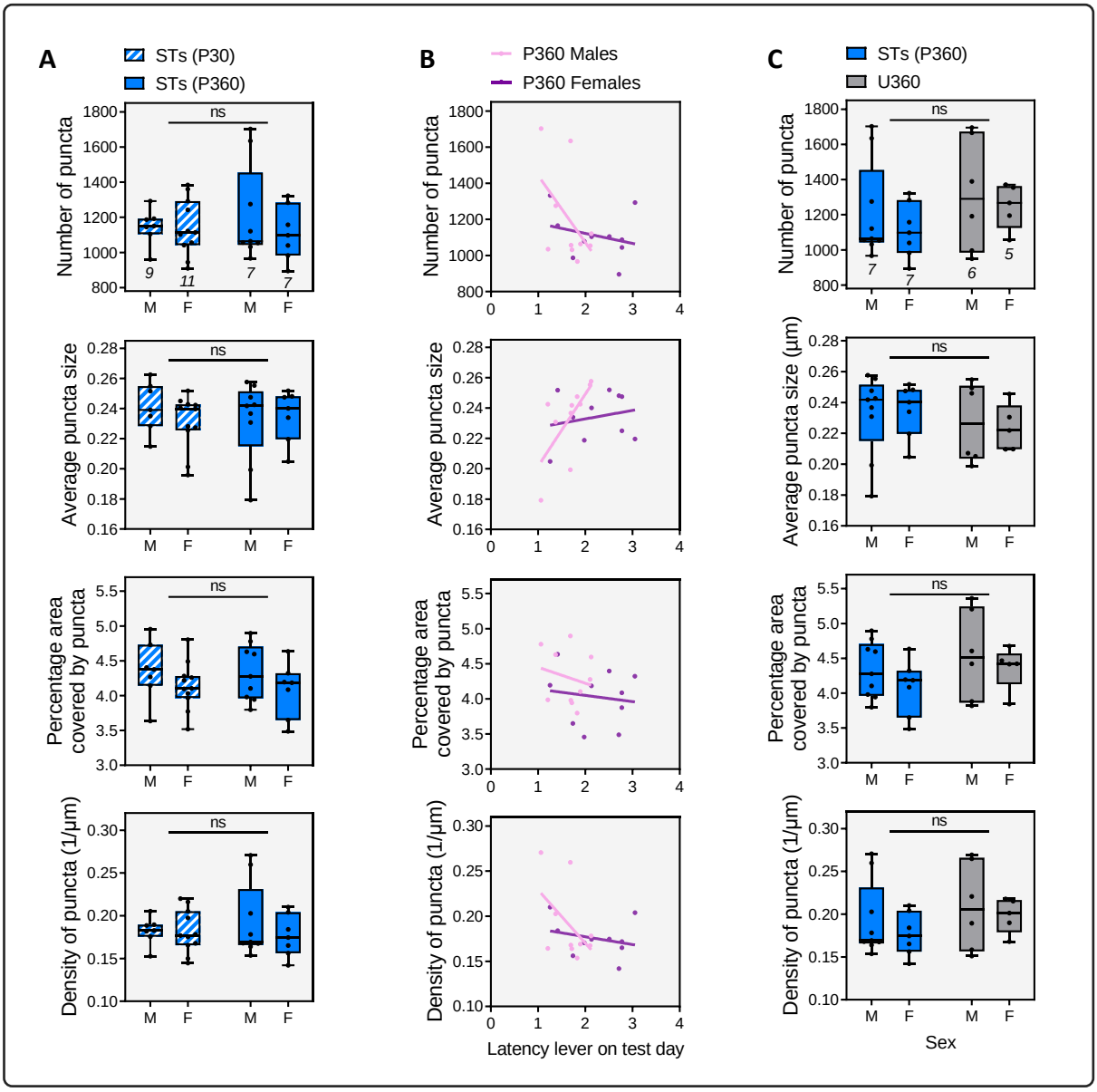


Figure III.1.8. Homer1 staining in the NAc core. (A) Number of homer1 puncta, average puncta size, percentage area covered by homer1 puncta, and density of puncta of male and female sign-trackers perfused 30 minutes after cue re-exposure and 360 minutes after cue re-exposure. (B) Relationship between the latency to first contact the CS during cue re-exposure and homer1 staining in male and female paired rats. (C) Comparison of homer1 staining between male and female sign-trackers perfused 360 minutes after cue re-exposure and male and female unpaired rats also perfused 360 minutes after cue re-exposure.

Table III.1. Recapitulative of results found in Experiment 1 between paired animals perfused 360 minutes after cue re-exposure and unpaired animals. (↑) Greater than the opposite group (*i.e.*, paired vs. unpaired). (=) Similar to the opposite group. (Positive correl.) A positive correlation was found for that group; no comparison is made between paired and unpaired. (No) No correlation was found for that group; no comparison is made between paired and unpaired. Males and females are not directly compared.

	Paired		Unpaired	
Spines	**Males**	**Females**	**Males**	**Females**
Density			↑	↑
Head diameter	=	=	=	=
Length	=	=	=	=
Latency x Density	Positive correl.	Positive correl.	No	No
Latency x Diameter	No	No	No	No
Latency x Length	Positive correl.	Positive correl.	No	No
Synaptophysin				
Number of puncta	↑	↑		
Puncta size	=	=	=	=
% area	↑	↑		
Density	↑	↑		
Latency x Number	No	No		
Latency x Size	No	No		
Latency x % area	No	No		
Latency x Density	No	No		
Homer1				
Number of puncta	=	=	=	=
Puncta size	=	=	=	=
% area	=	=	=	=
Density	=	=	=	=
Latency x Number	No	No		
Latency x Size	No	No		
Latency x % area	No	No		
Latency x Density	No	No		

Experiment 2. Impact of reward presence or absence during cue re-exposure on conditioned behaviour and neurobiology

Introduction

To assess whether omitting the reward in Experiment 1 resulted in a negative RPE, which is typically accompanied by a decrease in dopamine signalling (Schultz et al., 1997), in Experiment 2 rats were re-exposed to both the predictive cue and the reward. Baseline and cue-induced postsynaptic morphological alterations as well as presynaptic and postsynaptic activity in the NAc core were examined using Golgi-cox staining, synaptophysin and homer1 staining. Pavlovian behaviour prior to the re-exposure to the cue and the reward along with sex differences were considered.

Materials and methods

Animals

Male (n=18) and female (n=18) Lister Hooded rats (outbred; Charles River, Kent, UK) aged from four to six weeks upon arrival (males 150-225g, average 181g; females 74-146g, average 113g) were housed and handled in the same conditions than Experiment 1. The housing room was set on a reverse light and dark cycle of 14h-light and 10h-dark (dark at 08:00). They were tested when they reached adulthood, between 11 and 12 weeks of age (males 331-447g, average 386g; females 197-253g, average 226g). All procedures were approved by Institutional Ethical Review Committee at the Open University (The Animal Welfare and Ethics Research Board; PPL numbers 70/7995 and PABC1F4D1) and were

carried out in accordance with the Animals [Scientific Procedures] Act (1986) and EU Directive 86/609/EEC.

Rewarded cue re-exposure

To study the involvement of the reward in dopamine signalling, on day 8 animals were re-exposed to both the discrete cue and the reward (Figure III.2.1): after a contextual extinction of 5 minutes in the conditioning chamber, the lever was extended 10 times for 4 seconds in a random variable 90-seconds ITI (30-150 seconds range), after which one food pellet was delivered.

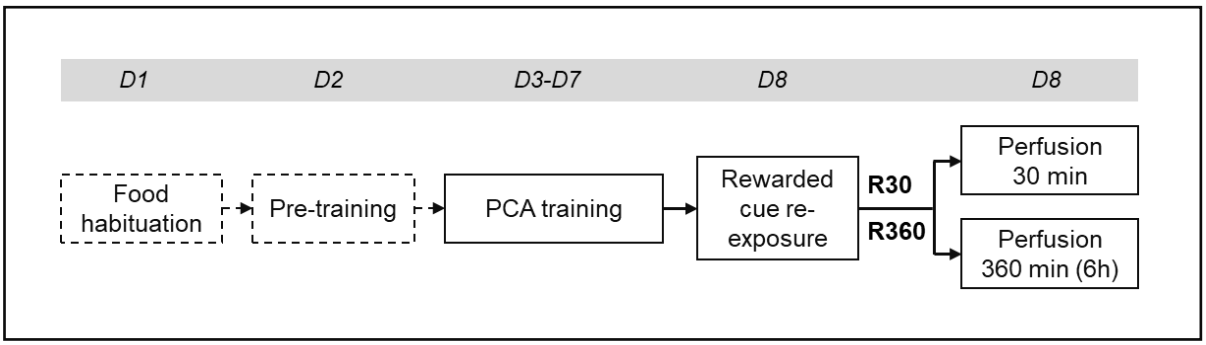


Figure III.2.1. Outline of the procedure used in Chapter III, Experiment 2.

Histology

Similar to Experiment 1, a first group (**R30**: females n=9, males=10) was perfused 30 minutes after the re-exposure session (D8), whereas a second group (**R360**: females n=9, males n=8) was returned to homecages after the test session and left undisturbed for 360 minutes (6 hours) before being perfused. Brains were subsequently sliced, stored and processed as described in the Histology section of Chapter II.

Results

PCA training: behavioural phenotypes and the effects of sex

The majority of rats was classified as STs (males = 55.56%, females = 70.59%). A third of animals did not express an explicit preference towards the cue or the reward location

('intermediate'; males = 33.33%, females = 23.53%), and a small percentage was identified as GTs (8.49% on average). A Chi-square test of independence did not reveal any significant effect of sex in the phenotypic repartition ($\chi^2 = 3.115$, df = 2, p = 0.211). The distribution of PCA scores across conditioning sessions still shows the same shift from the central part to the highest range of the score for both sexes, thereby illustrating the development of a core sign-tracking population (Figure III.2.2-B). The propensity to sign-track develops earlier than for females and can already be observed on day 2 for males (Figure III.2.2-B). The development of a sign-tracking CR was very similar between male and female STs apart from slight but significant differences in the probability (Figure III.2.3-A: Effect of sex: $F_{1,24}$ = 4.450, p = 0.0455) and latency to approach the lever (interaction sex x session: $F_{4.96}$ = 2.553, p = 0.0455), but only at the beginning of the training (lever probability: Šídák session 2, p = 0.0062; lever latency: Šídák session 1, p = 0.0400, Šídák session 2, p = 0.0284). No sex difference was observed for food-cup directed behaviours (all $F_{1, 24} < 2.473$, all p > 0.1289). When taking into consideration all rats regardless of their behavioural pavlovian bias (Figure III.2.3-B), females contacted the food-cup significantly more than males outside of CS presentation during the first session, before learning occurred (effect of sex: $F_{1, 24}$ = 5.391, p = 0.0290; Šídák session 1, p = 0.0025), but both sexes reduced their visits at a similar rate in subsequent sessions. When comparing STs' interaction towards the lever between the test session of Experiment 1, during which the cue was presented without the reward, and Experiment 2, during which the reward was delivered after each lever extension (Figure III.2.3-C), no difference was found in lever contacts (Mann-Whitney: U = 418.5, p = 0.5989), lever probability (Mann-Whitney: U = 4.505, p = 0.4719) or lever latency (unpaired *t*-test: t=1.616, df=59, p = 0.1114).

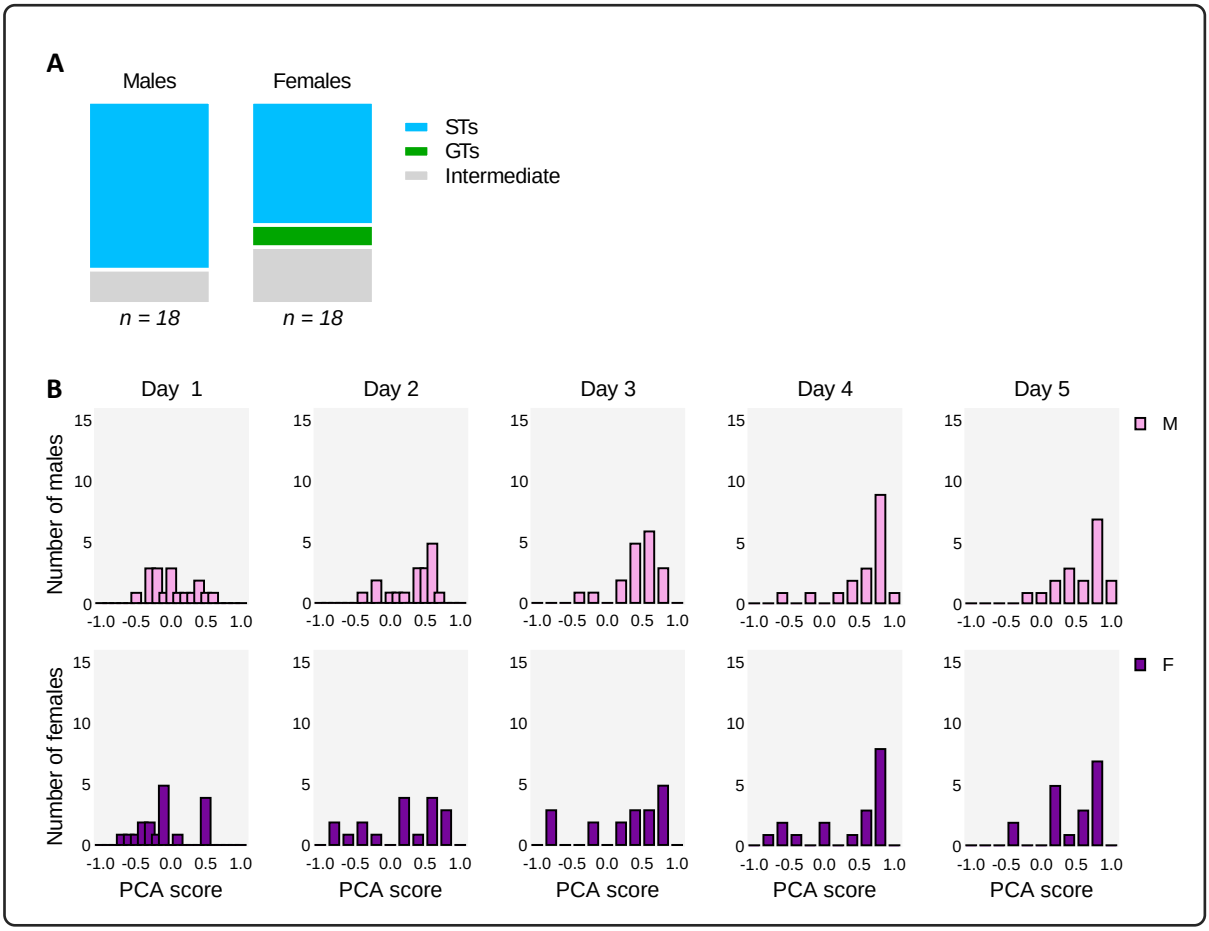


Figure III.2.2. Pavlovian phenotypic repartition of Experiment 2 and evolution across training. (A) Repartition of sign-trackers, goal-trackers and intermediate male and female rats. (B) Distribution of the PCA score for each training session in male (M) and female (F) rats.

Cue re-exposure: effect of reward

The re-exposure session of this experiment was different to the session of Experiment 1 in that whilst rats were re-exposed to the lever in the presence of the reward in the same conditions than during paired training, the lever was extended for a shorter time and the total number of trials was lower. The number of contacts with the lever (Spearman $r = 0.6928$, $p < 0.0001$), the probability (Spearman $r = 0.6022$, $p = 0.0001$) and latency (Spearman $r = 0.7447$, $p < 0.0001$) to first approach the lever during re-exposure were significantly correlated to the same measures during the last training session (Figure III.2.4). These results were confirmed by the Bonferroni correction for multiple correlations. We therefore used behavioural bias during the test session as a reflection of animal's behavioural phenotypes.

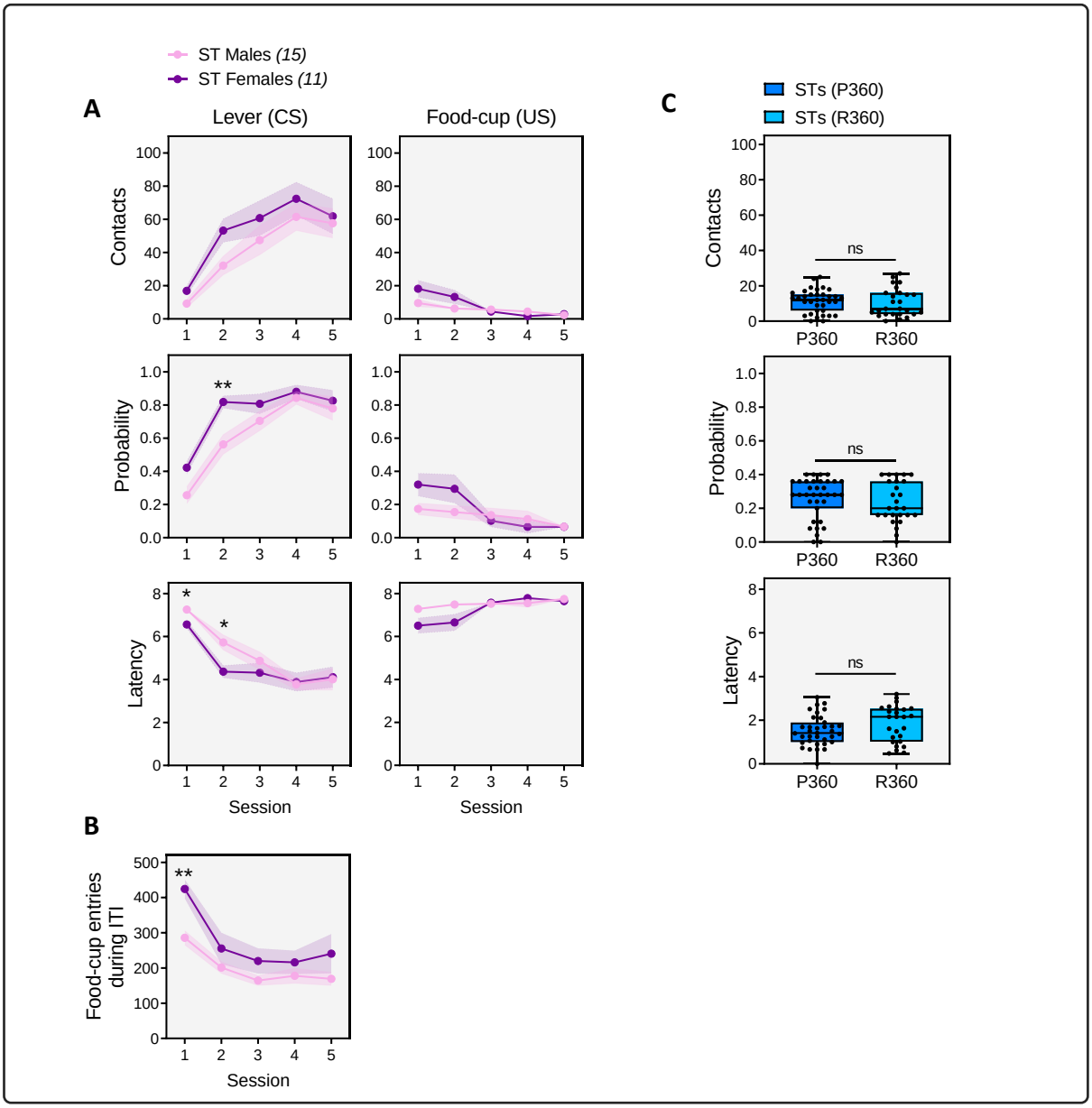


Figure III.2.3. Interaction with the CS+ and the US during the pavlovian procedure. (A) Comparison between male and female sign-trackers for each session. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation. (B) Food-cup entries during the inter-trial interval of male and female sign-trackers. (C) Comparison between sign-trackers of Experiment 1 – P – perfused 360 minutes after being re-exposed to the cue alone, and sign-trackers of Experiment 2 – R – perfused 360 minutes after being re-exposed to the cue and the reward. Males and females combined. (* $p \leq 0.05$, ** $p \leq 0.01$).

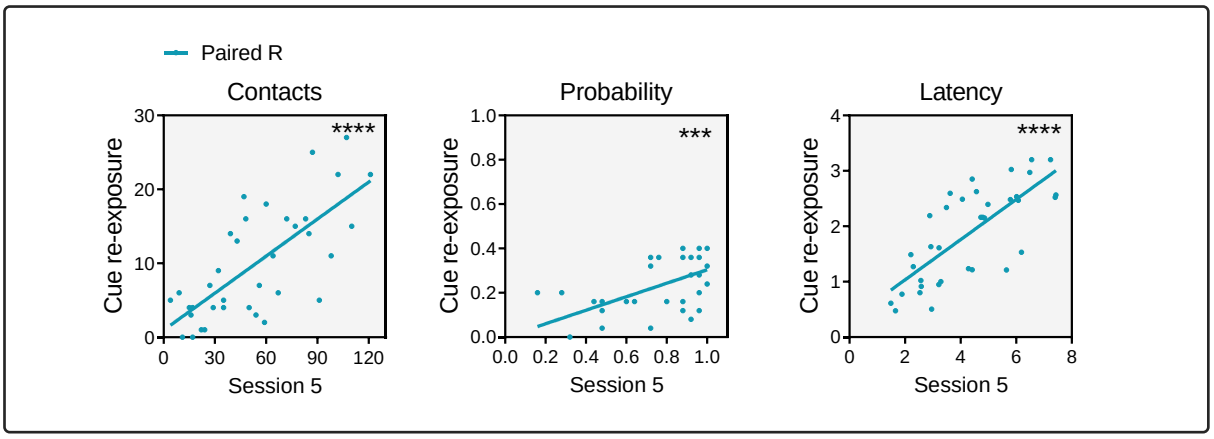


Figure III.2.4. Correlation between conditioned responses of the last training session and the rewarded cue re-exposure. Number of contacts with the lever during CS presentation, probability to contact the lever during CS presentation, latency to first contact the lever during CS presentation. (*** $p \leq 0.001$, **** $p < 0.0001$).

Spine morphology: effects of time, sex and reward

A total of 191 first-order dendrites taken from random locations in the nucleus accumbens core were reconstructed (5 to 6 per rat; average diameter = 0.97 µm; length ranging from 35 to 201.9 µm, average length = 85.78 µm). A two-way ANOVA showed that the group of STs perfused immediately after cue re-exposure and the group of STs perfused 360 minutes (6 hours) later did not differ in their spine density, spine head diameter or spine length (Figure III.2.5-A). No sex difference was observed. The speed at which paired animals contacted the cue during the re-exposure session was not associated with spine changes 360 minutes after (Figure III.2.5-B: spine density, Pearson r = -0.3948, p = 0.1302; head diameter, Pearson r = 0.1916, p = 0.4771; spine length, Pearson r = -0.3759, p = 0.1513). A two-way ANOVA indicates a difference in spine length (effect of reward: $F_{2, 33} = 4.281$, p = 0.0222; Tukey NR experiment vs. R experiment: p = 0.0171), but none in spine density ($F_{2, 33} = 6.881$, p = 0.0032; Tukey NR experiment vs. R experiment: p = 0.3783) or spine head diameter (main effect of reward: $F_{2, 33} = 2.330$, p = 0.1131) between STs who were only presented with the lever on the test session and STs whose re-exposure session included the food reward (Figure III.2.5-C). Paired STs from Experiment 2 did not significantly differ from the unpaired group in their spine density (Figure III.2.5-C; Tukey R experiment vs. unpaired: p = 0.01158).

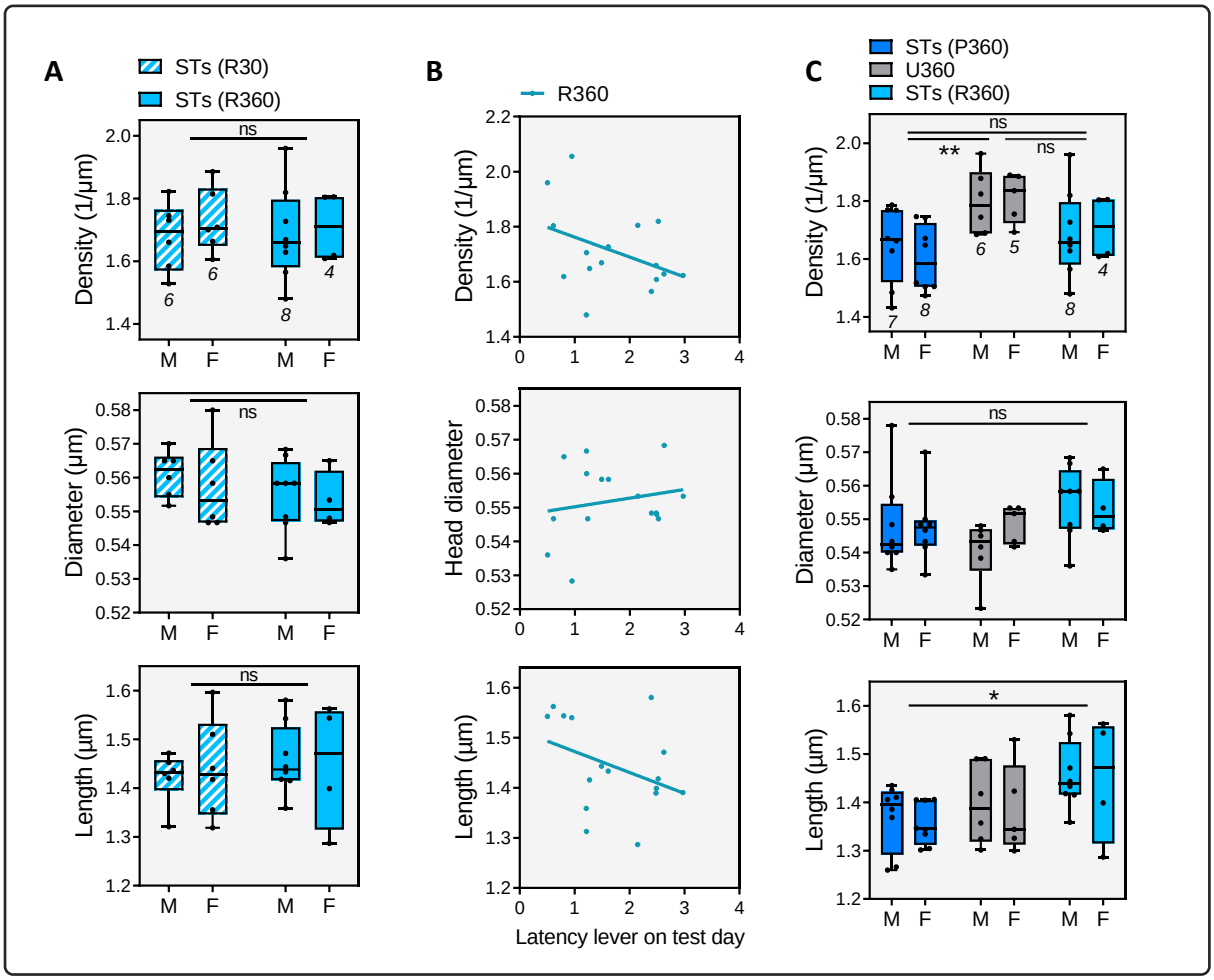


Figure III.2.5. Dendritic spine morphology in the NAc core. (A) Density of spines, average spine head diameter and average length of spines in male and female sign-trackers that undertook a rewarded cue re-exposure and were perfused 30 minutes and 360 minutes after cue re-exposure. (B) Correlation analysis between the latency to approach the CS during the re-exposure and the density, diameter and length of spines, in all animals from the rewarded experiment. (C) Density of spines, average spine head diameter and average length of spines in male and female sign-trackers from Experiment 1 perfused 360 minutes after cue re-exposure, male and female unpaired rats perfused 360 minutes after cue re-exposure, and males and females from Experiment 2 perfused 360 minutes after re-exposure to the cue and the reward. Effect of the conditioning. (** $p \leq 0.01$).

Plasticity mechanisms: effects of time, sex and pairing

Synaptophysin: No variation was found in synaptophysin staining between animals perfused at different times (Figure III.2.6-A; all $F_{1, 22} < 2.153$, all $p > 0.1564$). Although a visual tendency for animals who approach the lever faster to have more numerous and denser synaptophysin puncta six hours after the test can be observed (Figure III.2.6-B), no significant relationship was found for the number (Pearson r = -0.04541, p = 0.0671), the size (Pearson r = 0.2115, p = 0.4152), the area covered by (Pearson r = -0.3011, p = 0.2405) and the density of (Pearson r = -0.4541, p = 0.0671) puncta. A two-way ANOVA comparing

synaptophysin staining between STs of Experiment 1 and 2 revealed reward x sex interactions (Figure III.2.6-C). Indeed, female STs from the NR360 group had significantly more synaptophysin puncta (interaction, $F_{1, 24} = 6.890$, $p = 0.0148$; Šídák, $p = 0.0173$), a wider area covered by puncta (interaction, $F_{1, 24} = 2.239$, $p = 0.0411$; Šídák, $p = 0.0481$) and a higher density of synaptophysin puncta (interaction, $F_{1, 24} = 2.966$, $p = 0.0148$; Šídák, $p = 0.0473$) than female STs who were re-exposed to both the CS and the reward. No difference

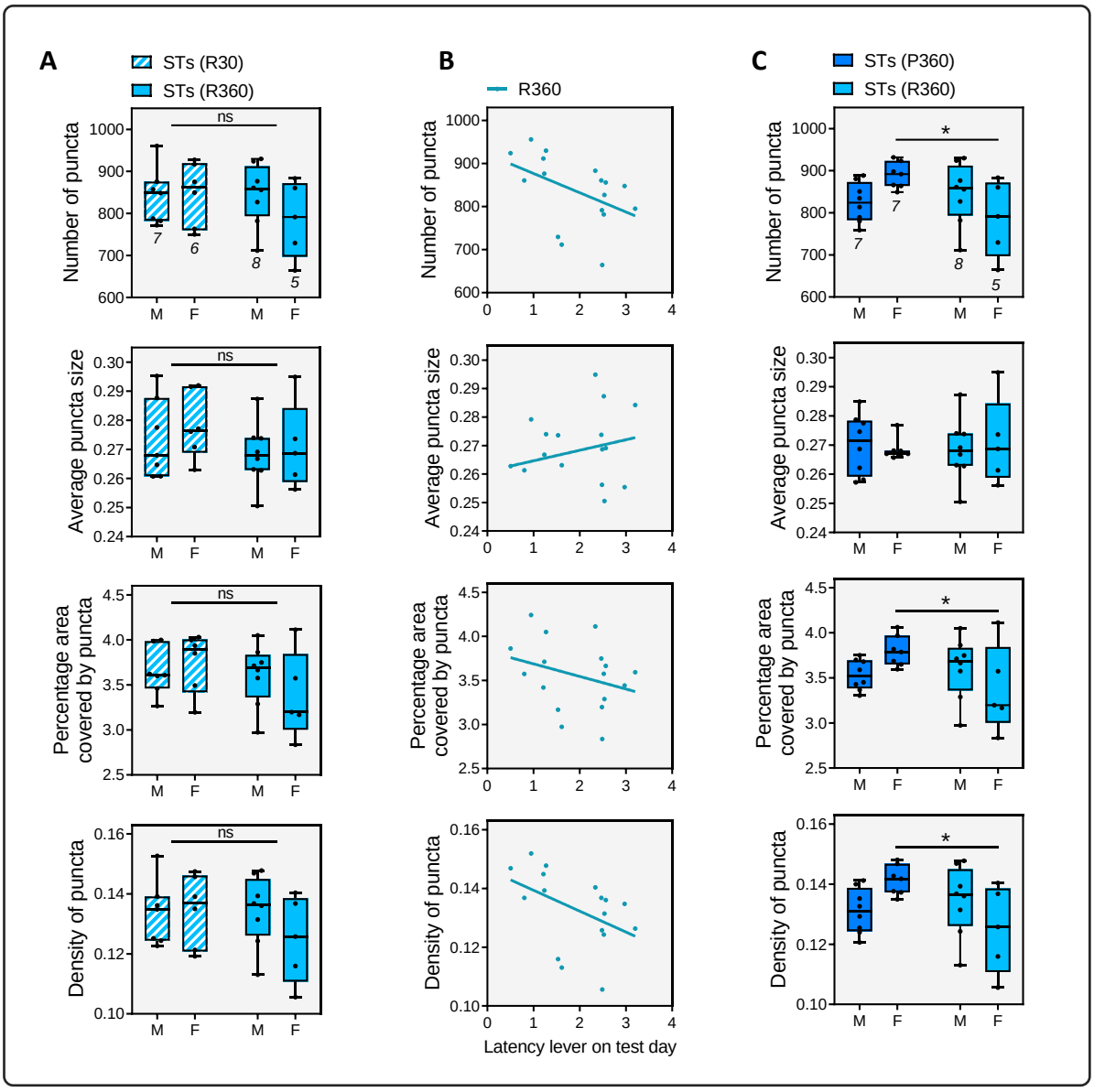


Figure III.2.6. Synaptophysin staining in the NAc core. (A) Number of synaptophysin puncta, average puncta size, percentage area covered by synaptophysin puncta, and density of puncta of male and female sign-trackers perfused 30 minutes or 360 minutes after being re-exposed to the cue and the reward. (B) Relationship between the latency to first contact the CS during cue and reward re-exposure and synaptophysin staining. (C) Comparison of synaptophysin staining between male and female sign-trackers of Experiment 1 perfused 360 minutes after cue re-exposure, and male and female sign-trackers of Experiment 2 perfused 360 minutes after being re-exposed to the cue and the reward. Effect of the reward, effect of sex. (* $p \leq 0.05$).

was found in the puncta size ($F_{1, 24}$ = 0.007634, p = 0.9311). No difference was found in males, and no main effect of reward was found between both STs of Experiment 1 and 2 (all $F_{1, 24}$ < 2.964, p > 0.0980).

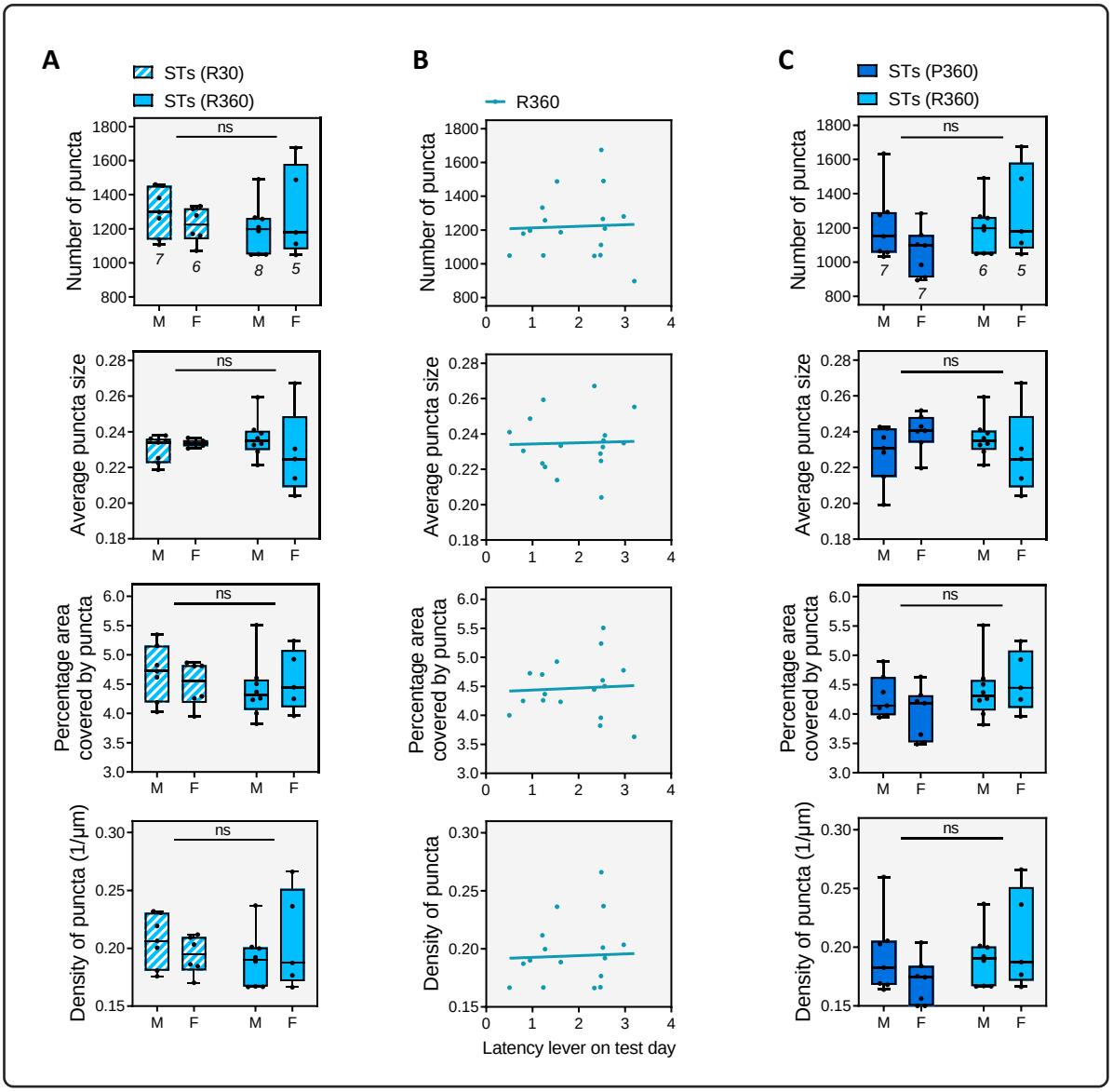


Figure III.2.7. Homer1 staining in the NAc core. (A) Number of homer1 puncta, average puncta size, percentage area covered by homer1 puncta, and density of puncta of male and female sign-trackers perfused 30 minutes or 360 minutes after being re-exposed to the cue and the reward. (B) Relationship between the latency to first contact the CS during cue and reward re-exposure and homer1 staining. (C) Comparison of homer1 staining between male and female sign-trackers of Experiment 1 perfused 360 minutes after cue re-exposure, and male and female sign-trackers of Experiment 2 perfused 360 minutes after being re-exposed to the cue and the reward.

Homer1: No difference in Homer1 staining was found between animals perfused immediately after cue re-exposure and rats perfused 360 minutes after, regardless of their sex (Figure III.2.7-A; all $F_{1, 22}$ < 0.3263, all p > 0.5737). No relationship was found between

the bias towards the predictive cue and Homer1 staining (Figure III.2.7-B; all Pearson r > 0.03387, all p > 0.8234). A two-way ANOVA showed that 360 minutes after the test session, the presentation of the reward did not lead to a change in Homer1 number (effect of reward: $F_{1.25} = 1.062$, p = 0.3126), size ($F_{1.25} = 0.03599$, p = 0.8511), percentage area ($F_{1.25} = 2.697$, p = 0.0.1131) or density ($F_{1.25} = 1.062$, p = 0.3126) of puncta in the nucleus accumbens core (Figure III.2.7-C).

Table III.2. Recapitulative of results found in Experiment 2 between paired animals perfused 360 minutes after cue re-exposure in the presence and absence of reward. (↑) Greater than opposite group (*i.e.*, rewarded vs unrewarded group). (=) Similar to the opposite group. (Positive correl.) A positive correlation was found for that group; no comparison is made between rewarded and unrewarded. (No) No correlation was found for that group; no comparison is made between rewarded and unrewarded. Males and females are not directly compared.

	No reward		Reward	
Spines	**Males**	**Females**	**Males**	**Females**
Density	=	=	=	=
Head diameter	=	=	=	=
Length	=	=	=	=
Latency x Density	No	No	No	No
Latency x Diameter	No	No	No	No
Latency x Length	No	No	No	No
Synaptophysin				
Number of puncta	**=**	↑		
Puncta size	=	=	=	=
% area	**=**	↑		
Density	**=**	↑		
Latency x Number	No	No	No	No
Latency x Size	No	No	No	No
Latency x % area	No	No	No	No
Latency x Density	No	No	No	No
Homer1				
Number of puncta	=	=	=	=
Puncta size	=	=	=	=
% area	=	=	=	=
Density	=	=	=	=
Latency x Number	No	No	No	No
Latency x Size	No	No	No	No
Latency x % area	No	No	No	No
Latency x Density	No	No	No	No

Experiment 3. Relationship between oestrous cycle and incentive salience

Introduction

Sex hormones are robust modulators of structural changes. The reproductive cycle of the female rat lasts from 4 to 5 days and is typically separated into four stages: proestrus – at the end of which females ovulate, oestrus, metestrus and diestrus (Feder, 1981). Oestrogen and progesterone fluctuate across the cycle, producing transient behavioural and physiological variations which are at their highest during the proestrus phase, before ovulation (Becker *et al.*, 2005; Steiner *et al.*, 1981). Spine densities appear to be higher in proestrus females than in males (Woolley *et al.*, 1990), suggesting that data are not comparable between sexes when ignoring the oestrous cycle. More specifically, females in proestrus or exposed to oestrogen exhibit an increased LTP and more density of spines in the hippocampus (Shors *et al.*, 2001; Warren *et al.*, 1995; Woolley *et al.*, 1990) as well as in the NAc core (Forlano and Woolley, 2010; Wissman *et al.*, 2012) – although other studies found that oestrogen led to a *decrease* in spine density in this area (Peterson *et al.*, 2015). Conversely, research suggests that dendritic spine density is intermediate and less plastic during diestrus and metestrus (Alexander *et al.*, 2018; Woolley *et al.*, 1990). This difference in spines observed throughout the cycle might be explained by the effect of oestrogen and progesterone on dopaminergic activity, including in the striatum (Becker, 1999): indeed, these sex hormones have been shown to induce changes in neuronal excitability through modulation of MSN receptors and increase of dopamine release. It is also possible that oestradiol influences spine density by increasing the density of NMDA receptors or enhancing neurons' sensitivity to inputs mediated by NMDA receptors (Weiland, 1992; Woolley and McEwen, 1994).

Thus, as a secondary analysis of behavioural data obtained in Experiment 1 and 2, the following analysis describes sex differences in pavlovian conditioned approach with more scrutiny by distinguishing females in proestrus from other females. Previous studies have detected no difference in the expression of this specific behaviour across the oestrous cycle (Pitchers *et al.*, 2015), and our data partially replicated this result.

Materials and methods

Animals

As described in the literature (Feder, 1981), most of our females had 4-days long oestrous cycles (Table III.3). One female from Experiment 1 ('paired-P' group) later classified as 'intermediate' did not 'dance' during pavlovian training. However, her former regularity allowed us to extrapolate her stages and perfuse her during her estimated diestrus. A female from Experiment 2 ('paired-R') group did not exhibit obvious cycle stages; as this animal was categorised as 'intermediate' as well, this did not influence behavioural and neurobiological results.

Data organisation and statistical analysis

The following results combine animals from Experiment 1 (paired-P) and Experiment 2 (paired-R) as they undertook identical training sessions, and very few females were in proestrus during sessions 1 and 5 of Experiment 2 (R: Table III.3, Figure III.3.1-A) which would have decreased the robustness of the analysis. It is important to highlight that females from the 'proestrus' and 'not proestrus' groups are not the same for each datapoint; instead, each session contains individuals that were or were not in proestrus on that specific day. Analyses are therefore simple two-way ANOVAs and not repeated measures.

Table III.3. Quantification of the oestrous cycle in females of Experiments 1 and 2.

	Average length	Proestrus Session 1	Proestrus Session 2	Proestrus Session 3	Proestrus Session 4	Proestrus Session 5
Paired (P)	4 days	7 (29.2%)	8 (33.3%)	5 (20.8%)	3 (12.5%)	7 (29.2%)
Paired (R)	4 days	2 (11.1%)	6 (33.3%)	3 (16.6%)	7 (38.9%)	2 (11.1%)
Paired (all)	4 days	9 (21.4%)	14 (33.3%)	8 (19%)	9 (21.4%)	9 (21.4%)
Unpaired	4 days	3 (50%)	2 (33.3%)	1 (16.6%)	0	3 (50%)

Results

PCA training: effects of sex and the oestrous cycle

Examining sign-trackers whilst separating females in proestrus from females not in proestrus, Figure III.3.1-B shows that females in proestrus diverged significantly in their PCA index score (effect of sex, $F_{2, 290} = 4.100$, $p = 0.0175$) during the first conditioning session (Tukey: session 1 males vs. proestrus, $p = 0.0004$; session 1 proestrus vs. non-proestrus, $p = 0.0060$). However, at the end of the training, once the association between the CS and the US was learnt, males and females at all oestrus stages had similar PCA index scores (Figure III.3.1-B). The interaction with the lever was similar between sign-tracking males and sign-tracking females in proestrus or in other stages through conditioning sessions (Figure III.3.1-C; all $F_{2, 290} < 1.455$, all $p > 0.2352$). Interestingly, the difference observed in Figure III.1.4-B (Experiment 1, paired-P rats) wherein female STs interacted more than male STs with the food-cup during CS presentation at the beginning of the conditioning (Figure III.1.4-B) appears to be true for all females regardless of their oestrous stage. Indeed, during the first session, females in proestrus contacted the food-cup significantly more (Figure III.3.1-C; effect of sex: $F_{2, 290} = 5.210$, $p = 0.0060$; Tukey, $p = 0.0008$) and faster (effect of sex: $F_{2, 246} = 8.055$, $p = 0.0004$; Tukey, $p = 0.0002$) than males, and females in other oestrous stages visit the food magazine more (Tukey: $p = 0.0048$) and faster (Tukey: $p = 0.0.0173$) as well; whereas females in proestrus and in other stages did not differ from one another in their

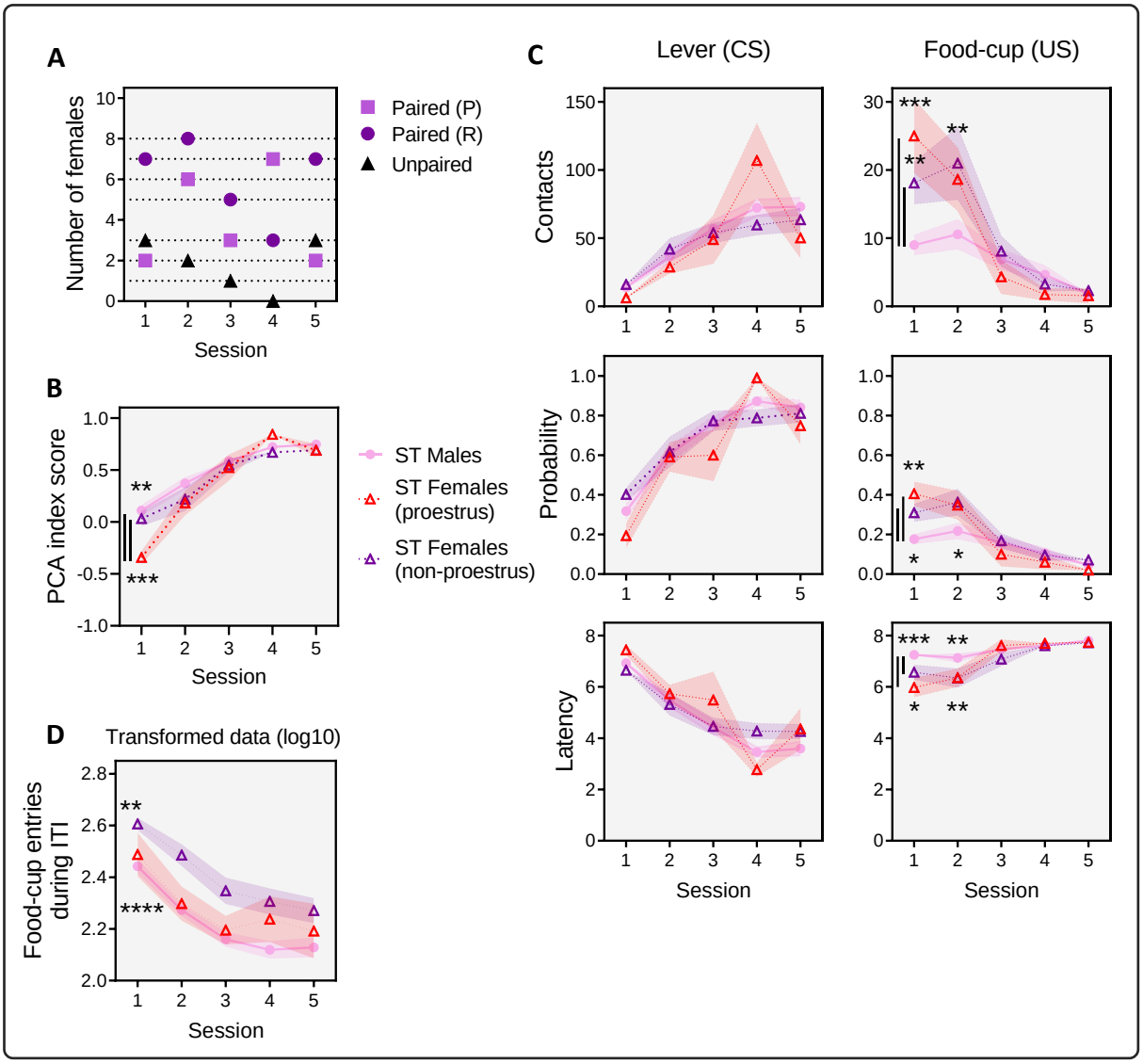


Figure III.3.1. Conditioned responses across the oestrous cycle for paired animals. Datapoints from female groups are all composed of different individuals and are therefore not repeated measures. (A) Number of females in the paired groups from Experiment 1, Experiment 2, and in the unpaired group, that were in the proestrus stage of the oestrous cycle in each of the five training sessions. (B) Evolution of the PCA index score of all male sign-trackers from Experiment 1 and 2 combined, of all female sign-trackers from Experiment 1 and 2 combined that were in proestrus during each specific session, and of all female sign-trackers from Experiment 1 and 2 combined that were in any other stages of the oestrous cycle during each specific session. Difference between the proestrus group and both other groups. (C) Comparison of the number of contacts with the lever and the food-cup, the probability to contact the lever and the food-cup, and the latency to first contact the lever and the food-cup during lever presentation, between female sign-trackers in proestrus, female sign-trackers in other oestrous stages, and male sign-trackers. Difference between male group and both other groups for each session. (D) Number of food-cup entries during inter-trial intervals in female sign-trackers in proestrus, female sign-trackers in other oestrous stages, and male sign-trackers. Difference between the non-proestrus group and both other groups for each session. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

behaviour (Food-cup contacts: Tukey, $p = 0.2747$; Food-cup latency: Tukey, $p = 0.2121$). Here again, at the end of the pavlovian conditioning, all STs displayed the same behaviour regardless of the oestrous cycle.

Rats that did not undertake associative conditioning (unpaired) interacted with the

lever at a comparable rate regardless of their sex and oestrous cycle (Figure III.3.2-A; all $F_{2, 48} < 1.141$, all $p > 0.3280$). However, Figure III.1.4-B (Experiment 1) showed that females tended to interact more with the food cup than males. When distinguishing females depending on their oestrus stage, a two-way ANOVA revealed that this difference was essentially due to females not in proestrus who contacted the magazine more (Figure III.3.2-A; effect of sex: $F_{2, 48} = 40.85$, $p < 0.0001$; non-proestrus vs. males: Tukey, $p < 0.0001$; non-proestrus vs. proestrus: Tukey, $p < 0.0001$) and faster (effect of sex: $F_{2, 48} = 18.05$, $p < 0.0001$; non-proestrus vs. males: Tukey, $p < 0.0001$; non-proestrus vs. proestrus: Tukey, $p < 0.0029$). An effect of sex was also detected in the probability to contact the lever ($F_{2, 48} = 25.11$, $p < 0.0001$) between females not in proestrus and males (Tukey, $p < 0.0001$) and females in proestrus (Tukey, $p = 0.0017$).

Locomotion and activity: effects of sex and the oestrous cycle

Previous results have shown that female STs visited the food-cup outside of the lever presentation significantly more than males in the middle of the training (Figure III.1.4-D, Experiment 1). Figure III.3.1-D suggests that females that are not in proestrus are responsible for this difference (effect of sex: $F_{2, 390} = 42.70$, $p < 0.0001$; non-proestrus vs. males: Tukey, $p < 0.0001$; non-proestrus vs. proestrus: Tukey, $p = 0.0059$). The same result can be observed in unpaired animals (Figure III.3.2-B; effect of sex: $F_{2, 48} = 35.39$, $p < 0.0001$; non-proestrus vs. males: Tukey, $p < 0.0001$; non-proestrus vs. proestrus: Tukey, $p = 0.0005$).

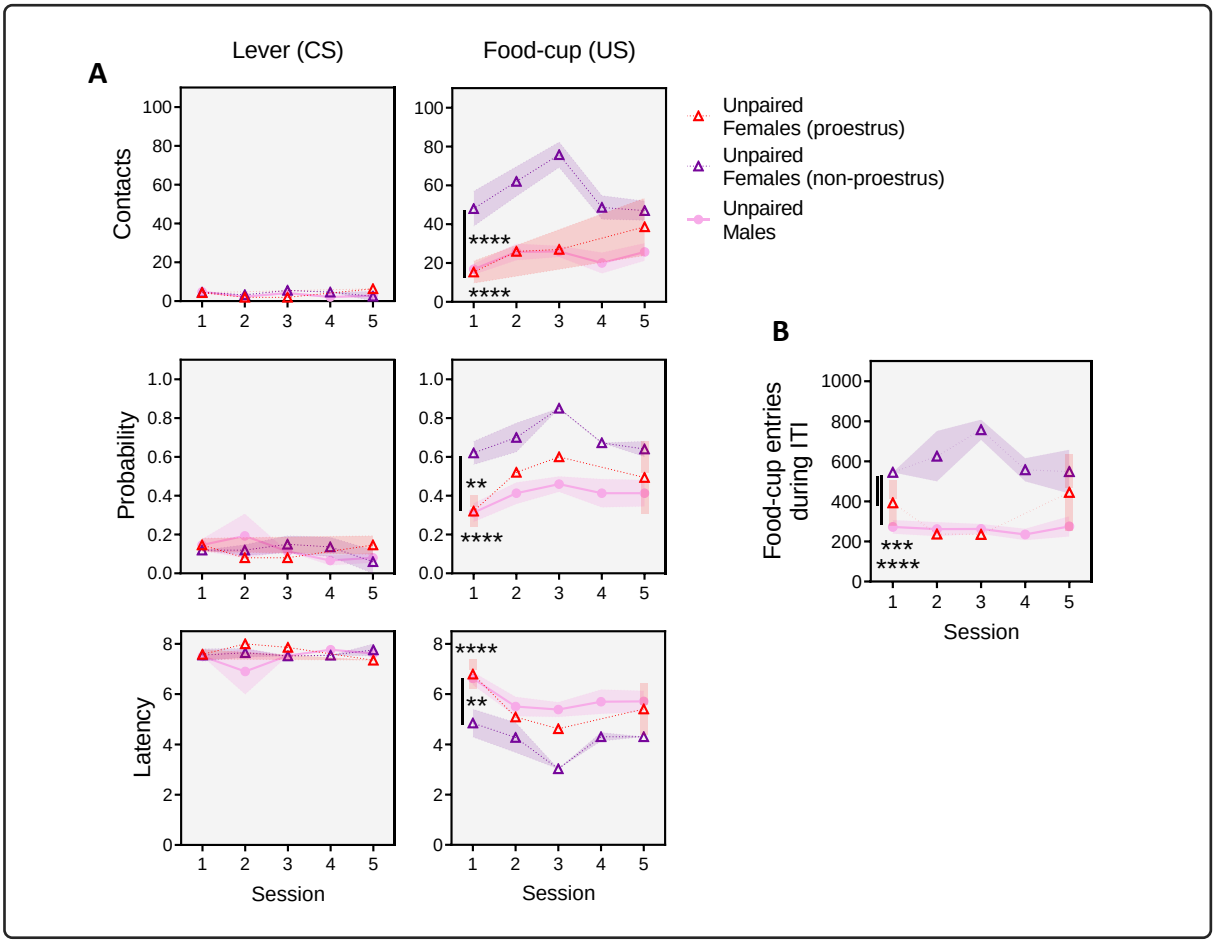


Figure III.3.2. Conditioned responses across the oestrous cycle for unpaired animals. Datapoints from female groups are all composed of different individuals and are therefore not repeated measures. (A) Comparison of the number of contacts with the lever and the food-cup, the probability to contact the lever and the food-cup, and the latency to first contact the lever and the food-cup during lever presentation, between all female sign-trackers in proestrus, all female sign-trackers in other oestrous stages, and all male sign-trackers. Difference between the non-proestrus group and both other groups. (B) Number of food-cup entries during inter-trial intervals in female sign-trackers in proestrus, female sign-trackers in other oestrous stages, and male sign-trackers. Difference between the non-proestrus group and both other groups. (** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$).

Discussion Chapter III

Chapter III investigated baseline and cue-induced postsynaptic structural plasticity, presynaptic and postsynaptic activity associated with individual differences in incentive salience attribution in the NAc core of male and female rats. Experiment 1 and 2 examined the impact of associative conditioning and rewarded cue re-exposure on the striatal neurobiology, whereas Experiment 3 took a closer look at the effect of the oestrous cycle on the development of sign- and goal-tracking.

Degree of individual variation in conditioned responding

Over five conditioning days, rats learnt to associate the extension of a lever with the delivery of a food pellet in order to identify individuals assigning motivational value to the predictive cue. Strikingly, in both experiments, most of our animals fell into this category (from 55% to 77%). There is a lot of variation amongst publications regarding the proportion of sign- and goal-trackers within a study sample; this of course depends on the classification used by researchers, which can be similar to that of the present study (*e.g.*, a threshold of the final PCA index score: Fitzpatrick *et al.*, 2019; Meyer *et al.*, 2012; Yager and Robinson, 2013) or another alternative (*e.g.*, a set percentage of animals with the highest mean number of lever presses: Flagel *et al.*, 2007 and 2008; Robinson *et al.*, 2009), but the length of the ITI, the vendor, the strain/stock and possibly the sex also have an influence on the development of learning phenotypes (Fitzpatrick *et al.*, 2013; Lee *et al.*, 2018; Pitchers *et al.*, 2015). The vast majority of studies reporting their classification worked with male Sprague-Dawley rats and used a PCA index threshold of <0.5 for goal-trackers, and >0.5 for sign-trackers. Within these experiments, the sign-tracking proportion varies from 21% to 37%, and 30% to 38% of animals are categorised as goal-trackers (Fitzpatrick *et al.*, 2019; Meyer *et al.*, 2012a; Morrow *et al.*, 2015; Saunders and Robinson, 2012; Singer *et al.*, 2016a). Researchers using a lower PCA index threshold of 0.3 or 0.4 found 30% to 52% of

sign-trackers, and from 14% to 39% of goal-trackers (Meyer *et al.*, 2014; Yager and Robinson, 2013). Yet, even when transforming data from Experiments 1 and 2 to categorise our animals using the stricter PCA index criterion of 0.5 and considering only males to allow a better comparison with published literature, an average of 79.8% developed a sign-tracking phenotype whereas 2.5% acquired a goal-tracking phenotype. This noticeable difference might be due to outbred Lister Hooded rats which are often described as particularly inquisitive, exploratory and for the most part more similar behaviourally to wild rats (Clemens *et al.*, 2014; Mitchell, 1976). Another factor that has been shown to affect the development of sign-tracking behaviour is early-life experience; environmental enrichment and positive social interactions decreases sign-tracking probability, whereas stressors tend to increase its likelihood (Beckman and Bardo, 2012; Fitzpatrick *et al.*, 2019; Lomanowska *et al.*, 2011; Vigorito *et al.*, 2022). Although transportation is stressful for animals, especially at a young age, the branch of Charles River from which the youngsters of the present study were delivered is less than three hours away from our facilities. Animals were received at three weeks old and developed together for 6 weeks in the animal housing, where their welfare and their environment were monitored. This period of acclimatisation and handling was longer than what is often described in publications and should have reduced the stress of animals. Other laboratories working with Lister Hooded rats and raising them in a different environment might corroborate or invalidate this higher propensity to sign-track.

Another interesting aspect hinted in Experiment 1 is the tendency for goal-trackers to increase their interaction with the predictive cue – and to interact less with the food magazine – during the last conditioning session. Although less substantial, the same inclination was observed in Experiment 2 and Chapter IV (data not shown). This raises the interesting possibility that with more training sessions, our goal-trackers might have shifted their attentional bias. Shelly Flagel reported that, in an unpublished experiment in which rats were trained for 16 conditioning sessions, goal-trackers maintained their behaviour over

time, but individuals not belonging to either category tended to develop sign-tracking inclinations (Flagel *et al.*, 2009). When another team trained rats for 12 sessions, 92% of them were classified as sign-trackers with a PCA index threshold of 0.3 (Roughley and Killcross, 2019, supplementary materials). It is worth noting however that each conditioning session consisted of 28 pairings lever-food instead of 25 in classical PCA procedures. Similarly, a pavlovian procedure consisting of 8 conditioning sessions produced an approximate average of 72% of sign-trackers against 4% of goal-trackers in Sprague-Dawley rats (Pitchers *et al.*, 2015). The theory that in certain conditions and over time non-sign-tracking individuals might develop an increasing interaction towards the predictive cue is intriguing; more experiments would be needed to determine whether this is indeed the case, and if for these animals the cue truly becomes a conditioned reinforcer and induces conditioned motivation.

The widely used technique of classification discussed above allows to reliably distinguish animals for which the discrete cue not only has predictive value but has also become an incentive stimulus capable of exerting control over motivated behaviour, providing a useful tool capable of accounting for variations in the prevalence of sign- and goal-trackers (Meyer *et al.*, 2012a). However, as with every protocol, it does have limitations in that it considers a limited number of aspects and neglects others that could be indicators of incentive value attribution. A conditioned response is complex and composed of numerous features: an individual for which the lever possesses motivational value might approach it, turn or display agitation, but not necessarily press it, therefore not being detected as a sign-tracker. In conditioned place preference, the topography of CRs differs depending on the type of reward; morphine can trigger sniffing and rearing behaviours, whereas food has been shown to induce rotations (see Uslaner *et al.*, 2006). When the pavlovian cue is not a discrete and manipulable object – for example, a light – sign-trackers sometimes exhibit an ‘orientation’ behaviour (Olshavsky *et al.*, 2014), demonstrating that conditioned

responses also vary depending on the environment. Including an analysis of the ensemble of behaviours exhibited by animals during conditioning sessions would bring valuable additional insight.

In this Chapter, both male and female rats were investigated, and disparities found between both sexes varied from one experiment to the other. Results suggested that females might attribute incentive salience to the reward cue in a slightly lesser extent than males, as illustrated by the phenotype repartition in Experiment 2, as well as the interaction towards the magazine and towards the lever in Experiment 1 and 2. However, these differences in the speed or the strength of the sign-tracking response were neither severe nor constant across both experiments. Furthermore, in Chapter IV, the repartition is inverted with sign-trackers being mostly males, and no observed sex difference in the development of conditioned responses. Thus, disparities between males and females in the acquisition and expression of sign- and goal-tracking behaviours in our experimental conditions are minor, not consistent, and might simply be due to sample variations. Very few studies have investigated the influence of sex in this behaviour, and those who did yielded mixed results such as female rats displaying a slightly greater sign-tracking behaviour than males (Fuentes *et al.*, 2018; King *et al.*, 2016), or acquiring sign-tracking behaviour faster than males but developing a similar PCA index score at the end of the conditioning (Pitchers *et al.*, 2015). Taken together, these elements suggest that the assignment of motivational value to discrete reward cues is robust enough to equalise potential innate sex differences in behaviour.

The only consistent distinction between males and females in the present study is the number of food-cup entries during inter-trial intervals, which was higher in females in all experiments of Chapter III, including for control animals who did not undertake conditioning, which indicates that this sex difference might have been innate or related to food-reward learning but not to *cue*-reward learning. In addition to being used to measure the acquisition of conditioning – due to animals decreasing their visit to the food-cup as they

learn that the reward only follows cue presentation – visits to the food-cup between trials are sometimes considered as an indirect indication of non-specific behavioural activity. Surprisingly, most previous research – apart from one publication (Hughson *et al.*, 2019) – has found no sex difference in magazine entries during ITI (Fuentes *et al.*, 2018; King *et al.*, 2016; Pitchers *et al.*, 2015) despite the fact that females have been extensively described as more active in multiple behavioural procedures including open fields and mazes (Archer, 1975; Hyde and Jerussi 1982; Tropp and Markus 2001). This suggests that locomotion and level of activity might not be the main elements involved in checking the food-cup between trials. For example, 'distracted' rats have been observed to rear up and down and increase their amount of grooming behaviour, which might reduce the level of checking. On the other hand, checking might not at all be the behaviour involved in our results in that a conditioned appetitive and consummatory responses towards the food-cup (*e.g.*, gnawing, biting) would also result in a higher count of magazine 'entries'. Observing how rats behave using recordings and directly quantifying locomotor activity instead of simply assessing their interaction with the food magazine might help disentangling individual variation in non-specific behavioural activity.

Following pavlovian conditioning and the development of conditioned responses, animals were initially re-exposed to the lever in the absence of reward to trigger neuronal activation in the networks specifically involved in processing the cue (Flagel *et al*., 2011a; Yager *et al.*, 2015). It might be argued that extending the discrete cue repeatedly without delivering the associated reward might result in extinguishing conditioned responses; additionally, in order for each female to be tested and perfused during the targeted diestrus stage of the oestrous cycle, re-exposure sessions were held at various days after the last conditioning session, which could have decreased the strength of the association. However, studies demonstrated that sign-tracking behaviour remains robust over time and resists extinction procedures far more substantial than 10 lever presentations (*i.e.*, 4 to 29 extinction

sessions of 25 reward presentations: Ahrens *et al.*, 2016; Beckman and Chow, 2015; Gillis *et al.*, 2019; Fitzpatrick *et al.*, 2019). It is to be noted, however, that in these experiments authors subjected animals to a greater number of training sessions (from 7 to 14). The duration of cue presentation, which was shortened from 8 seconds to 4 seconds in order to limit extinction (Flagel *et al*., 2011a; Yager *et al.*, 2015), might have also prompted a new learning and affected the resulting neurobiology due to conditions being different from those of training. Nonetheless, the fact that interactions towards the lever were relatively unchanged when the reward was presented in Experiment 2 suggests that no major extinction took place in our experimental conditions. Furthermore, it is not uncommon to leave conditioned rats undisturbed to recover for days to weeks after invasive procedures without observing a disappearance of learnt associations (Singer *et al.*, 2016a). Behavioural measurements were nonetheless compared to confirm that the propensity to attribute incentive salience to the lever was conserved in conditioned animals between the re-exposure and the last conditioning session. Females particularly retained the level of interaction with the reward cue compared to males. Although used by other teams in previous studies (Flagel *et al.*, 2011a; Yager *et al.*, 2015), this procedure is imperfect and one might wonder whether it is effective in isolating the predictive cue and the networks involved in its processing – this shall be discussed in the following paragraph.

No impact of the timing of perfusion

Structural plasticity and synaptic mechanisms involved in processing the reward-associated cue were studied at two different timepoints after cue re-exposure. A subset of rats was perfused 30 minutes after the test session to observe a ‘baseline’ plasticity evoked by the development of different learning strategies over conditioning sessions, whereas another group was perfused 360 minutes (6 hours) later to compare with the plasticity specifically induced by the cue during the last re-exposure. Unexpectedly, this procedure did

not succeed in discerning both, as evidenced by the absence of differences in spine characteristics, pre- and postsynaptic staining between P30-P360 and R30-R360 groups. The first hypothesis as to why plasticity appeared similar in these groups is that structural changes might have already occurred 30 minutes after the test session. In both cell culture and *in vivo*, spinogenesis has been shown to become obvious approximately 30 minutes after induction of LTP (Engert and Bonhoeffer, 1999; Jourdain *et al.*, 2003), with morphological alterations starting to be visible a few minutes after stimulation but stabilising and becoming functional over a few hours (Abraham and Williams, 2003; Lamprecht and LeDoux, 2004); however, studies have reported that *in vivo* spine growth was slightly slower, and could only be detectable hours after stimulation (see De Roo, 2008). Another possibility might be that our re-exposure conditions failed in reactivating and isolating networks involved in processing the cue; instead, we might still be observing a 'baseline' plasticity, certainly distinct between sign- and goal-trackers – but nonetheless only induced by the training regardless of the time of perfusion. However, Shelly Flagel and Lindsay Yager, who re-exposed their rats to the predictive cue using the same procedure in order to isolate its ability to induce neuronal activity in the NAc core and other regions involved in motivation, successfully demonstrated that such activity was greater in sign-trackers. Authors processed brains between 30 minutes (Flagel *et al.*, 2011a) and 60 minutes (Yager *et al.*, 2015) after cue re-exposure, which corresponds to the timing of stimulus-elicited c-fos mRNA expression; as mentioned above, the timing of spine plasticity might have been miscalculated in our experiment.

Neurobiology underlying variations in incentive salience attribution

Golgi staining was utilised to reconstruct dendritic spines in the NAc core after behavioural training, and other brain slices were processed to detect pre- and postsynaptic markers of plasticity (synaptophysin and homer1, respectively). The low number of goal-

trackers did not allow a strict comparison of the postsynaptic neurobiology underlying both phenotypes. However, correlation analyses revealed that animals who approached the lever faster during the re-exposure session – and therefore supposedly attributed more incentive salience to the reward cue – had more spine density and longer spines in the NAc core. This effect was especially prevalent in males, but no further sex difference was observed. Spine retraction, which is often thought to support rewiring of existing connections, could explain some aspects of the observed spine changes in that it would also result in thinner and longer spines, however the higher density indicates that this might not be the only phenomenon involved. Alternatively, an increase in spine density combined with the longer spines suggests that spinogenesis, and possibly synaptogenesis, might have occurred in these animals, for mature spines tend to be shorter. Newly formed filopodia-like spines, characterised by long necks without head compared to mature and established mushroom spines which require a wider head to form strong synaptic connections, initiate contact with presynaptic terminals of nearby axons and are believed to be the precursors of excitatory spines (Hering, 2001; Ziv and Smith, 1996). The formation of new spines indicates a need for more connections towards pre-existing presynaptic buttons and the creation of a new network; of course, such spines must then stabilise into mature elements for said network to be functional (Dailey and Smith, 1996, Ziv and Smith, 1996). Interestingly, the absence of variation in average head diameter despite the fact that thin new spines would bring the average global diameter down suggests that established spines of sign-tracking individuals might have been larger and compensate for the thin new ones. Regrettably, due to technical constraints, the ratio of spine types was not quantified – but future research might allow to explore these hypotheses further. Furthermore, future experiments could investigate synaptic morphology and plasticity in distal dendrites to compare with the current results and determine whether plasticity mechanisms differ depending on the arborisation of MSNs.

Previous work demonstrated presynaptic signalling differences in STs compared to GTs (Flagel *et al*., 2011; Sarter and Phillips, 2018; Singer *et al.*, 2016a); we were unable to make this comparison due to a low number of GTs. In the present work, the tendency to sign-track inferred through the speed at which animals interacted with the CS+ was not associated a specific presynaptic activity. However, markers of presynaptic activity appeared to be greater in females compared to males. Similar patterns have been described in past studies (Bangasser *et al.*, 2011) and might be related to sex differences in the processing of emotionally relevant information (Bangasser *et al.*, 2011).

The PSD wherein proteins such as homer1 reside follows the size of the dendritic spine it is attached to; in the absence of change in spine diameter, and given that newly formed spines do not possess a PSD, it is coherent that said density would remain equivalent between sign- and goal-trackers – and so would homer1 puncta. Of course, if established spines of sign-trackers were effectively larger than those of goal-trackers, differences in homer1 staining might be expected. What is more, if synaptic alterations occurred after re-exposure to the cue, proteins composing the PSD might not have had the time to change in response to these new inputs.

Effect of associative conditioning

To untangle structural plasticity elicited by CS-reward pairing from plasticity evoked by non-associative sensory exposure to the reward or the lever, a separate subset of rats was trained under unpaired conditions during which the lever and food pellets were not contingent. As extensively described in previous literature (Flagel *et al.*, 2007, 2010 and 2011a; Lomanowska *et al.*, 2011; Meyer *et al.*, 2012a; Robinson and Flagel, 2009; Saunders *et al.*, 2018; Singer *et al.*, 2016a; Uslaner *et al.*, 2006; Yager and Robinson, 2013), these animals did not develop a conditioned response, hence confirming that a stimulus not associated with a reward does not acquire incentive salience.

In contrast to sign-trackers, rats who did not learn an associative conditioning had a greater density of spines in the NAc core accompanied by a lesser amount of synaptophysin. The previous section led us to hypothesise that compared to goal-trackers, learning a sign-tracking CR was associated with either the formation of new spines or spine shrinkage in the NAc core; it is striking that the absence of associative learning resulted in an even greater increase in spine density. Being able to directly compare goal-trackers and unpaired animals would have been greatly informative. Fewer proteins involved in presynaptic activity may be linked to the fact that similar to goal-trackers, unpaired animals have been shown to lack the increase in c-fos mRNA levels (Flagel *et al.*, 2011a) as well as the dopamine release induced by the CS (Flagel *et al.*, 2011b) observed in the NAc core of sign-trackers. On the other hand, because we ignore in which type of cells the synaptophysin is located, the decrease in vesicle proteins might be explained by a reduced release of dopamine caused by an upregulation of *inhibitory* neurons, which would support changes in spine structural organisation.

Influence of the reward on test day

It could be argued that re-exposing the animals to the lever in the absence of reward during the test session could initiate an extinction of the CS-US association, or lead to a negative prediction error which could, in turn, affect dopaminergic activity (Chang *et al.*, 2016; Schultz *et al.*, 1997). In Experiment 2, rats undertook the ultimate test session in the presence of both the predictive cue and the reward. As mentioned earlier in the discussion, sign-trackers' interaction towards the lever and towards the food-cup was comparable during this last session regardless of whether the reward was present or not which suggests that the strength and the nature of the association remained the same. Supporting the absence of behavioural differences, the morphology and number of dendritic spines and the amount of postsynaptic marker homer1 in the NAc core was similar for sign-trackers in both conditions.

However, presynaptic activity appeared to be greater in female STs when the reward was omitted, which is in line with the hypothesis of a recruitment of an inhibitory network.

Effect of the oestrous cycle on conditioned responding

Reproductive hormones cause great fluctuations in female's behaviour and physiology (Becker *et al.*, 2005; Steiner *et al.*, 1981). The pavlovian conditioned approach has not been found to vary across the oestrous cycle (Madayag *et al.*, 2017; Pitchers *et al.*, 2015), however authors only compared the average coefficient of variance between males and females or the PCA score across the four oestrous stages. In the present study, pavlovian data from Experiment 1 and Experiment 2 were combined and re-analysed by separating females in proestrus from females in other stages. We report that sign-trackers in proestrus displayed less sign-tracking tendencies than other females and males at the beginning of training, before learning the association CS-US. This however did not translate into a lower interaction with the lever nor a higher approach towards the food-cup; on the contrary, only general sex differences were observed for the latter, suggesting an inherent sexual dimorphism rather than an influence of reproductive hormones. Strikingly, the higher 'goal-tracking-like' response of unpaired females previously observed did not appear to originate from females in proestrus, but from females in other stages. Moreover, as noted earlier, the most consistent difference was found in the number of visits to the magazine during inter-trial intervals: and here again, in both the conditioned and the unpaired groups, the variation observed between males and females seems attributable to females not in proestrus. It is conceivable that in some instances, a potentially innate sex difference might have been compensated by other behavioural mechanisms during proestrus and might have therefore disappeared. During proestrus, females tended to considerably jump and turn in the conditioning chambers, which could have resulted in a reduced checking of the food magazine between trials. Because the detectors only measure magazine entries, it is also

possible that animals simply exhibited a specific conditioned response driving them to stay near the food-cup. Explanations can be manifold and depend on what this measure really indicates for each individual, and most studies have not found the same sex disparity (Fuentes *et al.*, 2018; King *et al.*, 2016; Pitchers *et al.*, 2015 – but see Hughson *et al.*, 2019). As mentioned previously, studying recordings of the conditioning sessions would allow to give a better description of behaviour and might provide valuable insight.

Remarkably, despite all the aforementioned elements, male and female sign-trackers always exhibited similar behaviours at the end of the training and no difference was detected in lever-oriented interaction across the oestrous cycle. The development of a sign-tracking CR therefore appears robust and potentially capable of erasing initially existing sexual disparities. It is tempting to hypothesise that because behaviours were similar once the association was learnt, the neurobiology of females might be the same regardless of their oestrous stage, however the rate of learning was not comparable – and literature demonstrated that spine characteristics drastically fluctuated throughout the cycle in the hippocampus and the NAc core (Shors *et al.*, 2001; Forlano and Woolley, 2010; Warren *et al.*, 1995; Wissman *et al.*, 2012; Woolley and McEwen, 1994; Woolley *et al.*, 1990). Future experiments should investigate this matter by collecting the brains at different stages of the oestrous cycle after pavlovian conditioning.

Chapter IV
Modulation of the motivational value of the predictive cue

Introduction

Even after consolidation, a memory trace remains dynamic in order for the organism to respond appropriately to an ever-changing environment. Once 'reactivated' by re-exposure to one of its elements, the stable trace enters an active and flexible state and can subsequently be weakened, reinforced or updated (Alberini and LeDoux, 2013). Memories then return to a stable and 'inactive' state to be stored into long-term memory through a process called reconsolidation (Figure IV.1) which, similar to consolidation, requires *de novo* protein synthesis and LTP induction (Nader *et al.*, 2000). During this phase of destabilisation, the memory trace can be disrupted (Przybyslawski and Sara, 1997); this has been used in both non-human (Nader *et al.*, 2000; Dębiec and LeDoux, 2004) and human (Kindt *et al.*, 2009) animals to reduce the strength of maladaptive memories such as fear conditioning or Post-Traumatic Stress Disorder. More specifically, altering reconsolidation does not appear to erase the aversive memory trace fully, but rather the expression of fear (Kindt *et al.*, 2009; Przybyslawski *et al.*, 1999), in other words the saliency of the emotional aspect. Using propranolol, a β-adrenergic antagonist commonly used to affect reconsolidation in both aversive conditioning (Kindt *et al.*, 2009; Przybyslawski *et al.*, 1999) and appetitive conditioning (Milton *et al.*, 2008; Lee and Everitt, 2008; Schramm *et al.*, 2016), Elizabeth Cogan and her team succeeded in reducing the motivational/incentive value of the discrete cue (*i.e.*, lever) in a pavlovian conditioning task, thereby decreasing the expression of sign-tracking behaviour (Cogan *et al.*, 2019). Propranolol administered after retrieval sessions also inhibited the neuronal activation typically observed in sign-trackers following presentation of the reward cue, but did not affect conditioned orienting – indicating that the predictive association between the lever and the reward was not affected. Other studies have demonstrated that the injection of propranolol to target aversive memories

prevents the increase of spine density following such conditioning (Comas Mutis *et al.*, 2021; Vetere *et al.*, 2013).

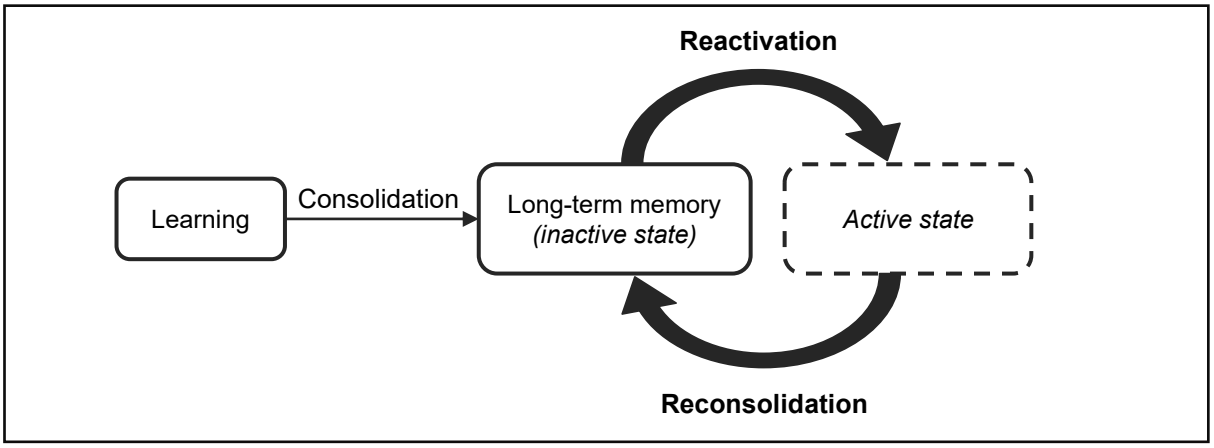


Figure IV.1. Illustration of the stages which are thought to be involved in learning and memory. A stable memory can return to an active and labile state when reactivated under the proper conditions, and requires reconsolidation to be stabilised into long-term memory again. *Modified from Silva and Soares, 2018.*

The initial aim of Chapter IV was therefore to investigate whether propranolol might degrade the motivational impact of pavlovian cues by reversing synaptic changes that occur for sign-trackers in the NAc core during training. However, despite using the same technique as Elizabeth Cogan, animals displayed a transient but noticeably abnormal behaviour after propranolol injection, which shall be discussed in the following sections. Whilst they did not appear in any distress during or afterwards, it was deemed cautious to modify our paradigm, which unfortunately meant that we were unable to replicate her results and thus did not reconstruct dendritic spines nor investigate our hypothesis.

Materials and methods

Animals

Male (n=18) and female (n=18) Lister Hooded rats (outbred; Charles River, Kent, UK) aged from four to six weeks upon arrival (males 127-230g, average 172g; females 110-153g, average 130g) were housed and handled in the same conditions described in

Experiment 1. They were tested at 12 weeks of age (males 373-457g, average 421g; females 214-264g, average 238g). All procedures were approved by Institutional Ethical Review Committee at the Open University (The Animal Welfare and Ethics Research Board; PPL number PABC1F4D1) and were carried out in accordance with the Animals [Scientific Procedures] Act (1986) and EU Directive 86/609/EEC.

Drugs

Drugs were administered intra-peritoneally (i.p.) on the right side at the dose of 1 ml/kg. Sodium chloride (Dental Sky Wholesaler Ltd, UK) was used as a control treatment. In a first batch, propranolol hydrochloride ≥99% (Sigma Aldrich, UK) was diluted at 20 mg/kg in sodium chloride 0.9% (Cogan *et al.*, 2019). However, dosage was subsequently decreased due to treatment affecting the motricity, and in a second part propranolol was diluted at 10 mg/kg (Schramm *et al.*, 2016). Animals were familiarised with the injection handling technique (body manipulation, use of a towel for transportation and gentle restraint) three weeks prior.

Memory destabilisation

A first group of rats (**RET20** or **NORET20**; females n=3, males n=3) was initially administered propranolol at a dose of 20 mg/kg as described by Cogan and colleagues (Cogan *et al.*, 2019). However, when checked in their homecages 30 minutes after injection, rats exhibited transient lethargy and difficulty moving, although no pain nor avoidance behaviour were observed. It was therefore decided to decrease to a dose of 10 mg/kg (**RET10** or **NORET10**; Schramm *et al.*, 2016) for the remainder of the experiment, which unfortunately meant that group sizes are small. Sample sizes showed in the following section are part of the second cohort injected with the lower dose. Data of rats administered with a dose of 20 mg/kg are included in the results section and discussed appropriately.

Retrieval sessions. A subgroup of rats (**RET**) was given two additional sessions to induce memory retrieval (D8 and D9; Figure IV.2). As with the PCA training, it consisted of 25 presentations of the lever followed by the delivery of a food pellet. However, immediately at the end of the session, rats were injected with either propranolol (females n=6, males n=6) or saline (females n=3, males n=4). Injections took place in a different room in which they were transported in a towel in order to reduce anxiety and dissociate this aversive procedure from the behavioural session. Rats were then brought back to their homecages and left undisturbed.

Non-retrieval control. A separate cohort of rats (**NORET**) serving as control without memory retrieval did not undertake additional sessions but were only taken into the injection room with the 'retrieval' rats (Figure IV.2) and injected with either propranolol (females n=4, males n=3) or saline (females n=2, males n=2). They were then taken back to their homecages and left undisturbed.

Test session. Finally, during a final test day (D10), all animals were re-exposed to the discrete cue in the absence of reward to observe potential behavioural consequences of the injections (Figure IV.2). After a contextual extinction of 5 minutes in the chamber to minimise contextual cues, the lever was extended 10 times for 4 seconds in a random variable 90 seconds ITI (30-150 seconds range). No food pellets were delivered following the lever retraction.

PCA index. Because the aim of the present experiment was to observe the relationship between dendritic spines and modulation of the incentive value of the cue in sign-trackers specifically, the PCA index threshold used to categorise animals into learning phenotypes was changed to -1.0/-0.3 (GTs) and 0.3/1.0 (STs) to increase the number of subjects per group.

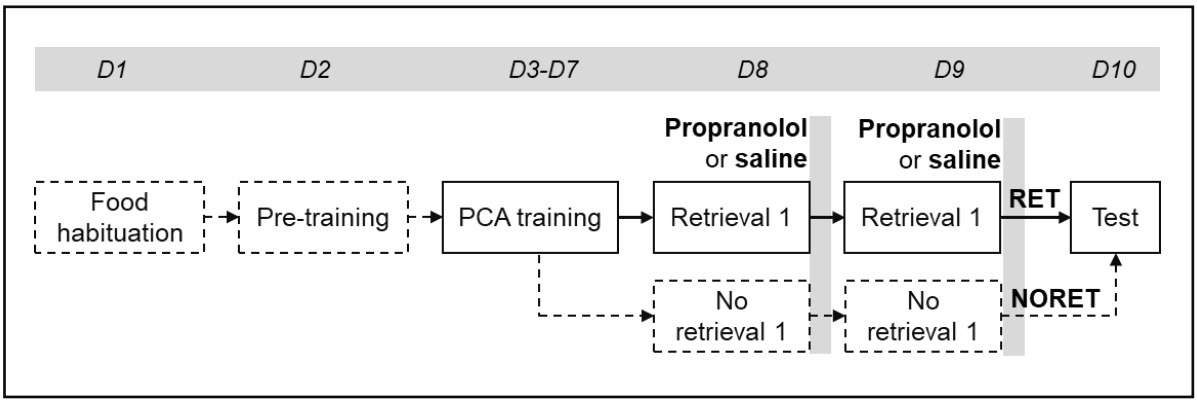


Figure IV.2. Outline of the procedure used in Chapter IV.

Dendritic spine morphology

After the final test session (D10), animals were left undisturbed for 360 minutes (6 hours). They were subsequently anaesthetised with isoflurane 5%, injected with pentobarbitone sodium and perfused as described in Experiment 1. Fixed brains then underwent Golgi-cox staining, however stained neurons from the NAc core were not subsequently quantified.

Results

PCA training: behavioural phenotypes and the effect of sex

Most animals were classified as sign-trackers (Figure IV.3-A: 55.6% of males, 70.6% of females), and very few as goal-trackers (between 5.9% and 11.1%). A third of females (33.3%) and 23.5% of males displayed an intermediate behaviour. The pattern of repartition did not significantly differ between males and females ($\chi^2 = 1.125$, df = 2, p = 0.570). As expected, sign-trackers and goal-trackers developed distinct conditioned responses across conditioning sessions (Figure IV.3-B). Two-way repeated measures ANOVAs did not find any variance in animal's PCA index scores when combining phenotypes (Figure IV.4-A; $F_{1, 34} = 0.1250$, p = 0.7259), nor any difference between sign-tracking males and females in their

interaction towards the lever (Figure IV.4-B; all $F_{1, 20} < 0.3904$, all $p > 0.5391$) and the food-cup (all $F_{1, 20} < 0.7907$, all $p > 0.3845$).

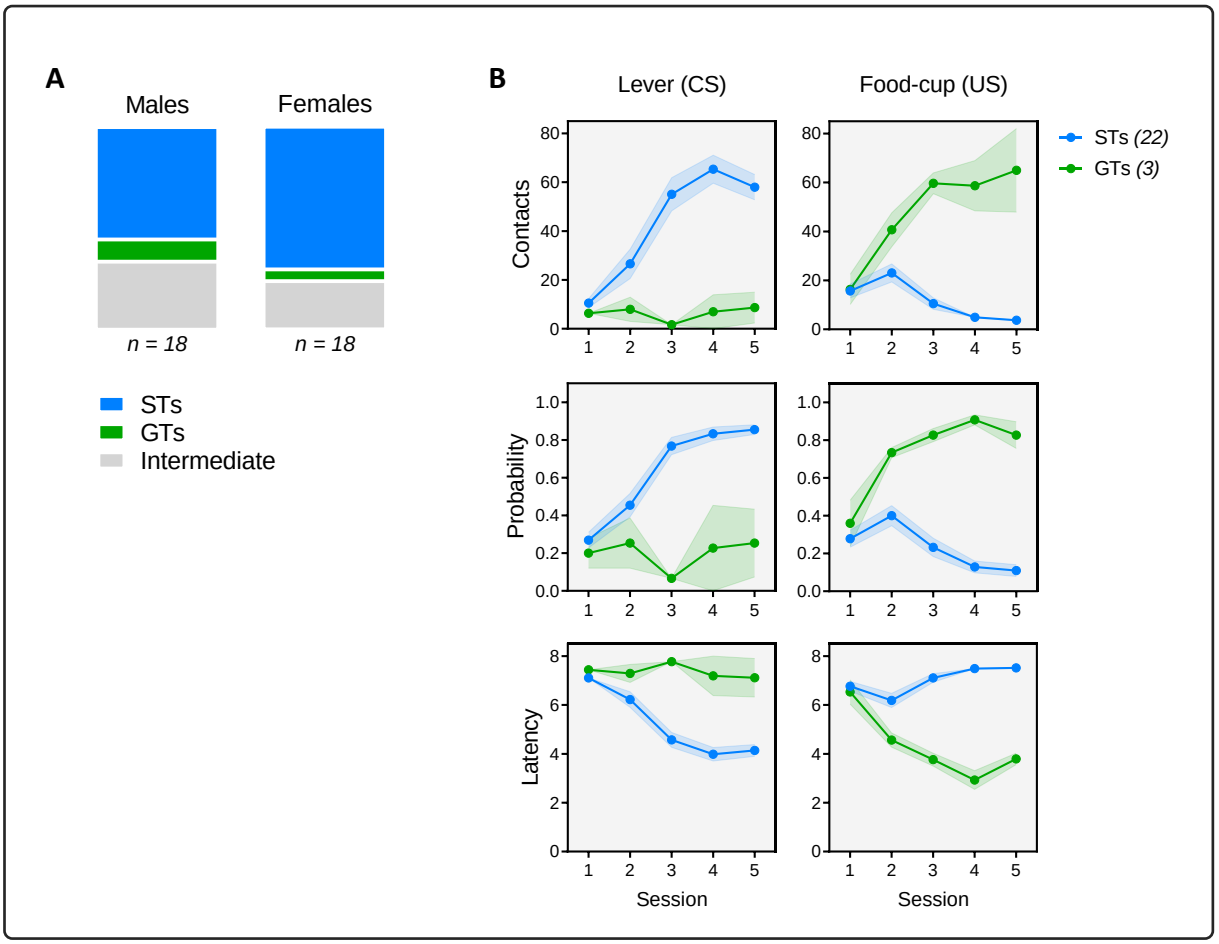


Figure IV.3. Pavlovian phenotypic repartition and conditioned responses. (A) Repartition of sign-trackers, goal-trackers and intermediate male and female rats. (B) Comparison between sign-trackers and goal-trackers. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation. Males and females combined.

First dosage: Effects of propranolol and retrieval

Following training, a first cohort of rats was injected with either 20 mg/kg of propranolol (RET20) to disrupt memory reconsolidation or saline after each of two retrieval sessions, before undertaking a final test session. Due to the low number of rats in propranolol (n=3) and saline (n=5) groups, only results combining sign-trackers males and females are presented in this section. Figure IV.5-A and two-way ANOVAs revealed an effect of

treatment in sign-trackers on the probability to contact the lever during the test session (Figure IV.5-A; $F_{1, 7} = 6.375$, $p = 0.0395$), an effect of treatment ($F_{1, 7} = 18.31$, $p = 0.0037$), retrieval ($F_{1, 7} = 14.62$, $p = 0.0065$) and an interaction retrieval x treatment ($F_{1, 7} = 12.39$, p

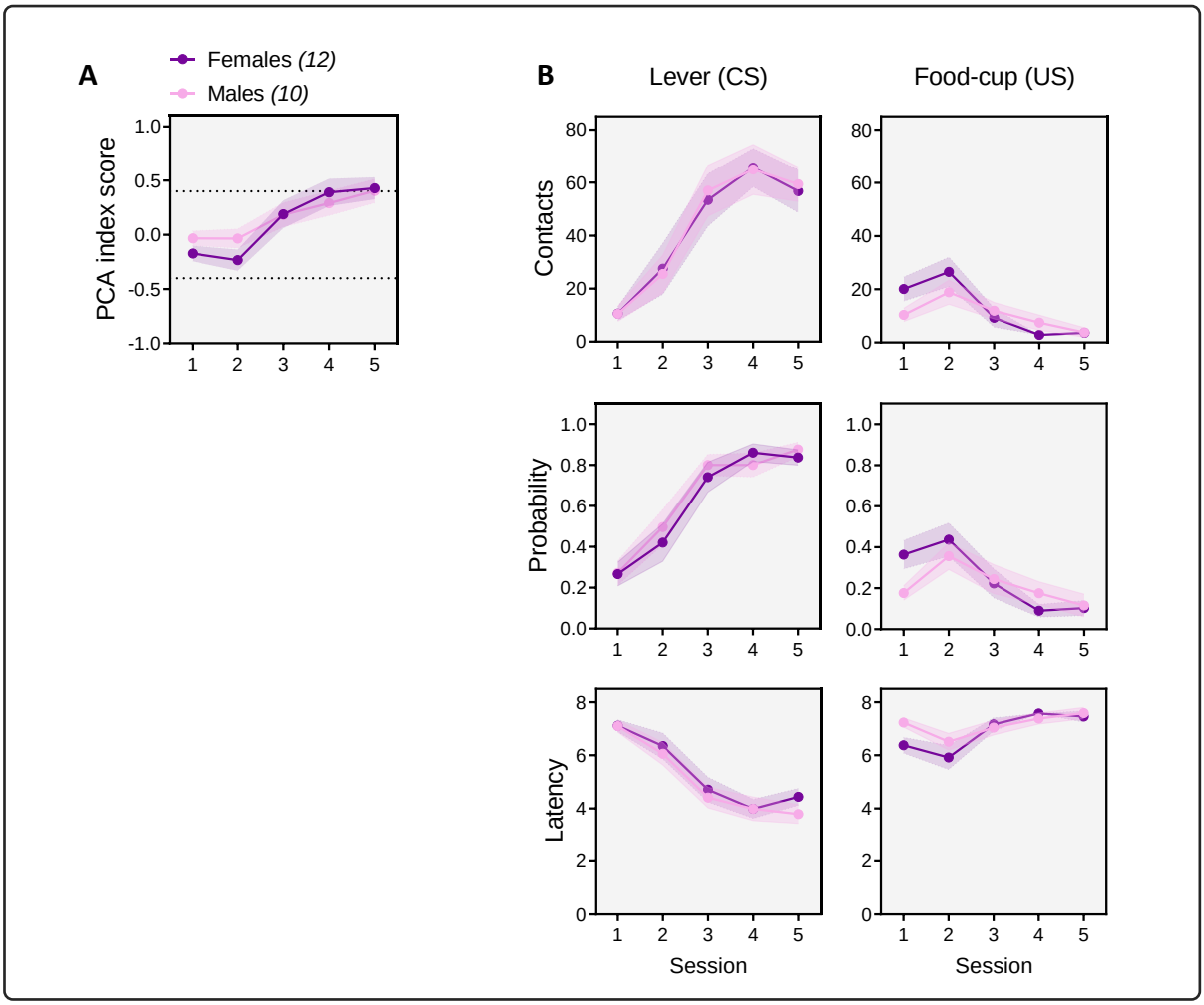


Figure IV.4. Absence of sex differences in conditioned responses. (A) Evolution of the PCA score between males and females. (B) Comparison between male and female sign-trackers. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation.

= 0.0097) for the probability to contact the lever during the last session; but no effect of the propranolol on the number of contacts to the lever ($F_{1, 7} = 2.191$, $p = 0.1824$) or on food-cup directed behaviours (all $F_{1, 7} < 2.423$, all $p > 0.1706$). Within the retrieval group, sign-trackers injected with propranolol scored a significantly lower PCA score than control animals (Figure IV.5-B: unpaired *t*-test: t=3.210, df=6, $p = 0.0184$). It is worth noting that the variability appears greater in animals treated with propranolol.

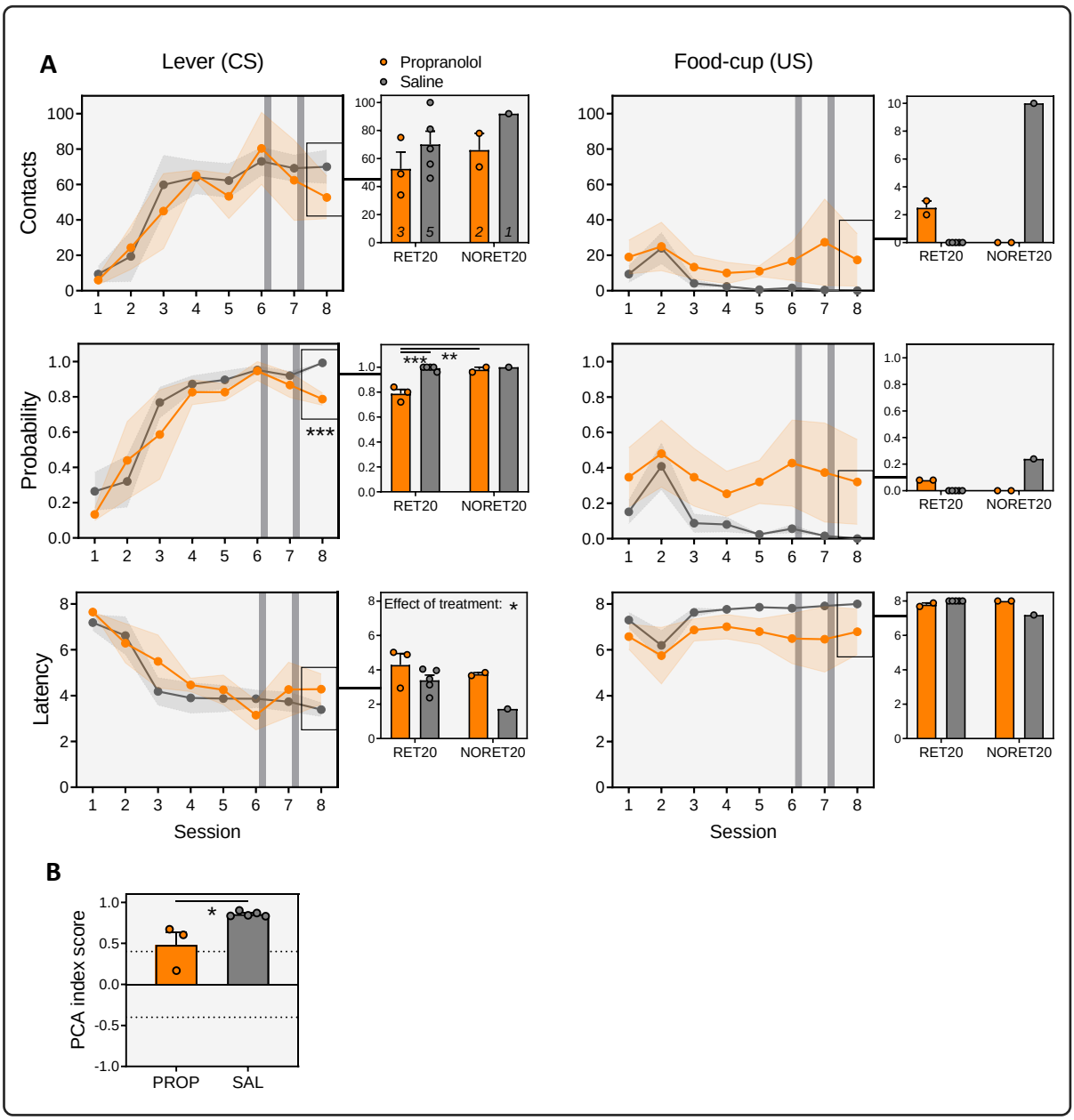


Figure IV.5. Effect of post-retrieval 20 mg/kg propranolol on conditioned responses. (A) Line graphs: Comparison between 20 mg/kg propranolol and saline i.p. injections after two retrieval sessions (grey bands, session 6 and 7) in sign-trackers, sexes combined. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation. Bar graphs: Test session 8. Comparison between sign-trackers administered 20 mg/kg propranolol and saline after retrieval sessions and sign-trackers injected without retrieval sessions. Effect of the treatment, effect of the retrieval. (B) Effect of 20 mg/kg propranolol and saline on the PCA index score of sign-trackers during the test session. (* $p \leq 0.05$, *** $p \leq 0.001$).

Second dosage: Effects of propranolol, retrieval and sex

Due to transient change in behaviour and locomotion observed in animals in the half-hour following propranolol injection, the remaining rats (RET10 or NORET10) were administered with a decreased dose of 10 mg/kg. When combining male and female sign-trackers,

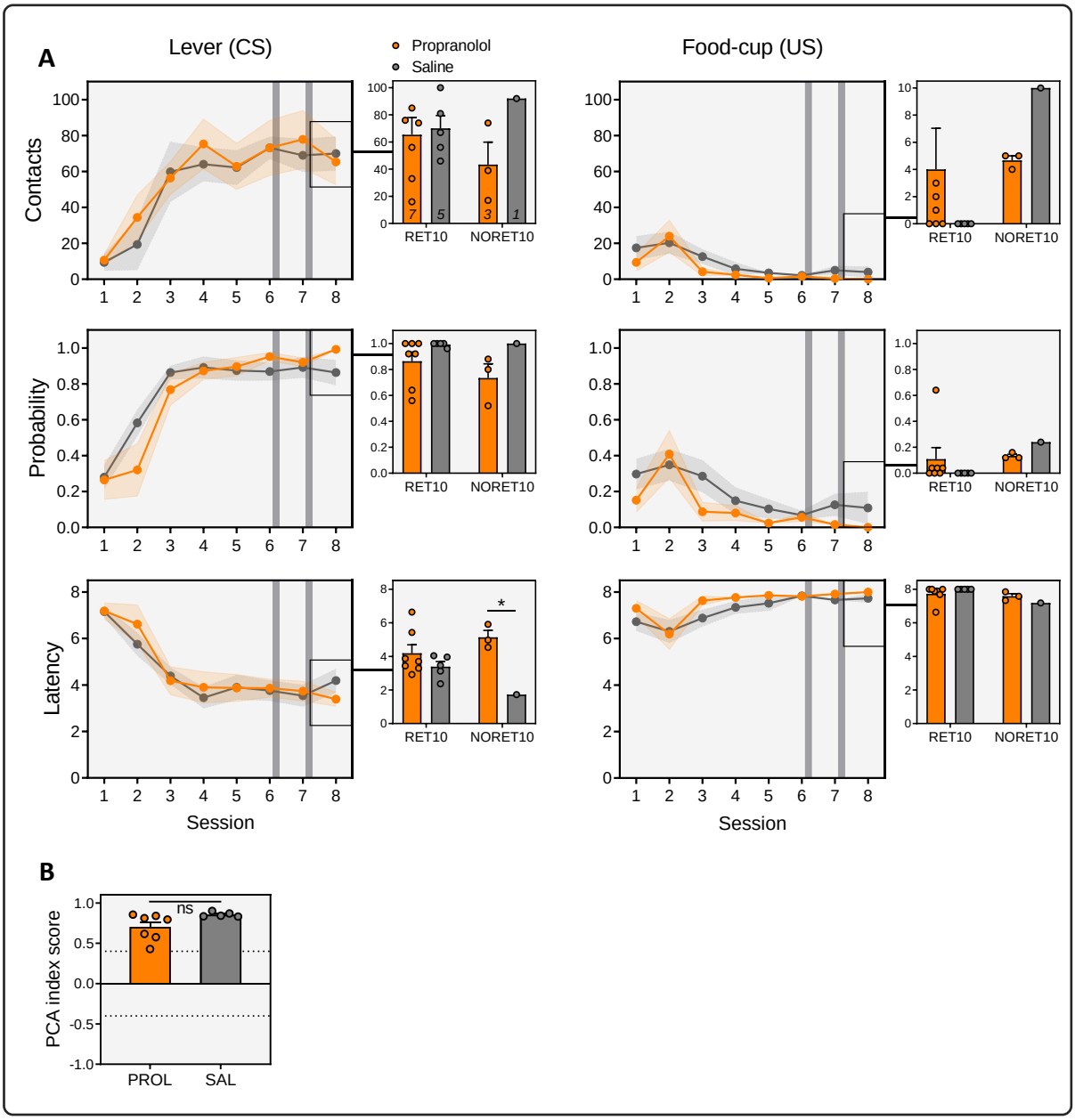


Figure IV.6. Effect of post-retrieval 10 mg/kg propranolol on conditioned responses. (A) Line graphs: Comparison between 10 mg/kg propranolol and saline i.p. injections after two retrieval sessions (grey bands, session 6 and 7) in sign-trackers, sexes combined. Number of contacts with the lever and the food-cup during CS presentation, probability to contact the lever and the food-cup during CS presentation, latency to first contact the lever and the food-cup during CS presentation. Bar graphs: Test session 8. Comparison between sign-trackers administered 10 mg/kg propranolol and saline after retrieval sessions and sign-trackers injected without retrieval sessions. (B) Effect of 10 mg/kg propranolol and saline on the PCA index score of sign-trackers during the test session. (* $p \leq 0.05$).

the only effect of treatment can be observed in the latency to first contact the lever (Figure IV.6-A; $F_{1, 12} = 9.264$, $p = 0.0102$), with a surprisingly higher latency in animals who were *not* subjected to retrieval sessions (Šídák, $p = 0.0337$) but not for the retrieval group (Šídák, $p = 0.4039$). The treatment had no effect on the number of lever contacts ($F_{1, 13} = 0.7840$, $p = 0.3920$) or the probability to contact the lever ($F_{1, 13} = 4.089$, $p = 0.0643$) during retrieval

sessions, nor on food-cup directed behaviours (all $F_{1, 13} < 0.5507$, all $p > 0.4712$). No difference in PCA index score was found between sign-trackers treated with propranolol and controls (Figure IV.6-B; unpaired *t*-test: t=2.027, df=10, p = 0.0702). The variability of animals injected with propranolol was greater than for animal injected with saline. Propranolol produced a similar effect in male and female sign-trackers (Figure IV.7) for lever-directed behaviours (effect of sex: all $F_{1, 6} < 0.5987$, all $p > 0.6044$) and food-cup directed behaviours (effect of sex: all $F_{1, 6} < 0.3868$, all $p > 0.5569$).

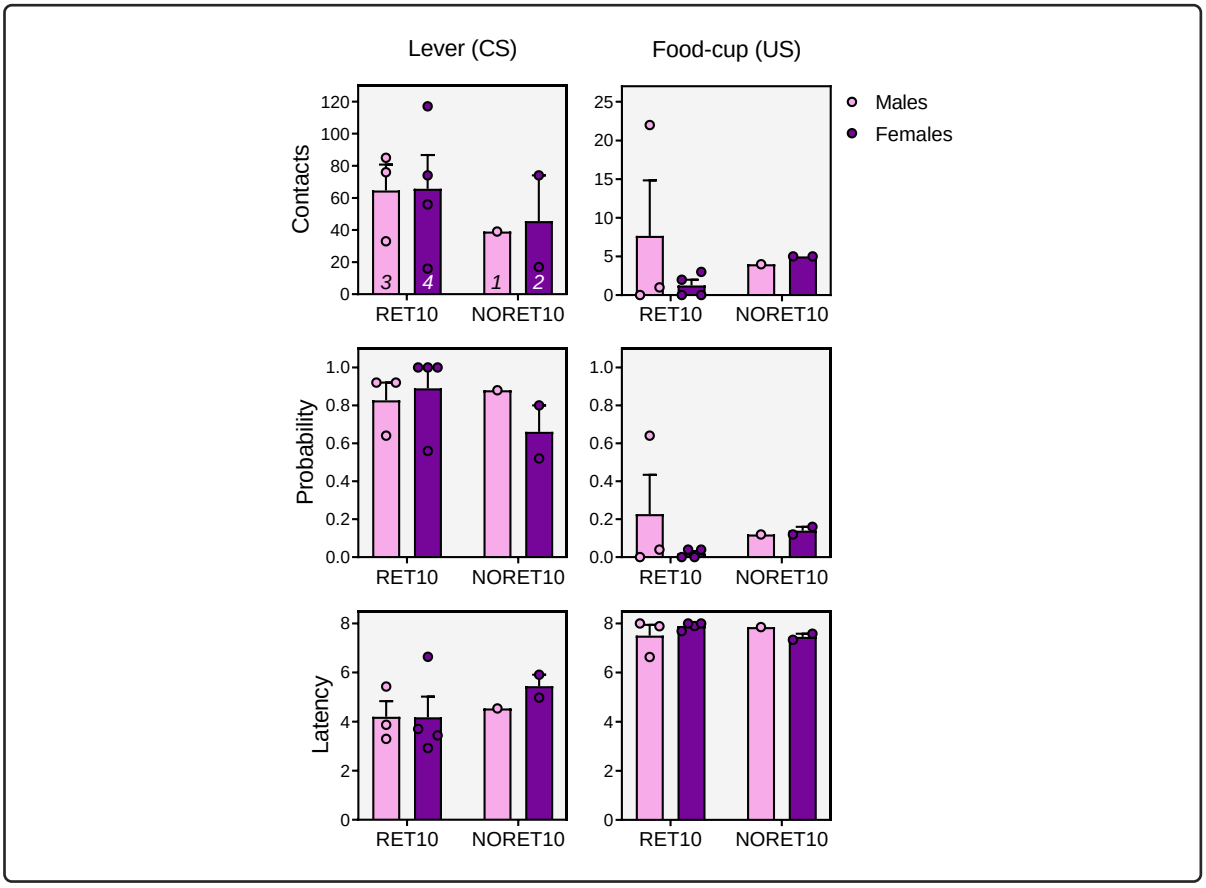


Figure IV.7. Sex modulation of post-retrieval 10 mg/kg propranolol on conditioned responses. Test session 8. Comparison between male and female sign-trackers administered 10 mg/kg propranolol or saline after retrieval sessions, and male and female sign-trackers injected without retrieval sessions.

Discussion

In order to investigate whether interfering with the reconsolidation and thus the expression of sign-tracking behaviour led to a modification of this phenotype's specific post-synaptic neurobiology, rats received i.p. injections of propranolol, a noradrenergic β-blocker, immediately after each of two retrieval sessions following the initial pavlovian conditioning. As discussed in Chapter III, despite slight variations in the development of distinct learning strategies between experiments, Lister Hooded rats raised in our housing conditions predominantly assigned motivational value to the food-associated cue and developed their conditioned response at an approximate rate regardless of their sex.

Cogan and her team showed that disrupting the reconsolidation using propranolol (20 mg/kg) decreased all three measured aspects of lever-directed behaviours – contacts, latency and probability – exclusively in sign-trackers without affecting magazine-directed interactions (Cogan *et al.*, 2019). However, in the present study, when checked for welfare in their home cages after the injections, rats treated with propranolol exhibited transient sluggishness, slower response time and minor paralysis of the hind leg on the side of injection. After dissipation of this effect, approximately thirty minutes after the onset of symptoms, no change of behaviour was observed; animals moved with ease and ate at their normal rate. They did not display avoidance towards the experimenter and behaved in the conditioning chambers as they did prior to injections, which suggested that the experience might not have been aversive. In experiments involving fear conditioning, systemic propranolol at such a dose does not seem to affect immediate locomotion in rats or mice in such a drastic manner (Rodriguez-Romaguera *et al.*, 2009; Stuchlik *et al.*, 2009; Sun *et al.*, 2011). A literature search about reports of this effect in other studies proved unfruitful as some researchers might not inspect their animals after the end of their experiment, or at least not include such minor changes in demeanour in their publication. When informally asked

for their opinion and experience in this matter, other researchers who worked with Lister Hooded reported that animals who were given a dose of 10 mg/kg appeared light-headed five minutes after the injection, without leg unresponsiveness. It is therefore possible that this rat stock might have a different reaction to the drug or the dose. Future studies investigating spine changes associated with the manipulation of the incentive salience attributed to pavlovian cues may need to be undertaken with Sprague-Dawley rats.

Despite our rats seeming untroubled by this unusual post-propranolol experience, it appeared prudent to decrease the dose to match other studies involving reconsolidation in Lister Hooded rats (10 mg/kg: Milton *et al.*, 2008; Schramm *et al.*, 2016). Although this unfortunately meant that very few animals undertook the procedure that the current study attempted to replicate (propranolol group: females n=3, males n=3), the disruption of the reconsolidation by 20 mg/kg of propranolol did decrease sign-tracking behaviour and this effect was, at least partially, retrieval-dependent. However, with the lower dose, the reconsolidation of sign-tracking behaviour was not altered to the same extent. Male and female sign-trackers contacted the lever less readily, but at the same frequency, and this effect was not contingent upon memory retrieval.

Pavlovian cues are powerful in controlling behaviour when imbued with motivational value, making sign-tracking phenotype particularly robust. Studies attempting to extinguish this learning bias showed that compared to goal-tracking, multiple sessions were necessary and yet failed in impacting the initial pavlovian association completely (Ahrens *et al.*, 2016; Beckman and Chow, 2015; Gillis *et al.*, 2019; Fitzpatrick *et al.*, 2019). Pavlovian cues engage the mesolimbic dopaminergic circuit more in sign-tracker individuals compared to goal-trackers, and the activity evoked by these cues in the NAc core is more resistant to extinction for the former (Gillis *et al.*, 2019). Moreover, outcome devaluation does not affect sign-tracking to the same extent as goal-tracking (Morrison *et al.*, 2015).

Considering these elements, it is coherent that a higher dose might be required for the motivational value of food-associated cues to be reduced.

Chapter V
Conclusion of Section One

In Chapter III, baseline and cue-induced synaptic structural plasticity as well as presynaptic and postsynaptic activity in the NAc core were investigated in male and female rats. Chapter IV presented preliminary results of an investigation into the relationship between cue value and spine changes in the NAc core.

Results presented in Chapter III suggest that there may be differences in postsynaptic excitability reflecting individual variation in the tendency to imbue pavlovian cues with motivational value. Spine retraction or spinogenesis might have occurred after presentation of the cue in individuals with a tendency to sign-track, regardless of whether the reward was also displayed during test day. The timing of this plasticity needs to be further investigated to see whether sign- and goal-trackers inherently differ, or if plasticity was altered as a result of pavlovian training. Quantifying the ratio of spines (*e.g.*, mature mushroom spines, young filopodia…) might also allow to establish whether sign-trackers do possess larger spines than other animals in the NAc core after re-exposure to the pavlovian cue. Pharmacologically inhibiting dendritic spine structural plasticity through actin destabilisation could provide a means to distinguish alterations *resulting from* learning and structural changes regulating behaviour. Chapter III also examined the impact of the oestrous cycle on the propensity to attribute incentive salience to pavlovian cues, and revealed that the development of pavlovian conditioned behaviours appeared robust enough to neutralise minor and inconsistent variations initially observed between sexes and across the oestrous cycle.

A key aspect that was not taken into account in this thesis was the natural rat behaviour (*e.g.*, type of approach, head movements, turning, grooming…). Qualitative observations would have added valuable nuances into behavioural analyses in that different indicators of incentive salience attribution to reward cues might have been detected, which would have broadened the categorisation of conditioned responses. Non-specific behavioural activity during inter-trial intervals and the locomotor activity of females across oestrous

stages would have also benefitted from further characterisation. Future work should aim to include such elements to describe behaviour more finely.

Different rat strains (*i.e.*, inbred) and stocks (*i.e.*, outbred) can vary in their behaviour, cognitive aptitudes, and responses to pharmacological agents (Andrews *et al.*, 1995; Kearns *et al.*, 2006). Therefore, the most suitable stock differs depending on each specific research question. Chapters III and IV demonstrated that Lister Hooded rats might be advantageous to work with in experiments in which many sign-trackers are needed, particularly if animals have the opportunity to play and grow in the animal housing for a few weeks instead of starting behavioural testing a week after being ordered. In contrast, when a substantial goal-tracking group is required to allow direct comparison with sign-trackers, the present studies suggest that other stocks such as Sprague Dawley or Long-Evans might be more appropriate to minimise the number of animals. Additionally, because of their transient but intense response to propranolol at a dose of 20 mg/kg, Lister Hooded rats might not be the most suitable stock to replicate experiments from Cogan and her team (Cogan *et al.*, 2019) and investigate neurobiological mechanisms associated with the alteration of the incentive value of pavlovian cues.

In summary, these studies provide insight into the neurobiological processes contributing to individual disparities in the vulnerability to develop some disorders, particularly conditions involving anomalies in motivated behaviours and dopaminergic circuitry.

Chapter VI
Translational pavlovian procedures: Introduction

Although spontaneity can be regarded as a positive and adaptable behavioural feature, impulsivity seldom is. Impulsive individuals lack the behavioural inhibition required to make appropriate and measured decisions, often leading to detrimental consequences (American Psychiatric Association, DSM-5-TR, 2022). Whilst impulsiveness is considered a stable personality trait exhibited by some individuals more than others ('trait' impulsivity), it can also be sporadically determined by internal states, such as anxiety or desire (Wingrove and Bond, 1997), or environmental variables, and can thus vary depending on the situation at hand ('state' impulsivity). Evidence from animal behaviour and neurobiology has led researchers to subdivide this heterogeneous construct into several components: typically, *action* impulsivity and *choice* impulsivity. The former relies on subjects' inability to inhibit premature or poorly timed responses, whereas the latter prevents individuals from delaying gratification and spurs them to make disadvantageous choices (Evenden, 1999; Dalley *et al.*, 2011; Jentsch *et al*., 2014). It is important to distinguish impulsivity from compulsivity which, although being linked to a dysfunction in impulse control, more specifically designates *repetitive* behaviours performed stereotypically despite potential undesirable effects (Dalley *et al.*, 2011).

As such, trait impulsivity is a symptom expressed in several externalising disorders including substance use disorder. Impulsive individuals are more likely to initiate drug taking, have more difficulty cutting down drug use despite adverse consequences, and are more prone to relapsing (Crews and Boettiger, 2009; Dalley *et al*., 2011; De Wit, 2009; Jentsch *et al*., 2014). For example, impulsive rats self-administer drug and alcohol more readily (Belin *et al.*, 2008; Perry *et al.*, 2005; Poulos *et al.*, 1995) and have a higher rate of reinstatement after extinction (Economidou *et al.*, 2009). Impulsivity is a core feature of Attention Deficit/Hyperactivity Disorder (ADHD; Nigg, 2001; Urcelay *et al*., 2011), and pathological gamblers diagnosed with ADHD were found to be more impulsive than gamblers not affected by this condition (Rodriguez-Jimenez *et al.*, 2006; Stanford *et al.*,

2009), which indicates a cumulative effect of these different maladaptive symptoms. Additionally, alcohol and drug misusers as well as individuals with ADHD score significant higher on self-report questionnaires of impulsivity (Stanford *et al.*, 2009).

As described in Chapter I, animal studies indicate that individual differences in reward-cue processing are associated with a pattern of other behavioural characteristics. Multiple elements suggest that sign-trackers in non-human animals may be particularly vulnerable to impulse control disorders. Firstly, this behaviour has been depicted as persistent and difficult to suppress, as illustrated by the perseveration of cue-directed responses despite repeated omissions of the reward (Brown and Jenkins, 1968; Fitzpatrick *et al.*, 2019; Schwartz and Williams 1972) and their resurgence after changing pairing rules between the CS+ and the food (Kearns and Weiss, 2007; Epstein and Skinner, 1980; Lindblom and Jenkins, 1981). The seemingly irresistible attentional bias that sign-trackers display towards reward – or drug – cues (Flagel *et al.*, 2009; Flagel and Robinson, 2017; Saunders and Robinson, 2010; Tomie *et al.*, 2008) is arguably caused by a poor attentional control ('bottom-up' processing) due to an imbalance in cholinergic activity in relevant structures such as the prefrontal cortex. This theory is supported by studies revealing that a higher propensity to sign-track was associated with stronger attentional control deficits modulated by acetylcholine, including during impulsivity and attention tasks (Koshy Cherian *et al.*, 2017; Paolone *et al.*, 2013; Pitchers *et al*., 2017). Moreover, impulsivity appears to be a significant factor involved in the degree of reactivity to food cues (Hou *et al.*, 2011; Tetley *et al.*, 2010), and such degree of reactivity can be used to predict impulse control disorders (Colaizzi *et al.*, 2020; Phillips and Sarter, 2020; Saunders and Robinson, 2013). Additionally, and most significantly, animals for whom reward cues acquire motivational value exhibited stronger *action* impulsivity in reaction time tasks and differential reinforcement tasks in which they needed to withhold a response to obtain the reward (Flagel *et al.*, 2010; King *et al.*, 2016; Lovic *et al*., 2011). Sign-trackers were not

more impulsive on tasks assessing choice impulsivity in these studies; however, it is worth noting that other experiments found that rats who performed more 'autoshaping' conditioned response chose immediate small food rewards over a delayed but larger gratification (Tomie *et al.*, 1998), and that lesions of the subthalamic nucleus negatively affected both autoshaping responses and delay discounting (Winstanley *et al.*, 2005).

Sign-tracking therefore emerges as an endophenotype relevant to several disorders including substance use disorder and other addictions, obesity, post-traumatic stress disorder and depression. Being able to detect individual variation in responses to appetitive conditioned stimuli might help developing screening tools to predict abnormal appetitive processing and risk profiles for the aforementioned maladaptive conditions, as well as identifying biomarkers or other means to assess treatments. Furthermore, individuals combining a propensity to attribute incentive salience to reward cues and a greater impulsivity might possess an increased vulnerability to developing other related disorders. Investigating these matters with non-human animal models is valuable to assess underlying mechanisms and ensure results are comparable with the existing literature, however extending studies to humans in order to verify their utility is equally essential (Colaizzi *et al.*, 2020).

In the 1970s' and 1980s', researchers started to examine autoshaping behaviour in humans (Deckner *et al.*, 1980; Newman *et al.*, 1980; Pithers, 1985; Siegel, 1977; Wilcove and Miller, 1974). Wilcove and Miller instructed participants to sit in front of a device with a lever and a slot delivering pennies and noticed that subjects produced a conditioned autoshaping response when the appearance of the lever was paired with the reward, but did not when these elements were not contingent (Wilcove and Miller, 1974). They also noted a possible confusion about environmental events being controlled by participants' responses. Using a similar paradigm in which participants were not instructed to perform a particular response – apart from being advised not to collect coins which would have prevented the

emergence of a goal-tracking CR – Pithers observed similar results: a subset of participants developed an autoshaping response when the lever was paired with the reward independently of their interaction, and this behaviour was not impacted by the nature of the CS (*e.g.*, tone vs. light) as long as the lever itself was 'localisable' – and therefore, manipulable (Pithers, 1985). In another vastly different experiment in which participants were asked to learn a prediction and to provide a response in an attempt to gain or lose poker chips depending on their accuracy, authors remarked that individuals contacted the predictive cue increasingly throughout training despite the absence of explicit reinforcement (Newman *et al.*, 1980).

More recently, several studies investigating sign- and goal-tracking in humans employing various methods have arisen; detailed descriptions of their procedures shall be provided in subsequent chapters. Most used computerised stimuli (Cherkasova *et al.*, 2018 and 2021; Garofalo and di Pellegrino, 2015; Schad *et al.*, 2020; Wardle *et al.*, 2018) whereas others designed apparatuses that participants could physically interact with (Colaizzi *et al.*, 2022, and see Joyner *et al.*, 2018; Cope *et al.*, 2022). Participants were categorised into sign- and goal-tracking phenotypes using their gaze and/or their approach behaviour, but all experiments outwardly succeeded in detecting variations in cue-induced motivation. Other studies did not investigate sign- and goal-tracking in humans *per se* – at least not using the pavlovian conditioned approach technique widely used in rodents (Meyer *et al.*, 2012a) – but have examined individual differences in attentional biases towards reward cues. In an experiment, sign-trackers were inferred from participants who were distracted by 'high-value' reward-associated cues as opposed to 'low-value' cues, and therefore produced slower response times (Duckworth *et al.*, 2022). Other authors using this 'value-modulated attentional capture' – which measures the response time between two distractors paired with low vs. high rewards – have concluded that neutral stimuli imbued with value through association with the reward were able to 'capture' and drive attention allocation (Albertella *et al.*, 2019; Anderson *et al.*, 2011; Le Pelley *et al.*, 2015; Watson *et al.*, 2019). In 2016,

Francesco Versace extrapolated incentive salience attribution from individuals who exhibited higher EEG brain reactivity to food-associated cues (Versace *et al.*, 2016). These 'sign-trackers' were also shown to have specific activation in structures involved in motivation and reward such as the right putamen, the pallidum, the amygdala and the orbito-frontal cortex (Duckworth *et al.*, 2022); furthermore, a reward-prediction error signature in the nucleus accumbens was observed in sign-trackers but was absent in goal-trackers (Schad *et al.*, 2020).

Amongst the experiments cited above, several have reported a link between the tendency to sign-track and impulsivity, either using self-report questionnaires (Albertella *et al.*, 2019; Anderson *et al.*, 2011; Colaizzi *et al.*, 2022; Garofalo and di Pellegrino, 2015; Cope *et al.*, 2022; Wardle *et al.*, 2022) or behavioural tasks (Colaizzi *et al.*, 2022; Wardle *et al.*, 2018). Reward cues also appeared to promote risky choices (Ludvig *et al.*, 2015), although another study observed that *goal-tracking*, and not sign-tracking, was associated with cue-induced suboptimal decision-making (Cherkasova *et al.*, 2021).

With the objective to build on the existing literature, Chapters VII, VIII and IX aimed to investigate the relationship between different aspects of state and trait impulsivity – assessed respectively by behavioural tasks and self-report questionnaires – and the propensity to allocate reward cues with motivational value in three different settings, thereby examining whether the same variation in conditioned responses can be found across paradigms. In line with results obtained in the publications cited above, human sign-trackers were hypothesised to have a higher degree of impulsivity in self-report questionnaires and tasks evaluating action and/or choice impulsivity.

Chapter VII
Computerised image-based pavlovian procedure

Introduction

Chapter VII focusses on computer-based versions of pavlovian conditioning in which, contrary to rodent procedures, participants do not interact with physical and manipulable objects. Amongst the first to attempt to identify variations in incentive salience attribution in humans, a team used a computerised pavlovian conditioning to assess subsequent cue-induced instrumental learning (Pavlovian-to-Instrumental transfer) (Garofalo and di Pellegrino, 2015). Visual stimuli – a predictive cue and a distractor – were presented on top of the screen, followed by a patch and the presentation of either the rewarded (leading to an actual monetary recompense) or the unrewarded outcome. Male and female participants were instructed that the outcome was not contingent upon their response but were still directed to press a key after disappearance of the CSs. The experimenters used an eye-tracking device to calculate an index based on participant's dwell time towards the CS minus the US over the total dwell time at the end of the conditioning to subsequently isolate sign- from goal-trackers. The presence of the reward-paired cues in the PIT increased responding for reward specifically in sign-trackers. Analyses led authors to determine that individual variation observed were related to distinct learnt conditioned responses and not simple spatial biases, and that sign-trackers scored a higher level of self-report impulsivity as measured by the Barratt Impulsiveness Scale (BIS-11). Gender differences were not investigated. In a similar fashion, another team paired visual stimuli with virtual money, but also neutral images or negative images (*i.e.*, loss of money) and added specific tones to each association to assist the predictive value (Schad *et al.*, 2020). Participants were all 18 year old males, and were not directed to perform any particular action apart from memorising pairings. Once more, sign- and goal-trackers were categorised using an eye-tracker – this time measuring the *probability* to gaze at the CS minus the US – and eye-tracking responses in humans were deemed equivalent to behavioural conditioned responses observed in

animals, which was confirmed in a PIT task. Using the same paradigm and measures as Garofalo and di Pellegrino (2015) as well as pupillary dilatation responses, another experiment also effectively identified individual variation in reward-learning processing (Cherkasova *et al.*, 2021).

The aforementioned publications presented only limited gender comparisons and only investigated one type of impulsivity. The following experiment used an eye-tracking device to assess individual variation in attentional bias in both male and female participants, and study its relationship with trait self-report impulsivity, trait/state action impulsivity and trait/state choice impulsivity.

Materials and Methods

Participants

A total of 34 participants (Table VII.1) recruited via institutional recruitment circular at King's College London took part in this study. All had normal or corrected to normal vision, no neurological or psychiatric conditions and no learning disabilities. Information about participants' education (Table VII.1), current status (*i.e.*, student or not, neuroscience / psychology field or not) and dominant hand was requested in order to account for any population bias and ensure comparison between experiments. Participants gave their written informed consent and received an £8 Amazon voucher for completion of the experiment. All procedures were approved by the Research Ethics Committee of King's College London (MRPP-19/20-14902) and conducted in accordance with the General Data Protection Regulations.

Table VII.1. Demographics of Chapter VII.

	Males (n = 12)	**Females** (n = 22)	***p*-value**
Age (years)	24.3 ±*1.73*	22.9 ±*1.02*	*t*-test: 0.6222
Completed Education			χ^2: 0.611
GCSE	0%	0%	-
A-levels	58.3%	59.1%	-
Undergraduate	33.3%	18.2%	-
Postgraduate	8.3%	18.2%	-
Other	0%	4.5%	-
Status			χ^2: 0.347
Student: Psycho- or neuro-related	0%	27.3%	-
Student: Other	8.3%	4.5%	-
Not student: Psycho/neuro-related	66.7%	59.1%	-
Not student: Other	8.3%	9.1%	-
Dominant hand			Fisher: 0.529
Right	100%	90.9%	-
Left	0%	9.1%	-

Experimental procedure

The study took place at King's College London. The entirety of the experiment was computer-based and conducted using the Gorilla experiment builder (https://gorilla.sc/; Anwyl-Irvine et al., 2020). Tasks are described in more details in the next sections. Participants first completed the Barratt Impulsiveness Scale questionnaire (VII.1), after which three impulsivity tasks were then conducted in random orders (Delay Discounting Task, Go-NoGo and Iowa Gambling Task; latin square). Participants then underwent a pavlovian procedure during which eye movements were recorded a built-in Gorilla eye-tracker, and the study ended by a survey designed to gain feedback on the task by assessing strategies implemented by participants as well as their perception and comments on the study. The entire study lasted approximately an hour.

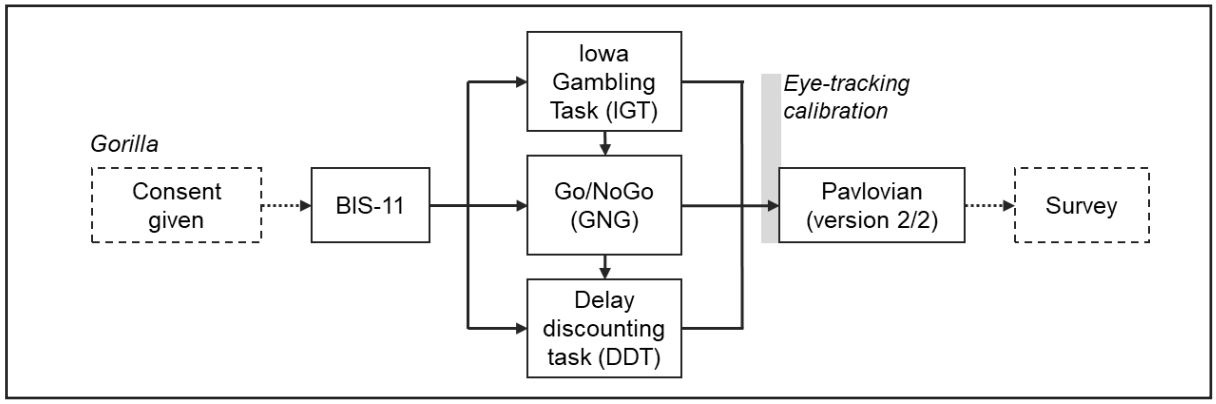


Figure VII.1. Outline of the study undertaken in Chapter VII.

Measure of impulsivity

Barratt-Impulsiveness Scale 11 (BIS-11). The BIS-11 is a well-established self-report questionnaire of trait impulsivity widely used in both healthy and clinical populations (Patton *et al.*, 1995; Stanford *et al.*, 2009) possessing a robust internal consistency (Stanford *et al.*, 2009; Total score: Cronbach's α of 0.83; Attentional: Cronbach's α of 0.74; Motor: Cronbach's α of 0.59; Non-planning: Cronbach's α of 0.72). The questionnaire is presented in Appendix A. Participants answered thirty questions designed to assess second-order factors of impulsivity ('Attentional' which evaluates the ability to concentrate; 'Motor' which estimates unprompted actions; and 'Non-planning' as the absence of forethought) and associated first-order factors of impulsivity (attention and cognitive instability; motor and perseverance; self-control and cognitive complexity). Answers were chosen amongst 'rarely / never', 'occasionally', 'often' and 'almost always / always'. Responses were subsequently rated from 1 to 4 respectively (11 questions were reversely rated), and a global score as well as scores for each second-order categories were calculated. Subjects with global scores above 71 were considered highly impulsive, whilst a score below 52 suggested over-controlled or dishonest individuals (in line with Stanford *et al.*, 2009).

Delay-Discounting Task (DDT). This task assesses the tendency to prefer small immediate rewards over greater but delayed rewards and can be used to provide a measure of choice impulsivity (Evenden, 1999). Instructions can be found in Appendix B. Participants were repeatedly asked to select between two choices: an amount of virtual money 'available immediately', displayed in a box on the left-hand side, or another virtual amount available after a specified delay displayed on the right-hand side (Figure VII.2; '1 week', '2 weeks', '2 month', '6 months', '1 year', '3 years', '10 years'). The offered sums ranged from £1 to £1000. The choice was offered a total of 364 times with increasing and decreasing combinations, allowing the participants to choose each combination twice. Two parameters were subsequently calculated: an indifference point for each of seven delays defined as 'the amount that the participant chooses equally often as the delayed reward', which was used to trace a discounting curve, and an area under the curve (AUC) relative to the degree of discounting (Hurst *et al.*, 2011) which provided a model-free approach not based on assumptions about the discount function (Meyerson *et al.*, 2001). Greater impulsivity was associated with a smaller AUC, which indicates a greater discounting. Participants were excluded if they selected more than two 'switch points' at any delay and if the average IF of the first three delays was lower than the average of the last three delays (Hurst *et al.*, 2011).

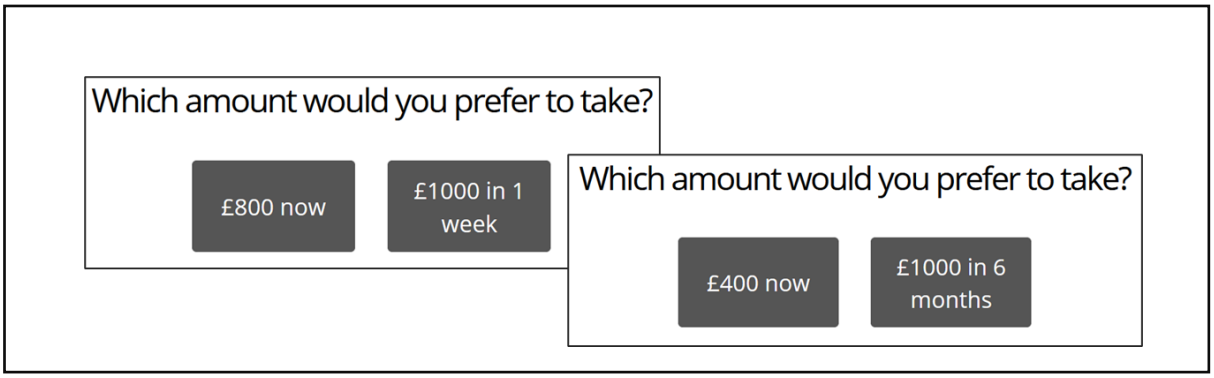


Figure VII.2. Delay discounting task (DDT). Participants must select between an immediate amount of hypothetical money and a delayed amount.

Go-NoGo (GNG). The GNG evaluates impulsive actions through response inhibition. A black square was displayed every 1900 ms for 100 ms on a white background, either at the top-centre or at the bottom-centre of the screen (Figure VII.3). Participants were instructed (Appendix C) to press the spacebar as fast as possible with their dominant hand when the stimulus appeared at the top of the screen ('Go' trial), and to withhold their response when the square appeared at the bottom ('No-go' trial). The task was composed of two blocks: during the first part 23% of trials were 'Go' conditions (randomised 30 'Go' and 100 'No-go'), whereas in the second part both conditions were shown 50% of the time (randomised 50 'Go' and 50 'No-go'). This task is often used to estimate inattention by looking at reaction time or omission errors (failure to respond to a 'Go'), but it can also provide a measure of impulsivity – or response inhibition – when observing commission errors ('false alarms'; Gay *et al.*, 2008; Aichert *et al.*, 2012). This type of error was therefore compared between the first and the second block to evaluate the stability of performances.

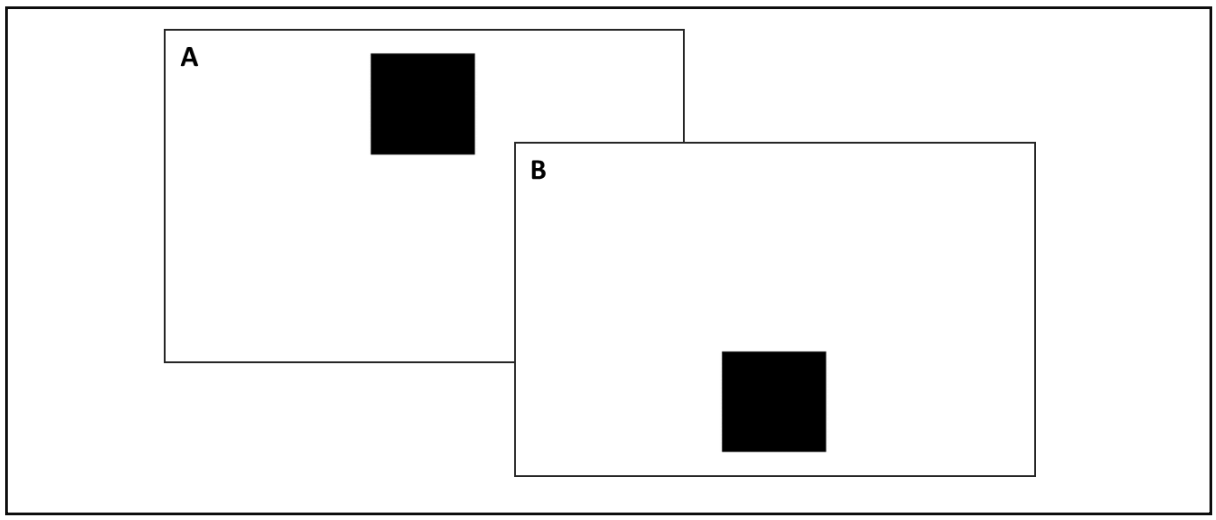


Figure VII.3. Go-NoGo task (GNG). (A) 'Go' signal prompting participants to press the spacebar. (B) 'No Go' signal indicating that participants have to withhold their response.

Iowa Gambling Task (IGT). This test, which assesses decision-making in both healthy and psychiatric populations (Bechara *et al.*, 1994), is also believed to indirectly evaluate choice impulsivity (Franken *et al.*, 2008; Upton *et al.*, 2011). Four cells representing card decks (A, B, C, D) were displayed at the bottom of the screen (Figure VII.4).

Participants were told to attempt to 'win' as much virtual money as possible (Appendix D) by selecting a hundred times from the decks; cards picked could either make them win a reward or pay a fee. Decks A and B contained cards considered 'disadvantageous' in the long term: both decks had a substantial reward of £100, but also had large fees (A: 25 fees of £150-350; B: 5 fees of £1250). Decks C and D, on the other hand, were considered 'advantageous': both decks had reduced rewards of £50, but held smaller fees (C: 25 fees of £25-75; D: 5 fees of £250). After each choice a white screen displayed the reward and the potential penalty, after which the four decks were presented again along with the updated sum of virtual money won at the top of the screen. A net score as well as a score for the last 40 trials – when participant's strategy was considered acquired – were calculated. A score of 100 was considered optimal (*i.e.,* the participant chose only from decks C and D), whereas a score of -100 was deemed a bad score, (*i.e.,* the participant chose only from decks A and B). Altogether, higher scores indicated a lower impulsivity and better performances, whereas negative scores suggested a greater propensity to persevere in detrimental and risky choices.

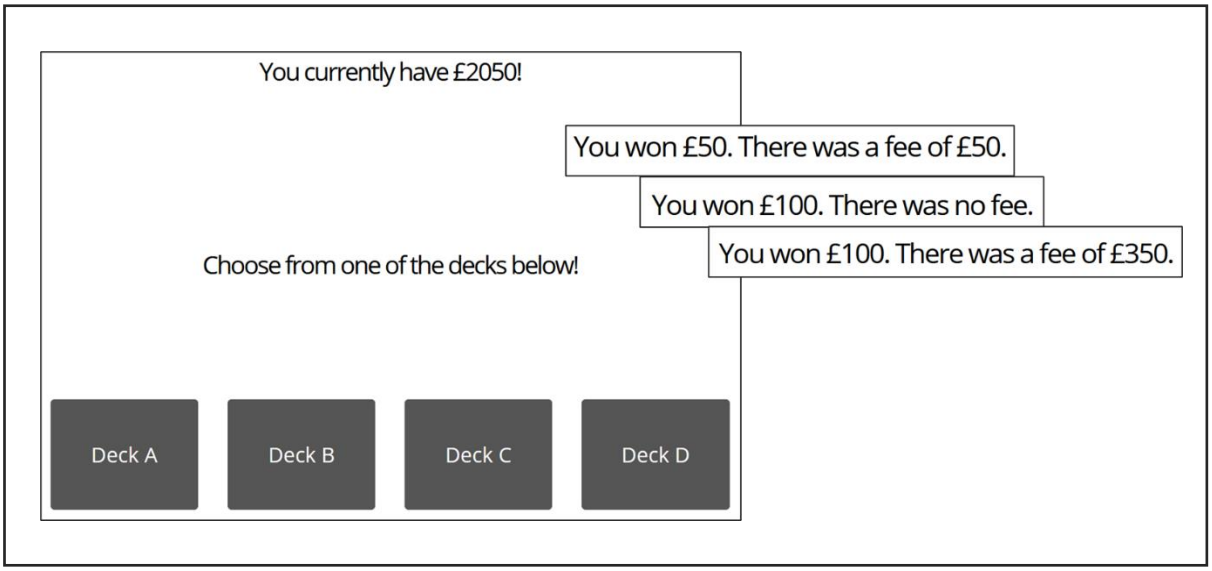


Figure VII.4. Iowa Gambling Task (IGT). Participants must choose from four decks of cards, two of which are disadvantageous.

Pavlovian conditioning

The task, inspired by Garofalo and di Pellegrino (Garofalo and di Pellegrino, 2015), was composed of 41 randomised trials of 4 steps. Two blank cells were displayed one at the top, one at the bottom, in the centre of the screen. The CS+ or CS- stimuli were first presented for 2000 ms in the top cell, then disappeared (Figure VII.5-A,B). A grey square was shown in place of the bottom cell and was replaced after 2000 ms by the outcome stimulus of same duration underneath the CS (Figure VII.5-C). Following a CS+ stimulus the rewarded outcome was represented by virtual money (US), whilst a CS- stimulus resulted in an unrewarded neutral symbol. Following the disappearance of the outcome image, both cells were cleared, and a new

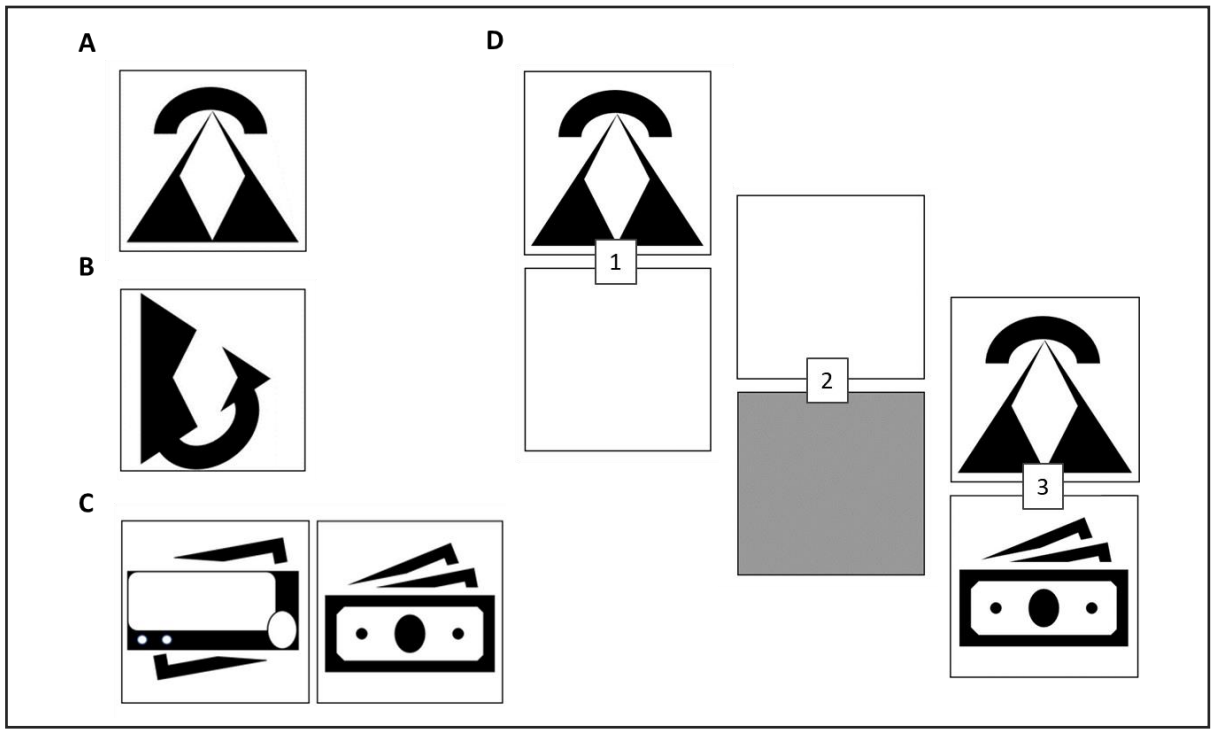


Figure VII.5. Pavlovian procedure of Chapter VII. (A) CS+ image, rewarded. (B) CS- image, unrewarded. (C) Left: neutral outcome. Right: Rewarding outcome. (D) Trial sequence on the screen. 1: Conditioned stimulus. 2: Grey square during which participants are instructed to press the spacebar. 3: Conditioned stimulus and outcome.

trial commenced after a 2000 ms ITI. In order to focus participant's attention and facilitate the monitoring of their gaze during the task, they were instructed (Appendix E) to press the spacebar with their dominant hand when the grey square was presented between the CS and

the outcome, regardless of their nature (CS+/US or CS-/neutral). Their response did not affect the value of the outcome.

Eye-tracking

The position of the participant's gaze during the pavlovian task was measured using the webcam-based Gorilla eye-tracking. This eye-tracking, only available in beta version on Gorilla at the time of testing, used the WebGazer.js library (Papoutsaki et al., 2018) which extrapolates the position of the gaze on the screen in real time. Individual calibration of the eye-tracking prior to the task consisted in following fixation points appearing in different areas of the screen; detection was considered satisfactory if circles around fixation points were small and separated. The head was stabilised with a head rest throughout the procedure, but the accuracy of head movements was monitored throughout the task and a 'face confidence' threshold was calculated based on the consistency of head mapping, with 1 suggesting a high confidence and 0 signifying that the position was lost and not reliable. Trials for which the threshold did not exceed 0.5 were excluded. Accordingly, participants with an insufficient number of remaining trials (due to the face confidence threshold or to missing data) were excluded as well.

Sign- and goal-tracking classification

To calculate an index score approaching that used in rodent procedures and identify participants who might have attributed the predictive cue with incentive salience, we used participant's gaze as an indicator of appetitive responses and measured the total proportion of time spent fixating the cell containing the CS+ during a whole CS+ trial (or the total proportion of time spent fixating the cell containing the CS– during a whole CS– trial), and the total proportion of time spent fixating the cell containing the US during a whole trial, for every trial of the second half of the conditioning (block 2; to measure conditioned responses once the association is learnt). These measures were then averaged for each participant. It is

worth noting that in this pilot experiment designed to assess the feasibility of the procedure, the classification differed from rodent paradigms in that responses were recorded within a whole trial instead of being monitored specifically during CS+ presentation; future experiments should adjust the analysis accordingly to obtain more comparable and precise data. A 'Tracking index' was calculated during the second half of the pavlovian conditioning (block 2) as the average gaze time on the CS+ zone minus the average gaze time on the US zone divided by the total gaze time: *(CS gaze time – US gaze time) / (Total gaze time)*. We obtained a score ranging from -1.0 to 1.0 (Garofalo and di Pellegrino, 2015). Participants scoring from 1.0 to 0.3 were classified as sign-trackers whereas those scoring from -0.3 to 1.0 were considered goal-trackers. Participants with intermediate scores were not included in this study as we wanted to compare more radical learning strategies. We determined this threshold to ensure each group would be composed of sufficient participants.

End-of-experiment survey

The final survey aimed to assess participants' strategies as well as their perception and comments on the study (Appendix F). Participants were asked to rank their desire ('I wanted to interact / to obtain…') as well as their pleasure ('I liked to interact…') to interact with the elements composing the pavlovian conditioning. Questions were also asked about their understanding of the task, such as the relationship between the CSs and the US, and whether their strategy evolved throughout the sessions. In a separate part, experimenters inquired about the feelings participants had whilst undertaking the study (*i.e.*, to rank their enjoyment, challenge, boredom, frustration and confusion).

Statistical analysis

GraphPad Prism (versions 8 and 9; GraphPad Software Inc.; San Diego, CA, USA) was used for ANOVAs, correlations and independent group comparisons. Chi-square and Fisher analyses were conducted on SPSS (versions 27 and 28; IBM Corp.; Chicago, IL,

USA). All group comparison results are presented as mean + SEM. Statistical significance was set at 0.05. Measures were all checked for normality using the Shapiro-Wilk test, and non-parametric tests were used when appropriate. Phenotypic repartition between males and females as well as demographic data (*i.e.*, current status, education) were compared using the Chi-square test of independence. When groups contained two categories (*i.e.*, dominant hand), which violated assumptions of the Chi-square's test, Fisher's exact test was conducted. Repeated measures such as behaviour evolution between block 1 and block 2, the interaction between CS+ and CS-, the preference for a specific zone of the screen, or impulsivity measures such as IGT choice distribution and GNG commission errors between blocks 1 and 2, were analysed using two-way RM ANOVA. When significant, RM ANOVAs were followed by post-hoc Šídák multiple comparison's correction tests to determine in which specific attentional bias group the differences in CR evolution and CS+ discrimination were detected. Comparisons between two independent groups such as gender differences in attentional bias or in impulsivity scores were investigated using the parametric independent *t*-test, or the non-parametric Mann-Whitney test. Correlations analyses were conducted using Pearson's correlations when the dataset met normality assumptions, or Spearman's correlations when it did not. A Bonferroni correction was applied after multiple correlations to reduce the risk of type I error. Participants were excluded from the analysis because of incomplete and unreliable eye-tracking datasets, as well as for having more than two switch points in the DDT. All figures were produced on GraphPad Prism.

Results

Pavlovian conditioning: behavioural phenotypes, learning and gender differences

In order to assess the degree of individual variation in learning strategies and attentional bias, participants undertook a pavlovian task after testing the impulsivity. Nine participants were excluded from behavioural analyses (1 female and 3 males from the first

task, 2 females and 1 male from the second task, 1 female and 1 male from both) due to a combination of incomplete data returned by the software and unstable eye-tracking camera calibration preventing gaze measures to be reliable. A Tracking index was calculated during the second half of the *second* Pavlovian task (*cf.* Methods) based on the proportion of time fixating the zones containing the CS+ and the US, and was used to categorise participants into 'sign-tracking' and 'goal-tracking' groups. Figure VII.6-A shows that a consistent 30% of participants for both genders mostly gazed towards the predictive cue, whereas 40% of men and 21% of women spent more time fixating the reward zone, and from 30% to 47% of

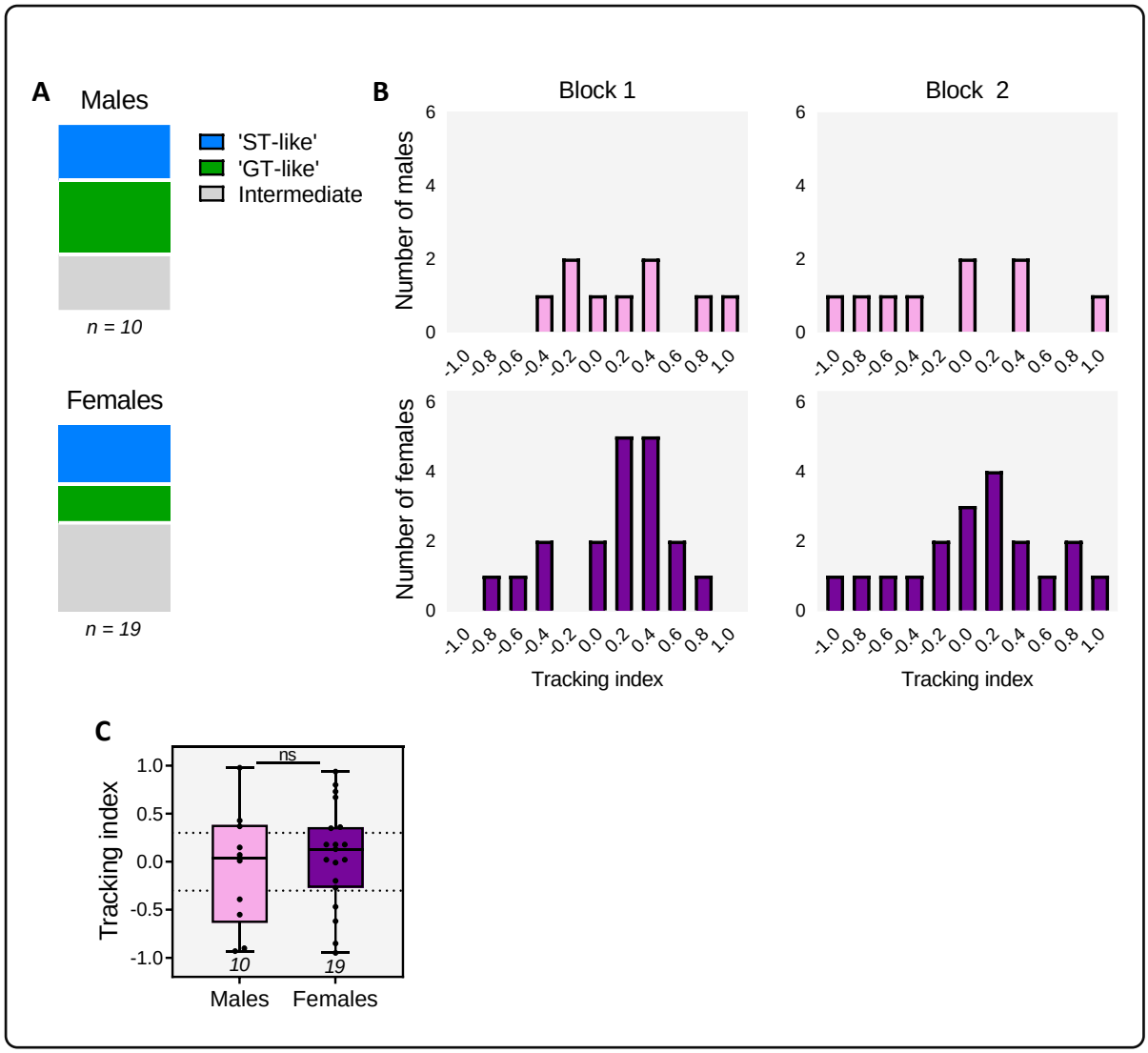


Figure VII.6. Phenotypic repartition and evolution across blocks. (A) Repartition of male and female participants displaying an attentional bias towards the predictive cue, towards the reward location, or an intermediate behaviour. (B) Distribution of the Tracking index for each block in male and female participants. (C) Comparison between the Tracking index of male and female participants.

participants did not show a preference towards either. Phenotypic repartition was similar between both genders ($\chi^2 = 1.336$, df = 2, p = 0.513). The distribution of the Tracking index was not skewed towards a specific zone (Figure VII.6-B), and no gender difference was observed in the average Tracking index (Figure VII.6-C; unpaired *t*-test: t = 0.6316, df = 27, p = 0.533).

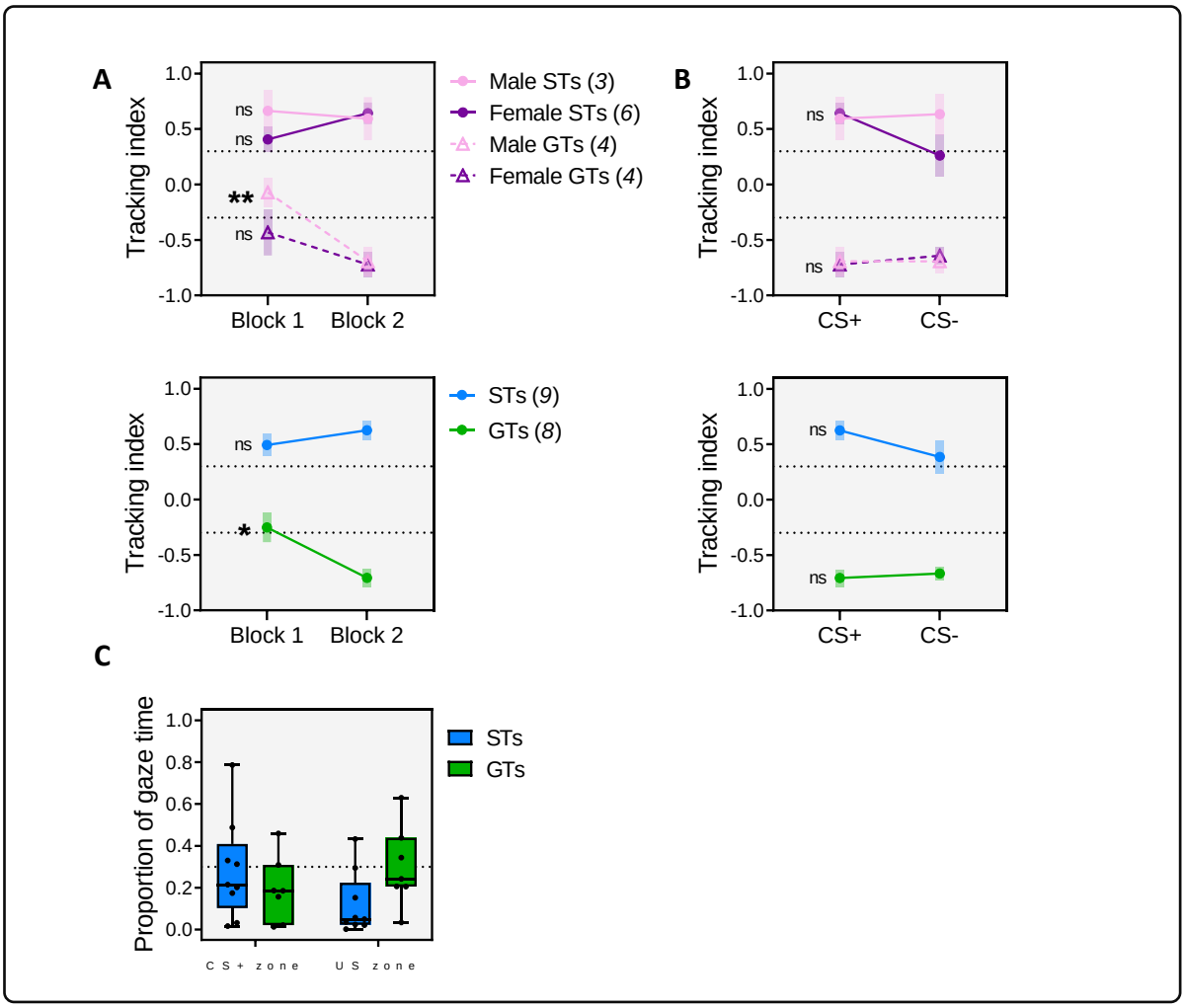


Figure VII.7. Indicators of pavlovian learning. (A) Top: Evolution of the Tracking index across blocks between males and females displaying an attentional bias towards the cue, and males and females displaying an attentional bias towards the reward. Effect of sex, effect of the block. Bottom: Evolution of the Tracking index between both attentional biases, genders combined. Effect of the block. (B) Top: Assessment of the response towards the rewarded cue CS+ and towards the distractor CS- during the second block between males and females displaying an attentional bias towards the cue or towards the reward. Effect of sex, effect of the block Bottom: Assessment of the response towards the CS+ and the CS- during the second block between both attentional biases, genders combined. Effect of the block. (C) Proportion of time spent gazing at the zone containing the CS and towards the zone containing the US during the first block, when participants are naive. (* p ≤ 0.05, ** p ≤ 0.01).

Sign- and goal-tracking are two conditioned responses that develop as the individual learns the association between the predictive cue and the reward availability. However, Figure VII.7-A illustrates that the behaviour of sign-trackers did not develop between the first and the second block of the second pavlovian task: a two-way repeated measures ANOVA with gender and sign-tracking as between-subjects factors and block as a within-subject factor revealed no effect of block for participants with an attentional bias towards the cue ('sign-trackers': $F_{1, 7} = 0.6349$, $p = 0.4517$). Another ANOVA with gender and goal-tracking as between-subjects factors and block as a within-subject factor showed that males tended to increase their goal-tracking with extended training (Effect of block: 'goal-trackers': $F_{1, 6} = 28.67$, $p = 0.0017$; Šídák, $p = 0.0042$), but not females ($p = 0.1020$). No effect of gender was found for 'sign-trackers' ($F_{1, 7} = 0.3772$, $p = 0.5585$) or 'goal-trackers' ($F_{1, 6} = 1.021$, $p = 0.3512$). Accordingly, when combining genders, an effect of block ($F_{1, 15} = 4.866$, $p = 0.0434$; 'goal-trackers', $p = 0.0013$) and an interaction block x group ($F_{1, 15} = 16.28$, $p = 0.0011$) can be observed (Figure VII.7-A). Another characteristic of sign- and goal-tracking phenotypes is that their relative CRs are expressed preferentially during CS+ trials, as opposed to CS- trials. When examining the Tracking index during CS+ trials and another equivalent index calculated with the interaction towards CS- during CS- trials, the CR did not appear to be preferentially expressed during CS+ trials over CS- trials regardless of the gender or the attentional bias (Figure VII.7-B, top; effect of the CS: $F_{1, 26} = 0.3660$, $p = 0.5504$), or when combining participants from both genders (Figure VII.7-B, bottom; effect of the CS: $F_{1, 15} = 1.701$, $p = 0.2119$). Interactions towards the CS+ and the CS- were similar during the first block for both STs and GTs, and they did not differ during the second block either (data not shown). To explore the aforementioned results further, the proportion of time naive participants spent gazing at the CS+ zone and the US zone during the first block of the first Pavlovian conditioning, thus before associative learning occurred, was measured between participants later allocated to the 'sign-tracking' and 'goal-tracking' groups (Figure

VII.7-C). No significant inherent preference was observed for either group (effect of zone: $F_{1, 14} = 0.1471$, $p = 0.7071$). All together, these elements suggest that in our testing conditions the pavlovian conditioning was not completely effective or sufficient in developing sign-tracking conditioned responses; instead, we might have observed inherent individual variation in attentional bias.

Impulsivity: Relationship between tests, age and gender differences

Before the behavioural task participants filled in the self-report BIS-11 questionnaire, from which four measures were extracted: a total score and three subscores assessing attentional, motor and non-planning impulsivity. Although literature has shown that the BIS-11 had a robust internal consistency (Cronbach's α of 0.83), the reliability of the scales was calculated in the present experiment. The non-planning subscale did not meet the consistency criterion of <0.5 and was therefore excluded from subsequent analyses (Table VII.2). Three additional impulsivity tasks were undertaken between the first and the second behavioural task. From the Iowa Gambling Task (IGT), designed to test decision-making and choice impulsivity, an average score of the last 40 trials – once the strategy is established – was calculated. Go-NoGo (GNG) responses were measured using commission errors during the first and second block. Finally, the area under the curve (AUC) was used to estimate impulsivity in the Delay Discounting Task (DDT); two participants were excluded from this test due to having more than two 'switch points'. These impulsivity measures were not found to be related to each other (Figure VII.8: all Pearson or Spearman $r > -0.06514$, all $p > 0.2145$).

Table VII.2. Internal reliability of the bis-11 total scale and subscales.

	No. of items	Cronbach's α
Total score	30	0.717
Attentional	8	0.615
Motor	11	0.595
Non-planning	11	0.408

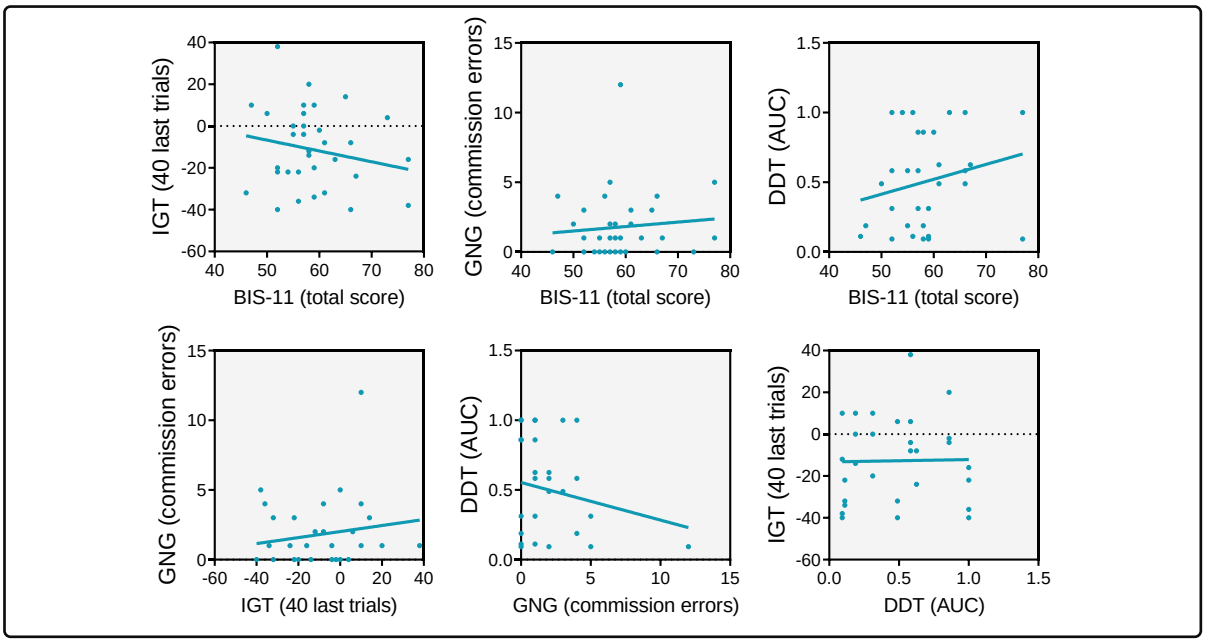


Figure VII.8. Relationship between impulsivity tests. Correlations between the BIS-11 total score, the last block of the IGT, commission errors during the second block of the GNG, and the area-under-the-curve of the DDT.

The only gender difference observed in impulsivity measures was found in the IGT, in which males made significantly more impulsive choices during the 40 last trials (Table VII.3 and Figure VII.9-A; unpaired *t*-test: t = 2.516, df = 32, p = 0.0171). This was supported by the higher selection of deck B (unpaired *t*-test: t = 3.105, df = 3230 p = 0.0041), which held disadvantageous cards, and the lesser selection of favourable decks C (unpaired *t*-test: t = 2.388, df = 30, p = 0.0234) and D (Mann-Whitney U = 49, p = 0.0070), compared to females (Table VII.3). Scores obtained in all other impulsivity tasks were similar for both genders (Table VII.3 and Figure VII.9-A; all unpaired *t*-test: t > 0.5457, df = 32, p > 0.2521; DDT: Mann-Whitney U = 106.5, p = 0.6062; GNG: $F_{1, 32} = 0.2874$, p = 0.5957). Older males

showed a tendency to be more impulsive than their younger counterparts on the second half of the Go-NoGo task, although the low number of male participants along with the necessity of using a potentially less powerful non-parametric test might have impacted the significance (Figure VII.9-B, middle; Spearman r = 0.6298, p = 0.0565). Age was otherwise not related to specific impulsivity measures (Figure VII.9-B; all Pearson or Spearman r > -0.5887, all p > 0.0791).

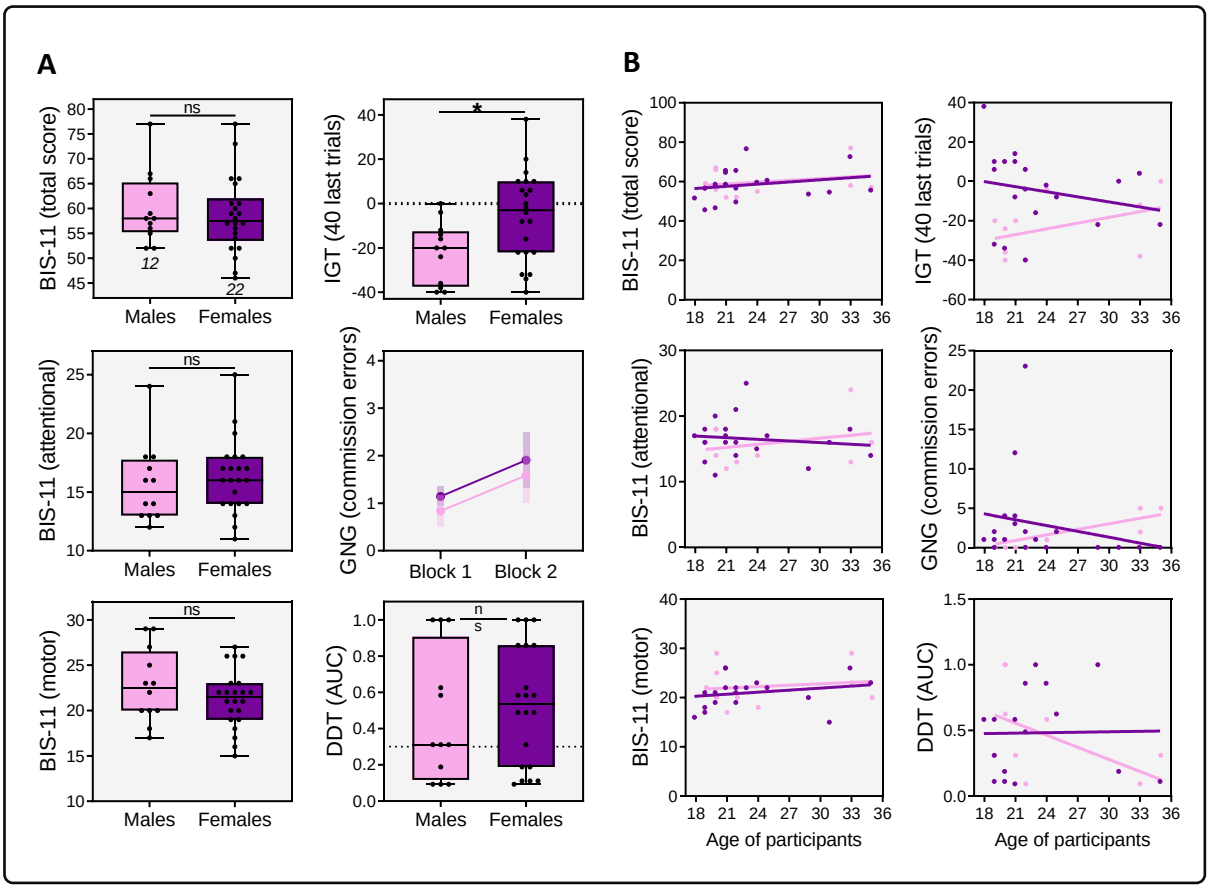


Figure VII.9. The impact of gender and age on impulsivity measures. (A) Comparison between male and female participants in all impulsivity tests. (B) Relationship between impulsivity measures and the age of participants for men and women. (* p ≤ 0.05).

Table VII.3. Impulsivity measures and gender (* p ≤ 0.05).

	Males (n = 12)	**Females** (n = 22)	***p*-value** (*t*-test / M-W)
Barratt Impulsiveness Scale 11			
Total score	60 ±*2.08*	58.5 ±*1.7*	0.5909
Attentional scale	15.67 ±*0.96*	16.15 ±*0.72*	0.5731
Motor scale	22.75 ±*1.17*	20.8 ±*0.67*	0.2521
Go-NoGo			
Block 1: Commission errors	0.83 ±*0.32*	1.3 ±*0.29*	0.8779
Block 2: Commission errors	1.58 ±*0.57*	3 ±*1.21*	0.8690
Iowa Gambling Task			
% Deck A	17.5 ±*3.54*	17.9 ±*1.7*	0.9191
% Deck B	59.4 ±*5.51*	37.7 ±*4.15*	0.0041**
% Deck C	10.3 ±*2.26*	17 ±*1.66*	0.0234*
% Deck D	12.9 ±*3.37*	27.5 ±*3.36*	0.0070**
Score of 40 last trials	-22 ±*4*	-7.1 ±*4.55*	0.0171*
Delay Discounting Task			
AUC	0.467 ±*0.1*	0.53 ±*0.07*	0.6062

Relationship between impulsivity and attentional bias: gender differences

No significant difference was found in the decks selected during the IGT (Figure VII.10-A; $F_{1, 15} = 0.000$, $p > 0.999$), the commission error rate of the second block of the GNG ($F_{1, 15} = 0.01174$, $p = 0.9152$) or the discounted values of the DDT ($F_{1, 15} = 0.5300$, $p = 0.4778$) between participants who spent more time looking at the CS+ zone and participants who fixated the US zone more. Because the Tracking index ranges from negative to positive scores, the proportion of time participants spent gazing at the zone containing the CS+ was used to further investigate the relationship between impulsivity and attentional bias. Indeed, both measures were highly correlated (Figure VII.10-B; Pearson $r = 0.7347$, $p < 0.0001$). A correlation was found between women who spent less time gazing at the CS+ zone and higher impulsivity in the DDT (Figure VII.10-C; Pearson $r = 0.4862$, $p = 0.0478$). Additionally, men who spent more time looking at the CS+ zone were more impulsive in the

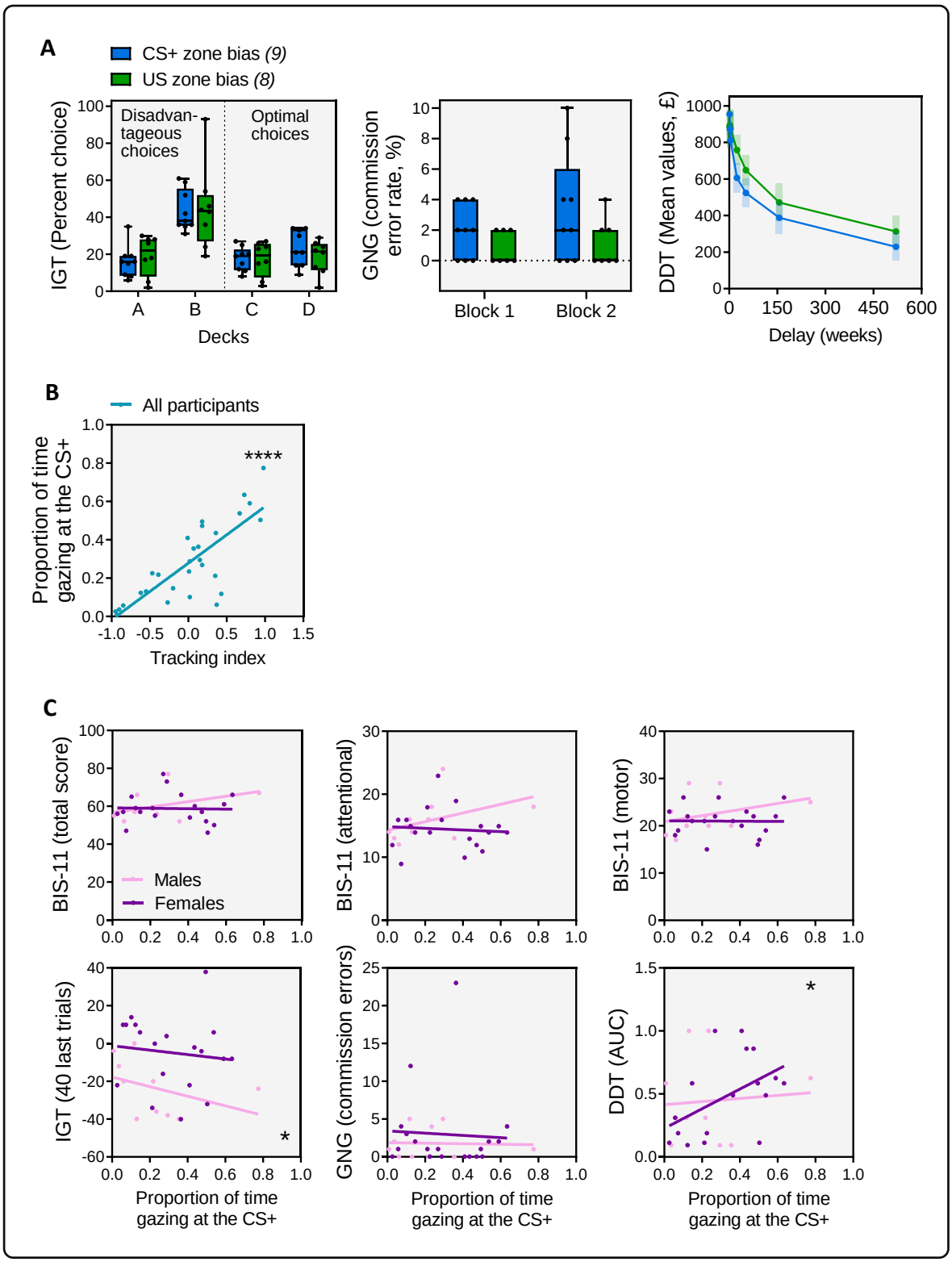


Figure VII.10. Relationship between impulsivity and behavioural responses. (A) Comparison of impulsivity performances between sign-trackers, goal-trackers, and participants who thought they had to perform an action, genders combined. (B) The Tracking index is correlated to the proportion of time participants spent gazing at the CS+ zone. (C) Relationship between impulsivity measures the attentional bias in male and female participants. (* $p \leq 0.05$, **** $p < 0.0001$).

IGT (Figure VII.10-C; IGT 40 last trials: Spearman r = -0.6829, p = 0.0343). However, neither of these results were confirmed after the Bonferroni correction for multiple correlations. No other relationship was found between participants' gaze and impulsivity scores (all Pearson or Spearman r > 0.3696, all p > 0.0577).

Motivation engendered by the pavlovian task

In the end-of-experiment survey designed to gain insight into participant's experience and thought processes during the study, 73.5% of participants reported taking the task (*i.e.*, looking at the symbols and pressing the spacebar) automatically without focussing on the outcome, 47% were 'indifferent to the outcome', 64.7% felt 'bored' and had 'extraneous thoughts', and 50% believed that pressing the spacebar triggered a specific outcome.

Discussion

In rats, hyperresponsivity to reward cues appears to be associated with higher levels of impulsivity (Flagel *et al.*, 2008; Lovic *et al.*, 2008; Tomie *et al.*, 1998). Pavlovian procedures using computerised stimuli (Garofalo and di Pellegrino, 2015) or built apparatuses (Colaizzi *et al.*, 2022; Cope *et al.*, 2022) with human participants have yielded mixed results regarding this relationship. As all these studies measured impulsivity with self-report questionnaires, Section Two of the present thesis aimed to investigate this hypothesis further by asking participants to undertake various impulsivity tasks assessing different aspects of this personality trait. As expected, due to the study taking place within a university, 79% of participants were taken from a student population and 82% had completed undergraduate studies.

Eye-tracking devices have been widely used to measure appetitive associations, including sign- and goal-tracking behaviours (Cherkasova *et al.*, 2021; Cope *et al.*, 2022;

Garofalo and di Pellegrino, 2015; Schad *et al.*, 2020; Wardle *et al.*, 2018), and both accuracy and consistency are required to ensure that collected responses are reliable. When the present study was designed and carried out, the experiment builder Gorilla (www.gorilla.sc) had started to implement a beta version of a rudimentary webcam-based eye-tracking (Anwyl-Irvine *et al.*, 2018), and it was decided to make use of this convenient tool included in the platform hosting the other tasks. However, the device was still in development and despite the efficiency and responsivity of Gorilla staff, severe trial loss and missing files led to the exclusion of several participants. The eye-tracker has since then been optimised and appears viable (Calabrich *et al.*, 2021; Greenaway *et al.*, 2021).

The behavioural paradigm used in Chapter VII was not effective in identifying individual differences in incentive salience attribution to reward-associated cues. Variations emerge in animal studies as the animals learn that the initially neutral cue predicts the availability of the reward, leading to the development of a sign-tracking, goal-tracking or intermediate profile. As such, the degree of interaction towards the cue or the reward location should progress throughout training as the individual assimilates the CS-US association, and the expression of conditioned responses is expected to be limited to rewarded trials (CS+) over unrewarded trials (CS-) or inter-trial intervals. Instead, human participants did not appear to discriminate between conditioned stimuli and only the group interacting more with the US intensified said interaction during the second block; what is more, the two groups appeared to diverge prior to the pavlovian association being learnt. Although a slight difference at the beginning of training can sometimes be found for lever-directed behaviours between sign- and goal-tracker rats (Flagel *et al.*, 2009; Meyer *et al.*, 2012a; also see Chapter III), the combined lack of these two learning indicators – CS discrimination and evolution of CR – strongly suggests that the discrete phenotypes detected were inherent attentional biases rather than pavlovian learning profiles and, consequently, observations drawn from this experiment did not allow to answer the initial research question or compare our results

with the work of Garofalo and di Pellegrino (2015). Given that attentional biases influence the propensity to develop attraction for reward-associated stimuli (Anselme and Robinson, 2020), it is still possible that the two behaviours we observed might have contributed to a probability for individuals to be sign- and goal-trackers in different conditions.

The setup of the behavioural task might explain the failure to measure sign- and goal-tracking phenotypes. An important limitation when attempting to replicate animal paradigms is to motivate human participants to an appropriate and equivalent extent, and testimonies obtained in the final survey suggest that many participants were unfortunately 'indifferent to the outcome' and 'bored'. Primary rewards, such as food or mating, are necessary to ensure the survival of individuals and thus the propagation of replicators, whereas secondary rewards derive their value from natural rewards when associated with them (Schultz, 2015). In many human cultures money has become as potent as primary rewards and is considered to activate the same dopaminergic mesolimbic circuit (Breiter *et al.*, 2001; Knutson *et al.*, 2001 – however, see Sescousse *et al.*, 2013 who shows that different rewards activate specific regions, and Wardle *et al.*, 2018 who discussed the translational validity of using secondary reinforcers instead of biologically significant ones), which is why numerous human studies use monetary gratification (Cherkasova *et al.*, 2021; Colaizzi *et al.*, 2022; Garofalo and di Pellegrino, 2015) to circumvent the obvious ethical and practicality matter of delivering food repeatedly to participants with diverse dietary requirements. The current study attempted to induce motivation by associating the CS+ symbol with a 'virtually rewarding' outcome of an illustration representing a bank note, and by verbally stressing its appetitive aspect when describing the experiment to participants ('The objective of this task is to obtain as many bank notes as possible'). This evidently proved insufficient to assign motivational value to the rewarding outcome and, together with the length and pace of the task, led to participants feeling detachment and boredom. Offering a real reward as well as cutting the behavioural

task into several sessions might improve participant's incentive to learn and would also be more comparable to the procedure given to rats.

Although results obtained in the present chapter did not assess whether human sign- and goal-tracking phenotypes were associated with distinct levels of impulsivity, they still enabled the comparison between individual variation in attentional biases and several aspects of impulsivity. Whilst it is commonly accepted that the Go-NoGo can reflect response inhibition (Aichert *et al.*, 2012; Lane *et al.*, 2003) and that the Delay Discounting Task evaluates impulsivity through the ability to delay gratifications (Lovic *et al.*, 2011; Tomie *et al.*, 1998), whether the Iowa Gambling Task directly assesses choice impulsivity is less evident. Human and rodent studies suggest that poor decision-making in this task can be positively correlated to impulsivity (Barrus *et al.*, 2015; Burdick *et al.*, 2013; Franken *et al.*, 2008; Upton *et al.*, 2011), potentially because making optimal decisions requires the ability to develop a flexible strategy by considering the consequences of previous choices, which is more difficult for individuals with a poorer impulse control who might instead focus on selecting greater and immediate rewards. However, as performance in the IGT was not correlated to impulsivity measured using the other tests, it should be kept in mind that we might have only observed decision-making using this task, and not choice impulsivity.

Participant's gaze was not skewed towards any particular object (CS+ or US) and were similar between genders. Male participants made more disadvantageous choices than females on the Iowa Gambling Task, which is in line with the fact that men have been shown to display more problem gambling in most countries (Calado and Griffith, 2016), but strangely discordant with previous findings reporting that females consistently chose more disadvantageous strategies in this specific behavioural task in both human (Bechara and Martin, 2004; Reavis and Overman, 2001; van den Bos *et al.*, 2013) and rodent studies (van den Bos *et al.*, 2006; van den Bos *et al.*, 2013). It is also worth noting that the score of men was significantly poorer than what is commonly observed in healthy participants. This

discrepancy might be due to the low number of male participants or due to the age which, especially in males, has been associated with more detrimental decision-making on the Iowa Gambling Task (Reavis and Overman, 2001) – although our male participants ranged from 19 to 35 years of age with an average of 24 years old, which should not justify such an extreme difference. In addition, no such difference between genders was found in Chapter IX wherein participants undertook the same decision-making task which suggests that results might not be fully reliable, possibly due to the imbalance between male and female samples or to the other tests undertaken during the study impacting the results. Men and women of the present experiment scored similarly in all the scales of the self-report impulsivity questionnaire BIS-11, in the inhibitory motor task Go-NoGo, and in the Delay Discounting Task. These impulsivity tests returned mixed results in literature depending on the study; a meta-analysis revealed higher impulsivity scores from male participants in the BIS-11 total score and subscales, no gender difference in the Go-NoGo inhibitory control task nor for delay discounting (Cross *et al.*, 2011) whereas other studies found no BIS-11 gender differences (see review from Stanford *et al.*, 2009). These distinct assessment methods provide unique information about the multiple aspects involved in impulsive behaviour, which is reflected by the absence of correlation found in our tests and often in the literature as well as between self-report and behavioural measures (Anker *et al.*, 2009; De Wilde *et al.*, 2013; Lane *et al.*, 2003; Sanchez-Kuhn *et al.*, 2017), although performances do sometimes relate (Enticott *et al.*, 2006; Ortner *et al.*, 2003; Sweitzer *et al.*, 2008). The reason for which the non-planning subscale of the BIS-11 did not meet the reliability criterion is unknown, but the Cronbach alpha has been reported to vary depending on testing conditions and the audience undertaking the test instead of being inherent to a specific scale (Brown, 2002).

Female participants who gazed more towards the outcome stimulus were found to have slightly more difficulties delaying gratification in the DDT; on the other hand, men –

who made more disadvantageous choices than females on the IGT – were also more impulsive on this task when exhibiting a greater attentional bias towards the predictive cue. These results would deserve to be repeated to examine whether they are robust. As males did not spend a higher proportion of time than females looking at the CS+ stimulus, the variation in attentional bias alone cannot account for this gender difference in impulsivity scores. No other correlations were found between participant's attentional preferences and impulsivity measures. In 2018, Wardle and colleagues compared the proportion of time looking at a CS+ picture to performances in a Stop Signal Reaction Task which, similar to the Go-NoGo task, measures response inhibition, and to BIS-11 scores, and found that a biased attention for the CS+ was associated with a greater impulsivity on both tasks (Wardle *et al.*, 2018). Impulsivity has also been shown to be related to the strength of appetitive conditioning including by moderating attentional bias towards reward cues, as illustrated by studies assessing the link between self-report impulsivity and food-cue reactivity (Hou *et al.*, 2001; Tetley *et al.*, 2010), although impulsivity measured by self-report scales and response inhibition performances do not appear to be involved in the acquisition of appetitive responses themselves (Papachristou *et al.*, 2013). However, it is crucial to remember that the present experiment did not truly measure appetitive responses; participants did not successfully learn that the cue predicted the rewarding outcome and, most of all, did not report *wanting* said rewarding outcome in the end-of-experiment survey. As such, no positive valence was attributed to the 'reward', and attentional biases should not be considered as indirect incentive measures. Instead, it is possible that participants varied in their focus with some individuals heeding the stimulus that experimenters had described as being an 'outcome' or an 'objective' even in the absence of motivation and learning, and others choosing to absently fixate the other stimuli. Alternatively, participants might have simply been biased towards the bottom half of or the top half of the screen based on other unknown parameters, irrespective of instructions and stimuli.

The experiment of Chapter VII is limited in its interpretations. Using a more reliable eye-tracking device, establishing a stronger motivation for the rewarding outcome, and finally augmenting the sample size – and particularly the number of male participants – to increase the power of the experiment, might allow to replicate previous findings and investigate whether sign-tracking behaviour is associated with different types of trait impulsivity.

Chapter VIII
Virtual room-based pavlovian environment

Introduction

Computerised pavlovian procedures are rather remote from their rodent counterparts in that subjects are not truly interacting with the stimuli; the nature of the expressed conditioned responses is therefore not directly comparable and can vary depending on the measures taken. By allowing participants to visualise objects, to interact with them, and to move in space, computer-generated virtual environments recreating real-life setups might enable the development of more natural and viable conditioned responses associated with the reward. Such virtual environments have been shown to successfully elicit conditioned place preference (Astur *et al.*, 2015) and extinction of aversive conditioning (Alvarez *et al.*, 2007) as well as illustrating various neutral and appetitive cognitive processing in invertebrates (Buatois *et al.*, 2020); however, no publication involving pavlovian conditioning was uncovered. Additionally, testing participants using a digital environment allows to make the study available to a greater number of subjects, thereby increasing the sample size and improving the generalisability of research findings.

Chapter VIII therefore describes a virtual pavlovian procedure designed to evaluate whether individual differences in response strategies observed in animal studies can be replicated in humans, and whether such phenotypes are also linked to impulsivity and addictive behaviours (Flagel *et al.*, 2009; Lovic *et al.*, 2011; Robinson & Berridge, 1993; Saunders and Robinson, 2013; Tomie *et al.*, 2008).

Materials and Methods

Participants

A total of 327 participants (Table VIII.1) took part in this study (a priori power analysis: N = 347; d = 0.15, $\alpha = 0.05$; $\beta = 0.80$). They were recruited through Prolific (https://prolific.co/; version 2022) which allowed us to offer the study to participants who

indicated having no neurological or psychiatric conditions, no learning disabilities, and no medical condition that could cause sensitivity to flashing lights onscreen or to motion cues (*e.g.,* epilepsy, travel sickness or vertigo). Informed consent was given through the same recruiting platform. Participants indicated their current status (*i.e.*, student or not, field of neuroscience / psychology or not), their past education and their dominant hand. The study was remunerated £4.38 upon completion with an average of £11.53 per hour. This work was approved by the Open University Human Research Ethics Committee (HREC/4012/Rostron) as well as King College London's Ethic Committee and conducted in accordance with the General Data Protection Regulations.

Experimental procedure

Participants took the entire study on their own devices but were instructed to use computers instead of tablet or mobiles in order to standardise responses and reaction times and allow better viewing conditions. After giving their consent on Prolific, they were redirected to Qualtrics (Qualtrics; Provo, Utah, USA; version 2021) where three forms were presented: a self-assessed impulsivity questionnaire, an alcohol use disorder identification test, and a drug abuse screening test (Figure VIII.A). After completing these questionnaires, participants were sent to a third server hosting a virtual pavlovian conditioning task designed by researchers at the Open University and programmed by Gwyneth Morgan, an Open University interactive media developer. Participants subsequently answered three questions designed to gain feedback on the task, and were finally redirected back to Prolific to be remunerated. The time taken to complete the study ranged between 16 and 66 minutes, with an average of 25 minutes.

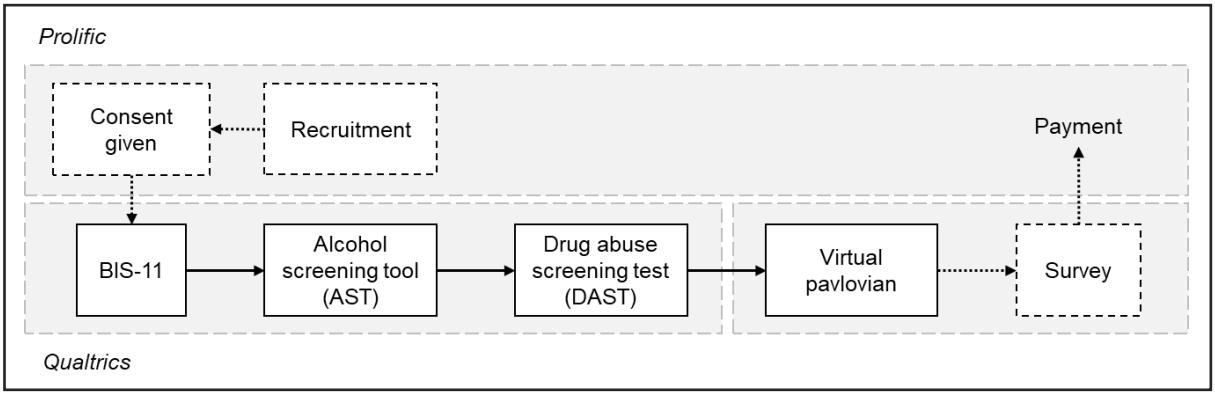


Figure VIII.1. Outline of the experiment undertaken in Chapter VIII.

Table VIII.1. Demographics of Chapter VIII.

	Males (n = 177)	**Females** (n = 150)	***p*-value**
Age (years)	25.9 ±*0.36*	26.7 ±*0.38*	*t*-test: 0.1293
Completed Education			χ^2: 0.712
GCSE	4.5%	2.7%	-
A-levels	27.7%	20.7%	-
Undergraduate	40.7%	41.3%	-
Postgraduate	24.9%	33.3%	-
Other	2.3%	2%	-
Status			χ^2: 0.622
Student: Psycho- or neuro-related	6.2%	6.7%	-
Student: Other	49.1%	41.3%	-
Not student: Psycho/neuro-related	6.1%	7.3%	-
Not student: Other	34.4%	44.7%	-
Dominant hand			Fisher: 0.479
Right	87.6%	90%	-
Left	10.7%	10%	-
Ambidextrous	1.7%	0%	-

Measures of impulsivity and substance use

Barratt-Impulsiveness Scale 11 (BIS-11). As in Chapter VII, participants answered a thirty-questions form in which they self-assessed several subtypes of impulsivity. A global score and second-order scores (*i.e.,* attentional, motor, non-planning) were calculated, and participants with global scores greater than 71 were considered highly impulsive.

Alcohol screening tool (AST or AUDIT). The AST, developed by the World Health Organisation (Saunders *et al.*, 1993) and reported to have a high internal consistency (Cronbach's α of 0.80+; Allen *et al.*, 1997), is composed of ten questions and helps identifying risky and harmful drinking behaviours that occurred in the twelve months preceding the assessment (Appendix G). Our participants had to select the appropriate answer amongst five or three possible event recurrences. Responses were scored from 0 to 5 for the 5-scales questions, or 0/2/4 for the 3-scales questions. When the questionnaire is carried out in a medical context, the health professional starts to discuss the harms associated with alcohol consumption for a score above 7. In the present study, we used scores both as a continuous scale and as categories (0-7 'low risk', 8-12 'moderate risk', 13+ 'high risk').

Drug abuse screening test (DAST). The DAST (Skinner, 1982) is similar to the AST in that it determines the participant's involvement with both medical and non-medical drugs excluding alcoholic beverages (*e.g.,* cannabis, solvent, tranquilisers, barbiturates, cocaine, stimulants, hallucinogens and narcotics) during the last year (Appendix H). The scale has been described as reliable in literature (Cronbach's α of 0.82; Shields *et al.*, 2004). For each of the ten questions, participants had to indicate whether or not they experienced the event stated. Each question was scored 1 point for a 'yes' apart from one reversed question. When undertaken in a medical context, a total score equal or superior to 3 must prompt the assessor to investigate further in order to help the participant with a potentially harmful pattern of behaviour. In our study, we considered the scores both as a continuous scale and as categories (0-2 'low risk, 3-5 'moderate risk', 6-10 'high risk').

Pavlovian conditioning

Stimuli. This task was designed to virtually simulate the paradigm widely used in rodent studies (Flagel *et al.*, 2009) and allow the expression of conditioned responses mirroring those elicited in animal studies. Participants entered a virtual room and were able

to navigate using a mouse, a keyboard, a trackpad or a touchpad. On each wall was a pulling lever and a light button which illuminated in yellow or blue for 5 seconds in a counterbalanced manner during CS+ or CS- trials (Figure VIII.2). The CS+ illumination – independent of participants' action – was always followed by the delivery of a golden coin (US) into a bowl located on top of a table against the centre wall; this lever was therefore a predictive cue. Illumination of the CS- was not followed by any reward ('distractor').

Procedure. As in Chapter VII, participants were instructed to earn and collect as many coins as possible to increase the rewarding value of the US and provide an objective to focus on within the task (Appendix I). Participants were otherwise encouraged to freely explore the environment by moving their viewpoint and interact with the objects within it. Screenshots of the virtual environment can be found in Figure VIII.2. Immediately after entering the room, one 'free' coin was delivered and participants were familiarised with the settings for 4 trials during which data were not recorded, after which the training started. The conditioning session itself consisted of 24 randomised trials (12 CS+/US pairings and 12 CS- illuminations) with a CS duration of 5 seconds and a random ITI of 7, 8 or 9 seconds. Coins were immediately delivered at the end of CS+ illuminations. A click on the coin bowl 'collected' the available coins; however, if the coin dispenser was clicked, the levers were moved up and down or the lights were pressed, it had no consequence. A banner with a coin counter was displayed at all times at the bottom of the screen. At the end of the 24 trials, participants were prompted to exit the virtual room by clicking on the door and answered questions about the method used to perform the task (*i.e.,* mouse, keyboard, trackpad, touchpad, changed method), whether any action allowed to increase the speed at which coins were delivered (*i.e.,* 'nothing made a difference', 'interacting with the US' or 'the CS+' made a difference), and about their strategy within the task (*i.e.,* 'switching the viewpoint', 'keeping it on the US' or 'CS+').

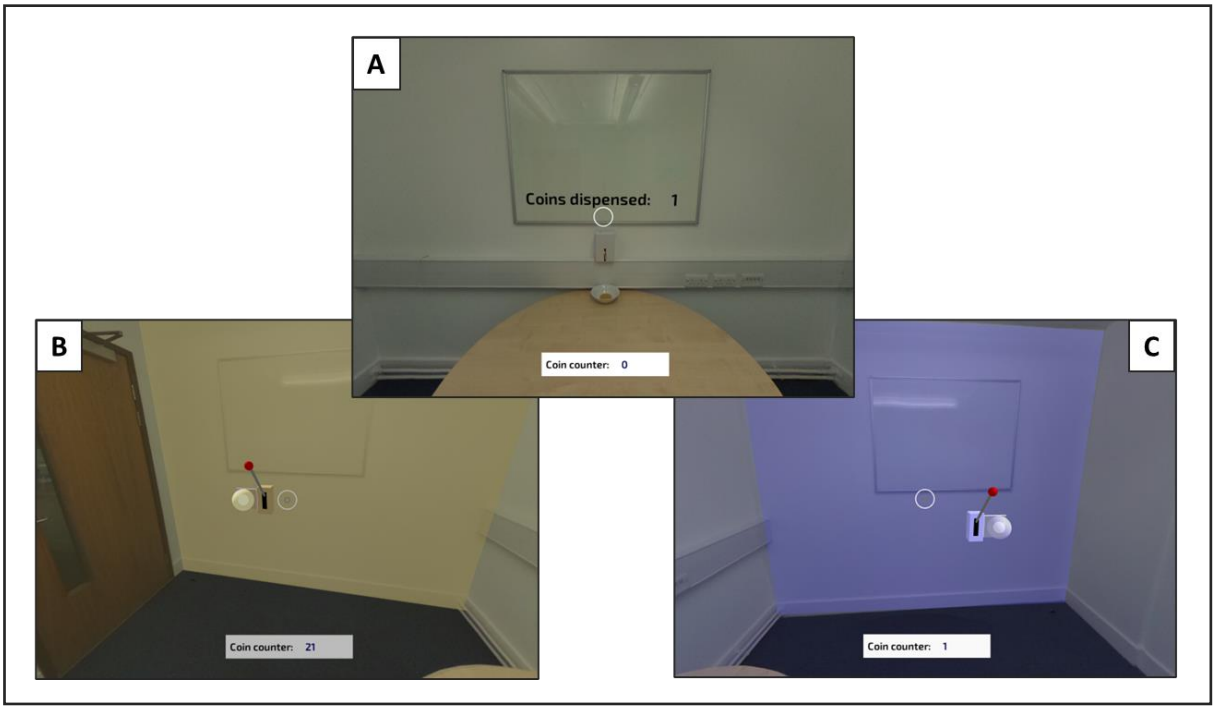


Figure VIII.2. Screenshots of the pavlovian virtual environment. (A) Slot delivering coins (US) into a bowl. (B) Counterbalanced CS+ or CS- light, illuminated in yellow when active in this example. (C) Counterbalanced CS+ or CS- light, which illuminated for 5 seconds in blue when active in this example. Participants interacted with the elements by placing the circle on the target object and 'selecting'.

Sign- and goal-tracking classification

Participants' conditioned responses were evaluated using their interaction with the CS+ (lever and light) and the US (coin dispenser and bowl) at the end of training; both elements forming the CS+ and US were systematically combined as they were considered to be part of the same object. Similar to the rodent PCA index (Meyer *et al.*, 2012a), during the second half of the conditioning session (block 2) the number and latency of clicks on the CS+ and the US during the CS+ illumination were extracted, which allowed to calculate (1) the probability of clicking on the lever/light and the coin dispenser/bowl; (2) the response bias to click the lever/light and the dispenser/bowl during the CS+ period; and (3) the average latency to first click on the lever/light and the dispenser/bowl. We averaged these elements and determined a 'virtual' PCA index score ranging between -1.0 and 1.0 (Figure VIII.3). Participants with PCA index scores ranging from -1.0 to -0.3 were considered goal-trackers, and those with scores greater than or equal to 0.3 were classified as sign-trackers.

Participants with intermediate scores were not included in this study as we wanted to compare more radical learning strategies.

Probability:	$P(CSplus) - P(US)$
Response bias:	$(CSplus\ contacts\ -\ US\ contacts)/(CSplus\ contacts\ +\ US\ contacts)$
Latency:	$((US\ contact\ latency\ -\ CSplus\ contact\ latency))/5$
PCA score:	$((probability\ score\ +\ response\ bias\ score\ +\ latency\ score))/3$

Figure VIII.3. Detail of the 'virtual' PCA score calculation. *Inspired by Meyer et al., 2012*. 'CSplus' indicates clicks on both the CS+ lever and the CS+ light button during CS+ illumination. 'US' designates clicks on both the coin dispenser and the bowl during CS+ illumination.

End-of-experiment survey

At the end of the pavlovian conditioning, participants were asked which method they used to engage with the task (*i.e.*, mouse, keyboard, trackpad, touchscreen, or changed method during the task). Experimenters also inquired about participants' understanding of the relationship between the CSs and the US ('did anything you did within the task caused the delivery of the coins') and whether they developed a strategy during the task. Specific questions can be found in Appendix J.

Statistical analysis

GraphPad Prism (versions 8 and 9; GraphPad Software Inc.; San Diego, CA, USA) was used for ANOVAs, correlations and independent group comparisons. Chi-square and Fisher analyses were conducted on SPSS (versions 27 and 28; IBM Corp.; Chicago, IL, USA). All group comparison results are presented as mean + SEM. Statistical significance was set at 0.05. Measures were all checked for normality using the Shapiro-Wilk test, and non-parametric tests were used when appropriate. Phenotypic repartition between males and females, demographic data (*i.e.*, current status, education) and techniques used to take the study were compared using the Chi-square test of independence. When groups contained two categories (*i.e.*, dominant hand), Fisher's exact test was conducted. Repeated measures such as behaviour evolution across blocks and the interaction between CS+ and CS- were

examined using two-way RM ANOVA. When significant, RM ANOVAs were followed by post-hoc Šídák multiple comparison's correction tests to determine in which specific phenotype the evolution of CR across sessions and the differences in CS+ discrimination were detected. The relationship between impulsivity, phenotypes and genders was analysed with two-way ANOVAs, and significant effects were investigated using Tukey's multiple comparison's correction to assess in which phenotype or which gender the difference was found. Comparisons between two independent groups such as gender differences in phenotype or in impulsivity scores were investigated using the parametric independent *t*-test, or the non-parametric Mann-Whitney test. Correlations analyses were conducted using Pearson's correlations when the dataset met normality assumptions, or Spearman's correlations when it did not. A Bonferroni correction was applied after multiple correlations to reduce the risk of type I error. As detailed hereinbelow (Table VIII.5), participants were excluded from the main analysis or processed separately if data was missing, if they reported an instrumental contingency, or if they did not validate discrimination criteria. All figures were produced on GraphPad Prism.

Results

Scales of impulsivity and drug/alcohol use: interrelationship, age and gender differences

Before being trained in the virtual pavlovian task, all participants filled three questionnaires designed to assess their impulsivity, the extent of their alcohol use and of their drug use. A global score as well as three sub-scores were calculated for the Barratt Impulsiveness Scale 11 (BIS-11), and a single score was extracted from the Drug Abuse Screening Tool (DAST) and from the Alcohol Screening Tool (AST). Table VIII.2 demonstrates that all scales were reliable and were therefore included in the study. Of the 329 participants, only individuals who did not identify as either male or female were

excluded in the present section (Table VIII.3: 327 participants remaining, 177 males and 150 females).

Self-report measures of drug use were positively correlated with scores of alcohol use (Figure VIII.4; Spearman r = 0.3731, p <0.0001). A greater drug use was also associated with a higher global score of impulsivity (Spearman r = 0.1647, p = 0.0027), and a high impulsivity was correlated with more alcohol use (Spearman r = 0.1785, p = 0.0011). All these measures survived the Bonferroni correction for multiple correlations. A Chi-square

Table VIII.2. Internal reliability of the BIS-11, the AST and the DAST scales.

	No. of items	Cronbach's α
BIS-11		
Total score	30	0.817
Attentional	8	0.682
Motor	11	0.635
Non-planning	11	0.700
AST	10	0.833
DAST	10	0.609

Table VIII.3. Measures of impulsivity, drug use and alcohol use between genders.

	Males (n = 177)	**Females** (n = 150)	***p*-value** (*t*-test / M-W)
Barratt Impulsiveness Scale			
Total score	59.7 ±*0.68*	58.4 ±*0.78*	0.1666
Attentional scale	16.2 ±*0.26*	15.4 ±*0.27*	0.0633
Motor scale	21.2 ±*0.29*	20.8 ±*0.33*	0.2057
Non-planning scale	22.3 ±*0.33*	22.2 ±*0.36*	0.8807
Alcohol Screening Tool	5.8 ±*0.35*	5.6 ±*0.44*	0.3468
Drug Abuse Screening Test	0.8 ±*0.1*	0.8 ±*0.09*	0.7915

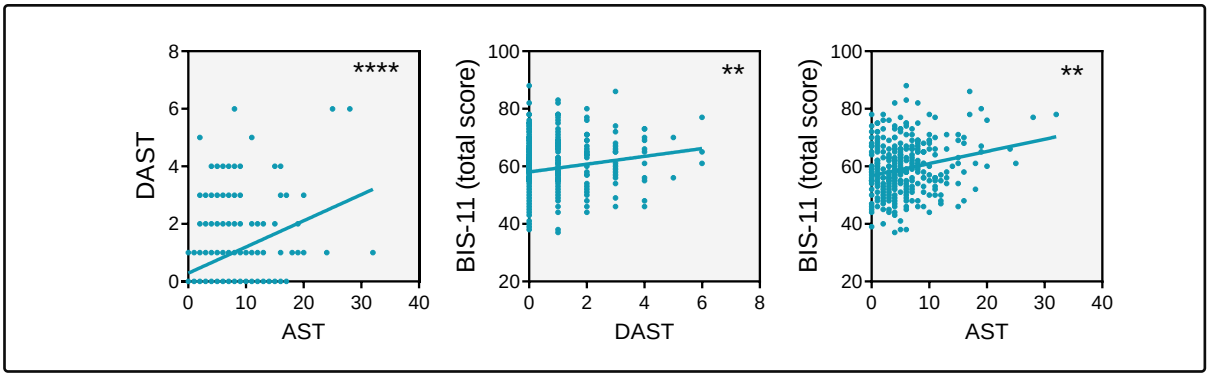


Figure VIII.4. Relationship between traits in participants. Correlation between self-report impulsivity, self-report drug use and self-report alcohol use in all participants, genders combined. (** p ≤ 0.01, **** p < 0.0001).

analysis confirmed a statistically significant association between drug use and alcohol use categories ('low risk', 'moderate risk', 'high risk' of addiction) for all participants (χ^2 = 23.782, df = 4, p <0.001). No gender difference was found in impulsivity, drug use or alcohol use (Table VIII.3). However, a strong relationship was found between impulsivity and age, with younger men scoring more impulsive on the general BIS-11 score (Table VIII.4; Spearman r = -0.3504, p <0.0001), the attentional subscale (Spearman r = -0.2475, p = 0.0009), the motor subscale (Spearman r = -0.2774, p = 0.0002) and the non-planning subscale (Spearman r = -0.2594, p = 0.0005). This effect was observed in a lesser extent in women for the attentional (Spearman r = -0.1894, p = 0.0200) and non-planning (Spearman r = -0.1652, p = 0.0433) subscales, however it should be noted that women's significant results did not survive the Bonferroni correction for multiple correlations.

Table VIII.4. Relationship between age, impulsivity and substance use.
(* p ≤ 0.05, *** p ≤ 0.001, **** p < 0.0001).

	Age	
	Males	**Females**
BIS-11		
Total score	<0.0001****	0.0750
Attentional	0.0009***	0.0200*
Motor	0.0002***	0.9975
Non-planning	0.0005***	0.0433*
AST score	0.5715	0.9963
DAST score	0.0356*	0.2977

Pavlovian conditioning: behavioural phenotypes, learning and gender differences

In a computerised virtual environment, presentation of a light (CS+) was repeatedly paired with the delivery of a coin (US) whereas the presentation of another light (CS-) was not linked to any outcome. Table VIII.5 illustrates participants included in the pavlovian analysis. Out of 329 participants, two did not identify as either male or female and were not analysed due to a small sample size. Datasets of 4 participants (1 male, 3 females) were incomplete and were excluded from pavlovian analysis as well, leaving a final sample of 323 participants. In rodents, sign- and goal-tracking are only and strictly measured using a pavlovian – as opposed to instrumental – setting. Whilst it is difficult to determine whether all rats do learn a pavlovian association or if some individuals learn an instrumental pairing due to the lack of understanding researchers truly have on the experience of animals, it *is* possible to ask human participants. Thus, to assess and compare conditioned responses in these two very distinct learning settings, participants were asked whether they thought any of their actions 'caused' the release of the coin to separate individuals who undertook a pavlovian conditioning from those who believed the reward was dependent upon their behaviour ('instrumental' conditioning). Only participants trained in a pavlovian conditioning were considered to ensure that the identification of learning strategies would be rigorously comparable to rodents; participants who learnt an instrumental task were treated separately. Amongst the 323 remaining participants, 200 did not learn a pavlovian task, and 125 were successfully trained in pavlovian conditioning (66 males and 59 females). Additionally, within the same pool of 323 participants, 145 did not discriminate the CS+ from the CS- (the discrimination rationale is detailed in the next paragraph) and were excluded from the behavioural analysis. Consequently, a total of 65 participants, 40 men and 25 women, were considered compatible with our research question and were compared in the following section.

Table VIII.5. Participants excluded from the behavioural analysis.

Exclusion rationale	Number of excluded participants	Remaining participants	Proportion (%)
	-	329	
'Other' gender (not comparable)	2	327	99.4%
Missing data in the behavioural task	4	323	98.2%
Instrumental or other conditioning	200	-	37.4%
Validated <1 discrimination criterion	145	-	54.1%
Included in the behavioural analysis	65		19.8%

A crucial indicator of whether individuals correctly learnt the pavlovian association is to assess the discrimination between the CS+ and the CS-. This can be determined by (1) measuring the *interaction towards the CS+ and towards the CS-* during their respective trials, and by (2) measuring the *interaction towards the US* during CS+ and CS- trials. If the association is learnt, individuals should exhibit a marked preference towards the CS+ (for sign-tracking strategies) or the US location (for goal-tracking strategies) during CS+ trials. A percentage of discrimination was therefore calculated for both conditions, and each criterion was validated when the CS+ discrimination was above 50%. Participants learnt the task in different ways as they validated either one discrimination criterion, both, or none, and in order to ensure the successful learning of the pavlovian association, only participants who validated one or two discrimination criteria were investigated. As illustrated in Figure VIII.5-A, participants allocated to the 'sign-tracking' and 'goal-tracking' groups *who validated at least one discrimination criterion* (1DC, full lines) interacted with the object of interest of their CRs – the CS+ and the US location, respectively – preferentially and to a greater extent during rewarded trials (CS+ vs. CS-: Interaction CS x Group, $F_{2,62} = 14.92$, $p < 0.0001$; Šídák STs $p < 0.0001$; US: Interaction CSs x Group, $F_{2, 60} = 12.43$, $p < 0.0001$; Šídák GTs $p < 0.0001$). To further illustrate the effect of discrimination criteria on each relevant CR, the interaction towards CSs of STs who did not validate any criterion (NoDC) was compared to STs who did (1DC; Figure 2, left, dotted lines) and the interaction towards the

US of GTs who did not validate criteria (NoDC) was compared to GTs who did (1DC; Figure VIII.5-B). As expected, these participants did not appear to prefer CS+ trials over CS- trials (STs NoDC: p = 0.2271; GTs NoDC: Šídák p = 0.5124).

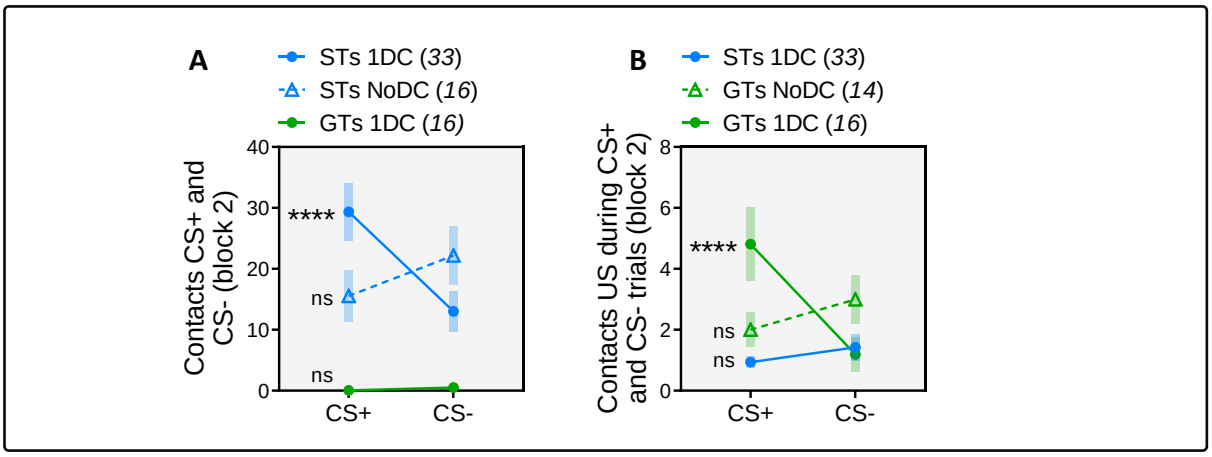


Figure VIII.5. Conditioned responses depending on the number of discrimination criteria validated by participants. (A) Comparison between the number of contacts with the predictive cue and with the distractor during their respective trials on the second training block, genders combined. Sign-trackers who validated at least one discrimination criterion and sign-trackers who did not validate any discrimination criterion. Although the conditioned response of goal-trackers is directed towards the US and not the CS, goal-trackers who validated at least one discrimination criterion are included for comparison. (B) Comparison between the number of contacts with the US during CS+ and during CS- trials on the second training block, genders combined. Goal-trackers who validated at least one discrimination criterion and goal-trackers who did not validate any discrimination criterion. Although the conditioned response of sign-trackers is directed towards the CS and not the US, sign-trackers who validated at least one discrimination criterion are included for comparison. (**** $p < 0.0001$).

Interactions towards the lever and towards the coin dispenser were measured to calculate a PCA score similar to the index used in rodent experiments. Amongst the pavlovian group, 57.5% of males and 40% of females displayed sign-tracking-like tendencies whereas 20% of males and 32% of females showed a goal-tracking phenotype; 25% on average did not favour either learning strategy (Figure VIII.6-A). A Chi-square analysis indicated that phenotypic repartition was not significatively different between genders ($\chi^2 = 2.017$, df = 2, p = 0.365). Pavlovian learning was not skewed towards a particular phenotype (Figure VIII.6-A-B). In contrast, participants who believed they needed to perform an action to obtain the reward ('instrumental') showed a marked attentional bias towards the CS+ (Figure VIII.6-A; 62.5-72.1%) or no preference at all (16.3-22.5%). Very few participants displayed a preference towards the reward location during CS+ presentation ('goal-tracker-like'; 11.6-15%). Despite a slight propensity for men to exhibit a stronger

preference towards the predictive cue, no major gender difference was observed in the PCA index score of 'pavlovian' participants (Figure VIII.6-C; Mann-Whitney U = 374.5, p = 0.0913) or 'instrumental' participants (Figure VIII.6-D; Mann-Whitney U = 751.5, p = 0.3255). When taking all participants together, no difference was found between behaviours expressed by men and women (χ^2 = 1.993, df = 2, p = 0.380).

In rodents and human studies, sign- and goal-tracking are described as learnt conditioned responses that develop throughout training. The virtual pavlovian conditioning was separated into two halves, or 'blocks' in order to compare participant's behaviours at the beginning and at the end of the training. Because of violations of ANOVA assumptions, each group of Figures VIII.6-E (right and left) was analysed separately using the non-parametric Wilcoxon test. Although the PCA score of sign- and goal-trackers from the pavlovian group appeared to diverge increasingly across training with no gender difference at the end of the training, only male goal-trackers significantly increased their phenotype's score across block 1 and 2 (Figure VIII.6-E; Z = 8, p = 0.0156). It is also important to note that both groups appeared to inherently differ in their attentional biases during block 1, when phenotypes were not expected to have yet emerged. Strikingly, a visual assessment suggests that participants from the instrumental group exhibiting an attentional bias towards the CS+ ('STs-like') and towards the US location ('GTs-like') appeared to behave equivalently to their pavlovian counterparts (Figure VIII.6-E). Pavlovian GTs (Z = 16, p = 0.0040), 'STs-like' instrumental participants (Z = 70, p = 0.0001) and 'GT-like' instrumental participants (Z = 13, p = 0.0188) increased their specific behaviour across training blocks (Figure VIII.6-E), but not pavlovian sign-trackers (Z = 32, p = 0.1205).

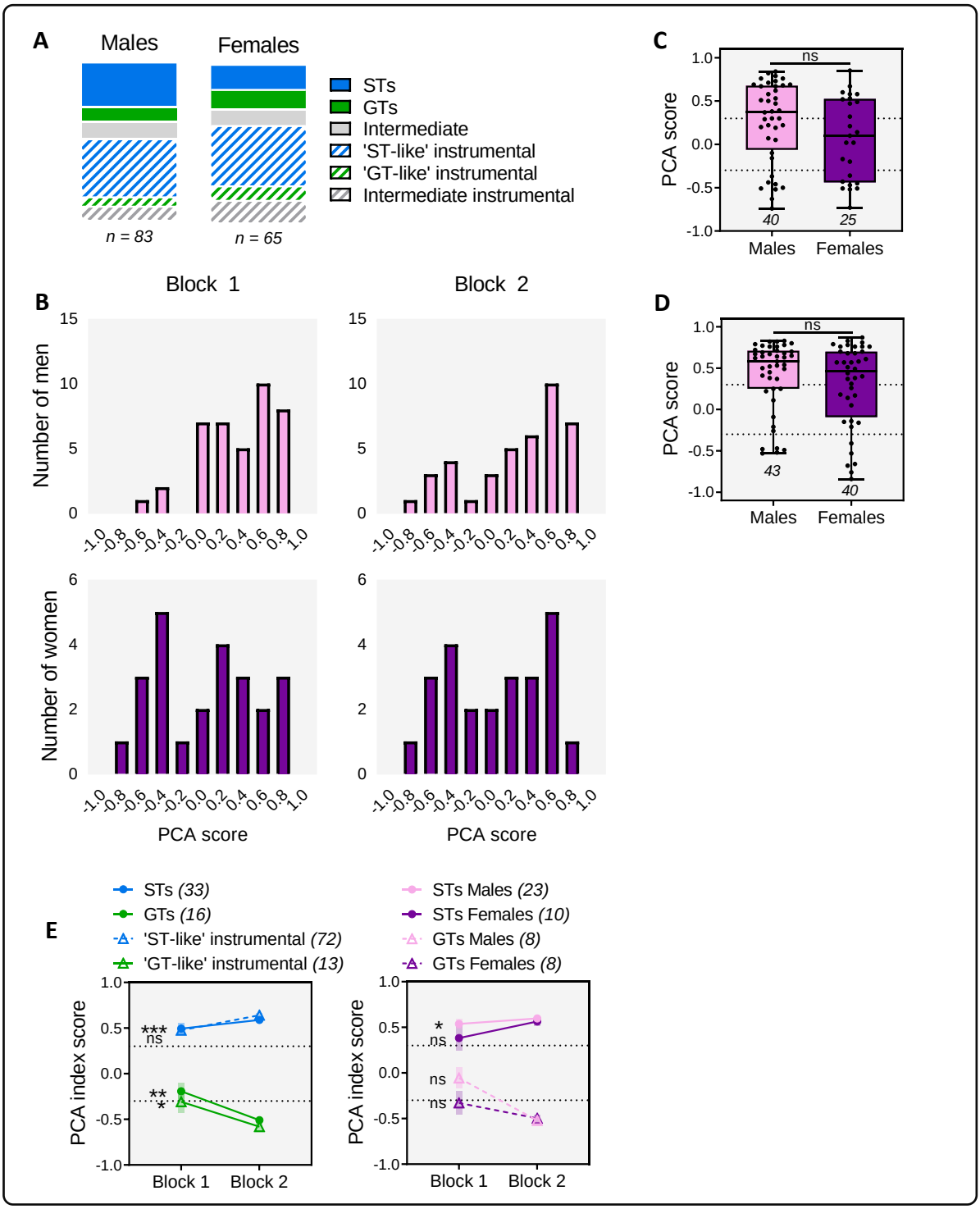


Figure VIII.6. Phenotypic description of participants and indicators of learning. (A) Repartition of male and female participants who believed they were undertaking a pavlovian learning and were thus categorised as sign-trackers, goal-trackers and intermediate; and repartition of male and female participants who thought they needed to perform an action to obtain the reward and were thus classified as 'sign-tracking-like instrumental', 'goal-tracking-like instrumental', and 'intermediate instrumental'. (B) Distribution of the PCA index for each block in male and female participants. (C) Comparison between the PCA index of male and female participants who learnt a pavlovian association. (D) Comparison between the PCA index of male and female participants who thought they needed to perform an action to obtain the reward. (E) *Left*: Comparison between the PCA index during block 1 and block 2 for sign-trackers, goal-trackers, and instrumental participants with an attentional bias towards the CS+ or the US, genders combined. *Right*: Comparison between the PCA index during block 1 and block 2 in male and female pavlovian sign-trackers and goal-trackers. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Relationship between pavlovian learning strategies and impulsivity, drug and alcohol use

To investigate the relationship between tracking phenotypes and self-reports of impulsivity, drug use and alcohol use, the latency to first contact the CS+ was used as an indicator of participant's learning strategy (Figure VIII.7-A: PCA index score x CS+ latency; Spearman r = -0.7109, p <0.0001), to replace the PCA index score which has a positive and negative scale. Only the 65 participants who were included in the initial pavlovian analysis were examined. Apart from a greater report of drug use in women who did *not* approach the CS+ quickly (Figure VIII.7-B; Spearman r = 0.4795, p = 0.0153), which did not survive the Bonferroni correction for multiple correlations, no relationship was found between the interaction towards the CS+ (Figure VIII.7-B), which might reflect the tendency to sign- or goal-track, and impulsivity, drug or alcohol use (all Spearman r > -0.2303, all p > 0.1529). Accordingly, no difference was observed between participants categorised as sign-trackers and those classified as goal-trackers, nor between genders (Figure VIII.7-C; effect of the CR: all $F_{1, 45} < 1.934$, all p > 0.1712).

Unexpectedly, relationships were observed within the instrumental group (Figure VIII.8). Indeed, when combining genders, 'instrumental' women who contacted the CS+ faster were less impulsive in the general score (Figure VIII.8-A; Pearson r = 0.4026, p = 0.0100), the motor subscale (Spearman r = 0.3167, p = 0.0465) and non-planning subscale (Pearson r = 0.3358, p = 0.0341) of the BIS-11 questionnaire. However, only the general BIS-11 score was confirmed after Bonferroni correction. Moreover, females who contacted the CS+ faster reported using less alcohol (Spearman r = 0.3790, p = 0.0159) which, again, did not survive the Bonferroni correction.

No association was found between 'instrumental' male's phenotype and impulsivity, drug or alcohol use (Figure VIII.8-A; all Pearson or Spearman r > -0.004895, all p > 0.2163). A two-way ANOVA showed a main effect of gender in the BIS-11 general score (Figure VIII.8-C; $F_{1, 63} = 4.072$, p = 0.0479), a main effect of gender and an interaction gender x

attentional bias in the motor subscale (gender: $F_{1, 63} = 4.492$, $p = 0.0380$; interaction: $F_{1, 63} = 4.017$, $p = 0.0493$), as well as an effect of the attentional bias in the AST score ($F_{1, 63} = 4.674$, $p = 0.0344$).

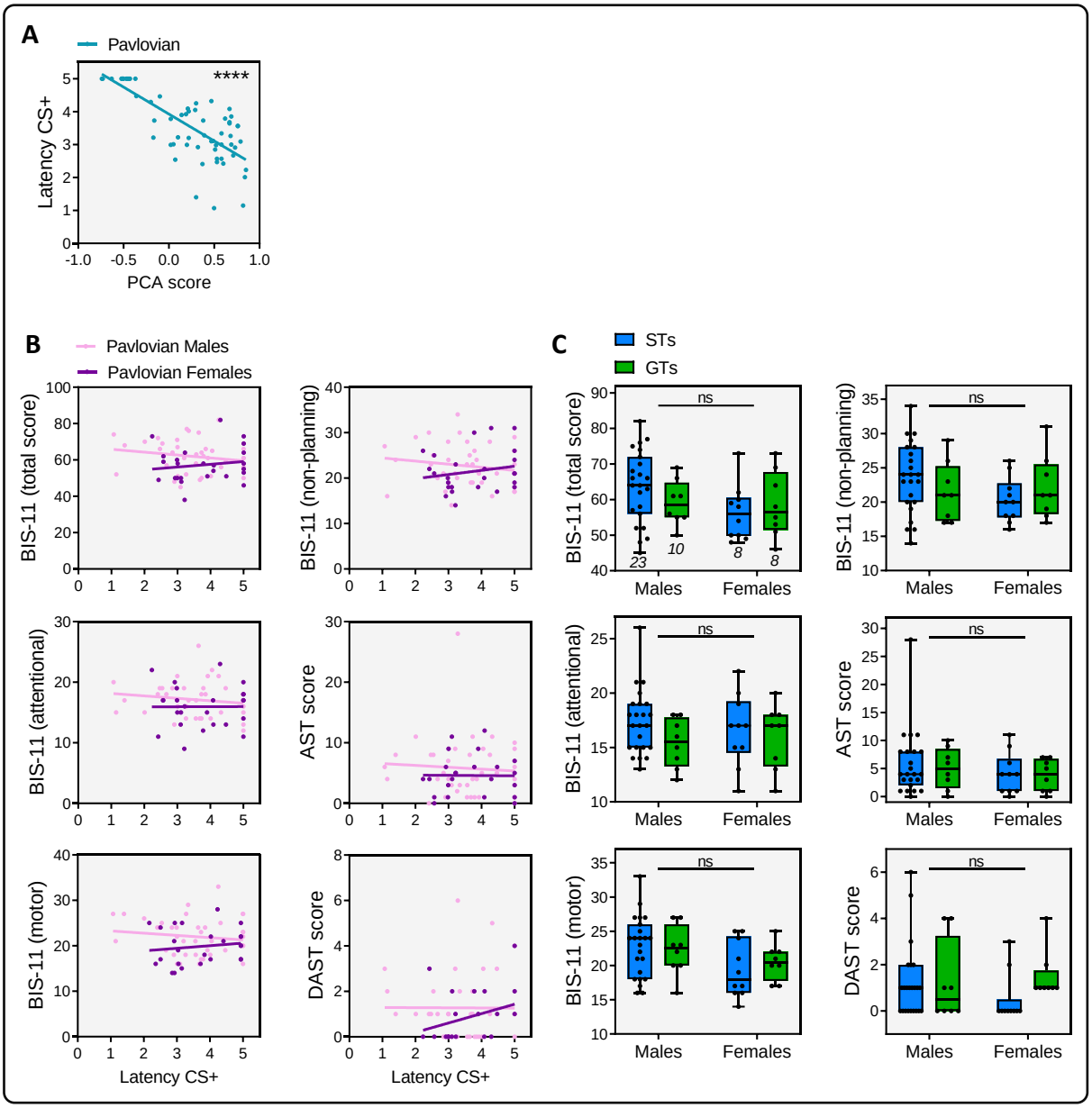


Figure VIII.7. Relationship between impulsivity, drug use, alcohol use and phenotype in pavlovian participants. (A) The latency to first contact the CS+ is correlated with the PCA score, genders combined. (B) Correlations between the latency to first contact the CS+ and impulsivity measures, self-reports of drug use and self-reports of alcohol use in male and female participants. (C) Comparison between conditioned responses and impulsivity, drug and alcohol use in male and female participants. Effect of phenotype and gender. (**** $p < 0.0001$).

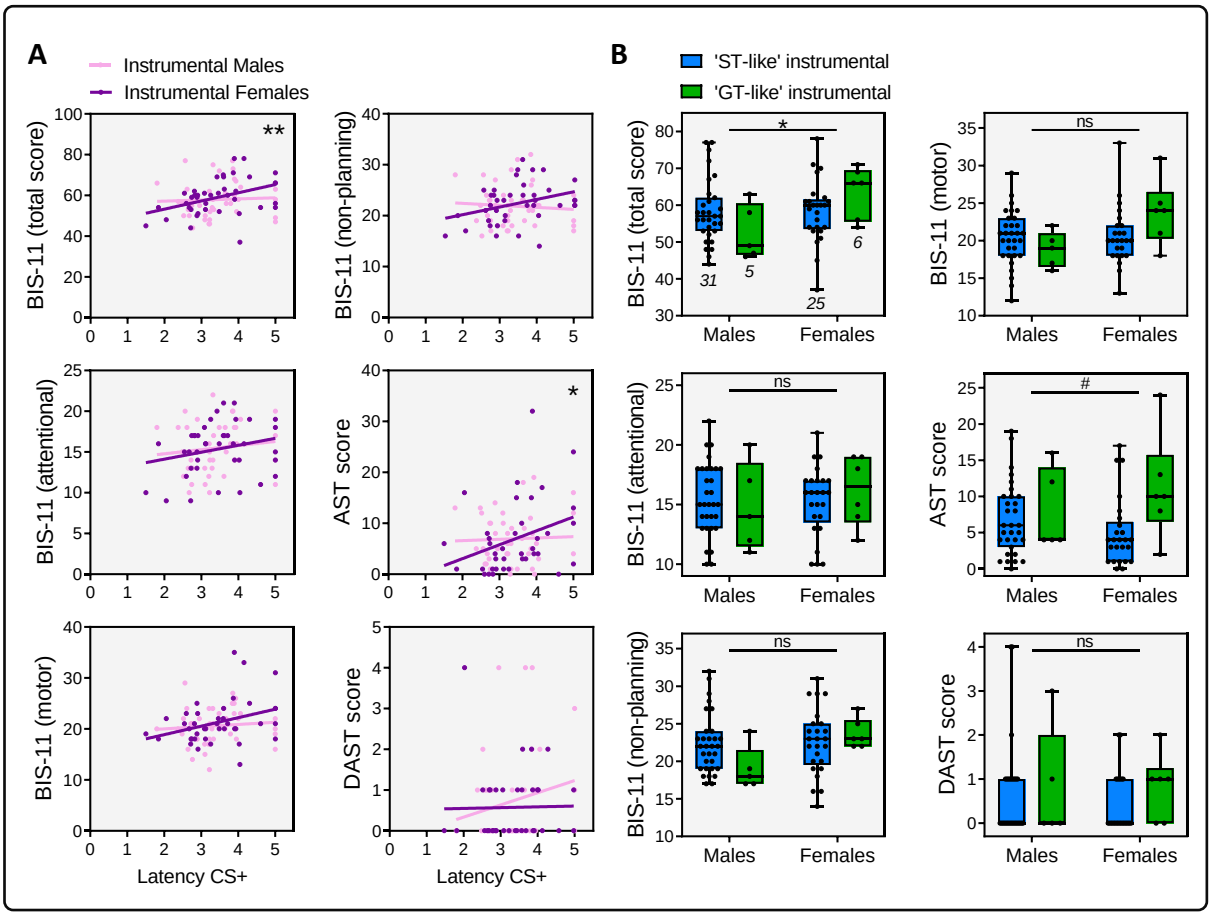


Figure VIII.8. Relationship between impulsivity, drug use, alcohol use and phenotype in 'instrumental' participants. (A) Correlations between the latency to first contact the CS+ and impulsivity measures, self-reports of drug use and self-reports of alcohol use in male and female participants who believed they needed to perform an action to obtain the reward. (B) Comparison between behavioural responses and impulsivity, drug and alcohol use in male and female participants who believed they needed to perform an action to obtain the reward. Effect of phenotype and gender. (Gender: * p ≤ 0.05, ** p ≤ 0.01. phenotype: # p ≤ 0.05).

Exploratory analysis: How devices influenced the type of learning and the conditioned response

The following section is an additional exploratory analysis aimed at dissecting participants' behaviour further. Due to the high navigational requirements of the virtual behavioural task and in the interests of standardising data, participants were instructed to take the study on a laptop or desktop as opposed to a mobile or a tablet. Notwithstanding this precaution, steering could still be achieved through multiple methods: using a mouse, a keyboard, a trackpad or a touchscreen. Men and women significantly differed in their choice of methods (χ^2 = 53.611, df = 8, p<0.001). 0.666). Participants of both genders had a predilection for the mouse (Figure VIII.9-A; 44% on average), but this preference was

mostly imputable to males (52.3% over 35.4% for females). Moreover, males seemed to have changed their input method in a greater extent than females (23.3% vs. 11.6%). Female participants utilised the trackpad markedly more than males (24.5% vs. 4%). The touchscreen was the least favourite technique for both genders (5.1% on average).

In males – who primarily took the virtual task using a mouse or a keyboard, the trackpad (Figure VIII.9-B; 71% out of 7 participants) followed by the keyboard (46.7%) were associated with the highest proportion of pavlovian learning, whereas the touchscreen (60% out of 5 participants) and the mouse (47.7%) were accompanied by a greater instrumental approach. On the other hand, females – who mostly used a mouse, a keyboard or a trackpad – learnt a pavlovian association to a greater extent with a keyboard (54.4%) and a mouse (40%) and developed an instrumental training at a higher rate when using a touchscreen (63.6% out of 11 participants) and a trackpad (55.6%). Participants of both genders who changed their technique during the task were those for which the indecision about the learning type was the highest (21% on average). Figure VIII.9-B also assesses whether the technique used to undertake the virtual task might have had an impact on the CR developed within the pavlovian group. It is worth noting that the touchpad, touchscreen and 'changed' groups comprise very little participants. Remarkably, a dichotomy can be observed for the preferred input method (the mouse) which yielded more sign-trackers in males (71.9%) and more goal-trackers in females (52.4%). The keyboard, which is the second method used by males and the third used by females, was associated with a high rate of undetermined conditioned response (70.6% for females, 42.7% for males).

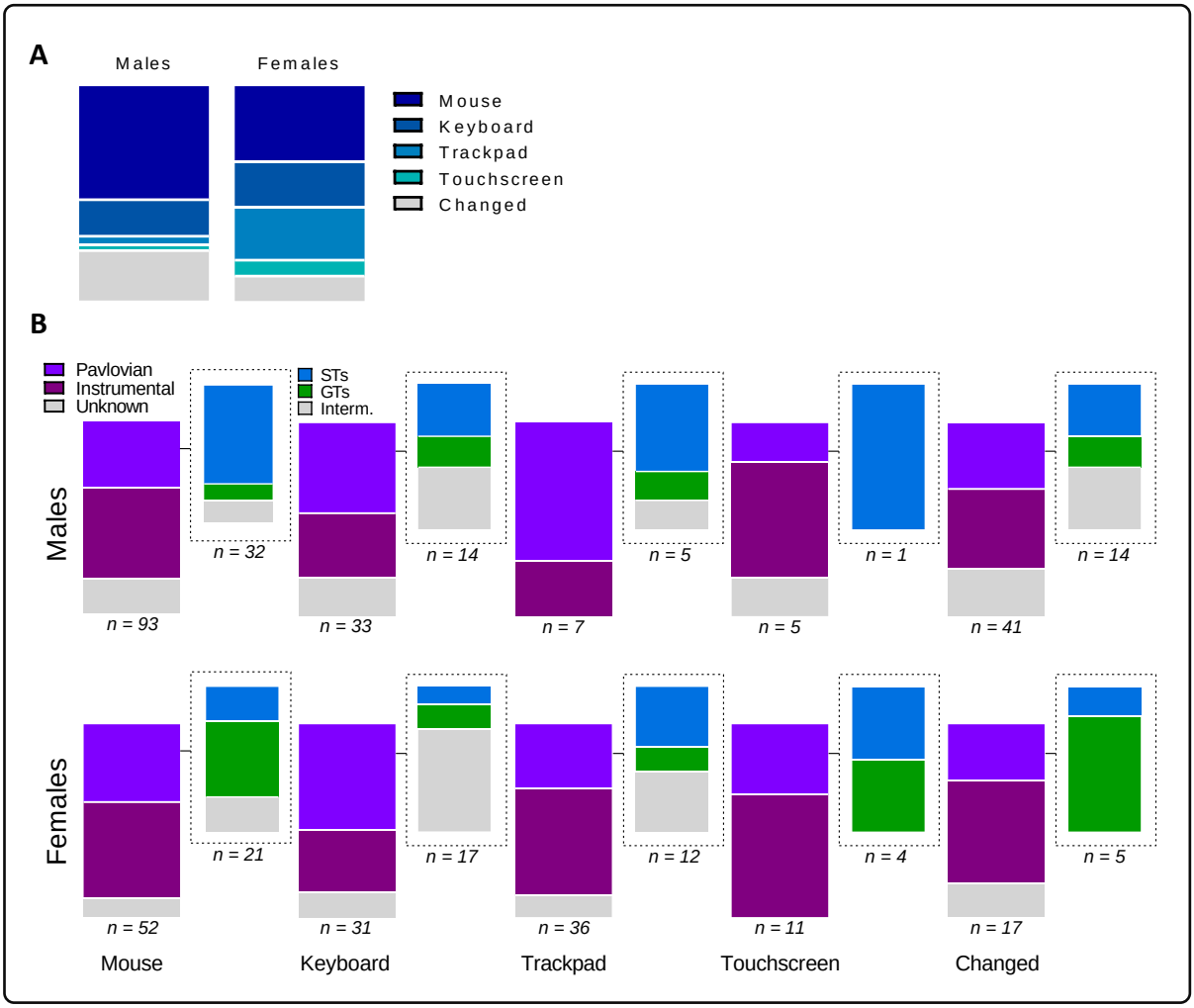


Figure VIII.9. Impact of the device used by participants on learning. (A) Repartition of the devices used by male and female participants to take the study. (B) Left: Repartition of the type of learning (pavlovian, instrumental, other) for each device used by male and female participants. Right: Within participants who learnt a pavlovian association, repartition of conditioned responses (sign-tracking, goal-tracking, intermediate) for each device used by male and female participants.

Discussion

Due to the lack of direct interaction with stimuli, computer-based pavlovian procedures are not truly comparable to rodent paradigms. Offering participants the opportunity to navigate in a virtual environment in which they can – virtually – interact with the CS and collect the reward brings experimenters one step closer to mimicking the animal paradigm whilst limiting physical constraints, allowing to gather a large amount of data and facilitating data processing. Compared to Chapters VII and IX, where participants were taken from a university, half of the population of Chapter VIII was not composed of students,

participants were older on average (26 years old) and only 13% worked in the field of neuroscience or psychology. The sample, although too small to hold robust power and despite being self-selecting, might still be more balanced and more representative of the general population.

In order to relate trait impulsivity and substance use – which have been shown to be accompanied by specific behavioural profiles (Anselme and Robinson, 2020) – to individual variation in our participant's pavlovian conditioned responses, participants were asked to report on their alcohol use and their drug use in the year preceding the study as well as about their impulsive tendencies. Consistent with the wider literature, greater alcohol consumption was associated with more drug use and more self-assessed impulsivity, and impulsive participants reported using more drugs as well. Both trait and state impulsivity increase the vulnerability to substance – alcohol and drug – use as it participates in the probability of initiating and acquiring the substance, the escalation into substance use disorder, the maintenance and the relapse (Jentsch *et al.*, 2014), and substance users have been shown to score higher in all subscales of the BIS-11 questionnaire (Stanford *et al.*, 2009). What is more, alcohol and drug use are common co-occurring conditions (Stinson *et al.*, 2005), which supports the positive association found above. Participants of the present experiment do not, for the most part, qualify as substance misusers; only 8.5 to 9.3% of participants were classified as being at a high risk of developing alcohol misuse, and 0.7 to 1.1% reported using drugs in ways which may be considered harmful or problematic according to the survey – which is lower than the ONS bulletin of 2019 stating that 9.4% of adults surveyed reported using illicit drugs (ONS, 2019). On the other hand, 73% of participants were at low risk of developing alcohol-related disorders (with 10% not drinking alcohol at all) and 89% were at low risk of developing substance use disorders (55% did not use drug at all in the past twelve months).

Gender and sex differences in impulsivity are subject to variations depending on the test subject and the paradigm used. In non-human animals, males either exhibit more action impulsivity than females (Jentsch and Taylor, 2003; Weafer and de Wit, 2014) or perform similarly (Burton and Fletcher, 2012), and females make more impulsive choices than males, but results are rather mixed in humans (Weafer and de Wit, 2014). Male and female participants reported an equivalent impulsivity in all the scales of the BIS-11 in the present experiment, with a comparable 10% of men and 8% of women categorised as highly impulsive (< 72 in the global score). In substance abuse, however, gender differences are slightly less ambiguous; as mentioned in Chapter I, men report more substance-related issues but women are more sensitive to the effect of drugs and alcohol (Agabio *et al.*, 2017; Carroll *et al.*, 2004; Erol and Karpyak, 2015; Volkow *et al.*, 2011b). Yet, here, male and female participants reported similar levels of drug and alcohol consumption. This might be a consequence of the specific type of population registered on academic recruitment platforms such as Prolific, which might represent a more levelled sample compared to the general population.

In contrast to Chapter VII, young participants were on average more impulsive than older subjects on all scales of the questionnaire which is in line with previous studies in human and non-human animals describing a development of self-assessed and behavioural impulsivity with age, including between 18 and 30+ years old (Burton and Fletcher, 2012; Doremus-Fitzwater *et al.*, 2012; Stanford *et al.*, 2009; Steinberg *et al.*, 2008). However, this result was not repeated when the sample size was reduced in the pavlovian group, which either suggests that the relationship was not robust or that age is not a relevant factor for participants who learnt a pavlovian association. No such age-related differences were observed in reports of substance use despite young humans using more drugs than older ones worldwide (UNODC, World drug report, 2022) which might, again, be due to the means of recruitment.

As with the experiment of Chapter VII, within-participant control variables were used as indicators that participants did learn a pavlovian association necessary for the acquisition of a conditioned response and, ultimately, the attribution of motivational value to reward predictive cues. Because the study was time-consuming and was undertaken from participant's own homes as opposed to being supervised by experimenters, subjects were not asked to fill the lengthy survey investigating their motivation. However, two related discrimination criteria were examined: the interaction with the CS+ vs. CS- virtual levers during their respective rewarded or unrewarded trials, and the interaction with the coin dispenser during rewarded vs. unrewarded trials. Participants who did not learn that the CS+ was associated with rewarded trials (*i.e.*, coin delivery from the dispenser) would be expected to interact with the CS+ and the CS- in a similar manner and to contact the coin dispenser equivalently during rewarded and unrewarded trials; in other words, to validate neither discrimination criteria. Remarkably, a dichotomy was found in the criterion validated by other participants in that individuals with an attentional bias towards the predictive cue consistently favoured interacting with said CS+ over the CS-, and individuals who interacted more with the reward location did so significantly more during rewarded trials, reinforcing the confidence in the validity of both discrimination criteria as CR-specific markers of learning; it is worth noting that 10.3% of participants validated both criteria, but one criterion was deemed sufficient. A gradual development of sign- and goal-tracking behaviours as individuals progressively learnt the predictive value of the cue, less drastic than the separation observed in rodent paradigms but similar to that of other human pavlovian procedures (Colaizzi *et al.*, 2022 – in youth), was also observed in our participants. Contrary to Chapter VII, a subset of participants therefore appeared to have learnt conditioned responses and individuals with an attentional bias towards the reward cue might have truly attributed it with incentive value.

Male participants were classified as sign-trackers to a greater extent than females, who were homogeneously categorised as sign- and goal-trackers. It is interesting to note that the most widely used tool to take the study for both genders, the mouse, was associated with a far greater sign-tracking CR in males, and a practically mirrored goal-tracking CR in females. Further investigations would be needed to shed light on this phenomenon. However, both genders scored equivalent PCA index scores on average and developed conditioned responses in a comparable way across blocks. Studies using rodents as well as experiments included in the first section of the present thesis have shown inconsistent and thus perhaps negligeable sex differences in pavlovian learning profiles (Fuentes *et al.*, 2018; King *et al.*, 2016; Pitchers *et al.*, 2015). Moreover, sign- and goal-tracking research on humans that included both genders have not compared them (Cherkasova *et al.*, 2021; Garofalo and di Pellegrino, 2015) or have found no difference in the propensity to attribute incentive salience to reward cues regardless of the procedure used (Colaizzi *et al.*, 2022; Duckworth, 2017; Versace *et al.*, 2016; Wardle *et al.*, 2018). Another interesting result is the seemingly intrinsic divergence between participants categorised as sign- and goal-trackers from the beginning of the training. Whilst most studies in human and non-human animals described a differential development of these learning profiles from initially similar performances, a few publications showed a minor separation before the initial acquisition, usually in the probability to first contact the CS+ or the number of contacts to the CS+ (Flagel *et al.*, 2009; Meyer *et al.*, 2012a).

Although men and women did not diverge in their propensity to attribute incentive salience to the reward cue, it is still possible that the visuospatial nature of the pavlovian task might have impacted their performances differentially. Indeed, numerous studies have shown that, due to a combination of brain structures, sex hormones, but also learning opportunities and cultural stereotypes (Halpern and Collaer, 2005), men had faster cognitive processing and reaction time to visual stimuli, were more accurate in movement tracking,

oculomotor measures and visuomotor tasks, whereas women were more cautious, proactive and favoured contextualised activities (Bianco *et al.*, 2020; Cazzato *et al.*, 2010; Mathew *et al.*, 2020). Moreover, both genders rely on different information to navigate and, whilst gender differences tend to disappear after familiarisation with the task, males seem to exhibit better spatial ability and virtual navigation than females on an initial session of virtual environment (Ross *et al.*, 2006). Finally, the nature of the instructions ('collect the coins') combined with the premise men might be slightly more self-confident with technology (Cai *et al.*, 2017) might have led women to display a disproportionate and rigid focus on the target (the reward) and therefore a decreased inclination to switch the viewpoint to track the CS. All the aforementioned elements might have been negligeable in the present setup especially given that participants registered on the recruiting platform Prolific might be familiar with visual manipulations required in computerised psychology tasks, which could explain the absence of behavioural difference between male and female participants; but it is also conceivable that a potential variance was concealed or cancelled out by gender disparities in visuomotor demands.

Female participants who appeared to attribute more motivational value to the CS+ reported using less drug in the year preceding the study. Despite the extensive literature describing the relationship between the tendency to attribute incentive salience to pavlovian cues and substance use (Anselme and Robinson, 2020; Flagel *et al.*, 2009 and 2010; Saunders and Robinson, 2012; Tomie *et al.*, 2008) as well as impulsivity (Cope *et al.*, 2022; Garofalo and di Pellegrino, 2015; Lovic *et al.*, 2011), no such association was found in the present study, regardless of gender. Another recent publication from Janna Colaizzi and her team observed no difference in self-report and behavioural measures of impulsivity across groups (Colaizzi *et al.*, 2022), however the experiment was undertaken with children and the impulsivity questionnaire was different than that of the present study.

Because the virtual behavioural task allowed participants to navigate and interact with the stimuli without constraint or specific instructions as opposed to a simpler computerised task in which the only room for manoeuvre is to look at a screen and press a key, nearly half of participants misjudged the association between the light and the reward and believed that the delivery of coins was contingent upon their *action*, thereby developing an instrumental learning. Several aspects might explain this level of confusion. Instructing participants of the lack of influence their action had on reward availability would have undeniably ensured the absence of instrumental influence (Garofalo and di Pellegrino, 2015), but it was considered more important to allow free exploration of the environment (Cope *et al.*, 2022) to allow conditioned responses to form naturally and encourage participants to pay attention to both the US and the CS+ instead of potentially neglecting the latter. Another element that might have impacted the understanding of the task is the tool used to undertake the study. Indeed, when the viewpoint of the screen was focussed on the light or on the coin dispenser, the rest of the environment was hidden and participants needed to switch the viewpoint to witness events – although light activation generated a specifically-coloured illumination of the screen which was visible when the viewpoint was fixed on the coins. If navigating in the virtual room was made more difficult by the tool used, participants might have missed associated events and misinterpreted outcomes and consequences. Between all the techniques listed – mouse, keyboard, trackpad or touchscreen – the mouse, followed by the touchscreen, were indubitably the most efficient, fluid and precise, whereas the keyboard and trackpad were probably less manoeuvrable and more cumbersome (also see Kar *et al.*, 2015). Surprisingly however, the mouse and the touchscreen were both associated with a higher rate of instrumental learning; and the keyboard and trackpad yielded more pavlovian learning particularly in males. A substantial variable that was not controlled in this study and that might have shed light on gender disparities in the use of input devices and their consequences was individual's personal computer-related habits. On average, men are more

self-confident in their abilities regarding technology (Cai *et al.*, 2017), they play more video games involving visuospatial manipulations (Bonanno and Kommers, 2010; Brown *et al.*, 1997) and perform better than women – particularly when stereotypes are reinforced prior to the experiment (Cruea and Park, 2012; Hamlen, 2010). It might therefore be plausible that male participants, *on average*, were able to choose a more efficient tool to undertake the virtual task, as they even changed and adapted the method in a larger proportion than females. Although men and women adopted relatively similar strategies in our paradigm, inquiring about participant's gaming history might have allowed to support or disprove this hypothesis and to draw a more complete picture of the results.

Similar to the experiment of Chapter VII, and despite their PCA index scores being equivalent to those of 'pavlovian' participants, the behaviour of individuals who did not learn a pavlovian conditioned response should be referred to as attentional bias as opposed to sign- and goal-tracking. In line with participant's conviction that interacting with the virtual lever next to the light was necessary to obtain the reward, most of them showed an attentional bias towards said lever, especially in males although no significant gender difference was observed. Female participants were more impulsive than males on the BIS-11 global score and motor subscale. Female participants who clicked on the lever faster reported less alcohol consumption and a lower impulsivity; it is possible non-impulsive women were simply faster overall, potentially because of a better attention than their impulsive counterparts, but the limited data available does not allow to conclude on this result.

The present experiment aimed at investigating the relationship between impulsivity, substance use and the attribution of incentive salience to pavlovian cues, however it encountered several limitations. A large number of participants was tested but only a small subset was considered suitable to study the question at hand. Increasing the sample size by ensuring all participants learn a pavlovian association instead of an operant association

would allow a stronger analysis. The ultimate objective is to compare different pavlovian settings – a simple eye-tracking, a virtual environment in which participants can navigate using the computer, virtual reality during which they can move in space, and finally a real-life setup comparable to that used in rodents – and see if the same robust propensity to sign-track can be observed in male and female participants or whether, on the other hand, it varies depending on the paradigm and associated constraints.

Chapter IX
‘Real-life’ pavlovian procedure

Introduction

In our quest to explore variations in conditioned responses across pavlovian settings, the ultimate aspiration was to conceive a procedure comparable to that used in rodents in an attempt to verify whether the same behaviours and phenotypes were expressed in both species. At the time when the project of this thesis was designed and conducted, no publication studying sign-tracking behaviour in human using a physical approach paradigm was yet published; however, two very interesting experiments undertaken in Ann Arbor, Michigan emerged as the thesis was being composed.

Cope and her team (Cope *et al.*, 2022) built a console with an extendable lever paired with the delivery of tokens leading to real monetary reward during a single session composed of 63 trials. Participants from both genders were equipped with eye-tracking devices measuring the direction of their gaze, and their physical interaction with the levers were recorded as well. They were not given specific instructions and instead, were encouraged to behave as they wanted. Instead of exclusively calculating an index to classify participants, phenotypes were determined by running a series of models based on participant's interaction with the CS as well as their gaze towards the lever and magazine; this allowed experimenters to identify the number of profiles that best fitted observable data. Three classes emerged that mapped animal studies surprisingly well, with participants interacting more with the lever and not fixating the token magazine, others contacting the lever very rarely but gazing at the magazine a lot, and participants in an intermediate class. Control analyses confirmed that these behaviours appeared to be learnt, which reinforced the validity of the experiment. In addition to establishing whether individual differences in learning profiles could be observed, authors sought to investigate their relationship with impulsivity, drug and alcohol use through self-report questionnaires and found that the 'sign-tracking' class was associated

with a higher behavioural impulsivity than ‘goal-trackers’. However, drug and alcohol use scores were either too low to be investigated or were similar across profiles.

The objective of Colaizzi’s team was to develop a procedure allowing to measure externalising behaviours in children as they have been shown to predict impulse control disorders in adulthood (Colaizzi *et al.*, 2022). They used an apparatus which they introduced a few years prior (see Joyner *et al.*, 2018) consisting of colourful blocks, an extendable lever and a reward tray delivering beads leading to monetary reward. Interestingly, despite participants being children from 9 to 12 years old, authors realised that treats were not incentive enough, which is why the experiment of 2022 used money instead. At the end of the 40 trials composing the single session, they identified learning profiles using participant’s behavioural responses and noticed that none had an attentional bias towards the reward location. Children with a propensity to sign-track reported more attentional deficits and had less inhibitory control; moreover, experiments discovered that brain activation was dominated by motivational networks in sign-trackers whilst other participants’ main neural activation was located in cortical structures.

The preliminary experiment of Chapter IX took a step further in mimicking the pavlovian procedure used in rodents. Instead of restricting participants’ movements to a single device, the conditioned stimulus and the reward dispenser were distributed in space in such a way to allow the expression of more complex conditioned responses including approach and orienting behaviours. The relationship between variations in attraction to reward cues and specific subtypes of impulsivity was also evaluated using the same questionnaire and behavioural tasks as those of Chapter VII.

Materials and Methods

Participants

A total of 38 participants recruited through institutional recruitment circular at King's College London took part in this experiment (Table IX.1). Participants indicated having normal or corrected to normal vision and hearing, no neurological or psychiatric conditions, no learning disorders. The study also required them to have typical mobility levels to ensure navigating between items would be possible during the pavlovian conditioning task. Participants were asked about the education they completed, their current work status (*i.e.*, student or not, field of neuroscience / psychology or not) and their dominant hand. Informed consent was given at the beginning of the study, and a £8 Amazon voucher was delivered upon completion. This research was approved by the Research Ethics Committee of King's College London (MRM-21/22-14902) and conducted in accordance with the General Data Protection Regulations.

Experimental procedure

The study took place at King's College London. The Gorilla experiment builder (https://gorilla.sc/; Anwyl-Irvine et al., 2020) was used to create computerised questionnaires and impulsivity tests. The pavlovian device was assembled by David Pound from S.T.E.M. Electronics Facility & Open Engineering Labs at the Open University. The structure of the experiment was similar to Chapter VII (Figure IX.1). After completing the BIS-11 impulsivity questionnaire, participants underwent a first session of pavlovian conditioning during which they were able to navigate in the room between different stimuli and collect marbles. They subsequently had to take the same three impulsivity tests in randomised orders (Delay Discounting Task, Go-NoGo and Iowa Gambling Task) before undertaking a second pavlovian session (as per rodent procedures involving multiple sessions). Finally, participants responded to a survey allowing the experimenter to gain

feedback on the study and participant's strategies and were offered a food reward. The experiment lasted approximately fifty minutes.

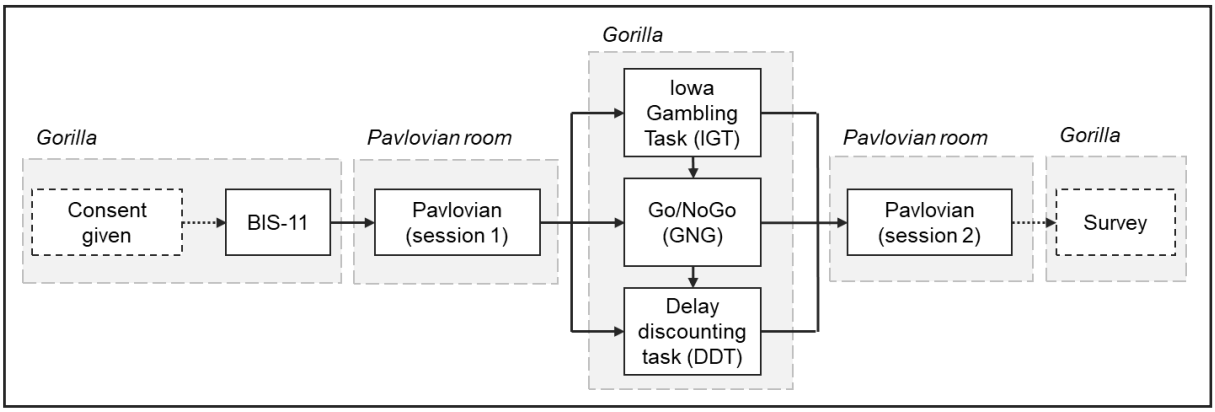


Figure IX.1. Outline of the experiment undertaken in Chapter IX.

Table IX.1. Demographics of Chapter IX.

	Males (n = 8)	**Females** (n = 30)	***p*-value**
Age (years)	21.6 ±*1.15*	20.6 ±*0.6*	*t*-test: 0.3017
Completed Education			χ^2: 0.666
GCSE	12.5%	3.33%	-
A-levels	62.5%	80%	-
Undergraduate	12.5%	6.67%	-
Postgraduate	12.5%	10%	-
Other	0%	0%	-
Status			χ^2: 0.868
Student: Psycho/neuro-related	25%	40%	-
Student: Other	0%	3.33%	-
Not student: Psycho/neuro-related	75%	56.67%	-
Not student: Other	0%	0%	-
Dominant hand			Fisher: 0.381
Right	87.5%	96.67%	-
Left	12.5%	3.33%	-

Measure of impulsivity

Barratt-Impulsiveness Scale 11 (BIS-11). A global score and second-order scores (*i.e.,* attentional, motor, non-planning) were calculated, and participants with global scores greater than 71 were considered highly impulsive.

Delay-Discounting Task (DDT). Participants with a smaller Area Under the Curve were considered more impulsive.

Go-NoGo (GNG). Commission errors during the first and the second block were calculated.

Iowa Gambling Task (IGT). A score for the last 40 trials was calculated, with a higher score indicating a greater impulsivity.

Pavlovian conditioning

Apparatus. The experiment took place in a small rectangular room (263 x 282 cm) in which three electronic devices were connected to each other: a reward dispenser (41 x 27 x 26 cm; Treat & Train™ remote reward dog trainer) on the centre wall 90 cm from the floor and two white 'buttons' (150 x 220 x 40mm; Philips LED orientation lights 5LM) located on each side wall at shoulder/chest height, approximately 190 cm from the dispenser – sufficient to allow navigation and therefore the expression of conditioned approach behaviours (Figure IX.2). LED strips (12V) were inserted in the lamp modules and were driven from a wireless 12V relay attached to each button. The three components were remotely controlled via RF transmitters (433MHz RF link) mounted on a PCB, itself wired on a Veroboard. The transmitters signalled the lamps to turn on and off, and the dispenser to deliver marbles in a timely fashion. A MBED NXP LPC1768 socketed on the board was powered by a USB interface which was also used to communicate with a host computer. The laptop (Debian 8, GNU/Linux) contained three scripts allowing to drive the unit: a 'sequence' script (MBED CLI toolkit in C/C++) designed to setup parameters and variables, a 'record keeping' script running the sequence script and creating files for each iteration, and

an 'abort' script. The light buttons were ornamented by two plastic patterns – a dark red octagon and a green star – which were counterbalanced between participants; shapes were markedly different to allow for potential dyschromatopsia (colour vision deficiency). The buttons had the possibility to be pressed to ensure similarity with the manipulable lever used in the rodent paradigm, which is a desirable characteristic to develop sign-tracking (Meyer *et al.*, 2014). When activated by the controller, the buttons illuminated and produced a noticeable clicking noise. Colourful plastic marbles were delivered from the treat dispenser into a small well on the front, which caused a rattling noise. A wicker basket was provided next to the dispenser to collect the marbles.

Figure IX.2. Setup of the pavlovian procedure of Chapter IX. (A) Marble dispenser and a basket to collect marbles. (B) CS pushing button, CS+/CS- and left/right counterbalanced between participants. Illuminated during CS+ or CS- trials. (C) Conditioned stimuli on either side of the US in a room in which the participant can navigate. (D) CS pushing button, CS+/CS- and left/right counterbalanced between participants. Illuminated during CS+ or CS- trials.

Instructions. Before the pavlovian training, participants were instructed to freely navigate in the room and interact with its components. To invigorate desire to obtain the marbles (*secondary reward*), and to focus participants' attention on the task, they were encouraged to collect the marbles and informed that they would have the possibility to

exchange them for a treat of their choice at the end of the study (*primary reward*; clementines, bananas, apples, cereal or fruit bars, chocolate bars, sweets). The wide assortment of treats allowed to accommodate all tastes, diets, allergies and intolerances, and participants were assured that they did not have to take anything if they did not want to. Subjects were then reminded that they would be recorded through a camera worn on a chest harness and a distal camera positioned on the other side of the room – this requirement was included in the information sheet participants read before applying for the study, and written consent was gained at the beginning of the experiment.

Procedure. After explaining the procedure to participants, the experimenter exited the conditioning room and manually launched the script from the host computer located in another room and connected to the controller. Each pavlovian block was composed of 20 randomised trials: One of the light buttons illuminated for 10 seconds (CS+; predictive cue) after which a marble (US) was immediately released from the treat dispenser. Interacting with the CS+ did not affect marble delivery. The illumination of the second button was not paired with the delivery of a marble (CS-; distractor). The side and the pattern of the CS+ were counterbalanced between participants. Each trial was separated by a variable ITI of 15 to 25 seconds designed to incite participants to move between objects and therefore interact with them, whilst being short enough to maintain their focus. The first and the second block were identical in that the CS+ and CS- were the same, however the order of rewarded vs. unrewarded trials was randomised. At the end of the second session, participants filled the final survey and were then given the opportunity to select from the food rewards as desired.

Sign- and goal-tracking classification

Each pavlovian session was recorded through a chest camera and a distal wide-angle webcam which gave indications about hand movements as well as body position and navigation. The software BORIS (Behavioural Observation Research Interactive Software;

Friard and Gamba, 2016) was used to manually code live videos of sessions and quantify participant's interactions with the CSs and the US. More specifically, we monitored the *number* of contacts with the CS+, the CS-, the marble dispenser and the marbles, during CS+ illumination. The *latency* to first contact the CS+, the CS-, and the marble dispenser during CS+ illumination, and the *time* spent looking at the CS+, the CS- and the marble dispenser during CS+ illumination, were also extracted. During the second session, we calculated a PCA index score with participants ranging from -1.0 to -0.3 classified as goal-trackers, and participants ranging from 0.3 to 1.0 as sign-trackers (Figure IX.3). Participants with intermediate scores were not included in this study as we wanted to compare more radical learning strategies. To obtain a more comprehensive understanding of the characteristics of conditioned responses, a 'Directional index' was also calculated as the average time directed towards the CS+ during CS+ presentation, minus the average time directed towards US during CS+ presentation, divided by the total time of CS+ presentation (Figure IX.3; see Garofalo and di Pellegrino).

Probability:	$P(CSplus\ button) - P(dispenser\&marbles)$
Response bias:	$\frac{(CSplus\ contacts\ - dispenser\&marbles\ contacts)}{((CSplus\ contacts + dispenser\&marbles\ contacts))}$
Latency:	$((dispenser\&marbles\ latency\ - CSplus\ button\ latency))/10$
PCA score:	$((probability\ score\ +\ response\ bias\ score\ +\ latency\ score))/3$
Directional index:	$(CS\ gaze\ time\ -\ US\ gaze\ time)/(total\ gaze\ time)$

Figure IX.3. Detail of the 'virtual' PCA index score calculation and the Directional index score calculation. *Inspired respectively by Meyer et al., 2012 and Garofalo and di Pellegrino, 2015.*

End-of-experiment survey

Participants were asked to rank their desire ('I wanted to interact / to obtain…') as well as their pleasure ('I liked to interact…') to interact with the elements composing the pavlovian conditioning. Questions were also asked about their understanding of the task,

such as the relationship between the CSs and the US, and whether their strategy evolved throughout the sessions. In a separate part, experimenters inquired about the feelings participants had whilst undertaking the study (*i.e.*, to rank their enjoyment, challenge, boredom, frustration and confusion). All questions can be found in Appendix K.

Statistical analysis

GraphPad Prism (versions 8 and 9; GraphPad Software Inc.; San Diego, CA, USA) was used for ANOVAs, correlations and independent group comparisons. Chi-square and Fisher analyses were conducted on SPSS (versions 27 and 28; IBM Corp.; Chicago, IL, USA). All group comparison results are presented as mean + SEM. Statistical significance was set at 0.05. Measures were all checked for normality using the Shapiro-Wilk test, and non-parametric tests were used when appropriate. Participants' behaviour was manually coded using the BORIS software: specific events (*i.e.*, CS+ on and off, CS- on and off, marble delivery), actions (*i.e.*, contacts with CS+, CS- and the marble dispenser, marble collection) and body positions (*i.e.*, turn towards the CS+, the CS- or the marble dispenser) were allocated to specific keys on a computer keyboard and the experimenter manually pressed the appropriate key for each occurrence, thereby allowing the subsequent reconstruction of each participant's training session. Phenotypic repartition between males and females and demographic data (*i.e.*, current status, education) were analysed using the Chi-square test of independence. When groups contained two categories (*i.e.*, dominant hand), Fisher's exact test was used. Repeated measures such as behaviour evolution across blocks, the interaction between CS+ and CS-, the selection of decks during the IGT, or the commission errors during the GNG, were investigated using two-way RM ANOVA. Significant effects were followed by post-hoc Šídák multiple comparison's correction to determine in which phenotype or in which gender differences were found. The relationship between impulsivity, phenotypes and the type of learning (*i.e.*, pavlovian or instrumental) was examined with the

non-parametric Kruskal-Wallis tests, and when significant groups were compared using Dunn's multiple comparison's correction test. Comparisons between two independent groups such as gender differences in phenotype or in impulsivity scores were investigated using the parametric independent *t*-test, or the non-parametric Mann-Whitney test. Correlations were analysed using Pearson's correlations when the dataset met normality assumptions, or Spearman's correlations when it did not. A Bonferroni correction was applied after multiple correlations to reduce the risk of type I error. Participants were excluded from analyses for not conducting the tasks to a satisfactory standard (*e.g.*, pavlovian conditioning and IGT, as shall be described hereunder), as well as for having more than two switch points in the DDT. All figures were produced on GraphPad Prism.

Results

Pavlovian conditioning: behavioural phenotypes, learning and gender differences

In an attempt to mirror the pavlovian procedure used in rats to identify sign- and goal-trackers, participants were asked to interact with an apparatus composed of two manipulable buttons located on opposite walls (CS+ and CS-) and with a dispenser delivering marbles (US) after the CS+ illumination. Two participants were excluded due to dismissive conducts (*i.e.*, not focussing on the task and not interacting with the buttons or marble dispenser). Amongst the 36 remaining participants, 15 implemented a learning strategy akin to a 'pavlovian' conditioning according to the end-of-experiment survey (Figure IX.4: 11 females, 4 males) and 21 adopted a strategy resembling an 'instrumental' task – as described in Chapter VIII (17 females, 4 males). Figure IX.4 illustrates the repartition of phenotypes according to two different categorisation criteria, the PCA score and the Directional index. The PCA index yielded a more balanced repartition within pavlovian learning between genders in that both sign- and goal-trackers were identified (Figure IX.4-A), whereas the Directional index only categorised goal-trackers in males

(Figure IX.4-B). When investigating the attentional bias of participants from the 'instrumental' group, the PCA score detected that 86% interacted preferentially with the CS+ (which is coherent with the fact that participants thought they needed to interact with the cue in order for the reward to be delivered) whilst the Directional index distinguished individuals who interacted more with the CS+ (19%) and with the US location (38%). Male and female participants exhibited a significantly different repartition of phenotypes when taking all participants ($\chi^2 = 8.339$, df = 2, p = 0.015) and the pavlovian group ($\chi^2 = 6.563$, df = 2, p = 0.0.38).

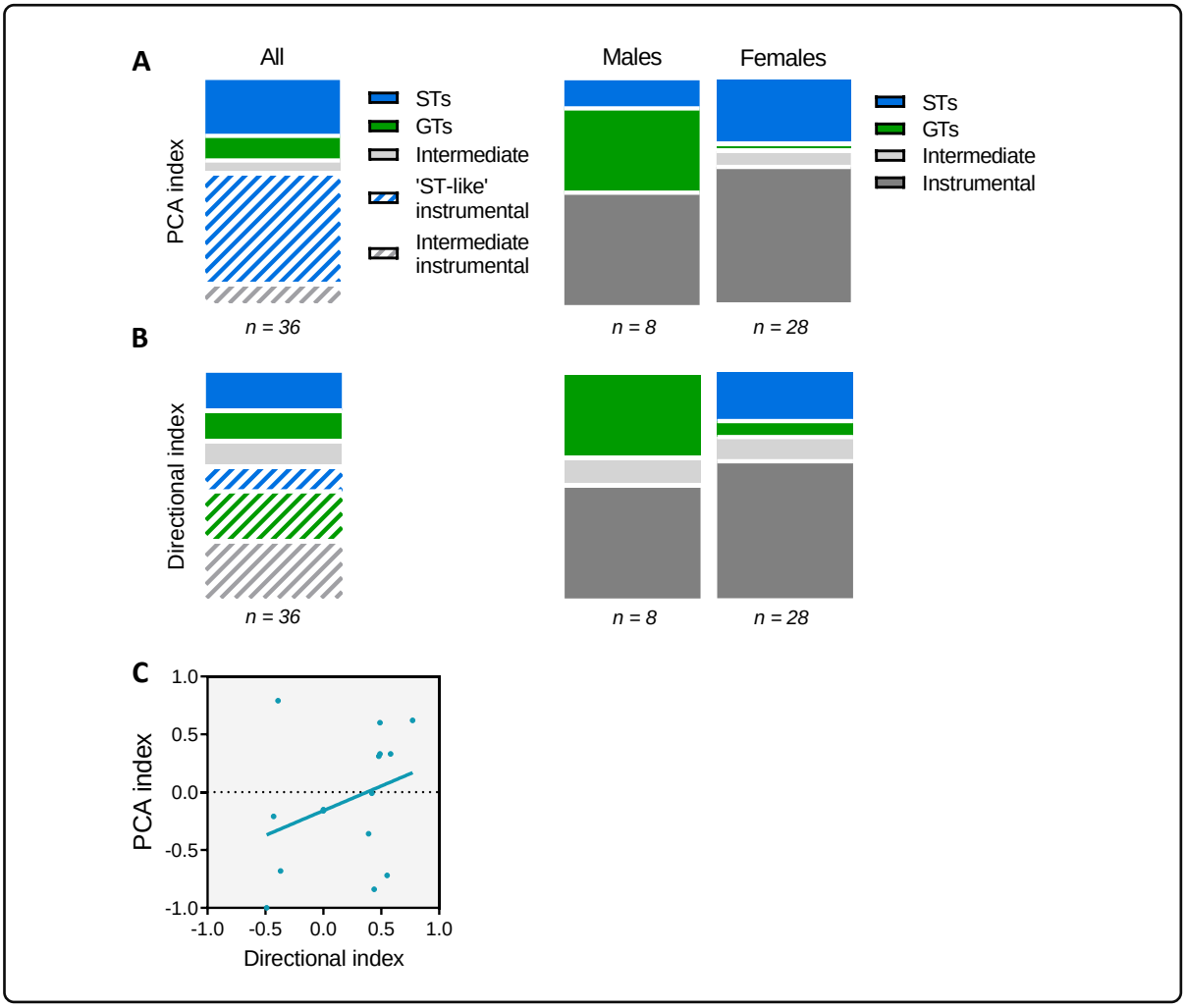


Figure IX.4. Comparison between two indices of phenotypic categorisation. (A) Repartition of responses classified using a PCA index score for all, male then female participants who learnt a pavlovian association, and for all, male then female participants who believed they needed to perform an action to obtain the reward. (B) Repartition of responses classified using a Directional index score for all, male then female participants who learnt a pavlovian association, and for all, male then female participants who believed they needed to perform an action to obtain the reward. (C) The scores of the PCA index and the Directional index are not related in participants who learnt a pavlovian association.

In accordance with the distinction in phenotype repartition, no correlation was found between the scores of the PCA index and the Directional index in the pavlovian group (Figure IX.4-C: Spearman r = 0.4056, p = 0.1335).

Sign- and goal-tracking behaviours categorised using the PCA index score both evolved between the first and the second pavlovian session (Figure IX.5-A; Effect of session: $F_{1, 30} = 37.13$, p <0.0001; Šídák STs p = 0.0040, GTs p <0.0001), and only goal-tracking interaction developed using the Directional index score (Figure IX.5-B; Effect of session: $F_{1, 11} = 16.96$, p = 0.0017; Šídák STs p = 0.0651, GT p = 0.0135). Unexpectedly, instead of increasing from the first session to the second as is typically observed in sign-trackers, the PCA index *decreased*. Furthermore, it should be noted that participants allocated to the sign-tracking and goal-tracking groups at the end of the pavlovian training appeared to diverge from the beginning. Statistical analysis of the instrumental group was impossible due to violations of ANOVA assumption; however, the Directional index was visually similar between participants with a marked bias towards the CS+ and sign-trackers, and between participants interacting more with the US and goal-trackers (Figure IX.5-B). On the other hand, when classified using the PCA index score, participants from the instrumental group did not follow the same trend as those from the pavlovian group as their behaviour remained constant between sessions (Figure IX.5-A). A repeated measures ANOVA with gender and behaviour as between-subjects factors and session as a within-subject factor showed no effect of gender for sign-trackers ($F_{1, 7} = 1.866$, p = 0.2142) and goal-trackers ($F_{1, 2} = 3.582$, p = 0.1989) of the pavlovian group classified with the PCA index, nor for goal-trackers categorised using the Directional index ($F_{1, 3} = 4.868$, p = 0.1145).

As described in Chapters VII and VIII, another important element allowing to determine whether individuals learnt the pavlovian association is to compare interactions towards the CS+ and the US during CS+ trials and CS- trials. A minor but significant CS+ discrimination was observed for sign-trackers of the pavlovian group (Figure IX.5-C; Effect

of CS: $F_{1, 28} = 8.709$, $p = 0.0063$; Šídák $p = 0.0154$) and, as expected, for participants from the instrumental group with an attentional bias towards the predictive cue who believed they needed to interact with the CS+ to obtain the reward ($p < 0.0001$); however, no such discrimination was found for goal-trackers (Šídák $p = 0.4404$). Because of violations of ANOVA assumptions, both pavlovian and both instrumental groups were analysed separately using the non-parametric test Mann-Whitney in the following part. Female participants from the pavlovian group showed significantly more sign-tracking tendencies than males (Figure IX.5-D; Mann-Whitney $U = 4$, $p = 0.0154$) contrary to participants from the instrumental group who did not differ between genders (Mann-Whitney $U = 22$, $p = 0.2554$), which is in line with the fact that the 'instrumental' group largely exhibited an

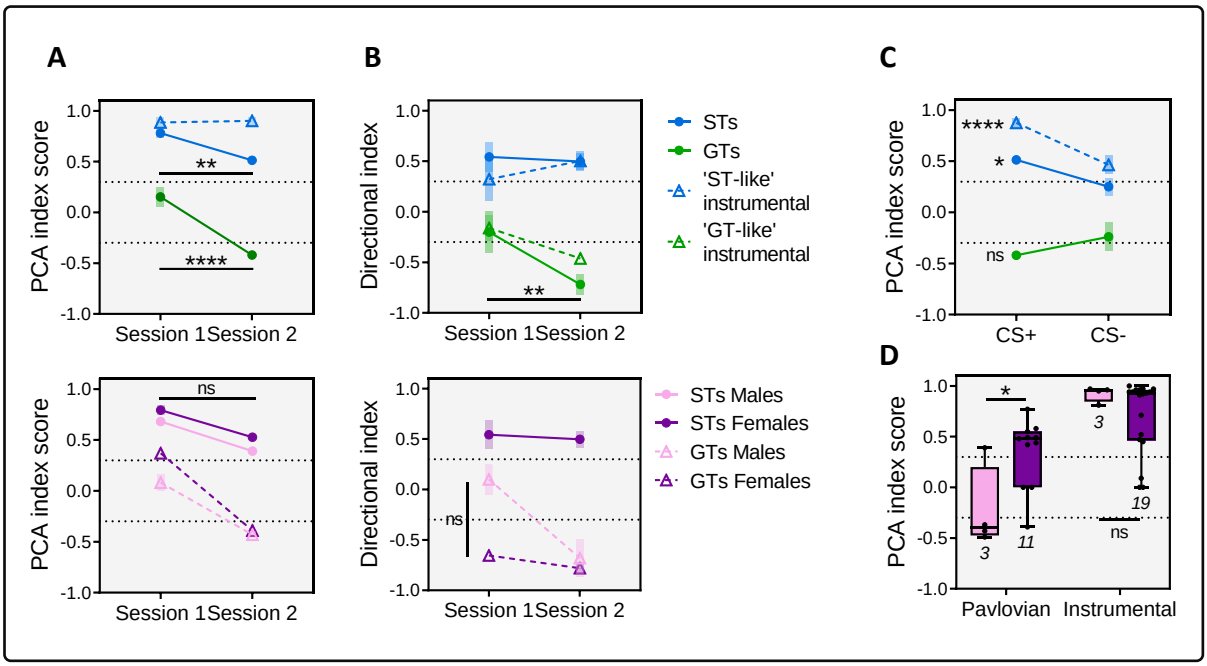


Figure IX.5. Indicators of pavlovian learning: comparison between two indices of phenotypic categorisation. (A) Top: Evolution of conditioned responses calculated using the PCA index across training sessions between sign-trackers, goal-trackers, 'instrumental' participants with an attentional bias towards the CS+. Effect of the session. Bottom: Evolution of conditioned responses between male and female sign-trackers, and male and female goal-trackers. Effect of the session. (B) Top: Evolution of conditioned responses calculated using the Directional index across training sessions between sign-trackers, goal-trackers, 'instrumental' participants with an attentional bias towards the CS+ or towards the US. Effect of the session. Bottom: Evolution of conditioned responses between female sign-trackers, male and female goal-trackers. Effect of the session. (C) Comparison between a PCA index score calculated using responses towards the CS+ during CS+ trials and a score calculated using responses towards the CS- during CS- trials. Sign-trackers, goal-trackers, and 'instrumental' participants with an attentional bias towards the CS+. (D) Gender comparison. PCA index scores of male and female pavlovian participants, and male and female participants who believed they needed to perform an action to obtain the reward. (* $p \leq 0.05$, ** $p \leq 0.01$, **** $p < 0.0001$).

attentional bias towards the cue – the object of their operant association – compared to the pavlovian group which also included goal-trackers.

Based on the presence of sign- and goal-trackers in both genders, the absence of gender difference within phenotypes, and the distinct repartition and evolution of pavlovian-related behaviour across sessions compared to the 'instrumental' group, the PCA index score was deemed more suitable than the Directional index to analyse the current experiment. Following results thus solely consider sign- and goal-trackers classified using this categorisation tool.

Gender and age differences in impulsivity

As in Chapter VII, participant's impulsivity was assessed using the Barratt Impulsiveness Scale 11 self-report questionnaire and three computerised impulsivity tasks (Go-NoGo, Iowa Gambling Task and Delay Discounting Task). One female from the pavlovian group as well as one female and one male from the instrumental group were excluded from the DDT analysis due to a number of switch points superior to 2, and one pavlovian female was excluded from the IGT analysis for selecting the decks in a regular pattern and therefore not developing a strategy. Table IX.2 shows that the motor subscale of the BIS-11 did not meet the consistency criterion and was therefore excluded from our analyses. Results presented in this section include all participants regardless of their pavlovian or instrumental learning strategy. No relationship was found between the different measures of impulsivity when combining the genders, and scores of male and female participants were similar in all impulsivity tasks (Table IX.3). Contrary to what was observed in Chapter VIII, age did not have a differential impact on impulsivity measures (data not shown).

Table IX.2. Internal reliability of the BIS-11 total scale and subscales.

	No. of items	Cronbach's α
Total score	30	0.822
Attentional	8	0.758
Motor	11	0.460
Non-planning	11	0.713

Table IX.3. Impulsivity measures and gender.

	Males (n = 8)	Females (n = 30)	*p*-value (*t*-test / M-W)
Barratt Impulsiveness Scale 11			
Total score	60.25 ±*1.69*	60.37 ±*1.08*	0.9755
Attentional scale	17 ±*1.08*	17 ±*0.88*	0.6515
Non-planning scale	21 ±*1.38*	22.67 ±*0.82*	0.0889
Go-NoGo			
Block 1: Commission errors	0.62 ±*0.65*	0.8 ±*1.45*	0.7109
Block 2: Commission errors	1.12 ±*1.28*	1.03 ±*1.58*	0.6923
Iowa Gambling Task			
% Deck A	18.6 ±*4.42*	17.55 ±*2.48*	0.7376
% Deck B	34 ±*5.96*	45.41 ±*3.08*	0.0950
% Deck C	19.6 ±*6.52*	18.34 ±*1.91*	0.7969
% Deck D	27.7 ±*9.04*	18.69 ±*1.96*	0.4084
Score of 40 last trials	-0.5 ±*9.13*	-5.93 ±*3.68*	0.5246
Delay Discounting Task			
AUC	0.12 ±*0.15*	0.16 ±*0.2*	0.2192

Relationship between pavlovian learning strategies and impulsivity

Due to the low number of male sign- and goal-trackers and the absence of gender differences in behaviour and impulsivity, males and females were combined in these groups. As with previous experiments, the PCA index was closely and significantly related to the latency to first contact the CS+ (Figure IX.6-A; Spearman r = -0.8867, p <0.0001) and the latter was therefore used in subsequent correlation analyses. Figure IX.6-B illustrates the absence of relationship in the pavlovian group between the latency to first approach the CS+ and impulsivity measures (Figure IX.6-B; all Spearman r > -0.3813, all p > 0.1596). No

correlation between behaviour and impulsivity was observed in the instrumental group either (Figure IX.6-B; all Spearman r > 0.1248, all p > 0.0771), which was anticipated as participants believed the delivery of the reward was contingent on their interaction with the CS+. Sign-trackers, goal-trackers and 'instrumental' participants had similar scores in the general BIS-11 scale (Figure IX.6-B; $F_{2, 28} = 0.5179$), the BIS-11 attentional scale (Kruskal-Wallis $H_2 = 1.287$, $p = 0.5254$) and non-planning scale ($F_{2, 28} = 0.6524$, $p = 0.5285$). The three groups selected decks in a comparable manner in the IGT ($F_{2, 27} = 0.000$, $p > 0.999$) and obtained a similar IGT score during the 40 last trials ($F_{2, 27} = 0.2265$, $p = 0.7988$). Participants had a similar rate ($F_{2, 28} = 0.07429$, $p = 0.9286$) and number (Kruskal-Wallis $H_2 = 0.1588$, $p = 0.9237$) of commission errors during the second block of the GNG. Finally, no difference was detected between the three groups in their discounting values ($F_{2, 25} = 0.3147$, $p = 0.7328$) or their AUC ($F_{2, 25} = 0.4091$, $p = 0.6686$).

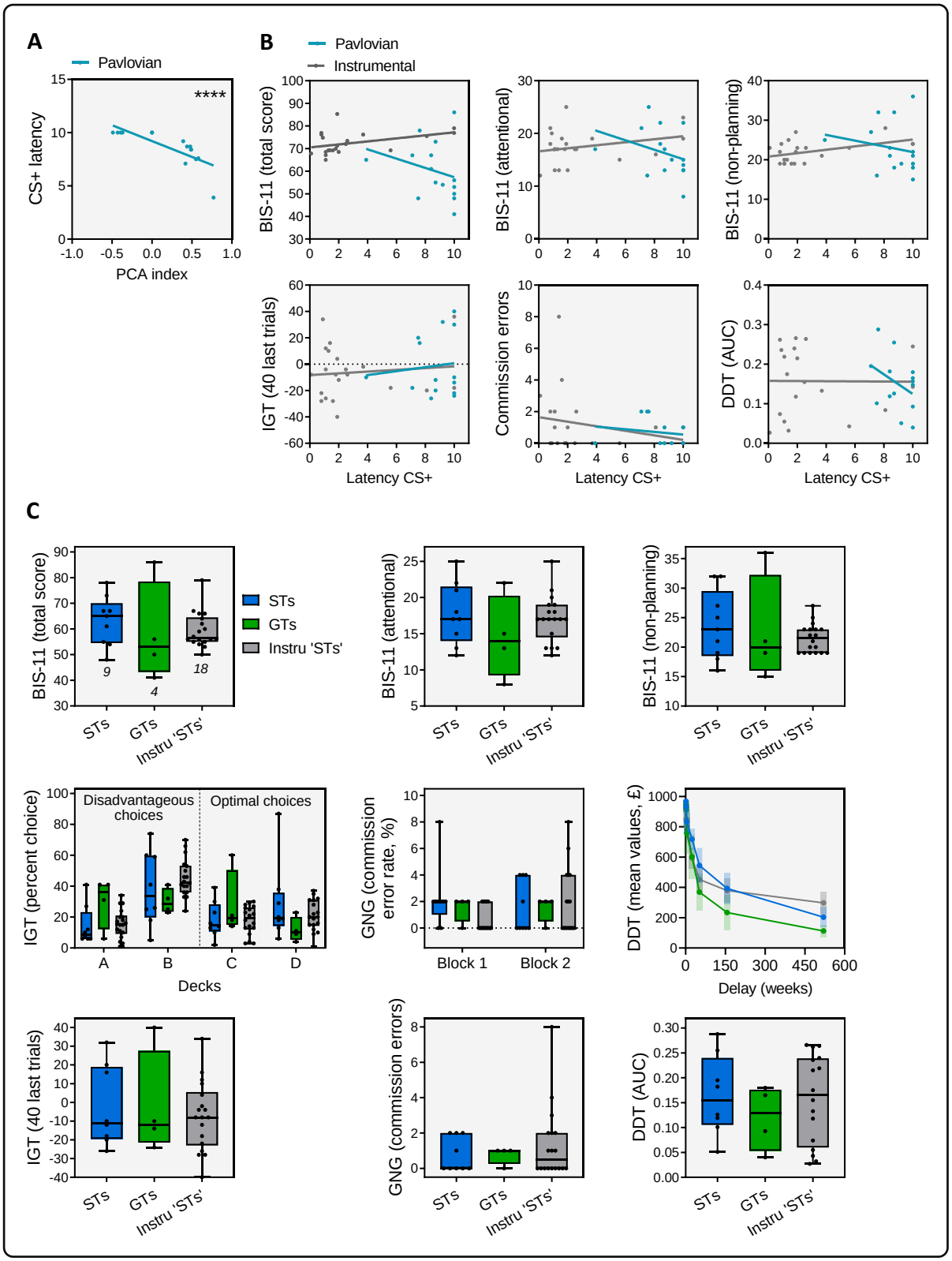


Figure IX.6. Relationship between impulsivity and conditioned responses. (A) The latency to first contact the CS+ is correlated to the PCA index in pavlovian participants. (B) Correlations between impulsivity measures and the latency to first contact the CS+ in pavlovian participants and in participants who thought they had to perform an action to obtain the reward, genders combined. (C) Comparison of impulsivity measures between sign-trackers, goal-trackers, and participants who thought they had to perform an action, genders combined. (** $p \leq 0.01$, **** $p < 0.0001$).

Discussion

After exposing participants to a computerised pavlovian conditioning based on eye-tracking, then to a virtual environment in which they were able to virtually interact with stimuli, the remaining step was to design a paradigm replicating the rodent pavlovian procedure wherein individuals could navigate in a real room and manipulate tangible objects. Coincidently, in 2022, two similar experiments were undertaken by independent teams that devised small apparatuses using retractable levers as predictive cues and tokens leading to money as reward; they calculated an index score based on participant's physical interaction towards these elements and, in one case, eye-tracking technology (Colaizzi *et al.*, 2022, and see Joyner *et al.*, 2018; Cope *et al.*, 2022). The present experiment independently took a slightly different approach. Instead of building a single device presented in front of the participant, the conditioned stimuli were located on two walls surrounding a central reward dispenser in such a way that individuals, as in rodent procedures, would need to move to interact with the CS and the US. Distances between objects as well as inter-trial intervals were carefully designed in order to encourage exploration and all elements were manipulable (Meyer *et al.*, 2014) to allow conditioned responses to naturally evolve and ensure individual variation in behaviours expressed in the learning process. Contrary to Chapters VII and VIII in which no primary reward was issued, the predictive cue was paired with food – although indirectly through the collection of marbles – to stimulate appetitive conditioning. As a result, and whilst it is important to recognise that experimenter's expectations might have subconsciously impacted participant's responses in the final survey, 100% of participants reported wanting to obtain the marbles, 81% stated that they liked getting the marble, and a mere 35% admitted being bored compared to 65% in Chapter VII. Wardle and her team (Wardle *et al.*, 2018) independently used a similar rewarding technique in which participants were able to select a savoury or sweet snack from their choice. It is also interesting to note

that Colaizzi and her team decided to use monetary incentives after realising that in pilot studies, participants did not demonstrate a particular motivation towards food rewards (Colaizzi *et al.*, 2022 – about Joyner *et al.*, 2018).

Before discussing the quantitative results obtained using this 'real-life' pavlovian procedure, it might be valuable to describe behaviour using a qualitative perspective; indeed, participant's behaviour was measured with a coding software using videos taken from a distal camera, but the impressive multiplicity of responses that unfolded ought to be detailed despite the absence of formal data. In comparison with computerised tasks, which promote restricted options and a limited set of unconscious behaviours, by attempting to make the procedure ecologically viable more variables were introduced thereby making the task more complex and leading to a range of off-task behaviours. All participants behaved similarly at the beginning of the session. Immediately after guiding participants through the instructions, the experimenter left the room to manually launch the script. During this short interval, participants started to explore the environment by looking at the buttons (CSs) and the marble dispenser (US location) and, probably due to the fact that directives were to 'collect as many marbles as possible', tried to press the buttons or, less frequently, to touch the marble dispenser. When the first trial commenced – either a rewarded CS+ trial or an unrewarded CS- trial, different reactions related to the action the participant was engaged in and to which button was activated first were triggered, initiating the development of several categories of behaviour which, as in previous experiments, we shall divide in two categories: (1) pavlovian and (2) instrumental.

(1) Pavlovian: Participants understood that their interaction with the elements composing the room did not modify the rate and the timing of reward delivery, either because the first rewarded trial occurred before they had time to interact, either because they were initially pushing the buttons for a few trials but then took the initiative to attempt to withhold their response. This learning interpretation was confirmed by participant's responses in the

end-of-experiment survey. These individuals progressively stopped interacting with the buttons, especially during the second training session, and mainly stayed by the CS+ button, by the US, walked or stood in between; most still collected the marbles but a few did not. Measuring the development of their CR acquisition was more complex as only body position and eye gaze revealed whether their attention was biased towards the predictive cue or towards the reward location, which is why evaluating the discrimination between CS+ and CS- using a PCA index score was not effective in goal-trackers, and why the latency to first contact the CS+ (used in correlation analyses) started 4 seconds after illumination of the button. Interestingly, a subset of participants 'evidently' *knew* that the delivery of the marbles was not contingent upon their interaction with the CS+ (no surprise was displayed when they did not press the button and reward still followed), but still did so on a few trials. This behaviour might have been the expression of a human sign-tracking response (after all, contrary to levers, buttons are hardly nibbleable), or the result of boredom, or alternatively individuals might have been verifying that the rule did not change or that they did not fail to understand a more complex pattern of response.

(2) Instrumental: Participants believed that pressing the CS+ button led to the delivery of the marbles. Even without confirming their interpretation of the procedure (*i.e.*, pavlovian or instrumental) using the survey, videos clearly showed individuals looking expectantly at the button, hastening to press it as soon as it illuminated as though the procedure was timed, then immediately turning to the marble dispenser to wait for the marbles; which is why, this time, the latency to first contact the CS+ on correlation analyses is particularly fast. Moreover, after a few seconds, participants often appeared troubled when the reward was not yet delivered and pressed the button again, with a demeanour vastly different from the supposed sign-trackers from the previous paragraph. It is within this instrumental group that various fascinating behaviours emerged. (i) *Simple instrumental*: Participants exclusively pressed the CS+ button, once or a few times, immediately after its

illumination. (ii) *Simple sequence*: Individuals believed they needed to press the CS- button as well as the CS+ button to obtain the marbles, usually in this succession in order for the CS- to 'activate' the 'real' CS+ button. (iii) *Complex sequence*: Four participants out of thirty (13.3%) walked back and forth between CS buttons and pressed both several times in different patterns (*e.g.*, twice the CS-, once the CS+, once the CS-). Importantly, individuals using instrumental sequences were more likely to be stuck in patterns and failed to deviate from them when they did not meet their expectations (in other words, when their sequence was not followed by marbles), ignoring variations and finding explanations for them: 'I did not press hard enough', 'it was a technical problem'. What is more, when participants who thought marbles were triggered by CS sequences omitted one and noticed that the reward still followed CS+ illumination, they persisted in their sequences and exhibited confusion during unrewarded trials.

How and why these complex responses emerged is intriguing. First and foremost, it is evident that in the absence of instructions, human participants have a tendency to overthink, overcomplicate in an attempt to find meaning. Instead of considering that the illumination of a button was predictive of an event regardless of their action, many participants assumed that they were undertaking the task incorrectly and that they needed to find how to '*make the CS- work*'. Still when participants understood that marbles were delivered after the illumination of the CS+ button regardless of their action, a small subset appeared perplexed when the CS- was not followed by the reward and started pressing the buttons again.

In 1961, Bruner and Revusky discussed that literature tended to only report behaviours directly leading to the reinforcement instead of also recording associated, seemingly irrelevant responses produced by test subjects; when doing so in an instrumental conditioning paradigm, it was discovered that human participants believed the reward could only be obtained by 'a pattern of responses on at least one collateral [object] in order to 'set

up' the reinforced [object]' (Bruner and Revusky, 1961). Similarly, in a study in which no instruction was given and the reward was not dependent on any response, but manipulable objects were presented, 15% of participants – percentage reminiscent of the present experiment – developed persistent 'superstitious' complex response patterns (Ono, 1987). This phenomenon has also been observed in various non-human animals who sometimes develop sequences of irrelevant and stereotyped behaviours which are hypothesised to be accidentally maintained by the reward being delivered at the end of the chain (Kellog, 1949; Skinner, 1948). As stated in Chapter I, pavlovian learning is a fundamental and adaptive process by which animals learn to associate environmental stimuli to events to signal the presence of dangers and the availability of rewards, leading to avoidance of the former and attraction to the latter and thereby increasing the animal's chances of survival. Accordingly, instrumental learning is evolutionarily essential to increase the frequency of specific responses to obtain rewards and decrease the likelihood of producing others to avoid punishments. Detecting correlations between events is primordial to learn either of the aforementioned associations; the stimulus/response and the outcome must be contingent and contiguous, which means that when two events keep occurring together, a causal relationship is instinctively inferred. This gives rise to cognitive biases such as the illusion of causality – or tendency to extrapolate false causal relationship, which is enhanced when the outcome is desired and when the participant's behaviour is the suspected cause of said outcome, possibly due to an increased production of responses preventing the individual to learn the true reinforcement (Alloy *et al.*, 1979; Matute, 1996 and 2015). Causality bias is thought to underlie superstitious thinking and ritualistic behaviours such as performing rain dances or sacrifices to control the weather, in that repeated coincidences lead to an overestimation of contingency and the detection of false patterns. These connections were particularly relevant when individuals needed a sense of predictability as they provided explanations to and control over natural events (Alloy *et al.*, 1979; Blanco and Matute, 2018; Matute *et al.*, 2015;

Pinto, 2022). Ignoring infrequent variations and remaining oblivious to negative feedback might be less adaptively detrimental than failing to recognise a potentially life-threatening or rewarding causal relationship, and less energetically demanding than learning a new association or seeking evidence (Haselton and Nettle, 2005; Pinto, 2022; Taylor and Brown, 1988). Within the context of the present experiment, it is thus plausible that participants who coincidently pushed the buttons in an increasingly complex sequence and saw their pattern repeatedly reinforced by the delivery of marbles – which was the instructed and desired outcome – simply developed a robust and persistent illusion of causality. A compelling example was a female participant who – considerably – misunderstood the instructions stipulating that computerised impulsivity tasks would be undertaken *after* the first behavioural session and commenced the tasks when the experimenter left the room to launch the pavlovian script. This subject later reported that her clicking of the computer whilst taking the Go-NoGo, the Iowa Gambling Task and the Delay Discounting Task were triggering the delivery of marbles. This participant was naturally excluded from the analysis, but her behaviour nonetheless deserved to be mentioned to illustrate the hypothesis of causality bias.

Another element as to why other participants might have shown a propensity to produce responses (*e.g.*, interacting with the CS- and the CS+ buttons, playing with the marbles and arranging them by colour…) despite witnessing that the reward was delivered irrespective of their action is boredom. Indeed, contrary to non-human animals whose time is partially spent resting, exploring and seemingly 'doing nothing', most humans from modern Western societies are not comfortable standing in a room and waiting without constant stimulation and engagement – particularly younger individuals who are used to continuous exposure to external stimuli (Crampton and Hodge, 2009; Pielot *et al.*, 2015; Vodanovich and Kass, 1990). In this situation, purposeful interaction can be difficult to separate from activities undertaken because of ennui. The recent publication by Colaizzi

(Colaizzi *et al.*, 2022) mentions video examples of sign- and goal-tracking behaviours in their Supplementary Materials, however at the time of writing the videos were not yet available. Comparing the behaviour of participants from both experiments would provide valuable insight into paradigm-induced differences.

The multiplicity of behaviours expressed by humans combined with the unrestricted setup of the experiment made the categorisation into sign- and goal-trackers complex. Participant's responses in the final survey provided a standardised and reliable measure to separate individuals who learnt an instrumental association or a pavlovian association however, as described in the paragraphs above, both the direction (Directional index) and the physical interaction with stimuli (PCA index) appeared relevant to identify potential variations in attraction to reward cues. In rodent studies, only the PCA index is taken into account as animals produce clear consummatory-like responses towards the reward location and the reward-paired cue which are simple to measure, although conditioned responses are composed of additional associated behaviours that would deserve to be evaluated as well (Uslaner *et al.*, 2006). Humans, on the other hand, sometimes did not interact with objects but fixated on the reward location or the reward-paired cue during cue presentation instead, particularly when they learnt a pavlovian association and understood that they did not need to interact to obtain the marbles. Another difference with rodent experiments is that the CS+, CS- and the US (collected marbles) were available at all time, which means that participants were able to interact and 'play' with them when bored; this would have affected the PCA score. Incorporating *both* gaze and physical interaction into a single score would thus provide a more complete description of individual conditioned responses; however, this proved to be too complex for the present analysis. Colaizzi and her team classified participants using a score based on the rodent PCA index and a threshold of -0.5 and 0.5 (Colaizzi *et al.*, 2022), whereas Cope and colleagues used both a 'latent profile analysis' inspired by the PCA index – magazine gaze, lever gaze, lever presses – and a gaze index;

however these authors concluded that data-driven physical interaction (*i.e.* lever presses) resulted in a better separation amongst groups and was more useful to classify individuals (Cope *et al.*, 2022; supplementary materials). In the same way, in the present study, the PCA index score yielded a more balanced repartition of sign- and goal-trackers in both genders and reflected the instrumental learning more coherently in that participants exclusively interacted with the predictive cue during its illumination as opposed to the marble dispenser, and behaviour did not evolve from the first to the second session.

The same pavlovian learning indicators used in Chapters VII and VIII were employed in this experiment to estimate whether behaviours expressed by participants were legitimate conditioned responses. Only sign-trackers appeared to successfully discriminate between the CS+ button (rewarded) and the CS- button (unrewarded) contrary to goal-trackers who do not produce a significantly more goal-tracking response during CS+ trials, however this result needs to be delved into further. In the end-of-experiment survey, only three participants reported being confused about which button was predictive of reward delivery, which means that 97% *did* discriminate accurately. In the previous experiment, we observed a dichotomy between sign-trackers and goal-trackers whose discrimination was directed towards the object of their respective CR: the CS+ over the CS- for the former, and the US during CS+ trials over CS- trials for the latter. Here, only the PCA score was extracted and compared, which might give an incomplete representation of goal-tracker's responses. Additionally, human goal-trackers were naturally classified by considering their lack of interaction with the CS+ button and their more numerous contacts with the marble dispenser; still, the videos and the survey demonstrated than most did understand that the CS+ was rewarded. As such, it is possible that the few and only interaction with any button might have been with the CS- during its illumination in order to determine whether it could be turned into a rewarded switch as well, thus increasing their PCA index score during CS- trials. An index involving both physical interactions and gaze might have allowed to confirm

the discrimination in this group. The second marker of learning, the evolution of conditioned responses across sessions, returned surprising results as well. Sign- and goal-trackers did develop their behaviour from the first to the second session as they were learning about the association between CS+ and US, however both groups *decreased* their interaction – despite rodent literature showing an increase of sign-tracking behaviour at the end of training (Flagel *et al.*, 2009; Saunders and Robinson, 2012; Meyer *et al.*, 2012a; also see Chapters III and IV). All 'pavlovian' participants, regardless of their CR, appeared to have explored their environment during the first session and then settled once they understood that interacting with the items did not affect marble delivery. Notwithstanding the direction of behaviour evolution, these results are still indicative of learning, development of strategy and, therefore, of conditioned responses.

As already observed in Chapter VIII, and contrary to the majority of rodent studies, sign- and goal-trackers diverged from the beginning of training, which might suggest that variations in incentive salience attribution to reward cues are accompanied by inherent differences in attentional biases irrespective of appetitive learning. This trend was also found in Colaizzi's experiment, in which non-STs differed from STs at all stages (Colaizzi *et al.*, 2022). Female participants displayed considerably more sign-tracking tendencies than males, who were predominantly classified as goal-trackers, which differs from Colaizzi's experiment that either found no gender difference in the propensity to attribute incentive salience to pavlovian cues, or a tendency for males to be more sign-trackers than females depending on the measure used (Colaizzi *et al.*, 2022); it is however important to remember that participants were children and that their learning profiles might therefore be different than those of adults. In addition to sample composition and size (*i.e.*, very few males), the procedure used in the present experiment is not directly comparable to the two experiments using 'real-life' apparatuses mentioned at the beginning of this discussion, and the conditioned behaviours expressed might therefore deviate.

Participants who undertook the study were a few years younger on average than those of Chapter VII and, accordingly, 97% were students and 82% did not complete an undergraduate degree. Samples thus differ slightly and it is important to consider this element whilst comparing results. In line with earlier experiments of this thesis and possibly because of the low number of male participants, impulsivity performances were consistent across genders and ages. Sign-trackers were more impulsive than goal-trackers in the attentional subscale of the BIS-11 questionnaire, which assesses the ability to focus attention and concentrate. This result might echo previous reports of inferior performances from sign-trackers in attention tasks due to a 'bottom-up' analysis style caused by a relatively reduced cholinergic neuromodulation (Koshy Cherian *et al.*, 2017; Paolone *et al.*, 2013; Pitchers *et al.*, 2017). Moreover, in children, sign-tracking phenotype has been found to be associated with increased symptoms of attention deficits (Colaizzi *et al.*, 2022). Yet, instead of the expected relationship between sign-tracking behaviour and higher impulsivity (Garofalo and di Pellegrino, 2015; Lovic *et al.*, 2011), no correlation was found in our experiment. As remarked earlier, very few of the participants who learnt a pavlovian association – and therefore understood that they did not need to interact for marbles to be delivered – nonetheless continued to push the CS+ button. As a result, the latencies to first contact the CS+, which were used in correlation analyses, are skewed towards the higher end of the scale (from 7 to 10 seconds); it is worth noting that when CS+ latency is substituted with PCA scores, no correlation is found either. In contrast, Cole and her team found that self-assessed behavioural impulsivity was a significant predictor of sign-tracking (Cole *et al.*, 2022), and Colaizzi reported a reduced inhibitory control in sign-trackers – although without correlation with PCA score (Colaizzi *et al.*, 2022). Moreover, in line with results observed in the current experiment, no difference was found between sign- and goal-trackers in self-report measures of impulsivity (Colaizzi *et al.*, 2022). Disparities between experiments

might be due to differences in procedures or to the distinct population targeted (*i.e.*, children vs. adults) as impulsivity varies across the lifespan (Steinberg *et al.*, 2008).

Participants who believed that their action triggered the delivery of the marbles differed markedly from individuals who learnt a pavlovian association. Their interaction was essentially cue-directed, and their behaviour remained consistent across sessions; in other words, participants who developed either a simple instrumental response or a complex pattern maintained them until the end of the training in order to obtain the reward. Compared to pavlovian participants, and due to individuals responding immediately to the illumination of the cue, the latencies to first contact the CS+ in the instrumental group, whilst more spread over the scale, were mainly skewed towards the lower end (0 to 2 seconds). The level of impulsiveness was neither correlated to the speed at which participants contacted the CS+, nor to the type of instrumental response participants were performing (complex sequence vs. simple response; data not shown).

Chapter X
Conclusion of Section Two

Experiments from the Section Two sought to design different pavlovian procedures to identify sign-tracking and goal-tracking in human participants, and attempted to relate behavioural differences to several measures of impulsivity. Chapter VII involved a computerised pavlovian procedure in which phenotypes were inferred using a built-in webcam eye-tracker. In Chapter VIII, participants navigated in a virtual pavlovian environment; alcohol and drug use were assessed and compared to behavioural measures. Finally, the experiment developed in Chapter IX endeavoured to mimic rodent pavlovian procedures in allowing participants to interact with manipulable objects and offering them a real incentive.

Due to a combination of technical complications and imperfect procedure, the first online pavlovian paradigm was not effective in motivating individuals and identifying sign- and goal-trackers. In contrast, the virtual pavlovian environment and the 'rodent-like' pavlovian procedure did succeed in detecting phenotypes which validated criteria observed in animals attributing motivational salience to reward cues. Importantly, in both paradigms, a significant portion of participants behaved as if the task was instrumental, and qualitative analyses revealed a striking diversity of conditioned responses. Neither gender differences nor the relationship between impulsivity measures and behavioural phenotypes were consistent across the three procedures. Taken as a whole, and despite past research, results did not suggest any robust association between a higher impulsivity and sign-tracking behaviour as identified in these procedures. Impulsivity is a multi-faceted construct with not only inter- but also intra-individual variation (*i.e.*, state impulsivity) and although the studies described here assessed several types of impulsivity, measuring it artificially in an experimental room might not be as valid as if these measures had been taken at various moments in participants' natural environment (ecologically momentary assessments; McCarthy *et al.*, 2017).

All the experiments presented in this section must be interpreted with caution due to the low number of participants – including an imbalance between male and female participants – and the exploratory nature of the procedures. Research into human sign- and goal-tracking is lacking, and multiple approaches are needed to increase the 'ecological validity'. There is currently no consensus on the appropriate methodology to investigate individual variation in cue-induced motivation in humans, but the studies presented in Section Two as well as the wider literature did provide some useful insights. Because reward cues are only attributed with motivational value through their pavlovian association with the reward, experiments must ensure that the reward itself holds incentive value. Offering monetary or food rewards to participants instead of simple computerised illustrations might be a more reliable way to proceed. In both human and non-human animals, conditioned responses are complex and can be expressed in various ways (*e.g.*, direct interaction, gaze, body position, etc); Chapters VII, VIII and IX assessed several of these characteristics independently, and sign-tracking experiments should ideally consider them together going forward, either by creating an index including all aspects or by taking them all into account separately. Designing procedures allowing the expression and description of such composite responses by giving participants the possibility to physically interact with objects might help defining humans' specific cue-directed phenotypes, as they might be different than those of rodents, and ultimately find the most appropriate paradigm to identify sign- and goal-tracking in humans. However, limiting and controlling the range of behaviours might be desirable to prevent excessive off-task responses.

Once reliable paradigms to identify sign- and goal-trackers are developed, these phenotypes might benefit from being confirmed using a more direct measure of motivation such as a pavlovian-to-instrumental transfer procedure (Garofalo and di Pellegrino, 2015; Schad *et al.*, 2020) in which the reward cue should enhance instrumental approach

preferentially in sign-trackers. In non-human animals, sign- and goal-trackers do not only differ behaviourally and cognitively, but also neurobiologically. Although a few studies have investigated neuronal activity associated with variations in incentive salience attribution in humans (Colaizzi *et al.*, 2022; Duckworth, 2017; Schad *et al.* 2020; Versace *et al.*, 2016), none measured it during a pavlovian conditioning. Monitoring neuronal responses using fMRI or EEG during this task particularly in the nucleus accumbens would provide an interesting comparison and would also support translational research – although this would need to be undertaken using a simpler pavlovian task in which participants would be relatively restrained such as a computerised procedure (Schad *et al.* 2020) or, potentially, a virtual environment. Future research might want to explore whether pavlovian conditioned responses can be altered by manipulations as is the case in rodents (Cogan *et al.*, 2019), which could have relevance to alleviate maladaptive behaviours associated with sign-tracking such as aberrant incentive salience attribution to drug or trauma-associated cues.

Chapter XI
Synthesis

This thesis sought to extend the literature on rodent and human sign-tracking. **Section One** examined whether pre- and postsynaptic changes in the NAc core corresponded with variations in the motivation induced by pavlovian cues in rats. More specifically, this section investigated the structural organisation of dendritic spines as well as presynaptic and postsynaptic activity in the NAc core in male and female rats. The comparison between baseline and cue-induced plasticity, the impact of the reward on test day, the influence of sex and of the oestrous cycle were also studied. Finally, we attempted to replicate the modulation of sign-tracking responses by the β-adrenergic blocker propranolol in order to observe its effect on spine plasticity. Results obtained in this thesis suggest that there may be differences in postsynaptic structural organisation in the NAc core, and that such variations are associated with individual differences in the propensity to attribute food-predictive cues with motivational value.

In **Section Two**, three distinct pavlovian procedures designed to identify variability in incentive salience attribution in humans were assessed: an online paradigm, a virtual pavlovian environment, and a procedure resembling those used in rodents. The relationship between behavioural phenotypes as well as gender differences were analysed. Whilst procedures in which participants were able to interact with the components – the virtual pavlovian environment and the 'real-life' pavlovian procedure – were able to successfully detect individual differences in conditioned responses, further work is needed to enable comparison with animal studies. In contrast to the literature, no relationship was found between cue-induced motivation and impulsivity. Preliminary findings from Section Two provide guidance for future studies aiming to investigate sign- and goal-tracking phenotypes.

Translational work is essential to use the knowledge acquired with non-human animals to better understand the mechanisms underlying human functioning and, ultimately, improve human pathologies. Decades of research has shown that many factors played a role

in the success of cross-species procedures, that the outcome was often difficult to predict, and that some procedures translated better than others (Leenaars *et al.*, 2019). Within the theoretical scope of the present thesis, the dopamine-based distinction between 'wanting' and 'liking' has been demonstrated in rodents and in humans using equivalent dopamine manipulation techniques (Berridge and Valenstein, 1991; Leyton, 2010). The development of a virtual Morris water maze for humans allowed to robustly confirm the similarity between both species, in that the spatial cognition involved in completing this task requires an intact hippocampus and males consistently perform better than females (Astur *et al.*, 1998; Astur *et al.*, 2002; Sandstrom *et al.*, 1998). The study of emotional learning, aversive learning and anxiety in rodents successfully led to the development of treatments in humans (Fenster *et al.*, 2018; Milad and Quirk, 2012; Zuj and Norrholm, 2019). Researchers sometimes find elegant solutions to evaluate cognitive processes which, initially, do not appear to be easily accessible without direct report, as is the case with episodic memory (Ameen-Ali *et al.*, 2017). As illustrated above, translational procedures often attempt to develop similar procedures for all species, with a restricted set of variables in an effort to simplify the comparison. However, it appears critical to consider the limitations of such procedures when interpreting the underlying processes in each species. Many parallels can be drawn between human behaviour and that of other animals because they follow similar rules (*e.g.*, reinforcement); yet, many factors cannot be well-controlled in human studies, and people are also *aware* that they are being tested and might unconsciously adapt their behaviour or their responses. Thus, using identical experimental parameters in animals and people is challenging, and may not be an ideal approach. Another parallel aspect that might be interesting to explore to increase the translational validity of experiments is to modify laboratory procedures to assess more spontaneous and natural behaviours (Puścian and Knapska, 2022). With this objective in mind, researchers have recently developed a paradigm in which the propensity to attribute reward cues with incentive value was measured

in an enriched environment using more ecologically relevant stimuli instead of the traditional but artificial lever (Vigorito *et al.*, 2022).

The work of the present thesis provides further insight into aspects we might have overlooked in rodents because of the tendency to neglect qualitative observation of behaviour in operant chambers. Human pavlovian procedures exposed a wide array of different behaviours and ways of learning (*e.g.*, instrumental association). Because it is difficult to gain insight about what non-human animals are 'thinking', is conceivable that some rodent individuals learn a different association as well. This work highlights the importance of taking individual variation into account and acknowledge neurobehavioural differences in all species, to provide therapies better tailored to each individual's needs further along the line.

Concluding statement

Stimuli that are biologically relevant to the survival of organisms can possess incentive value, motivating animals to seek and approach them. Being able to appropriately respond to environmental elements signalling the presence of rewards is evolutionarily beneficial, and such pavlovian 'cues' sometimes acquire motivational value in their own right. However, *excessive* incentive salience can lead to disorders such as addiction, *insufficient* incentive salience can induce anhedonia in PTSD or depression, and *fearful* excessive incentive salience is thought to be involved in PTSD and symptoms of psychoses. Studying sign-tracking behaviour offers a mean to isolate the motivational aspect of pavlovian cues from the predictive component and examine how reward cues can, in some individuals, drive behaviour. To the best of our knowledge, the work undertaken in this thesis is the first to report specific postsynaptic characteristics associated with the attribution of incentive salience to food-predictive cues in rats. These findings can contribute to the understanding of the mechanisms underlying the vulnerability to develop some disorders.

The human pavlovian procedures discussed in this thesis also provide insights for the development of paradigms allowing to reliably recognise variations in appetitive responses to reward-related stimuli which could, ultimately, offer a mean to identify risk profiles for impulse control disorders.

Appendices

Appendix A

Barratt Impulsiveness Scale (BIS-11) used in Chapters VII, VIII and IX (Patton *et al.*, 1995; Stanford *et al.*, 2009).

Directions: People differ in the ways they act and think in different situations. This is a test to measure some of the ways in which you act and think. Read each statement and select the appropriate response. Do not spend too much time on any statement. Answer quickly and honestly.

1 – Rarely/Never 2 – Occasionally 3 – Often 4 – Always/Almost always

1.	I plan tasks carefully.	1	2	3	4
2.	I do things without thinking.	1	2	3	4
3.	I make-up my mind quickly.	1	2	3	4
4.	I am happy-go-lucky.	1	2	3	4
5.	I don't 'pay attention'.	1	2	3	4
6.	I have 'racing' thoughts.	1	2	3	4
7.	I plan trips well ahead of time.	1	2	3	4
8.	I am self-controlled.	1	2	3	4
9.	I concentrate easily.	1	2	3	4
10.	I save regularly.	1	2	3	4
11.	I 'squirm' at plays or lectures.	1	2	3	4
12.	I am a careful thinker.	1	2	3	4
13.	I plan for job security.	1	2	3	4
14.	I say things without thinking.	1	2	3	4
15.	I like to think about complex problems.	1	2	3	4
16.	I change jobs.	1	2	3	4
17.	I act 'on impulse'.	1	2	3	4
18.	I get easily bored when solving thought problems.	1	2	3	4
19.	I act on the spur of the moment.	1	2	3	4
20.	I am a steady thinker.	1	2	3	4
21.	I change residences.	1	2	3	4
22.	I buy things on impulse.	1	2	3	4
23.	I can only think about one thing at a time.	1	2	3	4
24.	I change hobbies.	1	2	3	4
25.	I spend or charge more than I earn.	1	2	3	4

26. I often have extraneous thoughts when thinking.	1	2	3	4
27. I am more interested in the present than the future.	1	2	3	4
28. I am restless at the theatre or lectures.	1	2	3	4
29. I like puzzles.	1	2	3	4
30. I am future oriented.	1	2	3	4

Appendix B

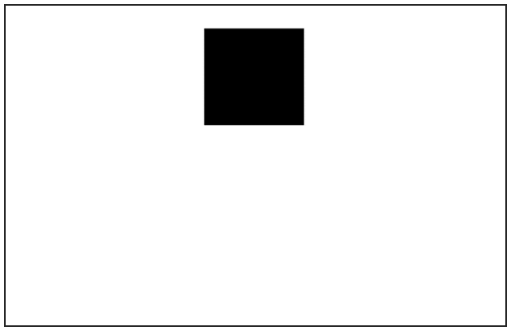
Instructions given to participants before the Delay Discounting Task.

‘In this task you will be asked to make some choices about money. You will not get the money that you choose, but make your choices as though you were really going to get the money. In each trial, you will be asked to choose between two different amounts of money, ranging from £1 to £1000. One amount will be available immediately and the second amount given after a specified delay. You must choose which amount you would prefer by clicking on it. After you make your choice, another set of money amounts will be presented until the task is complete.’

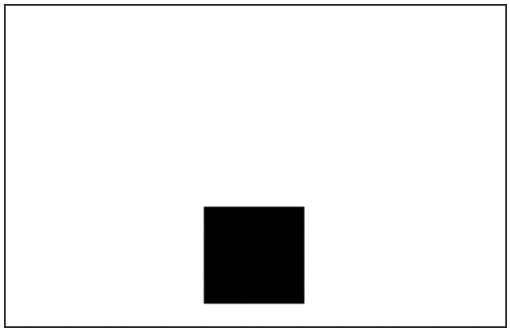
Appendix C

Instructions given to participants before the Go-NoGo.

‘In this task you will be asked to press the SPACE BAR when the black square appears at the top of the screen and not press anything if the black square appears at the bottom of the screen. Click NEXT to see an example.’



‘In this example, the square is to the top so you would press the space bar. Click NEXT to see another example.’



‘In this example, the square is to the bottom so you would NOT press the space bar. Click NEXT to continue.’

‘Are you ready to start the real thing? You will not be given feedback. Please be aware that this task will be fast-paced. Press START when you are ready to begin.’

Appendix D

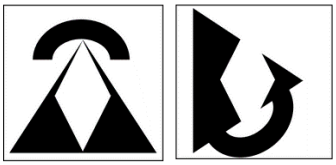
Instructions given to participants before the Iowa Gambling Task.

‘In this task, your goal is to win as much money as possible. You will start with £2000. You can earn money by selecting cards from different decks. Each card will give you a reward but sometimes you'll also have to pay a fee. You'll get to choose a total of 100 cards from across the four decks. Try to see how much money you can make!’

Appendix E

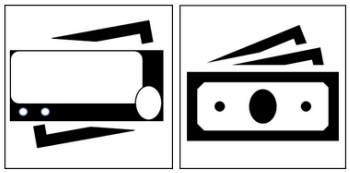
Instructions given to participants during the computerised image-based pavlovian procedure.

'One of these two targets will appear briefly at the top of the screen.'



'After some time, it will be followed by a grey square appearing at the bottom of the screen. Your task is to PRESS THE SPACE BAR with your dominant hand when you see the GREY square appear at the bottom of the screen. You will then see one of two possible outcomes appear in its place.'

'These are the two possible outcomes:'



'The one on the left is NEUTRAL and the one on the right is MONETARY. Please imagine that the monetary outcome represents actual money that you can win.'

'Please press NEXT to start practicing the task.'

(Practice trial)

'The outcome is presented at the bottom of the screen, in place of the grey square, irrespective of whether you press the space bar or not. Please ask the experimenter now if you have any questions relating to the task. While you complete this task, we will be monitoring your eye movements using eye tracking. The first step will be to calibrate the eye tracker. You will need to fixate a point on the screen and follow it around with your eyes as it is changing position. When you are ready to begin this, please press NEXT to start the calibration followed by the main task.'

Appendix F

End-of-experiment survey used in Chapter VII.

The first part concerns the eye-tracking tasks.

1. I would only receive the monetary outcome if I pressed the grey square.

Completely true *Completely false*

1 2 3 4 5

2. I wanted to obtain the monetary outcome.

Completely true *Completely false*

1 2 3 4 5

3. I felt indifferent as to whether I would receive the neutral or the monetary outcome.

Completely true *Completely false*

1 2 3 4 5

4. I was pressing the grey square automatically without thinking about the outcome.

Completely true *Completely false*

1 2 3 4 5

The following questions concern the whole study (questionnaires and tasks).

5. I enjoyed the experiments

Dislike *Like*

1 2 3 4 5

6. I felt the experiments were challenging

Completely true *Completely false*

1 2 3 4 5

7. I became bored throughout the experiments.

Completely true *Completely false*

1 2 3 4 5

8. I felt frustrated or confused throughout the experiments.

Completely true *Completely false*

1 2 3 4 5

9. Is it the first psychology experiment you have participated in at the University?

Yes *No*

10. The instructions and explanations were clear and provided me with enough information to understand the tasks.

Completely true *Completely false*

1 2 3 4 5

11. Did you encounter any technical problem during the experiments?

12. Were you nervous about having to come on campus for the study?

Completely true *Completely false*

1 2 3 4 5

13. Do you have something to add or to share with the experimenters? (Further comments or advice).

Appendix G

Alcohol Screening Tool (AST) used in Chapter VIII (Saunders *et al.*, 1993).

Answer the following questions about your alcohol use during the past 12 months. Select the box that best describes your answer for each question. Answer as accurately as you can.

1. How often do you have a drink containing alcohol?

Never *Monthly* *2-4 times a month* *2-3 times a week* *4+ times a week*

2. How many drinks containing alcohol do you have on a typical day when you are drinking?

1 or 2 *3 or 4* *5 or 6* *7 to 9* *10 or more*

3. How often do you have six or more standard drinks on one occasion?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

4. How often during the last year have you found that you were not able to stop drinking once you had started?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

5. How often during the last year have you failed to do what was normally expected of you because of drinking?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

6. How often during the last year have you needed a drink first thing in the morning to get yourself going after a heavy drinking session?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

7. How often during the last year have you had a feeling of guilt or remorse after drinking?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

8. How often during the last year have you been unable to remember what happened the night before because of drinking?

Never *Less than monthly* *Monthly* *Weekly* *Daily or almost daily*

9. Have you or someone else been injured because of your drinking?

No *Yes, but not in the last year* *Yes, during the last year*

10. Has a relative, friend, doctor or other healthcare worker been concerned about your drinking or suggested you cut down?

No *Yes, but not in the last year* *Yes, during the last year*

Appendix H

Drug Abuse Screening Test (DAST) used in Chapter VIII (Skinner, 1982).

The following questions concern information about your possible involvement with drugs *not including alcoholic beverages* during the past 12 months.
'Drug abuse' refers to 1) the use of prescribed or over-the-counter drugs in excess of the directions, and 2) any nonmedical use of drugs.
The various classes of drugs may include cannabis (marijuana, hashish), solvents (*e.g.*, pain thinners), tranquilisers (*e.g.*, Valium), barbiturates, cocaine, stimulants (*e.g.*, speed), hallucinogens (*e.g.*, LSD) or narcotics (*e.g.*, heroin). Remember that the questions *do not* include alcoholic beverages.
Please answer every question. If you have difficulty with a statement, then choose the response that is mostly right.

In the past 12 months…

1. Have you used drugs other than those required for medical reasons?

Yes *No*

2. Do you abuse more than one drug at a time?

Yes *No*

3. Are you unable to stop abusing drugs when you want to?

Yes *No*

4. Have you ever had blackouts or flashbacks as a result of drug use?

Yes *No*

5. Do you ever feel bad or guilty about your drug use?

Yes *No*

6. Does your spouse (or parents) ever complain about your involvement with drugs?

Yes *No*

7. Have you neglected your family because of your use of drugs?

Yes *No*

8. Have you engaged in illegal activities in order to obtain drugs?

Yes *No*

9. Have you ever experienced withdrawal symptoms (felt sick) when you stopped taking drugs?

Yes *No*

10. Have you had medical problems as a result of your drug use (*e.g.*, memory loss, hepatitis, convulsions, bleeding)?

Yes *No*

Appendix I

Instructions given to participants during the virtual pavlovian environment procedure.

'You are about to take part in a computerised psychological assessment task. The object of the task is to earn as many solid gold coins as you can, as quickly as you can. Please note that a coin is only counted as earned once it has been added to your coin counter. Please feel free to explore the task environment and to interact with the objects within it. Instructions on how to do this will be presented shortly. Please note that it is not possible to exit the task until all the coins have been collected, as shown by the onscreen coin counter so please make sure you have around 20-25 minutes to engage with the task in a quiet environment with minimal distractions. Please ensure you answer all onscreen questions at the end of the task.'

(Device-specific instructions)

Keyboard: 'Once inside the task environment you can use the arrow keys on your keyboard to move your viewpoint left, right, up or down. If you wish to 'click' on anything (for example to collect a coin or interact with something in the room) please use your arrow keys to bring the white viewpoint circle over the object you want to click, and then press your space bar key to click.'

Mouse: 'Once inside the task environment you can use your mouse to move your viewpoint. However, you must hold your left mouse button down whilst doing this. If you wish to 'click' on anything (for example to collect a coin or interact with something in the room) please use your mouse (holding down your left mouse button) to move the white circle viewpoint over the object you want to click, and then release and press your left mouse button again to click.'

Touchpad: 'Once inside the task environment you can use the touchpad to move your viewpoint. Simply move your finger around the touchpad to do this. If you wish to 'click' on anything (for example to collect a coin or interact with something in the room) please use your touchpad to move your viewpoint so that the white circle is sitting over the object you wish to click, and then tap your finger on the touchpad to click on it.'

Touchscreen: 'Once inside the task environment you can use your touchscreen to move your viewpoint on a horizontal plane only. Simply move your finger across your touchscreen device to do this. To move your viewpoint vertically (up and down) you will need to tilt your device. If you prefer you can also move your viewpoint horizontally by tilting your device. If you wish to 'click' on anything (for example to collect a coin or interact with something in the room) please use your touchscreen or tilt your device to move your viewpoint so that the white circle is sitting over the object you wish to click, and then tap your finger on the object to click it.'

Appendix J

Screenshot of the end-of-experiment survey used in Chapter VIII.

1. Did you stick to only one method of operating the task?

Yes, I used a touchscreen throughout ○
Yes, I used a keyboard throughout ○
Yes, I used a mouse throughout ○
Yes I used a laptop trackpad throughout ○
No, I changed method whilst doing the task ○

2. Did you do anything within the task that increased the speed of coins being dispensed from the coin dispenser slot

Yes, I clicked the left wall lever and this caused a coin to be dispensed from the slot ○
Yes, I clicked the left wall light and this caused a coin to be dispensed from the slot ○
Yes, I clicked the coin slot dispenser and this caused a coin to be dispensed from the slot ○
Yes I clicked the coin collection bowl and this caused a coin to be dispensed from the slot ○
No, nothing I did affected how quickly the coins were dispensed from the slot ○
I don't know ○

3. Did you adopt a strategy within the task?

Yes, I decided to keep my viewpoint mainly on the coin dispenser wall so that I could see when the coins had been dispensed ○
Yes, I decided to keep my viewpoint mainly on the left wall light as this predicted when the coins would be dispensed ○
Yes, I decided to keep switching my viewpoint between the left and front wall to track when coins might become available ○
No, I couldn't work out an effective strategy during the task as I wasn't sure what was going on ○

Appendix K

End-of-experiment survey used in Chapter IX.

This first part concerns the two experiments in which you were free to wander in the room and interact with the environment.

1. I wanted to interact with the light buttons.

Completely true *Completely false*

1 2 3 4 5

2. I wanted to interact with the marble dispenser.

Completely true *Completely false*

1 2 3 4 5

3. I would only receive the marbles if I pressed the light button.

Completely true *Completely false*

1 2 3 4 5

4. How much did you like to interact with the marble dispenser?

Dislike *Like*

1 2 3 4 5

5. I wanted to get the marbles.

Completely true *Completely false*

1 2 3 4 5

6. How much did you like to interact with the light buttons?

Dislike *Like*

1 2 3 4 5

7. How much did you like to get the marbles?

Dislike *Like*

1 2 3 4 5

8. I could ignore the light buttons and still obtain the marbles.

Completely true *Completely false*

1 2 3 4 5

9. The light buttons told me that I would be rewarded.

Completely true *Completely false*

1 2 3 4 5

10. I would only receive the marbles if I pressed the light button surrounded by a green star.

Completely true *Completely false*

1 2 3 4 5

11. I would only receive the marbles if I pressed the light button surrounded by a red octagon.

Completely true *Completely false*

1 2 3 4 5

12. I would only receive a marble after the red octagon button illuminated.

Completely true *Completely false*

1 2 3 4 5

13. I would only receive a marble after the green star button illuminated.

Completely true *Completely false*

1 2 3 4 5

14. The light buttons directed me to perform an action.

Completely true *Completely false*

1 2 3 4 5

15. My behaviour was different during the first and second marble experiment.

Completely true *Completely false*

1 2 3 4 5

16. Did you change your strategy during the second marble experiment?

Yes *No*

The following questions concern the whole study (questionnaire, marble experiments, computerised tasks).

17. I enjoyed the experiments

Dislike *Like*

1 2 3 4 5

18. I felt the experiments were challenging

Completely true *Completely false*

1 2 3 4 5

19. I became bored throughout the experiments.

Completely true *Completely false*

1 2 3 4 5

20. I felt frustrated or confused throughout the experiments.

Completely true *Completely false*

1 2 3 4 5

21. Is it the first psychology experiment you have participated in at the University?

Yes *No*

22. The instructions and explanations were clear and provided me with enough information to understand the tasks.

Completely true *Completely false*

1 2 3 4 5

23. Did you encounter any technical problem during the experiments?

24. Were you nervous about having to come on campus for the study?

Completely true *Completely false*

1 2 3 4 5

25. Do you have something to add or to share with the experimenters? (Further comments or advice).

References

Abraham, W. C., & Williams, J. M. (2003). Properties and mechanisms of LTP maintenance. *The Neuroscientist*, *9*(6), 463-474.

Agabio, R., Pisanu, C., Luigi Gessa, G., & Franconi, F. (2017). Sex differences in alcohol use disorder. *Current medicinal chemistry*, *24*(24), 2661-2670.

Ahrens, A. M., Singer, B. F., Fitzpatrick, C. J., Morrow, J. D., & Robinson, T. E. (2016). Rats that sign-track are resistant to Pavlovian but not instrumental extinction. *Behavioural brain research*, *296*, 418-430.

Aichert, D. S., Wöstmann, N. M., Costa, A., Macare, C., Wenig, J. R., Möller, H. J., ... & Ettinger, U. (2012). Associations between trait impulsivity and prepotent response inhibition. *Journal of clinical and experimental neuropsychology*, *34*(10), 1016-1032.

Aitken, T. J., Greenfield, V. Y., & Wassum, K. M. (2016). Nucleus accumbens core dopamine signaling tracks the need-based motivational value of food-paired cues. *Journal of neurochemistry*, *136*(5), 1026-1036.

Akam, T., & Walton, M. E. (2021). What is dopamine doing in model-based reinforcement learning?. *Current Opinion in Behavioral Sciences*, *38*, 74-82.

Alberini, C. M., & LeDoux, J. E. (2013). Memory reconsolidation. *Current Biology*, *23*(17), R746-R750.

Albertella, L., Le Pelley, M. E., Chamberlain, S. R., Westbrook, F., Fontenelle, L. F., Segrave, R., ... & Yücel, M. (2019). Reward-related attentional capture is associated with severity of addictive and obsessive–compulsive behaviors. *Psychology of Addictive Behaviors*, *33*(5), 495.

Albrechet-Souza, L., Schratz, C. L., & Gilpin, N. W. (2020). Sex differences in traumatic stress reactivity in rats with and without a history of alcohol drinking. *Biology of sex differences*, *11*(1), 1-11.

Aleman, A., Kahn, R. S., & Selten, J. P. (2003). Sex differences in the risk of schizophrenia: evidence from meta-analysis. *Archives of general psychiatry*, *60*(6), 565-571.

Alexander, B. H., Barnes, H. M., Trimmer, E., Davidson, A. M., Ogola, B. O., Lindsey, S. H., & Mostany, R. (2018). Stable density and dynamics of dendritic spines of cortical neurons across the estrous cycle while expressing differential levels of sensory-evoked plasticity. *Frontiers in Molecular Neuroscience*, *11*, 83.

Allen, J. P., Litten, R. Z., Fertig, J. B., & Babor, T. (1997). A review of research on the Alcohol Use Disorders Identification Test (AUDIT). *Alcoholism: clinical and experimental research*, *21*(4), 613-619.

Alloy, L. B., & Abramson, L. Y. (1979). Judgment of contingency in depressed and nondepressed students: Sadder but wiser?. *Journal of experimental psychology: General*, *108*(4), 441.

Alvarez, R. P., Johnson, L., & Grillon, C. (2007). Contextual-specificity of short-delay extinction in humans: Renewal of fear-potentiated startle in a virtual environment. *Learning & Memory*, *14*(4), 247-253.

Ameen-Ali, K. E., Norman, L. J., Eacott, M. J., & Easton, A. (2017). Incidental context information increases recollection. *Learning & Memory*, *24*(3), 136-139.

Anderson, B. A., Laurent, P. A., & Yantis, S. (2011). Value-driven attentional capture. *Proceedings of the National Academy of Sciences*, *108*(25), 10367-10371.

Andrews, J. S., Jansen, J. H. M., Linders, S., Princen, A., & Broekkamp, C. L. E. (1995). Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. *Physiology & behavior*, *57*(4), 785-790.

Anker, J. J., Zlebnik, N. E., Gliddon, L. A., & Carroll, M. E. (2009). Performance under a Go/No-go task in rats selected for high and low impulsivity with a delay-discounting procedure. *Behavioural pharmacology*, *20*(5-6), 406-414.

Anselme, P., & Robinson, M. J. (2020). From sign-tracking to attentional bias: Implications for gambling and substance use disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *99*, 109861.

Anwyl-Irvine, A. L., Massonnié, J., Flitton, A., Kirkham, N., & Evershed, J. K. (2020). Gorilla in our midst: An online behavioral experiment builder. *Behavior research methods*, *52*(1), 388-407.

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral biology*, *14*(4), 451-479.

Arellano, J. I., Benavides-Piccione, R., DeFelipe, J., & Yuste, R. (2007). Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies. *Frontiers in neuroscience*, 10.

Arnett, A. B., Pennington, B. F., Willcutt, E. G., DeFries, J. C., & Olson, R. K. (2015). Sex differences in ADHD symptom severity. *Journal of Child Psychology and Psychiatry*, *56*(6), 632-639.

Astur, R. S., Ortiz, M. L., & Sutherland, R. J. (1998). A characterization of performance by men and women in a virtual Morris water task:: A large and reliable sex difference. *Behavioural brain research*, *93*(1-2), 185-190.

Astur, R. S., Palmisano, A. N., Hudd, E. C., Carew, A. W., Deaton, B. E., Kuhney, F. S., ... & Santos, M. (2015). Pavlovian conditioning to food reward as a function of eating disorder risk. *Behavioural Brain Research*, *291*, 277-282.

Astur, R. S., Taylor, L. B., Mamelak, A. N., Philpott, L., & Sutherland, R. J. (2002). Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. *Behavioural brain research*, *132*(1), 77-84.

Averbeck, B. B., O'Sullivan, S. S., & Djamshidian, A. (2014). Impulsive and compulsive behaviors in Parkinson's disease. *Annual review of clinical psychology*, *10*, 553-580.

Bacharach, S. Z., Nasser, H. M., Zlebnik, N. E., Dantrassy, H. M., Kochli, D. E., Gyawali, U., ... & Calu, D. J. (2018). Cannabinoid receptor-1 signaling contributions to sign-tracking and conditioned reinforcement in rats. *Psychopharmacology*, *235*(10), 3031-3043.

Baker, D. A., Cornish, J. L., & Kalivas, P. W. (2002). Glutamate and dopamine interactions in the motive circuit. *Glutamate and Addiction*,143-156.

Barker, J. M., Torregrossa, M. M., & Taylor, J. R. (2012). Low prefrontal PSA-NCAM confers risk for alcoholism-related behavior. *Nature neuroscience*, *15*(10), 1356-1358.

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychological bulletin*, *121*(1), 65.

Barrett, D. (2010). *Supernormal stimuli: How primal urges overran their evolutionary purpose*. WW Norton & Company.

Barrus, M. M., Hosking, J. G., Zeeb, F. D., Tremblay, M., & Winstanley, C. A. (2015). Disadvantageous decision-making on a rodent gambling task is associated with

increased motor impulsivity in a population of male rats. *Journal of Psychiatry and Neuroscience*, *40*(2), 108-117.

Bayer, H. M., & Glimcher, P. W. (2005). Midbrain dopamine neurons encode a quantitative reward prediction error signal. *Neuron*, *47*(1), 129-141.

Bechara, A., & Martin, E. M. (2004). Impaired decision making related to working memory deficits in individuals with substance addictions. *Neuropsychology*, *18*(1), 152.

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*(1-3), 7-15.

Becker, J. B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology Biochemistry and behavior*, *64*(4), 803-812.

Becker, J. B., Arnold, A. P., Berkley, K. J., Blaustein, J. D., Eckel, L. A., Hampson, E., ... & Young, E. (2005). Strategies and methods for research on sex differences in brain and behavior. *Endocrinology*, *146*(4), 1650-1673.

Beckmann, J. S., & Bardo, M. T. (2012). Environmental enrichment reduces attribution of incentive salience to a food-associated stimulus. *Behavioural brain research*, *226*(1), 331-334.

Beckmann, J. S., & Chow, J. J. (2015). Isolating the incentive salience of reward-associated stimuli: value, choice, and persistence. *Learning & memory*, *22*(2), 116-127.

Belin, D., & Everitt, B. J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron*, *57*(3), 432-441.

Belin, D., Mar, A. C., Dalley, J. W., Robbins, T. W., & Everitt, B. J. (2008). High impulsivity predicts the switch to compulsive cocaine-taking. *Science*, *320*(5881), 1352-1355.

Berke, J. D. (2018). What does dopamine mean?. *Nature neuroscience*, *21*(6), 787-793.

Berlin, I., Givry-Steiner, L., Lecrubier, Y., & Puech, A. J. (1998). Measures of anhedonia and hedonic responses to sucrose in depressive and schizophrenic patients in comparison with healthy subjects. *European Psychiatry*, *13*(6), 303-309.

Berridge, K. C. (2000). Reward learning: Reinforcement, incentives, and expectations. *Psychology of learning and motivation*, 40, 223-278.

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*, *191*(3), 391-431.

Berridge, K. C., & Kringelbach, M. L. (2015). Pleasure systems in the brain. *Neuron*, *86*(3), 646-664.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?. *Brain research reviews*, *28*(3), 309-369.

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in neurosciences*, *26*(9), 507-513.

Berridge, K. C., & Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus?. *Behavioral neuroscience*, *105*(1), 3.

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'liking', 'wanting', and learning. *Current opinion in pharmacology*, *9*(1), 65-73.

Berridge, K. C., Venier, I. L., & Robinson, T. E. (1989). Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. *Behavioral neuroscience*, *103*(1), 36.

Bezdjian, S., Baker, L. A., Lozano, D. I., & Raine, A. (2009). Assessing inattention and impulsivity in children during the Go/NoGo task. *British Journal of Developmental Psychology*, *27*(2), 365-383.

Bianco, V., Berchicci, M., Quinzi, F., Perri, R. L., Spinelli, D., & Di Russo, F. (2020). Females are more proactive, males are more reactive: neural basis of the gender-related speed/accuracy trade-off in visuo-motor tasks. *Brain structure and function*, *225*(1), 187-201.

Bindra, D. (1978). How adaptive behavior is produced: a perceptual-motivational alternative to response reinforcements. *Behavioral and brain sciences*, *1*(1), 41-52.

Blaiss, C. A., & Janak, P. H. (2009). The nucleus accumbens core and shell are critical for the expression, but not the consolidation, of Pavlovian conditioned approach. *Behavioural brain research*, *200*(1), 22-32.

Blanco, F., & Matute, H. (2018). The illusion of causality: A cognitive bias underlying pseudoscience. *Pseudoscience: The conspiracy against science*, 45-75.

Boakes, R. A. (1977). Performance on learning to associate a stimulus with positive reinforcement. In H. Davis & H. Hurwitz (Eds.), Operant-Pavlovian interactions (pp. 6797). Hillsdale, NJ: Lawrence Erlbaum Associates.

Boileau, I., Dagher, A., Leyton, M., Gunn, R. N., Baker, G. B., Diksic, M., & Benkelfat, C. (2006). Modeling sensitization to stimulants in humans: an [11C] raclopride/positron emission tomography study in healthy men. *Archives of general psychiatry*, *63*(12), 1386-1395.

Bolles, R. C. (1972). Reinforcement, expectancy, and learning. *Psychological review*, *79*(5), 394.

Bonanno*, P., & Kommers, P. A. (2005). Gender differences and styles in the use of digital games. *Educational Psychology*, *25*(1), 13-41.

Bottjer, S. W., Scobie, S. R., & Wallace, J. (1977). Positive behavioral contrast, autoshaping, and omission responding in the goldfish (Carassius auratus). *Animal Learning & Behavior*, *5*(4), 336-342.

Bradberry, C. W., Barrett-Larimore, R. L., Jatlow, P., & Rubino, S. R. (2000). Impact of self-administered cocaine and cocaine cues on extracellular dopamine in mesolimbic and sensorimotor striatum in rhesus monkeys. *Journal of Neuroscience*, *20*(10), 3874-3883.

Bramham, C. R. (2008). Local protein synthesis, actin dynamics, and LTP consolidation. *Current opinion in neurobiology*, *18*(5), 524-531.

Brauer, L. H., & De Wit, H. (1997). High dose pimozide does not block amphetamine-induced euphoria in normal volunteers. *Pharmacology Biochemistry and Behavior*, *56*(2), 265-272.

Breiter, H. C., Aharon, I., Kahneman, D., Dale, A., & Shizgal, P. (2001). Functional imaging of neural responses to expectancy and experience of monetary gains and losses. *Neuron*, *30*(2), 619-639.

Breland, K., & Breland, M. (1961). The misbehavior of organisms. *American psychologist*, *16*(11), 681.

Brown, J. D. (2002). The Cronbach alpha reliability estimate. *JALT Testing & Evaluation SIG Newsletter*, *6*(1).

Brown, P. L., & Jenkins, H. M. (1968). Auto-shaping of the pigeon's key-peck. *Journal of the experimental analysis of behavior*, *11*(1), 1-8.

Brown, R. M., Hall, L. R., Holtzer, R., Brown, S. L., & Brown, N. L. (1997). Gender and video game performance. *Sex roles*, *36*(11), 793-812.

Bruner, A., & Revusky, S. H. (1961). Collateral behavior in humans. *Journal of the Experimental Analysis of Behavior*, *4*(4), 349.

Buatois, A., Laroche, L., Lafon, G., Avarguès-Weber, A., & Giurfa, M. (2020). Higher-order discrimination learning by honeybees in a virtual environment. *European Journal of Neuroscience*, *51*(2), 681-694.

Bullock, C. E., & Myers, T. M. (2009). Stimulus—food pairings produce stimulus-directed touch-screen responding in cynomolgus monkeys (macaca fascicularis) with or without a positive response contingency. *Journal of the experimental analysis of behavior*, *92*(1), 41-55.

Burdick, J. D., Roy, A. L., & Raver, C. C. (2013). Evaluating the Iowa Gambling Task as a direct assessment of impulsivity with low-income children. *Personality and individual differences*, *55*(7), 771-776.

Burns, M., & Domjan, M. (1996). Sign tracking versus goal tracking in the sexual conditioning of male Japanese quail (Coturnix japonica). *Journal of Experimental Psychology: Animal Behavior Processes*, *22*(3), 297.

Burton, C. L., & Fletcher, P. J. (2012). Age and sex differences in impulsive action in rats: the role of dopamine and glutamate. *Behavioural Brain Research*, *230*(1), 21-33.

Butz, M., Wörgötter, F., & van Ooyen, A. (2009). Activity-dependent structural plasticity. *Brain research reviews*, *60*(2), 287-305.

Byrnes, J. P., Miller, D. C., & Schafer, W. D. (1999). Gender differences in risk taking: A meta-analysis. *Psychological bulletin*, *125*(3), 367.

Cai, Z., Fan, X., & Du, J. (2017). Gender and attitudes toward technology use: A meta-analysis. *Computers & Education, 105*, 1-13.

Cajal, S. R. (1888). Estructura de los centros nerviosos de las aves. *Revista Trimestral de Histología Normal y Patológica*, *1*(1888), 1-10

Calabrich, S. L., Oppenheim, G. M., & Jones, M. W. (2021). Episodic memory cues in acquisition of novel visual-phonological associations: a webcam-based eye-tracking study. In *Proceedings of the annual meeting of the cognitive science society*, *43*, 43.

Calado, F., & Griffiths, M. D. (2016). Problem gambling worldwide: An update and systematic review of empirical research (2000–2015). *Journal of behavioral addictions*, *5*(4), 592-613.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience & Biobehavioral Reviews*, *26*(3), 321-352.

Carr, D. B., & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *Journal of neuroscience*, *20*(10), 3864-3873.

Carr, H., & Watson, J. B. (1908). Orientation in the White Rat. *Journal of Comparative Neurology & Psychology*, *18*, 27–44.

Carroll, M. E., Lynch, W. J., Roth, M. E., Morgan, A. D., & Cosgrove, K. P. (2004). Sex and estrogen influence drug abuse. *Trends in pharmacological sciences*, *25*(5), 273-279.

Catania, K. C. (2013). Stereo and serial sniffing guide navigation to an odour source in a mammal. *Nature Communications*, *4*(1), 1-8.

Cazzato, V., Basso, D., Cutini, S., & Bisiacchi, P. (2010). Gender differences in visuospatial planning: An eye movements study. *Behavioural Brain Research*, *206*(2), 177-183.

Chang, C. Y., Esber, G. R., Marrero-Garcia, Y., Yau, H. J., Bonci, A., & Schoenbaum, G. (2016). Brief optogenetic inhibition of dopamine neurons mimics endogenous negative reward prediction errors. *Nature neuroscience*, *19*(1), 111-116.

Chang, S. E., & Smith, K. S. (2016). An omission procedure reorganizes the microstructure of sign-tracking while preserving incentive salience. *Learning & Memory*, *23*(4), 151-155.

Cherkasova, M. V., Clark, L., Barton, J. J., Schulzer, M., Shafiee, M., Kingstone, A., ... & Winstanley, C. A. (2018). Win-concurrent sensory cues can promote riskier choice. *Journal of Neuroscience*, *38*(48), 10362-10370.

Cherkasova, M., Limbrick-Oldfield, E., Clark, L., Barton, J. J., Stoessl, A. J., & Winstanley, C. (2021). Risk-Promoting Effects of Reward-Paired Cues in Human Sign-and Goal-Trackers.

Chow, J. J., & Beckmann, J. S. (2018). NMDA receptor blockade specifically impedes the acquisition of incentive salience attribution. *Behavioural brain research*, *338*, 40-46.

Chow, J. J., Nickell, J. R., Darna, M., & Beckmann, J. S. (2016). Toward isolating the role of dopamine in the acquisition of incentive salience attribution. *Neuropharmacology*, *109*, 320-331.

Christiansen, D. M., & Berke, E. T. (2020). Gender-and sex-based contributors to sex differences in PTSD. *Current Psychiatry Reports*, *22*(4), 1-9.

Clemens, L. E., Jansson, E. K. H., Portal, E., Riess, O., & Nguyen, H. P. (2014). A behavioral comparison of the common laboratory rat strains Lister Hooded, Lewis, Fischer 344 and Wistar in an automated homecage system. *Genes, Brain and Behavior*, *13*(3), 305-321.

Cogan, E. S., Shapses, M. A., Robinson, T. E., & Tronson, N. C. (2019). Disrupting reconsolidation: memory erasure or blunting of emotional/motivational value?. *Neuropsychopharmacology*, *44*(2), 399-407.

Colaizzi, J. M., Flagel, S. B., Gearhardt, A. N., Borowitz, M. A., Kuplicki, R., Zotev, V., ... & Paulus, M. P. (2022). The propensity to sign-track is associated with externalizing behaviour and distinct patterns of reward-related brain activation in youth. *bioRxiv*.

Colaizzi, J. M., Flagel, S. B., Joyner, M. A., Gearhardt, A. N., Stewart, J. L., & Paulus, M. P. (2020). Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience & Biobehavioral Reviews*, *111*, 84-94.

Cole, P. D., & Adamo, S. A. (2005). Cuttlefish (Sepia officinalis: Cephalopoda) hunting behavior and associative learning. *Animal cognition*, *8*(1), 27-30.

Comas Mutis, R., Espejo, P. J., Martijena, I. D., Molina, V. A., & Calfa, G. D. (2021). Temporal dynamic of the hippocampal structural plasticity associated with the fear memory destabilization/reconsolidation process. *Hippocampus*, *31*(10), 1080-1091.

Cope, L., Gheidi, A., Martz, M., Duval, E., Khalil, H., Allerton, T., & Morrow, J. A Mechanical Task for Measuring Sign-and Goal-Tracking in Humans: A Proof-of-Concept Study.

Crampton, S. M., & Hodge, J. W. (2009). Generation Y: unchartered territory. *Journal of Business & Economics Research (JBER)*, *7*(4).

Crews, F. T., & Boettiger, C. A. (2009). Impulsivity, frontal lobes and risk for addiction. *Pharmacology Biochemistry and Behavior*, *93*(3), 237-247.

Cross, C. P., Copping, L. T., & Campbell, A. (2011). Sex differences in impulsivity: a meta-analysis. *Psychological bulletin*, *137*(1), 97.

Cunningham, C. L., & Patel, P. (2007). Rapid induction of Pavlovian approach to an ethanol-paired visual cue in mice. *Psychopharmacology*, *192*(2), 231-241.

Dailey, M. E., & Smith, S. J. (1996). The dynamics of dendritic structure in developing hippocampal slices. *Journal of neuroscience*, *16*(9), 2983-2994.

Daiwile, A. P., Jayanthi, S., & Cadet, J. L. (2022). Sex differences in methamphetamine use disorder perused from pre-clinical and clinical studies: Potential therapeutic impacts. *Neuroscience & Biobehavioral Reviews*, 104674.

Dalley, J. W., Chudasama, Y., Theobald, D. E., Pettifer, C. L., Fletcher, C. M., & Robbins, T. W. (2002). Nucleus accumbens dopamine and discriminated approach learning: interactive effects of 6-hydroxydopamine lesions and systemic apomorphine administration. *Psychopharmacology*, *161*(4), 425-433.

Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron*, *69*(4), 680-694.

Dalley, J. W., Lääne, K., Theobald, D. E., Armstrong, H. C., Corlett, P. R., Chudasama, Y., & Robbins, T. W. (2005). Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. *Proceedings of the National Academy of Sciences*, *102*(17), 6189-6194.

Danna, C. L., & Elmer, G. I. (2010). Disruption of conditioned reward association by typical and atypical antipsychotics. *Pharmacology Biochemistry and Behavior*, *96*(1), 40-47.

Davis, W. M., & Smith, S. G. (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *The Pavlovian journal of biological science: official journal of the Pavlovian*, *11*(4), 222-236.

De Roo, M., Klauser, P., & Muller, D. (2008). LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS biology*, *6*(9), e219.

De Wilde, B., Goudriaan, A., Sabbe, B., Hulstijn, W., & Dom, G. (2013). Relapse in pathological gamblers: A pilot study on the predictive value of different impulsivity measures. *Journal of Behavioral Addictions*, *2*(1), 23-30.

De Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction biology*, *14*(1), 22-31.

De Wit, H., & Wise, R. A. (1977). Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Canadian Journal of Psychology/Revue canadienne de psychologie*, *31*(4), 195.

Dębiec, J., & LeDoux, J. E. (2004). Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience*, *129*(2), 267-272.

Deckner, C. W., Wilcox, L. M., Maisto, S. A., & Blanton, R. L. (1980). Autoshaping of abnormal children. *Journal of abnormal child psychology*, *8*(3), 339-350.

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *The Lancet*, *379*(9810), 55-70.

Demeter, E., Guthrie, S. K., Taylor, S. F., Sarter, M., & Lustig, C. (2013). Increased distractor vulnerability but preserved vigilance in patients with schizophrenia: evidence from a translational Sustained Attention Task. *Schizophrenia research*, *144*(1-3), 136-141.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science*, *305*(5686), 1014-1017.

Di Chiara, G. (1998). A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. *Journal of psychopharmacology*, *12*(1), 54-67.

Di Ciano, P., & Everitt, B. J. (2004). Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology*, *47*, 202-213.

Di Ciano, P., & Everitt, B. J. (2005). Neuropsychopharmacology of drug seeking: Insights from studies with second-order schedules of drug reinforcement. *European journal of pharmacology*, *526*(1-3), 186-198.

Di Ciano, P., Cardinal, R. N., Cowell, R. A., Little, S. J., & Everitt, B. J. (2001). Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *Journal of Neuroscience*, *21*(23), 9471-9477.

Dichter, G. S., Smoski, M. J., Kampov-Polevoy, A. B., Gallop, R., & Garbutt, J. C. (2010). Unipolar depression does not moderate responses to the Sweet Taste Test. *Depression and anxiety*, *27*(9), 859-863.

Dickinson, A., Smith, J., & Mirenowicz, J. (2000). Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behavioral neuroscience*, *114*(3), 468.

Dickson, P. E., McNaughton, K. A., Hou, L., Anderson, L. C., Long, K. H., & Chesler, E. J. (2015). Sex and strain influence attribution of incentive salience to reward cues in mice. *Behavioural brain research*, *292*, 305-315.

DiFeliceantonio, A. G., & Berridge, K. C. (2012). Which cue to 'want'? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. *Behavioural brain research*, *230*(2), 399-408.

Doremus-Fitzwater, T. L., Barreto, M., & Spear, L. P. (2012). Age-related differences in impulsivity among adolescent and adult Sprague-Dawley rats. *Behavioral neuroscience*, *126*(5), 735.

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., & Fischman, A. J. (1999). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *The Lancet*, *354*(9196), 2132-2133.

Drevets, W. C., Gautier, C., Price, J. C., Kupfer, D. J., Kinahan, P. E., Grace, A. A., ... & Mathis, C. A. (2001). Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biological psychiatry*, *49*(2), 81-96.

Duckworth, J. J. (2017). *Human sign-tracking: an investigation into its mechanisms, measurements and neuropsychological correlates*. The University of Liverpool (United Kingdom).

Duckworth, J. J., Wright, H., Christiansen, P., Rose, A. K., & Fallon, N. (2022). Sign-tracking modulates reward-related neural activation to reward cues, but not reward feedback. *European Journal of Neuroscience*, *56*(7), 5000-5013.

Economidou, D., Pelloux, Y., Robbins, T. W., Dalley, J. W., & Everitt, B. J. (2009). High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. *Biological psychiatry*, *65*(10), 851-856.

Engert, F., & Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature*, *399*(6731), 66-70.

English, B. A., Hahn, M. K., Gizer, I. R., Mazei-Robison, M., Steele, A., Kurnik, D. M., ... & Blakely, R. D. (2009). Choline transporter gene variation is associated with attention-deficit hyperactivity disorder. *Journal of Neurodevelopmental Disorders*, *1*(4), 252-263.

Enticott, P. G., Ogloff, J. R., & Bradshaw, J. L. (2006). Associations between laboratory measures of executive inhibitory control and self-reported impulsivity. *Personality and Individual Differences*, *41*(2), 285-294.

Epstein, R., & Skinner, B. F. (1980). Resurgence of responding after the cessation of response-independent reinforcement. *Proceedings of the National Academy of Sciences*, *77*(10), 6251-6253.

Erol, A., & Karpyak, V. M. (2015). Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations. *Drug and alcohol dependence*, *156*, 1-13.

Evenden, J. L. (1999). Varieties of impulsivity. *Psychopharmacology*, *146*(4), 348-361.

Everitt, B. (1997). Craving cocaine cues: cognitive neuroscience meets drug addiction research. *Trends in cognitive sciences*.

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neuropsychological basis of addictive behaviour. *Brain research reviews, 36*(2-3), 129-138.

Fanselow, M. S. (2018). The role of learning in threat imminence and defensive behaviors. *Current opinion in behavioral sciences*, *24*, 44-49.

Fasano, C., Bourque, M. J., Lapointe, G., Leo, D., Thibault, D., Haber, M., ... & Trudeau, L. É. (2013). Dopamine facilitates dendritic spine formation by cultured striatal medium spiny neurons through both D1 and D2 dopamine receptors. *Neuropharmacology*, *67*, 432-443.

Feder, H. H. (1981). Estrous cyclicity in mammals. In *Neuroendocrinology of reproduction* (pp. 279-348). Springer, Boston, MA.

Fenster, R. J., Lebois, L. A., Ressler, K. J., & Suh, J. (2018). Brain circuit dysfunction in post-traumatic stress disorder: from mouse to man. *Nature Reviews Neuroscience*, *19*(9), 535-551.

Filbey, F. M., Toulopoulou, T., Morris, R. G., McDonald, C., Bramon, E., Walshe, M., & Murray, R. M. (2008). Selective attention deficits reflect increased genetic vulnerability to schizophrenia. *Schizophrenia research*, *101*(1-3), 169-175.

Fitzpatrick, C. J., Geary, T., Creeden, J. F., & Morrow, J. D. (2019a). Sign-tracking behavior is difficult to extinguish and resistant to multiple cognitive enhancers. *Neurobiology of learning and memory*, *163*, 107045.

Fitzpatrick, C. J., Gopalakrishnan, S., Cogan, E. S., Yager, L. M., Meyer, P. J., Lovic, V., ... & Morrow, J. D. (2013). Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. *PloS one*, *8*(10), e75042.

Fitzpatrick, C. J., Jagannathan, L., Lowenstein, E. D., Robinson, T. E., Becker, J. B., & Morrow, J. D. (2019b). Single prolonged stress decreases sign-tracking and cue-induced reinstatement of cocaine-seeking. *Behavioural brain research*, *359*, 799-806.

Flagel, S. B., & Robinson, T. E. (2017). Neurobiological basis of individual variation in stimulus-reward learning. *Current Opinion in Behavioral Sciences*, *13*, 178-185.

Flagel, S. B., Akil, H., & Robinson, T. E. (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. *Neuropharmacology*, *56*, 139-148.

Flagel, S. B., Cameron, C. M., Pickup, K. N., Watson, S. J., Akil, H., & Robinson, T. E. (2011a). A food predictive cue must be attributed with incentive salience for it to induce c-fos mRNA expression in cortico-striatal-thalamic brain regions. *Neuroscience*, *196*, 80-96.

Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., ... & Akil, H. (2011b). A selective role for dopamine in stimulus–reward learning. *Nature*, *469*(7328), 53-57.

Flagel, S. B., Robinson, T. E., Clark, J. J., Clinton, S. M., Watson, S. J., Seeman, P., ... & Akil, H. (2010). An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. *Neuropsychopharmacology*, *35*(2), 388-400.

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural brain research*, *186*(1), 48-56.

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology*, *191*(3), 599-607.

Forlano, P. M., & Woolley, C. S. (2010). Quantitative analysis of pre-and postsynaptic sex differences in the nucleus accumbens. *Journal of Comparative Neurology*, *518*(8), 1330-1348.

Franken, I. H., Hendriks, V. M., Stam, C. J., & Van den Brink, W. (2004). A role for dopamine in the processing of drug cues in heroin dependent patients. *European Neuropsychopharmacology*, *14*(6), 503-508.

Franken, I. H., van Strien, J. W., Nijs, I., & Muris, P. (2008). Impulsivity is associated with behavioral decision-making deficits. *Psychiatry research*, *158*(2), 155-163.

Fraser, K. M., & Janak, P. H. (2017). Long-lasting contribution of dopamine in the nucleus accumbens core, but not dorsal lateral striatum, to sign-tracking. *European Journal of Neuroscience*, *46*(4), 2047-2055.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in ecology and evolution*, *7*(11), 1325-1330.

Friedman-Hill, S. R., Wagman, M. R., Gex, S. E., Pine, D. S., Leibenluft, E., & Ungerleider, L. G. (2010). What does distractibility in ADHD reveal about mechanisms for top-down attentional control?. *Cognition*, *115*(1), 93-103.

Fuentes, S., Carrasco, J., Hatto, A., Navarro, J., Armario, A., Monsonet, M., ... & Nadal, R. (2018). Sex-dependent impact of early-life stress and adult immobilization in the attribution of incentive salience in rats. *PloS one*, *13*(1), e0190044.

Furukawa, E., Bado, P., Tripp, G., Mattos, P., Wickens, J. R., Bramati, I. E., ... & Moll, J. (2014). Abnormal striatal BOLD responses to reward anticipation and reward delivery in ADHD. *PloS one*, *9*(2), e89129.

Fusar-Poli, P., Rubia, K., Rossi, G., Sartori, G., & Balottin, U. (2012). Striatal dopamine transporter alterations in ADHD: pathophysiology or adaptation to psychostimulants? A meta-analysis. *American Journal of Psychiatry*, *169*(3), 264-272.

Gamzu, E., & Schwam, E. (1974). Autoshaping and automaintenance of a key-press response in squirrel monkeys. *Journal of the Experimental Analysis of Behavior*, *21*(2), 361-371.

Garofalo, S., & di Pellegrino, G. (2015). Individual differences in the influence of task-irrelevant Pavlovian cues on human behavior. *Frontiers in behavioral neuroscience*, *9*, 163.

Geinisman, Y., Berry, R. W., Disterhoft, J. F., Power, J. M., & Van der Zee, E. A. (2001). Associative learning elicits the formation of multiple-synapse boutons. *Journal of Neuroscience*, *21*(15), 5568-5573.

Gibbons, A., & Dean, B. (2016). The cholinergic system: an emerging drug target for schizophrenia. *Current pharmaceutical design*, *22*(14), 2124-2133.

Gillis, Z. S., & Morrison, S. E. (2019). Sign tracking and goal tracking are characterized by distinct patterns of nucleus accumbens activity. *ENeuro*, *6*(2).

Gipson, C. D., Kupchik, Y. M., Shen, H., Reissner, K. J., Thomas, C. A., & Kalivas, P. W. (2013). Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. *Neuron*, *77*(5), 867-872.

González-Martín-Moro, J., Hernández-Verdejo, J. L., & Clement-Corral, A. (2017). The visual system of diurnal raptors: updated review. *Archivos de la Sociedad Española de Oftalmología (English Edition)*, *92*(5), 225-232.

Greenaway, A. M., Nasuto, S., Ho, A., & Hwang, F. (2021). Is home-based webcam eye-tracking with older adults living with and without Alzheimer's disease feasible?. In *The 23rd International ACM SIGACCESS Conference on Computers and Accessibility*, 1-3.

Halpern, D. F., & Collaer, M. L. (2005). *Sex Differences in Visuospatial Abilities: More Than Meets the Eye*. Cambridge University Press.

Hamlen, K. R. (2010). Re-examining gender differences in video game play: Time spent and feelings of success. *Journal of Educational Computing Research*, *43*(3), 293-308.

Hart, A. S., Rutledge, R. B., Glimcher, P. W., & Phillips, P. E. (2014). Phasic dopamine release in the rat nucleus accumbens symmetrically encodes a reward prediction error term. *Journal of Neuroscience*, *34*(3), 698-704.

Haselton, M. G., & Nettle, D. (2005). The paranoid optimist: an integrative evolutionary model of cognitive biases. *Manuscript under Review*.

Hearst, E., & Jenkins, H. M. (1974). *Sign-tracking: The stimulus-reinforcer relation and directed action*. Psychonomic Society.

Hebb, D. O. (1949). The first stage of perception: growth of the assembly. *The Organization of Behavior*, *4*, 60-78.

Heinz, A., & Schlagenhauf, F. (2010). Dopaminergic dysfunction in schizophrenia: salience attribution revisited. *Schizophrenia bulletin*, *36*(3), 472-485.

Henry, D. J., & White, F. J. (1995). The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. *Journal of Neuroscience*, *15*(9), 6287-6299.

Hering, H., & Sheng, M. (2001). Dentritic spines: structure, dynamics and regulation. *Nature Reviews Neuroscience*, *2*(12), 880-888.

Hinshaw, S. P. (2018). Attention deficit hyperactivity disorder (ADHD): controversy, developmental mechanisms, and multiple levels of analysis. *Annual review of clinical psychology*, *14*, 291-316.

Holden, J. M., & Peoples, L. L. (2010). Effects of acute amphetamine exposure on two kinds of Pavlovian approach behavior. *Behavioural brain research*, *208*(1), 270-273.

Hollerman, J. R., & Schultz, W. (1998). Dopamine neurons report an error in the temporal prediction of reward during learning. *Nature neuroscience*, *1*(4), 304-309.

Hou, R., Mogg, K., Bradley, B. P., Moss-Morris, R., Peveler, R., & Roefs, A. (2011). External eating, impulsivity and attentional bias to food cues. *Appetite*, *56*(2), 424-427.

Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III—the final common pathway. *Schizophrenia bulletin*, *35*(3), 549-562.

Hughson, A. R., Horvath, A. P., Holl, K., Palmer, A. A., Solberg Woods, L. C., Robinson, T. E., & Flagel, S. B. (2019). Incentive salience attribution, "sensation-seeking" and "novelty-seeking" are independent traits in a large sample of male and female heterogeneous stock rats. *Scientific reports*, *9*(1), 1-16.

Hull, C. L., 1943. *Principles of behavior: an introduction to behavior theory*. Appleton-Century, Oxford, England.

Hurst, R. M., Kepley, H. O., McCalla, M. K., & Livermore, M. K. (2011). Internal consistency and discriminant validity of a delay-discounting task with an adult self-reported ADHD sample. *Journal of Attention Disorders*, *15*(5), 412-422.

Hyde, J. F., & Jerussi, T. P. (1983). Sexual dimorphism in rats with respect to locomotor activity and circling behavior. *Pharmacology Biochemistry and Behavior*, *18*(5), 725-729.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain research reviews*, *56*(1), 27-78.

Ikemoto, S. (2010). Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. *Neuroscience & biobehavioral reviews*, *35*(2), 129-150.

Janes, A. C., Pizzagalli, D. A., Richardt, S., Frederick, B. D., Chuzi, S., Pachas, G., ... & Kaufman, M. J. (2010). Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. *Biological psychiatry*, *67*(8), 722-729.

Jenkins, H. M., Barrera, F. J., Ireland, C., & Woodside, B. (1978). Signal-centered action patterns of dogs in appetitive classical conditioning. *Learning and Motivation*, *9*(3), 272-296.

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology*, *146*(4), 373-390.

Jentsch, J. D., & Taylor, J. R. (2003). Sex-related differences in spatial divided attention and motor impulsivity in rats. *Behavioral neuroscience*, *117*(1), 76.

Jentsch, J. D., Ashenhurst, J. R., Cervantes, M. C., Groman, S. M., James, A. S., & Pennington, Z. T. (2014). Dissecting impulsivity and its relationships to drug addictions. *Annals of the New York Academy of Sciences*, *1327*(1), 1-26.

Jourdain, P., Fukunaga, K., & Muller, D. (2003). Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *Journal of Neuroscience*, *23*(33), 10645-10649.

Joyner, M. A., Gearhardt, A. N., & Flagel, S. B. (2018). A translational model to assess sign-tracking and goal-tracking behavior in children. *Neuropsychopharmacology*, *43*(1), 228.

Jung, C. K., & Herms, J. (2014). Structural dynamics of dendritic spines are influenced by an environmental enrichment: an in vivo imaging study. *Cerebral cortex*, *24*(2), 377-384.

Kalmijn, A. J. (1971). The electric sense of sharks and rays. *Journal of Experimental Biology*, *55*(2), 371-383.

Kapur, S. (2003). Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *American journal of Psychiatry*, *160*(1), 13-23.

Kar, G., Vu, A., Juliá Nehme, B., & Hedge, A. (2015, September). Effects of mouse, trackpad and 3D motion and gesture control on performance, posture, and comfort. In *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, *59*(1), 327-331.

Kawa, A. B., Bentzley, B. S., & Robinson, T. E. (2016). Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. *Psychopharmacology*, *233*(19), 3587-3602.

Kearns, D. N., & Weiss, S. J. (2007). Recovery of Pavlovian sign-tracking (autoshaping) following the discontinuation of inter-trial interval food in rats. *Behavioural processes*, *75*(3), 307-311.

Kearns, D. N., Gomez-Serrano, M. A., Weiss, S. J., & Riley, A. L. (2006). A comparison of Lewis and Fischer rat strains on autoshaping (sign-tracking), discrimination reversal learning and negative automaintenance. *Behavioural Brain Research*, *169*(2), 193-200.

Keedwell, P. A., Andrew, C., Williams, S. C., Brammer, M. J., & Phillips, M. L. (2005). The neural correlates of anhedonia in major depressive disorder. *Biological psychiatry*, *58*(11), 843-853.

Keller, S. M., Schreiber, W. B., Staib, J. M., & Knox, D. (2015). Sex differences in the single prolonged stress model. *Behavioural brain research*, *286*, 29-32.

Kellogg, W. N. (1949). 'Superstitious' behavior in animals. *Psychological Review*, *56*(3), 172.

Killeen, P. R. (2003). Complex dynamic processes in sign tracking with an omission contingency (negative automaintenance). *Journal of Experimental Psychology: Animal Behavior Processes*, *29*(1), 49.

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. *Nature neuroscience*, *12*(3), 256-258.

King, C. P., Palmer, A. A., Woods, L. C. S., Hawk, L. W., Richards, J. B., & Meyer, P. J. (2016). Premature responding is associated with approach to a food cue in male and female heterogeneous stock rats. *Psychopharmacology*, *233*(13), 2593-2605.

Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *Journal of Neuroscience*, *21*(16), RC159-RC159.

Knutson, B., Bhanji, J. P., Cooney, R. E., Atlas, L. Y., & Gotlib, I. H. (2008). Neural responses to monetary incentives in major depression. *Biological psychiatry*, *63*(7), 686-692.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*, *24*(2), 97-129.

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry*, *3*(8), 760-773.

Koshy Cherian, A., Kucinski, A., Pitchers, K., Yegla, B., Parikh, V., Kim, Y., ... & Sarter, M. (2017). Unresponsive choline transporter as a trait neuromarker and a causal mediator of bottom-up attentional biases. *Journal of Neuroscience*, *37*(11), 2947-2959.

Krank, M. D., O'Neill, S., Squarey, K., & Jacob, J. (2008). Goal-and signal-directed incentive: conditioned approach, seeking, and consumption established with unsweetened alcohol in rats. *Psychopharmacology*, *196*(3), 397-405.

Kravitz, A. V., Tye, L. D., and Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nature Neuroscience. 15*, 816–818.

Kucinski, A., Lustig, C., & Sarter, M. (2018). Addiction vulnerability trait impacts complex movement control: Evidence from sign-trackers. *Behavioural brain research*, *350*, 139-148.

Kumar, P., Waiter, G., Ahearn, T., Milders, M., Reid, I., & Steele, J. D. (2008). Abnormal temporal difference reward-learning signals in major depression. *Brain*, *131*(8), 2084-2093.

Lajoie, J., & Bindra, D. (1976). An interpretation of autoshaping and related phenomena in terms of stimulus-incentive contingencies alone. *Canadian Journal of Psychology/Revue canadienne de psychologie*, *30*(3), 157.

Lamprecht, R., & LeDoux, J. (2004). Structural plasticity and memory. *Nature Reviews Neuroscience*, *5*(1), 45

Lane, S. D., Cherek, D. R., Rhoades, H. M., Pietras, C. J., & Tcheremissine, O. V. (2003). Relationships among laboratory and psychometric measures of impulsivity: Implications in substance abuse and dependence. *Addictive Disorders & Their Treatment*, *2*(2), 33-40.

Laruelle, M., Abi-Dargham, A., van Dyck, C. H., Rosenblatt, W., Zea-Ponce, Y., Zoghbi, S. S., ... & Innis, R. B. (1995). SPECT imaging of striatal dopamine release after amphetamine challenge. *Journal of Nuclear Medicine*, *36*(7), 1182-1190.

Le Pelley, M. E., Pearson, D., Griffiths, O., & Beesley, T. (2015). When goals conflict with values: counterproductive attentional and oculomotor capture by reward-related stimuli. *Journal of Experimental Psychology: General*, *144*(1), 158.

LeBlanc, K. H., Ostlund, S. B., & Maidment, N. T. (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behavioral neuroscience*, *126*(5), 681.

Lee, J. L., & Everitt, B. J. (2008). Reactivation-dependent amnesia in Pavlovian approach and instrumental transfer. *Learning & memory*, *15*(8), 597-602.

Lee, B., Gentry, R. N., Bissonette, G. B., Herman, R. J., Mallon, J. J., Bryden, D. W., ... & Roesch, M. R. (2018). Manipulating the revision of reward value during the intertrial interval increases sign tracking and dopamine release. *PLoS Biology*, 16(*9*), e2004015.

Leenaars, C. H., Kouwenaar, C., Stafleu, F. R., Bleich, A., Ritskes-Hoitinga, M., De Vries, R., & Meijboom, F. L. (2019). Animal to human translation: a systematic scoping review of reported concordance rates. *Journal of translational medicine*, *17*(1), 1-22.

Leger, M., & Neill, J. C. (2016). A systematic review comparing sex differences in cognitive function in schizophrenia and in rodent models for schizophrenia, implications for improved therapeutic strategies. *Neuroscience & Biobehavioral Reviews*, *68*, 979-1000.

Leuner, B., Falduto, J., & Shors, T. J. (2003). Associative memory formation increases the observation of dendritic spines in the hippocampus. *Journal of Neuroscience*, *23*(2), 659-665.

Levin, E. D., Conners, C. K., Sparrow, E., Hinton, S. C., Erhardt, D., Meck, W. H., ... & March, J. (1996). Nicotine effects on adults with attention-deficit/hyperactivity disorder. *Psychopharmacology*, *123*(1), 55-63.

Leyton, M. (2010). The neurobiology of desire: dopamine and the regulation of mood and motivational states in humans. In Kringelbach, M.L. & Berridge, K.C. (Eds), *Pleasures of the Brain*. Oxford University Press, Oxford, pp. 222–243.

Leyton, M., Boileau, I., Benkelfat, C., Diksic, M., Baker, G., & Dagher, A. (2002). Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C] raclopride study in healthy men. *Neuropsychopharmacology*, *27*(6), 1027-1035.

Leyton, M., Casey, K. F., Delaney, J. S., Kolivakis, T., & Benkelfat, C. (2005). Cocaine craving, euphoria, and self-administration: a preliminary study of the effect of catecholamine precursor depletion. *Behavioral neuroscience*, *119*(6), 1619.

Lindblom, L. L., & Jenkins, H. M. (1981). Responses eliminated by noncontingent or negatively contingent reinforcement recover in extinction. *Journal of Experimental Psychology: Animal Behavior Processes*, *7*(2), 175.

Lomanowska, A. M., Lovic, V., Rankine, M. J., Mooney, S. J., Robinson, T. E., & Kraemer, G. W. (2011). Inadequate early social experience increases the incentive salience of reward-related cues in adulthood. *Behavioural brain research*, *220*(1), 91-99.

Long, J. A., & Evans, H. M. (1922). *The oestrous cycle in the rat and its associated phenomena* (Vol. 6). University of California Press.

Lovic, V., Saunders, B. T., Yager, L. M., & Robinson, T. E. (2011). Rats prone to attribute incentive salience to reward cues are also prone to impulsive action. *Behavioural brain research*, *223*(2), 255-261.

Ludvig, E. A., Madan, C. R., & Spetch, M. L. (2015). Priming memories of past wins induces risk seeking. *Journal of Experimental Psychology: General*, *144*(1), 24.

Luman, M., Tripp, G., & Scheres, A. (2010). Identifying the neurobiology of altered reinforcement sensitivity in ADHD: a review and research agenda. *Neuroscience & Biobehavioral Reviews*, *34*(5), 744-754.

Madayag, A. C., Stringfield, S. J., Reissner, K. J., Boettiger, C. A., & Robinson, D. L. (2017). Sex and adolescent ethanol exposure influence Pavlovian conditioned approach. *Alcoholism: Clinical and Experimental Research*, *41*(4), 846-856.

Madras, B. K., Miller, G. M., & Fischman, A. J. (2002). The dopamine transporter: relevance to attention deficit hyperactivity disorder (ADHD). *Behavioural Brain Research*, *130*(1-2), 57-63.

Malone, J. C. (1975). The "paradigms" of learning. *The Psychological Record*, *25*(4), 479-489.

Mathew, J., Masson, G. S., & Danion, F. R. (2020). Sex differences in visuomotor tracking. *Scientific reports*, *10*(1), 1-12.

Matsuzaki, M., Honkura, N., Ellis-Davies, G. C., & Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature*, *429*(6993), 761-766.

Matute, H. (1996). Illusion of control: Detecting response-outcome independence in analytic but not in naturalistic conditions. *Psychological Science*, *7*(5), 289-293.

Matute, H., Blanco, F., Yarritu, I., Díaz-Lago, M., Vadillo, M. A., & Barberia, I. (2015). Illusions of causality: how they bias our everyday thinking and how they could be reduced. *Frontiers in psychology*, *6*, 888.

Mauldin, J. E. (1981). Autoshaping and negative automaintenance in the blue jay (Cyanocitta cristata), robin (Turdus migratorius) and starling (Sturnus vulgaris).

McCabe, C., Cowen, P. J., & Harmer, C. J. (2009). Neural representation of reward in recovered depressed patients. *Psychopharmacology*, *205*(4), 667-677.

McCarthy, D. E., Minami, H., Bold, K. W., Yeh, V. M., & Chapman, G. (2018). Momentary assessment of impulsive choice and impulsive action: Reliability, stability, and correlates. *Addictive behaviors*, *83*, 130-135.

McHugh, R. K., Votaw, V. R., Sugarman, D. E., & Greenfield, S. F. (2018). Sex and gender differences in substance use disorders. *Clinical psychology review*, *66*, 12-23.

Medvedev, N. I., Dallérac, G., Popov, V. I., Rodriguez Arellano, J. J., Davies, H. A., Kraev, I. V., Doyère V., & Stewart, M. G. (2014). Multiple spine boutons are formed after long-lasting LTP in the awake rat. *Brain Structure and Function*, *219*(1), 407-414.

Meredith, G. E., Baldo, B. A., Andrezjewski, M. E., & Kelley, A. E. (2008). The structural basis for mapping behavior onto the ventral striatum and its subdivisions. *Brain Structure and Function*, *213*(1), 17-27.

Meredith, G. E., Ypma, P., & Zahm, D. S. (1995). Effects of dopamine depletion on the morphology of medium spiny neurons in the shell and core of the rat nucleus accumbens. *Journal of Neuroscience*, *15*(5), 3808-3820.

Meyer, P. J., Cogan, E. S., & Robinson, T. E. (2014). The form of a conditioned stimulus can influence the degree to which it acquires incentive motivational properties. *PloS one*, *9*(6), e98163.

Meyer, P. J., Lovic, V., Saunders, B. T., Yager, L. M., Flagel, S. B., Morrow, J. D., & Robinson, T. E. (2012a). Quantifying individual variation in the propensity to attribute incentive salience to reward cues. *PloS one*, *7*(6), e38987.

Meyer, P. J., Ma, S. T., & Robinson, T. E. (2012b). A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. *Psychopharmacology*, *219*(4), 999-1009.

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: ten years of progress. *Annual review of psychology*, *63*, 129.

Milton, A. L., & Everitt, B. J. (2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *European Journal of Neuroscience*, *31*(12), 2308-2319.

Milton, A. L., Lee, J. L., & Everitt, B. J. (2008). Reconsolidation of appetitive memories for both natural and drug reinforcement is dependent on β-adrenergic receptors. *Learning & memory*, *15*(2), 88-92.

Mitchell, D. (1976). Experiments on neophobia in wild and laboratory rats: a reevaluation. *Journal of Comparative and Physiological Psychology*, *90*(2), 190.

Miyashita, Y., Nakajima, S., & Imada, H. (1999). Panel-touch behavior of horses established by an autoshaping procedure. *Psychological Reports*, *85*(3), 867-868.

Montague, P. R., Dayan, P., & Sejnowski, T. J. (1996). A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *Journal of neuroscience*, 16(5), 1936-1947.

Moretta, T., Buodo, G., Demetrovics, Z., & Potenza, M. N. (2022). Tracing 20 years of research on problematic use of the internet and social media: Theoretical models, assessment tools, and an agenda for future work. *Comprehensive Psychiatry*, *112*, 152286.

Morrison, S. E., Bamkole, M. A., & Nicola, S. M. (2015). Sign tracking, but not goal tracking, is resistant to outcome devaluation. *Frontiers in neuroscience*, *9*, 468.

Morrow, J. D., Maren, S., & Robinson, T. E. (2011). Individual variation in the propensity to attribute incentive salience to an appetitive cue predicts the propensity to attribute motivational salience to an aversive cue. *Behavioural brain research*, *220*(1), 238-243.

Morrow, J. D., Saunders, B. T., Maren, S., & Robinson, T. E. (2015). Sign-tracking to an appetitive cue predicts incubation of conditioned fear in rats. *Behavioural brain research*, *276*, 59-66.

Moss, F. A., & Thorndike, E. L. (1934). *Comparative psychology*. New York: Prentice-Hall.

Muller, D., Toni, N., & Buchs, P. A. (2000). Spine changes associated with long-term potentiation. *Hippocampus*, *10*(5), 596-604.

Munro, C. A., McCaul, M. E., Wong, D. F., Oswald, L. M., Zhou, Y., Brasic, J., ... & Wand, G. S. (2006). Sex differences in striatal dopamine release in healthy adults. *Biological psychiatry*, *59*(10), 966-974.

Myerson, J., Green, L., & Warusawitharana, M. (2001). Area under the curve as a measure of discounting. *Journal of the experimental analysis of behavior*, *76*(2), 235-243.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, *406*(6797), 722-726.

Nasser, H. M., Chen, Y. W., Fiscella, K., & Calu, D. J. (2015). Individual variability in behavioral flexibility predicts sign-tracking tendency. *Frontiers in behavioral neuroscience*, *9*, 289.

Nelson, A., & Killcross, S. (2006). Amphetamine exposure enhances habit formation. *Journal of Neuroscience, 26*(14), 3805-3812.

Nestler, E. J., & Carlezon Jr, W. A. (2006). The mesolimbic dopamine reward circuit in depression. *Biological psychiatry*, *59*(12), 1151-1159.

Newman, J. P., Wolff, W. T., & Hearst, E. (1980). The feature-positive effect in adult human subjects. *Journal of Experimental Psychology: Human Learning and Memory*, *6*(5), 630.

Nigg, J. T. (2001). Is ADHD a disinhibitory disorder?. *Psychological bulletin*, *127*(5), 571.

Nilsson, J., Kristiansen, T. S., Fosseidengen, J. E., Fernö, A., & van den Bos, R. (2008). Sign-and goal-tracking in Atlantic cod (Gadus morhua). *Animal Cognition*, *11*(4), 651-659.

Olds, J. (1956). Pleasure centers in the brain. *Scientific American*, *195*(4), 105-117.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of comparative and physiological psychology*, *47*(6), 419.

Olshavsky, M. E., Shumake, J., Rosenthal, A. A., Kaddour-Djebbar, A., Gonzalez-Lima, F., Setlow, B., & Lee, H. J. (2014). Impulsivity, risk-taking, and distractibility in rats exhibiting robust conditioned orienting behaviors. *Journal of the Experimental Analysis of Behavior*, *102*(2), 162-178.

Ono, K. (1987). Superstitious behavior in humans. *Journal of the experimental analysis of behavior*, *47*(3), 261-271.

Ortner, C. N., MacDonald, T. K., & Olmstead, M. C. (2003). Alcohol intoxication reduces impulsivity in the delay-discounting paradigm. *Alcohol and Alcoholism*, *38*(2), 151-156.

Panlilio, L. V., Yasar, S., Nemeth-Coslett, R., Katz, J. L., Henningfield, J. E., Solinas, M., ... & Goldberg, S. R. (2005). Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. *Neuropsychopharmacology*, *30*(2), 433-443.

Paolone, G., Angelakos, C. C., Meyer, P. J., Robinson, T. E., & Sarter, M. (2013). Cholinergic control over attention in rats prone to attribute incentive salience to reward cues. *Journal of Neuroscience*, *33*(19), 8321-8335.

Papachristou, H., Nederkoorn, C., Beunen, S., & Jansen, A. (2013). Dissection of appetitive conditioning. Does impulsivity play a role?. *Appetite*, *69*, 46-53.

Papachristou, H., Nederkoorn, C., Giesen, J. C., & Jansen, A. (2014). Cue reactivity during treatment, and not impulsivity, predicts an initial lapse after treatment in alcohol use disorders. *Addictive behaviors*, *39*(3), 737-739.

Papoutsaki, A., Gokaslan, A., Tompkin, J., He, Y., & Huang, J. (2018, June). The eye of the typer: a benchmark and analysis of gaze behavior during typing. In *Proceedings of the 2018 ACM Symposium on Eye Tracking Research & Applications* (pp. 1-9).

Parker, J. G., Zweifel, L. S., Clark, J. J., Evans, S. B., Phillips, P. E., & Palmiter, R. D. (2010). Absence of NMDA receptors in dopamine neurons attenuates dopamine release but not conditioned approach during Pavlovian conditioning. *Proceedings of the National Academy of Sciences*, *107*(30), 13491-13496.

Parkinson, J. A., Dalley, J. W., Cardinal, R. N., Bamford, A., Fehnert, B., Lachenal, G., ... & Everitt, B. J. (2002). Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. *Behavioural brain research*, *137*(1-2), 149-163.

Parkinson, J. A., Olmstead, M. C., Burns, L. H., Robbins, T. W., & Everitt, B. J. (1999). Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by d-amphetamine. *Journal of Neuroscience*, *19*(6), 2401-2411.

Pasquariello, K. Z., Han, M., Unal, C., & Meyer, P. J. (2018). Adrenergic manipulation inhibits Pavlovian conditioned approach behaviors. *Behavioural brain research*, *339*, 278-285.

Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt impulsiveness scale. *Journal of clinical psychology*, *51*(6), 768-774.

Pavlov, I. P. (1927) *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford University Press, London.

Pavlov, I. P. (1934). An attempt at a physiological interpretation of obsessional neurosis and paranoia. *Journal of Mental Science*, *80*(329), 187-197.

Pecina, S., Cagniard, B., Berridge, K. C., Aldridge, J. W., & Zhuang, X. (2003). Hyperdopaminergic mutant mice have higher “wanting” but not “liking” for sweet rewards. *Journal of Neuroscience*, *23*(28), 9395-9402.

Pelloux, Y., Everitt, B. J., & Dickinson, A. (2007). Compulsive drug seeking by rats under punishment: effects of drug taking history. *Psychopharmacology*, *194*(1), 127-137.

Perry, J. L., Larson, E. B., German, J. P., Madden, G. J., & Carroll, M. E. (2005). Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. *Psychopharmacology*, *178*(2), 193-201.

Peters, J., & De Vries, T. J. (2014). Pavlovian conditioned approach, extinction, and spontaneous recovery to an audiovisual cue paired with an intravenous heroin infusion. *Psychopharmacology*, *231*(2), 447-453.

Peterson, B. M., Mermelstein, P. G., & Meisel, R. L. (2015). Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. *Brain Structure and Function*, *220*(4), 2415-2422.

Phillips, K. B., & Sarter, M. (2020). Addiction vulnerability and the processing of significant cues: Sign-, but not goal-, tracker perceptual sensitivity relies on cue salience. *Behavioral neuroscience*, *134*(2), 133.

Pielot, M., Dingler, T., Pedro, J. S., & Oliver, N. (2015). When attention is not scarce - detecting boredom from mobile phone usage. *Proceedings of the 2015 ACM international joint conference on pervasive and ubiquitous computing*, 825-836.

Pinto, A. (2021). An Evolutionary Approach to the Adaptive Value of Belief. In *Evolutionary Psychology Meets Social Neuroscience*. IntechOpen.

Pitchers, K. K., Flagel, S. B., O'Donnell, E. G., Woods, L. C. S., Sarter, M., & Robinson, T. E. (2015). Individual variation in the propensity to attribute incentive salience to a food cue: influence of sex. *Behavioural brain research*, *278*, 462-469.

Pitchers, K. K., Kane, L. F., Kim, Y., Robinson, T. E., & Sarter, M. (2017a). 'Hot' vs. 'cold' behavioural-cognitive styles: motivational-dopaminergic vs. cognitive-cholinergic processing of a Pavlovian cocaine cue in sign-and goal-tracking rats. *European Journal of Neuroscience*, *46*(11), 2768-2781.

Pitchers, K. K., Phillips, K. B., Jones, J. L., Robinson, T. E., & Sarter, M. (2017b). Diverse roads to relapse: a discriminative cue signaling cocaine availability is more effective in renewing cocaine seeking in goal trackers than sign trackers and depends on basal forebrain cholinergic activity. *Journal of Neuroscience*, *37*(30), 7198-7208.

Pitchers, K. K., Vialou, V., Nestler, E. J., Laviolette, S. R., Lehman, M. N., & Coolen, L. M. (2013). Natural and drug rewards act on common neural plasticity mechanisms with ΔFosB as a key mediator. *Journal of Neuroscience*, *33*(8), 3434-3442.

Pithers, R. T. (1985). The roles of event contingencies and reinforcement in human autoshaping and omission responding. *Learning and Motivation*, *16*(2), 210-237.

Plichta, M. M., & Scheres, A. (2014). Ventral–striatal responsiveness during reward anticipation in ADHD and its relation to trait impulsivity in the healthy population: A meta-analytic review of the fMRI literature. *Neuroscience & Biobehavioral Reviews*, *38*, 125-134.

Pomerleau, O. F., Downey, K. K., Stelson, F. W., & Pomerleau, C. S. (1995). Cigarette smoking in adult patients diagnosed with attention deficit hyperactivity disorder. *Journal of substance abuse*, *7*(3), 373-378.

Pooley, A. E., Benjamin, R. C., Sreedhar, S., Eagle, A. L., Robison, A. J., Mazei-Robison, M. S., ... & Jordan, C. L. (2018). Sex differences in the traumatic stress response: PTSD symptoms in women recapitulated in female rats. *Biology of sex differences*, *9*(1), 1-11.

Potter, A. S., Schaubhut, G., & Shipman, M. (2014). Targeting the nicotinic cholinergic system to treat attention-deficit/hyperactivity disorder: rationale and progress to date. *CNS drugs*, *28*(12), 1103-1113.

Poulos, C. X., Parker, J. L., & Lê, D. A. (1998). Increased impulsivity after injected alcohol predicts later alcohol consumption in rats: evidence for" loss-of-control drinking" and marked individual differences. *Behavioral neuroscience*, *112*(5), 1247.

Przybyslawski, J., & Sara, S. J. (1997). Reconsolidation of memory after its reactivation. *Behavioural brain research*, *84*(1-2), 241-246.

Przybyslawski, J., Roullet, P., & Sara, S. J. (1999). Attenuation of emotional and nonemotional memories after their reactivation: Role of β adrenergic receptors. *Journal of Neuroscience*, *19*(15), 6623-6628.

Purdy, J. E., Roberts, A. C., & Garcia, C. A. (1999). Sign tracking in cuttlefish (Sepia officinalis). *Journal of Comparative Psychology*, *113*(4), 443.

Puścian, A., & Knapska, E. (2022). Blueprints for measuring natural behavior. *Iscience*, 104635.

Radevski, M. E., & Rice, B. A. (2022). Characterizing sign-tracking behavior in female Japanese quail (Coturnix japonica). *Experimental and Clinical Psychopharmacology*.

Reavis, R., & Overman, W. H. (2001). Adult sex differences on a decision-making task previously shown to depend on the orbital prefrontal cortex. *Behavioral neuroscience*, *115*(1), 196.

Reynolds, S. M., & Berridge, K. C. (2002). Positive and negative motivation in nucleus accumbens shell: bivalent rostrocaudal gradients for GABA-elicited eating, taste "liking"/"disliking" reactions, place preference/avoidance, and fear. *Journal of Neuroscience, 22*(16), 7308-7320.

Richter, C. P. (1927). Animal behavior and internal drives. *The Quarterly Review of Biology, 2*(3), 307-343.

Risher, W. C., Ustunkaya, T., Singh Alvarado, J., & Eroglu, C. (2014). Rapid Golgi analysis method for efficient and unbiased classification of dendritic spines. *PloS one*, *9*(9), e107591.

Robbins, T. W., & Everitt, B. J. (2007). A role for mesencephalic dopamine in activation: commentary on Berridge (2006). *Psychopharmacology, 191*, 433-437.

Robinson, M. J., & Berridge, K. C. (2013). Instant transformation of learned repulsion into motivational "wanting". *Current Biology*, *23*(4), 282-289.

Robinson, M. J., Anselme, P., Fischer, A. M., & Berridge, K. C. (2014). Initial uncertainty in Pavlovian reward prediction persistently elevates incentive salience and extends sign-tracking to normally unattractive cues. *Behavioural brain research*, *266*, 119-130.

Robinson, M. J. F., Fischer, A. M., Ahuja, A., Lesser, E. N., & Maniates, H. (2016). Roles of "wanting" and "liking" in motivating behavior: gambling, food, and drug addictions. *Behavioral neuroscience of motivation*, 105-136.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain research reviews*, *18*(3), 247-291.

Robinson, T. E., & Flagel, S. B. (2009). Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biological psychiatry*, *65*(10), 869-873.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology*, *47*, 33-46.

Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European journal of neuroscience*, *11*(5), 1598-1604.

Robinson, T. E., Jurson, P. A., Bennett, J. A., & Bentgen, K. M. (1988). Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. *Brain research*, *462*(2), 211-222.

Rodriguez-Jimenez, R., Avila, C., Jimenez-Arriero, M. A., Ponce, G., Monasor, R., Jimenez, M. E. E. A., ... & Palomo, T. (2006). Impulsivity and sustained attention in pathological gamblers: influence of childhood ADHD history. *Journal of Gambling Studies*, *22*(4), 451-461.

Rodriguez-Romaguera, J., Sotres-Bayon, F., Mueller, D., & Quirk, G. J. (2009). Systemic propranolol acts centrally to reduce conditioned fear in rats without impairing extinction. *Biological psychiatry*, *65*(10), 887-892.

Roshchina, V. V. (2010). Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. *Microbial endocrinology*, 17-52.

Ross, S. P., Skelton, R. W., & Mueller, S. C. (2006). Gender differences in spatial navigation in virtual space: implications when using virtual environments in instruction and assessment. *Virtual Reality*, *10*(3), 175-184.

Roughley, S., & Killcross, S. (2019). Differential involvement of dopamine receptor subtypes in the acquisition of Pavlovian sign-tracking and goal-tracking responses. *Psychopharmacology*, *236*(6), 1853-1862.

Rowntree, D. W., Nevin, S., & Wilson, A. (1950). The effects of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *13*(1), 47.

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential dopamine release dynamics in the nucleus accumbens core and shell reveal complementary signals for error prediction and incentive motivation. *Journal of Neuroscience*, *35*(33), 11572-11582.

Sala, C., & Segal, M. (2014). Dendritic spines: the locus of structural and functional plasticity. *Physiological reviews*, *94*(1), 141-188.

Salgado, S., & Kaplitt, M. G. (2015). The nucleus accumbens: a comprehensive review. *Stereotactic and functional neurosurgery*, *93*(2), 75-93.

Sánchez-Kuhn, A., León, J. J., Góngora, K., Pérez-Fernández, C., Sánchez-Santed, F., Moreno, M., & Flores, P. (2017). Go/No-Go task performance predicts differences in compulsivity but not in impulsivity personality traits. *Psychiatry Research*, *257*, 270-275.

Sarter, M., & Phillips, K. B. (2018). The neuroscience of cognitive-motivational styles: Sign- and goal-trackers as animal models. *Behavioral Neuroscience*, *132*(1), 1.

Sarter, M., Lustig, C., & Taylor, S. F. (2012). Cholinergic contributions to the cognitive symptoms of schizophrenia and the viability of cholinergic treatments. *Neuropharmacology*, *62*(3), 1544-1553.

Saunders, B. T., & Robinson, T. E. (2010). A cocaine cue acts as an incentive stimulus in some but not others: implications for addiction. *Biological psychiatry*, *67*(8), 730-736.

Saunders, B. T., & Robinson, T. E. (2012). The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. *European Journal of Neuroscience*, *36*(4), 2521-2532.

Saunders, B. T., & Robinson, T. E. (2013). Individual variation in resisting temptation: implications for addiction. *Neuroscience & Biobehavioral Reviews*, *37*(9), 1955-1975.

Saunders, B. T., O'donnell, E. G., Aurbach, E. L., & Robinson, T. E. (2014). A cocaine context renews drug seeking preferentially in a subset of individuals. *Neuropsychopharmacology*, *39*(12), 2816-2823.

Saunders, B. T., Richard, J. M., Margolis, E. B., & Janak, P. H. (2018). Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. *Nature neuroscience*, *21*(8), 1072-1083.

Saunders, B. T., Yager, L. M., & Robinson, T. E. (2013). Cue-evoked cocaine "craving": role of dopamine in the accumbens core. *Journal of Neuroscience*, *33*(35), 13989-14000.

Saunders, J. B., Aasland, O. G., Babor, T. F., De La Fuente, J. R., & Grant, M. (1993). Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction*, *88*(6), 791-804.

Savchenko, A., Müller, C., Lubec, J., Leo, D., Korz, V., Afjehi-Sadat, L., ... & Sukhanov, I. (2022). The Lack of Dopamine Transporter Is Associated With Conditional Associative Learning Impairments and Striatal Proteomic Changes. *Frontiers in Psychiatry*, *13*.

Schad, D. J., Rapp, M. A., Garbusow, M., Nebe, S., Sebold, M., Obst, E., ... & Huys, Q. J. (2020). Dissociating neural learning signals in human sign-and goal-trackers. *Nature human behaviour*, *4*(2), 201-214.

Scheres, A., Tontsch, C., Thoeny, A. L., & Kaczkurkin, A. (2010). Temporal reward discounting in attention-deficit/hyperactivity disorder: the contribution of symptom domains, reward magnitude, and session length. *Biological psychiatry*, *67*(7), 641-648.

Schettino, M., Ceccarelli, I., Tarvainen, M., Martelli, M., Orsini, C., & Ottaviani, C. (2022). From skinner box to daily life: Sign-tracker phenotype co-segregates with impulsivity, compulsivity, and addiction tendencies in humans. *Cognitive, Affective, & Behavioral Neuroscience*, 1-12.

Schlaepfer, T. E., Cohen, M. X., Frick, C., Kosel, M., Brodesser, D., Axmacher, N., ... & Sturm, V. (2008). Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology*, *33*(2), 368-377.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), 671-675.

Schramm, M. J., Everitt, B. J., & Milton, A. L. (2016). Bidirectional modulation of alcohol-associated memory reconsolidation through manipulation of adrenergic signaling. *Neuropsychopharmacology*, *41*(4), 1103-1111.

Schultz, W. (2015). Neuronal reward and decision signals: from theories to data. *Physiological reviews*, *95*(3), 853-951.

Schultz, W. (2022). Dopamine reward prediction error coding. *Dialogues in clinical neuroscience*.

Schultz, W., Apicella, P., & Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *Journal of neuroscience*, *13*(3), 900-913.

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science*, *275*(5306), 1593-1599.

Schuweiler, D. R., Athens, J. M., Thompson, J. M., Vazhayil, S. T., & Garris, P. A. (2018). Effects of an acute therapeutic or rewarding dose of amphetamine on acquisition of Pavlovian autoshaping and ventral striatal dopamine signaling. *Behavioural Brain Research*, *336*, 191-203.

Schwartz, B., & Williams, D. R. (1972). The role of the response-reinforcer contingency in negative automaintenance. *Journal of the Experimental Analysis of Behavior*, *17*(3), 351-357.

Scinska, A., Sienkiewicz-Jarosz, H., Kuran, W., Ryglewicz, D., Rogowski, A., Wrobel, E., ... & Bienkowski, P. (2004). Depressive symptoms and taste reactivity in humans. *Physiology & behavior*, *82*(5), 899-904.

Sescousse, G., Caldú, X., Segura, B., & Dreher, J. C. (2013). Processing of primary and secondary rewards: a quantitative meta-analysis and review of human functional neuroimaging studies. *Neuroscience & Biobehavioral Reviews*, *37*(4), 681-696.

Shields, A. L., Guttmannova, K., & Caruso, J. C. (2004). An examination of the factor structure of the Alcohol Use Disorders Identification Test in two high-risk samples. *Substance use & misuse*, *39*(7), 1161-1182.

Shors, T. J., Chua, C., & Falduto, J. (2001). Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *Journal of Neuroscience*, *21*(16), 6292-6297.

Siegel, R. K. (1977). Stimulus selection and tracking during urination: autoshaping directed behavior with toilet targets. *Journal of Applied Behavior Analysis*, *10*(2), 255-265.

Sienkiewicz-Jarosz, H., Scinska, A., Swiecicki, L., Lipczynska-Lojkowska, W., Kuran, W., Ryglewicz, D., Kolaczkowski, M., Samochowiec, J., & Bienkowski, P. (2013). Sweet liking in patients with Parkinson's disease. *Journal of the neurological sciences*, *329*(1-2), 17-22.

Silva, M. B., & Soares, A. B. (2018). Reconsolidation of human motor memory: From boundary conditions to behavioral interventions—How far are we from clinical applications?. *Behavioural Brain Research*, *353*, 83-90.

Simon, N. W., Mendez, I. A., & Setlow, B. (2009). Effects of prior amphetamine exposure on approach strategy in appetitive Pavlovian conditioning in rats. *Psychopharmacology*, *202*(4), 699-709.

Singer, B. F., Bryan, M. A., Popov, P., Robinson, T. E., & Aragona, B. J. (2017). Rapid induction of dopamine sensitization in the nucleus accumbens shell induced by a single injection of cocaine. *Behavioural brain research*, *324*, 66-70.

Singer, B. F., Bryan, M. A., Popov, P., Scarff, R., Carter, C., Wright, E., Aragona B. J., & Robinson, T. E. (2016a). The sensory features of a food cue influence its ability to act as an incentive stimulus and evoke dopamine release in the nucleus accumbens core. *Learning & Memory*, *23*(11), 595-606.

Singer, B. F., Bubula, N., Li, D., Przybycien-Szymanska, M. M., Bindokas, V. P., & Vezina, P. (2016c). Drug-paired contextual stimuli increase dendritic spine dynamics in select nucleus accumbens neurons. *Neuropsychopharmacology*, *41*(8), 2178-2187.

Singer, B. F., Guptaroy, B., Austin, C. J., Wohl, I., Lovic, V., Seiler, J. L., ... & Aragona, B. J. (2016b). Individual variation in incentive salience attribution and accumbens dopamine transporter expression and function. *European Journal of Neuroscience*, *43*(5), 662-670.

Singer, B. F., Tanabe, L. M., Gorny, G., Jake-Matthews, C., Li, Y., Kolb, B., & Vezina, P. (2009). Amphetamine-induced changes in dendritic morphology in rat forebrain correspond to associative drug conditioning rather than nonassociative drug sensitization. *Biological psychiatry*, *65*(10), 835-840.

Skinner, B. F. (1948). 'Superstition' in the pigeon. *Journal of experimental psychology*, *38*(2), 168.

Skinner, B. F. (1984). Selection by consequences. *Behavioral and brain sciences*, *7*(4), 477-481.

Skinner, H. A. (1982). The drug abuse screening test. *Addictive behaviors*, *7*(4), 363-371.

Soares-Cunha, C., Coimbra, B., David-Pereira, A., Borges, S., Pinto, L., Costa, P., ... & Rodrigues, A. J. (2016). Activation of D2 dopamine receptor-expressing neurons in the nucleus accumbens increases motivation. *Nature communications*, *7*(1), 1-11.

Solomon, R. L., & Corbit, J. D. (1974). An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychological review*, *81*(2), 119.

Sonuga-Barke, E. J., Sergeant, J. A., Nigg, J., & Willcutt, E. (2008). Executive dysfunction and delay aversion in attention deficit hyperactivity disorder: nosologic and diagnostic implications. *Child and adolescent psychiatric clinics of North America*, *17*(2), 367-384.

Staddon, J. E., & Simmelhag, V. L. (1971). The "supersitition" experiment: A reexamination of its implications for the principles of adaptive behavior.

Stanford, M. S., Mathias, C. W., Dougherty, D. M., Lake, S. L., Anderson, N. E., & Patton, J. H. (2009). Fifty years of the Barratt Impulsiveness Scale: An update and review. *Personality and individual differences*, *47*(5), 385-395.

Steinberg, E. E., Keiflin, R., Boivin, J. R., Witten, I. B., Deisseroth, K., & Janak, P. H. (2013). A causal link between prediction errors, dopamine neurons and learning. *Nature neuroscience*, *16*(7), 966-973.

Steinberg, L., Albert, D., Cauffman, E., Banich, M., Graham, S., & Woolard, J. (2008). Age differences in sensation seeking and impulsivity as indexed by behavior and self-report: evidence for a dual systems model. *Developmental psychology*, *44*(6), 1764.

Steiner, M., Katz, R. J., Baldrighi, G., & Carroll, B. J. (1981). Motivated behavior and the estrous cycle in rats. *Psychoneuroendocrinology*, *6*(1), 81-90.

Stewart, J., De Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological review*, *91*(2), 251.

Stinson, F. S., Grant, B. F., Dawson, D. A., Ruan, W. J., Huang, B., & Saha, T. (2005). Comorbidity between DSM-IV alcohol and specific drug use disorders in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Drug and alcohol dependence*, *80*(1), 105-116.

Stramek, A. K., Johnson, M. L., & Taylor, V. J. (2019). Improved timed-mating, non-invasive method using fewer unproven female rats with pregnancy validation via early body mass increases. *Laboratory animals*, *53*(2), 148-159.

Strauss, G. P., & Gold, J. M. (2012). A new perspective on anhedonia in schizophrenia. *American Journal of Psychiatry*, *169*(4), 364-373.

Stringfield, S. J., Madayag, A. C., Boettiger, C. A., & Robinson, D. L. (2019). Sex differences in nicotine-enhanced Pavlovian conditioned approach in rats. *Biology of sex Differences*, *10*(1), 1-11.

Stuchlik, A., Petrasek, T., & Vales, K. (2009). A dose–response study of the effects of pre-test administration of beta-adrenergic receptor antagonist propranolol on the learning of active place avoidance, a spatial cognition task, in rats. *Behavioural brain research*, *200*(1), 144-149.

Sun, H., Mao, Y., Wang, J., & Ma, Y. (2011). Effects of beta-adrenergic antagonist, propranolol on spatial memory and exploratory behavior in mice. *Neuroscience letters*, *498*(2), 133-137.

Sweitzer, M. M., Allen, P. A., & Kaut, K. P. (2008). Relation of individual differences in impulsivity to nonclinical emotional decision making. *Journal of the International Neuropsychological Society*, *14*(5), 878-882.

Swintosky, M., Brennan, J. T., Koziel, C., Paulus, J. P., & Morrison, S. E. (2021). Sign tracking predicts suboptimal behavior in a rodent gambling task. *Psychopharmacology*, *238*(9), 2645-2660.

Taylor, S. E., & Brown, J. D. (1988). Illusion and well-being: a social psychological perspective on mental health. *Psychological bulletin*, *103*(2), 193.

Tetley, A. C., Brunstrom, J. M., & Griffiths, P. L. (2010). The role of sensitivity to reward and impulsivity in food-cue reactivity. *Eating Behaviors*, *11*(3), 138-143.

Thorndike, E. L. (1898). Animal intelligence. *Nature*, *58*(1504), 390-390.

Timberlake, W., & Lucas, G. A. (1985). The basis of superstitious behavior: chance contingency, stimulus substitution, or appetitive behavior?. *Journal of the experimental analysis of behavior*, *44*(3), 279-299.

Tinbergen, N. (1951). *The study of instinct*. Oxford: Clarendon Press.

Tindell, A. J., Berridge, K. C., Zhang, J., Pecina, S., & Aldridge, J. W. (2005). Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. *European Journal of Neuroscience*, *22*(10), 2617-2634.

Tindell, A. J., Berridge, K. C., Zhang, J., Pecina, S., & Aldridge, J. W. (2005). Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. *European Journal of Neuroscience*, *22*(10), 2617-2634.

Toates, F. (1986). *Motivational Systems*. Cambridge: Cambridge University Press.

Tolin, D. F., & Foa, E. B. (2008). Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research.

Tomie, A., Aguado, A. S., Pohorecky, L. A., & Benjamin, D. (1998). Ethanol induces impulsive-like responding in a delay-of-reward operant choice procedure: impulsivity predicts autoshaping. *Psychopharmacology*, *139*(4), 376-382.

Tomie, A., Grimes, K. L., & Pohorecky, L. A. (2008). Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. *Brain research reviews*, *58*(1), 121-135.

Tomie, A., Lincks, M., Nadarajah, S. D., Pohorecky, L. A., & Yu, L. (2012). Pairings of lever and food induce Pavlovian conditioned approach of sign-tracking and goal-tracking in C57BL/6 mice. *Behavioural brain research*, *226*(2), 571-578.

Treadway, M. T., & Zald, D. H. (2013). Parsing anhedonia: translational models of reward-processing deficits in psychopathology. *Current directions in psychological science*, *22*(3), 244-249.

Treadway, M. T., & Zald, D. H. (2013). Parsing anhedonia: translational models of reward-processing deficits in psychopathology. *Current directions in psychological science*, *22*(3), 244-249.

Treit, D., & Berridge, K. C. (1990). A comparison of benzodiazepine, serotonin, and dopamine agents in the taste-reactivity paradigm. *Pharmacology Biochemistry and Behavior, 37*(3), 451-456.

Tropp, J., & Markus, E. J. (2001). Sex differences in the dynamics of cue utilization and exploratory behavior. *Behavioural brain research*, *119*(2), 143-154.

Tunstall, B. J., & Kearns, D. N. (2015). Sign-tracking predicts increased choice of cocaine over food in rats. *Behavioural brain research*, *281*, 222-228.

United Nations Office on Drugs and Crime. (2022). World Drug Report. Vienna, Austria. Retrieved from https://dataunodc.un.org/dp-drug-use-prevalence.

Upton, D. J., Bishara, A. J., Ahn, W. Y., & Stout, J. C. (2011). Propensity for risk taking and trait impulsivity in the Iowa Gambling Task. *Personality and individual differences*, *50*(4), 492-495.

Urban, N. B., Kegeles, L. S., Slifstein, M., Xu, X., Martinez, D., Sakr, E., ... & Abi-Dargham, A. (2010). Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a positron emission tomography imaging study with [11C] raclopride. *Biological psychiatry*, *68*(8), 689-696.

Urcelay, G. P., & Dalley, J. W. (2011). Linking ADHD, impulsivity, and drug abuse: a neuropsychological perspective. *Behavioral neuroscience of attention deficit hyperactivity disorder and its treatment*, 173-197.

Uslaner, J. M., Acerbo, M. J., Jones, S. A., & Robinson, T. E. (2006). The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. *Behavioural brain research*, *169*(2), 320-324.

Valenstein, E. S. (1971). Channeling of responses elicited by hypothalamic stimulation. *Journal of Psychiatric Research, 8*,335-344.

van den Bos, R., Homberg, J., & de Visser, L. (2013). A critical review of sex differences in decision-making tasks: focus on the Iowa Gambling Task. *Behavioural brain research*, *238*, 95-108.

Van den Bos, R., Lasthuis, W., Den Heijer, E., Van der Harst, J., & Spruijt, B. (2006). Toward a rodent model of the Iowa gambling task. *Behavior research methods*, *38*(3), 470-478.

Versace, F., Kypriotakis, G., Basen-Engquist, K., & Schembre, S. M. (2016). Heterogeneity in brain reactivity to pleasant and food cues: evidence of sign-tracking in humans. *Social cognitive and affective neuroscience*, *11*(4), 604-611.

Vetere, G., Piserchia, V., Borreca, A., Novembre, G., Aceti, M., & Ammassari-Teule, M. (2013). Reactivating fear memory under propranolol resets pre-trauma levels of dendritic spines in basolateral amygdala but not dorsal hippocampus neurons. *Frontiers in behavioral neuroscience*, *7*, 211.

Vigorito, M., Lopez, M. J., & Pra Sisto, A. J. (2022). Sign tracking in an enriched environment: a potential ecologically relevant animal model of adaptive behavior change. *Cognitive, Affective, & Behavioral Neuroscience*, *22*(4), 703-721.

Villalba, R. M., Lee, H., & Smith, Y. (2009). Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. *Experimental neurology*, *215*(2), 220-227.

Villaruel, F. R., & Chaudhri, N. (2016). Individual differences in the attribution of incentive salience to a Pavlovian alcohol cue. *Frontiers in Behavioral Neuroscience*, *10*, 238.

Volkow, N. D., Tomasi, D., Wang, G. J., Fowler, J. S., Telang, F., Goldstein, R. Z., ... & Wong, C. (2011b). Reduced metabolism in brain "control networks" following cocaine-cues exposure in female cocaine abusers. *PloS one*, *6*(2), e16573.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Wong, C., ... & Pappas, N. R. (1999). Reinforcing effects of psychostimulants in humans are associated with

increases in brain dopamine and occupancy of D2receptors. *Journal of Pharmacology and Experimental Therapeutics*, *291*(1), 409-415.

Volkow, N. D., Wang, G. J., Kollins, S. H., Wigal, T. L., Newcorn, J. H., Telang, F., ... & Swanson, J. M. (2009). Evaluating dopamine reward pathway in ADHD: clinical implications. *Jama*, *302*(10), 1084-1091.

Volkow, N. D., Wang, G. J., Newcorn, J. H., Kollins, S. H., Wigal, T. L., Telang, F., ... & Swanson, J. M. (2011a). Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. *Molecular psychiatry*, *16*(11), 1147-1154.

Vondanovich, S. J., & Kass, S. J. (1990). Age and gender differences in boredom proneness. *Journal of Social Behavior and Personality*, *5*(4), 297.

Wand, G. (2008). The influence of stress on the transition from drug use to addiction. *Alcohol Research & Health*.

Wang, H. D., & Deutch, A. Y. (2008). Dopamine depletion of the prefrontal cortex induces dendritic spine loss: reversal by atypical antipsychotic drug treatment. *Neuropsychopharmacology*, *33*(6), 1276-1286.

Wardle, M. C., Lopez-Gamundi, P., & Flagel, S. B. (2018). Measuring appetitive conditioned responses in humans. *Physiology & behavior*, *188*, 140-150.

Warren, S. G., Humphreys, A. G., Juraska, J. M., & Greenough, W. T. (1995). LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. *Brain research*, *703*(1-2), 26-30.

Watabe-Uchida, M., Zhu, L., Ogawa, S. K., Vamanrao, A., & Uchida, N. (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron*, *74*(5), 858-873.

Watson, P., Pearson, D., Most, S. B., Theeuwes, J., Wiers, R. W., & Le Pelley, M. E. (2019). Attentional capture by Pavlovian reward-signalling distractors in visual search persists when rewards are removed. *PLoS One*, *14*(12), e0226284.

Waxman, H. M., & McCleave, J. D. (1978). Auto-shaping in the archer fish (Toxotes chatareus). *Behavioral Biology*, *22*(4), 541-544.

Weafer, J., & de Wit, H. (2014). Sex differences in impulsive action and impulsive choice. *Addictive behaviors*, *39*(11), 1573-1579.

Weiland, N. G. (1992). Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology*, *131*(2), 662-668.

Weintraub, D., Koester, J., Potenza, M. N., Siderowf, A. D., Stacy, M., Voon, V., ... & Lang, A. E. (2010). Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Archives of neurology*, *67*(5), 589-595.

Whitton, A. E., Treadway, M. T., & Pizzagalli, D. A. (2015). Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Current opinion in psychiatry*, *28*(1), 7.

Wilcove, W. G., & Miller, J. C. (1974). CS-UCS presentations and a lever: Human autoshaping. *Journal of experimental psychology*, *103*(5), 868.

Williams, D. R., & Williams, H. (1969). Auto-maintenance in the pigeon: sustained pecking despite contingent non-reinforcement 2. *Journal of the experimental analysis of behavior*, *12*(4), 511-520.

Wingrove, J., & Bond, A. J. (1997). Impulsivity: a state as well as trait variable. Does mood awareness explain low correlations between trait and behavioural measures of impulsivity?. *Personality and Individual Differences*, *22*(3), 333-339.

Winstanley, C. A., Baunez, C., Theobald, D. E., & Robbins, T. W. (2005). Lesions to the subthalamic nucleus decrease impulsive choice but impair autoshaping in rats: the importance of the basal ganglia in Pavlovian conditioning and impulse control. *European Journal of Neuroscience*, *21*(11), 3107-3116.

Wise, R. A. (1982). Neuroleptics and operant behavior: the anhedonia hypothesis. *Behavioral and brain sciences*, *5*(1), 39-53.

Wise, R. A., Spindler, J., DeWit, H., & Gerberg, G. J. (1978). Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science*, *201*(4352), 262-264.

Wissman, A. M., May, R. M., & Woolley, C. S. (2012). Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity. *Brain Structure and Function*, *217*(2), 181-190.

Witteman, J., Post, H., Tarvainen, M., de Bruijn, A., Perna, E. D. S. F., Ramaekers, J. G., & Wiers, R. W. (2015). Cue reactivity and its relation to craving and relapse in alcohol dependence: a combined laboratory and field study. *Psychopharmacology*, *232*(20), 3685-3696.

Woolley, C. S., & McEwen, B. S. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *Journal of Neuroscience*, *14*(12), 7680-7687.

Woolley, C. S., Gould, E., Frankfurt, M., & McEwen, B. S. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *Journal of neuroscience*, *10*(12), 4035-4039.

Wyvell, C. L., & Berridge, K. C. (2000). Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. *Journal of Neuroscience*, *20*(21), 8122-8130.

Yager, L. M., & Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural brain research*, *214*(1), 30-34.

Yager, L. M., & Robinson, T. E. (2013). A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. *Psychopharmacology*, *226*(2), 217-228.

Yager, L. M., Pitchers, K. K., Flagel, S. B., & Robinson, T. E. (2015). Individual variation in the motivational and neurobiological effects of an opioid cue. *Neuropsychopharmacology*, *40*(5), 1269-1277.

Yao, W. D., Spealman, R. D., & Zhang, J. (2008). Dopaminergic signaling in dendritic spines. *Biochemical pharmacology*, *75*(11), 2055-2069.

Yeh, C. I., & Powers, A. S. (2005). Effects of blocking nitric oxide on learning in turtles (Chrysemys picta). *Behavioral neuroscience*, *119*(6), 1656.

Zener, K. (1937). The significance of behavior accompanying conditioned salivary secretion for theories of the conditioned response. *The American Journal of Psychology*, *50*(1/4), 384-403.

Ziv, N. E., & Smith, S. J. (1996). Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. *Neuron*, *17*(1), 91-102.

Zuj, D. V., & Norrholm, S. D. (2019). The clinical applications and practical relevance of human conditioning paradigms for posttraumatic stress disorder. *Progress in neuro-psychopharmacology and biological psychiatry*, *88*, 339-351.