Individual differences in the expression of Pavlovian-conditioned approach in response to a sexually-conditioned cue in male rats: A model of fetishistic behaviour

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ABSTRACT

Individual differences in the expression of Pavlovian-conditioned approach in response to a sexually-conditioned cue in male rats: A model of fetishistic behaviour

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Neutral stimuli that are repeatedly paired with reward act as predictors of impending reward, and can acquire incentive salient properties, measured using Pavlovian-conditioned approach. Individual differences in Pavlovian-conditioned approach have been observed in a subset of animals. In sign-trackers, the CS becomes 'attractive' and 'wanted', whereas in goal-trackers, the CS retains informational properties that signal the availability of reward. Most studies have investigated sign- and goal-tracking using food and drug reward, as these phenotypes may confer vulnerability or resistance to the development of addictive behaviours, respectively. To date, the expression of sign- and goal-tracking in response to a sexually-conditioned cue has been limited to male Japanese quail.

We are the first to assess individual differences in Pavlovian-conditioned approach in response to a cue paired with sexual reward leading to the ejaculatory state. We found evidence of sign-tracking, as subjects approached, engaged, and spent more time near the cue paired with the opportunity to ejaculate with a sexually-receptive female. Goal-tracking was also observed in subjects that approached and spent more time near the location where sexual reward was delivered.

Next, we compared the stability of sign- and goal-tracking to a sucrose- and sex-paired cue, and whether phenotypic differences are consistent across different types of natural reward. Sucrose goal-trackers fluctuated between cue- and goal-directed responses, though a statistical trend revealed a tendency to spend more time near the location where sexual reward was delivered. Sucrose sign-trackers appeared to 'shift' their behavioural phenotype, as they demonstrated goal-directed behaviour in response to a sexually-conditioned cue.

Lastly, we explored whether the chronic systemic administration of oxytocin influences the expression of Pavlovian-conditioned approach in response to a sexually-conditioned cue.

Oxytocin did not enhance nor diminish sign-tracking behaviour, however it potentiated goal-tracking responses in intermediate subjects that typically fluctuate between cue- and goal-directed behaviours.

Collectively, we demonstrate that individual differences in Pavlovian-conditioned approach develop in response to a sexually-conditioned cue in male rats, likely as they acquire incentive salience due to their pairing with sexual reward. Furthermore, sign- and goal-tracking are expressed differentially based on the type of natural reward and neuropeptide influence.

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Chapter 4: The effects of oxytocin administration on the expression of sign-tracking and intermediate behaviour in response to a sexually-conditioned cue in the male rat

Lindsay M. Sparks: Contributed to the experimental design, surgical procedures, drug preparation, hormone administration, behavioural training, data analyses, and to the interpretation of findings.

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LIST OF ABBREVIATIONS

μg……..……..……..... micrograms
6-OHDA……..…… 6-hydroxydopamine
ANOVA……..…… analysis of variance
bHR……..……high responders to novelty
bLR…… low responders to novelty
C...……..……..…… Celsius
CAM……..…… cue and manipulation
cm……..…… centimeter
CPP…… conditioned place preference
CS……..…… conditioned stimulus
D……..…… depth
D1…… dopamine D1-receptor family
D2…… dopamine D2-receptor family
DSM-5……. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
g……..…… grams
kg……..…….... kilograms
L……..……..…length
LLP……..…… late positive potentials
M...……..……..…… mean
m……..……..... meter
mA……..……..... milliamps
mg……..……..…… milligrams
ml……..……..... milliliters
n……..…… sample of the population
N……..…… total population
O-side……..……..... opposite side
PCA……..……..... Pavlovian-conditioned approach
RPM……..……..... revolutions per minute
S-R……..……..... stimulus-response
S-S……..…… stimulus-stimulus
S-side……..……..... same-side
SCS……..……..... Sexual Compulsivity Scale
SD……..…… standard deviation

SDMN……..…… social decision-making network
SEM……..…….. standard error of the mean
SSN……..……. social salience network
T-C……..……..... terrycloth-copulation
T-NoC……..…… terrycloth- no copulation
US……..…… unconditioned stimulus
W……..……..... width
w/*v*……..…… weight per volume

Chapter 1: General introduction

It starts with attraction. As humans, much of what we do depends on an initial attraction or fascination. The field of psychology, for instance, attracts countless students every academic year with an eagerness to understand human behaviour and mental processes, including attention, perception, decision-making, learning and memory. Similarly, our choice of partner typically begins with an attraction, physical or emotional, and the study of sexual behaviour attempts to elucidate the diversity of sexual responses, preferences and arousal states using a combination of cognitive, learning, biological and evolutionary perspectives in psychology. The application of learning theory and conditioning mechanisms has been particularly valuable in our understanding of individual differences in sexual preferences, which can, at times, lead to sexual dysfunction. A paraphilia is the experience of intense and persistent sexual arousal toward atypical objects, individuals, or situations, and may solely represent an unusual sexual preference without being pathological (McManus et al., 2013). In contrast, according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5; American Psychiatric Association, 2013), fetishistic disorder, a type of paraphilic disorder, involves the use of, or a specific focus on, non-living objects or non-genital body parts, and may cause distress, impairment, personal harm to the individual or a risk of harm to others.

Early studies have recognized the important roles of associative learning and conditioning mechanisms in the development of fetishistic behaviour in humans (Rachman & Hodgson, 1968). This has also been demonstrated in animal models in which sexually-salient cues have been shown to both guide and motivate sexual responses and preferences for partners and inanimate objects alike (Kippin et al., 1998; Köksal et al., 2004; Pfaus et al., 2013). Although the precise mechanisms that predispose certain individuals toward pathological behaviours remain unclear, many drug and food studies suggest that the subjective properties of a conditioned cue can affect the strength and vigor of responses (Flagel et al., 2009). For instance, a conditioned cue can possess predictive and informational properties for all subjects but may become ‘attractive’ and ‘wanted’ in some, but not others. These subjects will approach and engage with the conditioned cue, a response termed sign-tracking, which indicates that the conditioned cue has acquired incentive salience due to its association with reward. Sign-tracking behaviour has been well-documented using sucrose, food, and drug rewards (Morrison et al., 2015; Fitzpatrick & Morrow, 2016; Flagel et al., 2011; Yager, Pitchers et al., 2015), but there are only a limited number of studies evaluating sign-tracking using sexual reward. Importantly, it is theorized that the incentive properties of conditioned cues, in combination with a lack of inhibitory control observed in sign-trackers, is what makes the sign-tracking response pattern a model for addictive behaviours and

impulse control disorders (Tomie, 1996; Tomie et al., 1998). As such, it is useful to explore the development of individual variation in responding to sexually-salient cues, as it can enable a more comprehensive understanding of the etiology of paraphilias, and consequently, fetishistic disorder. Therefore, the goal of the current thesis is to examine the development of individual differences in responding to conditioned cues in male rats exposed to a sexual conditioning paradigm.

A brief history on the role of conditioned stimuli in reward learning: From conditioned reflexes to incentive salience, 'liking' and 'wanting'

For over a century, the principles of learning have been used to investigate the causes of, and the wide range of stimuli that provoke many aspects of behavioural responses. From Pavlov's original studies, we learned that a neutral stimulus (e.g., metronome) paired with a biologically potent stimulus (unconditioned stimulus [US], e.g., food) can elicit a conditioned response (e.g., salivation) similar to the unconditioned response that is naturally produced by the US (Pavlov & Anrep, 2003). Since then, many prominent researchers have developed compelling theories to explain the role of the conditioned stimulus (CS) in Pavlovian conditioning.

Early drive-reduction theories centered around the concept of homeostasis and explained behaviour as motivation to maintain equilibrium. According to Hull (1943), biological and physiological needs (e.g., hunger, thirst, sex) create tension or arousal, referred to as *drives*; humans and animals then seek out ways to reduce these drives. Here, the reduction of the drive acts as a reinforcement for a behaviour, which increases the likelihood that the same behaviour will be expressed in response to the biological or physiological need in the future by learning the stimulus-response (S-R) relationship. Therefore, Hull proposed that drive reduction *was reward*, and food, water, and sex become reinforcers when used to reduce hunger, thirst, and sexual drive states, respectively. By the 1970s, several brain stimulation studies found that brain sites in which electrical stimulation induced feeding behaviour were often sites that could also support brain stimulation reward; in other words, brain stimulation could induce motivated behaviour, suggesting that drive and reinforcement might reflect the same internal state (Olds & Milner, 1954; Valenstein, 1976; Valenstein et al., 1970). Consequently, the explanation of reinforcement by drive reduction was largely replaced by alternative concepts which focused on incentive reward processes and motivation.

Bolles (1972), a major opponent of drive-reduction theory, proposed that observations of operant *stereotypies*, sometimes referred to as *misbehaviour*, further contradicted Hullian theory. First, in one study, a sample of racoons underwent training, where the deposit of a wooden coin resulted in the delivery of food reward (Breland & Breland, 1961; Breland & Breland, 1966).

Though the subjects learned the task, they also became increasingly preoccupied with the coin, treating it as a morsel of food; they chewed, licked, rubbed, and washed the coin, and repeatedly deposited the coin into the slot but then pulled it back without releasing it. Second, several studies reported the development of operant responses in the absence of reinforcement by drive reduction. For example, a sample of pigeons were trained that the presentation of a light stimulus predicted the availability of food reward, independent of a response. Interestingly, subjects began to peck the light stimulus once illuminated, even though pecking behaviour had not been specifically reinforced (Brown & Jenkins, 1968). Based on these observations, Bolles refuted the notion that reinforcers act to reduce tension produced by internal states; the racoons' engagement with, and reluctance to deposit the coin into the slot in fact delayed or prevented the receipt of food reward, and the pigeons' key-peck operant response developed in the absence of reinforcement. As such, Bolles proposed that subjects learned a stimulus-stimulus (S-S) association, where the first stimulus (CS, e.g., light) elicited an *expectation* of reward (e.g., access to food reward) rather than a response, and the second stimulus was simply hedonic in value (US, e.g., food).

By 1974, Bindra proposed a theory in which the CS *becomes* the incentive, thus building upon Bolles' earlier framework. According to Bindra, a CS can induce a central motivational state comparable to the hedonic stimulus that caused pleasure (US). As a result, the subject may attribute specific incentive properties from the hedonic stimulus to the CS, thereby attracting the animal and influencing its behaviour. Therefore, his interpretation of Breland and Breland's studies (1961, 1966) explained that the wooden coin (CS), through its pairing with a morsel of food, acquired the same hedonic properties as the food reward, leading racoons to view the CS as 'food-like', resulting in a consummatory attempt. However, Bindra's approach did not explain the influence of drive states in guiding behaviour, such that a CS, acting as an incentive, should produce a response irrespective of the subject's internal drive state.

By 1986, Toates extended Bindra's theory, emphasizing the importance of perception and sensation, and suggested that conditioned stimuli can act as incentives depending on our internal drive state. This phenomenon, termed *alliesthesia*, explains that the sensation of pleasure (e.g., a sweet treat) is contingent on our physiological state (e.g., hunger, satiety) despite that the sensory characteristic of sweetness remains unchanged (Cabanac, 1979). In other words, one cookie might induce hedonic pleasure, but ten cookies will change the pleasure of the sensation, even though the first and last cookie are just as sweet. Furthermore, Toates (1986) proposed that the internal drive state could potentiate the hedonic value of rewards, and vice versa. For example, food tastes better when we are hungry, but it might also potentiate appetite for more

food when satiated. This phenomenon, termed priming, refers to an enhancement of responses following response-independent presentations of a reinforcing stimulus. For instance, when food 'primes' are delivered by an experimenter to non-food deprived rats trained to lever press for food, such primes are sufficient to increase the likelihood of subsequent lever responses, referred to as *motivational aftereffects* (Eiserer, 1978). As such, Toates proposed that the incentive value of a CS and US can fluctuate depending on physiological need or internal drive state, that the internal drive state can potentiate responding to an incentive or to a stimulus paired with an incentive, and that the interaction between external incentive stimuli and physiological drive can trigger motivated behaviour. Importantly, he contended that both are *equally* necessary to create a motivational state. Numerous studies have since supported Toates' theory of the reciprocal relationship between incentive cues and internal drive state. For example, in a study designed to disentangle the role of incentive taste cues from physiological need, Bédard and Weingarten (1989) found that neither oral stimulation nor drive reduction were capable of satiating hunger, and that subjects' appetite for a second meal was only supressed by a combination of sham feeding and intragastric meal, supporting Toates' view of the synergistic interaction between incentive cues and internal drive states.

By 1989, Berridge and colleagues introduced a modified Bindra-Toates model, in order to differentiate *liking* from *wanting*. According to the authors, 'liking' refers to the unconscious core processes underlying conscious liking, as reactions to hedonic stimuli may occur implicitly without conscious awareness. In contrast, 'wanting' refers to the core process of desire elicited by an incentive (Berridge, 1999). Therefore, 'wanting' *is* incentive salience, exemplified by a type of motivation that promotes approach, engagement, and the consumption of reward (Berridge et al., 2009). While 'wanting' can apply to biologically-relevant incentive stimuli (i.e., US), it can also occur in response to learned stimuli (i.e., CS) that signal the availability of reward through Pavlovian stimulus-stimulus (S-S) associations. Specifically, the CS can acquire incentive motivational properties through repeated pairings with an inherent incentive stimulus (US), resulting in a CS that is both 'attractive' and 'wanted'.

Berridge et al. (2009) identified a set of 'wanting' properties that can be triggered by reward-related cues. First, the 'motivational magnet' property of incentive salience states that an incentive CS becomes a 'motivational magnet', meaning that it fascinates and preoccupies the subject, and can elicit approach, engagement, and consummatory responses. This property was first evidenced by Uslaner et al. (2006), who reported that pairings of intravenous delivery of cocaine with an illuminated lever (CS) resulted in both approach and investigation of the CS, with increasing rapidity, compared to an unpaired condition. The second property, cue-triggered US

‘wanting’, indicates that encountering a CS paired with reward will trigger ‘wanting’ for the US, which likely occurs as incentive salience is transferred to learned representations of the absent reward. This property has been evidenced in studies using Pavlovian-instrumental transfer, where Pavlovian associations and operant responses are first trained in separate experimental phases; then, operant behaviour is tested in both the absence and presence of the Pavlovian CS to assess whether the cue enhanced or diminished instrumental responding (Cartoni et al., 2016). Specifically, presentations of a sucrose-paired cue have been shown to significantly increase lever pressing when compared to instrumental responding measured in the absence of sucrose or cue reinforcement, suggesting that the CS triggered ‘wanting’ for sucrose reward leading to an invigoration of operant responses (Wyvell & Berridge, 2000). Lastly, the conditioned reinforcer property of incentive salience indicates that a ‘wanted’ CS can reinforce the learning of new instrumental responses in the absence of reward by acting as a conditioned reinforcer. For instance, Di Ciano and Everitt (2004) showed that a light-CS paired with cocaine, heroin or sucrose can facilitate the acquisition of new lever responding compared to when the light-CS and reward were unpaired, thereby serving as a conditioned reinforcer to support the acquisition of a new response. Collectively, the properties of ‘wanting’ and their supporting studies provided a strong indication for the motivational influence of conditioned cues, and their ability to guide behavioural outcomes.

In addition to their extension of the Bindra-Toates model, Berridge et al. (2009) presented compelling evidence that the CS can become ‘wanted’ and ‘attractive’ following repeated pairings with reward, thereby influencing conditioned responding. Here, Pavlovian-conditioned approach (PCA) is a paradigm used as an objective measure to determine whether conditioned cues have acquired incentive salience. In some rats, the CS acts as a ‘motivational magnet’ eliciting approach, engagement and consummatory responses, all of which can be invigorated by multiple presentations of the cue (Uslaner et al., 2006; Flagel et al., 2009). This conditioned response is referred to as sign-tracking (Hearst & Jenkins, 1974; Flagel et al., 2007). Under identical experimental conditions, other subjects will instead approach the location where reward is delivered (e.g., food magazine) with increasing vigor upon repeated CS presentations (Uslaner et al., 2006; Flagel et al., 2009). Here, the CS has not acquired incentive salience, rather, it remains informative in nature and the conditioned response is referred to as goal-tracking (Boakes, 1977; Flagel et al., 2007). Lastly, intermediate responders demonstrate both cue- and goal-directed conditioned responses and vacillate between approaching the CS and the location of US delivery. Importantly, the CS is equally predictive of the US in sign-, goal-tracking and intermediate subjects, it simply triggers different conditioned responses. Interestingly, these

findings are consistent with the views initially proposed by Bolles (1972), Bindra (1974) and Toates (1986), in that conditioned cues can develop incentive salience. However, Berridge and Robinson's extension further characterized the *meaning* of incentive salience as the psychological component of 'wanting' and qualified the development of sign-tracking behaviour in response to incentive cues, which develops in some, but not all animals. Such differences in the development of sign- and goal-tracking in response to reward-related cues have led to animal models of addiction, in which conditioned cues become imbued with incentive salience and contribute to the instigation, maintenance, and reinstatement of maladaptive behaviours (Flagel et al., 2009; Everitt & Robbins, 2000; Schindler et al., 2002; Kruzich et al., 2001), and may help explain why some individuals are at greater risk for addictive behaviours or impulse control disorders (Tomie, 1996; Tomie et al., 1998).

***Misbehaviour* and the consummatory responses directed toward conditioned stimuli: Evidence from food, water, and sexual reward studies**

The first extensive study of cue-directed responses was conducted by Brown and Jenkins (1968) in a sample of pigeons, where food grain served as the US. Briefly, pigeons were trained to associate a CS (i.e., key illumination) with the response-independent delivery of food grain. With extended training, subjects displayed approach and engagement with the CS, and began to peck the illuminated key. At the time, Brown and Jenkins (1968) referred to this behavioural response as autoshaping, as the procedure was both automatic and could be automated, and because subjects had shaped their behaviour to exhibit a response that would normally require experimental manipulation. In 1974, Hearst and Jenkins coined the term *sign-tracking* to refer to this learned phenomenon which has been largely investigated using natural reward, such as food, sucrose, water, and sex.

Many studies have since established a pattern in the form of the conditioned responses that can develop following Pavlovian conditioning. In sign-trackers, the conditioned response toward the CS mimics the consummatory response that is typically exhibited toward the US. In studies using food reward, rats are trained that the presentation of a lever (i.e., CS) predicts the delivery of a food pellet, and sign-trackers will grasp, sniff, bite and gnaw at the lever as though it were food itself (Peterson, 1975; Gillis & Morrison, 2019). Similarly, in pigeons trained to associate an illuminated key with the delivery of water, sign-tracking subjects display a drinking-specific gullet movement pattern directed toward the key-light (Jenkins & Moore, 1973). Lastly, when male Japanese quail learn that the presentation of a terrycloth object predicts the opportunity to copulate with a sexually-receptive female, half of subjects display sign-tracking copulatory responses, and will grab, mount, and make a cloacal contact with the terrycloth object (Köksal et

al., 2004). Importantly, approach and engagement directed toward the CS suggest that cues paired with different types of natural reward acquire incentive motivational properties in a variety of species. Furthermore, the sign-tracking conditioned response appears to be linked to the nature of the associated reward, as subjects often display consummatory behaviours directed toward the CS.

In addition to discovering phenotypic differences in conditioned responding, several studies have attempted to disentangle the incentive and predictive properties of food-paired cues. For example, Robinson and Flagel (2009) investigated the development of sign- and goal-tracking using food reward. Here, subjects underwent Pavlovian conditioning, where an illuminated retractable lever (CS) predicted the delivery of a single food pellet (US) into a food magazine. In a subset of subjects, the illuminated lever elicited a sign-tracking conditioned response, as evidenced by approach and engagement with CS. In other subjects, the CS produced a goal-tracking conditioned response, as animals displayed approach behaviour toward the food magazine. Next, Robinson and Flagel (2009) compared the illuminated lever's ability to act as a conditioned reinforcer in sign- and goal-trackers, by testing whether the lever could support the acquisition of a nose poking instrumental response. Sign-trackers made significantly more nose pokes into the active port compared to goal-trackers, indicating that the CS also acted as a conditional reinforcer due to the acquisition of incentive salience in sign-trackers, and retained only predictive properties in goal-trackers. Importantly, the distinction between the incentive and predictive properties of reward-related cues suggests the differential involvement of specific neural systems in learning versus incentive motivation.

'Addicted' to the cue: The study of incentive salience in animal models of addiction

In humans, the treatment of drug addiction poses many challenges. Among these, perhaps the greatest problem is the tendency for addicts to relapse following a period of abstinence. It is estimated that 21.6% Canadians meet the criteria for substance use disorder during their lifetime (Statistics Canada, 2012), and in 2017, the Canadian Centre on Substance Use and Addiction reported that nearly half of respondents in active recovery had experienced at least one relapse, with the highest percentage of respondents reporting two to five relapses (McQuaid et al., 2017). There is mounting support that relapse can be facilitated by exposure to environmental stimuli that have been paired with substance use, such as people, places, or drug paraphernalia (O'Brien et al., 1992; Childress et al., 1993) as these cues are 'wanted' once imbued with incentive salience through their association with reward (Bolles, 1972; Bindra, 1978; Toates, 1986; Berridge, 2001). The role of incentive salience in drug addiction has been well-established in animal models, and several studies have identified three properties that

characterize incentive stimuli. Specifically, incentive cues can bias attention, become desirable, and can invigorate reward-seeking behaviour (Berridge, 2001; Berridge & Robinson, 2003; Meyer et al., 2012; Saunders & Robinson, 2013) which is reminiscent of drug addiction and relapse.

Tomie (1996) was perhaps the first to form a parallel between sign-tracking responses and the symptoms of drug abuse, proposing that locating the cue at the site of the instrumental response manipulandum (an arrangement referred to as *cue and manipulation*; CAM) may lead to the development of a response pattern that can be qualified as both excessive and compulsive. Here, the reward cue is described as a discriminative stimulus that reliably predicts the delivery of positive reinforcement, and response manipulandum is defined as an object that, when manipulated, characterizes the performance of the instrumental response. In an appetitive learning paradigm, CAM is arranged when subjects receive positive reinforcement following contact or manipulation with the reward cue. Breland and Breland's (1961) study can be used to exemplify CAM arrangement. Briefly, racoons were given a wooden coin, which reliably predicted the delivery of food reinforcement, and were required to contact, carry, and deposit the coin to earn food reward. Consequently, the wooden coin functioned both as a discriminative stimulus and response manipulandum; it was predictive of reinforcement, and when manipulated (e.g., contacted, carried, and deposited) earned reinforcement. Initially, subjects learned the reinforcement contingency, and deposited the coin to receive a morsel of food. However, with extended training, the racoons appeared to be preoccupied by the wooden coin, handling it with their forepaws, chewing, licking, rubbing, and washing it, and inserting it into the slot only to quickly pull it out again. As such, the *misbehaviour* induced by CAM was characterized as both excessive and problematic; it caused greater physical output and delay or forfeiture of reward. Furthermore, animals appeared to be unable to stop these responses despite the contingent loss of reinforcement, which suggests that their *misbehaviour* is a compulsive response pattern.

According to Tomie (1996), an important mediator of the relationship between CAM and excessive and compulsive responding can be explained using the sign-tracking response. Sign-tracking involves approach and engagement with the CS, which may at times be presented at a far distance from the location of reward. Therefore, sign-tracking removes the subject from the goal area and serves no purpose other than to delay or reduce access to reward. Tomie (1996) emphasizes that the environmental arrangement of CAM elicits several behaviours that are similar to the symptoms of drug abuse, through the pairing of the drug-taking implement (e.g., wine glass, hypodermic syringe; CS) with the drug's rewarding effects (US). For example, the drug-taking implement can elicit conditioned physiological responses related to those induced by the drug itself, subjective-emotional states (e.g., craving), and complex motor and consummatory

responses which appear similar to those performed during drug-taking. In sum, when CAM is arranged, several subjects develop a sign-tracking conditioned response, which can be characterized as involuntary, reflexive, and analogous to the human addict.

There is a growing body of evidence that sign-tracking behaviour develops in response to drug-paired cues, which has been studied in Pavlovian, instrumental, and self-administration paradigms. For example, Krank et al. (2007) investigated the behavioural influence of cues paired with ethanol. Subjects were initially trained to self-administer a 10% ethanol solution using a matching schedule of reinforcement, which employed a concurrent variable-interval-20-seconds on two distinct levers. Next, during Pavlovian conditioning, the levers were removed, and animals were assigned to paired and unpaired conditions. For paired subjects, each light-CS presentation preceded the delivery of 0.2 ml of ethanol solution, whereas ethanol was delivered at least 10-seconds after the light-CS presentation and 10-seconds before the next light-CS presentation in unpaired subjects. Following Pavlovian conditioning, the levers were reintroduced for a transfer test session, and the light-CS was presented using the same schedule as during Pavlovian conditioning. The light-CS presentations alternated from right to left sides, and lever responses were categorized according to period and light-CS location (i.e., inter-trial interval [time between separate cue trials], pre-CS [10-seconds before cue], right cue, left cue). The development of sign-tracking behaviour was observed in a subset of paired subjects who displayed significant levels of approach toward, and contact with, the light-CS compared to unpaired subjects, suggesting that discrete cues paired with drug reward can acquire incentive salience. Similarly, sign-tracking behaviour has been shown to develop for a number of addictive substances, including cocaine (Uslaner et al., 2006; Saunders & Robinson, 2010; Yager & Robinson, 2013), heroin (Peters & De Vries, 2013), remifentanil (Yager, Pitchers et al., 2015) and nicotine (Palmatier et al., 2012).

You don't always 'like' what you 'want', or 'want' what you 'like': The neurobiological correlates of incentive salience and Pavlovian-conditioned approach

The dissociation between 'liking' and 'wanting' first began with the dopamine-pleasure hypothesis, which proposed that the reduction of dopamine neurotransmission caused a loss of pleasure (Wise, 1980). At the time, researchers theorized that the brain's dopaminergic systems were activated in response to rewarding stimuli, and that manipulation of the dopamine system could influence how much a reward was 'wanted', measured as preference, approach or consummatory behaviour (Koob & Le Moal, 1997; Wise, 1985). 'Liking' and 'wanting' were believed to be proportional in nature; a 'liked' stimulus was also 'wanted', and vice versa.

In 1989, a classic study changed our interpretation of rewarding stimuli, suggesting that 'liking' and 'wanting' were distinctive components of reward. In their study, Berridge and colleagues (1989) focused on the mesostriatal dopamine system, which is comprised of both the mesolimbic and nigrostriatal pathways (Molochnikov & Cohen, 2014). The mesolimbic pathway features projections of dopamine neurons from the ventral tegmental area to the ventral striatum, which is composed of the nucleus accumbens and the olfactory tubercle and plays an important role in motivation cognition and reinforcement learning (Nestler et al., 2015; Berridge & Kringelbach, 2015; Alcaro et al., 2007). The nigrostriatal dopamine pathway originates in the substantia nigra pars compacta and sends its projections to the caudate and putamen of the dorsal striatum and is strongly implicated in the control of procedural aspects of movement, and motivated behaviours (Hull & Rodríguez-Manzo, 2009). Berridge et al. (1989) evaluated taste reactivity using solutions reflecting a range of taste palatability and intensity (i.e., sucrose, sodium chloride, hydrochloric acid, quinine hydrochloride) in rats with varying degrees of 6-hydroxydopamine-induced aphagia. Specifically, they hypothesized that 6-hydroxydopamine (6-OHDA) lesions of the mesostriatal dopamine system would reduce objective measures of 'liking', reflecting a reduction of hedonic orofacial expressions elicited by sucrose. Interestingly, the 6-OHDA rats demonstrated normal 'liking' reactions despite dopamine depletion; they displayed comparable levels of ingestive consummatory responses (e.g., paw licking, lateral tongue protrusions, tongue protrusions) compared to control animals. However, the mesolimbic 6-OHDA lesions appeared to significantly abolish motivation or 'wanting', as subjects no longer sought or consumed food reward, suggesting that the mesolimbic dopamine system mediates 'wanting' or desire for the incentive, but not 'liking' for the same reward stimulus. Next, Berridge and Valenstein (1991) investigated whether electrical stimulation of the mesolimbic dopamine system via the lateral hypothalamus could enhance 'liking' of food reward by measuring hedonic taste reactivity (e.g., lateral tongue protrusions, rhythmic tongue protrusions, paw licking) and feeding actions (e.g., grasping, carrying, licking food, chewing, ingestion). The data suggested that electrical stimulation of the lateral hypothalamus did not produce an overall enhancement of positive taste hedonics, however it did enhance taste for normally unpalatable stimuli, such as concentrated quinine. Interestingly, electrical stimulation of the lateral hypothalamus led to a fourfold increase in feeding actions compared to when stimulation was not delivered. The authors concluded that stimulation of the mesolimbic dopamine system elicited 'wanting' but not 'liking' further differentiating between the two components of rewarding stimuli.

In the last few decades, many studies have since clarified the neurobiology of incentive salience and identified the mesocorticolimbic dopamine pathway as the mechanism underlying

motivational processes and control. The mesocorticolimbic circuit includes both the previously described mesolimbic pathway, and the mesocortical pathway, which includes dopamine projections from the ventral tegmental area to the prefrontal cortex, and is thought to be involved in motivation, cognitive control, and executive function (Cools, 2008). Importantly, a vast array of mesocorticolimbic brain regions is involved in the processing of incentive stimuli and have been shown to be activated during sign-tracking behaviour (Fitzpatrick, 2019; Flagel et al., 2011; Yager, Garcia et al., 2015).

Berridge argues that incentive salience is not simply learned and elicited by the CS due to its associative relationship with reward, rather, he proposes that it can fluctuate with neurobiological factors (Mahler & Berridge, 2009). Specifically, the mesocorticolimbic pathway is sensitive to each re-encounter with conditioned stimuli, therefore regenerating the incentive salience of reward-related cues. This introduces a second source of motivation, referred to as the neurobiological state factor. A first example relates to the state of the mesolimbic dopamine pathway once the CS is re-encountered in one's environment; Wyvell and Berridge (2000, 2001) report that the elevation of dopamine levels in the nucleus accumbens induced by amphetamine use and neural sensitization can drastically enhance the conditioned incentive salience for sucrose reward following re-encounters of the conditioned cue. Second, incentive salience can also fluctuate due to changes in one's physiological state, such as hunger, thirst, and salt appetites (Fudim, 1978; Berridge & Schulkin, 1989; Tindell et al., 2009), a phenomenon first described as *alliesthesia* by Toates (1986). Third, the neurobiological state factor includes short- and long-term consequences of drugs of misuse, such as intoxication-priming following drug intake, and withdrawal, which can produce sensitization of mesolimbic dopamine transmission and lead to an enhancement of incentive salience (Wyvell & Berridge, 2000, 2001; Mahler & Berridge, 2009; Smith et al., 2011). For example, in amphetamine-sensitized rats, the neuronal firing pattern in the ventral pallidum shifts away from prediction signal encoding toward incentive encoding, without changes in hedonic impact encoding. In other words, mesolimbic dopamine activation has been shown to specifically intensify 'wanting' of conditioned cues, and not 'liking', though the predictive value remains unchanged (Tindell et al., 2005). Lastly, stress, as a neurobiological state can influence incentive salience; for instance, elevated levels of the peptide hormone corticotropin-releasing factor in mesocorticolimbic circuits can mediate dopamine release in the nucleus accumbens, triggering 'wanting' (Peciña et al., 2006; Berridge et al., 2010; Dallman, 2010). Collectively, such neurobiological states all share a common ability to both trigger and amplify 'wanting', with each subsequent re-encounter of reward-related cues. As such, the interaction between conditioned stimuli, neurobiological and physiological states, and incentive

salience provides a comprehensive demonstration for the role of reward-related cues in motivated behaviour.

To date, the neurobiological correlates that contribute to sign-tracking behaviour have focused on dopamine activity in the nucleus accumbens (Day & Carelli, 2007; Everitt & Robbins, 2005; Tomie et al., 2008). For instance, in an autoshaping task, rats were trained to discriminate between a visual conditioned stimulus (CS+) predictive of food reward, and a visual CS- which was not followed by food delivery; subjects selectively approached the CS+ before shifting toward the food magazine to consume the reward, indicating the development of a sign-tracking response (Cardinal et al., 2002). Next, subjects received selective excitotoxic lesions to the nucleus accumbens core, the anterior cingulate cortex, or the central nucleus of the amygdala, which have been shown to be required for the acquisition of autoshaping (Parkinson et al., 2000; Bussey et al., 1997). The findings revealed that lesions to the nucleus accumbens core and to the anterior cingulate cortex significantly impaired performance of the autoshaped response; lesioned subjects approached the CS+ significantly less than control subjects and failed to discriminate between the CS+ and CS-, and these effects were more severe and persistent following lesions to the nucleus accumbens core. Lesions to the central nucleus of the amygdala had no effect on performance. Such findings have also been reported by Parkinson et al. (2002), who found that 6-OHDA lesions to the nucleus accumbens core resulted in long-term neuroadaptations in dopamine function, and significantly impaired the acquisition and expression of sign-tracking behaviour. Collectively, these data reveal that the nucleus accumbens core and the anterior cingulate cortex are required for both the acquisition and the expression of the autoshaping response, whereas an intact central nucleus of the amygdala is important for learning the stimulus-reward association, specifically.

Dopamine receptor blockade has also been shown to differentially affect sign- and goal-tracking. For example, Flagel et al. (2011) studied rats that were selectively bred to display differences in locomotor behaviour to a novel environment, where high responders to novelty (bHR rats) consistently acquire a sign-tracking response pattern compared to low responders to novelty (bLR rats) who typically learn a goal-tracking conditioned response (Flagel et al., 2010). Phasic dopamine release was recorded in the nucleus accumbens core using fast-scan cyclic voltammetry during PCA training, where bHR and bLR rats rapidly approached and engaged with the lever-CS (sign-trackers) and food magazine (goal-trackers), respectively. During the acquisition of PCA, CS-evoked dopamine release in the nucleus accumbens core increased in bHR rats in comparison to an unpaired control group, an effect that was not observed in bLR rats, suggesting that sign- and goal-trackers produce significantly different patterns in phasic dopamine

release in response to reward-related stimuli. In the same study, Flagel et al. (2011) also examined whether the acquisition and expression of sign- and goal-tracking conditioned responses were differentially impacted by dopamine receptor blockade using flupenthixol, a non-selective dopamine-receptor antagonist. The findings revealed that flupenthixol attenuated the performance of conditioned responding in both bHR and bLR rats, which was most evident during PCA training. Furthermore, during a drug-free test session (i.e., no flupenthixol injection), bHR rats were still incapable of displaying sign-tracking behaviour, suggesting that intact dopamine transmission is required for both the acquisition and expression of a sign-tracking conditioned response. In contrast, there was no effect of flupenthixol on the acquisition or expression of the goal-tracking conditioned response, as bLR rats continued to display a fully developed goal-tracking conditioned response during a drug-free test session. Together, these data highlight the role of dopamine in the nucleus accumbens core in the performance of sign- and goal-tracking conditioned responses. Importantly, however, intact dopamine transmission appears to only be required for the acquisition of sign-tracking behaviour, suggesting that individual differences in the learning of conditioned responses may be mediated by distinct neural systems.

The influence of conditioned cues in sexual arousal and preferences: From rodents to humans

A growing number of neuroimaging studies propose that food, drugs, and sex share a 'reward network' of interacting neural systems, which includes the prefrontal cortex (e.g., orbitofrontal, insula, anterior cingulate cortex), the nucleus accumbens, the ventral pallidum, and the amygdala (Berridge & Kringelbach, 2015; Georgiadis & Kringelbach, 2012; Kringelbach et al., 2012). However, in comparison to food and drugs, investigations into incentive salience, sign- and goal-tracking, and sexual reward have been relatively limited despite evidence of the latter serving as an effective reward state (Ågmo & Berenfeld, 1990; Kippin et al., 1998; Pfaus et al., 2001). In human sexual conditioning research, the US is typically a sexually-arousing stimulus as opposed to copulation with a sexually-receptive partner in animal studies. Like animals, humans can detect the contingency of the CS-US relationship and will adopt a conditioned response, which can be measured using physiological metrics (e.g., changes in genital response, erection) or behavioural recordings (e.g., erectile responsiveness, penile volume, circumference, rigidity) of sexual arousal (Janssen et al., 2000; Janssen et al., 2006). Due to the wide diversity of human sexual responses, and given our emphasis on male subjects, the following section will focus male sexual behaviour, exclusively.

Several studies have shown that Pavlovian conditioning can enhance male sexual arousal. In one study, Lalumière and Quinsey (1998) exposed a group of male participants to

pairings of a slide depicting a moderately attractive, nude female (CS) and a highly arousing film of heterosexual sexual interactions (US), and a second group of male subjects were only presented with the slide. Changes in penile circumference were measured and compared between sexual and neutral stimuli. In all subjects, penile responses to sexual slides exceeded responses to neutral slides, and 80% of participants achieved near full or full erection to the film. Furthermore, participants exposed to the sexual slide and film showed an increase in penile response to the CS compared to other test stimuli, while those who viewed the slide alone displayed an inverse pattern. According to the authors, it may be that the CS-US contingency resulted in a penile response inhibition to stimuli that was not associated with the US; conversely, those exposed to the sexual slide alone may have experienced inhibition due to habituation or the Coolidge effect. Importantly, such findings demonstrate that a CS paired with sexual reward can increase sexual arousal, but that male subjects may also habituate to sexual stimuli when presented repeatedly.

Though incentive salience theory has not been directly applied to sexual conditioning in humans, its emphasis on neurophysiological mechanisms of learning and reward may be beneficial to the understanding of sexual dysfunctions, such as paraphilic or fetishistic disorders. As such, a recent study used a form of Pavlovian conditioning, referred to as evaluative conditioning, which posits that hedonic value is transferred to an initially neutral stimulus following its repeated pairing with the US, or a 'liked' stimulus (De Houwer et al., 2001). In their study, Hoffman et al. (2014) assessed whether male subjects, rating high in sexual compulsion, are more responsive to sexual cues and more sensitive to sexual conditioning, compared to males with lower ratings of sexual compulsion. Participants completed the Sexual Compulsivity Scale (SCS; Kalichman et al., 1994; Kalichman & Rompa, 1995) to evaluate high-risk sexual behaviour prior to the experimental session. They were then equipped with a genital device to measure changes in penile circumference and exposed to baseline presentations of olfactory stimuli (i.e., basil, geranium, lemon odour). The conditioning group received presentations of the basil or geranium odour (CS+) followed by an erotic film clip; CS- presentations were interspersed among the trials and consisted of whichever odour was not used as the CS+. A control group received unpaired presentations of the CS and erotic film clips. Participants then completed a risk-taking task and an affective priming task. The risk-taking task involved the presentation of attractive faces, accompanied by high- or low-risk profiles (e.g., number of sexual partners ranging between 2-19, consistency of condom use). Participants were asked to imagine that the high- or low-risk person was interested in intercourse, and to rate the likelihood that they would engage in sexual activity; increased likelihood scores were interpreted as a measure of increased sexual motivation

or risk-taking. The affective priming task was designed to measure affective change; participants rated odour pleasantness prior to and after conditioning as a measure of explicit odour preference. For implicit odour preferences, participants were exposed to positive and negative words in the presence of the CS+ and CS- odour cues. They were then asked to categorize the words as positive or negative; congruent pairs were expected to be rated more quickly (i.e., CS+, positive word; CS-, negative word) compared to incongruent pairs, and faster reaction times were suggestive of an implicit odour preference. The data revealed that high and low SCS participants showed comparable genital responses to the erotic film, and a statistical trend for high SCS males to show higher levels of conditioned arousal and increased sexual motivation to conditioned cues compared to low SCS males. In the presence of the CS+, high SCS males showed significantly greater intent to engage in sexual activity with both low- and high-risk sexual partners compared to the control group. The high SCS males also experienced increased implicit 'liking' for the odour that was paired with the erotic film. Though 'liking' and 'wanting' have been characterized as separate components of rewarding stimuli, 'liked' stimuli can also be 'wanted' (Berridge & Robinson, 2016), which may have contributed to the increased sexual motivation and intent to engage in high-risk sexual activity in high SCS males. Importantly, these findings further emphasize that conditioned cues paired with sexual reward can both increase arousal and sexual motivation in human subjects and highlight the role of Pavlovian conditioning in sexual compulsivity.

Sexual behaviour and conditioning have been extensively studied using animal models, which provide an important link to human studies in terms of the nature of the CS in eliciting sexual arousal and preferences. For instance, in their classic study, Kippin et al. (1998) investigated the role of Pavlovian conditioning in the development of partner preference using olfactory stimuli. During training sessions, male rats were exposed to scented or unscented females that were either sexually-receptive or unreceptive. At test, copulatory preferences were measured by comparing the frequency of consummatory sexual responses (e.g., mounting, intromissions, ejaculations) toward a scented and an unscented female, and the choice of female for first mount, intromission, and ejaculation. Interestingly, males that received training with a scented-receptive female developed a conditioned ejaculatory preference for the scented female, and males trained with scented-unreceptive or unscented-receptive females developed a conditioned ejaculatory preference toward unscented females. These data suggest that olfactory cues that are paired with sexual reward can produce a conditioned ejaculatory preference and can facilitate ejaculation toward cues previously paired with a sexually-receptive female.

The ability for conditioned cues to induce a reward state has also been supported by studies using conditioned place preference (CPP), which can be used to evaluate incentive salience by measuring approach responses to conditioned cues paired with reward (McKendrick & Graziane, 2020). Typically, subjects explore two separate, distinctive compartments of the apparatus, and a baseline preference is measured. Next, subjects are exposed to the US in their non-preferred compartment (e.g., copulation to ejaculation with a sexually-receptive female), and the control manipulation is presented in their preferred compartment. At test, subjects are again allowed to explore both compartments freely, and CPP is theorized to develop when subjects spend more time in the compartment that was paired with reward. Ågmo and Berenfeld (1990) used CPP to evaluate the rewarding aspect of the ejaculatory state. During conditioning sessions, male subjects copulated to one ejaculation in a mating-test cage and were then transferred to the non-preferred compartment of a CPP apparatus for 30-minutes. Indeed, male rats developed a place preference to the compartment paired with a single ejaculation, further emphasizing that conditioned cues can guide and impact behaviour, and that ejaculation can serve as a powerful reward state.

Though many studies have determined that olfactory stimuli paired with sexual reward can induce conditioned partner and place preferences, investigations into other types of sensory cues have been limited. In perhaps the first of its kind, Pfaus et al. (2013) introduced a rodent model of fetish development, by investigating the ability of a somatosensory cue to influence sexual arousal and copulation in male rats. In one experiment, sexually-naïve male rats were given their first copulatory experience with a sexually-receptive female; one group wore the somatosensory cue during copulation (i.e., rodent tethering jacket), and the other group copulated without the jacket. At test, half of the rats in each group were tested with the jacket on or off. Rats that were trained and tested under congruent conditions (i.e., trained and tested with somatosensory cue, trained and tested without somatosensory cue), and rats that were trained without but tested with the jacket were found to copulate normally, as measured by mounting, intromission and ejaculatory latencies, and ejaculation frequencies. In contrast, rats trained with the jacket but tested without the somatosensory cue displayed longer mount, intromission, and ejaculation latencies, fewer ejaculations, and significantly fewer rats were able to copulate to ejaculation. In a second experiment, one group of sexually-naïve male rats were given two types of conditioning sessions; in one session, the jacket was associated with sexual reward (i.e., copulation to ejaculation with a sexually-receptive female), and in an alternate session, no jacket was paired with sexual inhibition (i.e., unsuccessful attempts at copulation with a sexually non-receptive female). A separate group of sexually-naïve male rats were exposed to the opposite order of association,

meaning that the somatosensory cue was associated with sexual inhibition, and no jacket was paired with sexual reward. At test, males in both groups were tested wearing the jacket in the presence of a sexually-receptive female. The group of males trained to associate the jacket with sexual arousal exhibited normal levels of copulatory behaviour, whereas males trained to associate the somatosensory cue with sexual inhibition experienced fewer ejaculations and longer ejaculation latencies.

Collectively, these data highlight that sexual behaviour can be influenced by contextual and discrete cues associated with the physiological state of sexual arousal, which can lead animals to direct appetitive responses toward incentives that are predictive of reward. These findings may also point to the learning mechanisms that contribute to the development of sexual fetishes in humans. As explained previously, most theories explain fetishistic development as an interaction between Pavlovian conditioning processes between objects or body parts and sexual arousal or orgasm during one's early sexual experiences (McGuire et al., 1964; Pfaus et al., 2013). In the absence of social constructs of 'normal' sexual behaviour and preferences, these findings may explain the development of fetishes as the simple biproduct of Pavlovian conditioning and incentive salience during first sexual experiences with conditioned stimuli, sexual arousal, and the ejaculatory reward state.

Sexual behaviour and motivation: An interplay of neuroanatomical, neurochemical, and neuroendocrine processes

The expression of male sexual behaviour stems from an interaction between neuroanatomical, neurochemical, and neuroendocrine systems, and the processing of sensory stimuli (Breedlove & Watson, 2020). For example, hypothalamic neuroendocrine cells synthesize gonadotropin-releasing hormones, which are released from axons that terminate on the hypophyseal portal veins, thereby travelling to the anterior pituitary. The hormone-producing cells of the anterior pituitary then respond to gonadotropin-releasing hormones by either increasing or decreasing the release of tropic hormones, such as luteinizing hormone and follicle-stimulating hormone. Luteinizing hormone and follicle-stimulating hormone, which produce testosterone and control the production of sperm respectively, then travel through the circulatory system to regulate the secretion of sex steroid hormones, such as androgens (e.g., testosterone) from the testes.

In male rats, the ability to copulate is dependent on circulating levels of androgens (Hull et al., 2002), and androgen receptors in the medial preoptic area of the hypothalamus are necessary for copulatory performance (Harding & McGinnis, 2004). Neuroanatomically, the medial preoptic area integrates olfactory inputs from both the bed nucleus of the stria terminalis and the amygdala, and somatosensory inputs from the genital area via the central tegmental field,

which is highly active during penile stimulation and erection (Hashikawa et al., 2016). The medial preoptic area directs copulatory behaviour by sending axons to the ventral midbrain, which in turn, projects to the basal ganglia to coordinate mounting, and to the spinal cord, via several brainstem nuclei that regulate copulatory reflexes. One of these brainstem nuclei, the paragigantocellular nucleus in the pons, sends serotonergic fibres down into the spinal cord, where they inhibit a circuit responsible for penile erection. Therefore, erections occur when the medial preoptic area inhibits the inhibitory paragigantocellular nucleus projection, thereby releasing the spinal circuit to enable an erection (Breedlove & Watson, 2020).

Several brain lesion and stimulation studies have provided evidence for the role of the medial preoptic area in male sexual behaviour. An early study by Larsson and Heimer (1964) reported that electrolytic lesions to the medial preoptic area impaired copulation in 38% of subjects, and completely abolished sexual behaviour in half of subjects. In a separate study, Heimer and Larsson (1967) made smaller electrolytic lesions to the medial preoptic anterior region of the hypothalamus, which led to a decrease in copulatory behaviour in 45% of subjects. Similar findings have since been reported in a variety of species, including fish, reptiles, birds, and mammals (Hull & Dominguez, 2003; Hull et al., 2002, 2006). Several studies have also suggested that electrical stimulation of the medial preoptic area can facilitate sexual performance, resulting in an increased frequency of ejaculations in a timed test, and a reduction in the number of intromissions preceding an ejaculation and time required to achieve ejaculation (Malsbury, 1971; Rodríguez-Manzo et al., 2000; Vaughan & Fisher, 1962).

Though research highlights the role of the medial preoptic area in copulatory behaviour, it is important to note that medial preoptic area lesions do not inhibit the *motivation* to copulate with a sexually-receptive female. Using an instrumental procedure, Everitt and Stacey (1987) measured sexual motivation by training males to lever-press for a sexually-receptive female under a second-order schedule of reinforcement. Following training, male rats received excitotoxic lesions to the medial preoptic area, and sexual motivation was again assessed, post-operatively. As previously demonstrated by Larsson and Heimer, medial preoptic area lesions significantly abolished copulatory responding; out of 7 medial preoptic area-lesioned males, only two mounted, one intromitted, and no subjects ejaculated. Interestingly however, medial preoptic area lesions appeared to affect consummatory behaviours, specifically, and not sexual motivation. Subjects continued to lever-press for access to the sexually-receptive female and displayed a marked increase in the frequency of anogenital investigations, which is an appetitive behaviour. Though incapable of successful mounting, medial preoptic area-lesioned males climbed on top of the sexually-receptive female but were unable to thrust or palpate with their forelimbs. Collectively,

these findings provide important evidence that the medial preoptic area is an important brain region in the production of male sexual behaviour, but that sexual motivation likely relies on other systems, most likely the dopamine reward pathway.

As previously discussed, the mesolimbic dopamine pathway is fundamental to the regulation of motivation, incentive salience and reward, and is activated by drugs of abuse, feeding, drinking, and sexual behaviour. Furthermore, many aspects of male copulatory behaviour (e.g., mounts, intromissions, ejaculation) can be characterized as being rewarding and reinforcing, as evidenced by the development of conditioned partner (Kippin et al., 1998) and place preferences (Ågmo & Berenfeld, 1990), and operant tasks demonstrating that male rats will perform an instrumental response to gain access to a sexually-receptive female (Everitt & Stacey, 1987). Several studies have measured dopamine release in the mesolimbic pathway at various timepoints during male appetitive and consummatory behaviours. For example, microdialysis studies have shown that dopamine is released into the nucleus accumbens upon presentation of a sexually-receptive female, and levels remain elevated throughout the expression of sexual responses (Pfaus et al., 1990; Pfaus & Phillips, 1991; Damsma et al., 1992; Wenkstern et al., 1993; Balfour et al., 2003). Furthermore, when infused directly into the nucleus accumbens, dopamine-receptor agonists and antagonists facilitate and inhibit the initiation of sexual behaviour, respectively (Everitt et al., 1989; Pfaus & Phillips, 1989). Given that the mesolimbic dopamine pathway has also been characterized as the 'wanting' system (Berridge & Robinson, 2016; Brom et al., 2014), it is likely that the attribution of incentive salience to contextual and discrete cues may mediate approach behaviour during sexual interactions.

Sexually-conditioned cues become 'attractive' and 'wanted' by some, but not others

The ability of conditioned cues to guide and motivate sexual responses and preferences is unquestionable, and copulation is always dependent on approach behaviour toward a sexually-receptive partner. Conditioned cues, imbued with incentive salience, can also induce approach behaviours measured using PCA. However, there is limited knowledge on the ability of conditioned cues to elicit PCA using a sexual conditioning paradigm.

Domjan et al. (1986) first investigated sexually-conditioned PCA responses in male Japanese quail. Subjects were divided into two conditions: a paired and an unpaired group. For paired subjects, a red light was illuminated for 30-seconds prior to the start of the mating trial with a sexually-receptive female, which remained on for the first 10-seconds after the door the female's compartment was opened. For unpaired subjects, the red light was illuminated for 40-seconds and scheduled 3-5 hours following the mating trial. Pavlovian-conditioned approach was observed in male Japanese quail, who spent an increasing amount of time near the conditioned light

stimulus across mating trials compared to unpaired subjects. The paired groups also displayed shorter latencies to initiate copulation with the sexually-receptive female, which suggests that the CS acquired incentive salience, given its influence on sexual motivation.

In a follow-up study, Burns and Domjan (1996) investigated individual differences in conditioned responding, now referred to as sign- and goal-tracking, using a visuo-tactile cue. As in the previous study, subjects were assigned to a paired and an unpaired group. For the paired group, a wooden block (CS) was lowered to the floor of the test chamber for 30-seconds after which the door to the female's compartment was opened. The CS was then raised to the top of the cage, and the male and female quail could copulate. The unpaired group followed the same procedure, however the CS presentation occurred either 2-hours prior to, or after receiving access to the sexually-receptive female. The data revealed that the paired group spent significantly more time near the wooden block compared to the unpaired group, and that neither group spent much time in the goal area, which was the door in front of the female's compartment. As such, the results indicated that male Japanese quail display strong and persistent PCA and remain near a CS when paired with the opportunity to copulate with a sexually-receptive female, and that sign-tracking was the predominant response observed in this study.

An important link exists between the attribution of incentive salience or 'wanting' and impulsive action (Lovic et al., 2011). For example, several studies have characterized sign-trackers as impulsive with poor inhibitory control as shown by their inability to resist reward-related cues (Tomie et al., 2008). Furthermore, sign-tracking responses have been typified as persistent and resistant to extinction, likely due to action impulsivity (Beckmann & Chow, 2015; Fitzpatrick et al., 2019; Meyer & Tripi, 2018). Though not explicitly labeled sign- and goal-tracking behaviour, Köksal et al. (2004) investigated the persistence of conditioned responding to an inanimate terrycloth object during an extinction procedure in male Japanese quail. The acquisition and extinction of conditioned sexual approach were examined, where subjects were conditioned to associate a CS (terrycloth object or flashing light) with the opportunity to copulate with a sexually-receptive female. A control group received unpaired presentations of the terrycloth object and sexually-receptive female. In the terrycloth-CS condition, male Japanese quail displayed conditioned approach to the CS, and half of subjects also exhibited copulatory responses (e.g., grabs, mounts, cloacal contact) by the end of the acquisition phase. Thereafter, the terrycloth-CS condition was divided into two separate groups; those that displayed conditioned approach and copulatory responses toward the CS (Terrycloth-Copulation; T-C), and those that solely displayed conditioned approach (Terrycloth-No Copulation; T-NoC). In comparison, subjects exposed to the light-CS showed lower levels of conditioned approach with no copulatory responses, and the

unpaired group displayed minimal responding to the terrycloth-CS. During extinction, the terrycloth- and light-CS were presented in the absence of the sexually-receptive female. On the first session, the T-C, T-NoC and light-CS subjects showed similar levels of conditioned approach toward the CS; however, the T-NoC and light-CS groups showed substantial decreases in responding across extinction. In contrast, the T-C subjects continued to approach the CS throughout the extinction phase.

These findings further demonstrate that male Japanese quail can acquire conditioned approach toward a CS, and conditioned copulatory responses toward an inanimate object. Furthermore, the expression of conditioned copulatory behaviour toward the terrycloth object corresponds to the *misbehaviour* first evidenced by Breland and Breland (1961), in that subjects display consummatory behaviours toward the CS, like those directed toward the US. Importantly, these data are thought to model important aspects of fetishistic behaviour in humans; Pavlovian pairings of an inanimate object with sexual conditioning can result in consummatory responses toward a CS, and the resistance to extinction and persistence in conditioned approach are also thought to be major characteristics of fetishistic behaviour.

Overview of the thesis

There exists a large body of evidence that suggests that individual differences in PCA, expressed as sign- and goal-tracking, develop in response to food- and drug-paired cues. However, the incidence of sign- and goal-tracking for sex-paired stimuli has been mostly limited to studies using male Japanese quail, and has not been explored in rodents. Therefore, the aim of the present thesis was to investigate the development of individual differences in PCA using sexual reward in male rats.

Chapter 2 explored whether sign- and goal-tracking behaviours develop in response to a CS paired with the opportunity to copulate to ejaculation with a sexually-receptive female (US). Furthermore, we manipulated the spatial location of the CS relative to the US, based on previous reports that sign-tracking behaviour is highly sensitive to the spatial distance between the cue and reward in rats (Holland, 1980; Silva et al., 1992). In Experiment 1, we found that a subset of animals displayed conditioned approach responses toward the CS, suggestive of sign-tracking behaviour. In Experiment 2, most subjects displayed goal-tracking behaviour, as evidenced by a greater proportion of time spent near the door providing access to the female's compartment. Pavlovian-conditioned approach was not affected by spatial distance between the CS and US areas. Importantly, these data are the first to suggest that sexually-conditioned PCA responses can be modeled in rodents, and that conditioned cues can function as incentives due to their pairing with sexual reward in male rats.

Chapter 3 compared the stability of sign- and goal-tracking responses within individuals, in response to both sucrose and sexual conditioning, based on evidence that individual differences in PCA extend across different reward types (i.e., food and drug; Saunders & Robinson, 2010; Yager & Robinson, 2013). Based on a response bias score, we classified subjects as sign-, goal-trackers and intermediates using a sucrose conditioning paradigm. When exposed to sexual conditioning, a statistical trend suggested a tendency for sucrose goal-trackers to spend more time near the door providing access to the female's compartment, though this effect was not statistically significant indicating that sucrose goal-trackers fluctuated between the US and CS areas. Sucrose sign-trackers appeared to 'shift' their behavioural phenotype to goal-tracking, as they displayed greater conditioned approach toward the US as compared to the sexually-conditioned cue. Overall, these findings reflect a degree of instability in the expression of PCA responses between sucrose and sexual reward, suggesting variability in the incentive value attributed by individuals to stimuli paired with different types of natural reward.

Chapter 4 investigated whether the systemic administration of oxytocin could potentiate the expression of PCA toward a visuo-tactile cue, based on findings that oxytocin enhances a conditioned ejaculatory preference toward an olfactory cue (Ménard et al., 2019). The chronic administration of oxytocin did not further potentiate cue-directed approach in animals identified as sign-trackers following sexual conditioning. In intermediate subjects, known to vacillate between cue- and goal-directed approach responses, oxytocin potentiated the development of goal-tracking behaviour. These data further indicate that sexually-conditioned cues can acquire incentive salience, in that they can elicit approach behaviour in a subset of animals identified as sign-trackers. Furthermore, oxytocin might strengthen the CS-US association in intermediate subjects allowing for the development of a goal-tracking response pattern.

The use of animal models to study incentive salience is both useful and important, as these models allow researchers to elucidate the behavioural and neurobiological mechanisms that might predispose certain individuals toward addictive behaviours and impulse control disorders. Therefore, the overall aim of this thesis is to contribute to the literature by exploring the ability of sexually-conditioned cues to function as powerful incentives that guide behaviour, which can at times, lead to sexual dysfunction.

Chapter 2: The expression of sign- and goal-tracking in response to a sexually-conditioned cue in male Long-Evans rats

Abstract

Previous studies report that the pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US) results in individual differences in Pavlovian-conditioned approach behaviour toward either the CS (sign-tracking) or US (goal-tracking) using food and drug reward. Furthermore, increasing the spatial location of the CS relative to a food US can decrease the incidence of sign-tracking and increase goal-tracking responses in rats. At this time, no studies suggest whether sign- and goal-tracking develop following exposure to sexual reward in male rats, nor whether such differences in phenotypic expression are affected by the spatial location of the CS presentation. The current studies aimed to investigate whether male rats display sign- and goal-tracking behaviour in response to a sexually-conditioned cue paired with the sexual reward leading to ejaculation (US), and whether the spatial location of the CS relative to the US affects the expression of these phenotypes. Sexually-naïve, male Long-Evans rats received 12-13 Pavlovian-conditioning sessions in an open field chamber, where an orange cone CS (2-minute/presentation) predicted the opportunity to copulate to ejaculation (US) in a separate compartment with a sexually-receptive female. Sign- and goal-tracking were measured by the proportion of time spent in a pre-determined area centered around the CS or the sliding door to the female compartment respectively, in the absence and presence of the sexually-conditioned CS. In Experiment 1, a majority subset of rats (n = 8) spent significantly more time near the CS compared to the US, indicating sign-tracking behaviour following sexual conditioning. In Experiment 2, the spatial location of the CS presentation was manipulated, where it was positioned either in the opposite- or same-side corner of the individualized compartment of the open field chamber. A subset of rats (n = 6) spent significantly more time near the US compared to the CS, indicating goal-tracking behaviour. The proportion of time spent in the US- and CS-designated areas was not influenced by whether the CS was presented on the opposite- or same-side relative to the US. Overall, these findings suggest that individual differences in PCA can develop in response to a sexually-conditioned cue paired with sexual reward using a rodent model. Furthermore, the spatial location of Pavlovian cues relative to the US does not appear to affect the development of these behavioural phenotypes.

Introduction

The principles of learning have been studied extensively using aversive (e.g., fear, pain) and appetitive (e.g., food, drug reward) conditioning. Classically, Pavlovian conditioning occurs when a previously neutral stimulus (conditioned stimulus, CS) becomes associated with a salient, biological event (unconditioned stimulus, US). Following repeated pairings, the CS elicits a consummatory, conditioned response similar to the unconditioned response that is naturally produced by the US (Pavlov & Anrep, 2003).

Important constructs associated with Pavlovian conditioning include motivation and reinforcement learning. In Pavlovian conditioning, the outcome is response-independent; the reward is delivered regardless of the animal's actions. Therefore, any behavioural changes likely demonstrate intuitive responses to predictions of the outcome (Dayan & Balleine, 2002). Rewards can be characterized as incentive, environmental stimuli that have potent, biological value and motivational properties that can strengthen and direct subsequent behaviour. Hence, through incentive motivation, Pavlovian-conditioned approach (PCA) behaviours are generated.

Pavlovian-conditioned approach develops when the presentation of a CS (e.g., a response-independent lever) predicts the delivery of a US (e.g., food pellet), and is often used to measure whether a cue has acquired incentive motivational properties (Fitzpatrick & Morrow, 2016). Interestingly, many studies demonstrate that a CS becomes 'attractive' and 'wanted', as it functions to predict reward and can serve as an object of desire (Berridge, 1996). Once embedded with incentive salience, a previously neutral cue transforms into a salient cue that captures attention, elicits orientation and approach, and initiates behaviour directed toward it as a goal.

Based on the development of the conditioned response, PCA behaviour has been categorized in three ways (Flagel et al., 2009). Approximately one-third of rats display a preference in approach and vigorous engagement with the CS as it acquires incentive salience, a response defined as cue-directed or sign-tracking. By comparison, approximately one-third of rats will infrequently approach and engage with the CS. Rather, upon presentation of the CS, they display goal-directed behaviour or goal-tracking as they near the food receptacle to await delivery of the reward. Here, goal-tracking occurs due to the CS acting as a predictor of reward, however it does not adopt incentive properties (Petykó et al., 2015). The remaining one-third of rats exhibit neither a clear sign- nor goal-tracking response; instead, they fluctuate between cue- and goal-directed behaviours and are therefore identified as intermediates.

Individual differences in PCA have been widely investigated using food and drug reward. When a retractable lever (CS) predicted the availability of a food pellet (US), a subset of animals approached and interacted with the CS significantly more, and with increased rapidity, indicating

sign-tracking behaviour. Alternatively, other animals approached and engaged with, and displayed shorter latencies toward the food cup, reflecting goal-tracking behaviour. Similar findings have also been reported in studies using drug reward, where sign-trackers differentially attribute incentive salience to a cocaine- (Saunders & Robinson, 2010) and opioid-paired cues (Yager, Pitchers et al., 2015) in comparison to animals classified as goal-trackers. In an operant conditioning paradigm, Breland and Breland (1961) reported compulsive, consummatory behaviours in racoons that learned to deposit a coin in order to obtain a morsel of food. Here, subjects were described as being highly preoccupied with the coin, carefully washing it as though it were a morsel of food despite non-reinforcement of the behaviour or the delay/loss of the food reward. This effect, termed *misbehaviour* has also been replicated in pigs, chickens, otters, porpoises, and whales (Breland & Breland, 1961). Collectively, these studies suggest that individual differences in PCA responses can be observed across different types of reward (e.g., natural, drug) and conditioning paradigms with varying species.

Investigations into sign- and goal-tracking using sexual reward have been rather limited in comparison. In a classic sexual conditioning paradigm, researchers can measure male sexual behaviour directed toward a sexually-receptive female, when the female is presented following exposure to the CS. Likewise, PCA can be determined by the level of approach toward, and engagement with the CS and US location. In the first study of its kind, Domjan et al. (1986) investigated PCA using sexual conditioning in male Japanese quail. Here, PCA developed in male Japanese quail, as they approached and remained near the localized visual stimulus that predicted access to a female compared to males exposed to the CS at random intervals.

In a follow-up study, Burns and Domjan (1996) examined sign- and goal-tracking behaviour specifically, using copulation as the sexual reward. Individual differences in PCA responses were measured following sexual conditioning, where a wooden block served as the CS and the US represented the opportunity to copulate with a sexually-receptive female Japanese quail. It was noted that the predominant PCA response was sign-tracking, in that subjects approached and remained near the CS that was paired with the opportunity to copulate with a female Japanese quail. Not only has sexually-conditioned PCA been reported in male Japanese quail, but also in blue gourami fish (*Trichogaster trichopterus*). Here, Hollis et al. (1989) described that male blue gourami fish learned an anticipatory frontal display conditioned response, consisting of either cue-directed behaviour (i.e., immediate approach toward CS) or goal-directed behaviour (i.e., the location of the copulatory encounter).

In their study, Burns and Domjan (1996) also compared their sexual conditioning paradigm with studies of food reinforcement, which report that the strength of goal- and sign-tracking

behaviours is dependent on the spatial separation between the CS and food US in rats. Specifically, studies using food reward indicate that increasing the spatial separation between the CS and US decreases sign-tracking, while increasing a goal-tracking response (Holland, 1980; Silva et al., 1992). However, Burns and Domjan (1996) suggest that goal- and sign-tracking remain unaffected by this variable with sexual conditioning; sign-tracking remained the predominant response in male Japanese quail, and goal-tracking was not facilitated by increasing the spatial separation between the CS and US. Here, they propose that a non-stationary US might decrease the likelihood of goal-tracking in comparison to sign-tracking conditioned responses, as the location of the reinforcer is less distinctive compared to food pellets which are typically delivered into a stationary food cup.

Interestingly, though individual differences in PCA responses have been observed in avian and aquatic models using sexual conditioning, this phenomenon has yet to be examined in a rodent model. Though the spatial relationship between the CS and US did not appear to affect sign- and goal-tracking in male Japanese quail, it has been shown to affect individual differences in PCA in response to food-paired cues in rats (Holland, 1980; Silva et al., 1992). As such, it is also useful to explore whether spatial separation between the CS and US affects the expression of PCA in response to a sexually-conditioned cue in rats for methodological purposes. Therefore, the current studies aimed to investigate the existence of individual differences in PCA in male rats, using ejaculation which can serve as a powerful reward state (Ågmo & Berenfeld, 1990; Kippin et al., 1998; Pfaus et al., 2001), and to determine whether the spatial relationship between the CS and US might affect sign- and goal-tracking in a sexual conditioning paradigm.

Method

Subjects

Sexually-naïve male (225-250 g) and female (150-200 g) Long-Evans rats were obtained from Charles River Canada, Inc. (St-Constant, QC, Canada). Males were housed in groups of four, in polycarbonate (Plexiglas) gang-cages containing beta-chip bedding. Female rats were pair-housed in polycarbonate (Plexiglas) shoebox cages containing a mixture of beta-chip and corncob bedding. All rats were kept in an animal colony room which was maintained at a constant temperature of 21ºC, on a reversed 12-hour light/dark cycle (lights OFF at 0800; all procedures conducted during the dark phase). Access to standard rat chow (Charles River Rodent Animal Diet, St-Hubert, QC, Canada) and water were available *ad libitum* for the duration of the experiment.

Females underwent bilateral ovariectomy via lumbar incisions following intraperitoneal injections of a combination of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4

mg/kg) injected with a volume of 1 ml/kg of body weight. Females received 1-week recovery after surgery and were maintained on hormone replacement for the duration of the experiment using subcutaneous injections of estradiol benzoate (10 µg in 0.1 ml of sesame oil) administered every 48-hours, and progesterone (500 µg in 0.1 ml of sesame oil) 3 to 4 hours prior to conditioning sessions. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Apparatus

Behavioural conditioning sessions were conducted using an open field chamber (120 L x 120 W x 60 D cm), which contained two compartments separated by an opaque polycarbonate (Plexiglas) divider. The divider featured a sliding door (15 cm) that was slid vertically by pulling a 4.5 m nylon cord, which served to provide male rats' access to the female rat for copulation during sessions. The larger compartment of the open field (120 L x 80 W x 60 D cm) was the area where the male rat was presented with the conditioned stimulus (i.e., orange cone; CS), while the smaller compartment of the open field (120 L x 41 W x 60 D cm) was the area where the male rat accessed a sexually-receptive female rat for copulation.

Sign- and goal-tracking were measured by the proportion of time spent in the pre-determined CS Zone (e.g., Experiment 2, area situated in the opposite- or same-side corner, relative to the sliding door that provided access to the receptive female) and the pre-determined US Zone (e.g., area situated in front of the sliding door that allowed access to the receptive female). The pre-determined CS and US Zones each spanned an area of 38 W x 38 L cm. The locations of the CS and US Zones alternated across sessions in order to control for place preferences. The CS was an orange cone (14 L x 14 W x 23 W cm) which was presented in the CS Zone during a two-minute exposure, after which the sliding door opened to provide access to the sexually-receptive female. The US was the male rat's ejaculation and subsequent post-ejaculatory period (2-minute duration). A video camera (Sony Handy Cam, model DCR-SR68) was used to record the behavioural conditioning sessions, which were then scored by trained experimenters. For scoring purposes, the CS and US Zones were analogously indicated on an acetate sheet, which was secured to a computer screen during scoring. This ensured that time spent in the US and CS Zones were consistently measured across subjects during each conditioning session.

Procedure

Subjects were given two daily sessions to habituate to the open field chamber for a period of 20-minutes. The behavioural conditioning sessions were conducted for a total of 12-13 sessions (Experiment 1 and 2, respectively) over the course of seven consecutive weeks. Each

session was scheduled at four-day intervals, and included two individual trials (e.g., total of two ejaculations per session).

At the beginning of each session, rats were placed in the large compartment of the open field chamber in order to habituate to the context for 5-minutes (Figure 1). During this period, the orange cone (CS) was not presented, nor was the female rat placed in the smaller adjacent compartment. The proportion of time spent in the CS and US Zones was measured, in the absence of the CS. Following the habituation period, the male rat was presented with the CS in the pre-determined CS Zone for 2-minutes located in the opposite-side corner relative to the sliding door that provided access to the female. The proportion of time spent in the CS and US Zones was measured. Following the CS presentation, the sliding door was slid open, which allowed the male rat to access the compartment that would contain the sexually-receptive female rat. Once he entered this compartment, the sliding door was closed, and the female rat was placed in the smaller compartment for copulation. The male rat then copulated with the receptive female rat until ejaculation and remained with the female rat for a 2-minute post-ejaculatory period. Afterward, the female rat was removed from the small compartment, and the experimenter returned the male rat to the larger compartment for the second trial. If the male rat did not reach ejaculation within 30 minutes of copulation with the sexually-receptive female, then the sexual conditioning session was immediately terminated.

In Experiment 2, the sexual conditioning procedure was identical to Experiment 1, however the spatial location of the CS manipulated. The CS was presented in either the opposite- or same-side corner relative to the US in order to determine its potential influence on sign-, goal-tracking and intermediate behaviour.

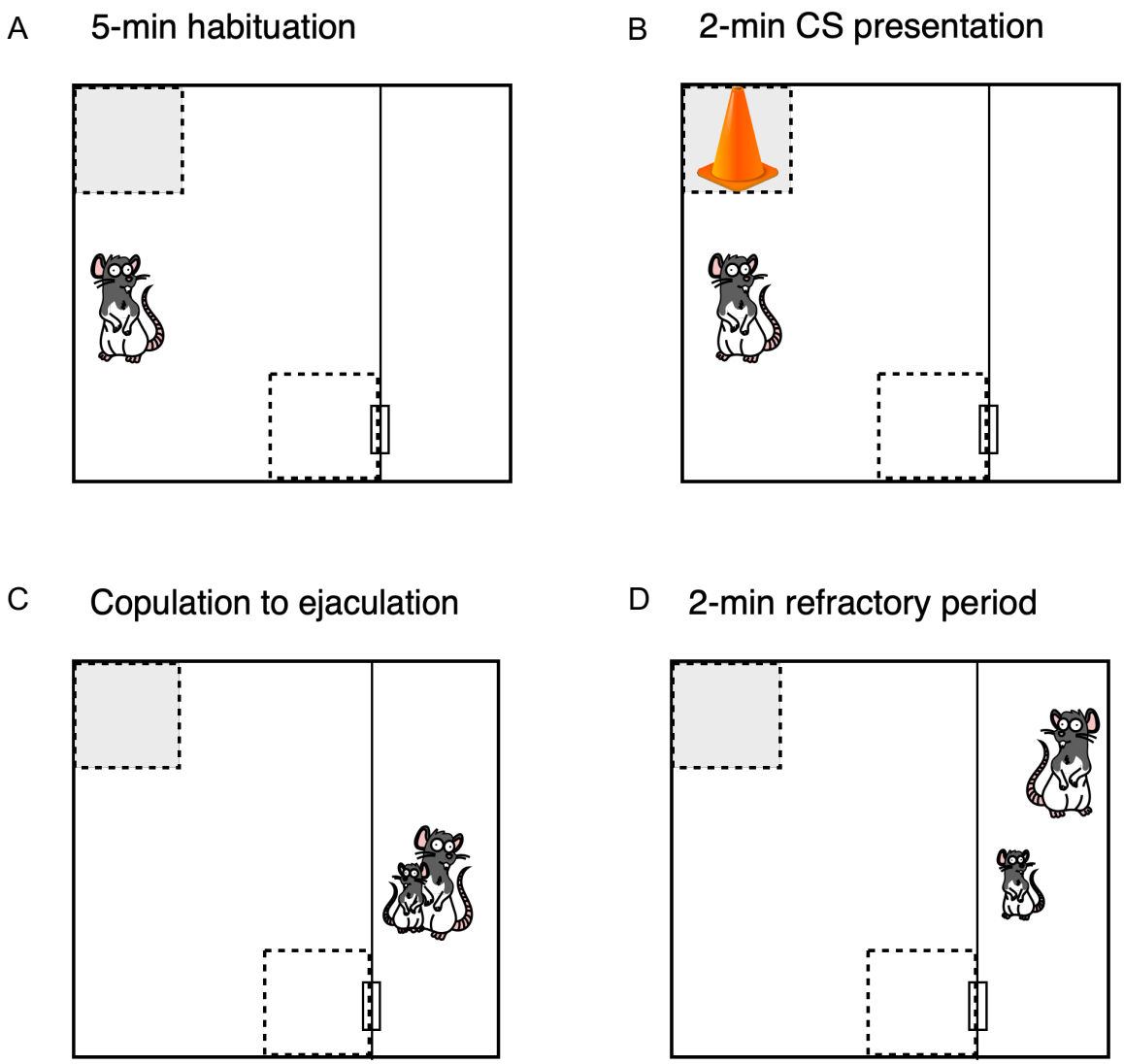


Figure 1. Experimental apparatus used for sexual conditioning. The apparatus was an open field chamber, divided into larger and smaller compartments. (A) The subject was placed in the larger compartment of the open field chamber for a 5-minute habituation period. (B) A bright orange cone, which served as the CS, was presented for 2-minutes in the furthest diagonal corner relative to a sliding door that provided access to a smaller compartment. (C) The CS was removed, and a sexually-receptive female was placed into the smaller compartment of the open field chamber. The door was slid open, which allowed the male to enter. He then copulated with the female to ejaculation, and (D) remained with her for a 2-minute refractory period. The female was then removed from the smaller compartment, and the male was returned to the larger compartment for a second trial.

Statistical analyses

The dependent measures included the proportion of time spent in the pre-determined CS and US Zones when the CS was absent (i.e., 5-minute habituation period), and the proportion of time spent in the pre-determined CS and US Zones when the CS was present, to represent PCA. A 15% CS-US difference score was calculated based on the final conditioning session as a preliminary criterion to identify rats displaying each phenotype.

Experiment 1: The acquisition of Pavlovian-conditioned approach. In order to measure phenotypic development over the course of the experiment, data from Pavlovian-conditioning sessions were analyzed using a repeated-measures analysis of variance (ANOVA) with Session (1, 6, 12; start, middle, end of training sessions, respectively) and Zone (US Zone, CS Zone [CS absent]; US, CS [CS present]) as within-subjects variables.

Experiment 2: The acquisition of Pavlovian-conditioned approach and the influence of CS-US spatial separation. Two rats were excluded due to insufficient levels of copulatory behaviour resulting in few ejaculations across sessions (e.g., > 10 subsequent trials with no ejaculation). Data from Pavlovian-conditioning sessions were analyzed using a repeated-measures analyses of variance (ANOVA) with Session (1, 7, 13) and Zone (US Zone, CS Zone [CS absent]; US, CS [CS present]) as within-subjects variables. In order to determine whether the location of the CS influenced sign-, goal-tracking and intermediate behaviour, the between-subjects variable of CS Location (opposite-side, same-side) was measured.

Results

Experiment 1: The acquisition of Pavlovian-conditioned approach following exposure to a sexually-conditioned cue

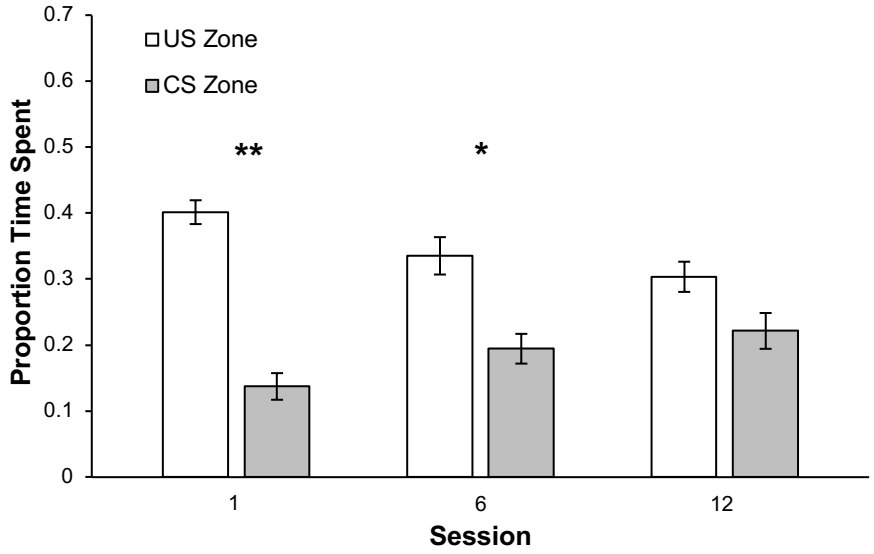
In order to determine the existence of sign-, goal-tracking and intermediate behaviour following exposure to a sexually-conditioned cue, the mean proportion of time spent in the CS and US Zones (CS absent) was compared in all rats across sessions 1, 6 and 12 (Figure 2A). A repeated-measures ANOVA on time spent in the US and CS Zones, in the absence of the CS, reflected a reduced preference for the US Zone as subjects progressed through sessions 1, 6 and 12. A statistically significant main effect of Zone, F (1, 11) = 39.202, $p < 0.001$, $\eta_p^2 = 0.55$ and Session x Zone interaction, F (2, 22) = 7.117, $p = 0.004$, $\eta_p^2 = 0.21$ indicated that subjects spent more time in the US Zone compared to the CS Zone across sessions, and that the relative amounts of time spent in the US versus CS Zone decreased and increased respectively, as a function of session. There was no significant main effect of Session, F (2, 22) = 0.075, $p = 0.928$. Follow-up paired-samples t-tests were conducted to compare the proportions of time spent in the US Zone and CS Zone on session 1, 6, and 12. On session 1, there was a statistically

significant difference in the proportion of time spent in the US Zone and CS Zone, $t(11) = 9.711$, $p < 0.001$, $d = 2.80$, where rats spent a higher proportion of time in the US Zone ($M = 0.403$, $SD = 0.625$) compared to the CS Zone ($M = 0.138$, $SD = 0.070$). There was also greater time spent in the US versus CS Zone during session 6, $t(11) = 3.331$, $p = 0.007$, $d = 0.96$ (US Zone: $M = 0.335$, $SD = 0.098$; CS Zone: $M = 0.193$, $SD = 0.079$). By session 12, there was no statistically significant difference in the proportion of time spent in the US Zone and CS Zone, $t(11) = 1.834$, $p = 0.094$. Here, rats displayed an initial preference for the US Zone compared to the CS Zone in the absence of the CS. Furthermore, as subjects learned the CS-US association, the proportion of time spent in the US Zone decreased while increasing in the CS Zone, eliminating the preference between Zones by the final session.

Next, we investigated the development of conditioned responding in the presence of the sexually-conditioned cue (Figure 2B). A repeated-measures ANOVA revealed a statistically significant main effect of Session, $F(2, 22) = 8.974$, $p < 0.001$, $\eta_p^2 = 0.07$, and Session x Zone interaction, $F(2, 22) = 22.063$, $p < 0.001$, $\eta_p^2 = 0.44$, suggesting changes in the proportion of time spent in both the US and CS Zones (CS present) across sessions, and that the proportion of time spent near the US and CS differed significantly across sessions 1, 6 and 12. There was no significant main effect of Zone, $F(1, 11) = 1.636$, $p = 0.227$.

Follow-up paired-samples *t*-tests were conducted to compare the proportion of time spent near the US and CS on session 1, 6, and 12. On sessions 1 and 6, there was no statistically significant difference in the proportion of time spent near the US and CS (Session 1, $t(11) = 1.918$, $p = 0.081$; Session 6, $t(11) = 1.318$, $p = 0.214$) indicating that rats spent a comparable proportion of time near each stimulus. By session 12, however, there was a statistically significant difference in the proportion of time spent in near the US and CS, $t(11) = -4.191$, $p = 0.002$, $d = 1.21$ where rats spent a higher proportion of time near the CS ($M = 0.527$, $SD = 0.158$) as compared to the US ($M = 0.203$, $SD = 0.117$). Therefore, the presence of the CS resulted in increased time spent in the CS Zone across sessions. This provides further evidence of a learned CS-US association, as the CS grew to function as a predictor for the opportunity to copulate with a sexually-receptive female.

A



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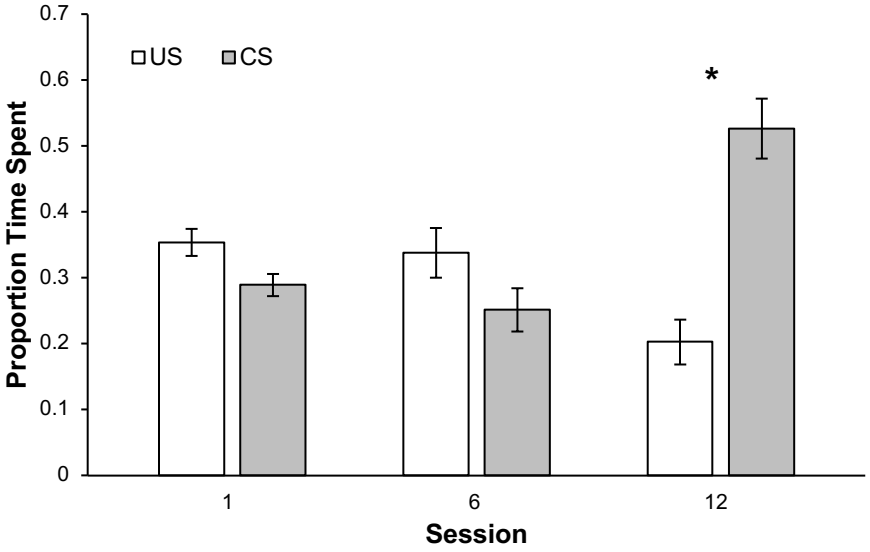


Figure 2. The development of individual differences in Pavlovian-conditioned approach following sexual conditioning in all subjects (N = 12). (A) Mean (± SEM) proportion of time spent in the US Zone (white bar) and CS Zone (grey bar) in the absence of the CS on sessions 1, 6 and 12, *p < 0.05. (B) Mean (± SEM) proportion of time spent near the US (white bar) and CS (grey bar) in the presence of the CS on sessions 1, 6, 12, **p < 0.001; *p < 0.01.

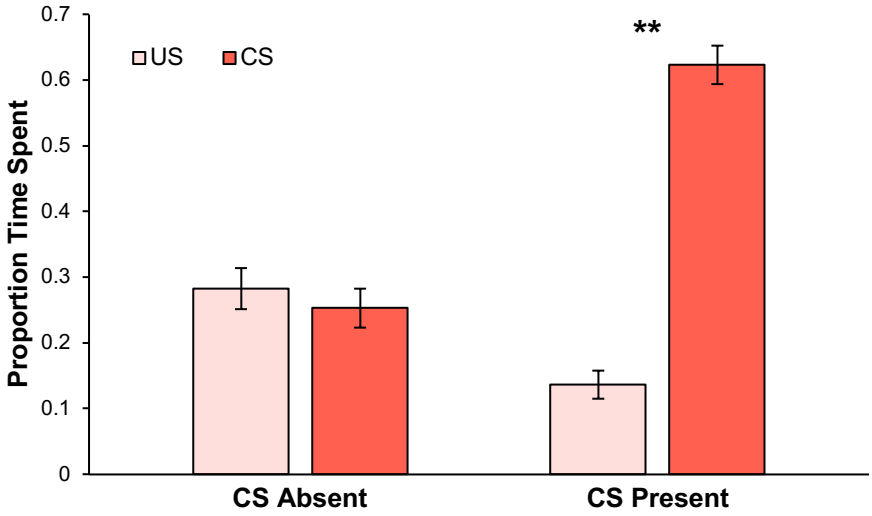
The classification of individual differences in Pavlovian-conditioned approach

Next, we focused on identifying whether individual rats displayed sign-, goal-tracking or intermediate behaviours based on the proportion of time spent near the US and CS (CS present). We first used a 15% CS-US difference score on session 12 as a criterion for preliminary phenotyping. Eight subjects met the 15% CS-US difference score criterion (i.e., time near CS > time near US) suggestive of cue-directed behaviour in our sample. No subjects satisfied the difference criterion score for goal-directed behaviour (i.e., time near US > time near CS), therefore these subjects were identified as intermediates (i.e., time near US ≈ time near CS; $n = 4$).

Animals that sign-track have the propensity to approach and engage with the CS (Flagel et al., 2009), therefore planned comparison *t*-tests were conducted on the mean proportion of time spent in the CS and US Zones in both the absence and presence of the CS during session 12 (Figure 3A). There was no statistically significant difference in the proportion of time spent in the US compared to CS Zone when the CS was absent, $t(7) = 0.533$, $p = 0.611$, and animals spent a significantly greater proportion of time near the CS when the CS was present, $t(7) = -10.207$, $p < 0.001$, $d = 3.61$. Subjects spent more time near the CS ($M = 0.624$, $SD = 0.083$) compared to the US ($M = 0.136$, $SD = 0.059$), which is consistent with the development of sign-tracking behaviour on session 12.

Animals that display intermediate behaviours display fluctuate between cue- and goal-directed behaviour (Flagel et al., 2009), therefore planned comparison *t*-tests were conducted on the mean proportion of time spent in the US and CS Zones in the absence and presence of the CS (Figure 3B). When the CS was absent, there was a statistically significant difference in the proportion of time spent in the US Zone compared to the CS Zone on session 12, $t(3) = 3.823$, $p = 0.032$, $d = 1.91$. The rats spent a significantly greater proportion of time in the US Zone ($M = 0.345$, $SD = 0.035$) compared to the CS Zone ($M = 0.160$, $SD = 0.089$). However, in the presence of the CS, a paired-samples *t*-test revealed no significant preference for either Zone, $t(3) = -1.015$, $p = 0.417$, suggesting a tendency to vacillate between the US and CS areas, which is indicative of intermediate behaviour.

A



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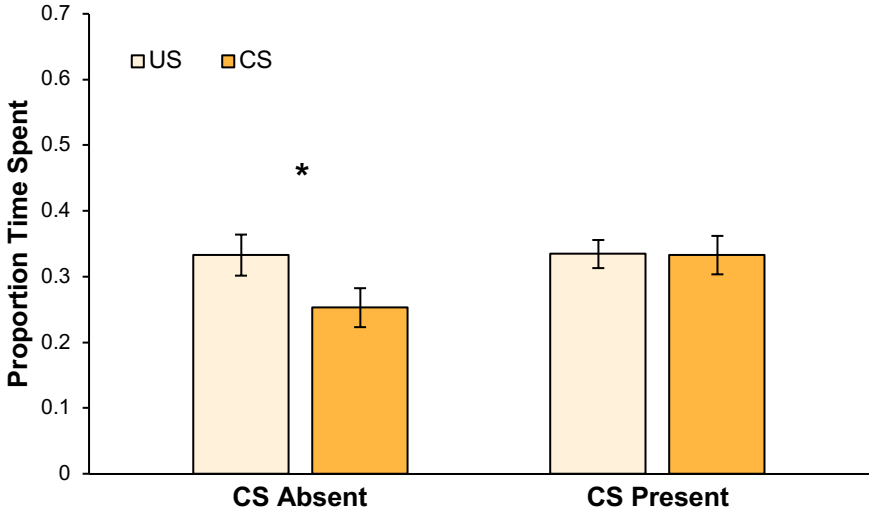


Figure 3. The development of sign-tracking and intermediate behaviour in response to a sexually-conditioned cue. (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence and presence of the CS on session 12 in animals identified as sign-trackers (n = 8). (B) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence and presence of the CS on session 12 in intermediate subjects (n = 4), **p < 0.001; *p < 0.05.

Experiment 2: The acquisition of Pavlovian-conditioned approach and the influence of CS-US spatial separation

The second experiment was conducted in order to determine whether the spatial location of the CS changes the development of each phenotype across Pavlovian-conditioning sessions. As in Experiment 1, preliminary phenotyping was identified based on the proportion of time spent near the US and CS (CS present) using a 15% CS-US difference score on session 13.

The classification of individual differences in Pavlovian-conditioned approach

Sign-tracking behaviour. A planned comparison, paired samples *t*-test revealed a statistical trend, t (2) = -3.755, p = 0.064 toward this subset of rats spending a greater proportion of time near the CS (M = 0.427, SD = 0.096) compared to the US (M = 0.177, SD = 0.025; n = 3). However, due to the non-significant statistical result, the behaviour of these subjects was not further analyzed.

Goal-tracking behaviour. Animals that goal-track have a propensity to spend more time near the goal area to await delivery of reward (Flagel et al., 2009), therefore planned comparison *t*-tests were conducted on the mean proportion of time spent in the US and CS Zones in the absence and presence of the CS (Figure 4A). A paired-samples *t*-test revealed goal-tracking in our sample (n = 6), as there was a statistically significant difference in the mean proportion of time spent in the US and CS Zone (CS absent) on session 13, t (5) = 4.611, p = 0.006, d = 1.88, with subjects spending significantly more time in the US Zone (M = 0.420, SD = 0.102) compared to the CS Zone (M = 0.160, SD = 0.047). There was also a statistically significant difference in the mean proportion of time spent near the CS and US on session 13, t (5) = 6.825, p < 0.001, d = 2.79, with rats spending a significantly greater proportion of time near the sliding door that provided access to the receptive female rat (M = 0.385, SD = 0.046) compared to the orange cone CS (M = 0.175, SD = 0.065).

Intermediate behaviour. A planned comparison, paired-samples *t*-test, t (4) = 2.121, p = 0.101 indicated intermediate behaviour in a subset of rats (n = 5), as subjects spent comparable proportions of time in the US Zone compared to the CS Zone in the absence of the CS on session 13 (Figure 4B). In the presence of the CS, subjects displayed a tendency to vacillate between the US and CS, t (4) = -0.571, p = 0.599.

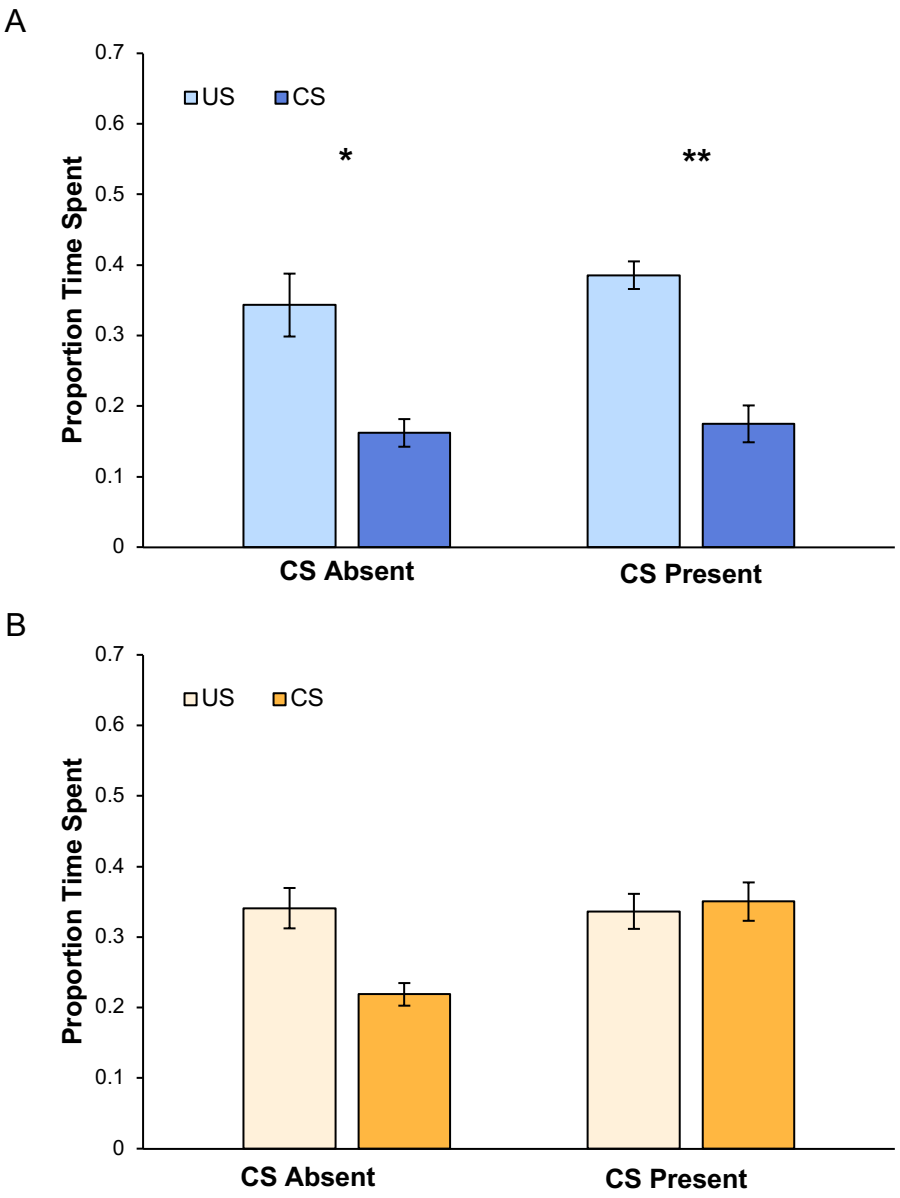


Figure 4. The development of goal-tracking and intermediate behaviour in response to a sexually-conditioned cue. (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence and presence of the CS on session 13 in animals identified as goal-trackers (n = 6), *p < 0.05. (B) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence and presence of the CS on session 13 in intermediates subjects (n = 5), **p < 0.001; *p < 0.01.

The influence of CS-US separation on the expression of Pavlovian-conditioned approach

The spatial location of the CS presentation does not affect goal-tracking behaviour. In order to examine whether the location of the CS relative to the US might affect goal-tracking behaviour, the mean proportion of time spent in the CS and US Zones (CS absent) was compared in rats exposed to the CS in the opposite- and same-side corners relative to the US across sessions 1, 7, and 13 (Figure 5A). A repeated-measures ANOVA revealed main effects of Session, F (2, 8) = 7.742, p = 0.013, η_p^2 = 0.31, and Zone, F (1, 4) = 31.425, p = 0.005, η_p^2 = 0.74, suggesting a statistically significant difference in the proportion of time spent in the US and CS Zones across sessions, and that subjects spent significantly more time in the US Zone (M = 0.379, SD = 0.025) compared to the CS Zone (M = 0.163, SD = 0.017) when the CS was absent. Furthermore, a statistically significant Session x Zone interaction, F (2, 8) = 4.671, p = 0.045, η_p^2 = 0.32, indicated that the proportion of time spent in the US and CS Zones increased and decreased respectively, as sessions progressed. The Session x Zone x CS Location interaction was close to significant, F (2, 8) = 4.440, p = 0.050, reflecting a statistical trend that the proportion of time spent in the US Zone across sessions increased when the CS was presented on the same-side versus the opposite-side relative to the US. However, the spatial location of the CS presentation did not appear to affect goal-tracking, as there was no significant main effect of CS Location, F (1, 4) = 2.467, p = 0.191. Non-significant Session x CS Location, F (2, 8) = 0.283, p = 0.761 and Zone x CS Location, F (1, 4) = 0.260, p = 0.637 interactions provide further evidence to suggest that the differences in proportions of time spent in the US and CS Zone were not affected by the location of the CS presentation.

Next, we compared the mean proportion of time spent near the US and CS (CS present) when the CS appeared in the opposite- and same-side corners relative to the US across sessions 1, 7 and 13 (Figure 5B). A repeated-measures ANOVA revealed main effects of Session, F (2, 8) = 5.554, p = 0.031, η_p^2 = 0.38, and Zone, F (1, 4) = 16.471, p = 0.015, η_p^2 = 0.39, indicating a statistically significant difference in the proportion of time spent near the US and CS across sessions, where subjects spent significantly more time near the US (M = 0.313, SD = 0.014) as compared to the CS (M = 0.175, SD = 0.029). The spatial location of the CS presentation did not appear to affect goal-tracking, as there was no significant main effect of CS Location, F (1, 4) = 0.585, p = 0.487, revealing that subjects responded equally to the CS presentation if it occurred on the opposite- or same-side as the US. Non-significant Session x CS Location, F (2, 8) = 0.116, p = 0.892, Zone x CS Location, F (1, 4) = 0.986, p = 0.377, Session x Zone, F (2, 8) = 1.306, p = 0.323, and Session x Zone x CS Location, F (2, 8) = 1.172, p = 0.358 interactions provide additional evidence that proportion of time spent in the US and CS Zones were not influenced by

the spatial location of the CS presentation. In accordance with the above findings, these data further validate the development of a goal-tracking phenotype, as subjects spent greater proportions of time near the US compared to the CS across sessions. Here, the CS presentation served as a predictor for the opportunity to copulate with a sexually-receptive female, and the spatial location of the CS did not affect their preference for the US Zone.

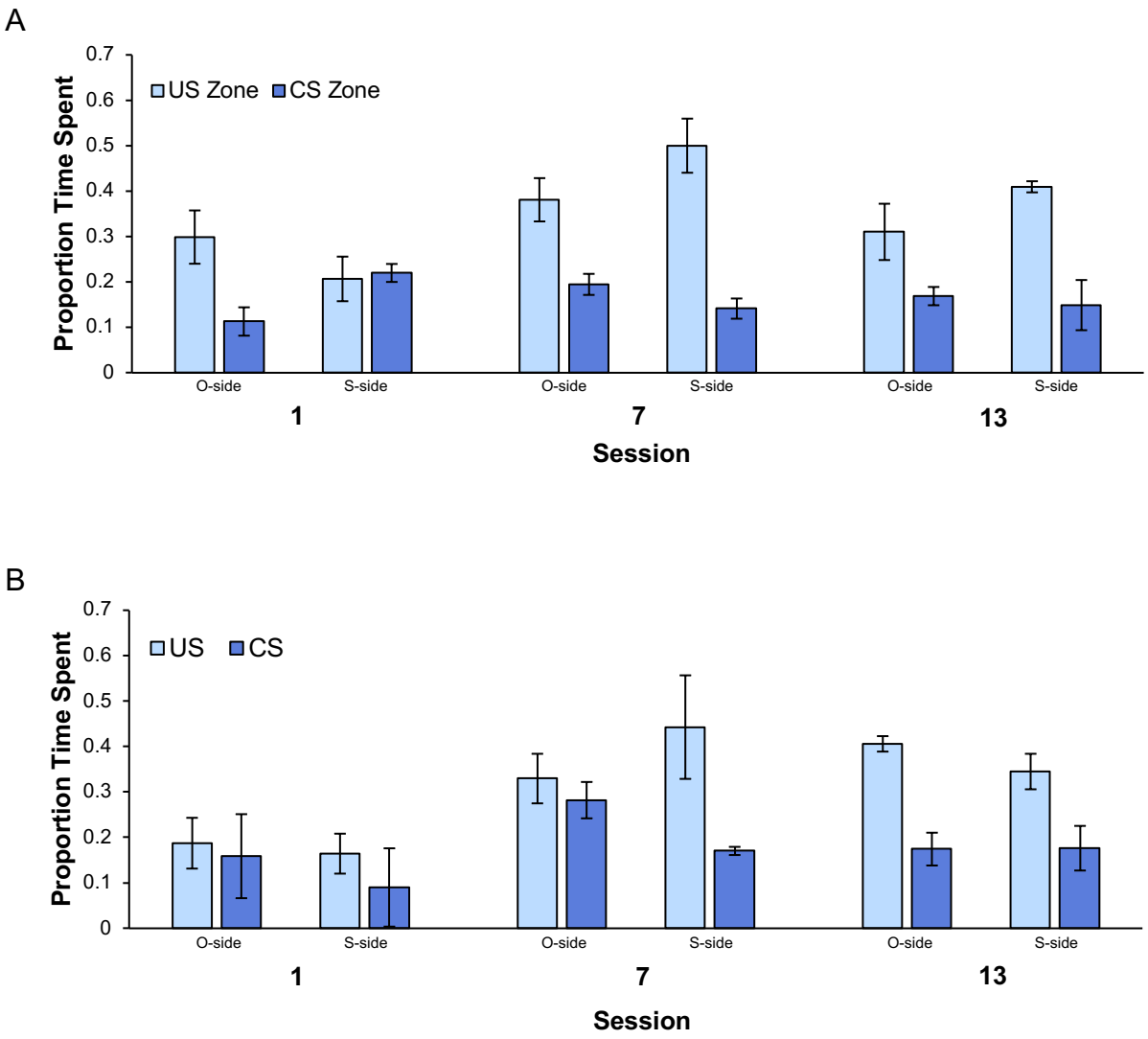


Figure 5. Goal-tracking behaviour is not affected by spatial separation between the sexually-conditioned cue and US. (A) Mean (± SEM) proportion of time spent in the US Zone (light bar) and CS Zone (dark bar) in the absence of the CS when it was presented on opposite- (O-side) versus same-side (S-side) as US on sessions 1, 7 and 13 in goal-trackers. (B) Mean (± SEM) proportion of time spent near the US (light bar) and CS (dark bar) in the presence of the CS when it was located on opposite- (O-side) versus same-side (S-side) as US on sessions 1, 7 and 13 in goal-trackers (n = 6).

The spatial location of the CS presentation does not affect intermediate behaviour. In order to examine whether the location of the CS relative to the US might affect intermediate behaviour, the mean proportion of time spent in the US and CS Zones (CS absent) was compared in rats exposed to the CS in the opposite- and same-side corners relative to the US across sessions 1, 7 and 13 (Figure 6A). A repeated-measures ANOVA revealed no significant main effects of Session, F (2, 6) = 0.581, p = 0.588 or Zone, F (1, 3) = 6.617, p = 0.082, indicating that subjects spent equal proportions of time in each Zone (US Zone: M = 0.292, SD = 0.042; CS Zone: M = 0.207, SD = 0.009) when the CS was absent, a finding that did not change across sessions. The spatial location of the CS presentation did not appear to influence intermediate behaviour, as there was no significant main effect of CS Location, F (1, 4) = 2.467, p = 0.191, nor were there any statistically significant interactions, Session x CS Location interaction F(2, 6) = 0.723, p = 0.523, Zone x CS Location interaction, F(1, 3) = 2.424, p = 0.217, Session x Zone, F(2, 6) = 0.123, p = 0.886, and Session x Zone x CS Location, F(2, 6) = 1.131, p = 0.383. These findings further support the propensity for intermediate subjects to vacillate between the US and CS Zones when the CS was absent, which was not affected by the location of the CS presentation.

In order to examine whether the location of the CS relative to the US might affect intermediate behaviour, the mean proportion of time spent near the US and CS (CS present) was compared in rats exposed to the CS in the opposite- and same-side corners relative to the US across sessions 1, 7 and 13 (Figure 6B). A repeated-measures ANOVA revealed no significant main effects of Session, F (2, 6) = 3.687, p = 0.090 or Zone, F (1, 3) = 0.377, p = 0.583, indicating that subjects spent equal proportions of time near the US (M = 0.297, SD = 0.019) and CS (M = 0.278, SD = 0.031), a pattern that did not change across sessions. The spatial location of the CS presentation did not appear to influence intermediate behaviour, as there was no significant main effect of CS Location, F(1, 3) = 1.427, p = 0.318, nor were there any statistically significant interactions, Session x CS Location, F(2, 6) = 0.135, p = 0.876, Zone x CS Location, F(1, 3) = 6.289, p = 0.087, Session x Zone, F(2, 6) = 0.057, p = 0.945, and Session x Zone x CS Location, F(2, 6) = 2.487, p = 0.163. Therefore, subjects spent equal proportions of time near the US and CS across sessions, regardless of whether the CS presentation occurred on the opposite- or same-side as the US. These data further validate an intermediate phenotype, as rats continued to oscillate near the US and CS when the CS was present. Moreover, whether the CS presentation occurred on the opposite- or same-side as the US had no impact on their behaviour.

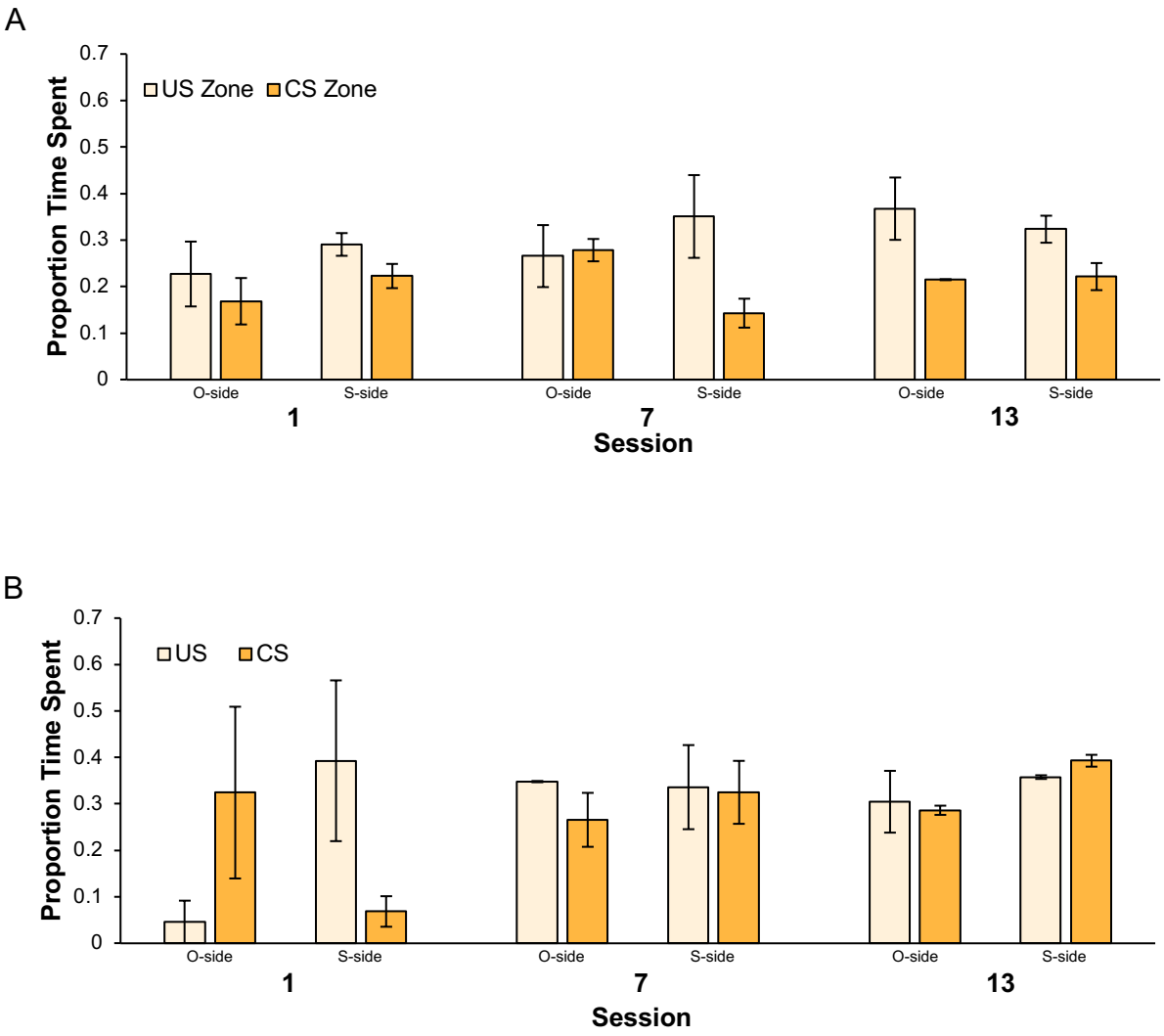


Figure 6. Intermediate behaviour is not affected by spatial separation between the sexually-conditioned cue and US. (A) Mean (± SEM) proportion of time spent in the US Zone (light bar) and CS Zone (dark bar) in the absence of the CS when it was presented on opposite- (O-side) versus same-side (S-side) as US on sessions 1, 7 and 13 in intermediate subjects. (B) Mean (± SEM) proportion of time spent near the US (light bar) and CS (dark bar) in the presence of the CS when it was located on opposite- (O-side) versus same-side (S-side) as US on sessions 1, 7 and 13 in intermediate subjects (n = 5).

Discussion

Individual differences in PCA responses displayed as sign- and goal-tracking have been well-established using food and drug reward, while such differences using sexual reward have only been studied in avian and aquatic models (Burns & Domjan, 1996; Hollis et al., 1989). Both phenotypes are learned responses that develop over repeated presentations of the CS and US, which then result in either sign- or goal-tracking behaviour, or an oscillation between the two. While increasing the spatial separation between a CS and food US can increase goal-tracking and decrease a sign-tracking response in rats using food reward (Holland, 1980; Silva et al., 1992), goal-tracking is not facilitated by increasing the spatial distance between a CS and US using a sexual conditioning paradigm in male Japanese quail, where sign-tracking emerges as the predominant response (Burns & Domjan, 1996). Whether sign- and goal-tracking for sexual reward can develop in a rodent model, and how US-CS spatial separation may influence the development of such phenotypes had been previously unclear. The identification of whether PCA responses occur in male rats following sexual reward provides the opportunity to elucidate these previously documented features in other species. Likewise, the influence of the US-CS spatial relationship on the development of sign- and goal-tracking in male rats is valuable for methodological purposes and future studies. As such, we investigated whether sign-, goal-tracking and intermediate behaviour can develop following sexual conditioning in male rats and tested whether the spatial separation between the CS and US might affect the development of these phenotypes.

The expression of sign-, goal-tracking and intermediate behaviours in response to a sexually-conditioned cue

The results obtained in both experiments suggest that individual differences in PCA responses exist in male rats exposed to sexual conditioning, as rats learned that the CS predicted the opportunity to copulate with a sexually-receptive female. Importantly, these data demonstrate the powerful role of the ejaculatory reward state in associative learning and are consistent with previous reports using conditioned partner and place preference paradigms (Kippin et al., 1998; Quintana et al., 2019; Ågmo & Berenfeld, 1990). For example, a neutral odour cue (e.g., lemon, almond) paired with the ejaculatory reward state can induce a conditioned ejaculatory preference toward a scented, sexually-receptive female compared to an unscented female (Kippin et al., 1998). Similarly, repeated pairings of a neutral somatosensory cue (i.e., rodent jacket) and the ejaculatory reward state results in a conditioned ejaculatory preference for a jacketed female compared to a non-jacketed female, reflected by greater ejaculation frequencies and choice of partner for first ejaculation (Quintana et al., 2019). The ejaculatory reward state can also be

conditioned to contextual stimuli; when male rats copulate to ejaculation in a separate mating cage and are then transferred to the non-preferred compartment of a conditioning cage, they spend more time in the initially non-preferred compartment indicating a conditioned place preference (Ågmo & Berenfeld, 1990). Collectively, the aforementioned studies provide irrefutable evidence that ejaculation generates an effective reward state to induce conditioning. Based on our findings, it appears that the CS may have a unique functional role in this relationship depending on the subject. In one experiment, a subset of animals was identified as displaying sign-tracking behaviour, as they spent a greater proportion of time in the CS area relative to the US area, both in the absence and presence of the sexually-conditioned cue. In a separate experiment, we identified goal-trackers, as animals spent greater proportions of time near the US compared to the CS under the same conditions. Alternatively, a number of animals displayed neither clear cue- nor goal-directed behaviour, and instead vacillated between both CS and US areas and were identified as intermediates. Lastly, we found that the spatial location of the CS relative to the US (opposite- or same-side) did not affect the expression of goal-tracking or intermediate behaviour.

The variability in the number of sign-, goal-trackers and intermediates across both experiments indicate that overall distributions can fluctuate from one study to another. Specifically, we report that there were 8 sign-trackers and 4 intermediate rats in Experiment 1, and 6 goal-trackers and 5 intermediate rats in Experiment 2. As mentioned previously, studies report that samples of Sprague-Dawley rats are composed of approximately one-third of each phenotype when using food reward (Flagel et al., 2009; Saunders & Robinson, 2010). In Sprague-Dawley rats, it has been suggested that differences in the ratio of each phenotype are observed in outbred rats acquired from different vendors and various colonies provided by the same vendor (Harlan Laboratories, Charles River Laboratories; Fitzpatrick et al., 2013). Specifically, Sprague-Dawley rats obtained from Harlan Laboratories exhibit more sign-tracking behaviour for food, whereas those acquired from Charles River Laboratories display more goal-tracking behaviour. In another study, Kearns et al. (2006) investigated inbred strain differences in sign-tracking behaviour. Using Lewis and Fischer rat strains, they discovered that Lewis rats acquired an autoshaping response (i.e., sign-tracking) more rapidly, and made significantly more lever contacts than Fischer rats, when the response-independent lever predicted the availability of food. Our data reflect that the number of sign-, goal-trackers and intermediate rats generally varied between experiments, and also in comparison to the aforementioned studies, perhaps due to a combination of differences in vendor, strain, and reward type.

The expression of sign- and goal-tracking may also depend on the type of natural reward and Pavlovian conditioning training methodology. In their study, Burns and Domjan (1996) found higher incidences of sign-tracking as compared to goal-tracking in male Japanese quail using sexual conditioning, while proportions of PCA for food US appear to be more balanced. The authors note that the 'goal' area is made less distinctive by a non-stationary US (i.e., sexually-receptive female) compared to a stationary food cup, which may contribute to the weaker formation of goal-tracking observed with sexual conditioning. Alternatively, sign-tracking remains unaffected by a non-stationary US, which is likely due to the behavioural characteristics of this phenotype. For example, sign-tracking has been characterized as a poorly controlled, robust, durable, and well-retained conditioned response in studies using retention intervals (Tomie et al., 2004), spontaneous recovery (Robbins, 1990) and rapid reacquisition (Tomie et al., 1980), and delay discounting procedures (Tomie et al., 1998), which confirms its rigidity and inflexibility as a behavioural outcome. It has also been suggested that variations in Pavlovian conditioning training may account for these differences. In magazine training, the food US is repeatedly presented independently in order to habituate subjects to the food delivery mechanism prior to conditioning, and as a result, rats become familiarized with the food source area (i.e., goal area). Though subjects are habituated to the apparatus in a sexual conditioning paradigm, the door to the female compartment remains closed and subjects are not exposed to the US prior to conditioning, which may weaken the development of a goal-tracking phenotype in some subjects, such as intermediates. Our findings are based on results from Long-Evans rats, and we report the variable incidence of both sign- and goal-trackers within our samples. In addition, the aforementioned studies highlight the factors that can contribute to variability in the distribution of phenotype across experiments.

The CS-US spatial separation does not affect the expression of goal- and intermediate responding in sexual conditioning

As evidenced by studies using food reward, the expression of sign- and goal-tracking responses are increased and decreased respectively by increasing the spatial separation between the US and CS. In our study, we were unable to determine the effect of CS location on sign-trackers due to their lack of representation. However, CS location did not appear to affect the development of goal-tracking or intermediate behaviour. In either phenotype, the proportion of time spent in the US and CS Zones (CS absent) or near the US and CS (CS present) was unaffected by the spatial location of the CS presentation (i.e., opposite- or same-side). In an early study using food reward, the development of sign-tracking appeared to be highly sensitive to the spatial location of the CS relative to the US. Specifically, Holland (1980) reported that sign-

tracking PCA responses could be inhibited by simply increasing the spatial distance between the CS-US by 30 centimeters in male rats. Alternatively, Burns and Domjan (1996) found no evidence of disrupted sign-tracking following sexual conditioning when the CS presentation was moved from 25 to 91 centimeters relative to the US in male Japanese quail. Therefore, CS spatial location does not appear to affect sign-tracking in an avian model, nor goal-tracking in a rodent model of sexual conditioning. Regrettably, we cannot compare our findings with those of Burns and Domjan (1996) because they could not confirm goal-tracking behaviour in their study, and we did not find evidence of sign-tracking in our sample.

In comparison to Experiment 1, the results obtained in Experiment 2 revealed only a statistical trend for sign-tracking behaviour. These data contradict previous reports from Burns and Domjan (1996) suggesting that sign-tracking is the predominant PCA response following sexual conditioning in male Japanese quail. A potential explanation for this discrepancy may be that subjects required additional sessions of sexual conditioning in our paradigm in order to develop sign-tracking. Specifically, the sexually-naïve rats used in Experiment 2 required extra sessions in order to learn how to copulate with a sexually-receptive female. Therefore, it is likely that a subset of animals tested received fewer sessions involving an ejaculation which serves as the reward state for the purpose of Pavlovian conditioning. This may have inadvertently influenced the development of a sign-tracking phenotype, and perhaps a greater number of sexual conditioning sessions would have strengthened the CS-US association in these animals, allowing sign-tracking to develop more robustly. This argument could also be applied to subjects identified as intermediates in either experiment, given reports that intermediate rats tend to display sign-tracking in response to a food-paired cue with extended training (Flagel et al., 2009).

An important limitation of these studies was the inability to reliably identify equal subsets of animals that represented sign-, goal-tracking and intermediate behaviour. This restricts the ways in which the results can be interpreted and makes it challenging to comprehensively study and compare the varying elements of these phenotypes in subsequent studies. However, an important implication of these studies is the empirical finding that individual differences in PCA exist following sexual conditioning in male rats. As mentioned previously, the existing literature was limited to avian and aquatic models (Domjan et al., 1986; Burns & Domjan, 1996; Hollis et al., 1989). To our knowledge, we are the first laboratory to investigate these behavioural phenotypes in a rodent model of sexual conditioning. From a methodological perspective, a secondary implication of this work includes the finding that the spatial separation between the CS and US does not affect the development of goal-tracking or intermediate behaviour. Lastly, our data adds to the literature by questioning contrasting reports of predominant phenotypes in sexual

conditioning, suggesting that neither sign- nor goal-tracking are more prevalent than the other in rats. For example, Burns and Domjan (1996) proposed that increasing and decreasing the spatial location of the CS presentation may have distracted goal-trackers from solidifying their PCA response, therefore resulting in sign-tracking as the predominant phenotype. However, our data revealed only goal-tracking and intermediate behaviour with no effect of CS spatial location. The nature of the CS used in the present studies may have affected the extent to which sign-tracking was observed. Though the data showed that it was effective in sexual conditioning and resulted in the expression of sign-tracking in one experiment, these findings were not replicated in a separate study. The CS, represented by a large, bright orange cone, was chosen based on a few criteria. First, Burns and Domjan (1996) reported the expression of sign-tracking in response to a visuo-tactile CS (i.e., wooden block) when using a sexual conditioning paradigm in male Japanese quail. Second, Holland (1980) demonstrated that a localized, visual CS positioned near the food US delivery area elicited greater sign- but less goal-tracking responses compared to a CS located farther from the food US delivery area. Lastly, a manipulable CS (e.g., lever-CS) paired with food US serves as a more effective conditioned reinforcer compared to an auditory CS (e.g., tone-CS), which exclusively elicits a goal-tracking phenotype (Meyer et al., 2014). Collectively, these findings validated the inclusion of visual and tactile properties, and the exclusion of auditory properties in selecting a neutral stimulus, which therefore should have served as an effective stimulus to elicit sign-tracking in Experiment 2.

Conclusions

The goal of the current studies was to investigate whether individual differences in PCA responses develop using a rodent model of sexual conditioning, and whether the spatial separation between the CS and US might affect sign-, goal-tracking and intermediate expressions of behaviour. We found that animals learned that a sexually-conditioned cue predicted the opportunity to copulate with a sexually-receptive female, further demonstrating the ejaculatory state serves as an effective sexual reward. We also found individual differences in PCA responses, showing that sign-, goal-tracking and intermediate behaviours develop in different subsets of male rats. Lastly, the spatial location of a CS presentation relative to the US does not appear to impact development of these phenotypes. Suggestions for future studies include assessing whether the variation in conditioned responses is influenced by the properties of the CS. For example, olfactory stimuli paired with copulation to ejaculation play an important role in the manifestation and modulation of male sexual behaviour and conditioned ejaculatory preference (Kippin et al., 2001). Furthermore, the use of alternative paradigms (e.g., food, sucrose conditioning) can be used to determine clearer subsets of goal-, sign-tracking and intermediate

rats prior to sexual conditioning. This would also allow for a unique opportunity to draw comparisons between the development of PCA phenotype as a function of natural reward type.

Chapter 3: Sign- and goal-tracking in response to a sucrose-paired cue is not predictive of Pavlovian-conditioned approach for a sex-paired cue in male rats

Abstract

Previous studies have indicated that environmental cues that have been associated with sexual reward can elicit Pavlovian-conditioned approach behaviours that can be classified as sign-tracking (i.e., cue-directed), goal-tracking (i.e., goal-directed), or intermediate (i.e., both cue- and goal-directed). Individual differences in PCA responses can be consistent across different types of reward (e.g., food and drug reward), but whether sign-, goal-tracking and intermediate phenotypes are stable across different types of natural reward remains unclear. Therefore, the purpose of this study was to compare the behavioural and phenotypic differences observed following sucrose conditioning to those that develop following sexual conditioning. In the first phase, male Long-Evans rats received 16 sucrose conditioning sessions, and were identified as displaying either sign-, goal-tracking, or intermediate behaviour, based on a response bias score and their tendency to approach and engage with a lever-CS (i.e., sign-tracking) or fluid port (i.e., goal-tracking). In accordance with their response bias scores, animals identified as sign-trackers (n = 7) made a significantly greater number of lever-CS contacts and displayed shorter latencies to make a lever-CS contact compared to goal-trackers (n = 13). Subjects classified as goal-trackers made a significantly greater number of port entries and displayed shorter latencies to make a port entry compared to sign-trackers. Only a minority of animals displayed intermediate responses (n = 3), fluctuating between cue- and goal-directed behaviours. In the second phase, sexually-naïve rats underwent 13 sessions of Pavlovian sexual conditioning in an open field, where an orange cone CS (2-minute/presentation) predicted the opportunity to copulate to ejaculation (US) in a separate compartment with a sexually-receptive female. Here, behaviours directed toward the CS (i.e., sign-tracking) and US (i.e., goal-tracking) were measured by the proportions of time spent in a pre-determined area centered around the CS, or the sliding door leading to the female's compartment, respectively, in the absence and presence of the sexually-conditioned CS. A statistical trend revealed that sucrose goal-trackers displayed a tendency to spend a greater proportion of time near the CS, however this did not reach statistical significance; therefore, they spent comparable proportions of time near the CS and US reflecting intermediate-like behaviour. Sucrose sign-trackers spent a significantly greater proportion of time in the US area compared to the CS area in both the absence and presence of the cue, indicating that the sign-tracking phenotype was not displayed following sexual conditioning; rather, subjects appeared to 'shift' toward a goal-tracking phenotype. Sucrose intermediate subjects continued to

display intermediate responses, vacillating between the US and CS areas in both the absence and presence of the sexually-conditioned cue. Overall, these findings suggest intra-individual differences in the basic mechanisms that mediate the development of goal- and sign-tracking behaviours across different types of natural reward.

Introduction

It has long been established that environmental stimuli paired with drug or natural rewards (e.g., food, sex, water) can both motivate and initiate behaviour. For example, conditioned stimuli (CS; drug-related cue) paired with cocaine (unconditioned stimulus; US) can trigger drug-craving in addicts, produce conditioned physiological responses, promote drug-taking, and increase relapse after a period of abstinence (Cascella et al., 1989). Similarly, in overweight individuals, food-related cues can elicit a heightened conditioned reactivity such as food-craving, thereby increasing the probability of food consumption and additional weight gain (Meyer et al., 2015). Lastly, sex-related cues have been shown to induce erections in response to unusual, non-sexual stimuli, and can become the focus of fetishistic behaviours (Rachman & Hodgson, 1968).

In animal models of drug- and food-seeking, conditioned stimuli have been theorized to become 'attractive', 'wanted', and act as 'motivational magnets' (Berridge, 2001). For instance, animals will 1) approach or consume the CS (Uslaner et al., 2006), 2) work to gain access to the CS, even in the absence of reward (Di Ciano & Everitt, 2004), and 3) increase and maintain drug/food-seeking and drug/food-taking behaviour in the presence of the CS (Schenk & Partridge, 2001; Marshall et al., 2018). Collectively, these behaviours and conditioned responses provide evidence that conditioned stimuli acquire incentive motivational properties due to their relationship with the US.

The conditioned response that results from CS-US pairing can provide insight into whether a CS has acquired incentive motivational value (Flagel et al., 2009) using a measure termed Pavlovian-conditioned approach (PCA; Fitzpatrick & Morrow, 2016). For example, if a response-independent lever (CS) is paired with reward (e.g., food pellet, US), a subset of animals will find it 'attractive' in that they will approach and engage with it, and work to gain access to it. In this case, the conditioned response is directed toward the cue or 'sign' of impending reward, which reflects the motivational significance of the cue; this behaviour is appropriately labelled as a sign-tracking response. However, under the same conditions, other animals may rarely approach or engage with the CS, although they do display a robust conditioned response directed toward the goal area where the reward will be delivered. Consequently, this type of behaviour has been labeled goal-tracking. Lastly, a proportion of animals can show fluctuations between both cue- and goal-directed behaviours and are classified as intermediates. Importantly, though all subsets of animals learn the predictive CS-US relationship, the CS acquires a reliable, incentive salience exclusively in sign-trackers (Robinson & Flagel, 2009).

Such individual differences in PCA responses have been shown to extend across different types of reward in several studies. For example, an animal that displays sign-tracking in response

to food reward is also more likely to sign-track for drug reward, and this could be an important factor in predicting vulnerability to drug addiction prior to experience with the drug. Saunders and Robinson (2010) demonstrated how sign- and goal-tracking can extend across different types of reward in a 2-phase study. First, a PCA procedure was used to identify sign-tracking and goal-tracking for the attribution of incentive salience to a response-independent lever-CS paired with food US. Following the identification of each behavioural phenotype for food reward, a drug self-administration paradigm was used featuring pairings of a light stimulus (CS) with the delivery of a drug reward (i.e., cocaine). Here, a nose-poke response in the active port resulted in an intravenous infusion of cocaine on a fixed-ratio schedule. Following each cocaine infusion, there was a 20-second timeout interval during which a light-CS was illuminated in the active nose-poke port. Therefore, the illumination of the light served as a predictive cue for cocaine delivery. After 14 sessions, the light-CS was removed for two subsequent sessions during which a nose-poke resulted in an infusion of cocaine without the illumination of the light. Next, the light-CS was reintroduced for an additional four sessions. Interestingly, rats identified as sign-trackers for food reward displayed a near 50% reduction in the number of self-administered infusions of cocaine per minute when the light was removed, as compared to rats that were identified as goal-trackers for a food US. Furthermore, reintroducing the light-CS resulted in a reduction of differences in the number of self-administered cocaine infusions between sign- and goal-trackers, as sign-trackers reinstated to baseline levels of self-administration within a few days. These results suggest that the subset of rats identified as sign-trackers for a food US associated the salient properties of cocaine to the light-CS, as the rate of cocaine self-administration depended on the presence of the light-CS.

Similar findings have also been reported using a classically-conditioned cue for cocaine and have shown consistency in sign- and goal-tracking for food and drug reward. Here, Yager and Robinson (2013) first used a PCA procedure to identify rats prone to attribute incentive salience to a lever-CS paired with food. Next, subjects underwent Pavlovian-conditioning training during which a light-CS predicted the delivery of a cocaine infusion. The extent to which the light-CS acquired incentive properties was measured using two separate features of the conditioned response: conditioned orientation and conditioned approach. A subject displayed conditioned orientation if he moved his head or body in the direction of the light-CS upon CS presentation, while conditioned approach was defined as movement toward the light-CS within a distance of 1 cm. These behaviours were analyzed separately to allow for the comparison of differences in associative learning and the attribution of incentive salience between sign-trackers (who orient and approach the light-CS) and goal-trackers (who orient toward the light-CS), and to address

the limitation of using an intravenous drug as the US in which there is no clear goal area. Though both sign- and goal-trackers learned a conditioned orientation response for a cocaine-paired cue, only sign-trackers approached and engaged vigorously with the light-CS. In sign-trackers, these behaviours were displayed in response to the light-CS, which demonstrates that the cocaine-paired cue became more 'attractive' and desired in subjects prone to attribute incentive salience to a food-paired cue. Importantly, these data provide evidence that individual differences in PCA responses can extend across different types of reward, in that sign-trackers for food US also display sign-tracking for drug US due to their intrinsic tendency to attribute incentive properties to reward cues. Furthermore, the reliability in phenotypic expression suggests it may be possible to predict subjects' susceptibility to drug-related cues based on their responses to food-related cues.

The stability of sign- and goal-tracking behaviours across different reward types reveals important and consistent individual differences in the manner in which CS-US relationships are learned, and how these associations guide behaviour. Furthermore, the persistence of sign- and goal-tracking in response to food- and drug-paired cues also suggests that individual variations in PCA are determined by common neural reward circuits (Berridge & Kringelbach, 2015; Georgiadis et al., 2012). Although animals can display sign- and goal-tracking for sexual reward, the extent to which they show clear individual consistencies in PCA responses is less marked than other reward types. For example, studies using a food US or drug US consistently report approximately one-third of animals display each of sign-tracking, goal-tracking and intermediate behaviours (Flagel et al., 2008; Flagel et al., 2009), whereas Burns and Domjan (1996) found high incidences of sign-tracking and no goal-tracking in response to a sexually-conditioned cue in male Japanese quail. Similarly, previous studies from our laboratory have demonstrated that individual differences in PCA using sexual reward in male rats may not be as reliable as those described using a food or drug US. In Chapter 2 of this thesis, we observed inconsistent incidences of each phenotype in separate experiments. Results showed that rats could learn that a CS predicted the opportunity to copulate to ejaculation with a sexually-receptive female, but sign-trackers (n = 8) and intermediates (n = 4) were observed in one experiment, while goal-trackers (n = 6) and intermediates (n = 5) were observed in another experiment.

In the present study, we first used sucrose conditioning to provide a clearer determination of subsets of animals displaying sign- and goal-tracking, and then evaluated whether the phenotypes expressed in response to sucrose reward would extend to sexual reward. The previous studies reviewed here (Saunders & Robinson, 2010; Yager & Robinson, 2013) have indicated that responsiveness to food-related cues can predict susceptibility to drug-related cues as both tend to acquire incentive salience in some individuals. We therefore examined whether

variation in the attribution of incentive salience to a sucrose-paired CS might be predictive of individual differences in the ability of a sexually-conditioned cue to motivate and guide behaviour.

Method

Subjects

Sexually-naïve male (175-200 g) and female (200-225 g) Long-Evans rats were obtained from Charles River Canada, Inc. (St-Constant, QC, Canada). Males were housed in polycarbonate (Plexiglas) gang-cages containing beta-chip bedding. Female rats were pair-housed in polycarbonate (Plexiglas) shoebox cages containing a mixture of beta-chip and corncob bedding and served as stimulus animals with which males could copulate. All rats were kept in an animal colony room which was maintained at a constant temperature of 21 ºC, on a reversed 12-hour light/dark cycle (lights OFF at 0800; all procedures conducted during the dark phase). Access to standard rat chow (Charles River Rodent Animal Diet, St-Hubert, QC, Canada) and water were available *ad libitum* for the duration of the experiment.

Females were ovariectomized bilaterally via lumbar incisions under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg), injected intraperitoneally at a volume of 1 ml/kg of body weight. Following a 1-week post-surgery recovery period, rats were maintained on hormone replacement with subcutaneous injections of estradiol benzoate (10 µg dissolved in 0.1 ml of sesame oil) 48-hours before each conditioning session, and progesterone (500 µg dissolved in 0.1 ml of sesame oil) 3 to 4 hours before each conditioning session to ensure they sexual receptivity. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Apparatus

Sucrose conditioning. Behavioural training was conducted using 12 operant conditioning chambers (32.8 L x 32.8 W x 32.8 D cm; Med Associates Inc., St-Albans, VT), each contained within a ventilated, sound-attenuating melamine cubicle (62.8 L x 68.2 W x 53.6 D cm). Each chamber was composed of a stainless-steel bar floor, paneled aluminum side-walls, and a clear, polycarbonate rear wall, ceiling, and front door. The right wall of each chamber featured a central fluid port (Med Associates Inc., ENV-200R3AM), which contained a circular fluid receptacle into which sucrose was delivered. The fluid port was connected to a polyethylene tube attached to a 20 ml syringe, which was attached to a syringe pump (Med Associates Inc., PMH-100, 3.33 rpm) located outside of the sound-attenuating cubicle. The left wall featured a white house-light (75 watts, 100 mA) to provide sufficient lighting during conditioning sessions. Port entries were measured by interruptions of an infrared beam across the entrance of the fluid port. Two stainless steel retractable levers (4.8 cm x 1.9 cm; ENV-112M) were situated 6.9 cm above

the grid floor, one on each side of the fluid port. A weight of 25 g applied to the lever was required to produce a recordable lever activation. The use of these two levers was counterbalanced; half of rats were exposed to the left lever; others were exposed to the right lever. Analyses revealed no statistically significant difference in left versus right lever-CS contacts across conditioning sessions, and subjects responded equally to the lever-CS regardless of whether the presentation occurred on the left or right side of the operant chamber (Session x Lever-CS interaction, F (15, 315) = 0.302, p = 0.730; Lever, F (1, 21) = 0.219, p = 0.645). The timing of experimental events and the recording of behavioural measures was controlled by a computer and Med PC-IV software (Med Associates, Inc.).

Sexual conditioning. Two open field chambers (120 L x 120 W x 60 D cm) were used for behavioural conditioning sessions. Each featured a large compartment (120 L x 80 W x 60 D cm) where the CS was presented, and a small compartment (120 L x 41 W x 60 D cm) where the subject could copulate with a sexually-receptive female. An opaque polycarbonate divider separated the compartments, which included a door (15 cm) that was slid vertically via a nylon cord (4.5 m) allowing the male to access the smaller compartment containing the female. Within the larger compartment, two pre-determined zones were used to measure sign- and goal-tracking behaviour. The CS Zone (38 W x 38 L cm) evaluated sign-tracking behaviour, and was located in the opposite, diagonal corner relative to the US Zone. The US Zone (38 W x 38 L cm) assessed goal-tracking behaviour and was located in front of the sliding door that provided access to the female. The locations of CS and US Zones was alternated across sessions in order to control for place preferences. The CS was an orange cone (23 H x 14 W x 14 L cm), which was placed in the CS Zone during a 2-minute CS presentation period after which the sliding door was opened to provide access to the sexually-receptive female. The US was defined as the male rat's ejaculation and subsequent post-ejaculatory period (2-minute duration). A video camera (Sony Handy Cam, model DCR-SR68) was used to record each session, which was then scored by trained experimenters. Both the CS and US Zones were marked on an acetate sheet secured to a computer screen during scoring. This ensured that time spent in the US and CS Zones were measured consistently across subjects during each conditioning session.

Procedure

Sucrose conditioning. Rats were subjected to two 24-hour sessions of sucrose exposure separated by 24-hours with no sucrose available. Here, they were presented with the sucrose solution and water bottle in their home-cage in order to acclimate to the taste of sucrose. The sucrose solution (10% *w/v*) was prepared by diluting sucrose in tap water. Following sucrose exposure, rats were habituated to the operant chambers for 20 minutes in order to diminish the

effect of a novel environment on behaviour. Sucrose conditioning sessions were conducted for 16 consecutive daily sessions.

Subjects were weighed prior to the start of each sucrose conditioning session. Sessions were 61.2-minutes in duration and began with the illumination of the house-light after a 120-second delay, which signaled the start of the session. Each trial consisted of a 10-second pre-CS interval, a 10-second CS presentation (during which the lever was extended and retracted), followed by the delivery of 0.2 ml of sucrose solution over a 6-second period (total = 2.4 ml per session), ending with a 10-second post-US interval (total duration, 36 seconds). Each session included 12 trials on a 240-second variable time schedule during which trials would begin randomly every 120, 240, or 360 seconds.

Sexual conditioning. The sexual conditioning procedure included 13 sessions scheduled over the course of seven weeks. Sessions were conducted once every four days and consisted of two individual trials. Each trial began with a 5-minute habituation period during which the male could explore the larger compartment in the absence of the CS and female rat. During habituation, the proportion of time spent in the CS and US Zones was measured. Next, the CS (i.e., orange cone) was placed in the CS Zone for a 2-minute presentation. Again, the proportions of time spent near the US and CS areas were measured while the CS was present. Following the CS presentation, the door was slid open to provide access to the small compartment. Once the male entered, a female rat was placed into the small compartment and the pair copulated until the male reached ejaculation. The male rat remained with the female for a 2-minute post-ejaculatory period which has been shown to induce conditioned ejaculatory preferences for odour and somatosensory stimuli associated with the female (Kippin et al., 1998; Quintana et al., 2018). The female was then removed from the small compartment, and the male returned to the larger compartment to begin the second trial. If the male did not ejaculate, the trial ended 25-minutes after entering the small compartment and the training session was terminated.

Statistical analyses

Sucrose conditioning. Data were collected using Med PC-IV software (Med Associates, Inc.). During sucrose conditioning sessions, the number of contacts with the lever during CS presentation was used as an indicator of sign-tracking behaviour, whereas the normalized number of port entries during the CS presentation served as the measure for goal-tracking behaviour. In order to control for individual differences in port entry behaviour not associated with the CS, normalized port entries were calculated by subtracting the number of port entries during the 10-second pre-CS interval from the number of port entries made during the 10-second CS presentation. Based on the number of lever-CS contacts and port entries made during the 10-

second CS presentation, response bias scores were calculated for each training session using the following formula: (lever-CS contacts – CS port entries)/(lever-CS contacts + CS port entries; Meyer et al., 2012; Villaruel & Chaudhri, 2016). Therefore, response bias scores ranged between -1.0 to +1.0; a negative value closer to -1.0 indicates a greater tendency to make a port entry during the lever-CS presentation (i.e., goal-tracking), and a positive value closer to +1.0 indicates a greater tendency to make a lever-CS contact (i.e., sign-tracking). In order to establish individual subjects' behavioural phenotypes, an average score was calculated across the late block of sucrose autoshaping (i.e., sessions 11-16). Response bias scores ranging from -1.00 to -0.36 were categorized as goal-trackers, -0.35 to 0.35 as intermediates, and +0.36 to +1.00 as sign-trackers (Villaruel & Chaudhri, 2016).

Repeated-measures analyses of variance (ANOVA) were used to assess sign- and goal-tracking behaviour based on dependent variables measured during the 10-second CS presentations across the 16 training sessions. Paired-samples *t*-tests were used to assess between-group differences across training sessions when statistically significant interactions were observed. The number of lever-CS contacts and normalized port entries were used as a measure of sign- and goal-tracking, respectively. Based on the number of CS presentation trials per session (i.e., 12 trials), the probability of a response, and latency to make a conditioned response, were also examined in order to better characterize each phenotype. Here, probability to make a port entry during the CS presentation was calculated as the number of lever-CS trials during which the subject made a port entry, divided by the total number of trials. Similarly, the probability to make a lever-CS contact was calculated as the number of trials during which the subject made a lever-CS contact, divided by the total number of trials. Latencies to make a port entry or lever-CS contact during a lever-CS trial were measured as the time in seconds after the onset of CS presentation. If subjects made no lever contacts or port entries during the 10-second lever-CS presentation, latencies were scored as being 10-second in duration.

Sexual conditioning. Dependent measures included the proportion of time spent in the pre-determined CS and US Zones when the CS was absent, (i.e., during the 5-minute habituation period) in order to assess baseline behavioural patterns when the CS was not present. Furthermore, the proportion of time spent in the pre-determined CS and US Zones was measured when the CS was present (i.e., during the 2-minute CS presentation); a greater proportion of time spent in the CS versus US Zone reflected sign- and goal-tracking behaviours, respectively. Data from Pavlovian-conditioning sessions were analyzed using a repeated-measures ANOVA with Session (1, 7, 13) and Zone (US Zone, CS Zone [CS absent]; US, CS [CS present]) as within-

subjects variables. Follow-up independent and dependent-samples *t*-tests were used to measure differences for statistically significant interactions, using a Bonferroni correction when appropriate.

Results

The acquisition of Pavlovian-conditioned approach in response to a sucrose-paired cue

During sucrose conditioning, there were marked increases in both normalized port entries (Figure 1A) and lever-CS contacts during the CS presentation (Figure 1B), indicating the development of both goal-directed and cue-directed conditioned responses. The normalized port entries reached maximal levels after 5 sessions of sucrose conditioning, and the number of lever-CS contacts during the CS presentation rose steadily across all 16 sucrose conditioning sessions. A repeated-measures ANOVA revealed a significant main effect of Session for both normalized port entries, F (15, 330) = 16.409, $p < 0.001$, $\eta_p^2 = 0.43$, and lever-CS contacts, F (15, 330) = 10.405, $p < 0.001$, $\eta_p^2 = 0.32$. Follow-up paired-samples *t*-tests conducted on sessions 1 and 16 indicated statistically significant increases in both normalized port entries, t (22) = -6.307, $p < 0.001$, $d = 1.32$ and lever-CS contacts, t (22) = -3.489, $p = 0.002$, $d = 0.73$, which demonstrates the acquisition of both goal- and cue-directed conditioned responses.

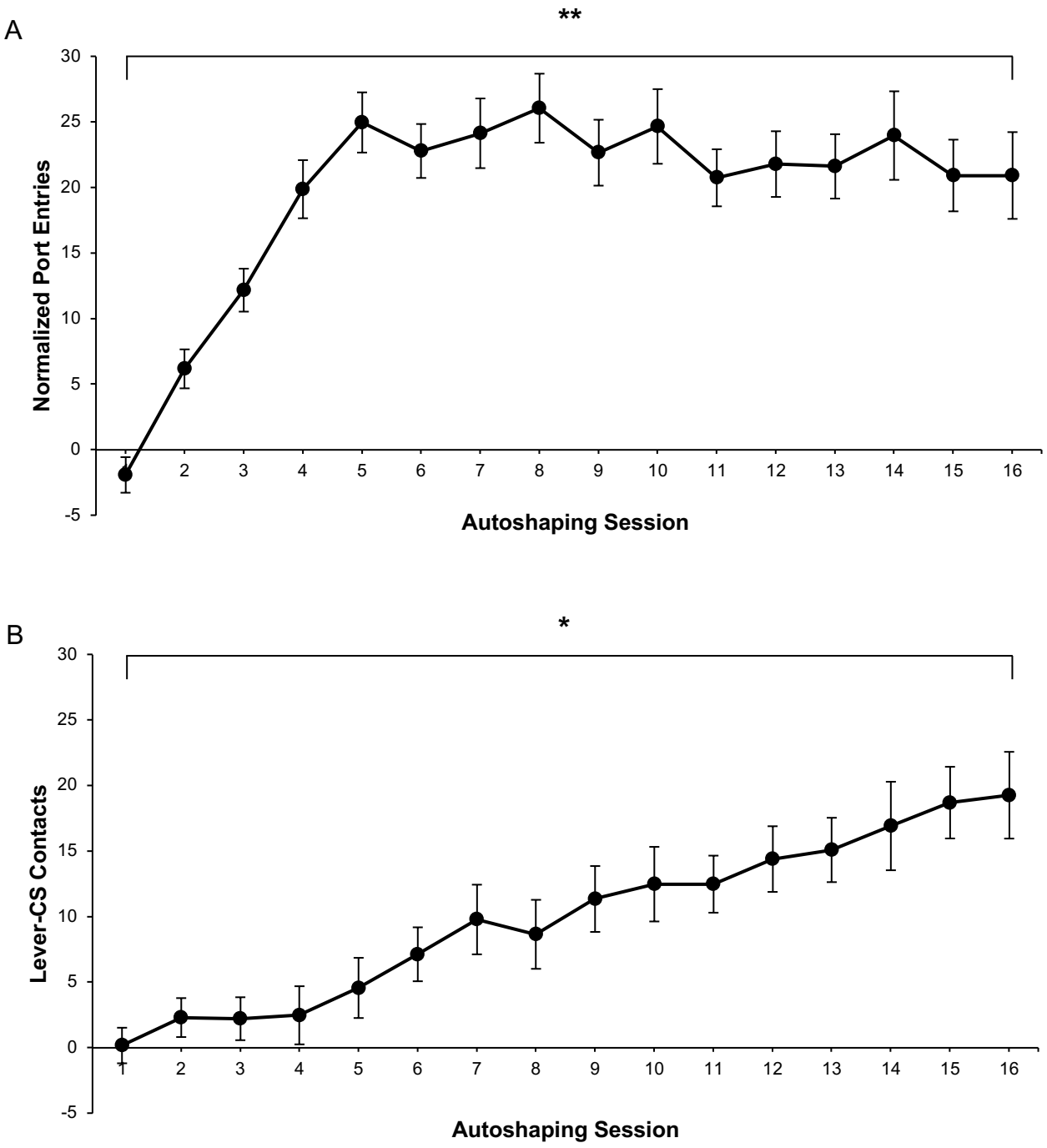


Figure 1. The acquisition of sucrose autoshaping in all subjects (*N* = 23). (A) Mean (± SEM) number of normalized port entries and (B) mean (± SEM) number of lever-CS contacts across 16 sucrose conditioning sessions, ** $p < 0.001$; * $p < 0.01$. The port entry data are normalized by subtracting port entries made during the 10-second interval preceding the lever-CS presentation from port entries made during the CS.

The characterization of individual differences in Pavlovian-conditioned approach

A response bias score, which reflects individual differences in the conditioned response to a lever-CS paired with the delivery of sucrose, was used in order to classify the behavioural phenotype of animals as sign-, goal-trackers, or intermediates. Response bias scores for the 23 subjects were calculated using the following formula: (lever-CS contacts - CS port entries)/(lever-CS contacts + CS port entries) and could therefore range between -1.0 to +1.0. Here, a positive value (i.e., closer to +1.0) indicates a greater tendency to make a lever-CS contact, which suggests a sign-tracking phenotype, and a negative value (i.e., closer to -1.0) indicates a greater tendency to make a port entry during the lever-CS presentation, which suggests a goal-tracking phenotype.

The sucrose conditioning sessions were divided into three blocks: an early block (i.e., sessions 1-5), a middle block (i.e., sessions 6-10), and a late block (i.e., sessions 11-16), and an average response bias score was calculated for each block. Phenotype classification was determined by the response bias score during the late training block. Specifically, subjects with a response bias score ranging from -1.00 to -0.36 were characterized as goal-trackers (n = 13), animals with scores between -0.35 and +0.35 were classified as intermediates (n = 3), and animals with scores from +0.36 to +1.00 as sign-trackers (n = 7).

Goal- and sign-trackers showed markedly different patterns of changes in response bias scores across the early, middle, and late blocks of sessions. Goal-trackers displayed low response bias scores across all session blocks (Figure 2A). Sign-trackers also showed low response bias scores in the early block, however these scores increased maximally during the late block (Figure 2B), indicating a slow acquisition of sign-tracking behaviour directed toward the CS. The small subset of three intermediate rats also showed low response bias scores during both early and middle blocks (Figure 2C). They showed their highest response bias scores during the late block, indicating some development of cue-directed responses late in training, which did not reach sign-tracking levels.

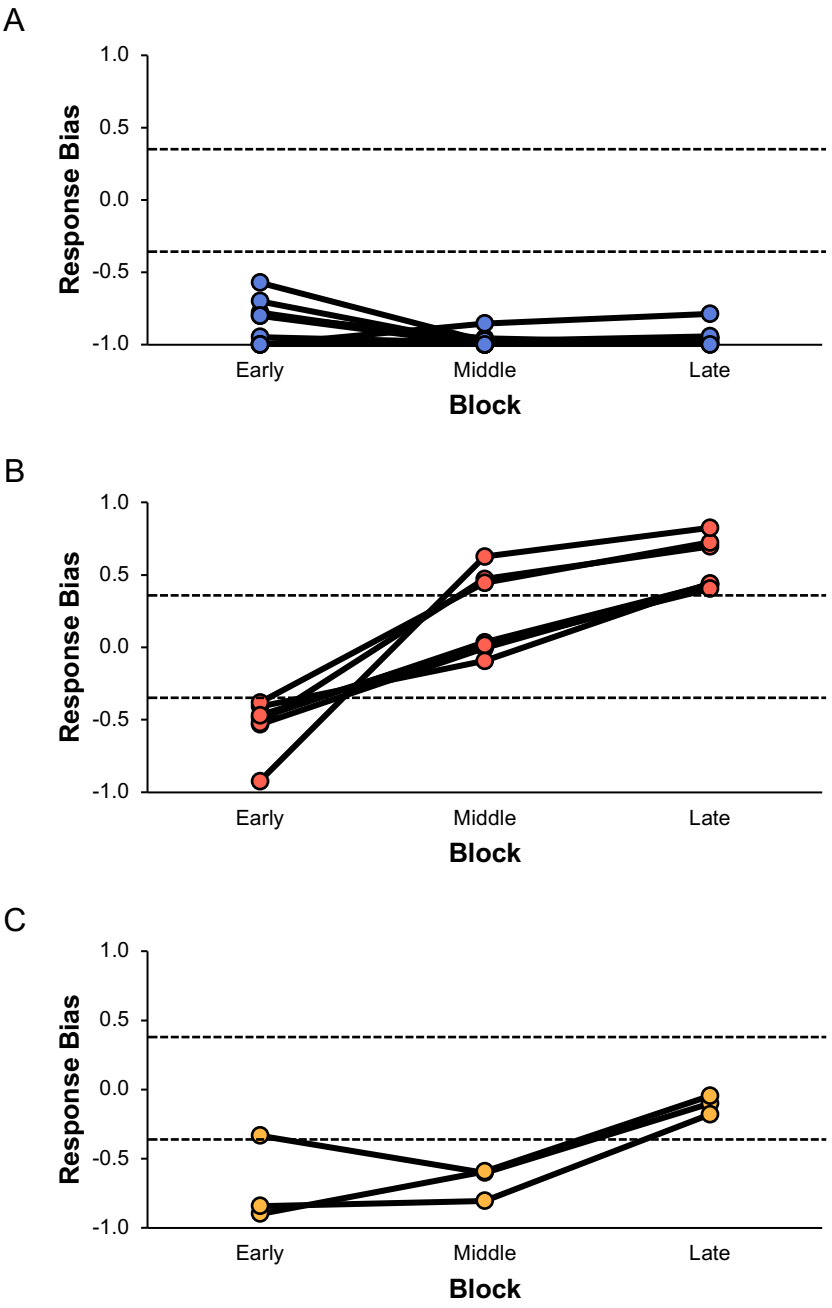


Figure 2. Response bias scores during early (sessions 1-5), middle (sessions 6-10) and late blocks (sessions 11-16) of sucrose autoshaping. Individual subjects were categorized based on response bias scores during the late block as either (A) goal-trackers (*n* = 13), (B) sign-trackers (*n* = 7), or (C) intermediate subjects (*n* = 3). Dashed lines indicate response bias score cut-off (+0.35 and -0.35) for phenotype classification.

US port entries made by sign-, goal-trackers and intermediate subjects across sucrose conditioning sessions

The number of port entries during autoshaping sessions was assessed in order to determine phenotypic differences in the ability to learn to approach the port during delivery of the sucrose US. The number of port entries varied across autoshaping sessions comparably in goal-trackers, sign-trackers, and intermediates, with the greatest number of port entries made during sessions 3 to 5 followed by an overall decrease as subjects learned the predictive relationship between the CS and sucrose delivery (Figure 3). Though a repeated-measures ANOVA revealed a main effect of Session, F (15, 300) = 8.272, $p < 0.001$, $\eta_p^2 = 0.29$, there was no main effect of Phenotype, F (2, 20) = 1.839, $p = 0.185$ nor a Session x Phenotype interaction, F (30, 300) = 1.164, $p = 0.304$. A follow-up paired-samples t-test comparing sucrose conditioning sessions 1 and 16 in all rats revealed that US port entries increased, t (22) = -2.564, $p = 0.018$, $d = 0.57$. Therefore, goal, sign-trackers and intermediate subjects learned to approach the fluid port following the retraction of the lever-CS when sucrose was delivered, across 16 sucrose conditioning sessions.

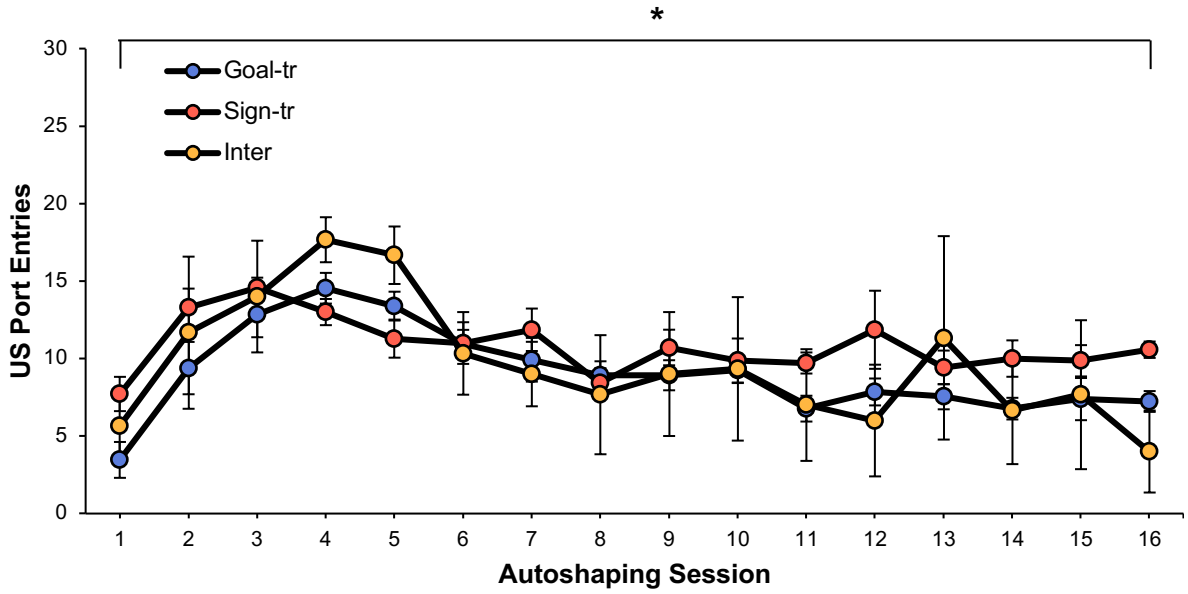


Figure 3. Goal-, sign-trackers and intermediate subjects increased the number of port entries made during sucrose delivery by the 16th autoshaping session. The data are expressed as mean (± SEM) US port entries made by goal-trackers (blue; n = 13), sign-trackers (red; n = 7), and intermediate subjects (orange; n = 3) in each session, *$p < 0.05$.

Individual variation in Pavlovian-conditioned approach across sucrose conditioning sessions

The relationship between behavioural phenotype and normalized port entries was also assessed, revealing a greater number of normalized port entries in goal-trackers, versus sign-trackers and intermediates, after 16 sessions of autoshaping. Here, changes in normalized port entries reflect the acquisition of a conditioned response, where goal-tracking is characterized by the number of normalized port entries and sign-tracking is characterized by the number of lever-CS contacts.

The acquisition patterns of normalized CS port entries across 16 sucrose conditioning sessions were compared in rats classified as goal-, sign-trackers and intermediates based on response bias scores (Figure 4A). A repeated-measures ANOVA revealed significant main effects of Session, F (15, 300) = 13.218, p < 0.001, η_p^2 = 0.40, and Phenotype, F (2, 20) = 4.311, p = 0.028, η_p^2 = 0.30, and a significant Session x Phenotype interaction, F (30, 300) = 3.425, p < 0.001, η_p^2 = 0.34. Consequently, follow-up analyses were conducted to analyze differences in normalized port entry acquisition patterns (i.e., goal-tracking response) for each phenotype. A Bonferroni correction was applied; therefore, all effects are reported at a 0.017 level of significance. Pairwise comparisons of session 1 and 16 revealed that rats characterized as sign-trackers and intermediates did not acquire a goal-tracking response pattern (sign-trackers: t (6) = -1.060, p = 0.330; intermediates: t (2) = -2.487, p = 0.131), but that goal-trackers displayed a significant increase in normalized port entries, t (12) = -11.868, p < 0.001, d = 3.29.

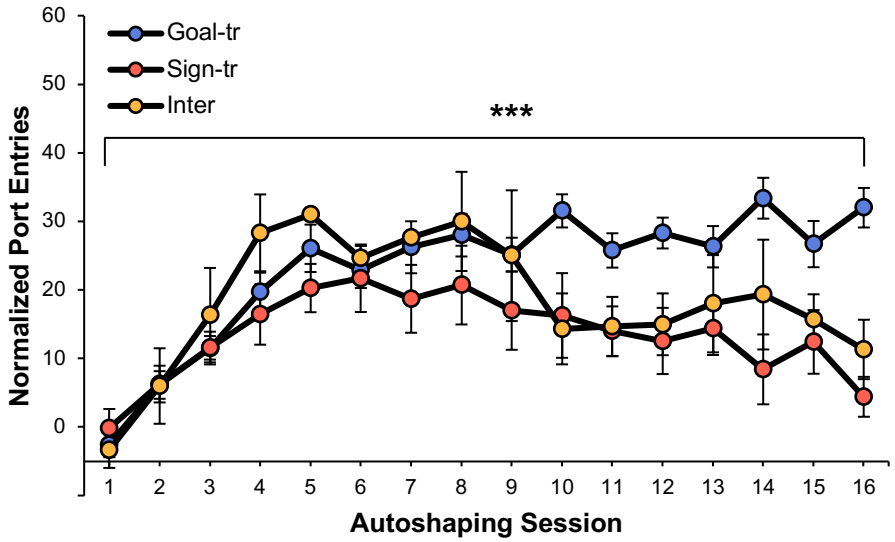
Next, a one-way ANOVA was conducted in order to compare phenotypic differences in normalized CS port entries on session 16 (Figure 4B), which revealed significant differences in normalized CS port entries between goal-, sign-trackers and intermediates, F (2, 22) = 21.398, p < 0.000, η_p^2 = 0.68. Lastly, follow-up independent-samples t-tests showed that goal-trackers made significantly more normalized port entries compared to sign-trackers, t (18) = 6.140, p < 0.001, d = 2.88, and intermediates, t (14) = 3.217, p = 0.006, d = 2.06 and no statistically significant differences in normalized CS port entries between sign-trackers and intermediates, t (8) = -1.308, p = 0.227 on session 16.

The acquisition patterns of lever-CS contacts across the 16 sucrose conditioning sessions were compared in rats classified as goal-, sign-trackers and intermediates based on response bias scores, with the greatest observable increases in sign-trackers (Figure 4C). A repeated-measures ANOVA revealed significant main effects of Session, F (15, 300) = 38.473, p < 0.001, η_p^2 = 0.66, and Phenotype, F (2, 20) = 82.301, p < 0.001, η_p^2 = 0.89, and a significant Session x Phenotype interaction, F (30, 300) = 23.776, p < 0.001, η_p^2 = 0.70. Therefore, follow-up analyses

were conducted in order to analyze differences in lever-CS contact acquisition patterns (i.e., sign-tracking response) for each phenotype. A Bonferroni correction was applied; therefore, all effects are reported at a 0.017 level of significance. Here, pairwise comparisons of session 1 and 16 revealed that rats characterized as goal-trackers and intermediates did not acquire a sign-tracking response pattern (goal-trackers: t (12) = -2.668, p = 0.020 (Early block, M = 0.17, SD = 0.55; Middle block, M = 0.26, SD = 1.05; Late block, M = 0.58, SD = 1.08); intermediates: t (2) = -5.116, p = 0.036 (Early block, M = 1.60, SD = 0.98; Middle block, M = 6.13, SD = 3.80; Late block, M = 17.61, SD = 6.03), which was indeed confirmed in sign-trackers, t (6) = -6.461, p = 0.001, d = 2.44 (Early block, M = 6.71, SD = 6.65; Middle block, M = 29.34, SD = 8.40; Late block, M = 44.40, SD = 18.39). Importantly, we found that sign-trackers and intermediates displayed increasing patterns of lever-CS contacts made during the CS presentation. This effect was not observed in goal-trackers, as the CS functions to provide information of impending reward and has not acquired incentive salience.

Lastly, a one-way ANOVA was conducted in order to analyze phenotypic differences in the number of lever-CS contacts on session 16 (Figure 4D), which revealed significant differences between goal-, sign-trackers and intermediates, F (2, 22) = 41.664, p < 0.001, η_p^2 = 0.81. Follow-up independent-samples t-tests indicated that sign-trackers made significantly more lever-CS contacts compared to goal-trackers, t (18) = -8.809, p < 0.001, d = 4.13, and compared to intermediates, t (8) = 2.493, p = 0.037, d = 1.72 and that intermediates made significantly more lever-CS contacts compared to goal-trackers, t (14) = -10.223, p < 0.001, d = 6.55.

A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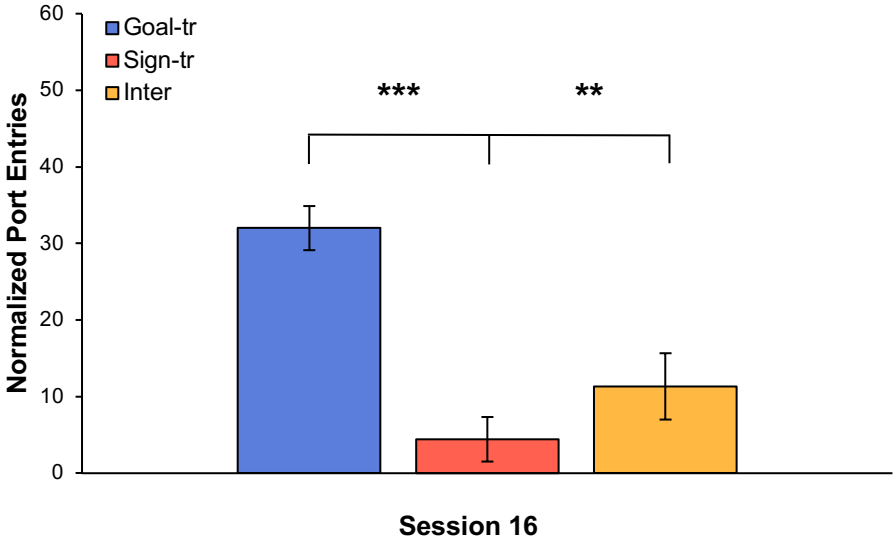
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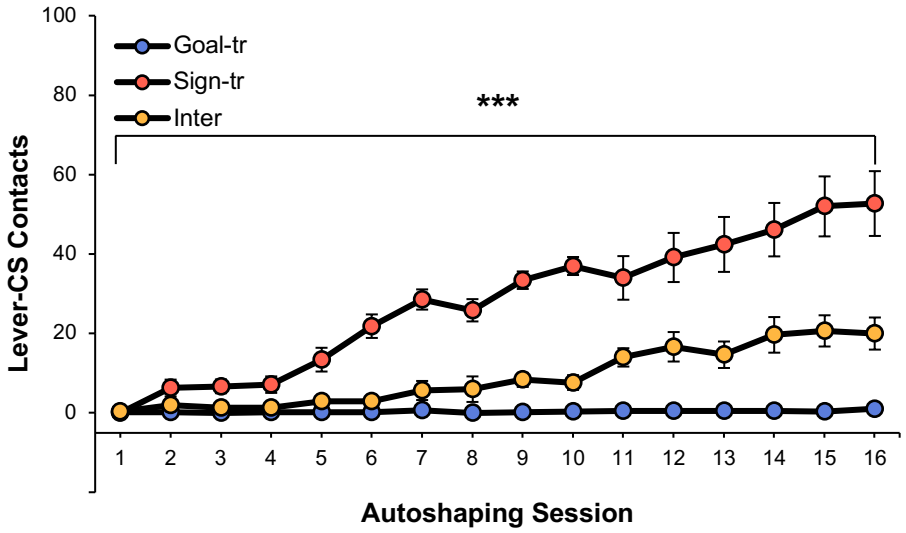
C



D

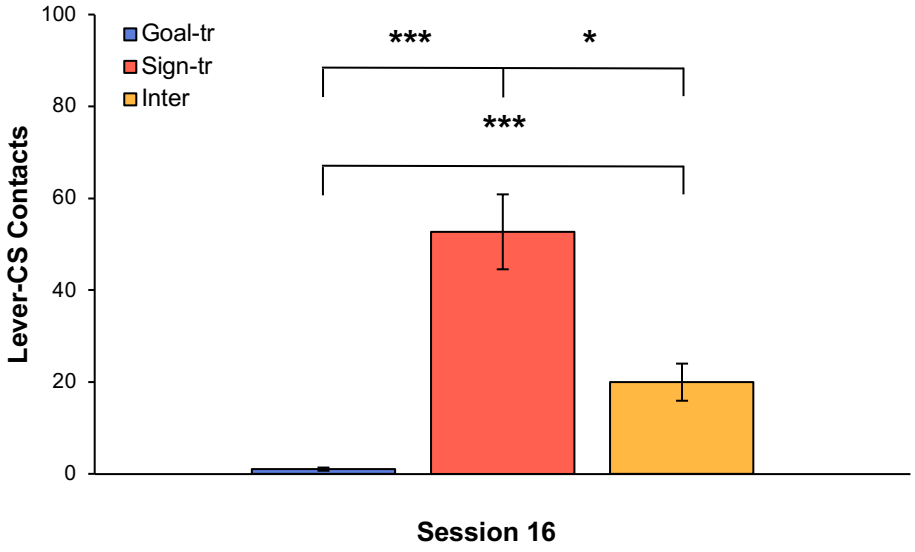


Figure 4. Individual differences in Pavlovian-conditioned approach during sucrose autoshaping. (A) Mean (± SEM) number of normalized port entries made during the lever-CS presentation by goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (B) Mean (± SEM) number of normalized port entries on the final session of sucrose autoshaping. (C) Mean (± SEM) number of lever-CS contacts made by goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (D) Mean (± SEM) number of lever-CS contacts on the final session of sucrose autoshaping, ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

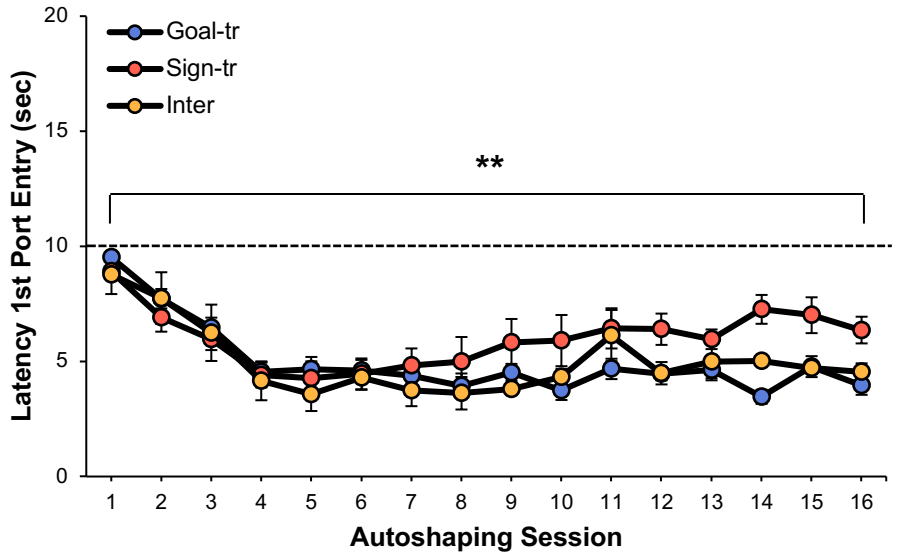
The mean latency to make a port entry was analyzed in sign-, goal-trackers and intermediates across the 16 sucrose conditioning sessions. All phenotypes displayed reductions in latencies across the first 4 autoshaping sessions (Figure 5A). A repeated-measures ANOVA revealed a significant main effect of Session, F (15, 300) = 17.456, p < 0.001, η_p^2 = 0.47, and Session x Phenotype interaction, F (30, 300) = 2.790, p < 0.001, η_p^2 = 0.22, however there was no significant main effect of Phenotype, F (2, 20) = 2.101, p = 0.149. Follow-up Bonferroni-corrected paired-samples t-tests (Bonferroni-adjusted α level = 0.017) revealed that subjects characterized as goal- and sign-trackers both displayed a shorter latency to make a port entry by the last sucrose conditioning session compared to the first session (goal-trackers: t (12) = 12.237, p < 0.001; d = 3.39 sign-trackers: t (6) = 4.798, p = 0.003, d = 1.81). In intermediates, the mean latency to make a port entry was similar to what was observed in goal-trackers on session 16, however the difference between the first and 16th session did not achieve statistical significance, t (2) = 4.539, p = 0.045.

Next, a one-way ANOVA was conducted to measure differences in latency to make a port entry in goal-, sign-trackers and intermediates on session 16 (Figure 5B), which revealed a statistically significant difference between the behavioural phenotypes, F (2, 22) = 6.371, p = 0.007, η_p^2 = 0.39. Follow-up independent-samples t-tests showed that goal-trackers displayed a significantly shorter latency to make a port entry compared to sign-trackers, t (18) = -3.410, p = 0.003, d = 1.60, but not intermediates, t (14) = -0.646, p = 0.528 on session 16. Lastly, sign-trackers and intermediates reflected comparable latencies to make a port entry, t (8) = 1.933, p = 0.089.

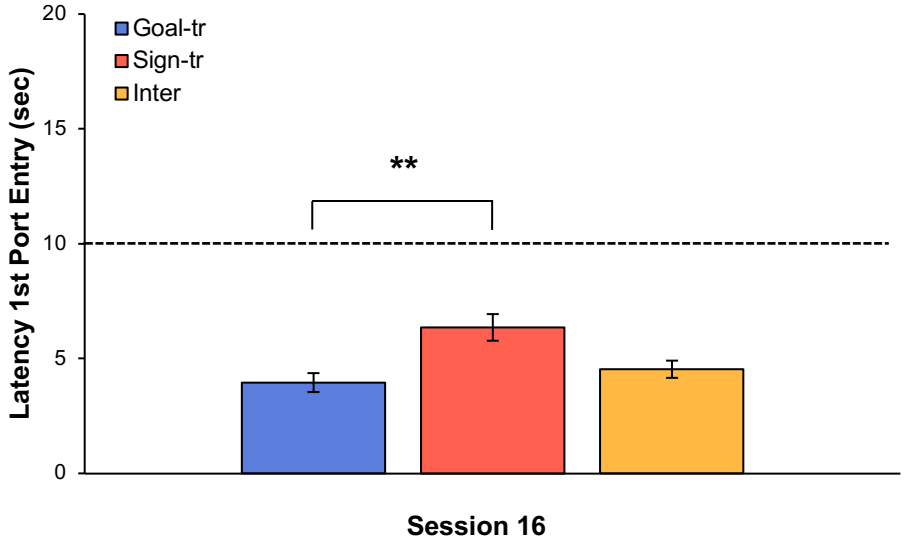
The mean latency to make a lever-CS contact was analyzed in goal-, sign-trackers and intermediates across 16 sucrose conditioning sessions, and the most marked reductions in latency to lever-CS press occurred in sign-trackers (Figure 5C). A repeated-measures ANOVA revealed significant main effects of Session, F (15, 300) = 10.300, p < 0.001, η_p^2 = 0.34, and Phenotype, F (2, 20) = 211.015, p < 0.001, η_p^2 = 0.96, and a significant Session x Phenotype interaction, F (30, 300) = 6.902, p < 0.001, η_p^2 = 0.41. Follow-up Bonferroni-corrected pairwise comparisons (Bonferroni-adjusted α level = 0.017) revealed that subjects classified as sign-trackers displayed a shorter latency to make a lever-CS contact by the last sucrose conditioning session compared to the first session, t (6) = 5.489, p = 0.002, d = 2.08. However, this rapid approach to the lever-CS was not observed in goal-trackers, t (12) = 2.743, p = 0.018, or intermediates, t (2) = 1.958, p = 0.189.

Next, a one-way ANOVA was used to compare phenotypic differences in the latency to make a lever-CS contact on session 16 (Figure 5D), which revealed a significant difference between the phenotypes, F (2, 22) = 24.519, $p < 0.001$, $\eta_p^2 = 0.71$. Follow-up independent-samples t-tests indicated that sign-trackers showed a significantly shorter latency to make a lever-CS contact compared to goal-trackers, t (18) = 6.484, $p < 0.001$, $d = 3.04$, and intermediates, t (8) = -3.050, $p = 0.016$, $d = 2.11$ on session 16, and no significant difference between goal-trackers and intermediates, t (14) = 0.211, $p = 0.836$.

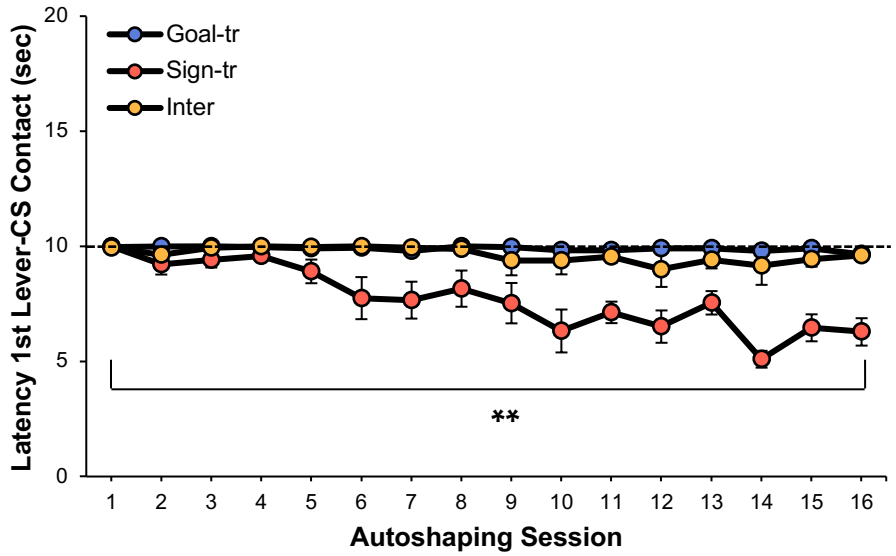
A


Goal-tr
Sign-tr
Inter
**
Latency 1st Port Entry (sec)
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Autoshaping Session

B


Goal-tr
Sign-tr
Inter
**
Latency 1st Port Entry (sec)
20
15
10
5
0
Session 16

C



D

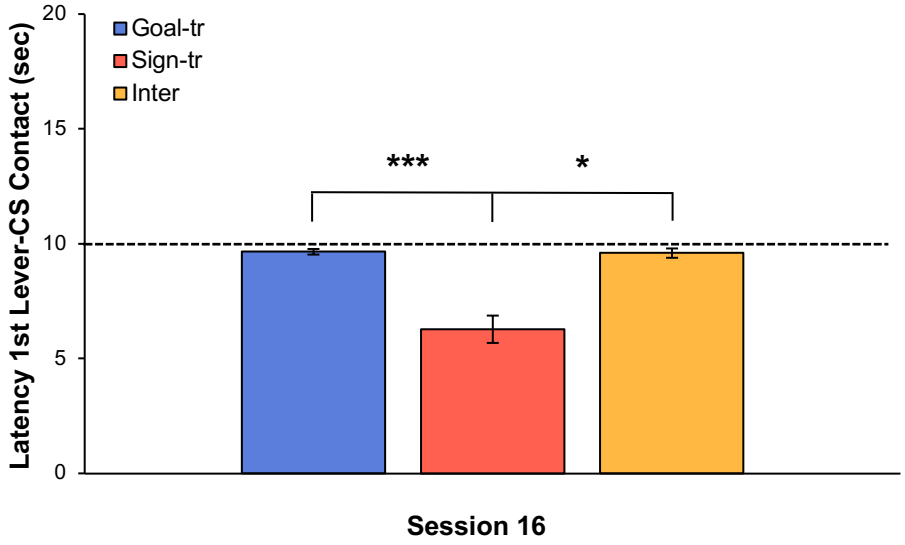


Figure 5. Individual differences in Pavlovian-conditioned approach during sucrose autoshaping. (A) Mean (± SEM) latency to first port entry during the lever-CS presentation by goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (B) Mean (± SEM) latency to first port entry on the final session of sucrose autoshaping. (C) Mean (± SEM) latency to first lever-CS contact made by goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (D) Mean (± SEM) latency to first lever-CS contact on the final

session of sucrose autoshaping, ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Dashed lines indicate the maximum latency to make a response or maximum recorded latency if no response was made.

The mean probability to make a port entry during the lever-CS trial was analyzed in goal-trackers, sign-trackers, and intermediates across 16 sucrose conditioning sessions. The probability increased in all behavioural phenotypes over the first four sessions, and although goal-trackers displayed the highest probability to make a port entry after 16 conditioning sessions, there were no significant differences between goal-trackers, sign-trackers, and intermediates (Figure 6A). A repeated-measures ANOVA revealed a significant main effect of Session, F (15, 300) = 22.210, $p < 0.001$, $\eta_p^2 = 0.53$, and a significant Session x Phenotype interaction, F (30, 300) = 3.152, $p < 0.001$, $\eta_p^2 = 0.24$, however there was no significant main effect of Phenotype, F (2, 20) = 1.604, $p = 0.226$. Follow-up Bonferroni-corrected paired-samples t-tests (Bonferroni-adjusted α level = 0.017) revealed that, compared to session 1, the probability of making a port entry during lever-CS trials increased by session 16 in both goal-trackers, t (12) = -20.042, $p < 0.001$, $d = 5.56$, and sign-trackers, t (6) = -4.612, $p = 0.004$, $d = 1.74$, but the increase was not statistically significant in subjects characterized as intermediates, t (2) = -5.152, p =0.036.

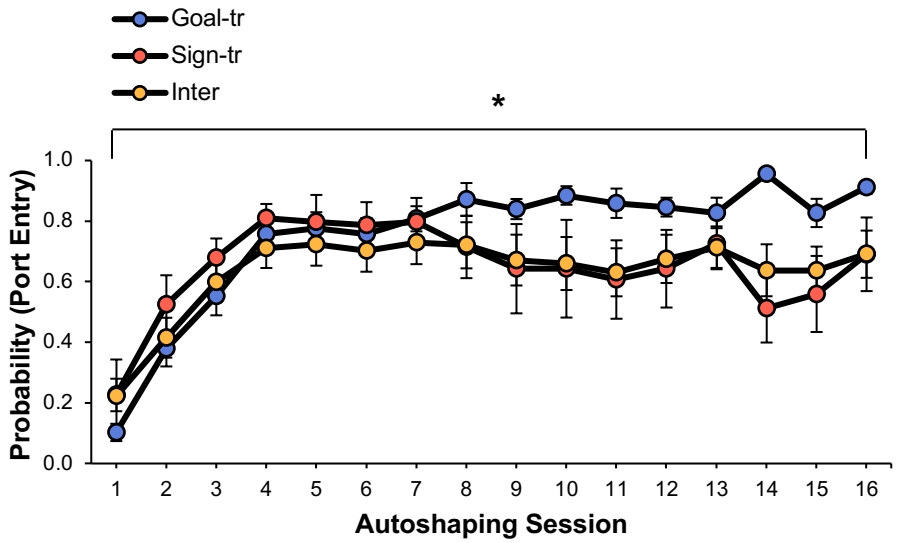
Lastly, a one-way ANOVA was conducted in order to assess phenotypic differences in the probability to make a port entry on session 16 (Figure 6B). This revealed no significant differences in the probability to make a port entry as a function of behavioural phenotype, F (2, 22) = 3.286, $p = 0.058$, though a statistical trend suggests a pattern for goal-trackers to show the highest probability to make a port entry (M = 0.91, SD = 0.07) compared to sign-trackers (M = 0.69, SD = 0.32) and intermediates (M = 0.89, SD = 0.01).

The mean probability to make a lever-CS contact was analyzed in goal-, sign-trackers and intermediates across 16 sucrose conditioning sessions (Figure 6C). The probability of making a lever-CS contact remained very low in goal-trackers over 16 conditioning sessions but increased in both intermediates and sign-trackers across sessions. A repeated-measures ANOVA revealed significant main effects of Session, F (15, 300) = 7.115, $p < 0.001$, $\eta_p^2 = 0.26$, and Phenotype, F (2, 20) = 41.558, $p < 0.001$, $\eta_p^2 = 0.81$, and a significant Session x Phenotype interaction, F (30, 300) = 5.940, $p < 0.001$, $\eta_p^2 = 0.37$. Follow-up Bonferroni-corrected pairwise comparisons (Bonferroni-adjusted α level = 0.017) revealed that sign-trackers displayed an increased probability to make a lever-CS contact by session 16 compared to session 1 of sucrose conditioning, t (6) = -7.775, $p < 0.001$, $d = 2.94$, which was not observed in subjects characterized as goal-trackers, t (12) = -2.598, $p = 0.023$, and intermediates, t (2) = -1.990, $p = 0.185$.

Next, a one-way ANOVA was used to compare differences in the probability to make a lever-CS contact on session 16 (Figure 6D), which revealed a statistically significant difference between goal-, sign-trackers and intermediates, F (2, 22) = 42.951, $p < 0.001$, $\eta_p^2 = 0.81$. Follow-

up independent-samples *t*-tests indicated that sign-trackers showed a greater probability to make a lever-CS contact compared to goal-trackers, t (18) = -8.685, p < 0.001, d = 4.07, and intermediates, t (8) = 4.219, p = 0.003, d = 2.91, with no significant difference between goal-trackers and intermediates, t (14) = -0.251, p = 0.806 on session 16.

A


Goal-tr
Sign-tr
Inter
*
Probability (Port Entry)
1.0
0.8
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0.4
0.2
0.0
1
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4
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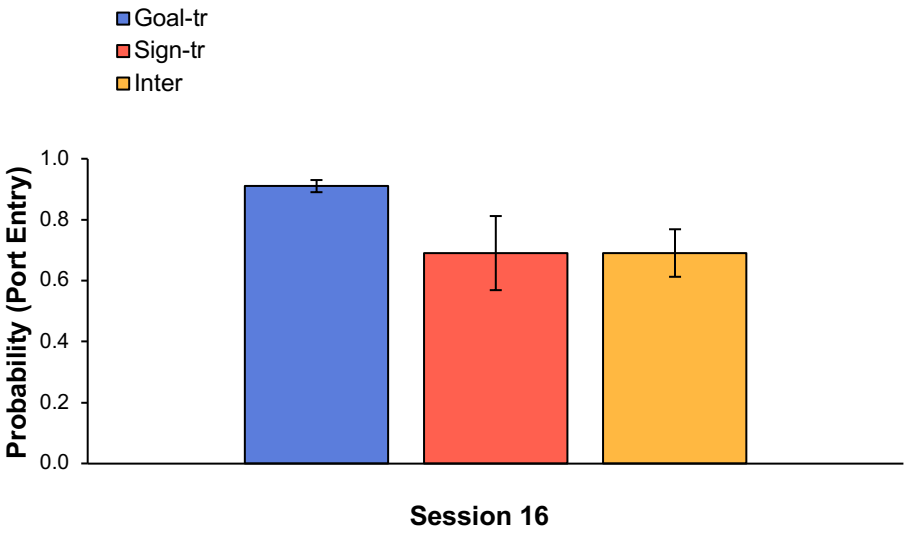
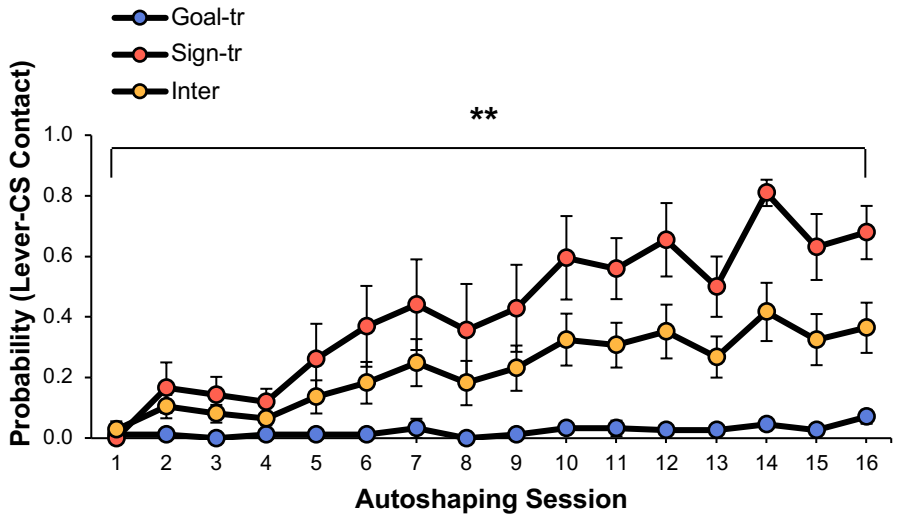
11
12
13
14
15
16
Autoshaping Session

B


Goal-tr
Sign-tr
Inter
Probability (Port Entry)
1.0
0.8
0.6
0.4
0.2
0.0
Session 16

C



D

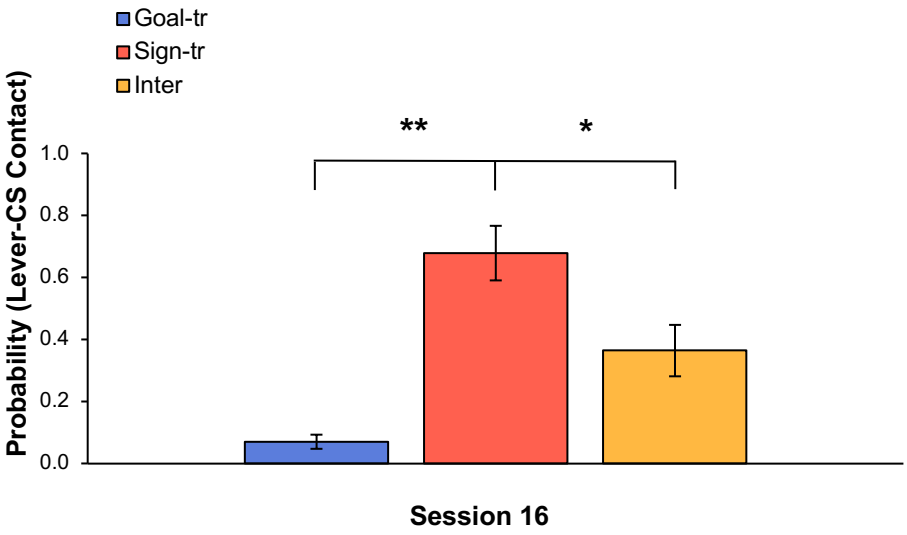


Figure 6. Individual differences in Pavlovian-conditioned approach during sucrose autoshaping. (A) Mean (± SEM) probability to make a port entry during the lever-CS presentation in goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (B) Mean (± SEM) probability to make a port entry on the final session of sucrose autoshaping. (C) Mean (± SEM) probability to make a lever-CS contact in goal-trackers (blue; *n* = 13), sign-trackers (red; *n* = 7) and intermediate subjects (orange; *n* = 3) across 16 sessions of sucrose autoshaping. (D) Mean (± SEM) probability to make a lever-CS contact on the final session of sucrose autoshaping, $^{**}p < 0.001$; $^{*}p < 0.01$.

In summary, rats identified as sign-trackers (*n* = 7) displayed a greater number of lever-CS contacts, a shorter latency to make a lever-CS contact, and a greater probability to make a lever-CS contact compared to goal-trackers (*n* = 13) and intermediate subjects (*n* = 3) by session 16. Animals characterized as goal-trackers showed a greater number of normalized port entries compared to sign-trackers and intermediate subjects, and a shorter latency to make a normalized port entry compared to sign-trackers by session 16. Though there were no significant differences in the probability to make a port entry across phenotypes, a statistical trend indicated that goal-trackers showed the highest probability to make a port entry by session 16 compared to sign-trackers but not intermediate subjects. This supports the classification of these animals as sign- and goal-trackers based on their respective response bias scores and allowed the evaluation of whether the expression of these phenotypes would extend to a sexual conditioning paradigm.

The acquisition of Pavlovian-conditioned approach in response to a sex-paired cue

Following sucrose autoshaping, two rats were excluded due to equipment malfunction during sexual conditioning. Consequently, the analyses were conducted on 21 rats (sucrose goal-trackers, *n* = 11; sucrose sign-trackers, *n* = 7, and sucrose intermediates, *n* = 3). In order to determine whether subjects developed PCA toward a sexually-conditioned cue, the mean proportion of time spent in the CS and US Zones while the CS was absent was compared in all rats across sessions 1, 7 and 13 in all sucrose subjects (Figure 7A). A repeated-measures ANOVA on time spent in the US and CS Zones, in the absence of the CS, suggests a consistent preference for the US Zone compared to the CS Zone across sessions 1, 7 and 13. A statistically significant main effect of Zone, F (1, 20) = 13.203, p = 0.002, η_p^2 = 0.26, indicated that subjects spent more time in the US Zone (M = 0.31, SD = 0.11) compared to the CS Zone (M = 0.19, SD = 0.09), and a non-significant Session x Zone interaction, F (2, 40) = 0.402, p = 0.672 revealed that this pattern was consistent across sessions. There was no main effect of Session, F (2, 40) = 1.443, p = 0.248.

Next, we compared the mean proportion of time spent near the US and CS in the presence of the CS in all rats across sessions 1, 7 and 13 in order to assess goal- and cue-directed behaviour (Figure 7B). A repeated-measures ANOVA revealed statistically significant main effects of Session, F (2, 40) = 8.232, p = 0.001, η_p^2 = 0.08, and Zone, F (1, 20) = 23.539, p < 0.000, η_p^2 = 0.18, and a significant Session x Zone interaction, F (2, 40) = 3.851, p = 0.030, η_p^2 = 0.08, indicating that subjects spent a greater proportion of time near the US (M = 0.353, SD = 0.017) compared to the CS (M = 0.228, SD = 0.015) and that changes in the proportions of time spent in each of these areas differed significantly across sessions 1, 7 and 13. Follow-up paired-samples *t*-tests were conducted to compare the proportion of time spent near the US and CS on

session 1, 7 and 13. On sessions 1 and 7, there were no statistically significant differences in the proportion of time spent near the US and CS (Session 1, t (20) = 2.089, p = 0.050; Session 7, t (20) = 0.744, p = 0.466) suggesting that subjects spent comparable proportions of time near each stimulus. However, there was a statistically significant difference in the proportion of time spent near the US and CS by session 13, t (20) = 6.580, p < 0.000, d = 1.44, where subjects spent a greater proportion of time near the US (M = 0.409, SD = 0.010) compared to the CS (M = 0.199, SD = 0.077).

Though the presence of the CS did not result in increased time spent near it across sessions, this does not indicate that subjects did not learn the predictive relationship between the CS and US. In order to determine whether subjects learned that the CS signalled the opportunity to copulate with a sexually-receptive female, the mean proportion of time spent in the US area, both in the absence and presence of the CS, was compared across sessions 1, 7 and 13 (Figure 7C). On sessions 1 and 7, Bonferroni-corrected planned comparison t-tests (Bonferroni-adjusted α level = 0.017) revealed no significant differences in the proportion of time spent near the US, in the absence and presence of the CS (Session 1, t (20) = -0.465, p = 0.647; Session 7, t (20) = -0.496, p = 0.625) indicating that rats spent a comparable proportion of time in the US area regardless of the CS' presence. By session 13, however, there was a statistically significant difference in the proportion of time spent near the US, in the absence and presence of the CS, t (20) = -3.541, p = 0.002, d = 0.77, where subjects spent a greater proportion of time in the US area when the CS was present (M = 0.410, SD = 0.010) compared to when it was absent (M = 0.311, SD = 0.095). This provides further evidence that rats learned the CS-US association across sexual conditioning sessions, as subjects spent less time in the US Zone when the CS was absent, and more time in the US Zone when the CS was present, as it marked the opportunity to copulate with a sexually-receptive female.

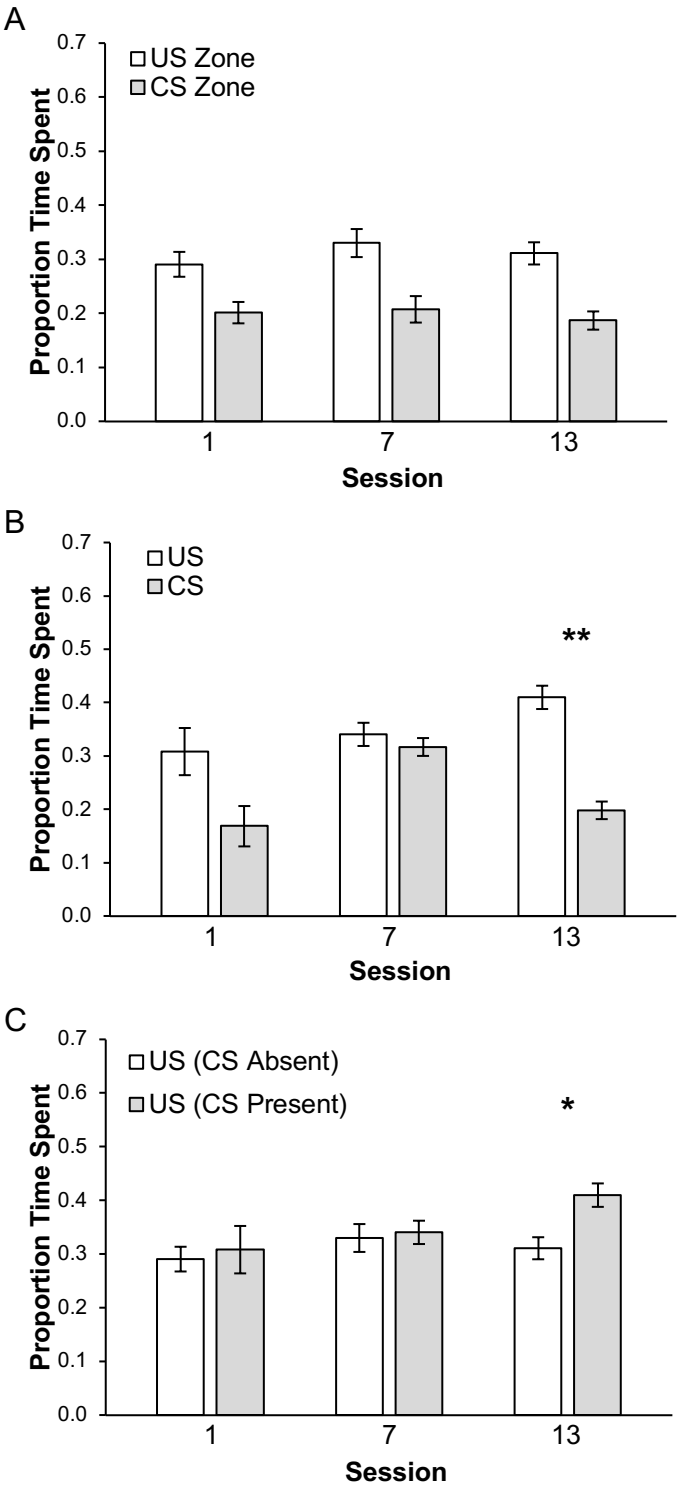


Figure 7. The development of individual differences in Pavlovian-conditioned approach following sexual conditioning in all sucrose subjects (*N* = 23). (A) Mean (± SEM) proportion of time spent in the US Zone (white bar) and CS Zone (grey bar) in the absence of the CS on sessions 1, 7 and

13. (B) Mean (± SEM) proportion of time spent near the US (white bar) and CS (grey bar) in the presence of the CS on sessions 1, 7 and 13. (C) Mean (± SEM) proportion of time spent in the US Zone when the CS was absent (white bar) and present (grey bar) on sessions 1, 7 and 13, $^{**}p < 0.001$; $^{*}p < 0.01$.

Phenotypic differences in responding to a sucrose- and sexually-conditioned cue

Next, we focused on examining whether the behavioural phenotypes established with sucrose conditioning predicted the phenotypes displayed in testing with sexual reward. Based on the response bias score obtained for each rat for sucrose conditioning, there were 11 goal-tracking rats, 7 sign-tracking rats, and 3 intermediate rats that underwent sexual conditioning. In order to provide a visual representation of sign-, goal-tracking and intermediate behaviour in response to sucrose and sexual reward, response bias scores were calculated for each (sucrose reward: [lever-CS contacts - CS port entries/lever-CS contacts + CS port entries]; sexual reward: [time spent near CS – time spent near US/total session time, in seconds]) during the late block of sucrose autoshaping, and the final session of sexual conditioning, respectively (Figure 8). In sucrose goal-trackers, there was no marked correlation between the response bias score for sucrose reward and sexual reward, r = 0.19, p (two-tailed) = 0.580. However, there was a significant relationship between sucrose sign-trackers and intermediate subjects' response bias scores for sucrose and sexual reward (sign-trackers: r = 0.86, p (two-tailed) = 0.013; intermediate: r = 1.00, p (two-tailed) = 0.007).

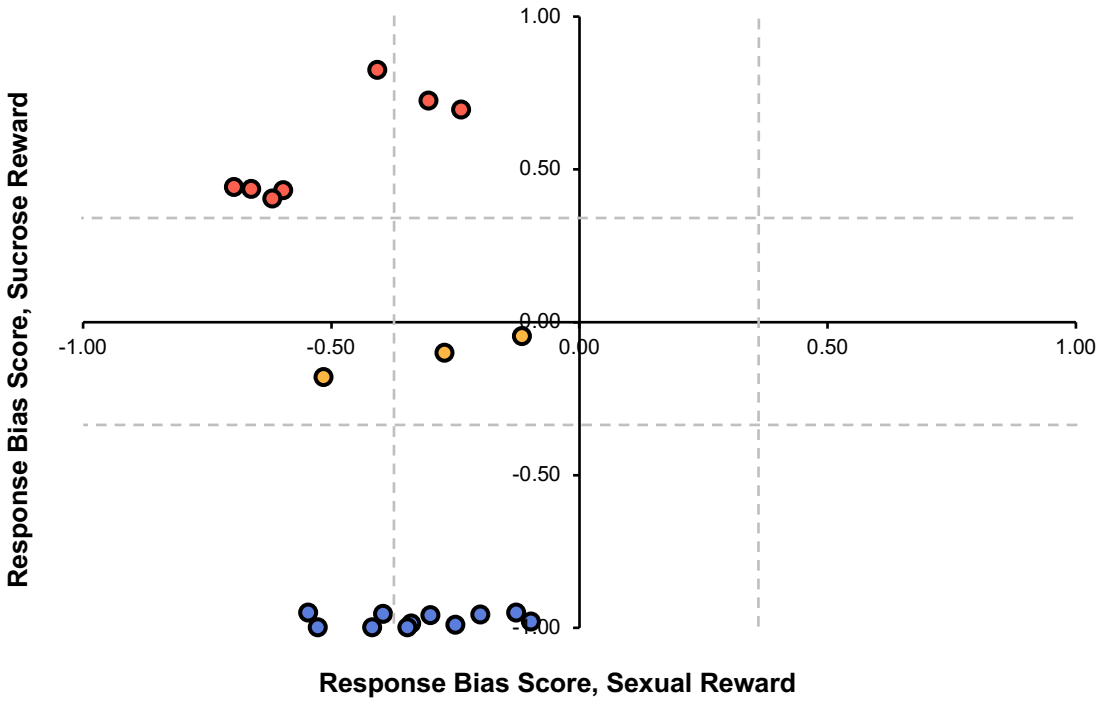


Figure 8. Individual response bias scores following sucrose autoshaping and sexual conditioning in subjects classified as sucrose goal-trackers (blue; *n* = 11), sucrose sign-trackers (red; *n* = 7) and sucrose intermediates (orange; *n* = 3). A response bias score between -1.00 and -0.36 suggests goal-tracking behaviour, a response bias score between -0.35 and +0.35 suggests intermediate behaviour, and a response bias score between +0.36 and +1.00 suggests sign-tracking behaviour. Following sexual conditioning, sucrose goal-trackers and intermediate subjects appeared to display intermediate-like behaviour, whereas sucrose sign-trackers appeared to 'shift' their behavioural phenotype to a goal-tracking response. Dashed lines indicate response bias score cut-off (+0.35 and -0.35) for phenotype classification.

Pavlovian-conditioned approach toward a sexually-conditioned cue in sucrose goal-trackers. In order to determine whether a goal-directed response for a sucrose-paired cue would also be displayed toward a sex-paired cue, the mean proportion of time spent in the US and CS Zones was first compared in sucrose goal-trackers across sessions 1, 7 and 13 when the CS was absent (Figure 9A). A repeated-measures ANOVA on time spent in the US and CS Zones, in the absence of the CS, indicated that goal-trackers spent a comparable proportion of time in the US and CS Zones across sessions 1, 7 and 13, as there were no significant main effects of Session, F (2, 20) = 1.695, p = 0.209 or Zone, F (1, 10) = 3.833, p = 0.079, and a non-significant Session x Zone interaction, F (2, 20) = 0.817, p = 0.456.

In the presence of the CS, sucrose goal-trackers also displayed comparable proportions of time near the US and CS across sessions 1, 7 and 13 (Figure 9B). Though a repeated-measures ANOVA revealed a non-significant main effect of Zone, F (1, 10) = 4.849, p = 0.052, there was a statistical trend toward this subset spending more time near the US (M = 0.338, SD = 0.027) compared to the CS (M = 0.238, SD = 0.027). There was no significant main effect of Session, F (2, 20) = 2.746, p = 0.088 nor a significant Session X Zone interaction, F (2, 20) = 0.545, p = 0.588. Therefore, it appears that sucrose goal-trackers may adopt intermediate-like behaviours in the presence of a sexually-conditioned CS.

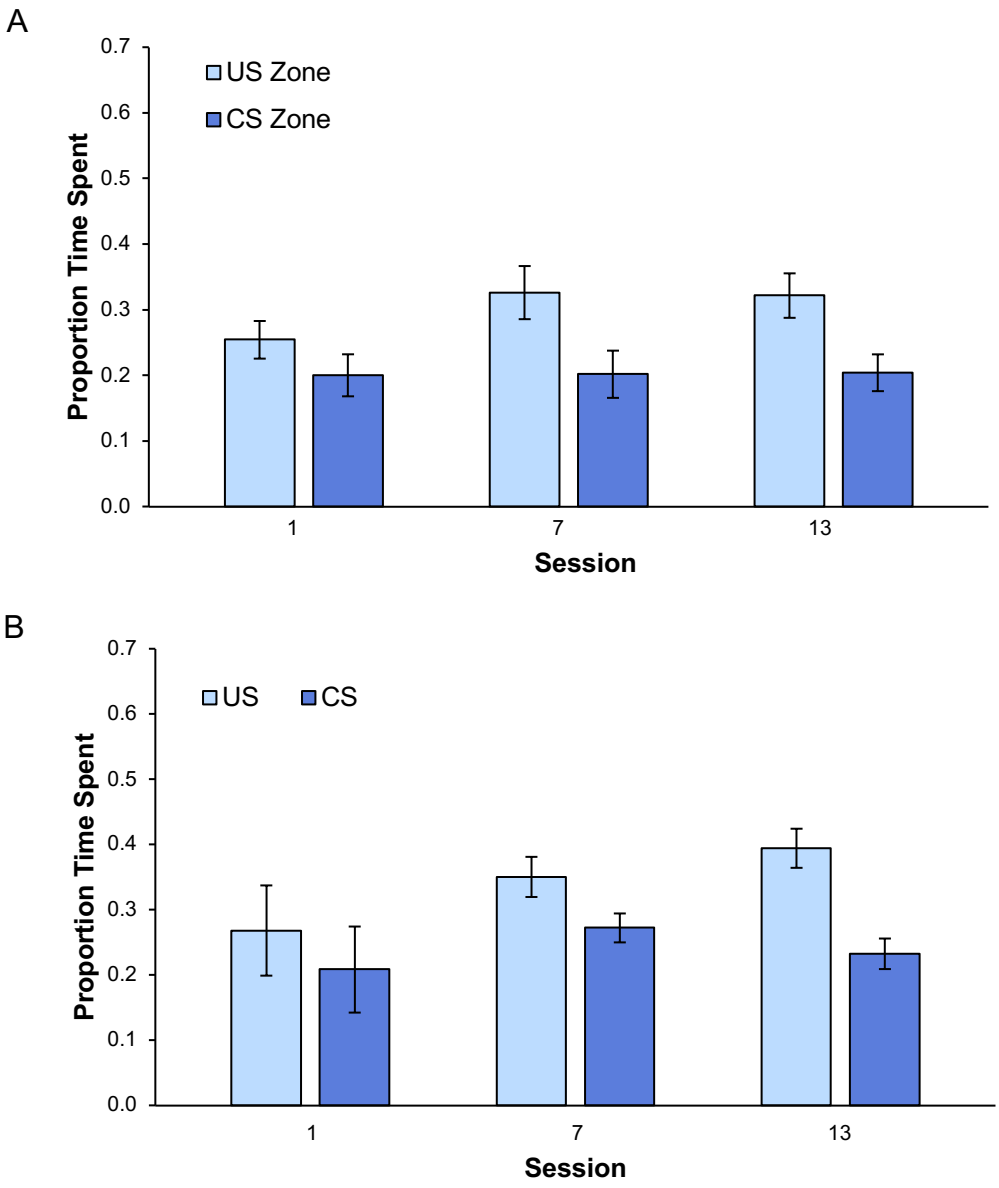


Figure 9. Sucrose goal-trackers display intermediate behaviour in response to a sexually-conditioned cue (*n* = 11). (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence of the CS on sessions 1, 7 and 13. (B) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the presence of the CS on sessions 1, 7 and 13.

Pavlovian-conditioned approach in response to a sexually-conditioned cue in sucrose sign-trackers. In order to assess whether subjects displaying a cue-directed response toward a sucrose-paired CS show a similar response pattern toward a CS associated with sexual reward, we compared the mean proportion of time spent near the US and CS across sessions 1, 7 and 13. First, the mean proportion of time spent in the US and CS Zones was compared in sucrose sign-trackers across sessions in the absence of the CS (Figure 10A). A repeated-measures ANOVA on time spent in the US and CS Zones revealed a significant main effect of Zone, F (1, 6) = 7.448, p = 0.034, η_p^2 = 0.40, indicating that overall, sign-trackers spent more time in the US Zone (M = 0.32, SD = 0.03) compared to the CS Zone (M = 0.19, SD = 0.02). There was no main effect of Session, F (2, 12) = 2.025, p = 0.175, nor a significant Session x Zone interaction, F (2, 12) = 1.700, p = 0.224.

When the CS was present, sucrose sign-trackers showed an increased preference in spending time near the US as they progressed through sessions 1, 7 and 13 (Figure 10B). A repeated-measures ANOVA on time spent near the US and CS, in the presence of the CS, revealed significant main effects of Session, F (2, 12) = 4.225, p = 0.041, η_p^2 = 0.15, and Zone, F (1, 6) = 98.641, p < 0.001, η_p^2 = 0.59, and a significant Session x Zone interaction, F (2, 12) = 11.581, p = 0.002, η_p^2 = 0.57, suggesting that subjects spent more time near the US compared to the CS, and that the proportions of time spent near the US versus the CS increased and decreased respectively across sessions. Follow-up paired-samples t-tests were conducted to compare the proportions of time spent near the US and CS on sessions 1, 7 and 13. On session 1, there was a statistically significant difference in the proportion of time spent near the US and CS, t (6) = 4.483, p = 0.004, d = 1.70, where subjects spent a higher proportion of time near the US (M = 0.42, SD = 0.15) compared to the CS (M = 0.11, SD = 0.01), however this preference was not observed on Session 7, t (6) = -1.598, p = 0.161 as rats showed an increase in the proportion of time spent near the CS (M = 0.37, SD = 0.04) compared to the US (M = 0.31, SD = 0.09). By session 13, there was a statistically significant difference in the proportion of time spent near the US and CS, t (6) = 6.007, p < 0.001, d = 2.27, where subjects spent more time near the US (M = 0.45, SD = 0.11) compared to the CS (M = 0.14, SD = 0.05). Though sucrose sign-trackers demonstrated an initial preference for the US area compared to the CS area, they displayed intermediate-like behaviours by the middle of sexual conditioning, and goal-directed behaviours by session 13.

In order to examine whether the development of goal-directed behaviour was specific to its predictive relationship with the CS, the mean proportion of time spent in the US area, both in the absence and presence of the CS, was compared across sessions 1, 7 and 13 (Figure 9C). A

Bonferroni correction was applied; therefore, all effects are reported at a 0.017 level of significance. On sessions 1 and 7, planned comparison *t*-tests indicated no statistically significant differences in the proportion of time spent near the US, in the absence and presence of the CS (Session 1, t (6) = -0.777, p = 0.467; Session 7, t (6) = -0.241, p = 0.817) suggesting that sucrose sign-trackers spent comparable proportions of time in the US area in both the absence and presence of the CS. However, by session 13, sucrose sign-tracking subjects spent more time in the US Zone during the CS presentation compared to when it was absent. A Bonferroni-corrected planned comparison (Bonferroni-adjusted α level = 0.017) revealed a statistically significant difference in the proportion of time spent near the US, in the presence and absence of the CS, t (6) = -3.457, p = 0.014, d = 1.31 where sucrose sign-trackers spent a greater proportion of time in the US area when the CS was present (M = 0.45, SD = 0.11) compared to when it was absent (M = 0.32, SD = 0.06). This provides additional evidence that rats learned the CS-US association across sexual conditioning sessions, as subjects spent less time in the US Zone when the CS was absent, and more time in the US Zone when the CS was present, as it marked the opportunity to copulate with a sexually-receptive female. Furthermore, as subjects learned the predictive relationship CS-US relationship, their behavioural phenotype shifted from cue-directed (i.e., sucrose-paired CS) to goal-directed (i.e., sex-paired CS) by session 13.

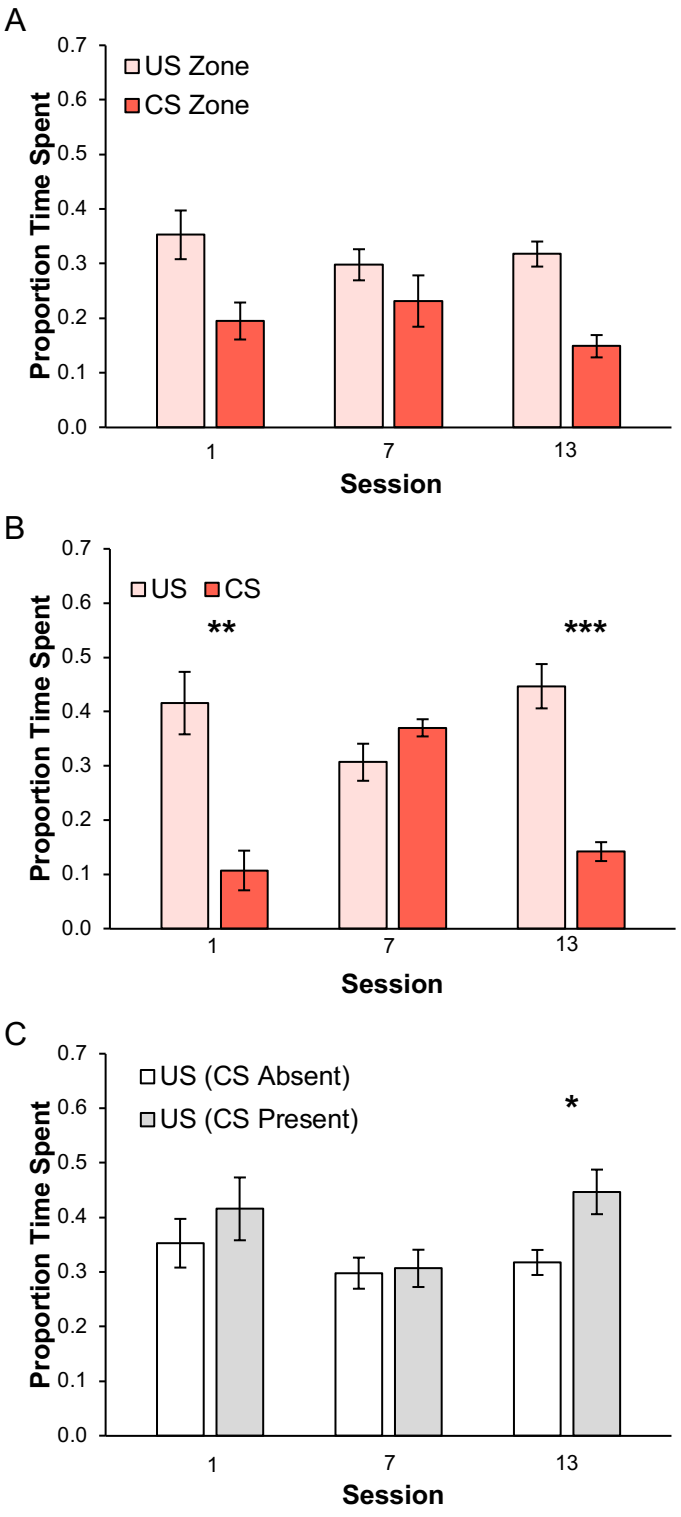


Figure 10. Sucrose sign-trackers shift toward a goal-tracking phenotype in response to a sexually-conditioned cue (*n* = 7). (A) Mean (± SEM) proportion of time spent in the US Zone (light bar) and CS Zone (dark bar) in the absence of the CS on sessions 1, 7 and 13. (B) Mean (± SEM)

proportion of time spent near the US (light bar) and CS (dark bar) in the presence of the CS on sessions 1, 7 and 13, *$p < 0.05$. (C) Mean (± SEM) proportion of time spent in the US Zone when the CS was absent (white bar) and present (grey bar) on sessions 1, 7 and 13, ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

Pavlovian-conditioned approach in response to a sexually-conditioned cue in sucrose intermediates. In order to investigate whether intermediate behaviour for a sucrose-paired CS developed in response to a sexually-conditioned cue, the mean proportion of time spent in the US and CS Zones (CS absent) was compared in sucrose intermediates across sessions 1, 7 and 13 (Figure 11A). A repeated-measures ANOVA on time spent in the US and CS Zones, in the absence of the CS, revealed that subjects spent comparable proportions of time in each Zone across sessions, as there were no significant main effects of Session, F (2, 4) = 5.097, p = 0.079 or Zone, F (1, 2) = 4.487, p = 0.168, nor a significant Session x Zone interaction, F (2, 4) = 2.979, p = 0.161. In the presence of the CS (Figure 11B), a significant main effect of Zone, F (1, 2) = 19.884, p = 0.047, η_p^2 = 0.22, suggests that sucrose intermediates showed an overall preference in spending time near the US (M = 0.32, SD = 0.01) compared to the CS (M = 0.24, SD = 0.01), however there was no main effect of Session, F (2, 4) = 4.241, p = 0.103 nor a Session x Zone interaction, F (2, 4) = 0.813, p = 0.505. Therefore, sucrose intermediate subjects continued to display an intermediate response in the presence of a sexually-conditioned cue.

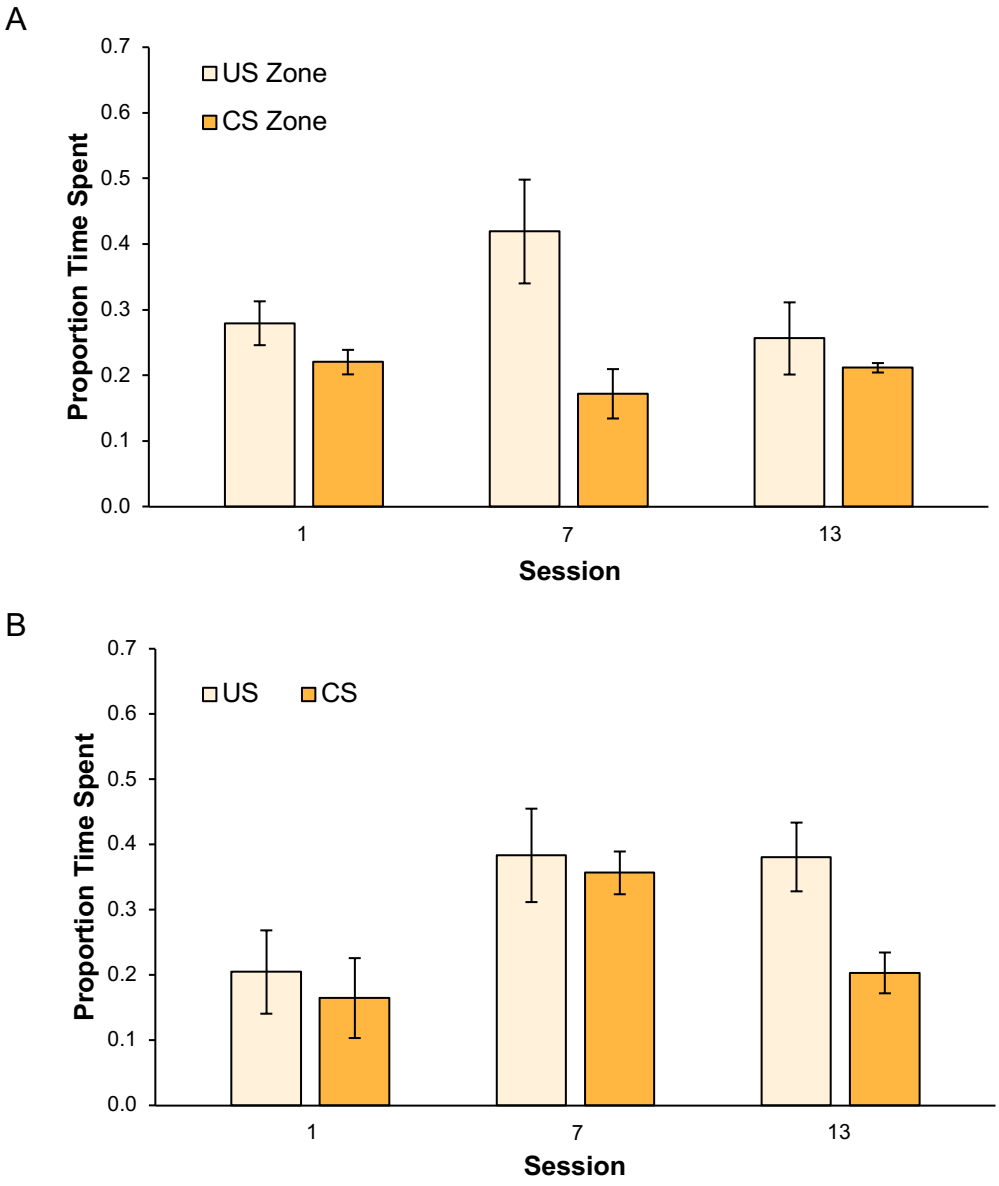


Figure 11. Sucrose intermediate subjects continue to express intermediate behaviour in response to a sexually-conditioned cue (*n* = 3). (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence of the CS on sessions 1, 7 and 13. (B) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the presence of the CS on sessions 1, 7 and 13.

Discussion

Individual differences in PCA displayed as sign-, goal-tracking and intermediate responses have been extensively studied using food and drug reward (Saunders & Robinson, 2010; Yager & Robinson, 2013; Villaruel & Chaudhri, 2016), and Chapter 2 of this thesis identified the development of both goal-tracking and sign-tracking using a sexual conditioning paradigm. Due to the variable frequencies of these phenotypes in the two experiments in Chapter 2, we sought to use a well-established sucrose conditioning paradigm to identify sign-, goal-tracking and intermediate behaviours prior to sexual conditioning, and this allowed us to investigate whether individual differences in PCA responses extend across different types of natural reward. Here, in a sucrose-conditioning paradigm measuring PCA behaviours, we found clear development of goal-tracking behaviour directed toward the fluid port and sign-tracking behaviour directed toward the lever-CS, and only a few subjects displayed intermediate behaviour. In our sexual conditioning paradigm measuring PCA, we then observed no sign-tracking toward the sexually-conditioned cue, and a robust expression of goal-tracking toward the door that provided access to the sexually-receptive female. Specifically, animals classified as sucrose sign-trackers did not display cue-directed behaviour in the sexual conditioning paradigm; rather, in the presence of the CS, they exhibited goal-directed behaviour reflected as a greater proportion of time spent near the US as compared to the CS during the final sexual conditioning session. In contrast, animals classified as sucrose goal-trackers showed a tendency to spend a greater proportion of time near the US, but this did not reach statistical significance, and therefore they displayed intermediate-like responses. Importantly, these data are the first to indicate that the behavioural phenotype expressed in response to a sucrose-paired cue may not extend to a sex-paired cue, revealing poor transference and differences in the basic mechanisms of conditioning to various types of natural reward.

The expression of sign-, goal-tracking and intermediate behaviours in response to a sucrose-paired cue

In the sucrose conditioning paradigm, our results revealed the development of individual differences in the attribution of incentive salience to a sucrose-paired CS. Specifically, we identified clear subsets of goal- and sign-trackers, and only a minority of animals displayed intermediate responses, which is consistent with previous studies using food and drug reward (Saunders & Robinson, 2010; Yager & Robinson, 2013; Villaruel & Chaudhri, 2016; Flagel et al., 2010; Yager, Pitchers et al., 2015). First, the tendency to sign- and goal-track was established for individual rats using a response bias score in early, middle, and late blocks of sucrose conditioning. In studies using a food pellet US, a PCA index is typically used to establish each

behavioural phenotype (Fitzpatrick & Morrow, 2016). However, Villaruel and Chaudhri (2016) suggest the use of response bias scores with a liquid US due to differences in the strength of conditioned responses that have been observed with solid and liquid reinforcers (Davey & Cleland, 1982), which can ultimately influence the PCA index. Therefore, 13 subjects were classified as goal-trackers, 7 subjects were identified as sign-trackers, and 3 subjects were characterized as intermediates based on their individual response bias scores in a sucrose conditioning paradigm.

An analysis of subjects' patterns of lever-CS contacts and normalized port entries indicated marked differences amongst these behavioural phenotypes, which is consistent with studies using a liquid US such as sucrose and ethanol (Morrison et al., 2015; Srey et al., 2015; Villaruel & Chaudhri, 2016). In comparison to sign-trackers, goal-trackers minimally approached and engaged with the lever-CS; they made significantly fewer lever-CS contacts and displayed longer latencies to make a lever-CS contact. In contrast, goal-trackers both approached and engaged with the fluid port where sucrose was delivered, as evidenced by increases in the number of normalized port entries across sucrose conditioning sessions and shorter latencies to make a port entry. Alternatively, sign-trackers both engaged with and approached the lever-CS, as lever-CS contacts increased across sucrose conditioning sessions, and they also displayed shorter latencies to make a lever-CS contact compared to goal-trackers. Animals identified as sign-trackers also made fewer normalized port entries and displayed longer latencies to make a port entry compared to goal-trackers. Rats classified as intermediates displayed neither a consistent goal- nor sign-tracking response pattern; though they displayed shorter latencies to make a port entry compared to sign-trackers, they made fewer normalized port entries compared to goal-trackers. Similar to goal-trackers, intermediate subjects also made fewer lever-CS contacts and displayed longer latencies to make a lever-CS contact compared to sign-trackers.

Our data also indicate that normalized port entry behaviour, indicative of a goal-tracking response, was acquired more rapidly compared to lever-CS contacts which indicate a sign-tracking response, which has also been reported by other studies using sucrose reward (Morrison et al., 2015). Overall, the increases in port entries and reduction in latencies to make a port entry were mainly observed across the first four sucrose conditioning sessions. The maximal number of normalized port entries occurred during sessions 5 and 6 in intermediate and sign-tracking subjects, respectively, and diminished and stabilized between sessions 8 to 16, whereas goal-trackers displayed a steady, increasing pattern of normalized port entries which was maximized by the end of training. In contrast, the overall acquisition pattern of lever-CS contacts developed steadily across sucrose conditioning sessions, which reflects a gradual associative learning of the

CS-US relationship. However, phenotypic differences emerged across training; sign-trackers displayed the highest number of, and greatest increases in, the number of lever-CS contacts, with shorter latencies to make a lever-CS contact, whereas goal-trackers consistently made very few lever-CS contacts and displayed longer latencies. Such phenotypic differences in the acquisition patterns of normalized port entries (i.e., goal-tracking) are consistent with previous studies using ethanol and sucrose, as mentioned previously (Morrison et al., 2015; Srey et al., 2015; Villaruel & Chaudhri, 2016). For example, following repeated pairings of a lever-CS and ethanol delivery, Villaruel and Chaudhri (2016) reported the development of cue- and goal-directed conditioned responses. Specifically, sign-trackers approached and engaged with the lever-CS and displayed shorter latencies to perform lever-CS contacts, whereas goal-trackers approached the fluid port where ethanol was delivered and displayed shorter latencies to make a port entry. Furthermore, each conditioned response was acquired early in training and remained stable across 24 sessions using an ethanol US, which is comparable to our subjects' acquisition patterns with sucrose reward. Lastly, we identified subset of intermediate subjects that fluctuated between cue- and goal-directed conditioned responses and never reached the level of responding observed in goal- and sign-trackers, which is in accordance with studies using food and drug reward (Flagel et al., 2008; Yager & Robinson, 2013; Srey et al., 2015). Interestingly, intermediate subjects also showed a steady delay in the onset and growth in latency and lever-CS contacts measure, which suggests that they form similar associations with the lever-CS compared to sign-trackers, but that this may be a slower process. Accordingly, Flagel et al. (2009) has reported that intermediate subjects may express sign-tracking behaviour toward a food-paired cue with extended training, though this develops more slowly and is less robust compared to animals that are initially classified as sign-trackers.

The expression of sign-, goal-tracking and intermediate behaviours in response to a sucrose-paired cue does not extend to a sex-paired cue

Next, we compared sucrose subjects' behavioural phenotype to the expression of PCA following sexual conditioning in order to determine whether sign- and goal-tracking persists across different types of natural reward. We found that sucrose goal-trackers (n = 11) spent comparable proportions of time in the US and CS Zones across sexual conditioning sessions. Though we also note a statistical trend for sucrose goal-trackers to spend more time near the US compared to the CS when the CS was present, our data suggest that these subjects displayed intermediate-like responses as this effect did not reach statistical significance. Interestingly, in sucrose sign-trackers (n = 7), we found a strong and statistically significant correlation between the response bias scores calculated for sucrose and sexual reward; specifically, all subjects response bias

scores moved from the sign-tracking toward the goal-tracking end of the response bias score continuum. Furthermore, upon inspection of individual data points, we note that animals on the lower end of sign-tracking for sucrose show the highest goal-tracking response for sexual reward, and animals on the higher end of sign-tracking for sucrose show the lowest goal-tracking response for sexual reward. Sucrose sign-trackers appeared to 'shift' their phenotype as they learned the associative CS-US relationship during sexual conditioning. Though subjects showed an initial preference for the US area on the first session of conditioning, they spent comparable proportions of time near the CS and US by the middle of training, suggesting that the cue may have started to acquire incentive salience due to its relationship with the US. However, by the final session of conditioning, sucrose sign-trackers once again spent more time near the US compared to the CS, indicating a final shift toward a goal-directed phenotype.

The existing literature has largely focused on comparing the expression of PCA between food and drug reward. Previously, studies have suggested that behavioural phenotypes are consistent and stable between food and cocaine; for instance, subjects identified as sign-trackers for food reward also attribute incentive salience to a cocaine-paired cue in both self-administration (Saunders & Robinson, 2010) and Pavlovian conditioning paradigms (Yager & Robinson, 2013). However, such findings compare natural to 'unnatural' reward; to our knowledge, we are the first to measure responses to two different natural rewards and to include sexual reward. Interestingly, our findings differ from Saunders and Robinson (2010) and Yager and Robinson (2013), suggesting that the stability of behavioural phenotype may be specific to reward type. Though food, drug and sexual reward share common reward networks (Berridge & Kringelbach, 2015), they are coded by specific neurons based on object value, action value, difference value and chosen value, and therefore differ in their subjective reward value and motivational properties (Schultz, 2015). For example, physiological drive states play an important role in incentive motivation and can be characterized as aversive or appetitive drive states. Therefore, the consumption of food, water and drug reduces the aversive, internal state of hunger, thirst, and craving, respectively (Kelley & Robinson, 2002; Volkow & Fowler, 2000).

Though Saunders and Robinson (2010) and Yager and Robinson (2013) did not use food deprivation, the US was not a standard rat chow food pellet. Instead, they used banana-flavoured food pellets, a form of sucrose pellet made from dextrose, sucrose, and corn syrup (BioServe, #F0059, Frenchtown, NJ, USA), which may induce behavioural responses that model 'craving' given the addictive-like properties of sucrose (Hoebel et al., 2009; de Macedo et al., 2016; Grimm et al., 2005). Though highly debated, several researchers propose significant overlap between intermittent sucrose consumption and drug addiction in its ability to produce addiction-like

behaviours such as bingeing, craving, tolerance, and withdrawal (Avena et al., 2008; DiNicolantonio et al., 2017; Kelley, 2004; Levine et al., 2003; Volkow & Wise, 2005) and that intense sweetness can even surpass cocaine reward (Lenoir et al., 2007). Therefore, the shared behavioural effects between sucrose and drug intake may explain the consistency in PCA for sucrose and cocaine reward.

In contrast, Singer and Toates (1987) propose that 'drive' refers to a metabolic disturbance, which creates an undesirable sensation, and can motivate behaviour in the absence of an incentive stimulus. Furthermore, the authors argue that sexual arousal does not produce an aversive state, rather, they characterize sexual arousal as an appetitive urge as *no organism would perform an arbitrary operant to dispel sexual arousal.* Accordingly, Both et al. (2007) have also argued against sexual motivation as an aversive, intrinsic drive state. Here, the authors suggest that sexual motivation does not develop through a deficit processed by the hypothalamus. Rather, they propose that sexual motivation evolves through the attractiveness of potential external rewards in one's environment; sex is not viewed as a biological need that requires satisfaction, in the same way as hunger and thirst. Therefore, it may be that the greater phenotypic variability in sign- and goal-tracking in a sexual conditioning paradigm reflects the appetitive nature of sexual motivation, as opposed to the consumption of food and drug reward which is induced by an aversive state of drive reduction.

The variability between sign- and goal-tracking for sucrose- and sex-paired cues may be affected by variations in incentive motivation, the initial value attributed to reward, and homeostatic changes and satiety following consumption. For example, both hunger and craving shape the reward system to increase the value and salience of food and drug, respectively, and can enhance behavioural responses to food- and drug-paired cues. Contrarily, satiety can reduce reward system sensitivity, resulting in a reduction of the value and salience of both food and non-food rewards, such as drugs or sex (Cassidy & Tong, 2017; Volkow & Fowler, 2000). Likewise, sexual desire and satiety operate similarly in their ability to influence the sensitivity of the reward system, value, and the incentive properties attributed to sexual reward (Phillips-Farfán & Fernández-Guasti, 2008). Therefore, it is important to consider differences in food versus sexual satiety when interpreting our results for sucrose and sexual conditioning and those obtained by Saunders and Robinson (2010) and Yager and Robinson (2013). For instance, many enzymes and hormones serve as satiety signals, including cholecystokinin (CCK), a peptide hormone, which is released from the gastrointestinal tract during meal consumption to produce sensations of fullness and satiety (Woods, 2004). Furthermore, protein, in the form of casein (an ingredient found in banana-flavoured food pellets) and sucrose have been shown to stimulate the pancreatic

release of CCK in rats (Douglas et al., 1988; Belissimo & Anderson, 2003). Lastly, the stimulation of CCK-A receptors by systemic and intracerebroventricular administration of a CCK-A receptor agonist has been shown to suppress food intake in both food-deprived and sated rats (Asin et al., 1992). Interestingly, this effect was observed within 15-minutes, suggesting that the satiety signal induced by CCK may occur within this timeframe. In contrast, sexual satiety, or the inhibition of mating behaviour following ejaculation, is dependent on the amount of sexual behaviour leading to satiety and the number of ejaculations (Phillips-Farfán & Fernández-Guasti, 2009). Specifically, studies show that male rats allowed to copulate *ad libitum* will experience an average of seven ejaculations and reach sexual satiety within a 4-hour timeframe (Rodríguez-Manzo & Fernández-Guasti, 1994). Therefore, it is possible that the 'shift' from sucrose sign-tracking to goal-tracking following sexual conditioning may be explained by the prolonged period needed to reach sexual satiety, resulting in a preoccupation toward the sexually-receptive female. Collectively, the differences in incentive value, motivation, physiological drive state and the time to reach food and sexual satiety may clarify the variance in phenotypes observed in our study and may also contribute to variations between PCA behaviours observed in our experiment compared to Saunders and Robinson (2010) and Yager and Robinson (2013).

Importantly, methodological differences may also explain inconsistencies between our data and the aforementioned studies (Saunders & Robinson, 2010; Yager & Robinson, 2013), as the characteristics of a CS can influence the form of a conditioned response (e.g., stimulus modality, manipulability; Holland, 1977). As in other food-, sucrose- and drug-reward studies (Yager & Robinson, 2010; Saunders & Robinson, 2010; Yager & Robinson, 2013; Villaruel & Chaudhri, 2016; Srey et al., 2015), we used a manipulable lever in our sucrose conditioning paradigm, which has been shown to act as an effective conditioned reinforcer and produce conditioned motivation (Meyer et al., 2014). In our sexual conditioning paradigm, however, an object served as the conditioned stimulus, and was placed in the region of the testing chamber opposite to the goal area. An object was selected as a CS based on its sensory properties (e.g., visual, tactile), and on the basis of findings that male Japanese quail predominantly display sign-tracking in response to an object-CS paired with sexual reward (Burns & Domjan, 1996). Though the inconsistencies between our data and Burns and Domjan (1996) may be species-specific, it may be useful to examine how the nature of the CS may affect its efficacy in producing a conditioned response. For instance, Holland and Rescorla (1975) have suggested that one measure of the association between a CS and US is the degree to which the CS can serve as a reinforcer in a second-order conditioning paradigm. Though an early study by Zamble et al. (1985) found that both a light and visuo-tactile CS (i.e., plastic toy fish) can establish a second-order

conditioned response when paired with non-contact sexual arousal, it would be important to measure whether a visuo-tactile CS can induce second-order conditioning with the additive effect of ejaculation in our paradigm. Everitt and Stacey (1987) report that instrumental responding for food or the opportunity to ejaculate with a sexually-receptive female is acquired at comparable rates, but that rats working for food under a second-order schedule tend to do so at a higher rate and more reliably compared to makes working for sexual reward. Therefore, it is possible that the nature of the CS and US may contribute to phenotypic variability in our experiment compared to studies using food-paired stimuli.

Conclusions

Due to inconsistencies in the expression of sign- and goal-tracking observed in Chapter 2, the current study first aimed to identify clearer PCA phenotypes using a sucrose conditioning paradigm and then investigated whether individual differences in PCA extended from sucrose to sexual reward. In a sucrose conditioning paradigm, we identified clear subsets of sign- and goal-trackers, as evidenced by their approach and engagement with the lever-CS (i.e., cue-directed) and fluid port (i.e., goal-directed), respectively, and a small number of intermediate subjects who displayed both conditioned responses. Following sexual conditioning, sucrose goal-trackers showed a tendency to spend more time near the US, though this did not reach statistical significance, and therefore their behaviour was described as intermediate-like. Sucrose sign-trackers appeared to have ‘shifted’ their phenotype, as they spent more time near the US-designated area compared to the CS. In individual subjects then, the expression of PCA phenotypes following sucrose conditioning did not predict PCA phenotypes for sexual reward. This may be due to variations in incentive motivation, the initial value attributed to reward, homeostatic changes and satiety following consumption or the nature of the CS. Future studies could include assessing whether the characteristics of the CS are sufficiently salient in eliciting PCA using a second-order conditioning paradigm. Furthermore, it may be useful to explore alternative manipulations to ‘stabilize’ the expression of phenotypes in response to a sexually-conditioned cue. For example, the neuropeptide oxytocin has been shown to enhance the acquisition of conditioned ejaculatory preferences for olfactory stimuli (Ménard et al., 2019). Therefore, it may be important to explore whether hormonal or neurochemical manipulations can potentiate the acquisition and expression of sign- and goal-tracking phenotypes during sexual conditioning.

Chapter 4: The effects of oxytocin administration on the expression of sign-tracking and intermediate behaviour in response to a sexually-conditioned cue in the male rat

Abstract

Repeated pairings of a conditioned stimulus (CS) with sexual reward (unconditioned stimulus; US) can result in Pavlovian-conditioned approach behaviours directed either towards the CS (sign-tracking) or US (goal-tracking). Intermediate subjects can show moderate approach behaviours toward both the US and the CS. Oxytocin enhances the acquisition of conditioned ejaculatory preference for olfactory stimuli, but it is not known whether oxytocin can potentiate cue- or goal-directed behaviours in a sexual conditioning paradigm. We examined whether oxytocin alters the expression of PCA toward a visuo-tactile cue in sign-, goal-tracking, and intermediate rats. Sexually-naïve, male Long-Evans rats received 13 Pavlovian conditioning sessions in one compartment of an open field chamber, where an orange cone CS (2-minute/presentation) predicted copulation to ejaculation in a separate compartment with a receptive female (US). On sessions 7-13, rats received repeated subcutaneous injections of saline or oxytocin (5 μg/kg) 4-hours, 2-hours, and 15-minutes prior to the start of the session. Sign- and goal-tracking were measured by the proportion of time spent in an area centered around the CS or near the door to the female compartment, respectively, both in the absence and presence of the cue. A subset of animals (*n* = 11) displayed sign-tracking behaviour, as they consistently spent a greater proportion of time near the CS compared to the US area, but the administration of oxytocin did not appear to further potentiate cue-directed responses in these subjects. A subset of animals (*n* = 11) showed intermediate response patterns, as they spent comparable proportions of time near the US and CS areas. Interestingly, the administration of oxytocin appears to have potentiated goal-directed responses in these animals, as they spent a greater proportion of time near the US area compared to saline-treated intermediate subjects. Overall, these findings provide further evidence that conditioned cues can acquire incentive motivational properties through Pavlovian-conditioning when paired with sexual reward leading to ejaculation. Furthermore, oxytocin may be implicated in the formation of Pavlovian associations between the CS and US and may shift intermediate subjects toward a goal-directed phenotype.

Introduction

The ability to establish predictive associations between environmental stimuli and rewarding outcomes is an important feature of learned behaviour and forms the basis of Pavlovian conditioning (Fanselow & Wassum, 2015). Traditionally, Pavlovian conditioning involves two distinct stimuli; a neutral stimulus, and an unconditioned stimulus (US) which may be aversive or appetitive. The US represents a biologically significant stimulus, in that it elicits a response naturally, without behavioural training. Following repeated pairings with the US, the neutral stimulus transforms into a conditioned stimulus (CS) and is capable of eliciting a conditioned response similar to the unconditioned response that is prompted by the US (Domjan, 2005). In the laboratory, Pavlovian conditioning and the study of conditioned stimuli have been widely applied to the investigation of fear, hunger, drug use and sexual behaviour, in humans and non-humans (Schachtman & Reilly, 2011).

Due to its associative relationship with a rewarding US, a CS can induce conditioned responding in the form of engagement and approach behaviours, referred to as Pavlovian-conditioned approach (PCA). Furthermore, PCA behaviours have been used to determine whether a CS has acquired incentive motivational properties, in which the CS becomes a 'motivational magnet' and is 'attractive' or 'wanted' much like the US (Fitzpatrick & Morrow, 2016; Berridge, 1996; Berridge, 2001). Several studies involving conditioning with food or drug rewards have demonstrated individual differences in the development of PCA. For example, in rats, only a subset of subjects demonstrates increases in cue-directed behaviour by preferentially approaching and engaging with the CS as it acquires incentive salience, and this conditioned response is referred to as sign-tracking (Hearst & Jenkins, 1974). In contrast, other rats display goal-directed behaviour; they infrequently approach and engage with the CS, and instead, when the CS is presented, will approach the area where the US is delivered. This conditioned response is referred to as goal-tracking (Boakes, 1977). Lastly, a proportion of subjects vacillate between cue- and goal-directed responses and are referred to as intermediates (Fitzpatrick & Morrow, 2016). Importantly, though they present differently, both sign- and goal-tracking are learned conditioned responses to the same cue; the CS is equally predictive of impending reward in both sign- and goal-trackers. However, the expression of these behavioural phenotypes can be differentiated based on incentive motivation and the cognitive expectation of reward; in sign-trackers, the CS is imbued with incentive salience, and in goal-trackers the CS carries informational properties that signal reward availability (Flagel et al., 2009; Toates, 1997).

In rodents, the development of sign-, goal-tracking and intermediate responses has been widely observed using food- and drug-paired cues, and general findings indicate that subject

samples include approximately one-third of each phenotype (Flagel et al., 2009). Furthermore, these responses have also been shown to extend from food to drug reward in both self-administration and Pavlovian paradigms (Saunders & Robinson, 2010; Yager & Robinson, 2013). Comparatively, we have found more variable incidences of sign- and goal-tracking responses when cues are paired with sexual reward; for example, we reported 8 sign-trackers and 4 intermediates, and 6 goal-trackers and 5 intermediates across two separate experiments in Chapter 2. Though we did not categorize subjects based on their responses to a sexually-conditioned cue in Chapter 3, we observed an overall tendency toward goal-tracking (n = 7) and intermediate responses (n = 16). Therefore, we have determined that both sign- and goal-tracking can occur using a sexual conditioning paradigm in the rat, however the frequencies of these phenotypes appear to be more variable, and the incidence of sign-tracking less robust.

Although the expression of sign- and goal-tracking for sexual reward is more variable in male rats in comparison to food and drug reward, it is likely that there exist commonalities in the brain reward circuitries that mediate rewarding stimuli, conditioned cues, and approach behaviours (Flagel & Robinson, 2017). Pavlovian associations depend on the interaction of neural reward circuits and their collective ability to integrate both sensory and motivational information to produce behavioural outcomes. The mesocorticolimbic projection, which includes the mesolimbic and mesocortical dopaminergic pathways, is thought to be central to the development of Pavlovian associations (Salamone & Correa, 2012; Flagel et al., 2010; Berridge, 2012). The mesolimbic pathway contains projections of dopamine neurons from the ventral tegmental area to the ventral striatum, which is composed of the nucleus accumbens core and shell regions and the olfactory tubercle and is strongly implicated in reward-related cognition (e.g., motivation, incentive salience; Nestler et al., 2015; Berridge & Kringelbach, 2015). In addition, the mesocortical dopamine pathway features projections of dopamine neurons from the ventral tegmental area to the prefrontal cortex and is strongly implicated in cognitive/executive functions such as associative learning, attention, planning, and working memory (Yager, Garcia et al., 2015; Kokane & Perrotti, 2020). These two dopaminergic pathways are believed to work in tandem to produce an integrative response to conditioned cues and rewarding stimuli. Specifically, it is hypothesized that dopamine is involved in attributing incentive salience from rewarding stimuli to conditioned cues via the mesolimbic projection from the ventral tegmental area to the shell region of the nucleus accumbens (Halbout et al., 2019), and that dopamine is also involved in updating the value of different goals based on these experiences via the orbital prefrontal cortex through the mesocortical pathway (Nestler et al., 2015). Dopamine is also thought to support memory consolidation of the CS-US association via the amygdala and hippocampus. These structures

are innervated by the ventral tegmental area, and can help encode new motor responses (e.g., approach behaviours) to facilitate future reward acquisition through the nucleus accumbens core subregion and dorsal striatum (Nestler et al., 2015).

The contribution of mesocorticolimbic dopamine in the development of PCA has been well-established (Cardinal & Everitt, 2004; Day & Carelli, 2007; Everitt & Robbins, 2005; Tomie et al., 2008), and several studies suggest that the tendency to attribute incentive salience to conditioned stimuli (i.e., sign-tracking) is linked to individual differences in dopaminergic projections from the ventral tegmental area to the nucleus accumbens core, specifically. For example, excitotoxic lesions of the nucleus accumbens core have been shown to severely impair the acquisition and expression of sign-tracking behaviour (Cardinal et al., 2002), and flupenthixol, a dopamine D1- and D2-receptor antagonist, significantly attenuates sign-tracking when infused into the nucleus accumbens core (Fraser & Janak, 2017). Furthermore, the development of cue- versus goal-directed Pavlovian-conditioned responses may cause distinct adaptations in the dopaminergic pathways. In one study, Flagel et al. (2007) sought to determine whether variations in gene expression may contribute to the development of sign- and goal-tracking responses, and, conversely, how the Pavlovian conditioning process may affect dopamine receptor expression by comparing dopamine D1- and D2-receptor mRNA levels on training sessions 1 and 5. Here, subjects were presented with an illuminated retractable lever (CS) followed by the response-independent delivery of a food pellet (US), and *in situ* hybridization was performed on brain tissue samples collected either on the first or fifth training session. As expected, a subset of subjects approached and engaged with the lever-CS (i.e., sign-tracking), whereas other subjects preferentially approached the area where the food pellet was delivered (i.e., goal-tracking). Following session 1, the authors reported that sign-trackers displayed elevated levels of dopamine D1-receptor mRNA in the nucleus accumbens core relative to goal-trackers, suggesting that the increased expression of dopamine D1-receptor mRNA may contribute to the development of cue-directed behaviour. Alternatively, after 5 training sessions, goal-trackers showed an elevated expression of tyrosine hydroxylase (i.e., an enzyme necessary for dopamine synthesis), dopamine transporter, and dopamine D2-receptor mRNA in comparison to sign-trackers, suggesting that the Pavlovian conditioning process may lead to distinct changes to dopamine receptor gene expression. Importantly, these data also suggest a differential role for dopamine D1- and D2-receptors in the development of sign- and goal-tracking behaviours, respectively.

Though dopamine is perhaps the most extensively studied neurotransmitter linked to motivation and reward, the neuropeptide oxytocin also collaborates with the neural pathways responsible for the processing of motivationally-relevant stimuli (Love, 2014). Oxytocin is first

synthesized in the paraventricular nucleus and supraoptic nucleus of the hypothalamus, and then transported within the long axons of these neuroendocrine cells for storage and release from the posterior pituitary into peripheral circulation (Nestler et al., 2015; Breedlove & Watson, 2020). Centrally, oxytocin neurons send axonal projections from the paraventricular nucleus of the hypothalamus to the amygdala, hippocampus, nucleus accumbens and ventral tegmental area (Ross & Young, 2009; Beier et al., 2015), and the activation of oxytocin neurons that project to the ventral tegmental area are thought to stimulate the release of dopamine in the mesocorticolimbic pathway (Melis et al., 2007; Melis et al., 2009; Succu et al., 2011). Oxytocin has been shown to play a central role in social behaviours (e.g., social memory and recognition, affiliation, sexual behaviour, aggression) and non-social behaviours (e.g., learning and memory; Lee et al., 2009), and it is hypothesized that oxytocin may increase motivation and the incentive salience of reward-related cues by modulating dopaminergic activity (Burkett & Young, 2012). For example, the administration of oxytocin has been shown to heighten attention toward social cues, the cognitive processing of social information and the amount of effort devoted to engaging in social behaviour (Love, 2014) by increasing reward sensitivity (Bethlehem et al., 2014; Strathearn, 2011) which is consistent with the known effects of oxytocin in enhancing dopaminergic activity in the mesolimbic system (Fitzpatrick & Morrow, 2020).

There is substantial evidence indicating that oxytocin may play an important role in sexual conditioning. For example, both male and female rats can be conditioned to display a sexual partner preference toward a mate bearing a neutral scent such as lemon or almond odour (Kippin et al., 1998; Coria-Avila et al., 2005), and males have been shown to exhibit a conditioned ejaculatory preference toward an olfactory cue when paired with the ejaculatory reward state (Kippin et al., 2001a, 2001b). For example, studies using the immediate-early gene c-Fos as a marker of neuronal activation suggest that the conditioned ejaculatory preference is mediated through an interaction of the mesocorticolimbic dopamine pathway and brain areas known to synthesize oxytocin. Specifically, neural pathways implicated in bonding and incentive motivation such as the nucleus accumbens core, olfactory tubercle, main (piriform) cortex, lateral hypothalamus, paraventricular nucleus and supraoptic nucleus of the hypothalamus and basolateral amygdala show significantly greater activation following exposure to a conditioned olfactory stimulus in paired versus unpaired male subjects (Kippin et al., 2003). Importantly, the activation of the paraventricular nucleus and supraoptic nucleus of the hypothalamus could reflect the involvement of oxytocin in sexual conditioning, as these regions contain cell bodies of oxytocin neurons that project to the posterior pituitary and other brain regions such as the ventral pallidum (Ménard et al., 2019; Groenewegen et al., 1999), an area involved in motivational salience and

reward via dopaminergic inputs from the ventral tegmental area (Smith & Kieval, 2000). The involvement of oxytocin neurons in sexual conditioning is further supported by findings that exposure to an olfactory cue paired with the ejaculatory reward state produces significantly greater activation of oxytocin neurons in the paraventricular nucleus of the hypothalamus compared to rats that ejaculate with an unscented female (Ménard et al., 2019). Furthermore, the administration of systemic oxytocin appears to enhance the acquisition of a conditioned ejaculatory preference for females bearing an odour paired with sexual reward, likely by enhancing the association between the olfactory cue and the ejaculatory reward state (Ménard et al., 2019).

The effectiveness of oxytocin in strengthening the development of a conditioned ejaculatory preference to an olfactory cue raises the possibility that oxytocin may also promote the sexual conditioning of a visuo-tactile cue that predicts access to a sexually-receptive female. Previously, we have shown that male rats can develop both goal- and sign-tracking behaviours following pairings of a cone with a sexually-receptive female, however the incidence of these phenotypes varied across experiments. If oxytocin promotes the conditioning of incentive salience through its interaction with the mesocorticolimbic dopamine pathways, and if this extends toward visuo-tactile cues, then the systemic administration of oxytocin during sexual conditioning might facilitate the development of a sign-tracking response. Therefore, in this study, male rats underwent sexual conditioning, and were identified as displaying either a goal-, sign-tracking or intermediate phenotype prior to the administration of oxytocin. Subjects then received injections of either saline or oxytocin for the remaining sexual conditioning sessions, and changes in the development of each phenotype was assessed.

Method

Subjects

Sexually-naïve male (225-250 g) and female (150-200 g) Long-Evans rats were obtained from Charles River Canada, Inc. (St-Constant, QC, Canada). All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee. Groups of four male subjects were housed in polycarbonate (Plexiglas) gang-cages containing beta-chip. Female rats were pair-housed in polycarbonate shoebox cages containing beta-chip and corncob bedding. All subjects were kept in an animal colony room on a reversed dark/light cycle (lights OFF at 8:00 AM) and all procedures were conducted during the dark phase. The animal colony room was maintained at a constant temperature of 21 ºC. Subjects had *ad libitum* access to water and rat chow (Charles River Rodent Animal Diet, St-Hubert, QC, Canada) for the duration of the experiment.

Female subjects underwent bilateral ovariectomy via lumbar incisions following anesthesia induced by intraperitoneal injections of a mixture of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) injected with a volume of 1 ml/kg of body weight. Following surgery, females were given 1 week for recovery. Subjects were then maintained on hormone replacement for the duration of the experiment using subcutaneous injections of estradiol benzoate (10 µg in 0.1 ml of sesame oil) administered every 48-hours, and progesterone (500 µg in 0.1 ml sesame oil) 3 to 4 hours prior to the start of the conditioning session. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Apparatus

Behavioural conditioning sessions were conducted in an open field chamber (120 L x 120 W x 60 D cm), which featured two compartments separated by an opaque polycarbonate divider. The divider included a sliding door (10 cm) which slid open vertically by pulling on a 4.5 m nylon cord to allow access between compartments. The conditioned stimulus (i.e., orange cone; CS) was presented in the larger compartment of the open field (120 L x 80 W x 60 D cm), and copulation occurred in the smaller compartment of the open field (120 L x 41 W x 60 D cm). Two pre-determined areas were used to assess differences in PCA within the larger compartment; the US Zone (38 W x 38 L cm, i.e., goal-directed behaviour) was located in front of the sliding door, and the CS Zone (38 W x 38 L cm, i.e., cue-directed behaviour) included the area surrounding the CS, which was presented in the opposite, diagonal corner relative to sliding door. In order to control for place preferences, the locations of the US and CS Zones were alternated across experimental sessions. An orange cone (23 H x 14 W x 14 L cm) served as the CS and was presented for a 2-minute period after which the sliding door was opened to provide access to a sexually-receptive female.

All conditioning sessions were recorded using a video camera (Sony Handy Cam, model DCR-SR68), and were then scored by trained experimenters. Both the US and CS Zones were marked on an acetate sheet, which was secured to a computer during scoring to ensure that behavioural measures were scored consistently across sessions.

Drug preparation and administration

Reagent grade oxytocin (Bachem, H2510) was dissolved in a 0.9% saline solution to obtain a dose of 5 µg/ml, which was administered subcutaneously (1 ml/kg of body weight) 4-hours, 2-hours, and 15-minutes prior to conditioning sessions. An equal volume of saline solution was administered to animals in the control group. The drug dose and injection schedule were selected based on a previous report demonstrating the effectiveness of oxytocin in inducing a

conditioned ejaculatory preference toward a female bearing an olfactory cue (Ménard et al., 2019).

Procedure

Subjects were given two daily 20-minute sessions in the larger compartment of the open field apparatus in order to habituate to the environment. Next, a total of 13 behavioural conditioning sessions were conducted, scheduled once every four days over the course of a seven-week period. Each conditioning session included two individual trials in which a total of two ejaculations could be obtained.

In order to habituate male subjects to subcutaneous injections, sham saline injections were administered 15-minutes prior to the start of behavioural conditioning sessions 1-6. On sessions 7-13, males received either an injection of oxytocin (5 µg/ml/kg) or saline vehicle, which was administered 4-hours, 2-hours, and 15-minutes prior to the start of the conditioning session.

Each conditioning session began with a 5-minute habituation period during which the male subject could navigate the larger compartment in the absence of the CS and sexually-receptive female. During this time, the proportions of time spent in the US and CS Zones were assessed in order to obtain a baseline behavioural measure. Next, the orange cone that served as the CS was placed in the center of the CS Zone for a 2-minute period, and the proportions of time spent near the US and CS were recorded. Following the CS presentation, the door separating the two compartments was slid open to enable the male to enter the smaller compartment. Once the male was inside the smaller compartment, the sexually-receptive female rat was placed into the compartment, and the pair copulated until the male reached ejaculation. The male subject's ejaculation and subsequent post-ejaculatory interval functioned as the US. The male rat remained with the female for a 2-minute post-ejaculatory period, which has been shown to induce conditioned ejaculatory preferences for both olfactory and somatosensory cues (Kippin et al., 1998; Quintana et al., 2018). The female was then removed from the smaller compartment, and the male subject was returned to the larger compartment to begin the second trial. If the male did not ejaculate after a 25-minute period with the female, the behavioural conditioning session was terminated.

Statistical analyses

Dependent measures included the proportion of time spent in the CS and US Zones, in the absence of the CS (i.e., during the 5-minute habituation period) in order to obtain a baseline measure of behaviour. The proportion of time spent in the US and CS Zones was also assessed in the presence of the CS (i.e., during the 2-minute CS presentation) to assess cue- and goal-directed behaviours. Here, a greater proportion of time spent near the CS was taken to reflect a

sign-tracking response pattern, whereas a greater proportion of time spent near the US was indicative of goal-tracking behaviour. A 15% CS-US difference score was calculated as a preliminary criterion on session 6 to identify rats displaying each phenotype. The acquisition of PCA was first analyzed using a repeated-measures analysis of variance (ANOVA) with Session (1, 6) and Zone (US, CS) as within-subjects variables. The influence of oxytocin on PCA was analyzed using a mixed-factorial ANOVA with session (7, 13) and Zone (US, CS) as within-subjects variables, and treatment (saline, 5 µg oxytocin) as a between-subjects variable. Follow-up paired-samples *t*-tests and planned-comparisons were conducted to measure differences for statistically significant interactions, and a Bonferroni correction was used for unplanned comparisons.

Results

The acquisition of Pavlovian-conditioned approach following exposure to a sexually-conditioned cue

In order to determine whether phenotypic differences develop following pairings of a visuo-tactile cue with sexual reward leading to ejaculation, we first assessed the development of PCA prior to the administration of oxytocin treatment. Here, the mean proportion of time spent in the US and CS Zones was compared in all rats on sessions 1 versus 6 when the CS was absent in order to evaluate their baseline behavioural pattern (Figure 1A). A repeated-measures ANOVA comparing time spent in the US and CS Zones, in the absence of the CS, revealed statistically significant main effects of Session, F (1, 23) = 34.694, $p < 0.001$, $\eta_p^2 = 0.40$, and Zone, F (1, 23) = 9.372, $p = 0.006$, $\eta_p^2 = 0.13$, and a significant Session x Zone interaction, F (1, 23) = 16.379, $p = 0.001$, $\eta_p^2 = 0.13$, indicating that the difference in the proportion of time spent in the US and CS Zones varied significantly between sessions 1 and 6. Consequently, follow-up analyses were conducted to analyze differences in proportion of time spent in the US and CS Zones on sessions 1 and 6; a Bonferroni correction was applied, therefore, all effects are reported at a 0.017 level of significance. Here, paired-samples *t*-tests revealed that subjects preferentially spent a greater proportion of time in the US Zone compared to the CS Zone on session 1, t (23) = 3.599, $p = 0.002$, $d = 0.74$, though this can likely be explained as exploratory behaviour in a novel environment. This preference dissipated by session 6, t (23) = 0.000, $p = 1.000$, as subjects spent a comparable proportion of time in the US Zone ($M = 0.20$, $SD = 0.05$) and CS Zone ($M = 0.20$, $SD = 0.06$) in the absence of the CS, suggesting rats learned that the significance of each zone exclusively depended on the presentation of the CS.

Next, we investigated the development of phenotypic differences in the presence of a sexually-conditioned cue in all rats on sessions 1 and 6, prior to the administration of oxytocin by

comparing the mean proportion of time spent near the US and CS (Figure 1B). A repeated-measures ANOVA revealed statistically significant main effects of Session, F (1, 23) = 20.368, p < 0.001, η_p^2 = 0.23, and Zone, F (1, 23) = 7.530, p = 0.012, η_p^2 = 0.13, and a significant Session x Zone interaction, F (1, 23) = 4.560, p = 0.044, η_p^2 = 0.04, suggesting that the difference in proportion of time spent near the US and CS differed between sessions 1 and 6. Therefore, follow-up analyses were conducted in order to measure differences in the proportion of time spent near the CS and US on sessions 1 and 6. Here, paired-samples *t*-tests revealed that subjects spent comparable proportions of time near the CS compared to US on session 1, t (23) = -2.338, p = 0.028; however, there was a statistically significant difference in the proportion of time spent between Zones by session 6, t (23) = -2.612, p = 0.016, d = 0.53, with subjects spending more time near the CS (M = 0.29, SD = 0.07) compared to the US (M = 0.21, SD = 0.11), suggesting the development of PCA toward a sexually-conditioned cue as subjects displayed an overall preference to spend time near the CS compared to the US by the middle of behavioural training.

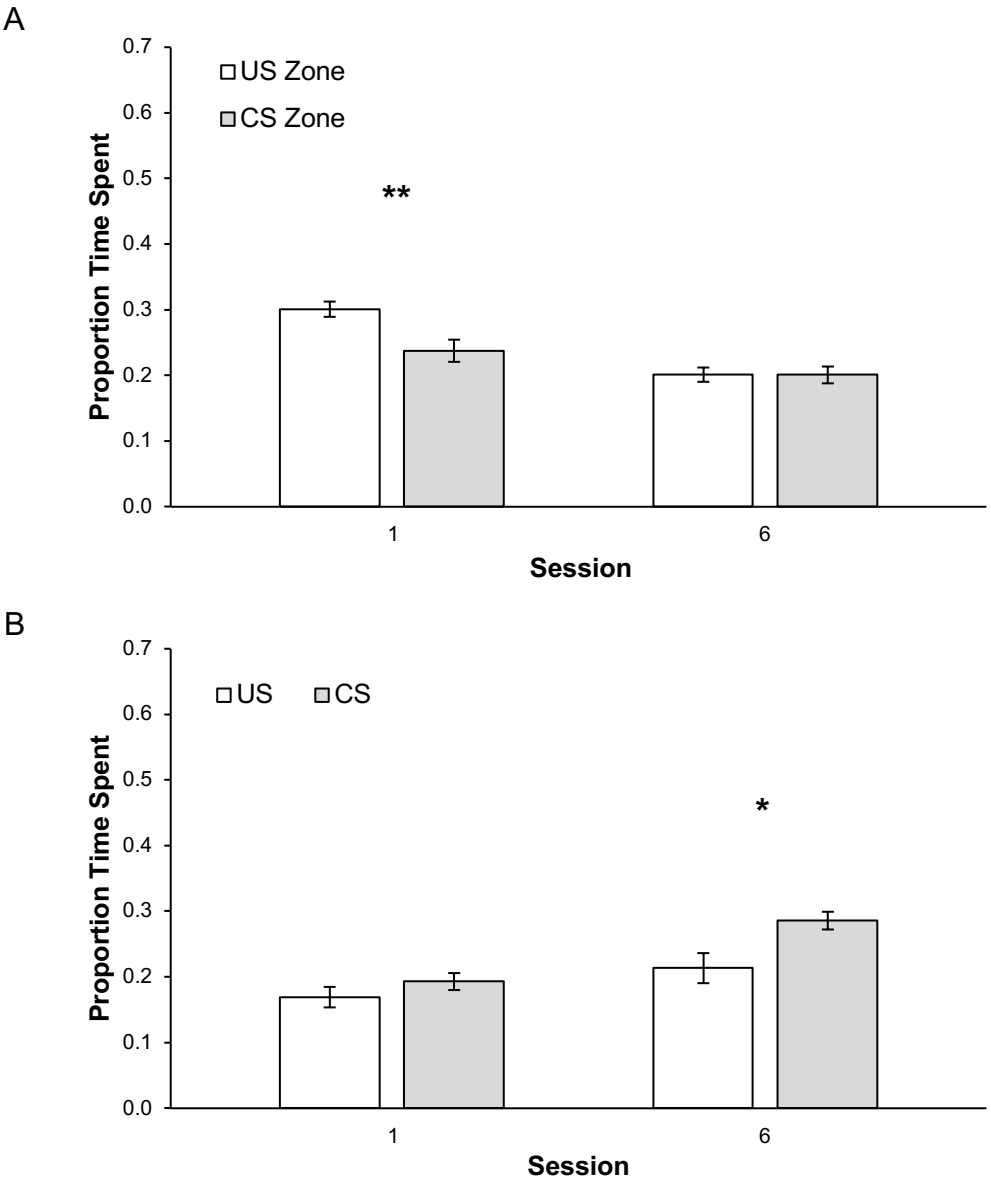


Figure 1. The development of individual differences in Pavlovian-conditioned approach following sexual conditioning in all subjects (N = 24). (A) Mean (± SEM) proportion of time spent in the US Zone (white bar) and CS Zone (grey bar) in the absence of the CS on sessions 1 and 6, prior to the administration of oxytocin. (B) Mean (± SEM) proportion of time spent near the US (white bar) and CS (grey bar) in the presence of the CS on sessions 1 and 6, prior to the administration of oxytocin, **p < 0.01; *p < 0.05.

The classification of individual differences in Pavlovian-conditioned approach

Based on the findings that subjects can display PCA toward a sexually-conditioned cue, we then classified subjects as displaying goal-, sign-tracking or intermediate response patterns by using the proportion of time spent near the US and CS when the CS was present. We first calculated a 15% CS-US difference score for preliminary phenotyping on session 6, as this session preceded the start of oxytocin administration. Eleven subjects reached the 15% CS-US difference score criterion for sign-tracking (i.e., time near CS > time near US) and two animals met the criterion for goal-tracking (i.e., time near US > time near CS), suggesting that cue-directed behaviour was more prominent than goal-directed behaviour in this study. Lastly, eleven rats did not reach the 15% CS-US difference score criterion (i.e., time near US ≈ time near CS) and were classified as intermediates. Due to the small number of goal-trackers identified in our sample, these two subjects were excluded from further statistical analyses.

Previous research has characterized sign-trackers by their tendency to approach and engage with the CS (Flagel et al., 2009). Therefore, a planned comparison *t*-test was conducted on the mean proportion of time spent near the CS compared to the US (CS present) on session 6 in animals that met the CS-US difference score criterion for sign-tracking behaviour (Figure 2A). There was a statistically significant difference in the proportion of time spent near the CS compared to the US, t (10) = -12.733, $p < 0.001$, $d = 3.84$, with subjects spending more time near the CS ($M = 0.32$, $SD = 0.05$) compared to the US ($M = 0.13$, $SD = 0.04$), which is indicative of cue-directed behaviour.

Animals that display intermediate behaviours tend to vacillate between cue- and goal-directed responses (Flagel et al., 2009). A planned comparison *t*-test was conducted on the mean proportion of time spent near the CS and US, while the CS was present on session 6 in animals that did not reach the CS-US difference score criterion for sign- or goal-tracking behaviour (Figure 2B). There was no statistically significant difference in the proportion of time spent near the CS and US, t (10) = -1.579, $p = 0.145$, indicating that subjects spent comparable proportions of time near the CS ($M = 0.27$, $SD = 0.07$) and US ($M = 0.26$, $SD = 0.09$), which is indicative of intermediate behaviour.

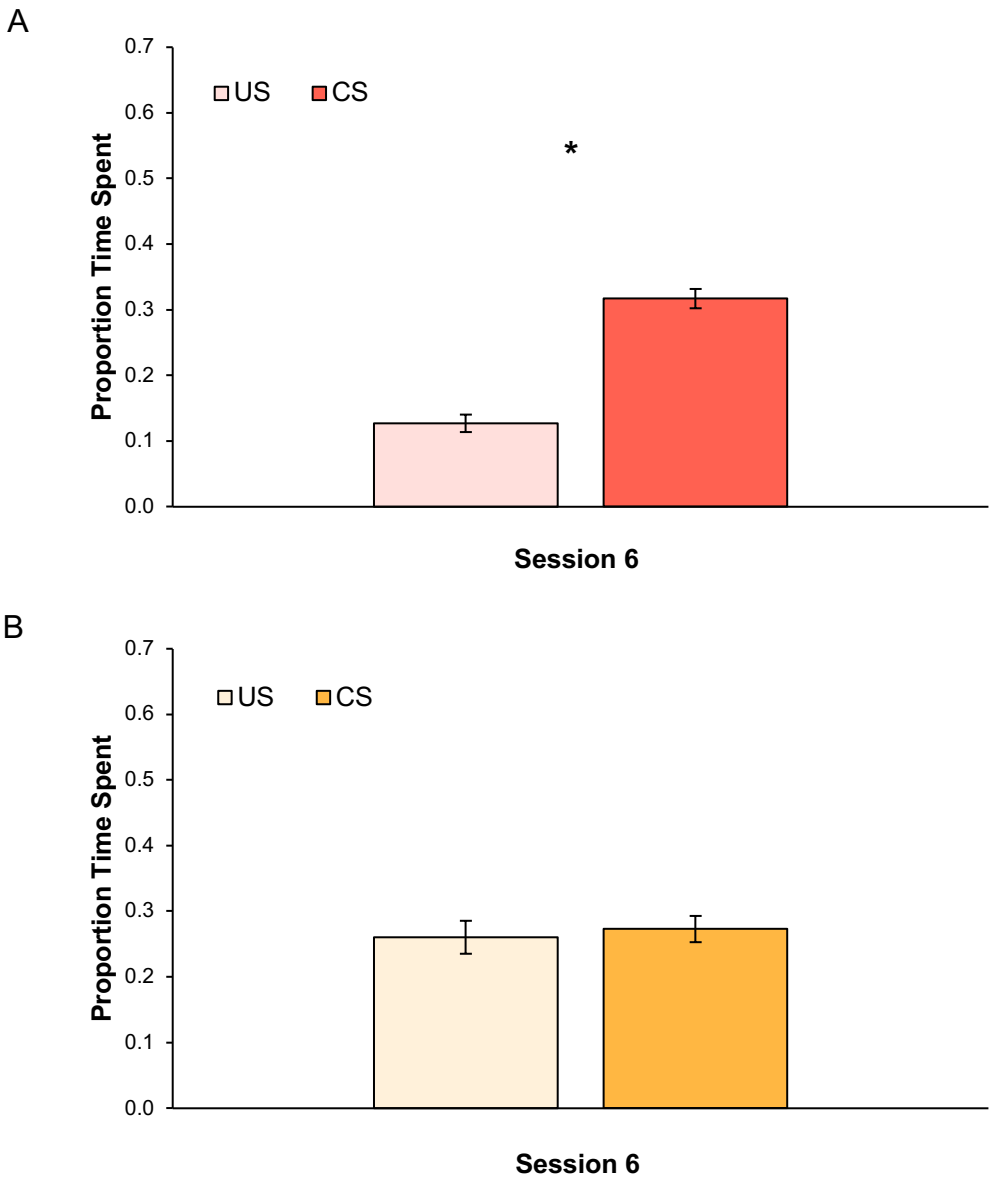


Figure 2. The development of sign-tracking and intermediate behaviour in response to a sexually-conditioned cue. (A) Mean (± SEM) proportion of time spent near the US (light bar) and CS- (dark bar) in the presence of the CS on session 6 in animals identified as sign-trackers (n = 11). (B) Mean (± SEM) proportion of time spent near the US (light bar) and CS- (dark bar) in the presence of the CS on session 6 in intermediate subjects (n = 11), *p < 0.001.

The influence of chronic systemic oxytocin administration on Pavlovian-conditioned approach in sign-trackers

Oxytocin may enhance the learning of, and motivation in responding to, Pavlovian-conditioned cues (Burkett & Young, 2012). Therefore, data were analyzed to determine whether oxytocin would affect the proportion of time spent in the CS and US Zones, both in the presence and absence of the CS. Data were compared on session 7, when animals first received injections of oxytocin or saline prior to conditioning, and at the end of behavioural conditioning on session 13. The mean proportions of time spent in the US and CS Zones (CS absent) were compared on sessions 7 and 13 in saline- and oxytocin-treated subjects, in order to determine if repeated administration of OT prior to conditioning might increase approach to the CS or CS Zone (Figure 3A). A mixed-factorial ANOVA revealed a statistically significant main effect of Zone, F (1, 9) = 15.105, p = 0.004, η_p^2 = 0.38, consistent with an increased proportion of time spent in the US Zone (M = 0.26, SD = 0.03) compared to the CS Zone (M = 0.16, SD = 0.03) but there was also a significant Session x Zone interaction, F (1, 9) = 11.076, p = 0.009, η_p^2 = 0.35, consistent with a decreased preference for the US Zone in session 13 versus session 7. Planned comparisons related to the Session x Zone interaction indicated that animals spent significantly more time in the US Zone compared to the CS Zone on session 7, t (10) = 4.565, p < 0.001, d = 1.38, however this difference dissipated by session 13, t (10) = 0.298, p = 0.772, as sign-trackers spent comparable proportions of time in the US (M = 0.20, SD = 0.06) and CS Zones (M = 0.19, SD = 0.13). There were no significant main effects of Session, F (1, 9) = 1.478, p = 0.255 or Treatment, F (1, 9) = 0.413, p = 0.537, nor a Session x Treatment, F (1, 9) = 0.073, p = 0.792, Zone x Treatment, F (1, 9) = 0.003, p = 0.956, or Session x Zone x Treatment interaction, F (1, 9) = 1.317, p = 0.281. Therefore, in the absence of the CS, both saline and oxytocin-treated sign-trackers showed a reduction in preference for the US Zone over the course of sexual conditioning, and similar proportions of time spent in both the US and CS Zones at the end of conditioning.

Next, we examined whether the administration of oxytocin might potentiate cue-directed behaviour in the presence of the CS by comparing the mean proportions of time spent near the US and CS on sessions 7 and 13 in saline- and oxytocin-treated subjects (Figure 3B). A mixed-factorial ANOVA revealed statistically significant main effects of Session, F (1, 9) = 10.126, p = 0.011, η_p^2 = 0.40, and Zone, F (1, 9) = 480.799, p < 0.001, η_p^2 = 0.93, and a significant Session x Zone interaction, F (1, 9) = 55.191, p < 0.001, η_p^2 = 0.47. This is consistent with subjects spending significantly more time near the CS (M = 0.43, SD = 0.01) compared to the US (M = 0.13, SD = 0.02) overall, and with an increase in the preference for the CS between session 7 and 13. These results did not depend on treatment with saline or oxytocin, and there was no main

effect of Treatment, F (1, 9) = 0.007, p = 0.933, nor interactions involving Session x Treatment, F (1, 9) = 0.001, p = 0.977, Zone x Treatment, F (1, 9) = 0.601, p = 0.458, or Session x Zone x Treatment, F (1, 9) = 0.106, p = 0.752. Thus, sign-trackers showed an increase in the time spent near the CS between sessions 7 and 13, however this increased preference for the CS did not depend on whether subjects were treated with saline or oxytocin prior to conditioning. Planned comparisons showed that there was a statistically significant difference in the proportion of time spent near the US and CS on both session 7, t (10) = -17.577, p < 0.001, d = 5.30, and session 13, t (10) = -19.173, p < 0.001, d = 5.78, with subjects consistently spending more time near the CS compared to the US which is in accordance with the sign-tracking phenotype.

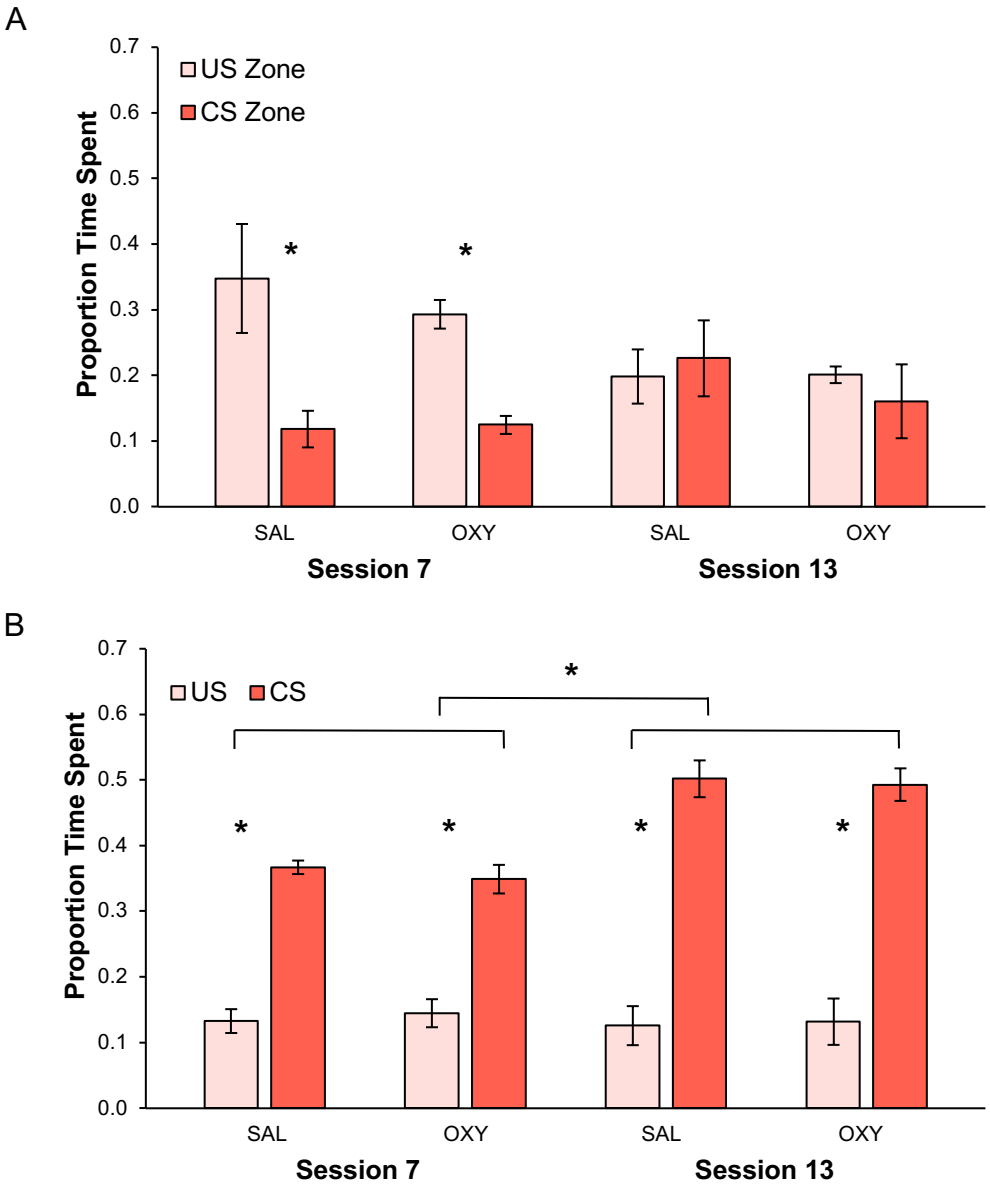


Figure 3. Oxytocin does not enhance nor diminish the expression of sign-tracking toward a sexually-conditioned cue in sign-trackers. (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence of the CS on sessions 7 and 13 in saline- and oxytocin-treated sign-trackers (n = 11). (B) Mean (± SEM) proportion of time spent near the US (light bar) and CS (dark bar) in the presence of the CS on sessions 7 and 13 in saline- and oxytocin-treated sign-trackers (n = 11), $^{**}p < 0.001$.

The influence of chronic systemic oxytocin administration on Pavlovian-conditioned approach in intermediate subjects

Intermediate behaviour is characterized by approach responses toward both the US and CS, and because oxytocin can facilitate a conditioned ejaculatory preference toward an olfactory cue (Ménard et al., 2019), we assessed whether the administration of oxytocin might promote the development of sign-tracking behaviours in the intermediate phenotype. The mean proportion of time spent in the US and CS Zones in the absence of the CS, was compared on sessions 7 and 13 in saline- and oxytocin-treated intermediate subjects (Figure 4A). A mixed-factorial ANOVA revealed a statistically significant main effect of Session, F (1, 9) = 21.430, p = 0.001, η_p^2 = 0.63, and a significant Session x Zone interaction, F (1, 9) = 5.475, p = 0.044, η_p^2 = 0.05, which is consistent with lower proportions of time spent in the US and CS Zones on session 13, and a somewhat greater reduction in the proportion of time spent in the CS Zone. Furthermore, a statistically significant Session x Treatment interaction, F (1, 9) = 22.200, p = 0.001, η_p^2 = 0.64, was consistent with a reduction in overall time spent in the US and CS Zones in oxytocin-treated subjects, and little change in the time spent in these zones in the saline-treated subjects. There were no main effects of Zone, F (1, 9) = 0.168, p = 0.692 or Treatment, F (1, 9) = 0.656, p = 0.439, nor a Zone x Treatment, F (1, 9) = 0.443, p = 0.522 or Session x Zone x Treatment interaction, F (1, 9) = 0.005, p = 0.947. Therefore, although there was some reduction in the times spent in the CS and US Zones in animals treated with oxytocin, there was no differential effect of treatment on time spent in the US versus the CS Zone.

In order to determine whether the administration of oxytocin enhanced the development of cue-directed behaviour in intermediate subjects, the mean proportion of time spent near the US and CS in the presence of the CS was compared on sessions 7 and 13 in saline- and oxytocin-treated rats (Figure 4B). Analysis using a mixed-factorial ANOVA showed no significant main effects of Session, F (1, 9) = 1.785, p = 0.214 or Treatment, F (1, 9) = 2.574, p = 0.143, nor a significant Session x Treatment interaction, F (1, 9) = 0.201, p = 0.665, but there was a statistically significant main effect of Zone, F (1, 9) = 183.165, p < 0.001, η_p^2 = 0.71, and a significant Zone x Treatment, F (1, 9) = 179.611, p < 0.001, η_p^2 = 0.71, Session x Zone, F (1, 9) = 215.382, p < 0.001, η_p^2 = 0.69, and Session x Treatment x Zone interaction, F (1, 9) = 210.990, p < 0.001, η_p^2 = 0.69. Planned-comparison t-tests were used to compare the proportions of time spent near the US and CS in saline- and oxytocin-treated subjects on the final behavioural conditioning session. In saline-treated subjects, there was no statistically significant difference in the proportion of time spent near the US and CS on session 13, t (4) = 0.161, p = 0.880, with rats spending comparable proportions of time near the US (M = 0.33, SD = 0.09) and CS (M = 0.33, SD = 0.08). In contrast,

there was a statistically significant difference in the proportion of time spent near the US and CS in intermediate rats that were treated with oxytocin, t (5) = 18.005, p < 0.001, d = 7.35, with subjects spending a much greater amount of time near the US (M = 0.58, SD = 0.05) as compared to the CS (M = 0.18, SD = 0.05). Therefore, the administration of oxytocin appears to have reduced the proportion of time spent near the CS, while increasing the proportion of time spent near the US by session 13. Interestingly, treatment with oxytocin did not enhance the development of cue-directed responses; rather, it appears to have potentiated goal-directed behaviour in intermediate subjects.

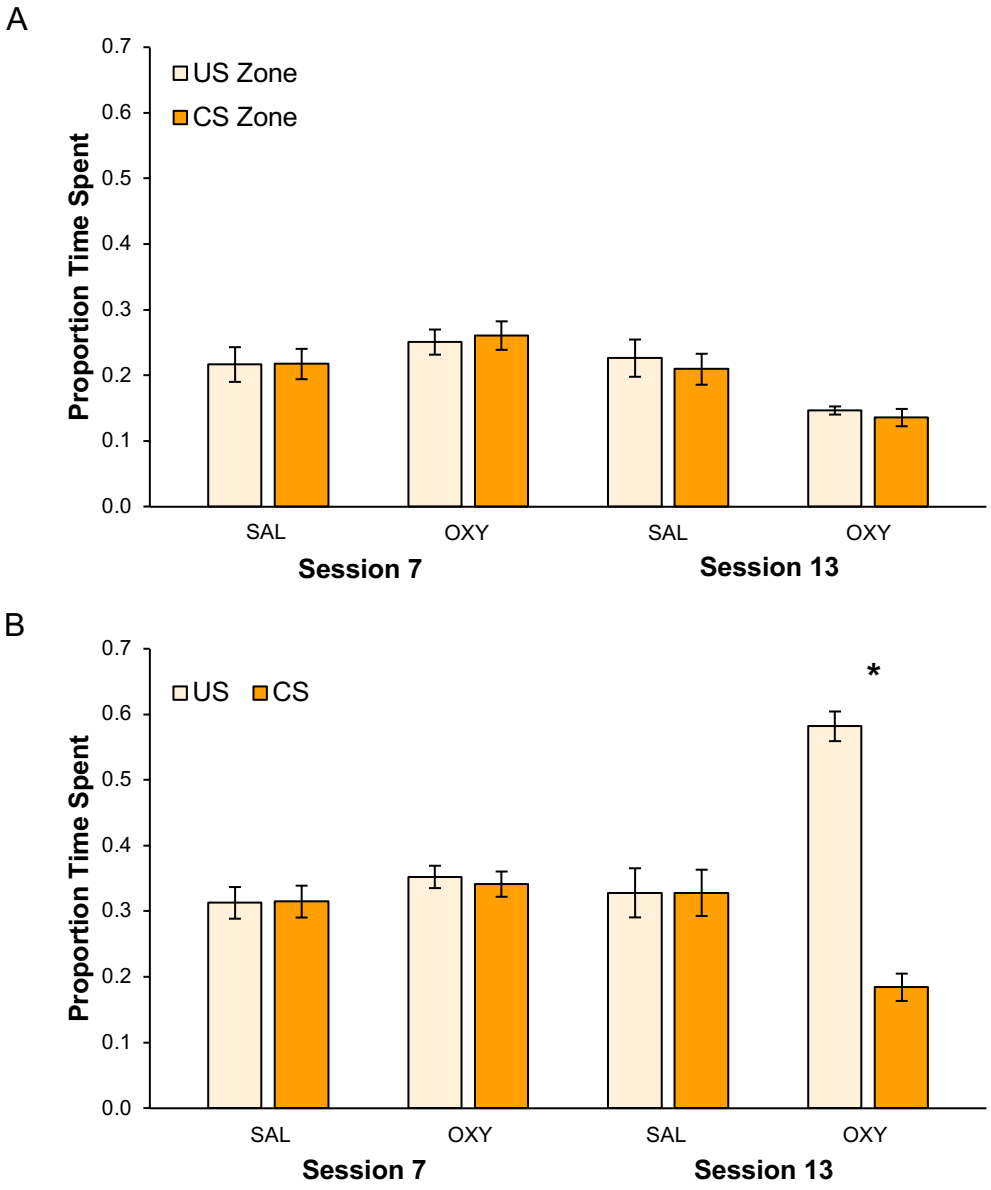


Figure 4. Oxytocin promotes the expression of goal-tracking in intermediate subjects exposed to sexual conditioning. (A) Mean (± SEM) proportion of time spent in the US-designated area (light bar) and CS-designated area (dark bar) in the absence of the CS on sessions 7 and 13 in saline- and oxytocin-treated intermediate subjects (n = 11). (B) Mean (± SEM) proportion of time spent near the US (light bar) and CS (dark bar) in the presence of the CS on sessions 7 and 13 in saline- and oxytocin-treated intermediate subjects (n = 11), *p < 0.001.

Discussion

Oxytocin is thought to have an important role in sexual conditioning. Previous research has investigated its involvement in both conditioned partner preferences and ejaculatory preferences using an olfactory cue (Kippin et al., 1998; Coria-Avila et al., 2005; Ménard et al., 2019). Oxytocin is likely to affect sexual conditioning through its interaction with the mesocorticolimbic dopamine pathway which contributes to the development of Pavlovian associations, including PCA (Flagel et al., 2010; Berridge, 2012; Everitt & Robbins, 2005; Tomie et al., 2008). The present experiment was conducted to determine whether the administration of oxytocin could promote PCA behaviours toward a visuo-tactile cue associated with sexual reward, and to determine whether oxytocin might facilitate sign-tracking behaviour by enhancing the incentive salience attributed to the CS. Our results demonstrated the development of sign-tracking and intermediate responses; although the administration of oxytocin did not facilitate the proportion of time spent near the CS in animals that sign-track, we found that oxytocin appears to increase the incidence of goal-directed behaviour in intermediate subjects. This suggests that oxytocin may strengthen the perceived value of sexual reward, rather than the incentive salience of the sexually-conditioned cue.

Previously, studies have found that the incidences of sign-, goal-tracking and intermediate responses to food- and drug-paired cues are similar, and relatively stable across studies, with subject samples yielding approximately one-third of subjects that can be classified as goal-trackers, sign-trackers, and intermediate subjects (Flagel et al., 2009). In contrast, we have reported more variable incidences of sign- and goal-tracking responses when using a sexual conditioning paradigm; we observed higher frequencies of goal-tracking and intermediate responses, and low incidences of sign-tracking behaviour to a sexually-conditioned cue in Chapters 2 and 3 of this thesis. In contrast, the present results identified 11 sign-trackers, 11 intermediates, and only two goal-tracking subjects. This highlights the variability in PCA behaviours across experiments involving a sexually-conditioned cue and suggests that multiple factors may contribute to the expression of PCA behaviours associated with sexual conditioning and reward.

Oxytocin does not enhance cue-directed behaviour in animals that sign-track for a sexually-conditioned cue

In the current experiment, we found the development of PCA toward a sexually-conditioned cue that was associated with sexual reward and the ejaculatory reward state. We identified 11 sign-trackers by the middle of sexual conditioning (i.e., session 6), that spent a greater proportion of time near the CS as compared to the US. Previous research from our

laboratory has shown that oxytocin can enhance the acquisition of a conditioned ejaculatory preference for olfactory stimuli (Ménard et al., 2019), and we therefore treated six sign-trackers with oxytocin and five sign-trackers with saline (4-hours, 2-hours, and 15-minutes prior to a sexual conditioning sessions 7 to 13), in order to determine the influence of oxytocin on PCA. Importantly, both saline- and oxytocin-treated subjects learned that the CS predicted the opportunity to copulate with a sexually-receptive female; although both saline- and oxytocin-treated sign-trackers spent a greater proportion of time near the US in the absence of the CS on session 7, this difference dissipated in both groups by session 13 as subjects learned that access to the sexually-receptive female depended on the *presence* of the CS. However, when the CS was present on sessions 7 and 13, both saline- and oxytocin-treated sign-trackers spent greater proportions of time near the CS as compared to the US. The administration of oxytocin, however, did not potentiate these cue-directed responses by session 13 as compared to subjects treated with saline. Therefore, oxytocin does not appear to enhance cue-directed behaviours in animals that sign-track for a cue associated with sexual reward.

In preclinical models of addiction, oxytocin has been proposed as a therapeutic agent because it effectively suppresses drug-primed and cue-induced reinstatement (Everett et al., 2019; Everett, Carey et al., 2020), potentially by reducing the incentive salience associated with drug-paired cues (Bowen & Neumann, 2017). Specifically, the administration of oxytocin appears to suppress cue-induced reinstatement to methamphetamine in sign-, but not goal-trackers, suggesting that the actions of oxytocin may be unique to brain regions which are sensitized in sign-trackers, such as frontal, striatal, and thalamic structures (Everett, Carey et al., 2020). Furthermore, studies report that drug- and food-paired cues elicit significantly higher dopamine release in the nucleus accumbens core in sign-trackers as compared to goal-trackers, and, given that the administration of oxytocin can modulate dopaminergic activity in the nucleus accumbens and the ventral tegmental area (Kohli et al., 2019; Melis et al., 2009; Peters et al., 2016), it is hypothesized that oxytocin might function to regulate this heightened cue-elicited dopamine signal more effectively sign-trackers (Everett, Baracz et al., 2020). Therefore, these data suggest that the administration of oxytocin suppresses cue-directed responses to drug-paired stimuli, likely by reducing the attribution of incentive salience. In contrast, the present study reports that oxytocin treatment did not enhance nor suppress sign-tracking behaviour toward a sexually-conditioned cue. A possible explanation for the lack of an effect of oxytocin may be due to the dosage administered. In two separate drug reinstatement studies, Everett, Baracz et al. (2020) and Everett, Carey et al. (2020) used intraperitoneal oxytocin doses of 0.3 or 1.0 mg/kg, whereas we used a subcutaneous dose of 5 μg/kg, administered 4-hours, 2-hours, and 15-minutes prior to the

sexual conditioning session. Consequently, the subjects used in the aforementioned studies were injected with 20 to 200 times the concentration of oxytocin compared to our subjects, and this could explain the lack of effect on sign-tracking subjects in our study.

Another explanation for our contrasting data relates to the interaction between oxytocin and stress, and how stress may be experienced differently in subjects exposed to drug reward versus sexual reward. In addition to its involvement in social behaviour, cognition, motivation and incentive salience, oxytocin has also been shown to regulate stress, anxiety, and fear, and both the synthesis and central release of oxytocin can be evoked by anxiogenic and stressful contextual and discrete cues (Neumann & Landgraf, 2012). Several studies have demonstrated the efficacy of oxytocin in the regulation of anxiety-related behaviour. For example, in a preclinical model of post-traumatic stress disorder (Janezic et al., 2016), subjects were first assigned to two groups: a shock group exposed foot shocks in a shock compartment, and a sham group received no foot shock. Both the shock and sham groups were then exposed to three short re-exposure sessions in the apparatus without shock, which served as situational reminders in the trauma-induction procedure. As a result, shock subjects exhibit contextual fear, as measured by a reduction in time spent in the shock compartment. The chronic administration of oxytocin decreased contextual fear, as shock subjects spent more time in the shock compartment as compared to their saline-treated counterparts. Though the shock oxytocin-treated animals spent less time in the shock compartment compared to sham oxytocin-treated animals during the first two situational reminder sessions, by the third situational reminder session they spent a comparable amount of time in the shock compartment as oxytocin-treated sham animals. These data suggest that the chronic administration of oxytocin produces a long-term reduction in stress and anxiety. If we apply these findings to the methamphetamine study described previously (Everett, Carey et al., 2020), it may be suggested that oxytocin effectively diminished cue-directed responses to drug-paired stimuli due to its anxiolytic effects. For example, methamphetamine has been shown to increase heart rate and blood pressure, and can increase the release of corticosterone, a stress hormone, via activation of the hypothalamic-pituitary-adrenal axis (Rusyniak et al., 2012; Zuloaga et al., 2015; Herring et al., 2008; Zuloaga et al., 2014). Accordingly, serum levels of corticosterone are shown to be elevated following autoshaping in animals that sign-track (Tomie et al., 2000). Though stress and sexual activity both induce an arousal state, sexual arousal and copulatory performance do not necessarily activate the hypothalamic-pituitary-adrenal axis similarly. Therefore, the efficacy of oxytocin's suppression of sign-tracking using methamphetamine could include an anxiolytic component; though our

subjects were aroused, they were not stressed, which might explain why oxytocin did not have an effect on sign-tracking behaviour.

Oxytocin has previously been shown to play a role in sexual conditioning using a partner preference paradigm. Briefly, male rats were trained to copulate with a sexually-receptive female bearing a neutral odour (i.e., lemon, almond), resulting in a conditioned ejaculatory preference toward scented females which develops through repeated pairings of the scent and the post-ejaculatory reward state (Kippin et al., 1998; Kippin & Pfaus, 2001a; Kippin & Pfaus, 2001b). Importantly, these male rats also show increased neuronal activation as measured by c-Fos in brain regions implicated in incentive motivation (i.e., nucleus accumbens core, olfactory tubercle, main olfactory [piriform] cortex, lateral hypothalamus, basolateral amygdala) and in areas involved in the synthesis and release of oxytocin, such as the supraoptic nucleus and paraventricular nucleus of the hypothalamus (Kippin et al., 2003). Furthermore, exposure to an olfactory cue paired with the post-ejaculatory reward state has also been shown to induce the activation of oxytocin neurons within the paraventricular nucleus of the hypothalamus, and the systemic administration of oxytocin appears to be important for the development of the conditioned ejaculatory preference as it enhances the association between an olfactory cue and sexual reward (Ménard et al., 2019).

However, we have reported that the chronic administration of oxytocin neither enhanced, nor diminished, sign-tracking behaviour toward a visuo-tactile cue paired with sexual reward, and this may be due in part to differences in the sensory properties of the CS (i.e., a visuo-tactile cue versus an olfactory cue; Gottlieb, 2012). For example, rodents depend extensively on their sense of olfaction, and an important component of male copulatory circuitry includes the vomeronasal system's ability to detect pheromones to activate male sexual arousal (Breedlove & Watson, 2020). Odour information is processed by mitral and tufted cells located in the main olfactory bulb via networks of interneurons (e.g., granule cells) which receive cortical top-down inputs from the anterior olfactory cortex; the mitral and tufted cells then relay sensory inputs directly to the olfactory cortex (Balu et al., 2007; Brunjes et al., 2005; de Olmos et al., 1978; Luskin & Price, 1983). Functional studies have also linked the rat's olfactory system to cognition and associative learning; specifically, cells in the olfactory cortex project to a portion of the thalamic mediodorsal nucleus that connects to the orbitofrontal cortex (Powell et al., 1965), which is involved in encoding and learning changes in contingencies, value, and inferred value (Fettes et al., 2017) and social motivation (Wilson et al., 2014). Further, the olfactory thalamocortical circuit, featuring connections to the amygdala, entorhinal cortex, and hypothalamus, plays an important role in odour discrimination and the processing of olfactory stimuli (McBride & Slotnick, 1997). Thus, the

powerful effects of oxytocin on sexual conditioning using an olfactory cue (Kippin et al., 1998) versus the visuo-tactile cue used in our study, may be explained by oxytocin's influence on olfactory circuitry in the brain. Several studies have also proposed a reciprocal relationship between the modulation of sensory processing via oxytocin, and the modulation of oxytocin signalling by sensory inputs (Grinevich & Stoop, 2018). Though the main olfactory bulb contains few oxytocin receptors, the anterior olfactory nucleus is among the brain regions with the highest expression of oxytocin receptors, and it is densely innervated by oxytocin neurons from the paraventricular nucleus of the hypothalamus (Freund-Mercier et al., 1987; Tribollet et al., 1988; Yoshimura et al., 1993; Knobloch et al., 2012). Interestingly, the systemic administration of oxytocin may act on oxytocin receptors peripherally, thereby stimulating the release of endogenous oxytocin in the brain by activating both autonomic and sensory neurons (Grinevich & Stoop, 2018). This amplification of oxytocinergic effects on brain areas involved in olfaction and reward may contribute to the enhanced associability of an olfactory cue with the ejaculatory reward state (Ménard et al., 2019). Therefore, it is possible that the role of oxytocin in enhancing conditioned responses may be specific to olfactory cues, which may explain the lack of effect in our study.

Oxytocin enhances goal-directed behaviour in animals that display intermediate responses toward a sexually-conditioned cue

Oxytocin is known to strengthen social affiliative behaviours (Lee, 2009), and it could therefore be expected that oxytocin might enhance the display of goal-tracking behaviours by promoting closeness or social bonding with the sexually-receptive female. In the current study, we found a subset of animals that vacillated between the cue- and goal-designated areas by the middle of sexual conditioning on session 6, prior to the administration of oxytocin, and these were classified as intermediate subjects. Interestingly, during subsequent conditioning, increased goal-directed, versus cue-directed, behaviour was observed in the oxytocin-treated, but not in the saline-treated intermediate subjects. This suggests that oxytocin might promote behaviours directed towards the US, and 'shift' intermediate subjects toward a goal-directed phenotype.

Though the exact mechanisms remain unclear, it is possible that oxytocin may have increased the rewarding effects of the social and sexual interaction with the sexually-receptive female in subjects that do not display a clear sign- or goal-tracking phenotype. For example, in one study, Ramos et al. (2015) used a social- conditioned place preference (CPP) paradigm to investigate the intrinsic rewarding effects of oxytocin, and whether such effects might be strengthened by the presence of a sex-matched conspecific, or the presence of a dynamic, tactile non-social object (i.e., a tennis ball). In a morning session, subjects received vehicle before being

placed in their preferred compartment with free access to the tennis ball. In the afternoon session, subjects were injected with oxytocin (0.5 mg/kg) and were placed in their non-preferred compartment with the sex-matched conspecific partner, who was also treated with oxytocin. Here, oxytocin induced a robust social-CPP, as subjects developed a strong preference for the environment in which they received oxytocin and access to a sex-matched conspecific. Furthermore, the data showed that these effects were enduring, as subjects continued to display strong social-CPP when tested 4-weeks after conditioning. It is also important to note that oxytocin alone did not induce a CPP, suggesting that oxytocin may specifically potentiate the rewarding effects of the social interaction. In our experiment, the sexually-conditioned cue acquired incentive salience in sign-trackers, and perhaps oxytocin was not sufficient to disrupt these properties. However, in intermediate animals that display neither clear sign- nor goal-tracking, oxytocin may have potentiated the rewarding effects of the social interaction with the sexually-receptive female, in addition to the experienced ejaculatory reward state, resulting in the expression of goal-tracking behaviour.

In a separate experiment, Ramos et al. (2015) also tested object-induced CPP to examine whether pairing oxytocin with a non-social, tactile, and dynamic object (i.e., a tennis ball) would induce a preference for the object-paired compartment versus an environment paired with a drug-free encounter with a sex-matched conspecific. Here, oxytocin induced an object-CPP, suggesting that the administration of oxytocin specifically enhanced the rewarding effects of the tennis ball stimulus, as subjects gradually increased their interaction with the tennis ball across all conditioning sessions while decreasing their social interaction with the sex-matched conspecific. In our study, the administration of oxytocin increased goal-directed behaviour, and not sign-tracking behaviour toward a visuo-tactile cue, as might be expected on the basis of the results of Ramos et al. (2015). However, this may be due to differences in the properties of the cone versus the tennis ball; Ramos et al. (2015) proposed that the increase in contact with the tennis ball may be due to enhanced tactile reward and object familiarity, and that oxytocin levels likely increase when touching a soft inanimate object such as the tennis ball or a teddy bear (Tai et al., 2011). It is possible that the use of a cone CS, which was stationary and made of hard plastic, may have limited the development of cue-directed behaviours, and allowed the display of goal-directed behaviours in intermediate subjects treated with oxytocin.

In experiments measuring PCA, intermediate subjects tend to vacillate between cue- and goal-directed responses. In the current study, we observed that saline-treated intermediate subjects continued to display intermediate responses, but that intermediate subjects treated with oxytocin developed goal-directed behaviour. As such, the administration of oxytocin may have

enhanced the predictive properties of the sexually-conditioned cue and strengthened the CS-US association, resulting in a greater proportion of time spent near the US in oxytocin-treated intermediate subjects. Though the vast majority of the oxytocin literature has focused on fear rather than appetitive conditioning, it is possible that oxytocin may enhance the association between discrete and rewarding stimuli similarly. For instance, multiple studies have provided evidence for the role of endogenous oxytocin in both the acquisition and extinction of fear, though these effects appear to be brain-region- and time-dependent. For example, in a fear extinction paradigm, micro-infusions of oxytocin into the basolateral amygdala have been shown to increase freezing behaviour and to impair fear extinction, suggesting that oxytocin can also enhance fear responses to contextual cues (Lahoud & Maroun, 2013). Furthermore, the bed nucleus of the stria terminalis, an area that receives dense oxytocinergic inputs from the paraventricular nucleus of the hypothalamus, and contains a high level of oxytocin receptors, may also be involved in the conditioned fear response to discrete cues (Gungor & Paré, 2016). Specifically, it has been suggested that the neurotransmission of oxytocin receptors in the dorsolateral bed nucleus of the stria terminalis might facilitate the acquisition of conditioned fear responses to a discrete cue, compared to an unpredictable threat (i.e., non-cued fear; Moaddab & Dabrowska, 2017). Therefore, it is possible that oxytocin, acting in the basolateral amygdala and the bed nucleus of the stria terminalis, may enhance the CS-US association by increasing the salience of the cue in a fear conditioning paradigm, thereby strengthening fear responses. Though the CS used in our experiment was not a fear-paired stimulus, research also suggests that oxytocin levels can be manipulated by Pavlovian conditioning (Onaka & Yagi, 1998) and may therefore indicate a role for oxytocin in the strengthening of associations between a Pavlovian-conditioned discrete cue and unconditioned stimuli. In contrast, studies investigating the influence of oxytocin in Pavlovian conditioning for reward appear to be much more limited. In the study by Ménard et al. (2019), the systemic administration of oxytocin 4-hours, 2-hours, and 15-minutes prior to a conditioning session strengthened the association between an olfactory cue and sexual reward by enhancing Pavlovian conditioning and odour discrimination, and potentially increasing the salience of the ejaculatory reward state. Therefore, it is possible that the administration of oxytocin may have enhanced the predictive value of the visual-tactile cue paired with sexual reward in intermediate rats in the present study. Saline-treated intermediate subjects continued to fluctuate between cue- and goal-directed responses throughout training, and this might reflect a weaker association between the sexually-conditioned cue and ejaculatory reward state. It is possible that oxytocin both strengthened the CS-US association, and the rewarding socio-sexual effects of interactions

with the sexually-receptive female, thereby resulting in the expression of goal-directed behaviour in oxytocin-treated intermediate subjects.

Conclusions

Though the development of PCA appears to be relatively stable in studies using drug and food reward (Morrison et al., 2015; Srey et al., 2015; Villaruel & Chaudhri, 2016; Saunders and Robinson, 2010; Yager and Robinson, 2013), previous findings reported in Chapters 2 and 3 suggest that the incidences of sign- and goal-tracking for sexual reward are much more variable across experiments. Interactions between oxytocin and the mesocorticolimbic pathway may promote the attribution of incentive salience (Burkett & Young, 2012), and the current study therefore aimed to identify whether the chronic systemic administration of oxytocin could potentiate sign-tracking for a sexually-conditioned cue. Subjects underwent Pavlovian training, during which a visuo-tactile cue predicted the opportunity to copulate with a sexually-receptive female. On session 6, phenotypic differences in PCA behaviour were identified; we observed 11 sign-trackers, 2 goal-trackers and 11 intermediate subjects. On sessions 7-13, subjects received chronic, systemic injections of either saline or oxytocin. In sign-trackers, we report that oxytocin did not enhance nor diminish cue-directed behaviour, suggesting that oxytocin might not potentiate cue-directed behaviour in animals that sign-track for a sexually-conditioned cue. Interestingly, the display of goal-tracking responses was increased in oxytocin- but not saline-treated intermediate subjects, which might reflect an additive effect of social bonding and the ejaculatory reward state following exposure to oxytocin in animals that otherwise fluctuate between cue- and goal-directed behaviour. Importantly, these data provide further evidence that conditioned cues can acquire incentive motivational properties in a subset of animals, through Pavlovian conditioning using an ejaculatory reward state. Furthermore, oxytocin may enhance the association between the CS and US and may ‘shift’ intermediate subjects toward a goal-tracking phenotype due to its role in social bonding and sexual behaviour.

Chapter 5: General discussion

The ability of conditioned cues to direct motivated behaviour has been researched extensively in studies that have used food and drug as a reward. The development of individual differences in PCA behaviours, as evidenced by sign- and goal-tracking responses, suggests the conditioned cues acquire incentive salience in certain subjects, and serve primarily as a predictor of reward in others. In contrast, the development of PCA in response to conditioned cues paired with sexual reward has been largely understudied and limited to avian models using male Japanese quail (Domjan et al., 1986; Burns & Domjan, 1996). Therefore, the overarching goal of this thesis was to determine whether sexually-conditioned cues can elicit sign- and goal-tracking behaviours in male rats.

Chapter 2 examined whether a conditioned cue paired with sexual reward could elicit individual differences in PCA, and whether such differences could be influenced by spatial distance between the CS and US. Results showed that sign-, goal-tracking and intermediate behaviours develop in response to a visuo-tactile cue paired with the opportunity to copulate to ejaculation with a sexually-receptive female, and that the spatial distance between the CS and US does not affect the expression of goal-tracking or intermediate responding. Chapter 3 investigated whether a sucrose conditioning paradigm could establish a clear determination of sign- and goal-tracking phenotypes prior to sexual conditioning, and whether the individual differences in PCA using sucrose reward would extend to sexual reward. We reported the development of sign-tracking, goal-tracking and intermediate behaviours following the pairing of a retractable lever with the delivery of sucrose but found that subjects' PCA phenotype for sucrose was not predictive of sign-, goal-tracking or intermediate behaviours during subsequent sexual conditioning. Lastly, Chapter 4 explored whether the chronic administration of oxytocin could enhance the expression of PCA using sexual reward. Treatment with oxytocin appeared to shift intermediate subjects toward a goal-tracking phenotype but had no significant effect in sign-trackers. Importantly, this thesis has provided evidence that sign-, goal-tracking and intermediate behaviours develop following pairings of a conditioned cue with the opportunity to copulate with a sexually-receptive female. Furthermore, we suggest that the expression of PCA may fluctuate depending on the type of natural reward and neuropeptide influences.

Sign- and goal-tracking behaviours develop in response to sexual reward

In male Japanese quail, previous reports have shown that sign-tracking is the predominant phenotype expressed in response to a sexually-conditioned cue, as subjects displayed conditioned approach and engagement toward the CS (Domjan et al., 1986; Burns & Domjan, 1996). The authors reported no incidences of goal-tracking or intermediate responses. In contrast,

we observed sign-, goal-tracking and intermediate behaviours in male rats in response to a cue paired with the opportunity to copulate to ejaculation with a sexually-receptive female. In Chapter 2, we identified 8 sign-trackers, 6 goal-trackers, and 9 intermediate subjects across two separate experiments. In Chapter 3, we did not initially classify subjects based on their responses to the sexually-salient cue, however we observed an overall tendency toward goal-tracking in 7 rats, and intermediate responses in 16 rats. Lastly, in Chapter 4, we categorized 11 subjects as sign-trackers and 11 subjects as intermediate responders. To our knowledge, these are the first data to provide evidence for the development of sign-, goal-tracking and intermediate behaviours in response to sexually-conditioned cues in male rats, and to find the occurrence of goal-tracking and intermediate responses when using sexual reward.

The results obtained in these experiments contribute to a growing literature on sexual conditioning in rats, and further validate the reward value of the ejaculatory state. From an evolutionary perspective, associative and reward learning are especially important, as both inform the behavioural consequences which are necessary for survival and reproduction. Both humans and animals rely on conditioned cues to become aroused and learn to identify external stimuli that predict the receptivity of a sexual partner (Pfaus et al., 2013). In rats, these conditioned responses have been modeled using conditioned partner and place preferences (Kippin et al., 1998; Ågmo & Berenfeld, 1990), and are contingent on sexual reward earned through copulation, which produces a preferred rate of intromissive penile stimulation leading to ejaculation in males (Pfaus et al., 2013). As such, several studies have identified the ejaculatory state as the most reinforcing element of sexual behaviour, as it has been found to be essential for the development of copulatory preferences (Coolen et al., 2004; Pfaus & Phillips, 1991; Tenk et al., 2009). Importantly, our data provide support that sexual reward leading to ejaculation serves as an effective reward stimulus, capable of transforming neutral stimuli into conditioned cues that predict positive outcomes.

Chapters 2 and 4 indicated that sign-tracking develops in response to sexually-conditioned cues, in accordance with Domjan et al. (1986) and Burns and Domjan (1996), who were the first to identify the development of sign-tracking responses to a sexually-salient cue. These findings indicate that copulation leading to ejaculation can also induce conditioned approach behaviour in male rats in addition to its ability to condition partner and place preferences (Ågmo & Berenfeld, 1990; Kippin et al., 1998; Quintana et al., 2019). The emergence of sign-tracking has been reliably demonstrated in response to food- and drug-paired cues (Robinson & Flagel, 2009; Flagel et al., 2010; Krank et al., 2007; Saunders & Robinson, 2010; Yager & Robinson, 2013; Yager, Pitchers

et al., 2015); as such, we propose the inclusion of sexual reward as a type of natural reward that is equally capable of eliciting the development of sign-tracking behaviour in male rats.

Chapters 2 and 3 revealed that certain subjects approached and spent a greater proportion of time near the US in response to a sexually-conditioned cue, similar to goal-tracking directed toward a food magazine (Robinson & Flagel, 2009) or ethanol delivery port (Krank et al., 2007) in food and drug reward studies, respectively. Interestingly, the incidence of goal-tracking had yet to be evidenced using sexual reward; Domjan et al. (1986) and Burns and Domjan (1996) found no indication of goal-tracking following sexual conditioning in male Japanese quail. In addition to differences in the experimental model, we propose that the inconsistencies between Domjan et al. (1986), Burns and Domjan (1996) and our findings may be species-specific. The copulatory behaviour of male Japanese quail and rats is characterized by a set of similar appetitive and consummatory responses, though certain distinctions may explain differences in goal-tracking incidence between species. In male Japanese quail, copulation begins when the male struts toward the sexually-receptive female; he stretches himself in such a way to position his beak, body, head, and neck parallel to the ground. He then erects his feathers and walks on his claws and digits with a stiffened gait to appear larger to the female. Subsequently, he will approach and mount the female, without any additional courtship or display behaviours. When mounting, the male uses his beak to grab the female's head or neck feathers and will position himself on the back of the female and spread his wings. He then arches his back and positions his cloaca onto the female's cloaca, which concludes in ejaculation (Mills et al., 1997). In male rats, copulation is initiated when the male investigates the sexually-receptive female's face and anogenital region. He will then approach the female from the rear, mount her, and deliver several rapid, shallow pelvic thrusts. If he contacts the female's vagina, he will exhibit a deeper pelvic thrust which will allow his penis to enter her vagina for 200-300 milliseconds (Beyer et al., 1981). He then springs off the female rapidly and grooms his genitals. The male will experience approximately 7 to 10 intromissions during which the penis enters the vagina at 1-to-2-minute intervals, ultimately leading to ejaculation (Hull & Dominguez, 2007).

If we compare copulatory responses, male Japanese quail appear to be less interactive with the female. As previously mentioned, a male Japanese quail will quickly mount in the absence of additional courtship and displays, whereas male rats initiate sexual behaviour by coming into proximity with the female and investigating her face and anogenital regions. Furthermore, the copulatory bout appears to be much shorter in duration in male Japanese quail compared to rats and has been described as brief (Domjan & Hall, 1986). In fact, in Domjan et al. (1986) and Burns and Domjan's (1996) studies, male Japanese quail were allowed 5 minutes to interact with the

female during which copulation was reported to occur, whereas in our experiments, male rats were given up to 30 minutes to copulate with the sexually-receptive female. Based on these factors, it is possible that social bonding and prolonged interaction between sexual partners contributes to the development of goal-tracking. It is well-known that rats are unquestionably social animals; they are well-adapted to group living and display complex social interactions (Lore & Flannelly, 1977). Therefore, it is possible that the development of goal-tracking behaviour may in part, depend on the synergistic value of social and sexual interactions with the sexually-receptive female. Male Japanese quail and rats are both social species; perhaps goal-tracking did not emerge in male Japanese quail as their social interaction with the female quail was limited, reducing her value as sexual reward. In our experiments, male rats spent a significantly longer period of time with the sexually-receptive female, which may have resulted in a synergy between social and sexual reward values, thus leading to the development of goal-tracking.

The inter-experimental variability in the incidence of sign- and goal tracking: Phenotypic sensitivity in food versus sexual reward studies

The vast number of studies investigating PCA in response to food- and drug-paired cues have allowed researchers to characterize the topographies and incidences of sign-, goal-tracking and intermediate responses in rats. Briefly, sign-trackers will vigorously approach and engage the CS with increasing rapidity upon its presentation, as the CS acquires incentive salience. In contrast, goal-trackers will approach the location where reward is delivered; the CS is primarily predictive of reward availability. Intermediate subjects exhibit neither a clear sign- or goal-tracking response; instead, they fluctuate between cue- and goal-directed conditioned responses. Furthermore, in studies using food reward, groups of subjects appear to be composed of approximately one-third of each phenotype (Flagel et al., 2009; Saunders & Robinson, 2010). The findings from Chapters 2, 3, and 4 of this thesis showed high variability in the numbers of sign-, goal-trackers and intermediate subjects across experiments, and the overall distributions of each phenotype fluctuated substantially from one study to another. Though more variable, we propose that the expression of sign-, goal-tracking and intermediate behaviour using sexual reward may be more stable in comparison to food reward following spatial manipulations to the CS-US relationship.

Several studies have proposed that sign- and goal-tracking conditioned response is dependent on several factors, one of which includes the spatial contingency between the CS and the food reward (Brown et al., 1993; Costa & Boakes, 2007; Holland, 1980; Silva et al., 1992; Timberlake & Lucas, 1985). For instance, early reports suggested that the distance between the CS and the location of food (US) delivery site influences the nature of the conditioned response

in pigeons (Peden et al., 1977) and rats (Karpicke, 1978). In a food reward study, Holland (1980) found that increased spatial distance between the CS and the US location led to a greater expression of goal-tracking behaviour in male rats, whereas sign-tracking behaviours were more frequent when the CS was presented closer to the food delivery site compared to a further location. Burns and Domjan (1996) investigated the manipulation of spatial distance between a sexually-salient cue and the door (US) providing copulatory access to a sexually-receptive female. Their findings revealed that increasing the spatial distance between the CS and US did not facilitate goal-tracking; in fact, they observed no goal-tracking in their experiments. In Chapter 2, we manipulated the spatial distance between the visuo-tactile cue and the door that provided the male access to the sexually-receptive female's compartment. Though we only observed a subset of goal-trackers and no sign-trackers, we found that the proportion of time spent in the CS- and US-designated areas was not influenced by the spatial location of the CS presentation. Collectively, these findings suggest that spatial distance affects PCA differently in studies using food and sexual reward. Specifically, sign- and goal-tracking in response to a cue paired with sexual reward appears to be more stable in terms of changes to the spatial arrangement of the CS and US, whereas individual differences in PCA using food appear to be more flexible and easily manipulated.

A comparison between sexual reward and other natural incentives may clarify the differences between the development of conditioned behaviours in response to sexually- or food-paired cues. For example, such variability may relate to the post-ingestive effects of reward following consumption. Eating, drinking and sexual activities are all rewarding given that their consummatory responses can effectively induce CPP, which measures the motivational effects of rewarding stimuli (Spyraki et al., 1982; Ågmo & Berenfeld, 1990; Tzschentke, 2007). However, important methodological differences may influence the interpretation of the rewarding value of food and sexual incentives. Specifically, most studies provide food reward *within* the place preference environment during the conditioning phase (Spyraki et al., 1982; Papp, 1988; Noye Tuplin & Holahan, 2018), which does not address whether the affective reaction produced by the rewarding stimulus participates in the development of CPP. For instance, sucrose reliably induces CPP whether it is consumed within the place preference environment or immediately before being placed into the CPP apparatus, indicating that sucrose activates positive affect that outlasts consummatory behaviour (Ågmo et al., 1995; White & Carr, 1985). In contrast, saccharin does not induce CPP when delivered immediately before place conditioning but can induce CPP as effectively as sucrose when consumed within the place preference environment (Stefaruk & van der Kooy, 1992; Ågmo & Marroquin, 1997) suggesting that presence of post-ingestive effects may

differentially contribute to conditioned responses. In comparison, sexual reward, and specifically ejaculation, can induce CPP independently of whether it is experienced within the CPP apparatus or in a separate mating cage prior to placement in the CPP apparatus (Ågmo & Berenfeld, 1990). Collectively, these findings suggest that sexual reward (and sucrose) induce a rewarding post-ingestive state that outlasts consumption; to date, it is unclear whether this applies to food-induced CPP as it is not addressed by current methodologies. In addition, most food reward CPP studies typically use food-deprived subjects; it may be that food reward induces CPP by reducing the hunger drive, or that the hedonic value of food is potentiated by subjects' internal drive state, as originally proposed by Hull (1943) and Toates (1986), respectively, whereas sexual motivation may not be similarly facilitated by an aversive drive state (Both et al., 2007). Based on these findings, the post-consummatory effects of sexual reward may explain why PCA using sexual stimuli is undisturbed by spatial manipulations to the CS-US relationship as demonstrated by our experiment and Burns and Domjan's (1996) study. We propose that the affective state outlasting consumption may contribute to the strength and stability of PCA in response to sexually-salient cues, particularly because there may not be an internal drive state to reduce (Both et al., 2007). In comparison, PCA in response to food-paired cues may be more susceptible to changes in spatial contingencies due to differences in post-ingestive effects of consummatory behaviour, combined with a need to reduce the hunger drive as subjects were food-deprived in the aforementioned studies (Holland, 1980; Costa & Boakes, 2007; Brown et al., 1993).

Differences in incentive salience and motivated behaviour induced by food, sex, and drugs

Several studies have proposed that it is possible to predict which individuals are likely to attribute incentive salience to reward-related cues. Importantly, such findings may shed light on those individuals who have difficulty resisting reward-paired cues, which makes them more susceptible to addiction. To date, these studies have exclusively investigated the propensity to attribute incentive salience to food- and drug-paired cues. Saunders and Robinson (2010) first tested this phenomenon using a cocaine self-administration procedure. Briefly, subjects were classified as sign- and goal-trackers based on their conditioned responses to a food-paired cue prior to cocaine self-administration, where a nose poke resulted in a cocaine infusion and the illumination of a light-CS. The findings revealed that subjects that attribute incentive salience to food-paired cues also attribute incentive salience to a cue paired with cocaine. Although food sign- and goal-trackers acquired cocaine self-administration at comparable rates, the removal of the light-CS severely decreased self-administration in food sign-trackers only; in food goal-trackers, removal of the light-CS resulted in a minor decrease in self-administration though this was not statistically significant. Food sign-trackers also showed a greater resistance under

extinction conditions compared to food goal-trackers; though both phenotypes slowly decreased their responses to the CS, food sign-trackers made more responses for the light compared to food goal-trackers during the first 4 extinction sessions. Importantly, these data highlight the propensity for sign-trackers to attribute incentive salience to food cues, and that this tendency extends to drug-paired cues even without prior drug experience.

Yager and Robinson (2013) reported similar results using a Pavlovian-conditioning procedure as opposed to self-administration. Specifically, the extent to which the light-CS elicited conditioned approach differed in food sign- and goal-trackers; across sessions, food sign-trackers reliably and persistently approached the light stimulus across sessions, whereas the probability to approach the light-CS decreased across sessions in food goal-trackers. In a separate experiment, food sign- and goal-trackers were trained to self-administer cocaine by making a nose poke response in the absence of any cue, followed by additional Pavlovian-conditioning sessions. Here, paired rats received presentations of an illuminated light-CS with a cocaine infusion, which were presented non-contingently in unpaired rats. Lastly, subjects underwent extinction training during which nose poke responses had no consequences and were then tested for Pavlovian cue-induced reinstatement, where a nose poke response resulted in the illumination of the cocaine-paired cue without cocaine infusion. There were no differences in the acquisition and extinction of cocaine self-administration in food sign- and goal-trackers, likely as these procedures did not include a cocaine-paired cue. All subjects showed cue-induced reinstatement when the light stimulus was paired with cocaine infusion; however, food sign-trackers in the paired condition showed greater reinstatement of responding to the cocaine-paired cue compared to food goal-trackers in the paired condition. Collectively, Saunders and Robinson (2010) and Yager and Robinson (2013) show that the tendency to attribute incentive salience to food-paired cues extends to drug-paired cues; compared to food goal-trackers, food sign-trackers displayed greater approach toward the cocaine-paired cue, resistance to extinction, and increased cue-induced reinstatement, suggesting that cocaine-paired cue was also imbued with incentive salience. Furthermore, though we previously proposed that the development of PCA in response to food-paired cues may be sensitive to the arrangement of the CS-US associative relationship, it appears to be stable across different types of reward.

Chapter 3 indicated the expression of sign-, goal-tracking and intermediate responses in response to a sucrose-paired cue following autoshaping. Specifically, sucrose sign-trackers displayed conditioned approach toward the lever-CS; they made significantly more lever-CS contacts, displayed a short latency to make a first lever-CS contact and showed high probability to make a lever-CS contact compared to other subjects. Sucrose goal-trackers displayed

conditioned approach toward the fluid port; they made significantly more normalized port entries, displayed a short latency to make a first port entry, and showed high probability to make a port entry compared to other subjects. In contrast to Saunders and Robinson (2010) and Yager and Robinson (2013), we found that subjects' sucrose phenotype was not predictive of sign- or goal-tracking in response to a sexually-salient cue. Specifically, sucrose sign-trackers displayed goal-directed behaviours in our sexual conditioning paradigm; they showed a tendency to approach the door that provided access to the sexually-receptive female and spent more time in the US-designated area. Though a statistical trend suggested a tendency for sucrose goal-trackers to approach and spend more time near the US, the analyses were non-significant; sucrose goal-trackers displayed intermediate-like behaviour, spending comparable proportions of time near the CS and US-designated areas. Therefore, our results are not consistent with those of Saunders and Robinson (2010) and Yager and Robinson (2013) as we found that the phenotypes expressed in response to a sucrose-paired cue do not predict PCA using sexual reward.

There is substantial overlap in the behavioural processes and brain mechanisms involved in feeding and those engaged by drugs of abuse, which may help to clarify the extension of sign- and goal-tracking behaviour in response to food- and drug-paired cues. For instance, the neural systems that evolved to respond to food are also activated by drugs of abuse (Avena et al., 2008). It has been well-established that addictive drugs activate the mesolimbic dopamine projection from the ventral tegmental area to the nucleus accumbens and that the nucleus accumbens is implicated in food-seeking and reinforcement learning, incentive motivation, and stimulus salience (Wise and Bozarth, 1984). Therefore, the unconditioned stimuli used in Saunders and Robinson (2010) and Yager and Robinson (2013) likely recruited similar neurological mechanisms. A banana-flavoured food pellet served as the food reward, which is a form of sucrose pellet made from dextrose, sucrose and corn syrup (Bio-Serv, #F0059, Frenchtown, NJ, USA), and standard rat chow, sugar, saccharin and corn oil have been shown to stimulate the release of dopamine in the nucleus accumbens (Bassareo and Di Chiara, 1997, Hajnal et al., 2004, Liang et al., 2006, Mark et al., 1991, Rada et al., 2005). Likewise, the use of cocaine as the drug reward directly amplifies mesolimbic dopamine signalling post-synaptically and increases dopamine concentration in the nucleus accumbens by inhibiting the presynaptic dopamine transporter thereby increasing dopaminergic concentrations in the synapse (Adinoff, 2004; Gerth et al., 2017).

Furthermore, a recent study by Bobadilla et al. (2020) reported that sucrose pellet- and cocaine-seeking both recruit similar neuronal networks by tagging activated cells in the nucleus accumbens core subregion following sucrose and cocaine self-administration. Using *in situ* hybridization, it was determined that cocaine and sucrose self-administration recruit one distinct

ensemble in the nucleus accumbens core; 66.7 ± 4.8% of cells recruited in the cocaine-seeking ensemble were D1-medium spiny neurons, and similarly, the sucrose-seeking ensemble was mostly composed of 54.08% ± 6.35% of D1-medium spiny neurons. The D1-medium spiny neurons are thought to mediate reinforcement learning and incentive salience attributed to rewarding stimuli (Baliki et al., 2013), as blocking D1-medium spiny neurons in the nucleus accumbens core has also been shown to inhibit the acquisition of sign-tracking responses (Macpherson & Hikida, 2018). Therefore, these data corroborate the role of the dopamine pathway in sucrose- and cocaine-seeking and identify shared recruitment of one distinct ensemble of D1-medium spiny neurons in the nucleus accumbens core, which may explain why individual differences in PCA extend from food to cocaine reward (see Rogers, 2017).

Cross-sensitization is a phenomenon thought to arise when different substances activate the same neural circuitry (Avena et al., 2008), and this offers a different potential explanation for the stability of sign- and goal-tracking in response to food- and drug-paired cues. Cross-sensitization occurs when the sensitization to one stimulus is generalized to other related stimuli, resulting in an amplification of responses to both the initial stimulus and related stimuli (Kalivas & Barnes, 1988; Robinson & Becker, 1986; Stewart & Badiani, 1993). Furthermore, reward cross-sensitization develops when sensitization to the rewarding properties of one substance extends to other substances of the same chemical class, or even certain natural rewards like sucrose. It is well-established that cross-sensitization occurs between many different types of addictive drugs, including amphetamine and cocaine (Ferrario & Robinson, 2007; Pierce & Kalivas, 1995), nicotine and ethanol (Blomqvist et al., 1996), and cocaine and ethanol (Itzhak & Martin, 1999), resulting in increased locomotor or consummatory behaviour (Piazza et al., 1989). Cross-sensitization is also reported to occur between sucrose, amphetamine, and cocaine (Avena & Hoebel, 2003; Gosnell, 2005).

The incentive-sensitization theory (Robinson & Berridge, 1993) proposes that chronic exposure to rewarding substances can sensitize the dopaminergic system and contribute to 'cue-triggered *wanting*' and a persistent compulsion to seek out other rewards. Specifically, microinjections of amphetamine into the nucleus accumbens shell subregion have been shown to potentiate the ability for a Pavlovian cue to elicit instrumental responding for sucrose reward (Wyvell & Berridge, 2001). Chronic exposure to rewarding substances can also cause adaptations to neurotransmitter release and synaptic plasticity (Gerdeman et al., 2003), and highly palatable diets have been shown to produce dopamine dysfunction. For instance, in rats given restricted daily access to sucrose at fixed intervals, there are changes in neural circuitry that resemble those observed following repeated amphetamine use (Avena et al., 2008; Furlong et al., 2014).

Specifically, the daily exposure to sucrose causes the repeated release of dopamine in the nucleus accumbens, as well as decreased D2-receptor and increased D1-receptor binding in the nucleus accumbens (Avena et al., 2008). The consumption of sucrose can also lead to cross-sensitization of dopamine-altering drugs; intermittent daily access to sucrose has been found to cross-sensitize with a low-dose of amphetamine up to 8 days post-consumption, as rats displayed an increased hyperactive response to amphetamine compared to control subjects. Gosnell (2005) reported similar findings between sucrose and cocaine; here, rats were first given 1-hour daily access to sucrose for 38 days, and then maintained on standard rat chow and water *ad libitum* for the remainder of the study. The data revealed that pre-exposure to sucrose produced sensitization to cocaine after repeated cocaine injections compared to standard rat chow, an effect which persisted up to 14 days. Together, these studies indicate that exposure to sucrose may produce alterations in the dopamine system, and suggest that sucrose, amphetamine, and cocaine activate similar brain mechanisms, leading to cross-sensitization. Furthermore, it may be that behavioural sensitization induced by banana-flavoured food pellets, as a form of sucrose pellet may contribute to 'wanting' or incentive salience to cocaine-paired cues thereby increasing the stability in sign- and goal-tracking for food- and cocaine-paired cues.

To our knowledge, no studies have investigated cross-sensitization between sucrose and sexual reward, or whether sexual responses are sensitized following repeated exposure to drugs of abuse. However, sexual reward also activates the mesolimbic dopamine pathway, as early studies report that dopamine is released into the nucleus accumbens following exposure to a sexually-receptive female, and elevated levels are maintained during the anticipatory and consummatory phases of sexual behaviour (Pfaus et al., 1990; Pfaus & Phillips, 1991; Damsma et al., 1992; Wenkstern et al., 1993; Balfour et al., 2003). Furthermore, sexual behaviour and sex-related environmental cues have been shown to activate dopamine neurons throughout the ventral tegmental area and increase neuronal activation in the nucleus accumbens core and shell subregions (Balfour et al., 2003). Though they share common neural mechanisms, several studies suggest differences in the adaptive properties of dopamine responsiveness between drug and natural rewards, which may contribute to variation in motivational attributes associated with different reward types. For instance, drugs of abuse and natural rewards both stimulate dopamine transmission in the nucleus accumbens shell subregion, however this effect habituates differently with repeated exposure to natural reward but not drug reward (Di Chiara, 2002).

In one study, Bassareo et al. (2002) exposed subjects to intraoral 20% sucrose and chocolate as an unfamiliar taste and measured extracellular dopamine in response to appetitive taste stimuli in the nucleus accumbens core and shell subregions and prefrontal cortex. In

comparison to water, 20% sucrose induced more pronounced appetitive reactions and increased dialysate dopamine in the prefrontal cortex, but not in the nucleus accumbens core or shell. In tests using chocolate, subjects were pre-exposed or naïve to chocolate in order to differentiate between stimulus novelty and appetitive taste stimuli. Chocolate increased basal dialysate dopamine in the nucleus accumbens core and shell, and this effect was more pronounced in the prefrontal cortex. Furthermore, 24-hours after pre-exposure, chocolate increased basal dialysate in the prefrontal cortex and in the nucleus accumbens core, but not shell subregion, and, in the nucleus accumbens core, chocolate increased extracellular dopamine to a greater extent in pre-exposed compared to naïve rats. In other words, the increase in dopamine transmission observed in the nucleus accumbens shell in response to chocolate intake habituated following a single pre-exposure to the same taste or food, though dopamine release remained elevated following repeated exposure to chocolate in the nucleus accumbens core and prefrontal cortex.

Therefore, extracellular dopamine transmission in response to motivational stimuli appears to differ between brain regions based on an interaction between motivational valence, novelty, and value; specifically, dopamine responsiveness in the nucleus accumbens shell subregion may be more sensitive to motivational valence and novelty, whereas it may reflect overall motivational value in the nucleus accumbens core and prefrontal cortex. According to Volkow et al. (2016), dopamine cells habituate after repeated consumption of natural rewards, such as food and sex, thereby satiating the drive to further pursue them. In contrast, addictive drugs bypass natural satiation and continue to directly increase dopamine release (Di Chiara, 2002; Wise, 2002), which can explain why individuals compulsively pursue drug but not natural reward. However, one might question the suitability of sucrose, banana-flavoured pellets, and chocolate as ‘natural’ rewards; in this context, perhaps food reward relates specifically to a standard rat chow diet. Therefore, evidence of cross-sensitization between sucrose and cocaine, in addition to the increased dopamine response following chocolate intake which does not habituate with repeated exposure may provide a further explanation for Saunders and Robinson (2010) and Yager and Robinson’s (2013) findings that sign- and goal-tracking for food extends to cocaine reward. In relation to our findings in Chapter 3, it is possible that sign- and goal-tracking for a sucrose-paired cue is stable, given it recruits a similar neuronal network to cocaine, and continues to elicit dopamine release without habituation in the nucleus accumbens core subregion. Though dopamine release is increased in the nucleus accumbens upon presentation of a sexually-receptive female and throughout the copulatory response, it may habituate upon repeated exposure as suggested by Volkow et al. (2016) and explain why sign- and goal-tracking for sucrose does not extend to sexual reward.

The role of oxytocin in social learning, and how it may stabilize the expression of goal-tracking in response to sex-paired cues

It is well-established that the mesolimbic dopamine pathway contributes to the attribution of incentive salience to reward-related cues, and consequently, to the development of individual differences in PCA. Furthermore, disruptions to the dopaminergic system are thought to mediate several behavioural disorders, including compulsive behaviours related to food, drugs, and sexual dysfunction (Johnson & Kenny, 2010; Di Chiara, 1998; Blum et al., 2012). Studies on the role and effects of neurocircuitries that influence these behaviours through their interactions with the mesolimbic dopamine system are relatively sparse, though the neuropeptide oxytocin has been proposed as an important mediator given its prevalent effects on the central nervous system (Baskerville & Douglas, 2010).

Both centrally and peripherally, the neuropeptide oxytocin is involved in a myriad of behaviours, including social memory, affiliation, sexual behaviour, stress, anxiety, and aggression (Lee et al., 2009; Johnson et al., 2016), and stimulation of central dopamine and oxytocin pathways produce similar effects on social and sexual behaviours (Baskerville & Douglas, 2008). Oxytocin neurons in the hypothalamus have also been shown to express dopamine receptors (Baskerville et al., 2009), suggesting a direct regulatory effect on sexual behaviour which is principally governed by the medial preoptic area (Larsson & Heimer, 1964; Malsbury, 1971; Rodríguez-Manzo et al., 2000; Vaughan & Fisher, 1962). In conjunction with the medial preoptic area, the paraventricular and supraoptic nucleus of the hypothalamus are implicated in the regulation of penile erection and copulation in male rats (Baskerville et al., 2009; Paredes & Ågmo, 2004; Pattij et al., 2005), and are also responsible for the synthesis and distribution of oxytocin (Nestler et al., 2015; Breedlove & Watson, 2020). The medial preoptic area, paraventricular and supraoptic nucleus of the hypothalamus are innervated by dopaminergic fibres from the incertohypothalamic system, a dopaminergic pathway from the zona incerta to the hypothalamus (Buijs et al., 1984; Decavel et al., 1987), and express dopamine D2-like receptors (Baskerville & Douglas, 2010), further suggesting a direct regulation of hypothalamic oxytocin by dopamine. Mesolimbic dopaminergic activity is also modulated by oxytocin; the ventral tegmental area receives oxytocin input from the paraventricular nucleus of the hypothalamus (Buijs, 1978; Sofroniew, 1983; Roeling et al., 1993) and contains oxytocin receptor mRNA (Freund-Mercier et al., 1987; Vaccari et al., 1998), and paraventricular nucleus of the hypothalamus oxytocin fibres lie in close apposition to dopamine cell bodies in the ventral tegmental area that terminate in the nucleus accumbens. As such, it has been proposed that during sexual arousal, stimulation of the mesolimbic dopamine pathway via oxytocin released in the ventral tegmental area activates the

incertohypothalamic dopamine fibres that innervate the medial preoptic area, paraventricular and supraoptic nucleus of the hypothalamus. In turn, oxytocin is thought to act within the hypothalamus, and in limbic brain structures (i.e., amygdala and hippocampus) and the spinal cord, thereby activating the mesolimbic dopamine pathways and the expression of penile erection (Baskerville & Douglas, 2010).

Several studies have identified an important role for oxytocin in sexual behaviour, and specifically, in the development of conditioned partner and ejaculatory preferences using olfactory stimuli (Kippin et al., 1998; Coria-Avila et al., 2005; Ménard et al., 2019). Based on neuroanatomical and neurochemical evidence linking the dopamine and oxytocin systems, it is hypothesized that the interaction of mesolimbic dopamine and oxytocin may contribute to Pavlovian conditioning, and perhaps PCA using sexual reward. As such, we investigated whether the chronic systemic administration of oxytocin could promote or potentiate the development of individual differences in PCA in response to a visuo-tactile cue paired with a sexually-receptive female. In Chapter 4, we reported the development of sign-tracking and intermediate behaviours in response to a sexually-conditioned cue; though the administration of oxytocin did not affect the expression of sign-tracking behaviour, we found that it facilitated a goal-tracking response in intermediate subjects.

In comparison to the literature on sign- and goal-tracking, there is relatively little information known about the intermediate phenotype. In a PCA procedure using food or drug reward, intermediate subjects tend to vacillate between cue- and goal-directed behaviours, thereby exhibiting both conditioned responses (Fitzpatrick & Morrow, 2016; Flagel et al., 2009). It is unclear why intermediate subjects do not show clear sign- or goal-tracking behaviour, but the emergence of this phenotype is not thought to reflect differences in learning ability (Harb & Almeida, 2014). It has been suggested that intermediate subjects may transition to sign-tracking in response to food-paired cues following extended training, though at a lower level compared to animals initially identified as sign-trackers (Flagel et al., 2009; Flagel et al., 2008). However, in our study, the chronic systemic administration of oxytocin shifted intermediate subjects toward a goal-directed phenotype.

Unlike studies using food and drug reward, the paradigm used here featured a strong social component, as a consequence of copulation with a sexually-receptive partner. In vertebrates, social behaviour is thought to be modulated by neuropeptide-sensitive neural networks (Johnson et al., 2017). Newman (1999) first introduced the brain's social behaviour network, currently referred to as the social decision-making network (SDMN), which is composed of six nodes: the extended medial amygdala (i.e., medial amygdala, medial bed nucleus of the

stria terminalis), the lateral septum, the preoptic area, the anterior hypothalamus, the ventromedial hypothalamus, and the midbrain (i.e., periaqueductal grey, various areas of the tegmentum). Each of these nodes is implicated in the control of multiple types of social behaviour, including appetitive and consummatory sexual responses, and work in tandem with other areas relevant to social behaviour, such as basal forebrain and cortical areas (Goodson, 2005; Coolen et al., 1997). According to Newman (1999), each node within the social behaviour network reacts to an assortment of different stimuli, and each social context and behavioural response is associated with a specific pattern of activity across the nodes. For example, the same nodes may show increased activation during instances of male sexual behaviour and aggression, however the overall response pattern across the nodes is specific to each social context.

Based on the relationship between neuropeptide signalling and functional connectivity between the SDMN, Johnson et al. (2017) proposed a social salience network (SSN) composed of several interconnected brain nuclei, which is thought to encode the valence and incentive salience of socio-sensory cues. The SSN includes several of Newman's (1999) SDMN nodes, and incorporates the paraventricular nucleus of the hypothalamus, the anterior olfactory nucleus, and the prefrontal cortex given socio-sexual learning and behaviours depend on activation of these areas. The SSN is proposed to assist in the encoding of valence and incentive salience in socio-sensory cues. In one study, Johnson et al. (2016) analyzed the expression of the immediate-early gene Fos, a marker for neuronal activation, transcriptional activity, and synaptic plasticity, across the oxytocin-receptor-expressing nodes of the SSN in male prairie voles following social isolation and two social contexts (socio-sexual interaction with copulation and socio-sexual interaction with copulation following central oxytocin-receptor blockade). They found that socio-sexual interaction and copulation strongly increased Fos expression across the SSN in comparison to socially-isolated subjects, and that there were no differences in Fos expression between the two social treatment groups. However, they found large differences in the patterns of correlated Fos expression across the SSN; vehicle-treated males showed a strong, positive correlation of Fos expression across SSN nuclei during socio-sexual interactions and copulation, which was disrupted by oxytocin-receptor antagonism. These data suggest that, in a social context, oxytocin-receptor signalling modulates the connection between the nodes of social processing networks, like the SSN.

In a separate study, Johnson et al. (2017) further explored how oxytocin receptor activation during a social context modulates the expression Fos across the SSN in the nucleus accumbens shell subregion of male prairie voles during socio-sexual interactions with a female. Neuroanatomically, the nucleus accumbens shell serves as an integration centre within the SSN.

Apart from the anterior olfactory nucleus, it receives direct projections from every other SSN node, and direct axonal oxytocinergic projections from the paraventricular nucleus of the hypothalamus. The data revealed that blocking oxytocin receptors in the nucleus accumbens shell did not influence copulation frequency, anogenital investigation, mounting, or intromission in male prairie voles, though previous reports suggested that intracerebroventricular injections of the oxytocin-receptor antagonist significantly reduces copulation frequency. This suggests that oxytocin receptors in other nuclei may be implicated in the modulation of sexual behaviour and is also consistent with previous studies in rats (Argiolas & Melis, 2013; Gil et al., 2011). Irrespective of treatment with the oxytocin-receptor antagonist or vehicle, increased Fos-ir was observed across all SSN nuclei in subjects that copulated with a female partner compared to socially-isolated subjects, further highlighting that socio-sexual interaction and copulation activate the SSN network. Furthermore, the expression of Fos in SSN nuclei strongly predicted Fos expression in the nucleus accumbens shell in control subjects, but not in males treated with the oxytocin-receptor antagonist. This indicates that, during socio-sexual interactions and copulation, nucleus accumbens shell oxytocin signalling robustly modulates how transcriptional activity and synaptic plasticity correlates with transcriptional activity and synaptic plasticity in other SSN nuclei, and further validates the activation of a social salience network induced by socio-sexual interactions and sexual reward.

Though the aforementioned studies used male prairie voles, several studies also report the central influence of oxytocin on sexual arousal, anticipatory and consummatory behaviour in male rats, and this likely arises through its ability to modulate the mesolimbic and mesocortical pathways as previously described (Argiolas & Melis, 2013; Gil et al., 2011; Melis et al., 2007, 2009, 2010; Succu et al., 2007, 2011; Sanna et al., 2012). Given the neuroanatomical and neurochemical parallels between rodent species, it is possible that socio-sexual interactions and copulation also activate the SSN in male rats, thereby controlling the consummatory (i.e., erection, ejaculation) and anticipatory (e.g., motivational, rewarding) aspects of male sexual behaviour in socio-sexual contexts. These studies also refer to centrally-released oxytocin specifically, however, it has been proposed that release of peripheral and central oxytocin may be coupled in vertebrates as evidenced in fish, birds, and mammals (Godwin and Thompson, 2012; Goodson and Kabelik, 2009; Knobloch et al., 2012; Mahoney et al., 1990; Ross, Cole et al., 2009; Saito et al., 2004).

The influence of oxytocin on mesolimbic and mesocortical dopamine may translate the motivational aspects of natural stimuli into goal-directed behaviours, which in the case of our study would be moving to the location of a sexual partner and copulation to reach ejaculation (Goto &

Grace, 2005). In Chapter 4, the administration of oxytocin did not affect the expression of sign-tracking in response to a sexually-conditioned cue but facilitated goal-tracking in intermediate subjects. Therefore, the actions of oxytocin appear to be specific in animals that have not attributed incentive salience to the cue paired with sexual reward. Sign- and goal-tracking have been shown to express different patterns of mesolimbic dopamine activity, as the presentation of reward-paired cues increase dopamine release in the nucleus accumbens in sign- but not goal-trackers (Flagel et al., 2011). Though not explicitly measured in intermediate subjects, one could assume that the release of dopamine in the mesolimbic pathway is highest in sign-tracking subjects compared to goal-trackers and intermediates, as its activation has been shown to intensify 'wanting' or incentive salience (Tindell et al., 2005). As such, it may be that the dose of oxytocin administered, may have been insufficient to modulate activity in an already amplified mesolimbic dopamine pathway in sign-trackers. In comparison, intermediate subjects fluctuate between cue- and goal-directed responses; they do not attribute incentive salience to reward-related cues, and therefore would not show an increase in mesolimbic dopamine release in response to a sexually-conditioned cue. Therefore, it is possible that oxytocin potentiated the value of socio-sexual interactions and copulation, potentially via the SSN, and modulated mesolimbic dopamine toward goal-directed behaviours in animals that otherwise do not show a clear conditioned response to a sexually-conditioned cue.

The implications and applications of sign- and goal-tracking to the understanding of human behaviour

The vast majority of research investigating individual variation in PCA has been conducted using animal models. Notably, these animal models offer an invaluable opportunity to elucidate the causal mechanisms that contribute to a variety of impulse control disorders in humans, including substance use disorder, eating disorders, and potentially paraphilic and fetishistic disorder. The ability to identify sign- and goal-tracking in responses to reward-related cues is especially useful, given that it may confer vulnerability or resistance to the development of maladaptive behaviours, respectively.

Prior to the term 'sign-tracking', autoshaping has been explored in pigeons, chickens, ring doves, racoons, reindeer, whales, pigs, and honeybees (Breland & Breland, 1961; Bitterman, 1988). The expression of sign- and goal-tracking has been investigated in several different species, including rodents, birds, and fish (Robinson et al., 2014; Brown et al., 1993; Domjan et al., 1986; Burns & Domjan, 1996; Hollis et al., 1989). Each species shares a common display of approach toward, and engagement with, a cue paired with reward or the location where reward is delivered, representing sign- and goal-tracking behaviours, respectively. Recently, translational

studies have attempted to explore sign- and goal-tracking in human subjects, given that the inherent characteristics of sign-tracking are associated with deficits in impulse control, 'obsessive', and 'compulsive' behaviours (Flagel & Robinson, 2017; Flagel et al., 2010; Lovic et al., 2011; Colaizzi et al., 2020). This has allowed researchers to identify overlapping constructs between rodent sign-tracking and human addiction. Briefly, in comparison to goal-trackers, sign-trackers exhibit poor performance in sustained attention tasks which require a behavioural response in the presence of distractors, which reflects a deficit in attentional control (Koshy Cherian et al., 2017; Paolone et al., 2013; Pitchers et al., 2017). Similarly, in humans, an attentional bias to reward-related cues is a characteristic of impulse control disorders (e.g., substance use), and analogous to sign-tracking behaviour in rodents (Le Pelley et al., 2015). In rats, novelty-seeking may exemplify risk-taking behaviour in humans; in rats selectively bred as high (bHR) and low (bLR) responders to novelty, bHR subjects display a sign-tracking response, whereas bLR subjects display a goal-tracking response. Furthermore, bHR rats exhibit several behaviours that parallel human addiction, including aggression, impulsivity, and an increased motivation for drug intake and propensity for relapse (Flagel et al., 2016, 2010; Flagel et al., 2014; Kerman et al., 2011). In terms of impulsive behaviour, bHR rats show a deficit in behavioural inhibition and in withholding responding for reward and display more impulsive actions compared to bLR rats (Flagel et al., 2010/11 bHR rats; Lovic et al., 2011). In humans, impulsivity is characterized by premature actions, rapid decision-making, and an inability to delay gratification, making it determinant of vulnerability to addiction (de Wit, 2008; Verdejo-García et al., 2008). Taken together, the parallels between animal models and the features of human addiction and compulsive behaviour are both important and useful, as they provide predictive insight into modelling risk for engaging in maladaptive behaviour.

To date, there is only preliminary evidence for the expression of sign- and goal-tracking in humans given the diversity in cognition, prefrontal control and psychopathologies that influence behaviour. In one of the first studies to measure sign- and goal-tracking in response to food-paired cues in humans, Versace et al. (2015) exposed lean (i.e., body mass index < 25 kg/m^2) and obese (i.e., body mass index > 29.9 kg/m^2) adults to food-related, pleasant (e.g., erotica, romantic), neutral (e.g., objects) and unpleasant (e.g., pollution, mutilation) emotional images while recording brain activity using electroencephalography. Late positive potentials (LLP) were used in order to measure the attribution of incentive salience to visual stimuli; LLP is an event-related potential that reflects facilitated attention to emotionally-salient stimuli (Dennis & Hajcak, 2009). In addition, subjects were required to complete a series of questionnaires designed to assess eating behaviours, mood, and hedonic capacity. Participants were classified as sign-trackers

based on significantly higher LLP responses to food-related stimuli and significantly blunted LLP responses to both high and low arousing pleasant stimuli. In sign-trackers, LLP responses to food-related stimuli were comparable in magnitude to LLP responses evoked by other emotional highly-arousing images. In contrast, the LLP responses to food-related stimuli were comparable to those induced by neutral images. While sign- and goal-tracking clusters were composed of both lean and obese subjects, there was a significantly higher proportion of obese subjects classified as sign-trackers compared to lean subjects. Lastly, obese individuals classified as sign-trackers self-reported a greater propensity for maladaptive eating behaviour, emotional eating, food cravings, and greater feelings of loss of control compared to lean sign-trackers. Importantly, these findings reflect individual differences in the propensity to attribute incentive salience to food-related cues relative to other pleasant stimuli, and greater susceptibility to overeating and obesity in human sign-trackers relative to goal-trackers.

The concepts of associative learning and conditioned cues have been extensively used to account for the wide diversity of human sexual responses, preferences, and arousal states. In some individuals, sexual preferences and behaviours can become impulsive and compulsive. Compulsive sexual behaviour is characterized by an inability to control sexual urges, behaviours, and thoughts despite negative consequences (Werner et al., 2018; Walton et al., 2017; Kuiper & Coolen, 2018). Paraphilic and fetishistic disorders can feature compulsive behaviour, though specifically toward atypical, non-living objects or non-genital body parts, and can cause distress, impairment and personal harm or risk of harm to others (DSM-5; American Psychiatric Association, 2013). Research into fetishistic behaviour is limited, though it is generally accepted that conditioning mechanisms play an important role (Rachman & Hodgson, 1968; Weinberg, et al., 1995). Specifically, fetishistic behaviour is thought to develop following a combination of initial experiences with the fetishized object and masturbatory imagery, which is subsequently reinforced by orgasm (McGuire et al., 1965). Though the precise mechanisms that turn an otherwise healthy fetish into fetishistic disorder remain unclear, studies investigating the attribution of incentive salience to sex-paired cues may provide a useful explanation.

Conclusions

Several preclinical studies suggest that the subjective properties of food- and drug-paired cues can strongly guide motivated behaviour (Flagel et al., 2009). In some subjects (i.e., sign-trackers), the conditioned cue becomes ‘attractive’ and ‘wanted’ as it acquires incentive salience; while in others (i.e., goal-trackers), it merely serves as a predictor for impending reward (Flagel et al., 2009). Interestingly, sign-trackers show poor attentional and impulsive control, and novelty-seeking, which are the defining characteristics of addiction, compulsive behaviour, and impulse

control disorders (Koshy Cherian et al., 2017; Paolone et al., 2013; Pitchers et al., 2017; Le Pelley et al., 2015; Flagel et al., 2010, 2014, 2016; Kerman et al., 2011; Lovic et al., 2011). Here, we proposed an animal model to examine PCA behaviours that could contribute to fetishism and investigated the development of sign- and goal-tracking behaviour in response to a cue paired with the opportunity to copulate to ejaculation with a sexually-receptive female, as orgasm is thought to reinforce fetishized objects (McGuire et al., 1965). We reported incidences of sign- and goal-tracking behaviour in response to a sexually-conditioned cue, and this indicates that sexually-salient cues can acquire incentive salience. Importantly, the association between sign-tracking and fetishistic disorder may provide a better etiological understanding of sexual dysfunction, and greater insight into the development of effective treatment interventions.

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