The role of oxytocin in the formation of conditioned mate preferences and the facilitation of reproductive responses in the female rat

Conall Eoghan Mac Cionnaith

A Thesis

In the Department

Of

Psychology

Presented in Partial Fulfilment of the Requirements

For the Degree of

Doctor of Philosophy (Psychology) at

Concordia University

Montréal, Québec, Canada

January 2023

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: *Conall Eoghan Mac Cionnaith*

Entitled: *The role of oxytocin in the formation of conditioned mate preferences and the facilitation of reproductive responses in the female rat*

and submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (*Psychology*)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

_____ Chair
Dr. William Zerges

_____ External Examiner
Dr. Fay Guarraci

_____ Examiner
Dr. Dave Mumby

_____ Examiner
Dr. Mark Ellenbogen

_____ Examiner
Dr. Andy Chapman

_____ Thesis Supervisor
Dr. Wayne Brake

Approved by

Dr. Andy Chapman
Graduate Program Director

17th January 2023 _____

Dr. Pascale Sicotte
Faculty of Arts and Science

Abstract

The role of oxytocin in the formation of conditioned mate preferences and the facilitation of reproductive responses in the female rat

Conall Eoghan Mac Cionnaith, Ph.D.

Concordia University, 2023

Oxytocin (OT) is known for its regulatory role in the formation of partner preference in the monogamous prairie vole. In contrast, female rats are considered promiscuous and typically copulate with many males during a mating bout. Despite this promiscuity, female rats selectively receive ejaculations from a dominant male and wait longer before resuming copulation following the receipt of a dominant male's ejaculation, allowing for sperm transport. A preference to solicit and receive a particular male's ejaculation can also be formed using Pavlovian conditioning in which females repeatedly pace copulation with males bearing a neutral scent cue. Pacing, the ability of a female to voluntarily initiate copulation and withdraw from a male, is known to be a potent sexual reward and facilitates pregnancy and reproductive responses. OT is known to regulate reproductive responses in female rats following copulation. The aim of the research in this thesis was to examine the role of OT in the formation of conditioned mate preferences, in addition to OT's effects on reproductive responses. In the first chapter, females received their first copulatory experiences with the same male under one of two different pacing conditions. It was found that the pacing condition associated with longer pacing intervals resulted in a preference to receive a paired male's ejaculation. Using immunohistochemistry, it was shown that preferred males activate OT neurons in three areas of the hypothalamus important for female sexual behaviours, the medial preoptic area, the ventromedial hypothalamus, and the paraventricular nucleus. The second chapter used a variety of behavioural and immunohistochemical assays to examine how OT transmission facilitates mate preference formation in rats. It was shown that peripherally-injected OT mimics the effects of vaginocervical stimulation (VCS) and facilitates mate preference formation. The effects of VCS were blocked by an OT receptor (OTR) antagonist that does not enter the brain, suggesting that peripheral OTRs are important for reproductive responses and preference formation following VCS. Taken together, these data indicate that OT transmission is required for sexual satiety, an important component of sexual reward, and early progestational responses, such as estrous termination. As such, these data suggest that sexual reward and enhanced reproduction are linked through Pavlovian processes that activate both central and peripheral OT.

Contributions of Authors

Chapter 1. Fos expression is increased in oxytocin neurons of female rats with a sexually conditioned mate preference for an individual male rat

Conall Mac Cionnaith. Designed and conceived experiment. Performed surgeries, conducted behavioural study, sectioned, and stained tissue. Collected data and ran all statistical analyses. Wrote and edited manuscript.

Alice Lemay. Helped with behavioural data collection and tissue sectioning.

Eamonn Gomez-Perales. Helped with behavioural data collection and tissue sectioning.

Genevieve Robert. Helped with behavioural data collection.

Rebecca Cernik. Helped with data behavioural data collection and tissue sectioning.

Wayne G.Brake. Helped to write and edit manuscript. Helped with immunostaining data analysis.

James G. Pfaus. Helped to design and conceive experiment. Helped to write and edit manuscript.

Chapter 2. Peripheral oxytocin signaling promotes estrous termination and conditioned mate preferences female Long-Evans rats

Conall Mac Cionnaith. Designed and conceived all experiments. Performed all surgeries. Conducted all behavioural experiments and conducted all immunoassays. Performed data analysis. Wrote manuscript.

Eamonn Gomez-Perales. Helped designed and conduct mate preference experiment.

Hanya Ismail. Helped conduct behavioural experiments and immunoassays.

Jesse Lacasse. Helped conduct immunoassays and created standard curves for ELISAs.

James G. Pfaus Helped design and conceive experiments. Helped write and edit manuscript.

Wayne G.Brake. Helped design and conceive experiments. Helped write and edit manuscript.

Table of Contents

List of Figures

Introduction

Chapter 1. Fos expression is increased in oxytocin neurons of female rats with a sexually conditioned mate preference for an individual male rat.

Chapter 2. Peripheral oxytocin signaling promotes estrous termination and conditioned mate preferences female Long-Evans rats

Discussion.

List of Abbreviations

AOB Accessory Olfactory Bulb

BNST Bed Nucleus of the Stria Terminalis

CLS Clitoral stimulation

DA Dopamine

D1R Dopamine D1-like receptor

D2R Dopamine D2-like receptor

EB Estradiol benzoate

FSH Follicle stimulating hormone

GnRH Gonadotropin releasing hormone

LH Luteinizing hormone

LM Lordosis magnitude

LQ Lordosis quotient

MeA Medial amygdala

MeApd Posterodorsal region of the medial amygdala

MHC Major histocompatibility complex

MOB Main olfactory bulb

mPOA Medial preoptic area

MUP Major urinary protein

NAcc Nucleus accumbens

NOS Nitric oxide synthase

OT Oxytocin

OTR Oxytocin receptor

P Progesterone

PEI Post-ejaculatory interval

PIDA Periventricular dopamine

POMC Proopiomelanocortin

PVN Paraventricular nucleus

SON Supraoptic nucleus

TIDA Tuberoinfundibular dopamine

VCS Vaginocervical stimulation

VMH/VMHvl/VMHdm Ventromedial hypothalamus/ventrolateral region/dorsomedial region

VTA Ventral tegmental area

ZI Zona Incerta

β-END Beta endorphin

μOR μ opioid receptor

General Introduction

Frame of reference.

Wild rats copulate in a seemingly indiscriminate manner. Many males copulate with a given female in a copulatory bout and, conversely, female rats may solicit and receive ejaculations from many males (McClintock, 1984). Thus, rats have been described as using a promiscuous mating strategy. Indeed, it has since been posited that mate choice may be random in female rats (Le Moëne & Snoeren, 2018). However, female rats selectively receive the ejaculations of dominant males and impose longer post-ejaculatory intervals after the receipt of a dominant male's ejaculation facilitating sperm transport (McClintock et al., 1982; McClintock, 1984). Moreover, Calhoun (1963) documented that dominant rats and their offspring tend to have greater reproductive success than subordinates. Creating large naturalistic environments in which a colony of rats can establish dominance hierarchies and tracking female mating preferences is difficult. Additionally, studying the underlying neurobiology of mate choice behaviours is difficult without standardized cues that can elicit patterns of neuronal activation.

Proximal experience-based preferences could offer a useful model with which to study female mate preferences and its underlying neurobiology. Coria-Avila et al. (2005) showed that female rats can form preferences for a type of male based on Pavlovian associations with the copulatory reward state induced by paced mating. Specifically, when females were given paced copulation trials with males bearing a neutral scent (i.e., natural almond extract), they preferentially solicited and received the first ejaculation from a scented male in an open field sexual preference test with two tethered males, one scented with the almond odour and the other unscented. Such an experientially-mediated preference allows for the use of a standardized cue that elicits preferential sexual behaviours.

In contrast to rats, the prairie vole is monogamous and forms long lasting affiliative pair bonds (Carter et al., 1995; Getz et al., 1981). OT is thought to regulate the formation of a partner preference in the prairie vole (Cushing & Carter, 2000; Young & Wang, 2004) Relative to the nonmonogamous montane vole, the prairie vole has increased OTR densities in several brain regions (Insel & Shapiro, 1992). Additionally, both systemically injected and centrally-infused OT is necessary and sufficient to induce a partner preference in prairie voles (Cushing & Carter, 2000). Whether OT also facilitates the formation of sexual preferences in the female rat has not yet been established.

OT has several important reproductive functions in the female rat. These range from maintaining the cyclicity of the estrous cycle (Johnston et al., 1990; Robinson & Evans, 1990), the facilitation of the lordosis reflex (Gorzalka & Lester, 1987), to the induction of the hormonal state required for pregnancy after copulation, i.e., the progestational state (Egli et al., 2006; Helena et al., 2011; Northrop & Erskine, 2008). Paced copulation, the type of copulatory stimulation that supports the acquisition of sexually conditioned partner preferences and a conditioned place preference (Coria-Avila et al., 2005; Paredes & Alonso, 1997), also facilitates the induction of the progestational state (Adler, 1969; Erskine, 1985; Kornberg & Erskine, 1994). Consequently, there is a link between the type of copulatory stimulation female rats find rewarding and reproductive outcomes.

The aim of this thesis was to examine whether OT regulates the formation and display of sexually conditioned preferences in the female rat and if so, by what mechanism. This research tests 1) whether OT neurons are activated by preferred mates; 2) whether exogenous OT facilitates the formation of a conditioned mate preference; 3) whether OT coordinates reproductive responses following copulatory stimulation; 4) whether there is an OT-mediated link between reproductive responses and preference behaviours.

Introduction

The survival of mammals depends on sexual reproduction. A defining characteristic of mammalian sexual reproduction is meiosis and the fusion of two haploid gametes (a male Y gamete and a female X gamete; as reviewed in Fusco & Minelli, 2019). The Y gamete is a sperm cell and the X is an ovum cell (Fusco & Minelli, 2019). At its core, the purpose of sexual reproduction is the recombination of genetic material which is thought to be evolutionarily beneficial because it can increase genetic variation in traits on which selection can then operate, over a shorter time-span relative to asexually reproducing organisms (Fisher, 1930, 1958; Weismann, 1889). However, genes are carried by hosts (i.e. individual animals), and the passing on of genetic material depends on how a host acts in its environment. Sexual behaviours animate the *telos* of genes to recombine and increase in frequency in a given population.

Males and females exhibit different sexual behaviours and physiologies (as reviewed in Segovia & Guillamón, 1993). As there are two sexually differentiated gametes, two sexually dimorphic phenotypes (both physical and behavioural phenotypes) have consequently emerged through sexual reproduction. Many sex differences, even including the phylogeny of differentiated genitalia, must be the result of natural selection. However, additional variation in sex-linked traits can be further selected for by differential success in mating (Darwin, 1871; Fisher, 1930, 1958; O'Donald, 1980). The selection of certain sex-linked traits is both a cause and a consequence of sexual reproduction and has been a primary driver of sexual dimorphism in animals
(Clutton-Brock, 2017; Darwin, 1859, 1871; Fisher, 1930, 1958). With each evolutionary iteration of sexual selection, the traits of the reproductively successful become more stable in a genetic population. Sexual selection has been pivotal in the selection of certain traits that increase an animal's success in mating. These evolutionary processes include mate choice and intrasexual-competition (Darwin, 1871). However, the consequences of sexual behaviours and reproduction are asymmetric for most mammals. Females bear the responsibility of gestation and mainly care for young offspring. As a result of this larger female investment (Trivers, 1972), it has been proposed that females of many species have been selected to be choosy when selecting a mate (P. Bateson, 1983; Fisher, 1930, 1958; Trivers, 1972).

Mate choice is one process by which sexual selection occurs. Theories of mate choice offer explanations for the frequency of certain traits that cannot be explained by natural selection alone. On a population level, it describes why some animals are compelled to mate preferentially with some individuals

over others (Halliday, 1983). Some theories of mate choice predict that there is an ultimate genetic and evolutionary causality that guides an animal to mate with one partner over another. Animals that express these certain selected traits will have increased success in mating than those who do not. Different theories of mate choice emphasize different mechanisms relating to how such preferences evolve, such as seeking to mate with an individual with a high genetic fitness value, seeking a mate with high fecundity, parental investment, or seeking a mate that can monopolize or guarantee resources. Regardless of the mechanism, whether animals that vary in certain traits have an increased likelihood of reproducing is always a species-specific empirical question (P. Bateson, 1983; Halliday, 1983).

At the same time, animals are particularly responsive to certain biologically relevant stimuli. These biologically relevant stimuli often have incentive properties that elicit an animal's motivation to either avoid or approach and consummate (Bindra, 1974; Toates, 1986). Stimuli that instigate behaviour in this manner are often referred to as a reward or hedonic (Bindra, 1974; Schultz, 2002) or primary reinforcer (Skinner, 1938; 1953; 1963). For example, when presented with the incentive stimulus of a potential mate, rats will readily cross an electrified grid to gain access to this mate (Anderson, 1937), indicative of the incentive properties of copulation. The pursuit of pleasure guides much of human sexual behaviour too, despite its sometimes negative outcomes. People sometimes destroy relationships and marriages, often upending their whole lives in the pursuit of sex. Incentive stimuli that induce appetitive and consummatory behaviours are often rewarding and experience with these stimuli increase or change an animal's subsequent seeking and consummation of an incentive stimulus. Importantly, animals learn about the responses required to approach and consummate a reward by operant associations, and cues that predict an incentive stimulus by Pavlovian associations.

Male macaques and capuchins trained to use tokens as a means of exchange quickly learn to trade tokens for sex or for an opportunity to view a female's genitalia (Addessi et al., 2007; Deaner et al., 2005). Such instrumental responses are reinforced, i.e., increased in frequency, by a response being followed by access to an incentive stimulus, following Thorndike's law of effect (Thorndike, 1911). Indeed, female rats can learn to nose-poke or lever press to gain access to a sexually vigorous male, in order to receive vaginocervical stimulation (VCS) and clitoral stimulation (CLS) from penile intromissions (Cummings & Becker, 2012; French et al., 1972; Pfaus et al., 2015). Though these operant responses are not inherently sexual, because they are made instrumentally by a female to consummate a sexual reward, they act as an

appetitive sexual response. Importantly, operant responses like those reported by Cummings and Becker (2012) allow a female to voluntarily impose an interval between intromissions by design, as the receipt of an intromission is conditional on a female's response. For female rats, the ability to impose an optimal interval between intromissions, i.e., pacing, is rewarding (Paredes & Alonso, 1997). In wild and semi-naturalistic environments, female rats pace by running away from males (Pfaus et al., 1999), exiting into, and then returning from burrow systems, or jumping on rocks or other obstacles that the male cannot get to (Calhoun, 1963; McClintock, 1984; McClintock & Adler, 1978).

Potential mates have unconditioned incentive properties that invigorate appetitive and consummatory sexual behaviours. Previously neutral cues, conditioned stimuli (CS), can themselves acquire incentive motivational properties based on an animal's experience with reward (Berridge & Robinson, 1998; Bindra, 1974; Toates, 1986). Through Pavlovian associations, animals form stimulus-stimulus associations that allow for the prediction of sexual outcomes, i.e., where to find a sexual partner (Paredes & Alonso, 1997), and also which partner to approach or avoid (Coria-Avila et al., 2005; Parada et al., 2011). Consequently, a previously neutral stimulus, e.g., an artificial scent, paired with an unconditioned stimulus, i.e., copulation with a mate, comes to elicit conditioned responses. This occurs because of its pairing with a subjective reward following paced copulation. The conditioned stimulus comes to predict or prepare an animal to make a conditioned response to experience the unconditioned stimulus (Hollis, 1984). Pavlovian sexual conditioning paradigms have been used to augment both the innate mating strategy and mate preferences of female rats (Coria-Avila et al., 2005, 2006; Holley et al., 2014, 2015; Quintana, Desbiens, et al., 2019). The repeated pairing of a cue, e.g., almond scent, the strain of a male, an individual male conspecific, or a somatosensory cue, with optimal copulation results in selective and preferential sexual behaviours. These preferential sexual behaviours can consist of selective solicitations and the tendency to receive ejaculations from a partner associated with paced copulation. In this case, optimal copulation refers to a female's ability to pace and voluntarily regulate the initiation of and receipt of penile intromissions that induce VCS and CLS (Paredes & Alonso, 1997). Paced copulation produces a subjective state of reward that is sufficient to support the formation of both conditioned place and partner preference (for a review see Pfaus et al., 2012; Quintana et al., 2022). Paced copulation induces a conditioned place preference equivalent to that induced by morphine administration. The selectivity in sexual behaviours demonstrated by sexually conditioned females is a marked shift from indiscriminate

mating. Excluding conditioning studies, it has been difficult to ascertain whether phenotypic cues guide a female's choice in mates. As such, the formation of proximal experience-based mating preference for cues and partners may be useful for studying the underlying neurobiology of mate preference behaviours in the rat.

Explanations of mating and sexual preferences that focus an animal's motivation to consummate a reward (Georgiadis et al., 2012; Toates, 1986) make strong predictions about future behaviour based on an individual's reward history. Reward and motivation-based explanations also explain why some animals are compelled to mate with certain partners over others. Additionally, a proximate experience-based explanation of sexual preferences can explain the formation of both reproductive and non-reproductive sexual preferences and practices, ranging from mate preferences to the acquisition of fetishes and paraphilias (Pfaus et al., 2012, 2020). Importantly, such a proximal experience-driven explanation does not necessarily conflict with evolutionary explanations of behaviours. Indeed, most would agree that the unconditioned sexual stimuli to which animals respond, their sexual behaviours and responses, and the presence of motivation and reward systems, are themselves all consequences of evolutionary processes.

For example, there are species-specific constraints on when and how sexual behaviours and mating occur. Mating strategies govern how animals reproduce and can vary qualitatively within and between species. The mating strategy an animal uses is evolutionarily selected for and, as a result, sets constraints on its sexual behaviours. Domesticated and wild Norway rats are described as promiscuous, meaning that they do not form pair bonds and when rats mate in groups they regularly switch sexual partners and appear to mate indiscriminately (McClintock, 1984). This mating strategy is a stark contrast to the rarer monogamous mating strategy which occurs in only 3% of mammals (Kleiman, 1977). Monogamous animals form long lasting breeding pairs, and both parents often engage in the parental care of offspring (Carter et al., 1995; Dunbar, 1982; Getz et al., 1981). Though mating strategies place constraints on how an animal mates, the same question can be applied to all to all mating strategies. That is, are animals more likely to mate with certain conspecifics compared to others? Or put in a manner relevant to this thesis, do female rats mate randomly or nonrandomly?

The idea that males mate indiscriminately and that females are "choosy" in their mates is rather intuitive to most but not always correct. Within many species intrasexual competition can determine who mates in a given breeding season. Female choice has been documented across species. That animals do not

mate randomly is a reasonable idea based on several theories and hypotheses of evolutionary mechanisms, and is also based on empirical observations in numerous species. As Halliday (1983) notes, the inherent plausibility of the hypothesis does not mean it is true for every species in every context and we ought to distinguish between intrasexual competition and mate choice. One may ask, do female rats mate indiscriminately with males? In order to answer this question, it is important to understand how a female rat copulates and whether it has the ability to selectively copulate with and be impregnated by one male over others.

Female rat sexual behaviours are influenced by multiple factors that in include but are not limited to: 1) the hormonal state of the female (Beach et al., 1942; Boling & Blandau, 1939); 2) prior experience with copulation (Carr et al., 1965; Gerall & Dunlap, 1973; Meerts et al., 2014); 3) recently received copulatory stimulation and also the type of stimulation (Blandau et al., 1941; Chester & Zucker, 1970; Coopersmith et al., 1996; Hardy & DeBold, 1972; Pfaus et al., 2000); 4) the environment in which a female copulates (Calhoun, 1963; McClintock, 1984; McClintock & Adler, 1978); and 5) Pavlovian associations made with cues that predict copulatory reward or inhibition (Coria-Avila et al., 2005, 2006; Pfaus et al., 2012, 2013). These proximate factors are not mutually exclusive, and as will be discussed in this review, are often inter-dependent in exerting their influence on female sexual behaviour and reward.

An overview of female rat sexual behaviour

There are some commonalities among mammals but also differences in reproductive physiology and behaviours. One commonality is that male sperm is inserted into the female vaginal canal and must cross the cervix in order to fuse with an ovum (as reviewed in Asdell, 1946; Bronson, 1989). However, ova do not remain in waiting to encounter sperm. Instead, ova are released from the ovaries during an ovulatory period, or alternatively, in response to the presence of males and subsequent sexual stimulation (as reviewed in Bronson, 1989). Thus, across most female mammals, sexual behaviours are inhibited until a female enters an ovulatory period in which fertilization can occur. Ovulation and the onset of sexual receptivity are often tightly coordinated to facilitate the likelihood of pregnancy after copulation.

Among female rats, this coordination is hormonally regulated. Briefly, kisspeptin and gonadotropin-releasing hormone (GnRH) exert top-down control on the release of the gonadotrophs, luteinising hormone

(LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (for a review see Conn & Crowley, 1994; Lehman et al., 2010). These gonadotrophs stimulate the maturation and release of ova as well as the release of ovarian hormones, namely progesterone and estrogens. Estrogens are a group of ovarian hormones that vary in both abundance and potency, and include 17-β estradiol, estrone, estriol. Ovarian hormones provide bottom-up feedback that in turn regulate the secretion of GnRH. The cyclical pattern of this hormonal feedback circuit results in a 4- to 5-day cycle, during which a female rat becomes sexual receptive during late-proestrus until the end of the estrus period (Komisaruk & Diakow, 1973).

Beach (1976) proposed that female rat sexual behaviour consists of three phases: attractivity, proceptivity, and receptivity. Attractive behaviours serve as incentive stimuli to entice males to the vicinity of a female. Such behaviours and attractive stimuli consist of scent markings and volatile estrous odours, and ear wiggling. Proceptive behaviours function to invigorate male pursuit and include full and partial solicitations. Solicitations are displayed as an approach and headwise orientation toward a male followed by a runaway, and hops and darts in front of the male, respectively. Receptivity refers to the lordosis reflex which occurs following tactile stimulation of the female's flank, perineum, or tail-base, causing a dorsiflexion of the spine and raising of the rump (Pfaff, 1980). This allows a male to mount and intromit to penetrate the vagina (Pfaus et al., 2015). The hormonal control of lordosis has been thoroughly studied in the female rat (see Micevych & Meisel, 2017; Pfaff, 1980; Pfaff et al., 2008). The removal of the ovaries, and consequently most ovarian hormones, completely inhibits the lordosis reflex (Beach et al., 1942). In laboratory settings a priming injection of estradiol benzoate (EB), 40 to 50 hours before sexual behaviour testing, followed by treatment with progesterone (P) 2-8 hours before sexual testing readily induces sexual receptivity (Parsons et al., 1980; Pfaff, 1980). It takes at least 16-hours after EB administration for a female to display lordosis (Green et al., 1970). Without the lordosis reflex a female cannot receive intromissions and ejaculations and consequently fertilization does not occur (as described in Pfaff, 1980).

Because the lordosis reflex is reliably inducible and relatively simple to quantify, much of the neural circuitry underpinning the reflex has been well-characterized. In contrast, the neural circuitry underlying proceptive and attractive behaviours have been examined less extensively. Early studies of female sexual behaviour often studied females copulating with one male in small, non-naturalistic enclosures. Thus, early laboratory studies could often portray female rats as playing a passive role in copulation (Kuehn & Beach,

1963), as only the lordosis reflex in response to male stimulation was examined. The focus on the lordosis reflex alone led some to believe a female was a passive recipient of a male's sexual behaviour (as discussed in (McClintock & Adler, 1978). However, once the laboratory study of female sexual behaviour expanded to observe females in semi-natural environments, the view of female passivity in copulation changed (McClintock, 1984). In comparison to the small testing chambers previously used, McClintock and Adler (1978) studied female rats freely behaving in a large semi-natural environment. They observed that the copulatory pattern of rats was in large part dependent on the behaviour of the female. Most penile intromissions made by males were preceded by a female solicitation. Like in pacing chambers, full solicitations in the large open field used by McClintock involve an approach and headwise orientation toward a male, followed by a runaway, which invigorates male pursuit (McClintock & Adler, 1978). Sometimes females make a partial solicitation, or hop and dart, in the vicinity of the male. These are initiated prior to lordosis and, in addition to enticing pursuit behaviour, solicitations produce more vigorous mounting on the part of the male. The tactile stimulation of the flanks and perineum, which also produces CLS, with male pelvic thrusts, culminates in VCS with multiple vaginal intromissions and ejaculations (Pfaus et al., 2015). The work of McClintock and Adler (1978) demonstrated that females were, in fact, active initiators of copulation, as the majority of intromissions are directly preceded by a solicitation.

In natural and semi-natural environments, the mating strategy of rats is described as non-monogamous and highly competitive (McClintock et al., 1982; McClintock, 1984). In larger groups, females take turns soliciting male pursuit followed by the receipt of a penile intromission and/or an ejaculation (McClintock et al., 1982; McClintock, 1984). After the receipt of an intromission, a female typically imposes an interval before soliciting again and resuming copulation. The cycle of solicitations and intromissions continues until a male ejaculates and enters a refractory period characterized by sexual inhibition. When a female receives an ejaculation, she enters a shorter period of quiescence during which the female does not solicit or accept mounts or intromissions. This quiescent period is necessary for sperm transport through the cervix (Matthews & Adler, 1977). A longer quiescent period increases the likelihood of impregnation as a female does not receive intromissions from another male that can dislodge the implanted sperm plug (Coria-Avila et al., 2004; Matthews & Adler, 1977).

Females can behave differently towards particular males in a way that increases the likelihood of being impregnated by a specific male. By selectively soliciting a particular male, a female can ensure it receives an ejaculation from that male. McClintock et al. (1982) also provided evidence of competition among females during mating. Dominant females often intercept and solicit a male being solicited by a competitor female to receive its ejaculation. Furthermore, when a female receives an ejaculation from a dominant male, the female imposes a significantly longer post-ejaculatory interval (PEI) compared to the PEI imposed after an ejaculation by a subordinate male (McClintock et al., 1982). As a male approaches ejaculation, he emits stereotyped 22 kHz ultrasonic vocalizations that become longer, louder, and more frequent (White et al., 1990). For a female, such vocalizations may function as a cue that signals when to selectively solicit a particular male in order to receive its ejaculation. The selective solicitation of dominant males, in conjunction with selectively receiving their ejaculations, increases a female's likelihood of being impregnated by a dominant male.

Although fundamental similarities exist in rat copulatory behaviour in the laboratory, semi-natural, and natural settings, some differences exist. Wild rats are social animals that live in colonies in a series of shared nesting and feeding sites (Barnett, 1958; Calhoun, 1963). Despite being a widely studied laboratory animal, knowledge of the social and sexual patterns of undomesticated rats is still lacking (Schweinfurth, 2020). To date, the most comprehensive work on understanding the sociosexual structure of rats remains the work of Calhoun (1963), which remains a widely ignored and understudied description of rat behaviour. Calhoun (1963) captured 14 Norway rats in a region of Baltimore and introduced them to a reasonably naturalistic outdoor pen. He then followed and observed the behaviours and structure of the colony from 1947-1949, approximating 3 years in total observation time. Within the pen, there were important differences in soil quality for burrowing and distance to the food pen. This led to differences in the favourability of the location of warrens, a network of burrows organized into social and familial groups, based on the distance to food and the paths subsequently built by the rats. Shortly after the introduction of the rats into the pen, a dominance hierarchy emerged that would determine, in most cases but not exclusively, the sociosexual interactions of the colony. The more dominant rats took up residence in the southeast portion of the pen, whereas the subordinate rats were relegated to the less favourable northwest side of the pen. This social structure was a significant determinant of mate preferences and reproductive capabilities in the colony.

In contrast to laboratory rats that often display a 4-5 day estrous cycle, Calhoun (1963) found that intervals between behavioural estrus were approximately 7-11 days. Within laboratory conditions, females cycle throughout the year, whereas Calhoun (1963) observed cycles that were photoperiod dependent and seasonal. No births were observed from October to the end of February. In fact, most males' testes were retracted to the abdominal cavity for this winter period. Despite similar temperatures occurring during October and April, males were more likely to have testes in the scrotum in April, suggesting that in wild rats mating cycles are seasonal and regulated by changes in the photoperiod.

In contrast to domestic rats, most rats in the colony did not have any offspring. This demonstrated that in semi-wild conditions rats do indeed have differential success in mating. The determinant of mating success was primarily attributed to a rat's position in the dominance hierarchy. Within the dominant groups, there tended to be a higher female to male ratio, with the resident male often forming a harem consisting of several females and their pups. In contrast, many of the subordinate males did not cohabitate with females and if they did, the sex ratio was always biased toward males. While it is commonly thought that female rats have synchronized estrous cycles (McClintock, 1978), Calhoun (1963) never observed such synchronization, as only one or few females entered estrus at the same time. Interestingly, a more rigorous laboratory study has failed to support estrous cycle synchronization (Schank, 2001). Based on Calhoun's observations, one female enters estrus typically lasting two nights. She would roam and mark various locations of the pen and burrows with her scent and urine to attract males. On the second night copulation would occur. The nature of the copulatory pattern that followed depended on the social group to which a female belonged. If the female belonged to a group with a dominant male, this male would fight and chase competitor males to monopolize access to the female. A female would enter and withdraw from its burrow, inciting pursuit from the male/s waiting outside the burrow entrance, a behaviour reminiscent of solicitation. In the case that the social group did not have a dominant male, males would fight for rights to remain at burrow entrances to pursue, mount, and intromit when a female left the burrow. Calhoun (1963) reported that a female could receive 1000 mounts over the course of a night until estrous behaviour was terminated, after which male pursuit and attempted mounts were met with aggression from the female.

One of the most important observations made by Calhoun (1963) suggested that mating success can be hereditary in wild rats. The dominant males and females residing in the southeast had significantly more

surviving offspring than the subordinates. The offspring of the dominant warrens later had greater litters and increased litter survival than those born in the subordinate side of the pen. The hierarchy-related increase in reproductive success was even evident in rats born in the dominant area that later migrated to the less favourable areas of the pen. Dominant rats in general could secure better access to the food pen, were larger and quicker to develop, and had less wounds from fighting.

Taken together, the observations of McClintock (1984) and Calhoun (1963) show that there is differential success in mating in rats and that most of this success can be attributed to the dominance hierarchy of the colony. Calhoun (1963) demonstrated that dominance is determined by the dam's (female rat parent) social standing, which directly affects the number of surviving litters and the subsequent growth and development of the offspring. Dominant males and females tended to have more litters together, indicative of assortative mating and mate choice. Assortative mating is the tendency for animals that are more alike in certain traits to mate at a frequency that is above chance. McClintock's (1984; McClintock et al., 1982) observations demonstrate how females exert this mate choice, by allowing more time for sperm transport from dominant males, and selectively soliciting and intercepting a dominant male to receive its ejaculation.

One may read the above descriptions of McClintock and Calhoun's observations and come to the conclusion that mate choice exists in rats. Indeed, both provide evidence of female choosiness and male intrasexual competition. Though social dominance and subsequent mating success appear to be heritable, it is not known to what extent these traits are heritable on a population level. Additionally, there are no estimates of the degree to which the variance in dominance and the female preference for dominant males can be attributed to genetic variation. As mentioned above, social dominance is a construct that encompasses, but is not limited to, the social standing of an offspring's dam, and the weight and age of a given rat. The extent to which variance in dominance traits predicts variance in mating success on a population level are not understood. Variance in particular traits and their relation to mating success on a population level are foundational to theories of mate choice. Furthermore, the extent to which the variation in mating success documented by Calhoun (1963) can be attributed to female choice over male intrasexual competition is also unclear. Theories of mate choice and laboratory studies that support or contradict mate choice in female rats are discussed below.

Mate choice in female rats: Theory and Praxis

Determining whether mate choice occurs in rats is problematic. Multiple theories and models of mate choice have been posited since Darwin's (1871) initial proposition that females have an aesthetic eye for beautiful or ornamented males, which then makes those males more successful in mating than males without ornaments. Consequently, male offspring inherit the propensity for ornamentation from their father, and will then be more successful in future mating.

In any species, the sum of all combined matings over a given period must be equal for each sex. Despite the equal sum of matings, there is an asymmetry between the sexes, such that the frequency of matings for females tends to be more equally distributed across individual females, whereas the distribution of mating success among males is unequal. A relatively small number of males tend to be quite successful relative to others. Bateman (1948) demonstrated this principle, stating that in non-monogamous species male reproductive success tends to vary much more than females when each sex displays classic sex roles (i.e., females providing greater parental investment, gestating offspring etc). An additional asymmetry related to Bateman's principle is that male gametes are cheap, motile, and plentiful, whereas female gametes are more costly to produce, immobile, and are fewer in number (for a discussion see Trivers, 1972). A consequence of this differential gametic investment is that males and females tend to invest differently in offspring (Trivers, 1972). In non-monogamous animals there is thus an asymmetry in investment before and after mating. For example, rat dams lactate and feed pups until they are weaned, providing maternal care until pups are mature enough to leave their birthing den. Though parental behaviours can be displayed by male rats in laboratory conditions (Brown & Moger, 1983), parental behaviours in wild male rats are sparsely documented. This is likely because wild females typically obstruct entrances to breeding demes (Calhoun, 1963), leaving the dam as the primary care giver. This difference in parental investment incurs a large energetic and reproductive cost on a female.

Though rats are a widely studied animal model, little to no research addresses sexual selection, female mate choice, or variance in reproductive success. However, female mate choice has been documented in numerous species (as reviewed in Andersson, 1994). In species that demonstrate greater sexual dimorphism it is expected that sexual selection operates or has operated more strongly. Given differences in parental

investment and the costs of mating and the promiscuous mating strategy of the rat, and the extent of sexual dimorphism, it is likely that mate choice exists in female rats.

Since Darwin, there have been numerous models and theories proposed as to how and when mate choice emerges (Kokko et al., 2006). Though Darwin's original conception of female choice has been influential, it initially fell out of favour and the role of female choice was ignored or considered unimportant (see Huxley, 1938). This occurred primarily because Darwin (1871) did not explain how female preferences emerged, but rather took a female's ability to aesthetically discriminate between attractive males as a given. Early conceptions of female mate choice lacked a clear mechanism for selection; however, the rediscovery of Mendelian genetics in the early part of the 20th century lay the foundations for theoretical advances. That certain traits were inherited by offspring from parents as reliable discrete units laid the foundation for the idea of a gene as the fundamental unit of inheritance. Though, as we now understand, many traits are not Medellian but are inherited across multiple gene loci. The understanding of linkage disequilibrium (i.e., the nonrandom heritability of multiple genes together) had a profound effect on models of mate choice.

Fisher (1930, 1958) described what has become known as the runaway hypothesis of sexual selection or the sexy-sons hypothesis. Building on Darwin's ideas, Fisher (1930) loosely provided a mechanism as to how female choice develops. That is, a female initially shows a preference to mate with a male favoured by natural selection or that has direct benefits such as access to resources. The sons of the male inherit the preferred traits, but the females will also inherit the mother's preference for the male phenotype. The male traits will thus increase in the population because of its increased reproductive success, and importantly the alleles for a female's preference become linked to the male's alleles for attractiveness and are selected for. This occurs because these traits are often polygenic and not specifically linked to sex chromosomes, i.e., they are inherited by both male and female offspring (Arnold, 1983). Lande's (1981) model demonstrated that linkage disequilibrium between the genes for female preferences and the preferred male traits is inevitable. Consequently, female preference and male attractivity becomes self-reinforcing and the initial direct benefits of the trait may be lost but the attractivity and the preference for it is retained.

Evidence for Fisherian selection in rats is lacking. Male rats bare no obvious sexually-selected ornaments. There may be ornamental traits that are obvious to female rats (e.g., major histocompatibility

complexes (MHCs) or pheromones; see below) but given that we have no access to the sensory world of a rat, such an observation is impossible to make.

Fisher's "sexy sons hypothesis" was later countered with ideas that females selected mates on the basis of a genetic advantage that their will offspring inherit. Thus, females select males that carry traits that indicate he has "good genes". A related hypothesis, the "handicap hypothesis," suggests that males are chosen by females based on traits and ornaments because they negatively affect adaptive fitness (Zahavi, 1975). A male that shows it can survive despite its ornamental handicap clearly has other beneficial traits that improve his fitness. Immunocompetence has been suggested as a trait on which good gene selection takes place (Faivre et al., 2003; Hamilton & Zuk, 1982). Because testosterone has immunosuppressant effects, it has been suggested that there is a trade-off for males between androgen-dependent sexual signalling and performance, sperm production, and immune function (Faivre et al., 2003). Consequently, males that can display androgen-linked traits, e.g., fighting/aggression, sexual arousal and motivation in male rats, signal increased immunocompetence and parasite resistance. Indeed, males tend to have greater parasite loads than females (Poulin, 1996). Males that can maintain androgen dependent signalling and behaviours despite this pathogen load are of intrinsically higher quality and are thus attractive and produce more competitive sperm (Anderson & Simons, 2006). Females thus obtain increased immunocompetency for their offspring by mating with males that have sufficient immunocompetence given the immunosuppressant effects of testosterone.

Indeed, females tend to avoid males infected with parasites (Hamilton & Zuk, 1982). Female mice can discriminate between parasite-infected males and prefer those who are not infected (as reviewed in Kavaliers & Choleris, 2017), though the evidence for parasite avoidance guiding mate choice in rats is more sparse. Interestingly, the parasite Toxoplasma Gondii makes male rats more sexually attractive to females (Dass et al., 2011). Though this parasite has other negative effects, it directly increases testosterone and spermatozoa production. As such, the parasite likely mimics an androgenic state of immunocompetency, as a means to spread as a sexually transmitted infection.

MHCs have been proposed as a sensory cue that signals a male's immune function, and whether a male is infected with a disease or parasite, and consequently its immunocompetence (see Kavaliers & Choleris, 2017). MHCs are volatile compounds that can act as chemical signals between animals. The genes that encode MHCs are highly variable across individual animals which allows for many unique combinations

on an individual level (Janeway et al., 2001). The wide variation in the genes related to MHCs has been hypothesized to be beneficial for immune function, and it has been shown in mice that females prefer to mate with MHC dissimilar males, to maintain this genetic variation (Potts et al., 1991; Roberts & Gosling, 2003). However, the extent to which this type of disassortative mating occurs in rats is not known.

Some have also proposed that MHCs may be used by females to detect males that are more genetically dissimilar as a mechanism to avoid inbreeding (Eklund, 1997). It has been widely assumed that, in general, animals avoid breeding with closely related conspecifics and that MHCs underpin inbreeding avoidance. Inbreeding avoidance has been theorized to be adaptive because it protects against a reduction in genetic variability within a population (Keller & Waller, 2002). However, a recent meta-analysis has shown there is miniscule effect size in favour of inbreeding avoidance across multiple species (de Boer et al., 2021), suggesting a negligible influence of inbreeding avoidance on mate choice. Such a finding questions the suggestion that females prefer males with dissimilar MHC profiles.

Although Fisherian and good gene selection appear to be contradictory, modelling studies have indicated that both occur on a continuum depending on the reproductive cost to a female (Kokko et al., 2006). Kokko et al. (2002) show that mate choice for attractiveness (i.e., sexy sons) or adaptive fitness (i.e., good genes-handicap) emerge based on the cost to a female, which they term the Fisher-Zahavi process. A meta-analysis combining data from multiple species suggests that both Fisherian and good gene traits both increase male attractiveness to a female (Prokop et al., 2012). The model proposed by Kokko et al. (2002, 2006) suggests that as male rats have no clear Fisherian ornaments, females likely choose males based on the fitness benefit for offspring. When female choice is cheap, male attractivity via ornaments occurs and can become negatively correlated with survivability and fitness (Kokko et al., 2002). Because male rats have no ornaments, this suggests that mating is evolutionarily costly for female rats. Indeed, Calhoun (1963) observed such high costs in that there were relatively high degrees of sterile mating, miscarriage, and pup mortality. These negative outcomes are not only energetically expensive for a female rat, but also temporarily inhibit reproductive capabilities through pregnancy or pseudopregnancy, i.e., a state resembling pregnancy lasting approximately 14-18 days that occurs after sterile mating (Swingle et al., 1951).

In contrast to the indirect genetic benefits a female may gain for its offspring from mate choice, the benefits for a female may be direct. This includes increased access to resources, particularly by males that

hold and defend territory of high value (Andersson & Simmons, 2006). Direct benefits are reminiscent of the ability of dominant males to control warrens in more favourable areas and closer to food sources (Calhoun, 1963). Similarly, McClintock (1984) observed that females preferentially mate with dominant males, and these males tend to have greater reproductive success. These direct benefits may be the most concise and obvious cue for mate choice in female rats. The dominance of a male depends on several factors related to its social environment but dominance in male rats is mostly related to weight and age (Macdonald et al., 1995). However, as Calhoun (1963) showed, the propensity toward dominance is heritable, whether indirectly through good genes that facilitate dominance, or through an improved development trajectory afforded to the offspring of dominant males. These offspring have an increased likelihood of survival after birth, greater growth, and increased weight gain. Indeed, laboratory studies have shown that females prefer to mate with dominant over subordinate males and spend more time with intact over castrated males (Carr et al., 1965; Moore & Wong, 1992).

The ability to discriminate between males of different androgen concentrations indicates the primacy of olfaction in guiding mate recognition and choice in female rats. It is well known that olfaction plays an important role in the social behaviours of rats. Carr et al. (1965) demonstrated that females prefer to spend time with an intact male over castrated males, although this preference was hormonally-dependent on priming with EB+P. A preference for dominant males was corroborated by Brown (1977). Scent marking is an important precopulatory behaviour for male and female rats (Calhoun, 1963) and castrated, androgen deficient male rats do not engage in scent marking (Taylor et al., 1987). Taylor et al. (1982) showed that females spend more time in the vicinity of castrated males injected with 800µg of testosterone compared to those injected with 200µg, suggesting males with higher circulating testosterone are more attractive. Interestingly, Taylor et al. (1982) found that the administration of 200µg of testosterone was not sufficient to restore scent marking fully following castration. Testosterone levels provide two types of olfactory cues to a female, a chemosensory pheromonal cue that can be carried as a male scent and the major urinary proteins produced during scent marking (Gawienowski et al., 1976), and potentially signals the immunocompetence of a male (Arakawa et al., 2011; Faivre et al., 2003). Galef et al. (2008) have shown that females prefer to copulate with males that have recently copulated. In this study, females did not witness the copulation. However, the preference was disrupted when females were made anosmic by the application of zinc sulfate to the nasal

epithelium, showing that olfaction is necessary for mate choice. However, Winland et al. (2012) allowed males to be classified as attractive or unattractive based on the amount of time multiple females spent with one of two males across four mating trials. Although attractiveness determined by the female's behaviour was consistent across females, there was no correlation between the male's attractiveness and testes weights or testosterone levels.

Major urinary proteins (MUPs) are volatile odours present in male scent markings. Similar to MHCs, there are many genes that code for MUPs allowing for wide genetic diversity (Beynon & Hurst, 2004). Kumar et al. (2014) showed that females spent more time with males whose urine contained more MUPs, and therefore these males were determined by the authors to be more attractive. However, the concentration of MUPs was not related to the number of offspring sired in a two male mating preference test. Consequently, the relation between markers of attractivity and reproductive success is unclear, or counterintuitively does not favour the attractive male. Interestingly, Winland and colleagues demonstrated that females solicited attractive males more than unattractive males. However, once again attractive males, i.e., a male that females spend more time with, did not sire more offspring (Winland et al., 2012; Zewail-Foote et al., 2009). In fact, Winland et al. (2012) showed that the unattractive males had a reproductive advantage. These studies highlight a nondirect and complex relation between attractivity and reproductive success.

Le Moëne and Snoeren (2018) have proposed that mate choice may be random in female rats. They demonstrated that female rats given access to three chambers containing male rats do not prefer the same male rat. When the male rats' positions in the chambers were switched, females visited the same chamber but not the same male. This finding is important because it suggests that when females are given free access to males, even if they vary in good gene traits and MHCs, it does not induce a consistent preference. This is opposite to the consistency in preference demonstrated by Zewail-Foote et al. (2009). Because the males used by Le Moëne and Snoeren (2018) were from a private breeder, one could question whether there was sufficient variability in MHC and MUP genes in commercially bred animals to allow for a preference. These data could be interpreted in support of choice for direct benefits when females' preference is determined by the direct benefits a male can offer, especially dominant male rats (Calhoun, 1963; McClintock, 1984). Examining mate choice based on social dominance was beyond the scope of Le Moëne and Snoeren's (2018) study.

A model for studying mate preferences.

To summarize, there are no obvious Fisherian ornaments carried by male rats that females could use to guide mate choice. Some have proposed that in species in which females mate with multiple males, as rats do, selection may occur at the level of spermatozoa (Keller & Reeve, 1995; Pizzari & Birkhead, 2002). However, more research needs to be conducted on cryptic, i.e., post-copulatory, mate choice in the female rat. Evidence for choice based on good gene traits is also inconclusive, though substantially more evidence exists in mice (see Ferkin, 2018).

In semi-natural and laboratory conditions there is reasonable qualitative evidence for mate preference based on direct benefits. The observations of Calhoun (1963) suggest a reproductive advantage of dominant males that confers a similar advantage to their offspring. McClintock (1984) corroborated this female preference for dominant males but did not test for increased reproductive success or fitness advantage of the offspring. At best, Calhoun (1963) indirectly observed that rats born in the dominant side of the pen had increased numbers of offspring. Dewsbury and Hartung (1980) demonstrated a similar preference for strain-linked dominance, as Long-Evans males tend to best F344 males in fights. Consequently, F344 females mated preferentially with male Long-Evans rats. However, creating large naturalistic environments in which a colony of rats can establish dominance hierarchies and tracking female mating preferences would be difficult. In addition to this issue, many of the studies that test female choice in rodents determine a preference based on the amount of time a female spends with or in the vicinity of a male (e.g., Salo & Dewsbury, 1995; White et al., 1986; Williams et al., 1994). As a result, we know even less about actual copulatory preferences of the female rat. Consequently, understanding whether female rats exercise mating preferences that impact their reproductive success, and the underlying neurobiology of these behaviours, has been difficult.

Proximal experience-based preferences could offer a useful model with which to study female mate preferences and its underlying neurobiology. Coria-Avila et al. (2005) showed that female rats can form preferences for a type of male based on Pavlovian associations with the copulatory reward state induced by paced mating. Specifically, when females were given paced copulation trials with males bearing a neutral scent (i.e., natural almond extract), they preferentially solicited and received the first ejaculation from a scented male in an open field sexual preference test with two tethered males, one scented with the almond odour and the other unscented. Such an experientially-mediated preference allows for the use of a

standardized cue that elicits preferential sexual behaviours. Using experimentally manipulated cues also allows researchers to observe patterns of neuronal activation in response to the cue alone, in addition to examining the effect of drugs that either facilitate or inhibit the formation of the preference. Sexual conditioning paradigms are not restricted to odours either. Coria-Avila et al. (2006) demonstrated that, although female rats preferentially copulate with males of the same strain, this innate preference could be reversed by pairing a different strain of male with the females' first experiences of paced copulation. Moreover, females also showed a preference to solicit and copulate with males bearing a visual and tactile cue, i.e., a rodent tethering jacket, that was explicitly paired with their first experiences of paced copulation (Quintana, Desbiens, et al., 2019).

In some forms of conditioning, the cue can simply be the familiar male without the addition of an odour or visual/tactile stimulus. For example, Holley et al. (2014) demonstrated that female rats can develop mate guarding of a familiar male paired with their first experiences of paced copulation. In this experiment, females were given either ten paced or unpaced copulation trials with either the same male (familiar) or novel males during each trial. This was followed by a competitive mating test with the familiar male and a competitor female. Females given paced copulation trials with the same familiar male showed spontaneous mate-guarding behaviours, including hovering and presenting behaviours close to the male, blocking behaviours where the female would impose herself between the familiar male and the competitor female, and female-female mounting behaviours if the competitor solicited the male. These behaviours were not displayed by females that had random-paired males or unpaced copulation with the same male. Female mate guarding is typically associated with monogamous species (Carter et al., 1995). The findings of Holley et al. (2014) suggest that females can recognize individual males and engage in mate guarding, to monopolize access to a male associated with paced copulation. Such behaviours are similar to the interception of a dominant male when he is solicited by a competitor female (McClintock & Adler, 1978).

The extent to which selective solicitations, selective receipt of ejaculations, and mate guarding relate to mate choice and differential reproductive success is debatable. The work of Windland and colleagues (2012) demonstrate that the preference to spend time with and solicit one male over another does not predict paternity. However, mate choice as a selective process cannot be determined across one generation (Arnold, 1983; O'Donald, 1980). Despite these caveats, an animal needs to copulate to exert a mate preference. The

degree of preference toward one male over others is likely the strongest factor that links mate preference to reproductive success. For example, should a female only receive ejaculations from one male, only one male can sire the offspring. Likewise, if she receives more than one ejaculation from the same male or imposes a lengthy interval between the first ejaculation and subsequent ejaculations, the first ejaculation will sire her offspring (Coria-Avila et al., 2004). Therefore, because partners and cues associated with previous sexual reward can induce a preference toward that partner, conditioning models may be useful for studying the mechanisms of mate preference in female rats.

Short- and long- term effects of copulation

Copulation induces neuroendocrine and neurobiological changes that influence a female's short- and long-term behaviours and vice-versa. These changes influence the likelihood of pregnancy but also future responding to partners and cues that become associated with rewarding copulation (Pfaus et al., 2012). Acutely, both CLS and VCS during copulation facilitate lordosis, but VCS increases the pacing interval between intromissions and facilitates sperm transport across the cervix (Adler, 1969; Coopersmith et al., 1996; Diakow, 1975; Edmonds et al., 1972). One neuroendocrine consequence of VCS is the release of LH, which may further facilitate the temporal coordination of copulation with ovulation, while also increasing the number of ova released (Sairam, 1980; Spies & Niswender, 1971).

While sperm deposition and transfer through the cervix is necessary for impregnation, it alone is not sufficient to induce pregnancy. For pregnancy to occur there needs to be an appropriate endocrine state that facilitates the implantation and maintenance of fertilized ova. The hormonal state that facilitates pregnancy, called the "progestational state", is dependent on the secretion of progesterone from the corpus luteum initially induced by a surge in prolactin (Goyeneche et al., 2003; Kornberg & Erskine, 1994; Terkel et al., 1990). Female rats do not form a fully functional corpus luteum that secretes sufficient progesterone to maintain pregnancy. Consequently, stimulation of the cervix during copulation initiates a neuroendocrine cascade that maintains the corpus luteum and inhibits luteolysis (Goyeneche et al., 2003; Kornberg & Erskine, 1994; McCracken et al., 1999; Terkel et al., 1990). Diurnal surges in prolactin occur for two weeks following copulation and are necessary for maintaining the corpus luteum and consequently, pregnancy (Freeman et al., 1974). VCS induces this progestational state (Adler, 1969; Chester & Zucker, 1970; Kornberg & Erskine,

1994). Male mounts alone without penile intromissions do not induce progestation (Hardy & Debold, 1971). Because male rats provide tactile stimulation of the clitoris, cervix, and the perineal area surrounding the vagina (Pfaff et al., 1977), the rate and type of copulatory stimulation are crucial to these short-term neuroendocrine changes that facilitate pregnancy (Adler, 1969; Chester & Zucker, 1970; Kornberg & Erskine, 1994). Fewer penile intromissions are required when the interval between intromissions is longer (i.e. paced intromissions; Edmonds et al., 1972). The fact that the number and timing of cervical stimulations induces a progestational state, indicates that sensory information relayed from the cervix to the central nervous system is integrated to induce a favourable endocrine state for pregnancy. The transection of the pelvic nerve that innervates the vagina and cervix inhibits the induction of this progestational state and inhibits pseudopregnancy after mating (Carlson & De Feo, 1965).

Vaginocervical stimulation induces activation in various brain regions associated with female sexual behaviour, as shown by Fos protein immunoreactivity (IR), an indirect marker of neuronal activation (Pfaus & Heeb, 1997). Both artificial VCS delivered with a lubricated glass rod and penile intromissions induce Fos protein expression in the medial preoptic area (mPOA), the ventrolateral ventromedial hypothalamus (VMHvl), and the posterior dorsal medial amygdala (MeApd), lateral septum, and bed nucleus of the stria terminalis (BNST; Pfaus et al., 1996; Polston & Erskine, 1995). Fos protein IR in many of these areas tends to become more abundant as the amount of VCS received increases (Pfaus et al., 1996). The transection of the pelvic nerve also attenuates Fos protein IR following VCS in the mPOA, MeApd, VMHvl, and BNST (Pfaus et al., 2006; Wersinger et al., 1993). While the mPOA, and VMHvl are sensitive to the amount of VCS, the mPOA, VMHvl, and MeApd show an activation pattern that is also sensitive to the number of ejaculations received (Coolen et al., 1996). The paraventricular nucleus of the hypothalamus (PVN), which is responsible for the synthesis and release of the hormone oxytocin (OT), shows significantly more activation of Fos in OT neurons following paced VCS (Flanagan et al., 1993). Diamond (1970) suggested there was a "vaginal code" in which the pattern and amount of VCS increases reproductive success in rodents. The neuronal activation following paced VCS shows that in rats the vaginal code induces a commensurate signature of neuronal neuroendocrine activation that ultimately facilitates pregnancy.

Although VCS initially facilitates lordosis and pacing behaviours, over a longer period of time, approximately 12-20 hours, VCS inhibits sexual receptivity (Pfaus et al., 2000). The pattern of activation

following VCS, approximately 75-90 mins after VCS in order to induce Fos protein, is likely part of an important neurobiological signal that a female has copulated successfully and no longer needs to be sexually receptive. Penile intromissions and artificial VCS induce a state of copulatory quiescence known as "estrous termination" (Hardy & Debold, 1971; Lodder & Zeilmaker, 1976; Pfaus et al., 2000). Sexual receptivity, measured by the presence of the lordosis reflex, has an average duration of 19 hours in unmated rats (Kuehn & Beach, 1963). However, the length of receptivity is significantly reduced depending on the amount of, and especially pacing of, VCS (Coopersmith et al., 1996; Erskine, 1985; Erskine et al., 1989; Erskine & Baum, 1982; Pfaus et al., 2000). As a female terminates estrous, solicitations are inhibited, pacing intervals between intromissions increase, and a female is more likely to aggressively reject male pursuits and mounts (Pfaus et al., 2000). Therefore, it is thought that estrous termination indicates a state of sexual satiety and inhibition following copulation. The amount and pacing of VCS sufficient to induce estrous termination, roughly 50 stimulations over the course of an hour, increases Fos protein IR in glutamatergic neurons in the VMHvl (Georgescu et al., 2009). During sexual receptivity glutamate transmission is reduced in the VMH (Georgescu et al., 2014) and GABA transmission is increased (McCarthy et al., 1990, 1991). Infusions of glutamate to the VMH inhibit lordosis (Georgescu & Pfaus, 2006a; Kow et al., 1985) and infusions of an AMPA/kainate receptor antagonist that inhibits glutamate signalling directly prior to the receipt of VCS, facilitates lordosis and solicitations, and inhibits estrous termination (Georgescu et al., 2012; Georgescu & Pfaus, 2006b). Thus, as a female receives VCS, the amount of excitation in the VMH increases in a manner that induces, or at least mediates, the induction of estrous termination and satiety that are required for progestation.

As noted above, the ability to pace the rate of genital stimulation is a critical mediator of the rewarding aspects of copulation for female rats. When a female can freely withdraw from and return to a male after receiving an intromission, both place and partner preferences can be formed (e.g., Coria-Avila et al., 2005; Jenkins & Becker, 2003; Paredes & Alonso, 1997). A female can learn to favour places associated with paced copulation and can direct sexual motivation toward males that have previously provided paced copulation. Although copulation increases dopamine (DA) release in the nucleus accumbens (NAcc) of female rats (Pfaus et al., 1995), paced copulation increases extracellular dopamine significantly more than unpaced copulation (Jenkins & Becker, 2003). Artificial VCS also induces a conditioned place preference that is not formed if the pelvic nerve is transected (Meerts & Clark, 2009). Interestingly, pelvic neurectomy also blocks

progestation (Carlson & De Feo, 1965; Helena et al., 2011). Cues that are associated with paced copulation induce Fos IR in many of the same areas that are related to the induction of estrous termination and progestational responses (viz. mPOA, PVN, lateral septum (LS), VMHvl; Coria-Avila & Pfaus, 2007). However, cues and partners paired with paced copulation also activate regions of the mesolimbic dopamine system and areas associated with female sexual motivation (viz. mPOA, VTA, and NAcc; Coria-Avila & Pfaus, 2007; Graham & Pfaus, 2010). Thus, by pairing neutral cues with paced copulation, sexual behaviours and preferences can be modulated by Pavlovian associations. Graham and Desjardin (1980) demonstrated that in males, cues that predict copulation induce reproductively relevant neuroendocrine responses. A scent paired with copulation to ejaculation significantly increased serum testosterone and LH. This suggests an intimate link between reproduction and reward. Such a link reminds us of the proximal and ultimate factors that guide reproductive success and mate preferences mentioned previously. Whether such links between reward and reproduction occur in females is unclear and one of the aims of this thesis is to provide further clarity. Altogether these findings suggest that copulation-induced patterns of neuronal activation and neuroendocrine changes facilitate reproductive success while also facilitating conditioned copulatory responses made to partner cues associated with rewarding copulation.

The multiple roles of oxytocin

Sexual reproduction far predates the speciation that gave rise to the earliest mammals. Leeches (*Hirrudo Verdana*) are a spontaneous hermaphroditic breeding species that produce both male and female germ cells (Wagenaar et al., 2010). Leeches use chemoreceptors to detect nearby mates, which is followed by a twisting of the body that allows it to either fertilize its partner, or be fertilized by its partner. This copulatory twist typically only occurs when a mate is present. Conopressin, a close analogue of both OT and arginine vassopressin (AVP), is a nine-chain amino acid peptide, present in gastropods and molluscs (Dutertre et al., 2008). Leeches injected with conopressin initiate this twisting that is only seen during mating and occurs even when a conspecific is not present. Both OT and AVP receptor antagonists disrupt the mating twist induced by an acute injection of conopressin (Wagenaar et al., 2010). The role of a common ancestor of OT and AVP, and the conopressin receptor, in controlling copulatory behaviours in sexually reproducing species is highly phylogenetically conserved.

In vertebrates, OT and AVP are two closely homologous nonapeptides that are derived from another phylogenetically ancient peptide, vasotocin. A duplication in the vasotocin gene resulted in the emergence mesotocin and isotocin, among other nonapeptides, that are present in fish, birds, and amphibians (Yamashita & Kitano, 2013). In mammals, a mutation in mesotocin resulted in the emergence of OT, along with mesotocin receptors becoming oxytocin receptors (OTRs). AVP is also derived from vasotocin, with the three AVP receptors V1a, V1b, and V2, tracing their lineage from the older vasotocin receptor subtypes. There is only one known OTR and its mechanism of signalling is complex, activating and recruiting various families of G proteins, Gi, Gq, and Go (Busnelli et al., 2012). These receptors are widely distributed in various peripheral organs and tissues, and in the central nervous system. As these tissues differentially express the G proteins that couple to the OTR, the effects of receptor activation can differ depending on the tissue or brain region. To further complicate matters, the concentration of OT may also determine aspects of OTR signalling. During relatively higher periods of OT signalling, such as lactation, OTR signalling activates beta-arrestin complexes causing an internalization of the receptor (Busnelli et al., 2012).

With the complexity of OTR signalling in mind, it should be noted that in rats the circuitry underpinning OT's central transmission and release from the posterior pituitary is no less complicated. Oxytocin neural projections from the PVN and OTR expression do not overlap in many brain areas (Knobloch & Grinevich, 2014). It is important to note that OT functions as a neuropeptide, a neurotransmitter, paracrine hormone, and as a classic endocrine hormone (Knobloch & Grinevich, 2014). It is synthesized in the same manner as canonical neuropeptides, such that it is cleaved from a large preproprotein. Oxytocin's precursor contains neurophysin, OT's carrier protein, and OT (Brownstein et al., 1980). This synthesis primarily occurs in magnocellular neurons located in the PVN and the supraoptic nucleus (SON), although OT is also found in parvocellular neurons. Approximately 97% of OT neurons in the PVN are magnocellular neurons (Althammer & Grinevich, 2018). OT's precursor protein is transcribed in the rough endoplasmic reticulum, and packed into large dense core vesicles in the Golgi body that are pooled in the either the soma or are transported to the axon terminus (Brownstein et al., 1980; Gainer et al., 1977). This allows for axonal and dendritic release (Ludwig et al., 2016) or the release of OT from the soma that can reach distal sites at least 100μm away (Son et al., 2013). Magnocellular neurons have projections to the portal veins of the median eminence allowing for endocrine release from the posterior pituitary (Silverman & Zimmerman, 1983). A very small amount of OT

fibers reach the ependymal layer of the 3rd ventricle, potentially allowing for some release into the ventricle and CSF (Knobloch & Grinevich, 2014). Many OT neurons are multipolar, and the axons often branch several times in horizontal, sagittal, and coronal planes (see Figure 1a; Grinevich et al., 2016). Some parvocellular neurons synapse onto magnocellular OT neurons that then project centrally (Eliava et al., 2016), in addition to magnocellular interneurons within the PVN (Van Den Pol, 1982), though many of the parvocellular projections are to hindbrain and spinal cites (Hancock, 1976; Swanson & Sawchenko, 1980). Tracing the projections of these neurons was difficult until the discovery of whole brain clearing and 3D imaging techniques such as CLARITY. In addition, the use of genetically encoded markers to label OT instead of classic immunolabeling has facilitated the discovery of previously unknown projections (see Figure 1b; Grinevich & Neumann, 2021). Consequently, the full extent of OT synaptic projections from the PVN in particular is not known.

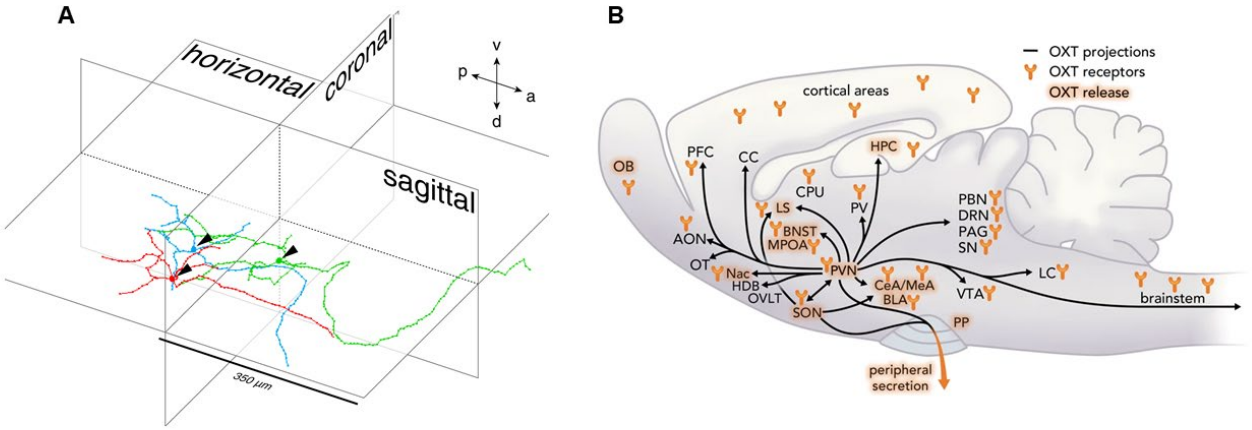


Figure 1: A) Fibers of three neurons were traced until the Venus signal became too faint to follow further. The tracing revealed four to five fibers emanating from the soma (g 5 4; b 5 4; r 5 5 main fibers) and if further branched, bifurcate one to four times along the fiber (g 5 8/0; b 5 3/2; r 5 1 total of subbranches/4 unbranched fibers). Venus fluorescence was recorded from the uncut translucent brain on a custom-built light-sheet microscope controlled by custom acquisition software based on Labview 8.6; mosaic tiles were stitched with a plugin written for ImageJ/Fiji and axons were traced with Knossos software in cooperation with Dr. Günter Giese, Max Planck Institute for Medical Research, Heidelberg, Germany. Scale bars represent and 350 µm. Somata indicated by circles. Reprinted from Grinevich et al**.** (2016) B) This scheme summarizes all available data from male and female including lactating rats regarding OXT neuronal projections, sites of OXT release, e.g., during stress exposure, mating, parturition, suckling, and OXT receptors within brain target regions, as outlined in detail in the text. AON anterior olfactory nucleus, OB olfactory bulb, OT olfactory tubercle, Nac nucleus accumbens, OVLT organum vasculosum laminae terminalis, SON supraoptic nucleus, PVN paraventricular nucleus, PP posterior pituitary, PFC prefrontal cortex, CC cingulate cortex, MPOA medial preoptic area, BNST bed nucleus of the stria terminalis, LS lateral septum, CPu caudate putamen, PV periventricular nucleus of the thalamus, CeA central amygdala, MeA medial amygdala, BLA basolateral amygdala, VTA ventral tegmental area, LC locus coeruleus, PBN parabrachial nucleus, DRN dorsal raphe nucleus, PAG periaqueductal gray, SN substantia nigra, HPC hippocampus, HDB nucleus of the horizontal limb of the diagonal band. Reprinted from Grinevich and Neumann (2021)

Oxytocin has received extraordinary amounts of attention due to its control of mating strategies in monogamous prairie voles. Much of the public attention OT has received has portrayed it over simplistically as a “love hormone”. This perception emerged from numerous studies examining the role of OT on the formation of partner preferences in the prairie vole. Partner preference in the prairie vole is thought to signify a pair bond (McGraw & Young, 2010; Young et al., 2005; Young & Wang, 2004). Pair bonds are defined by selective and somewhat exclusive mating, voluntary contact, mate guarding, coparenting, and negative affect when separated from the partner (Carter et al., 1995). However, many litters in the wild are sired by males not partnered to the female (Solomon et al., 2004). As mentioned above, in most studies pair bonds are inferred from partner preferences. In the prairie vole model, for example, a partner preference is a bias by an experimental animal to spend more time with a previously paired conspecific over a novel conspecific (Williams et al., 1992). One may wonder whether one behavioural measure, among many, is sufficient to support the existence of a latent variable like pair bonding. Though, a similar criticism can and should be applied to many studies of mate preferences in rats, especially those that do not allow the animals to copulate or mate. Moreover, how many facets of sexual behaviour ought to be preferentially directed toward one male over others, in order for a female preference to determined?

Partner preference in the prairie vole is readily induced by allowing copulation and/or a cohabitation period of 24-hours between the experimental animal and a conspecific (Williams et al., 1992). When prairie voles are allowed to copulate with a conspecific, partner preferences can be formed after as little as six hours (Williams et al., 1992). Experiences with reward, either social or sexual, determine pair bond formation in the prairie vole. One framework in which to view pair bonding is that a partner acts as a reinforcer, such that it invigorates motivation to spend more time with that conspecific over a novel animal. Because the time of cohabitation required for preference is shorter when copulation is allowed, copulation is a more potent reinforcer than social contact alone. The formation of experience-based partner preference in the vole further highlights the reproductive importance of experience with sexual reward.

Prairie voles are parentally monogamous, meaning that they mate and rear pups within a given pair. A monogamous mating strategy is thought to have been selected for due to the low population densities of voles in the North American prairies. It was advantageous and therefore reinforced by natural selection to pair, mate, and provide parental investment for pups in low resource and low population density environments. In

contrast, montane voles are close genetic relatives of the prairie vole. Prairie and montane voles look and behave similarly in many circumstances (Tamarin, 1985). However, montane voles are not monogamous and do not form pair bonds. The montane vole's mating strategy is ascribed to the fact that they live in higher population densities than their prairie vole relatives. Female prairie and montane voles have significantly different densities of OTRs in multiple brain regions that underpin this difference in mating strategy (Insel & Shapiro, 1992). Prairie voles show increased densities of OTRs in the NAcc, prelimbic cortex, LS, BNST, VMH, and lateral amygdala. Systemic injections of OT facilitate the formation of partner preference in female prairie voles (Cushing & Carter, 2000). Intracerebral infusions of OT also facilitate the formation of a preference, whereas infusions of an OTR antagonist block the formation of preference (Williams et al., 1994). Oxytocin binding in the NAcc core is also necessary for the formation of pair bonding in female prairie voles (Liu & Wang, 2003; Young et al., 2001). Therefore, OT signalling was identified as a potential regulator of a species' proclivity to a monogamous mating strategy, and consequently, its ability to form partner preferences.

Forming social bonds is a higher order cognitive ability. To state that OT is soley a "bonding hormone" ignores the fact that bonding is likely an emergent process of many simpler basic physiological and cognitive processes affected by OT signalling. After all, OT regulates a variety of physiological, neuroendocrine, and psychological processes. Oxytocin's oldest known function is the induction of uterine contractions during labour (Dale, 1906, 1909). During labour very little OT is released from the posterior pituitary and it is primarily synthesized and released locally in the uterus during labour (Fuchs et al., 1982; Petraglia et al., 1996). Thus, the brain monopolizes neither OT synthesis nor release. Oxytocin acts on myometrial muscular tissue in the uterus to induce uterine and cervical contractions (Dittrich et al., 2009). During labour, activation of OTRs induce the release of Ca^{2+} from the endoplasmic reticulum that then activates the myosin light-chain kinase, causing the muscle to contract (Gimpl & Fahrenholz, 2001). Milk ejection during lactation is regulated by centrally released OT through a similar second messenger cascade to OT's induction of uterine contractions (Leng & Sabatier, 2014).

Many of OT's roles are related to reproductive success, ranging from ovulation to its mediation of sexual arousal, receptivity, and other sexual behaviours, as well as the facilitation of pregnancy, and its peri- and postnatal effects on labour induction and lactation. As mentioned above, in laboratory rats, the estrous cycle is approximately 4 to 5 days in length. During late proestrus, circulating estradiol levels peak in blood,

and this peak in estradiol is followed by a surge in progesterone (Butcher et al., 1974). As circulating estradiol and progesterone increase so do circulating levels of OT (Robinson & Evans, 1990). During late proestrus the increase in circulating OT facilitates the release of LH (Robinson & Evans, 1990), which helps synchronize the onset of sexual receptivity with ovulation. Central infusions of OT induce the release of LH whereas the administration of an OTR antagonist inhibits the pre-ovulatory surge in LH (Johnston et al., 1990; Johnston & Negro-Vilar, 1988). It is thought that OT's effects on gonadotropin secretion are mediated by nitric oxide synthase (NOS; McCann & Rettori, 1996; Rettori et al., 1997). Many OT neurons in the PVN and SON are colocalized with NOS (Bredt et al., 1990) and one of the effects of OTR activation is an increase in the transcription of NOS (Melis et al., 1997). The increased release of nitric oxide (NO) through OT activation directly stimulates the release of LH (Rettori et al., 1997). As LH is released from the anterior pituitary, the corpus luteum begins to form. As mentioned above, if a female receives sufficient copulatory stimulation to induce progestation the corpus luteum will form fully. It functions as a paracrine gland that secretes progesterone to maintain pregnancy until the placenta is fully formed (Goyeneche et al., 2003; McCracken et al., 1999). However, when sufficient copulatory stimulation to induce pregnancy or pseudopregnancy is not received, the corpus luteum degrades and cyclicity resumes after approximately 12 days. Locally synthesized OT regulates the degradation of the corpus luteum, luteolysis, initiating the proceeding estrous cycle (McCracken et al., 1999). In addition to the changes in circulating OT, OTR mRNAs and OTR binding affinity in uterine tissue are highest during proestrus (Larcher et al., 1995).

In addition to the coordination of ovulation with the onset of sexual behaviours, genital arousal needs to be coordinated with sexual receptivity and motivation. Sexual arousal, receptivity, and motivation all interact in a manner that either facilitates or inhibits sexual function (Ågmo, 2011; Meston et al., 1996; Schober et al., 2011). Genital arousal is under complex control by descending motor and autonomic efferents that control genital musculature and blood flow, and genital afferents that convey sexual stimulation as sensory inputs back to the central nervous system (Giuliano & Julia-Guilloteau, 2006; McKenna et al., 1991; Normandin & Murphy, 2011). The autonomic nervous system is responsible for increasing blood flow to the genitalia which results in the engorgement and erection of the clitoris and increased blood flow to the vaginal canal. Increased blood flow allows heavily vascularized epithelial vaginal cells to produce a plasma-based mucus that lubricates the vagina (Giuliano et al., 2002). The vaginal epithelial cells that lubricate the vagina

are sensitive to the ovarian hormones, estradiol and progesterone. Consequently, the surface area of vaginal epithelial cells is highest when females are sexually receptive (Pessina et al., 2006). Sensory stimulation of the perineum, clitoris, and vulva activates much of the motoric and autonomic inputs that are required for genital arousal and vaginal lubrication (McKenna et al., 1991; Schober et al., 2011). The hormonal state that induces sexual receptivity also increases the sensory field of these tissues and their responsiveness to sexual stimulation (Komisaruk et al., 1972; Pfaff et al., 1977; Pfaff, 1980).

Sexual arousal, under autonomic control, is influenced by central and spinal excitation in which noradrenaline (NA), OT, NOS, and hypothalamic DA play important excitatory roles (for a review see Giuliano et al., 2002). Increased states of sexual arousal facilitate lordosis and proceptive sexual behaviours, such as solicitations and hops and darts (Meston et al., 1996). Because sexual stimulation can also facilitate lordosis quotients (Hardy & Debold, 1971), there is mutually reinforcing feedback between arousal, stimulation (i.e., VCS and CLS), receptivity, and motivation. Sensory information from the clitoris, vagina, and cervix (see Figure 2) are carried by the sensory branches of pudendal, pelvic, and hypogastric nerves (Peters et al., 1987). Retroactive tracers injected into the clitoris and vagina show that the PVN, among other regions, receives a substantial amount of stimulation from these sensory projections (Gelez et al., 2010; Normandin & Murphy, 2011). The PVN has projections to several hindbrain areas that regulate autonomic arousal (viz. the nucleus of the solitary tract (NTS), the rostral ventrolateral reticular nucleus (RVL), and the nucleus paragigantocellularis of the medulla (nPGi), in addition to spinal sympathetic and parasympathetic preganglionic fibers (Luiten et al., 1987; Normandin & Murphy, 2008; Saper et al., 1976; Sawchenko & Swanson, 1983; Swanson & Sawchenko, 1983).

The increase in OT in blood, the spine, and locally in the PVN during and after VCS (Nyuyki et al., 2011; Sansone et al., 2002) may be partly responsible for the facilitation of sexual arousal that follows genital and extragenital sexual stimulation. Although sympathetic nervous system activation is inhibitory to erectile responses in males, it has been shown that pharmacological activation of the sympathetic nervous system facilitates arousal in females (Meston et al., 1997; Meston & Heiman, 1998). Indeed, moderate increases in heart rate variability, an indicator of sympathetic activity, is associated with increased vaginal arousal (Lorenz et al., 2012). Pupil dilation, a result of sympathetic activity, is immediately increased following VCS in female

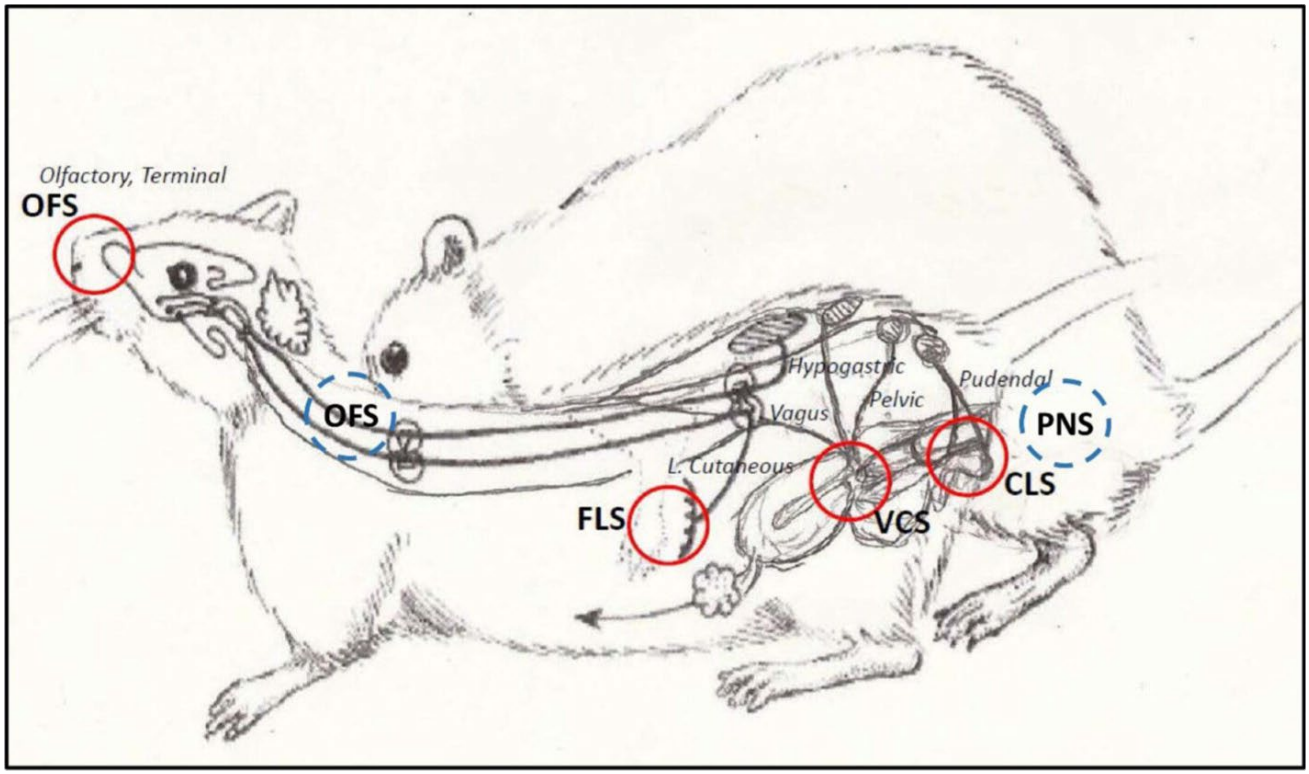


Figure 2: Sensory stimulation of female (red) and male (blue) rats during sexual interaction. OFS: olfactory stimulation. FLS: flank (tactile) stimulation. VCS: vaginocervical stimulation. CLS: clitoral stimulation. PNS: penile stimulation**.** Reprinted from Pfaus et al. (2016)**.**

rats (Sansone & Komisaruk, 2001). Oxytocin injected into the spinal cord induces this sympathetic response, which is also inhibited by an OTR antagonist (Sansone & Komisaruk, 2001).

This control of autonomic activation occurs because the PVN and mPOA control the motoric and autonomic inputs to genitalia (Normandin & Murphy, 2008). The mPOA integrates sensory inputs with autonomic inputs and outputs and stimulation of the mPOA directly increases intracervenous pressure in genital erectile tissue (Giuliano et al., 1996). The PVN receives substantial inputs from the mPOA (Silverman et al., 1981; Tribollet & Dreifuss, 1981), and its stimulation is known to induce erections in males (Chen et al., 1997). Both regions project to the nPGi which inhibits arousal through its descending inputs to the spine. The projections from the PVN to the nPGi are more numerous in females than in males (Marson & Foley, 2004; Normandin & Murphy, 2008). Therefore, the PVN's facilitation of sexual arousal may be by its direct activation from VCS (Nyuyki et al., 2011) and via the input it receives from the increased activation of the mPOA in response to CLS and VCS (Parada et al., 2010; Pfaus et al., 1996; Polston & Erskine, 1995). Increased PVN activation and release of OT into the spinal canal (Sansone et al., 2002) may thus act to facilitate female sexual arousal by directly stimulating sympathetic preganglionic fibers. However, the manner in which OT from the PVN augments the inhibitions of the nPGi is not fully understood.

Within the clitoris and vagina, it is thought that NO signalling via activation of NOS and vasointestinal protein (VIP) induce genital arousal. For blood flow to increase, the smooth muscle tissue of the clitoris and vagina need to relax. Both NO and VIP are thought to facilitate this smooth muscle relaxation (Munarriz et al., 2003). Estradiol increases the transcription of NOS, thus facilitating the potential for arousal. Smooth muscle relaxation is also facilitated by OT (Duridanova et al., 1997) through the increase of intracellular Ca^{2+}. Interestingly, OT also increases the production of NOS (Melis et al., 1997) and facilitates vasodilation through NO (Japundzic-Zigon, 2013). In summary, OT helps to coordinate sexual arousal with receptivity and ovulation. As OT is released into the periphery and spine during copulation, this increase in OT may work to potentiate sexual arousal through separate independent mechanisms.

When a female is sexually receptive, either during the late proestrus phase of the cycle, or when an ovariectomized female is injected with EB+P, OT regulates different aspects of female sexual behaviour and neuroendocrine responses to copulation. During periods of sexual receptivity induced by s.c. injections of EB+P, the binding affinity of OTRs in the mPOA (Caldwell, Walker, et al., 1994) and VMHvl increase

(Schumacher et al., 1989). Oxytocin binding in both areas facilitates lordosis (Caldwell, Johns, et al., 1994; Coirini et al., 1991). Within the VMHvl, it has been shown that the lordosis reflex is facilitated by OT because progesterone further increases the binding affinity of OTRs (Schumacher et al., 1990). Though the mPOA is typically associated with sexual arousal and a female's ability to solicit and pace the rate of VCS, its projections to the VMHvl are important for both lordosis and solicitations. A female cannot solicit and lordose at the same time. Thus, it has been proposed that a local dopaminergic circuit between the mPOA and VMHvl regulates the shift between solicitation and lordosis (Graham et al., 2015; Graham & Pfaus, 2010).

OT neurons of the PVN are sensitive to paced VCS. Fos IR in OT neurons in the PVN is increased following paced VCS (Flanagan et al., 1993). Flanagan and colleagues also demonstrated that Fos protein expression was also increased in neurons in the VMHvl that are directly adjacent to OT-containing axons. However, the PVN does not have direct OT projections to the mPOA (see Figure 1b) but has axons that project adjacent to the VMH (Canteras et al., 1994). Both hypothalamic regions contain relatively high amounts of OTRs (Caldwell, Walker, et al., 1994; Schumacher et al., 1990). Sexual receptivity induced with EB+P increases the surface area covered by OTRs surrounding the VMH, which may then come into contact with these adjacent projections from the PVN (Schumacher et al., 1990). In contrast, much of OT's actions in the mPOA during sexual receptivity must be from somatic and dendritic diffusion. Paced copulation significantly increases OT in dialysates taken from the PVN, relative to unpaced copulation (Nyuyki et al., 2011). VCS also increases OT in the spinal cord (Sansone et al., 2002), even in hypophysectomized females. Such increases in spinal OT may gate the excitability of the sensory input arriving from the genitalia, in addition to the motoric and autonomic outputs that facilitates lordosis and sexual arousal.

As mentioned above, following copulation with adequate stimulation, a female will either become pregnant, or pseudopregnant (Kornberg & Erskine, 1994). Oxytocin is thought to regulate the initiation of the progestational prolactin surge and as a result, some have proposed that OT acts as a prolactin releasing factor (Butcher et al., 1974; Freeman & Neill, 1972; Kennett & McKee, 2012). An infusion of the OTR antagonist, OTA, into the VMHvl completely inhibits the increase in prolactin following copulation (Northrop & Erskine, 2008). Prolactin secretion is tonically inhibited by incertohypothalamic DA (MacLeod et al., 1970). The inhibition of prolactin via OTR antagonism likely occurs by some convergent facilitation of incertohypothalamic DA release. One peripheral injection of OT is also sufficient to induce a nocturnal surge

in prolactin in unmated females (Egli et al., 2006). When the pelvic nerve that innervates the cervix is transected, this surge in prolactin induced by OT alone is blocked (Helena et al., 2011). The progestational changes induced by OT suggest that OT signalling during and after copulation induces long-term changes in reproductive capabilities.

The mPOA, VMH, and PVN are all sensitive to VCS and CLS. Both VCS and CLS increase Fos protein IR in the mPOA (Parada et al., 2010; Pfaus et al., 2006). As mentioned previously, Fos IR is increased in mPOA, VMH, MeApd, and BNST following paced intromissions and VCS (Pfaus et al., 1996, 2006; Polston & Erskine, 1995). Interestingly, the MeApd shows an increase in Fos protein that tracks the number of intromissions and ejaculations received (Coolen et al., 1996), and receives one of the few direct axonal projections from the PVN (Sofroniew, 1980). The MeApd also has reciprocal connections with the VMH, mPOA, and BNST (Akesson et al., 1988; Canteras et al., 1994). The increased activation and release of OT from the PVN may gate the reactivity of the MeApd to VCS and ejaculations, in conjunction with incoming input from mPOA, VMH, and BNST. This increased activation of the MeApd may then provide excitatory inputs to the mPOA and VMH that control estrous termination and progestation.

Taken together, the data reviewed above suggests that OT regulates reproductive capacity through its coordination of ovulation with the onset of sexual behaviours through to its facilitation of progestational responses. Oxytocin also likely facilitates a female's ability to recognize suitable mates, and also preferred sexual partners. Olfactory cues guide much of social recognition in rats. As mentioned previously, because of the variation in the genes that encode MHC proteins, it has been suggested that MHCs may signal an individual rat's identity (Keverne, 2004). The VNO has direct connections via the accessory olfactory bulb to the MeA and the mPOA, and its stimulation has been directly implicated in the onset of sexual behaviours by male rats in response to estrous odours (Bressler & Baum, 1996). In conjunction with MHCs, there are other volatile particles, pheromones, and major urinary proteins that provide olfactory cues about the sex of a conspecific. Pheromonal cues are detected by the VNO which then project to the accessory olfactory bulb. The accessory olfactory bulb contains a relatively large number of OTRs and plays an important role in social memory (Fang et al., 2008; Meddle et al., 2007). Activation of OTRs in the MeA which receives direct input from the accessory olfactory bulb and PVN (Meredith & Westberry, 2004) is necessary for a female to determine the sex of potential mates and promotes social memory (Lukas et al., 2013; Yao et al., 2017).

Attractive males that have greater amounts of MUPs also increase activation of the MeApd (Kumar et al., 2014).

Estradiol-sensitive neurons in the mPOA are particularly responsive to male odour cues (McHenry et al., 2017). The mPOA has direct projections to DA neurons in the VTA (Tobiansky et al., 2016). Thus, increased activation of mPOA neurons in response to male odours increases activation dopamine neurons in the VTA, which then increases the release of dopamine in the NAcc. This odour-induced release of dopamine in the NAcc has been shown to be necessary for a female to spend more time with a male conspecific over a female when sexually receptive. Ross et al. (2009) have shown that prairie voles and rats, but not montane voles, have OT projections from the PVN to the NAcc. Coria-Avila and Pfaus (2007) have shown increased Fos IR in PVN neurons following exposure to scent and strain cues associated with paced copulation. A substantial portion of those neurons likely contain OT. Coria-Avila and Pfaus also reported increased activation to preferred cues in the NAcc, mPOA, the VMH, and VTA, areas important for sexual motivation, solicitations, and lordosis. Therefore, the activation of OT neurons by preferred males associated with previous paced copulation likely induces the release of OT from these neurons in the PVN. It has been shown that activation of the OT pathway from the PVN to NAcc pathway is necessary for social reward (Dölen et al., 2013).

Holley et al. (2015) showed that the conditioned mate guarding of males associated with paced copulation is associated with increase OT neuronal activation in the PVN. This activation and release of OT from the PVN likely co-occurs with activation of the MeApd facilitating recognition, but also the conditional diffusion of OT into the mPOA and VMH where there are large amounts of OTRs. Conditional activation of these central OTRs and the gating of spinal excitation via OT may potentiate lordosis responses to preferred males. Furthermore, the direct OT projections from the PVN to the NAcc likely facilitate sexual motivation (and attention) toward preferred males. Concurrently, the increased excitation caused by OT diffusion into the mPOA may increase the excitability of its projections to the VTA, further influencing dopamine release in the NAcc.

The formation of mate guarding in female rats is facilitated by OT (Holley et al., 2015). Coria-Avila et al. (2005) demonstrated that when females are given one paced copulation with a scented male, it is not sufficient for the formation of sexual preferences. However, when females are injected systemically with OT,

one paced copulation is sufficient to induce mate guarding of a familiar male (Holley et al., 2015). This suggests that OT facilitates the formation of reproductive preferences similar to that shown in the prairie vole. Systemic injections of OT facilitate preference formation in voles too (Cushing & Carter, 2000). However, whether peripherally injected OT would facilitate the formation of mate preferences, such as the preference to solicit and receive ejaculations from a male associated with paced copulation, is not yet known. Oxytocin is too large and polar a peptide to cross the blood brain barrier (BBB) without a carrier protein. Some have suggested that a small amount of leakage into the brain occurs following a systemic injection, and the amount of leakage is suspected to be commensurate to the amount injected (Jin et al., 2007; Lim et al., 2005). Recently, putative evidence has emerged of the transport of OT across the BBB via RAGE proteins (Yamamoto et al., 2019). However, the amount of transported OT is relatively low. Leakage and transport of peripherally injected OT likely occurs (Ermisch et al., 1985), though the extent to which this augments behaviour is unclear. Thus, it is also possible that peripherally injected OT acts on peripheral OTRs which then facilitates central release and transmission.

Thesis aims

The overarching aim of this thesis is to further understand the link between reproductive success and the reinforcing properties of sex in the female rat by testing whether OT facilitates both mate preference and reproductive responses. Such a link would suggest that OT coordinates sexual reward with reproductive success, linking proximate and ultimate influences on mating behaviour.

As previously described, female rats can be sexually conditioned to preferentially solicit, selectively receive ejaculations from, and mate guard, a male associated with prior paced copulation (Coria-Avila et al., 2005; Holley et al., 2015). Importantly, the ability to pace the rate of intromissions during sexual conditioning is a determinant of whether a female biases its sexual behaviours toward a familiar male. Moreover, paced copulation can reliably induce a conditioned place preference equivalent to that of morphine (Paredes & Alonso, 1997). As reviewed above, the formation of partner and place preferences suggest that paced copulation is a potent sexual reward and that female rats can readily associate partners or places with this reward. And pacing serves a crucial reproductive purpose for the female rat given that paced copulation significantly increases the likelihood of a female becoming pregnant following sex (Edmonds et al., 1972; Erskine et al., 1989; Matthews & Adler, 1977).

Central OT transmission is necessary for the formation of partner preferences in prairie voles (Cushing & Carter, 2000; Williams et al., 1994). Despite not being monogamous, female rats can display selective sexual partner preferences like that of the prairie vole. It is unclear whether OT also controls the formation of mate preferences in female rats. If female rats display selective mating preferences, how do they do so?

Little is known about the role of OT transmission in the formation of sexually conditioned mate preferences. Because of OT's roles in progestation, sexual behaviours and arousal, and social recognition, it was hypothesized that OT plays an important regulatory role in the formation of mate preferences in female rats. Given the ability of pacing to facilitate both pregnancy and reward learning, reproduction and reward appear to be intimately connected.

The first aim of this thesis was to examine whether the selective preference behaviours shown by female rats is related to OT neuronal activation. Thus, in Chapter 1, we demonstrate that a female rat can be conditioned to selectively bias its sexual behaviours toward a specific male associated with copulation. Here, female rats repeatedly copulated with the same conspecific, and the degree to which a female could pace was varied. A preference for a familiar male was dependent on the type of paced copulation during sexual conditioning trials. Females that paced in conditions that are associated with longer pacing intervals (i.e., one-hole pacing divider; Ismail et al., 2009) formed preferences, whereas those that paced with shorter intervals (i.e., four-hole pacing divider) did not. It was then shown that preferred males (i.e., familiar conspecific associated with longer pacing intervals) induced more Fos protein IR in OT neurons in the mPOA, PVN, and the VMH. Chapter 1 demonstrates that female rats can display selective sexual preferences for individual conspecifics and that OT neurons are activated by these preferred conspecifics.

The aim of Chapter 2 was to investigate the specific role of OT signalling in the formation of sexually conditioned mate preferences. OT readily induces behavioural, neurobiological, and neuroendocrine changes that were hypothesized to facilitate the formation of a mate preference after only one copulatory experience. In this study, the effects of two different OTR antagonists, cligosiban that crosses the BBB, and L371,257 which does not, were examined. These drugs allowed us to determine the contributions of peripheral and central OT signalling to post-copulatory responses and mate preference formation.

First, we demonstrated that OT neurons in the VMHvl are sensitive to VCS, particularly when females are also injected systemically with OT. Using immunohistochemistry to detect Fos IR in OT neurons, it was shown that OT neuron activation was less abundant following VCS when females were injected with the OTR antagonists. Oxytocin alone and OT with VCS increased serum LH but not significantly relative to vehicle or the OTR antagonists. We also demonstrated that the density of OTRs in peripheral sexual organs, the clitoris and the cervix, and in various brain regions are hormonally dependent. These changes in OTR IR were also shown to be tissue dependent.

Using behavioural testing with pharmacological manipulations, we demonstrated that systemic OT readily induces estrous termination. While estrous termination is readily induced by VCS, we demonstrate that systemic OTR antagonists block estrous termination in response to VCS. The attenuation of estrous termination was stronger in females injected with the antagonist that does not cross the blood brain barrier. This led to the hypothesis that the induction of estrous termination is controlled primarily by OTRs outside the brain.

In order to examine the central vs peripheral control of estrous termination in response to VCS further, females were infused intracerebroventricularly with either OT, an OTR antagonist, or vehicle. Females were also systemically injected with the peripheral OTR antagonist or vehicle directly prior to VCS. Artificially increased central OT via an infusion was sufficient to induce estrous termination. The additional administration of a peripheral OTR antagonist did not attenuate estrous termination. However, because VCS initiates an increase in central OT transmission, these data still indicate a necessary role of peripheral OTRs in the induction of estrous termination, and subsequently, progestational responses. Finally, it was shown that systemic OT facilitates the formation of a mate preference after one paced conditioning trial. In contrast, administration of both OTR antagonists biased females to solicit a novel male during the partner preference test.

Chapter 1. Fos expression is increased in oxytocin neurons of female rats with a sexually conditioned mate preference for an individual male rat

Conall E. Mac Cionnaith[1], Alice Lemay[1], Eamonn L. Gomez-Perales[1], Genevieve Robert[1], Rebecca Cernik[1], Wayne Brake[1], James G. Pfaus[2].

[1]Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC H4B 1R6, Canada

[2]Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, VER 91193 México

Mac Cionnaith, C. E., Lemay, A., Gomez-Perales, E. L., Robert, G., Cernik, R., Brake, W. G., & Pfaus, J. G. (2020). Fos expression is increased in oxytocin neurons of female rats with a sexually conditioned mate preference for an individual male rat. *Hormones and Behavior*, *117*, 104612. https://doi.org/10.1016/j.yhbeh.2019.104612

Abstract

Evidence suggests an important role of Pavlovian learning in sexual partner selection. Female rats that experience paced copulation with a male scented with a neutral odor selectively solicit and receive ejaculations from the scented male relative to an unscented male. Exposure to the conditioned odor alone induces Fos protein in regions of the brain associated with sexual excitation. Here we tested whether female rats can be conditioned to show a sexual preference for an unscented male rat of the same strain. Female Long-Evans rats were given 10 copulatory trials with either a one-hole pacing divider or a four-hole pacing divider in a unilevel chamber with the same conspecific male (n = 16). Females were then given an open-field partner preference test with the paired male versus a novel male. After two reconditioning trials females were exposed to the partner or a novel male to induce Fos expression. Females that paced with the one-hole divider received the first ejaculation and more ejaculations overall from the paired compared to novel male. Fos immunoreactivity within oxytocin neurons in the PVN, mPOA, and VMH was increased in females with a preference that were exposed to the paired male. These data indicate that female rats can form selective sexual preferences for an individual conspecific and that their formation depend on the type of pacing during conditioning. These findings further suggest the involvement of oxytocin in the display of conditioned preferences. Thus, early copulatory experience appears to determine the mating strategy used by female rats.

Keywords : Conditioned partner preference; Paced copulation; Oxytocin; Sexual behavior

1. Introduction

In natural and semi-natural environments rats are generally considered promiscuous copulators. For example, within a group mating situation female and male rats change sexual partners regularly (McClintock, 1984). In contrast, monogamous prairie voles form long lasting breeding pairs and close social bonds (Getz et al., 1981). Pair-bonded prairie voles care for young offspring together, and are aggressive toward unfamiliar conspecifics, a behavior termed mate guarding (Carter et al., 1995; Getz et al., 1981). However, despite evidence of promiscuous copulation in rats, females exercise selectivity in their choice of male sexual partners. In the rat, female choice has an important influence on mating success such that a female can determine from which male it receives an ejaculation (McClintock & Adler, 1978), and females compete to receive the ejaculation of a dominant male. Indeed, in a group mating situation, dominant females have been observed to intercept a preferred male immediately prior to his ejaculation, and to take ejaculations selectively from a preferred male (McClintock, 1984). Copulatory experience and learning can also affect female sexual partner choices. For example, female rats can form sexual preferences for partner cues (e.g., odor, strain) and places associated with sexual reinforcement (Coria-Avila et al., 2005, 2006). This suggests that neural systems for sexual preferences based on novelty and familiarity exist and are modifiable by experience (Pfaus et al., 2012).

Sexually conditioned preferences depend on the pairing of a salient cue with sexually reinforcing sexual simulation (Coria-Avila et al., 2005, 2006; Paredes & Alonso, 1997). For female rats, the ability to pace the initiation rate at which they receive penile intromission (and thus both clitoral and cervical stimulation) is reinforcing. When female rats are allowed to pace their copulatory interaction with males, both place and partner preferences can be formed (Coria-Avila et al., 2005; Jenkins & Becker, 2003; Paredes & Alonso, 1997), indicating that paced sexual stimulation is rewarding. Neutral odor cues, such as almond or lemon scent, or strain cues, such as those that exist between albino and pigmented strains, have been used to condition sexual partner preferences (Coria-Avila et al., 2005, 2006). Females are given repeated, multiejaculatory paced copulations with either a scented male or one of a specific strain. When females are given the choice on a final test to copulate with a male bearing the pacing-related cue and a novel male, females solicit the pacing-related male more, and often choose from which male it receives ejaculations

(Coria-Avila et al., 2005, 2006). Thus, the cues that were present during the early experience of paced copulation come to guide female sexual partner choice.

Although much has been learned about the neurobiological basis of pair bonds in the prairie vole, the mechanisms by which sexual preferences for familiar cues are formed in the female rat are not fully understood. Pacing-related odor cues have been shown to increase Fos immunoreactivity (Fos IR), a marker of neuronal activation, in the piriform cortex (PirCtx), the Nucleus Accumbens (NAc), the medial preoptic area (mPOA), the paraventricular nucleus of the hypothalamus (PVN), and the ventral tegmental area (VTA; Coria-Avila & Pfaus, 2007). In contrast, when females were conditioned with a particular strain of rat, cues from a male of that strain increased Fos IR in the PirCtx, mPOA, the corticomedial amygdala (CoA), VTA, and the ventromedial hypothalamus (VMH). Thus, although both pacing-related odor and strain cues activate some brain areas in common, the sensory nature of the pacing-related cue appears to differentially activate other brain areas. Importantly, both odor and strain cues increased Fos in areas that have also been shown to be activated during partner preference formation in mated female prairie voles, i.e. the mPOA, Nac, and CoA (Curtis & Wang, 2003) .

Previous studies that conditioned partner preferences in female rats have used either salient odor cues or strain cues during conditioning (Coria-Avila et al., 2005, 2006). Female prairie voles form preferences for individual conspecific males (Getz et al., 1981) and female rats are also capable of forming such preferences. Holley et al. (2014) gave ovariectomized hormonally-primed sexually naïve female rats either 10 paced or non-paced copulatory trials with one particular male conspecific each. Females were given a test in an open field with their paired male in the presence of a sexually receptive intruder female. Females that received paced copulation with the same conspecific male showed three characteristic mate-guarding behaviors in the presence of the intruder female: hovering next to the male and presenting a pre-lordosis crouch, blocking access of the approaching intruder female by imposing themselves between it and the male, and agonistic mounting of the intruder female if it made an active solicitation (headwise orientation and runaway) of the male. Females that did not pace copulations during training did not display these behaviors. Moreover, paced females solicited and received more ejaculations from the pacing-associated males compared to non-paced females. Mate guarding has typically been associated with monogamous animals, such as the prairie vole.

However, these findings indicate that female rats can be conditioned to mate guard conspecific males that are associated with paced copulation.

Mate-guarding females exposed to their conspecific males had increased Fos IR in a number of brain regions, including the paraventricular nucleus (PVN), and the supraoptic nucleus (SON) of the hypothalamus. Both the PVN and SON contain large populations of parvocellular and magnocellular neurons that synthesize oxytocin (OT) or vasopressin (AVP) and send their projections to the rest of the brain or to the posterior pituitary, respectively (Swanson & Sawchenko, 1983). These neuropeptides have been shown to regulate pair bonding in prairie voles (Cho et al., 1999). Interestingly, Holley et al. (2015) pre-treated females with OT, AVP, or saline, during their first paced copulatory conditioning trial. Four days later, females were then given an open-field test with an intruder female and either the familiar male or a novel male. OT-treated females increased their hovering and presenting behaviors, whereas AVP-treated females increased their blocking behaviors, compared to saline-treated females. Females presented with a novel male competed with the intruder female as has been described previously (McClintock, 1984). Additionally, Fos IR was increased in OT and AVP neurons in the PVN and SON of mate-guarding females. Thus, central OT or AVP transmission may facilitate different aspects of mate guarding, with OT facilitating the formation of sexually conditioned preferences and affiliation, and AVP affecting the actual mate-guarding behavior of the female.

In previous studies of sexually-conditioned partner preferences in female rats (Coria-Avila et al., 2005, 2006), preference was assessed in a large open field with two males, one that had the cue (i.e. odor or strain) associated with pacing, and a novel male. In the absence of an externally applied odor like almond scent, it is not clear which cues guide female approach, solicitation, and selective choice of ejaculation. The strain of a male might be signalled by strain differences in certain pheromonal cues, such as major histocompatibility complexes (MHCs), the visible pigmentation of the male, and/or differences in ultrasonic vocalisation. Although the conditioned mate guarding studies of Holley et al. (2014, 2015) demonstrate that females can differentiate between two unscented males of the same strain, it is unclear whether females express this only in the presence of a competitor female, or whether they could show this preference in a copulatory choice test between two males of the same strain. Therefore, the aim of the current study was to assess whether female rats can be conditioned to prefer a conspecific male associated with paced copulation over a novel conspecific of the same strain. Additionally, we also examined whether sexually conditioned

females show increased Fos IR in OT neurons in areas previously associated with selective copulatory behaviors in the female rat.

2. Methods

2.1. Animals

Thirty-two Long Evans female rats weighing 175-200g purchased from Charles River were used in this experiment (Charles River, St-Constant, Qc, Canada). Females were sexually naïve. Thirty-two sexually vigorous Long-Evans males were used as sexual stimuli. The male rats were used in a previous experiment. All animals were housed in the Animal Care Facility at Concordia University at a constant temperature and humidity and given ad libitum access to standard laboratory chow (Charles River #5075, Montreal, Canada) and water. Female rats were paired-housed in Plexiglass shoebox cages and maintained on a 12h:12h reverse light cycle (8:00pm lights on). All procedures had ethical approval from the Concordia University Animal Research Ethics Committee.

2.2. Ovariectomy

Females were bilaterally ovariectomized to allow for the control of sexual receptivity with exogenous steroid hormone administration. Females were anaesthetized with a 4:3 mixture of ketamine hydrochloride (100mg/ml; Ketaset, Wyeth Cananda) and xylazine hydrochloride (20mg/ml; Rompum, Bayer Healthcare). Females were injected intraperitoneally with the ketamine: xylazine mixture at a dosage of 1ml/kg. Ovaries were removed through a lower-lumbar incision. Following surgeries, females were subcutaneously injected with 0.1ml Penicillin G (PenG, antibiotic), and given a non-steroidal anti-inflammatory drug, Ketoprofen (Anafen 100mg/ml, Boehringer Ingelheim) at a dosage of 0.03ml. Ketoprofen was administered at the same dosage for the next 3 days to provide analgesia to the female rats. Females were given one week to recover in their home cage before testing began.

2.3 Conditioning Apparatus

Females were sexually conditioned in Plexiglass unilevel pacing chambers (Height 38cm x Width 60cm x Length 38cm). Chambers contained a metal wire-mesh grid as a floor covered with a layer of woodchip bedding. Each conditioning chamber included a divider with either one hole or four holes (4cm x

4cm) large enough that the female could pass through but too small for the male to enter. The inclusion of the divider gave female rats the ability to pace copulation in the chamber with either the one-hole or four-hole divider, as has been done previously (Ismail et al., 2009).

2.4 Conditioning Procedure

Sexually naïve females were randomly assigned to paced copulation with either the one-hole or four-hole pacing condition (n = 16 per pacing group). Each female rat was paired with a specific male sexual partner. Females only copulated with the paired male during sexual conditioning trials. Fourty-eight hours prior to conditioning trials, females were subcutaneously injected with estradiol benzoate (EB) (10µg in 0.1ml seasame oil), followed by progesterone (P) (500µg in 0.1ml seasame oil) four hours before sexual conditioning. Hormonal priming with this dosage of EB and P has previously been shown to induce sexual receptivity (Jones et al., 2013). All conditioning trials were held during the middle third of the rats' dark circadian phase (2:00pm to 4:00pm). Female rats underwent ten sexual conditioning trials with the same male rat. All conditioning trials lasted 30 minutes and occurred once every four days for 10 trials. Before the commencement of each conditioning trial males were habituated to the conditioning chamber for five minutes. Reagent grade seasame oil was purchased from Sigma Aldrich (SigmaAldrich, Canada, Lot # MKBR2026V). Both EB and P were supplied by Steraloids INC (Newport, RI, USA).

2.5 Partner Preference Test

After ten sexual conditioning trials female rats were given a sexual partner preference test. In this test females could copulate freely with the paired male from the prior conditioning trials or a novel male. The partner preference test was held in an open field apparatus (123cm x 123cm x 46cm) with woodchip bedding covering the floor. The paired and novel males were tethered to opposite corners of the open field via a 30cm metal spring attached to a small jacket as described in Coria-Avila et al. (2005). The tethered males had a roaming range of approximately 45cm. Females were placed into a neutral corner of the open field and the partner preference test lasted 30 minutes. All trials were recorded with ceiling-mounted Sony Handicam and scored by a researcher who was blinded to the male type (paired or novel) and pacing condition.

The frequency of solicitations, hops and darts, and visits (entering the roaming space of the male) made toward the paired male and novel male by each female was recorded. The number of ejaculations the

female received from each male was scored, as well as the female's choice of male from which the female received the first ejaculation in the copulatory series. The time each female spent in each of the male's roaming space was also recorded.

2.6 Tissue Preparation

Females were given two reconditioning trials after the partner preference test. This was done to re-establish the association between the paired male and paced copulation because females also copulated with males that were not the paired male rat during the preference test. The reconditioning trials were conducted identically to the trials prior to the preference test.

To induce Fos protein, female rats from both pacing conditions were exposed to either the paired male rat or a novel male rat in the unilevel pacing chamber, n = 5 per group x2 (1-hole vs 4-hole), x2 (Paired vs Novel). Physical interaction was restricted by the inclusion of a wire mesh in place of the one/four-hole divider, allowing olfaction and auditory stimulation from the male. Females did not copulate as we aimed to induce Fos protein expression to a male associated with prior pacing. Exposure to a novel male was used as a control to compare Fos expression to a pacing associated- vs non-associated male. After one hour of exposure to a male, females rested for 15 minutes. Females were injected with an overdose of sodium pentobarbitol (120mg/kg; Euthanyl) and perfused intracardially with 250ml of phosphate buffered solution followed by 250ml of 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde for four hours, after which the brains were dehydrated in a 30% sucrose solution for 48 hours. Brains were then wrapped in aluminium foil and flash frozen on dry ice and stored at -80°c until sectioning. Brains were coronally sectioned at a thickness of 30µm using a Leica microtome. Five brains from each condition were sectioned and slices from the mPOA (B 0.00 to -1.32), PVN (B-1.08 to -1.80), SON (B-0.48 to -1.72), VTA (B-5.28 to -6.84), VMH (B-2.28 to -3.12) were selected for immunohistochemistry. The Paxinos and Watson (2006) rat brain atlas was used to identify brain regions. All sections were co-labelled for oxytocin and Fos protein expression.

2.7 Immunohistochemistry and Quantification

Sections were washed in fresh 0.9% tris-buffered saline (TBS; 22.27mmol Trizma Hydrochloride and 1.651mmol Trizma Base in 0.9% Saline), and quenched in 30% H_20_2 and TBS for 30 minutes at room

temperature. Sections were then pre-blocked with 3% Normal Goat Serum (NGS) in 0.2% Triton-TBS for 2-hours at room temperature. Tissue sections were incubated with the primary polyclonal anti-rat Fos antibody made in rabbit (1:20,000, Synaptic Systems, 226 003) with 3% NGS in 0.05% Triton-TBS for 72 hours at 4°c. The sections were then incubated with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories Canada, Burlington, ON; 1:200) in 3% NGS and 0.2% Triton TBS for one hour at 4°c. Sections were then incubated sequentially in 0.05% Triton-TBS with 3% NGS and avidin-biotinylated-peroxidase complex (Vectastain *ELITE*® ABC KIT, Vector Laboratories Canada; diluted 1:55) for two hours at 4°c. Between each incubation sections were washed three times in cold TBS for five minutes.

The sections were stained using a 3,3′-diaminobenzidine (DAB) to react the peroxidase, and nickel chloride to turn the nuclear reaction product blue-black. Sections were first washed in 50mM Tris for 10 minutes, followed by DAB in 50mM Tris (0.5mg/ml) at pH 7.6 for 10 minutes. Finally, 3% H_20_2 (0.1ml per 100ml of DAB solution) was added with 8% nickel chloride (400µl per 100ml of DAB/Tris buffer/ H_20_2) and the sections were washed in the solution for 10 minutes. The reaction was stopped by rinsing in cold TBS. To double label cells for cytoplasmic OT, the above steps were repeated, with the exception of the quenching and pre-blocking phase. Sections were incubated in a rabbit anti-rat oxytocin antibody (AB911; Millipore Sigma, 1:10,000) for 72 hours. During the DAB reaction, nickel chloride was not added which gave a reddish-brown stain to cytoplasmic OT-IR.

Sections were mounted onto gel-coated slides. Mounted sections were dehydrated with a 1-min wash in nanopure distilled water, then 10-min each in 75% ethanol, 90% ethanol, and 99% ethanol, followed by two hours in xylenes. The slides were then coverslipped using Permount (Fisher Scientific, SP15-500).

Photomicrographs of all brain regions of interest were captured using an Olympus light microscope at 20x magnification using Q-Capture Pro software. On average, 3 to 5 bilateral sections of each brain region of interest per rat were included in the analyses. Fos IR cells were identified by the dark-brown/black nuclear stain, and OT IR cells identified by the reddish-brown cytoplasmic stain (Fig 3). When the black Fos IR was found within an OT IR cell, it was counted as a colabelled Fos/OT cell (see Fig 3). ImageJ was used to identify OT IR cells. However, all identified OT-IR cells with Fos IR was counted manually by a researcher blinded to the conditions. Cell counts are reported as the number colabelled OT- and Fos- IR cells/mm².

2.8 Data Analysis

Data were analysed with R software Version 3.5.1 (R Development Core Team, 2018) through R Studio Version 1.1.456 (RStudio Team, 2016). All data and analysis scripts can be accessed at Open Science Framework, https://osf.io/ty9ma/. Video recordings from open field partner preference tests were scored by a researcher blind to both the pacing group of the female and the male to which the female directed her sexual behaviors. The female's choice of male for first ejaculation was calculated and analysed using a χ^2 test through base R (R Development Core Team, 2018). P-values from χ^2 tests were generated with a Monte Carlo simulation with 20,000 iterations, hence, as with all simulated p-values, degrees of freedom are not reported for these tests. All other behavioral data were analysed using mixed between-within 2x2 analysis of variance (ANOVAs). Each female's behavior toward the paired or novel male was treated as a within subject condition, a factor referred to as male type. The pacing group was a between-subjects condition in the mixed ANOVAs, with two levels, one-hole and four-hole. ANOVAs were conducted using the Analysis of Factorial Experiments (afex) package (Singmann et al., 2018). Levene's test, to assess the assumption of homogeneous variances was conducted with the car package (Fox & Weisberg, 2019). The assumption of homogeneous variances was not met for the number of solicitations but was met for all other ANOVA models.

Estimated marginal means for the ANOVA models were calculated using the emmeans: Estimated Marginal Means, aka Least-Squares Means package (Lenth et al., 2018). Planned comparisons were used to compare the means of behaviors made toward each male within each pacing condition, and also to contrast behaviors made to the paired male and the novel male across pacing conditions (1-hole vs 4-hole). The comparisons were as follows: 1) 1-hole: Paired vs Novel. 2) 4-hole: Paired vs Novel. .3) Paired Male: 1-hole vs 4-hole. 4) Novel male: 1-hole vs 4-hole. Multiple comparisons were adjusted for using the Holm correction (Aiken & Gensler, 1996). The same comparisons were used to compare Fos/OT-IR across groups. Generalised η^2 was calculated as an effect size and reported for all significant F-tests. Hedge's G_{avg} was used as an effect size for the planned comparisons, of within-subjects conditions. Hedge's G is reported as an effect sizes for between-subjects comparisons. Both Hedge's G_{avg} and Hedge's G were calculated with the supplementary materials of Lakens (2013). All data were visualised using the package ggplot2: Elegant Graphics for Data Analysis (Wickham et al., 2022).

3. Results

3.1. Open-field Partner Preference Test

Overall, females did not demonstrate increased appetitive sexual or social behaviors (i.e. time spent or visits) toward the male they were conditioned with over the novel male. However, females that paced with the one-hole divider during conditioning trials selectively received the paired males' ejaculations first and more frequently.

Females in the one-hole pacing condition received the first ejaculation significantly more often from the paired male compared to the novel male, $\chi^2 = 6.25$, $p = .019$ (Fig 1G). Moreover, 11 of 16 females only received ejaculations from the paired male. In the four-hole pacing condition, four females did not receive ejaculations during the partner preference test despite receiving intromissions and soliciting males. Therefore, the data from 12 females were used to compare the choice of first ejaculation in the four-hole pacing condition. Females did not receive the first ejaculation from the paired male more than the novel male.

There was a significant interaction between pacing (1- vs 4-hole) and male type (paired vs novel) for the number of ejaculations received, $F(1, 30) = 9.86$, $p = .004$, $\eta^2_G = .19$. Planned comparisons revealed that females in the one-hole pacing condition, but not four-hole pacing condition, received significantly more ejaculations from the paired male ($M = 2.125$, $SD = 1.09$) than from the novel male ($M = 0.56$, $SD = 1.03$), $t(30) = 3.26$, $p = .008$, $G_{avg} = .90$. It was also found that one-hole conditioned females received significantly more ejaculations from the paired male than four-hole conditioned females ($M = .69$, $SD = 1.2$), $t(50.14) = 3.61$, $p = .002$, $G = 1.22$ (Fig 1A).

No significant interaction, or main effect of pacing or male type were found for solicitations (Figure 1B). There was a significant interaction between pacing and male type on the frequency of visits to either male, $F(1, 30) = 7.82$, $p = .009$. However, the effect size was small for this interaction $\eta^2_G = .01$. Planned comparisons of the interaction with Holm adjustment revealed no mean group differences, (Fig 1E). A similar result was found for hops and darts. A significant interaction between pacing condition and male type was found, $F(1, 30) = 10.05$, $p = .004$, $\eta^2_G = .11$. The planned comparisons found that females conditioned with the one-hole pacing divider made more hops and darts toward the novel male ($M = 27.625$, $SD = 10.31$) compared to the paired male ($M = 20.125$, $SD = 7.43$), $t(30) = -2.663$, $p = .0493$, $G_{avg} = .79$ (Fig 1D).

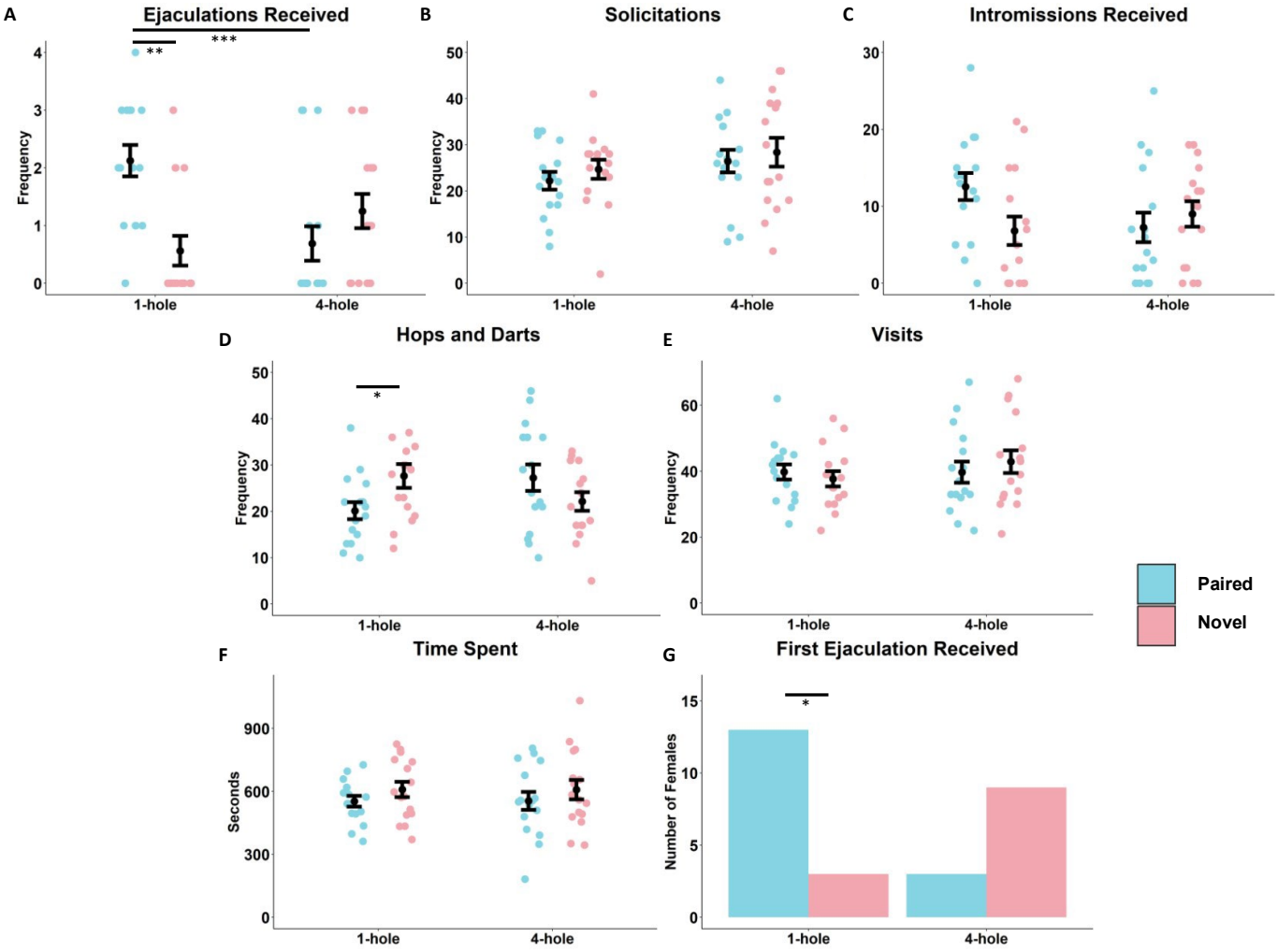


Figure 1: The distribution of data for each group displaying each individual data point. Black dots and bars represent the mean and standard error for each group. A) Displays the number of ejaculations received by a female from a paired and novel male. B) Displays the frequency of solicitations made by females toward the paired and novel males across pacing conditions. C) The number of intromissions received by each female for the paired and novel male in both pacing conditions. D) The number of hops and darts made by females toward a paired and novel male. E) No difference in visits, entries to a males' (paired and novel) roaming, ranges were observed. F) Across both pacing conditions, females did not spend time with the paired male over the novel male. G) The frequency of females by pacing group that received the first ejaculation of the trial from a paired or novel male. * $p < .05$, ** $p < .01$, *** $p < .005$

The mixed ANOVA found no interaction or main effects on the frequency of intromissions received by females (Fig 1C). There was also no significant interaction or main effects on the amount of time spent by females with each male (Figure 1F).

3.2. Fos and oxytocin co-localised immunoreactive cell counts

As females from both pacing groups were only exposed to either a paired male or a novel male to induce Fos protein, the reported omnibus tests are 2 (Paired/Novel Male) x 2 (1-hole/4-hole) factorial ANOVAs.

Overall, it was found that Fos/OT-IR was increased in the PVN in females conditioned using one-hole pacing divider and that were exposed to a paired male. Additionally, one-hole paced females had increased Fos/OT-IR in the mPOA and VMH when exposed to the paired male but not in females conditioned with the four-hole divider or those exposed to a novel male.

There was a significant interaction between male type and pacing on Fos/OT-IR in the PVN, $F(1, 16) = 11.87$, $p = .003$, $\eta^2_G = .43$. There were main effects of both pacing ($F(1,16) = 7.13$, $p = .02$, $\eta^2_G = .31$) and male exposure on Fos/OT-IR in the PVN ($F(1, 16) = 17.23$, $p = .0008$, , $\eta^2_G = .52$). Planned comparisons revealed that females in the one-hole pacing condition had significantly increased Fos/OT-IR when exposed to the paired male ($M = 17.06$, $SD = 4.11$) compared to females exposed to the novel male ($M = 5.51$, $SD = 3.53$), $t(16) = 5.37$, $p = .0002$, G = 2.72. Additionally, females in the one-hole condition exposed to the paired male had significant increased OT/Fos IR compared to four-hole condition females exposed to the paired male ($M = 7.76$, $SD = 3.74$), $t(16) = 4.325$, $p = .0016$, G = 2.13 (Fig 2A).

There was a significant interaction between pacing condition and male exposure on the number of Fos/OT-IR cells in the mPOA, $F(1, 16) = 5.73$, $p = .03$, $\eta^2_G = .26$. The ANOVA also revealed a significant main effect of pacing on the number of Fos/OT-IR neurons in the mPOA, $F(1, 16) = 11.49$, $p = .004$, $\eta^2_G = .48$. Planned comparisons revealed that females conditioned with the one-hole divider exposed to the paired male displayed significantly increased Fos/OT-IR cells ($M = 19.14$, $SD = 6.70$) compared to females conditioned with the four-hole pacing divider and exposed to a paired male ($M = 6.22$, $SD = 3.09$), $t(16) = 4.09$, $p = .0034$, $G = 2.24$ (Fig 2D).

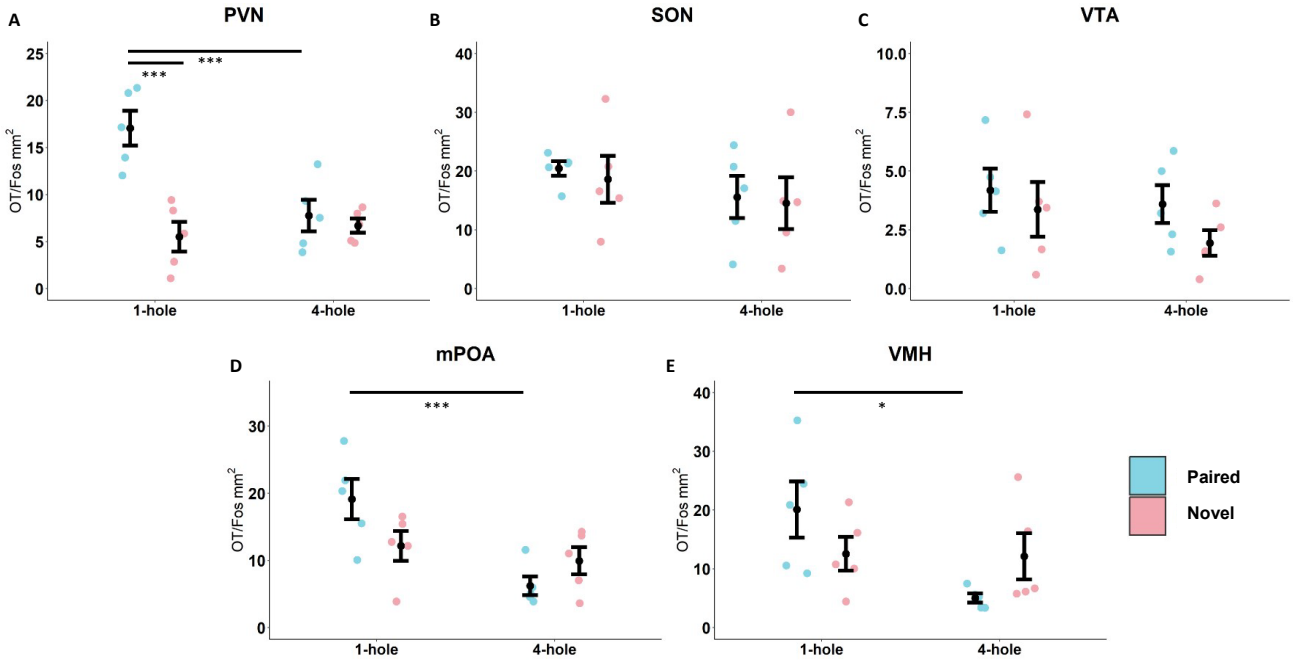


Figure 2: Displays individual data points of co-labelled Fos/OT IR cell per mm² across groups. Black dots represent means and black bars indicate standard error of the mean (n = 5). A) Fos/OT IR in the PVN was highest in females that were exposed to the paired male and conditioned with the one-hole pacing divider. One-hole conditioned females exposed to the paired male had significantly more Fos/OT IR compared to one-hole females exposed to a novel male, and four-hole conditioned females exposed to paired males. B) No differences in Fos/OT IR were found in the SON. C) Exposure to the paired male in one-hole paced females did not induce more Fos/OT IR in the VTA. D) In the mPOA females that paced with the one-hole had significantly more Fos/OT IR than females that paced with the four-hole divider. Additionally, Fos/OT IR was significantly greater in the one-hole females exposed to the paired male compared to four-hole females. E) In the VMH, females in one-hole condition exposed to the paired male showed significantly greater Fos/OT IR compared to four-hole conditioned females exposed to the paired males. * $p < .05$, ** $p < .01$, *** $p < .005$

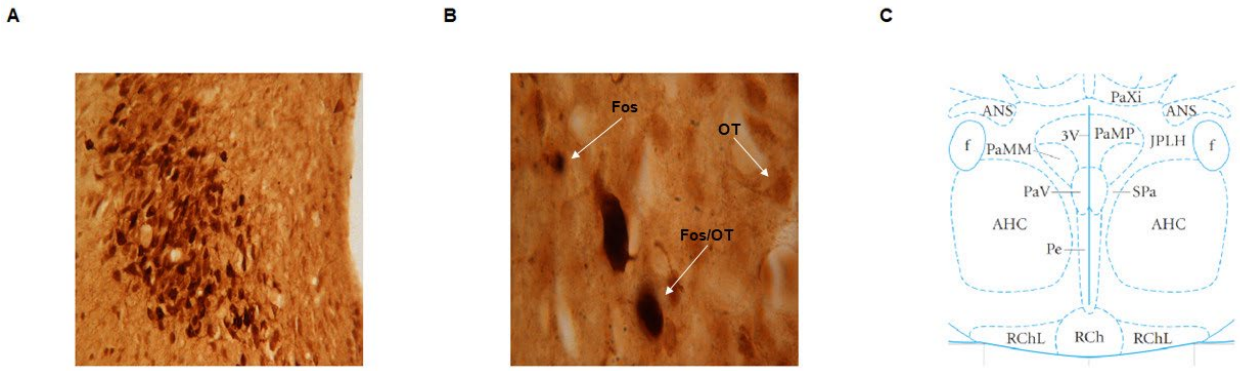


Figure 3. Photomicrographs demonstrating Fos/OT-IR in the PVN. A) A photomicrograph (x20) of the PVN. B) Demonstrates a Fos IR cell, identified by a round black stain. Fos/OT-IR is demonstrated by the round black stain within a dark brown cytoplasmic stain. OT-IR without Fos is shown as a dark brown stain with no black stain. C) A plate from the Paxinos and Watson brain atlas demonstrating one area in which the photomicrogrpahs were captured**.**

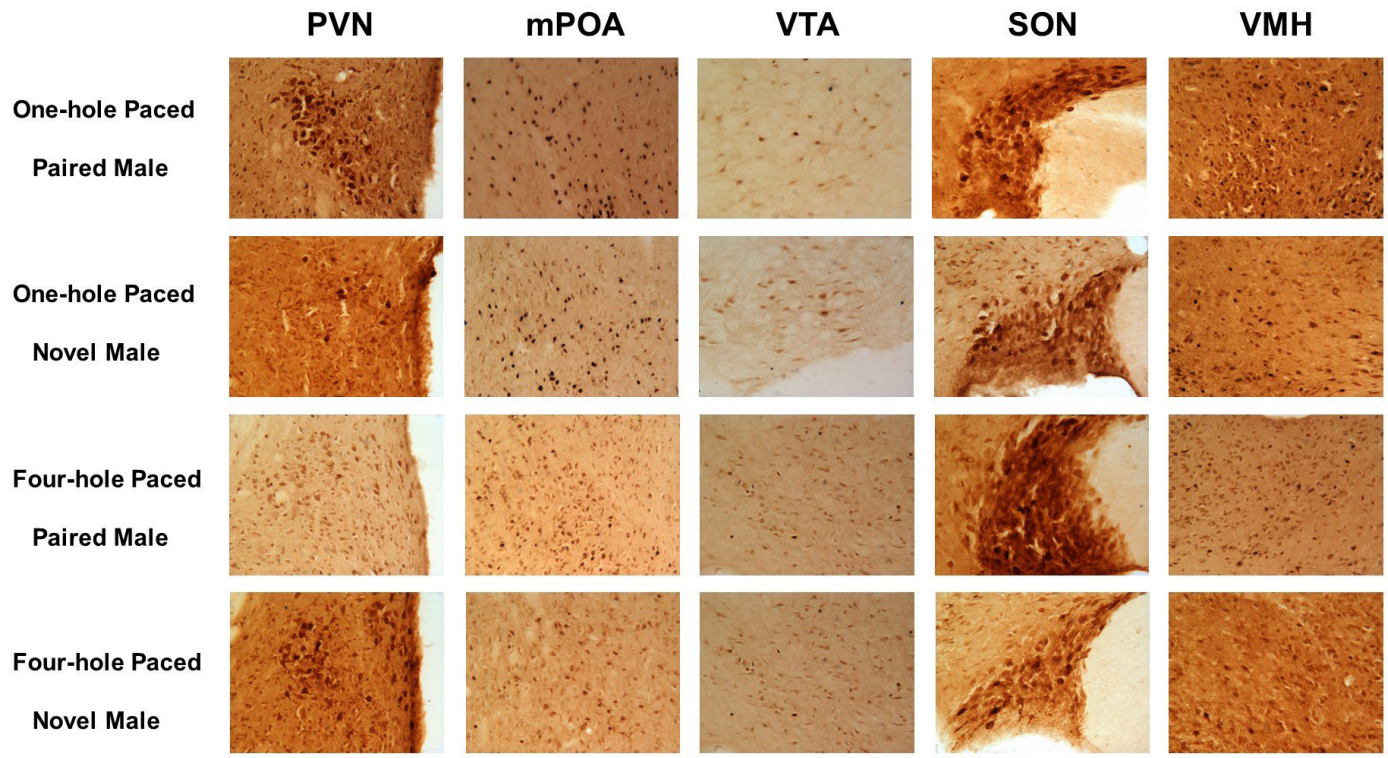


Figure 4. Displays representative photomicrographs of Fos/OT-IR in each brain region across all experimental conditions.

There was a significant interaction between pacing condition and male exposure on Fos/OT-IR cells within the VMH, $F(1, 16) = 4.55$, $p = 0.048$, $\eta^2_G = .22$. There was a significant main effect of pacing on Fos/OT-IR in the VMH, $F(1, 16) = 5.09$, $p = .038$, $\eta^2_G = .24$. The planned comparisons revealed that there were significantly more Fos/OT-IR cells in the VMH in one-hole conditioned females exposed to the paired male ($M = 20.09$, $SD = 10.71$) compared to females in the four-hole condition exposed to the paired male ($M = 5.03$, $SD = 1.71$), $t(16) = 3.104$, $p = .027$, $G = 1.77$ (Fig 2E). No other significant effects on OT/Fos IR cell densities were found in the VTA and SON (Figs 2B and 2C). See Fig 4 for representative photomicrographs of each brain region across all comparison groups.

4. Discussion

The present study was designed to examine whether female rats could be sexually conditioned to prefer one male of the same strain, relative to another male, without the addition of odors or somatosensory cues as conditioned stimuli. Female rats made preferential and selective sexual behaviors toward an individual male rat of the same strain associated with their experience of pacing conditions during conditioning trials. It was found that in the one-hole pacing condition, females were significantly more likely to choose the paired male for their first and subsequent ejaculations compared to the novel male. Females in the four-hole pacing condition did not display such a preference. Furthermore, there was a significant increase in Fos IR within OT IR neurons in the PVN, the mPOA, and the VMH, of females with a conditioned preference for the paired male relative to females in the other conditioning groups. Taken together, these data add to previous studies (e.g. Holley et al., 2015) suggesting that under certain pacing conditions (i.e. with a one-hole pacing divider) females can be conditioned to prefer an unscented but familiar individual male over another unscented novel male. Furthermore, the presentation of the preferred male activates significantly more OT neurons than does the presentation of a novel male.

Females that paced with the four-hole divider during conditioning trials did not form a preference for the pacing-associated male. This is an interesting finding given that previous studies have conditioned preferential and selective sexual behaviors with the four-hole divider (Coria-Avila et al., 2005, 2006; Holley et al., 2014, 2015). Moreover, the four-hole pacing condition was associated with less Fos/OT IR in the mPOA irrespective of the male to which females were exposed. Males used in the current study had prior sexual experience and were sexually vigorous during conditioning trials. However, Ismail et al. (2009) suggest that

the return latencies to the male's compartment are typically longer than with one-hole dividers compare to four-hole dividers (Ismail et al., 2009). Because a male can block a female's return to the other side of the pacing chamber when a one-hole divider is used, females can more easily pace with the four-hole divider. However, it appears that females pacing with the one-hole divider compensate by increasing the interval between intromissions. This resembles the proposal by McClintock and Adler (1978) that females regulate the number and timing of intromissions to maximise the likelihood of pregnancy.

Longer intervals between penile intromissions prior to ejaculation facilitates pregnancy (Edmonds et al., 1972). Both paced copulation and paced experimenter-administered artificial vaginocervical stimulation (VCS) or clitoral stimulation (CLS) are rewarding, as indicated by the induction of a conditioned place preference (Jenkins & Becker, 2003; Parada et al., 2010; Paredes & Alonso, 1997). Furthermore, paced copulation and both VCS and CLS induce a progestational state that facilitates successful pregnancy and hastens estrous termination (Cibrian-Llanderal et al., 2010; Erskine et al., 1989; Georgescu et al., 2012; Lehmann & Erskine, 2004; Pfaus et al., 2000). The rewarding and reproductive effects of paced copulation are thus inextricably linked. In the present study, we demonstrated that female rats can be conditioned to prefer an individual male rat paired with one-hole divider pacing. The reproductive and rewarding effects of paced copulation with the one-hole divider may drive the observed mate preference. Thus, Pavlovian preferences may depend not just on the reinforcing aspects of pacing, but also its pro-reproductive effects.

In semi-naturalistic and other environments, female rats pace by running away from males, imposing a distance of centimetres or even meters between themselves and a male, exiting into, and then returning from, burrow systems, or jumping on rocks or other obstacles that the male cannot get to them from (McClintock, 1984; McClintock & Adler, 1978; Pfaus et al., 1999). Even though pacing in the current study is non-naturalistic, the pacing conditions we imposed allowed females to control their interaction with a male. This ability serves the same function as pacing in naturalistic environments. In naturalistic mating encounters, females act in ways that increase the likelihood of being impregnated by a dominant male (McClintock et al., 1982). This requires the ability to recognise males that are dominant or subordinate and to adapt sexual behaviors toward dominant males. As dominance in male rats is related to the age of the male (Macdonald et al., 1995), female rats may use a flexible strategy for mate choice that is shaped by proximal factors. The data from the current study suggest that prior rewarding copulation with a specific male may be another proximal

factor that shapes female mate choice. In naturalistic environments, the copulatory patterns of specific males may also differ in reward value. The current study suggests Pavlovian associations formed during mating for different patterns of copulation may guide future mate choice.

We demonstrated that the pacing conditions during a female's early sexual experiences are an important determinant of whether it exhibits a future mate preference. Previous partner conditioning studies have given females copulatory experience with scented males in a unilevel pacing chamber with a four-hole divider versus unscented males in the same chamber with no divider (e.g.,Coria-Avila et al., 2005, 2006). Paired females (e.g., odor+pacing) displayed a preference to solicit and receive ejaculations from the scented versus unscented male. This conditioned sexual partner preference thus encompassed both appetitive and consummatory aspects of sexual behavior, and subsequent presentation of the odor alone activated significantly more Fos-labelled neurons in the PVN and SON (Coria-Avila & Pfaus, 2007). Another set of studies established that female rats display mate-guarding behaviors when given their first experiences of paced copulation in pacing chambers with a four-hole divider with a specific individual male. Females were subsequently presented with either their familiar male or a novel male along with a sexually receptive female conspecific in a large open field (e.g.,Holley et al., 2014, 2015). Paired females presented with the familiar male displayed mate guarding behaviors, whereas females presented with a novel male, and unpaired females (for whom the male was not associated with pacing), did not display these behaviors. As in the present study, paired females presented with their familiar male had increased numbers of double labelled Fos/OT neurons in the PVN and SON relative to unpaired females.

These data suggest that the intensity of pacing paired with various external cues forms a hierarchy of reward value resulting in the display of preference behaviors. First, only the one-hole pacing experience induced a preference to receive ejaculations from the unscented familiar male. This is reminiscent of the results of Ismail et al. (2009) who found that the formation of a conditioned ejaculatory preference in male rats for a familiar, almond-scented female occurred only if their early copulatory experiences were in unilevel pacing chambers with a one-hole, but not four-hole, divider. Second, four-hole pacing conditions are sufficient, relative to no pacing (removal of the divider), to induce a either preference for a male scented with a neutral odor, such as almond or lemon, or mate guarding of an unscented male. Finally, although pacing is rewarding to female rats as assessed by the induction of a conditioned place preference (Jenkins & Becker,

2003; Paredes & Alonso, 1997; Paredes & Vazquez, 1999), a place preference can be conditioned in female rats with non-paced copulation (Meerts & Clark, 2007) so long as a sufficient number of intromissions and ejaculations is delivered by the same male.

Within the framework of a hierarchy, it is possible that different types of rewarding stimulation come together to induce a place and/or partner preference. Artificial VCS or CLS activates Fos in regions of the mPOA and medial amygdala, respectively, but not in the PVN or SON (Parada et al., 2010; Pfaus et al., 1993). In contrast, nonpaced copulation with a male activated low amounts of Fos in the PVN, but not in the SON, whereas paced copulation in a bilevel chamber activated Fos moderately in OT neurons in the PVN and to a small but significant extent in the SON (Flanagan et al., 1993; Pfaus & Heeb, 1997). Paced copulation with scented males with the four-hole divider produced a partner preference to solicit and receive ejaculations selectively from the scented male. This condition also activated Fos to a much larger extent in both the PVN and SON, and such activation could be induced by presentation of the conditioned odor alone following conditioning (Coria-Avila & Pfaus, 2007). Although a four-hole pacing chamber is sufficient to induce conditioned mate-guarding for an unscented familiar male, in the present study only the one-hole divider was sufficient to induce a preference to receive ejaculations.

Numerous studies across different species have suggested that central OT transmission is involved in the formation of pair bonding and affiliative behaviors (Cushing & Carter, 2000; Holley et al., 2015; Insel & Shapiro, 1992; Liu & Wang, 2003; Ross, Cole, et al., 2009). In rats, oxytocin facilitates lordosis when infused into the mPOA (Caldwell et al., 1989). Furthermore, the ovarian steroids estradiol and progesterone, which are high during periods of sexual receptivity in the female rat, regulate the density of oxytocin receptors (OTRs) in the VMH and the mPOA (Caldwell et al., 1994; Coirini et al., 1991; Schumacher et al., 1989). In the current study, sexual receptivity during conditioning, partner preference testing, and partner exposure for Fos induction, was induced with estradiol benzoate and progesterone. Thus, there should have been induced a high density of OTRs in both areas. If preferred partners paired with paced copulation induces increased OT transmission in both areas, this may facilitate lordosis in the presence of that partner which would have the effect of facilitating the rate at which the male intromits and ejaculates. Neurons in the mPOA of female rodents are also highly responsive to male cues (McHenry et al., 2017). Increased activation of the mPOA

from the OT-projections of the PVN may drive sexual motivation toward the one-hole pacing-associated male (Xiao et al., 2017).

Although female rats have been described as promiscuous in their choice of copulatory partners, McClintock et al., (1982) demonstrated that in semi-natural environments females exercise selectivity during mating. Females compete more to receive the ejaculation of dominant males, and dominant females typically are more successful than subordinate females in this competition. In contrast, prairie voles are described as socially and parentally "monogamous", with both males and females showing a preference for the first individual they have sex with (Getz et al., 1981). Yet, many litters are sired by multiple males (N. G. Solomon et al., 2004). Indeed, whether pair-bonded prairie voles demonstrate similar sexual partner preferences to conditioned rats has not yet been studied. Given that female rats can show preferences for odor, strain, and individual conspecifics, the use of a Pavlovian conditioning procedure shows that more animals than would be predicted to be dominant made a selective mate choice. This suggests that females can use both either promiscuous or monogamous-like mating strategies, and the strategy used is modulated by social hierarchy and/or copulatory experience as we have shown (Coria-Avila et al., 2005, 2006; Holley et al., 2014, 2015). Females showing a Coolidge effect demonstrate an innate preference for novel partners (Ventura-Aquino et al., 2016). Pavlovian partner conditioning demonstrates that this innate preference is malleable by experience with copulatory reward.

Preface to Chapter 2

It was shown in Chapter One female rats can be conditioned to selectively receive the first, and more overall ejaculations from a familiar male. The formation of this preference depended on the pacing conditions experience by females during sexual conditioning. Specifically, pacing with the one-hole divider, associated with elongated pacing intervals (Ismail et al., 2009), resulted in a conditioned preference. As longer pacing intervals are also known to facilitate reproductive responses, i.e, a progestational state (Adler, 1969; Edmonds et al., 1972; Erskine et al., 1989), following copulation. This suggests an important link between sexual reward, required for preference formation, and enhanced reproductive responses. The ejaculatory preference shown by females was also accompanied by increased Fos IR in the OT IR neurons in the mPOA, VMH, and PVN. In this experiment, Fos protein, an indirect maker of neuronal activation (Pfaus & Heeb, 1997), was induced by noncontact exposure to a familiar male. Consequently, this pattern of activation gives some insight into the neurobiology involved in the display of preference. OT containing neurons in the mPOA, VMH, and PVN, are selectively activated by preferred males, this activation is likely important for incentive sexual motivation to be directed toward a specific male. However, apart from the contribution of pacing intervals to preference acquisition, Chapter One did not specifically examine the neurobiology underpinning preference formation. Therefore, Chapter Two addresses the role of OT transmission in preference acquisition, testing whether systemic OT facilitates the formation of a conditioned mate preference. In addition, Chapter Two examines whether peripherally injected OT enhances conditioned preference acquisition by its facilitation of an early reproductive response that follows VCS, i.e., estrous termination, a marker of sexual satiety.

Chapter 2

Peripheral oxytocin signaling promotes estrous termination and conditioned mate preferences female Long-Evans rats

Conall E. Mac Cionnaith[1*], Eamonn L. Gomez-Perales[1], Hanya Ismail[1], Jesse Lacasse[1], James G. Pfaus[2,3],

Wayne G. Brake[1]

[1]Center for Studies in Behavioral Neurobiology
Department of Psychology, Concordia University
Montréal, QC, Canada

[2]Department of Psychology and Life Sciences
Faculty of Humanities, Charles University
Prague, Czech Republic

[3]Division of Sexual Neuroscience
Center for Sexual Health and Intervention
Czech National Institute of Mental Health
Klecany, Czech Republic

Abstract

Vaginocervical stimulation (VCS) facilitates pregnancy in rats. Female rats form both conditioned place and mate preferences when they can pace, i.e., control the rate and timing of VCS, during copulation. Oxytocin (OT) is released centrally and peripherally during paced copulation and OT alone can induce a progestational state that is needed for pregnancy. However, this effect is blocked with the transection of the pelvic nerve, suggesting peripheral OT receptors (OTR) regulate post-copulatory reproductive responses. We show here that the expression of peripheral OTRs changes when females are sexually receptive and that antagonism of both peripheral and central OTRs reduces OT neuronal activation following VCS. We then demonstrate that systemic OT mimics the effects of VCS on inducing the copulatory quiescence that follows VCS, i.e., estrous termination. Estrous termination induced by VCS was completely reversed by a peripheral OTR antagonist, but not by a centrally infused antagonist. The formation of a conditioned mate preference is also facilitated by systemic OT and reversed by peripheral OTR antagonism. These results suggest that peripheral OT induces a state of sexual satiety, via estrous termination, and consequently, part of the rewarding effects of paced VCS. As such, OT may coordinate successful pregnancy with experientially learned mating preferences as a proximal means to guide mate choice.

Introduction

Sexual behavior is critical for the reproductive success of all mammals. A biological or genetic ultimate causality is thought to compel individuals to choose potential mates that have high fitness value (e.g., body types ideal for reproduction and/or physical strength; Buss, 1987) Ultimate evolutionary causes also give rise to species-specific patterns of copulation that guide successful reproduction, such as non-monogamous and monogamous mating strategies. On the other hand, there is a proximate causality that is more flexible and related to experientially-mediated processes involved in the perception of sexual reward and its association to a partner or cue (e.g., conditioned sexual preferences, paraphilias; Georgiadis et al., 2012). Although both can be considered different levels of analysis, there is still an unresolved debate concerning the relative degree to which they exert control on sexual behavior and mate choice. Indeed, both make vastly different predictions about the biological causes of nonreproductive sexual behaviors and their influence on sexual preferences of females and males.

The mating strategy of female rats is described as non-monogamous and highly competitive in group mating circumstances in the wild (McClintock, 1984). Despite their seemingly indiscriminate mating preferences, female rats can be conditioned in laboratory settings to selectively solicit and receive ejaculations exclusively from familiar males previously associated with paced copulation (Coria-Avila et al., 2005; Mac Cionnaith et al., 2020). When female rats are in heat, they solicit males by making a headwise gesture followed by a runaway which entices males to chase them (McClintock, 1984). The runaway stops when females display lordosis, the reflexive arching of the back in response to tactile flank stimulation that allows males to mount with penile intromissions. After several mounts with intromissions, a male ejaculates and females then enter a period of sexual quiescence that allows for sperm transport (Pfaus et al., 2015). The pattern of solicitation, runaway, lordosis, and post-ejaculatory quiescence allows females to control or pace, the initiation, and rate of copulatory stimulation (McClintock & Anisko, 1982). This consists of both clitoral stimulation (CLS) and vaginocervical stimulation (VCS). Allowing females to pace copulatory contact induces a sexual reward state that supports a conditioned place preference equivalent to that observed after morphine (Paredes & Alonso, 1997). Paced copulation also results in the formation of a conditioned mate preference for a familiar male and enhances the probability of pregnancy after copulation (Erskine, 1985; Mac Cionnaith et al., 2020). Female rats can also recognize prior mates and engage in mate guarding behavior with

a familiar male associated with paced copulation (Holley et al., 2014; Mac Cionnaith et al., 2020). Such mate preferences are reminiscent of the stable, long-term mating bonds observed in monogamous female prairie voles, and oxytocin (OT) plays an important regulatory role in this preference formation (Cushing & Carter, 2000; Insel & Shapiro, 1992).

In the non-monogamous female rat, OT regulates genital arousal (Gelez et al., 2010) and increases in the paraventricular nucleus of the hypothalamus (PVN; Nyuyki et al., 2011), the spinal cord, and in plasma after VCS (Sansone et al., 2002). OT is thought to initiate the progestational prolactin surge that follows copulation (Arey & Freeman, 1992). However, OT levels in plasma and in the spinal cord are uncorrelated (Sansone et al., 2002) and the increase of spinal OT after VCS occurs even in hypophysectomized females. This suggests that the increase of OT in the spinal cord after VCS is due to central release and not the diffusion of peripherally released OT into the spinal cord. Gelez and colleagues (2010) have shown that the clitoris and vagina project to OT neurons in the PVN and the medial preoptic area (mPOA). Both areas show increased neuronal activation after VCS (Flanagan et al., 1993; Pfaus et al., 2006). While one systemic injection of OT is sufficient to induce the progestational prolactin surge, transection of the pelvic nerve that innervates the vagina and cervix blocks this OT-induced prolactin surge and also neuronal activation after VCS (Helena et al., 2011; Pfaus et al., 2006). This suggests that systemic OT acts primarily at receptors in the vagina and cervix and this activation is relayed to the CNS via the pelvic nerve. Therefore, peripheral OT receptors (OTRs) are likely necessary for the neuroendocrine and behavioral responses to VCS, suggesting an important role for peripherally released OT during and after copulation.

Peripheral administration of OT increases central OT in the amygdala and hippocampus (Neumann et al., 2013), and Fos protein expression in the PVN, the supraoptic nucleus (SON) of the hypothalamus, and the amygdala (Hicks et al., 2016). That a peripheral injection of OT would induce Fos protein centrally is important because OT is too large and too polar a molecule to cross the blood brain barrier without the aid of a transporter. Although there is emerging evidence of active transport into the brain by RAGE proteins, the estimated concentration of transported OT is relatively low (Yamamoto et al., 2019). This indicates that the effects of systemically administered OT on the brain could be due to central feedback from its actions on OTRs in the vagina and cervix. The series of experiments reported here describe how OT signaling in the brain and, primarily, in the periphery, coordinates behavioral progestational responses to VCS and

experientially-acquired mate preferences in the female rat. Altogether, this potentiates the ability of female rats to be impregnated by preferred males, suggesting OT links an ultimate evolutionary goal, successful reproduction, with proximal reward-based preferences.

During the late proestrus stage of a rat's estrous cycle, circulating levels of endogenous estrogens peak and are followed by a surge in progesterone (Butcher et al., 1974). At the same time, luteinizing hormone (LH) concentrations in blood peak, stimulated in part by rising circulating OT (Robinson & Evans, 1990), to help synchronize ovulation with sexual receptivity. When pregnancy does not occur the increased circulating OT facilitates the degradation of the corpora lutea (McCracken et al., 1999), demonstrating that OT regulates aspects of reproductive physiology. Because females receive CLS and VCS during copulation (Pfaff et al., 1977; Pfaus et al., 2015), we hypothesized that these tissues would contain OTRs. Consequently, these tissues may be an important site of action for the behavioural effects of peripherally-injected OT.

Results

We demonstrate here that OTRs are expressed in the genitalia when ovariectomized females are hormonally primed for sexual receptivity, i.e., injected subcutaneously with estradiol benzoate and progesterone (EB+P). However, the abundance of OTR immunoreactivity (IR) depends on hormone priming and the type of tissue. In the cervix, sexually receptive females (EB+P) and vehicle injected females (oil + oil) do not differ and both show significantly less OTR IR than females injected with estradiol benzoate (EB+oil) alone (Figure 1K).

Sexually primed females (EB+P) had the least OTR IR in both the external and internal portions of the clitoris (Figure 1G and Supp Figure 2G). There was, however, no significant effect of hormone treatment on OTR IR in the vaginal canal (Figure 1H), dorsal lateral septum (Supp Fig 2A), the medial amygdala (Supp Fig 2C), the SON (Supp Fig 2D), the ventral tegmental area (Supp Fig 2E), and the dorsomedial region of the VMH (VMHdm; Supp Fig 2F). In the mPOA (Figure 1F) and the PVN (Figure 1G), vehicle injected females had significantly greater OTR IR than both females injected with EB and EB+P. Only in the VMHvl (Figure 1H) and the medial lateral septum (Supp Fig 2B) was OTR IR significantly greater in EB+P-injected females compared to females injected with EB alone. This shows an additive effect of P with EB to OTR IR in these regions, as has been previously reported (Schumacher et al., 1990). Our results broadly corroborate the

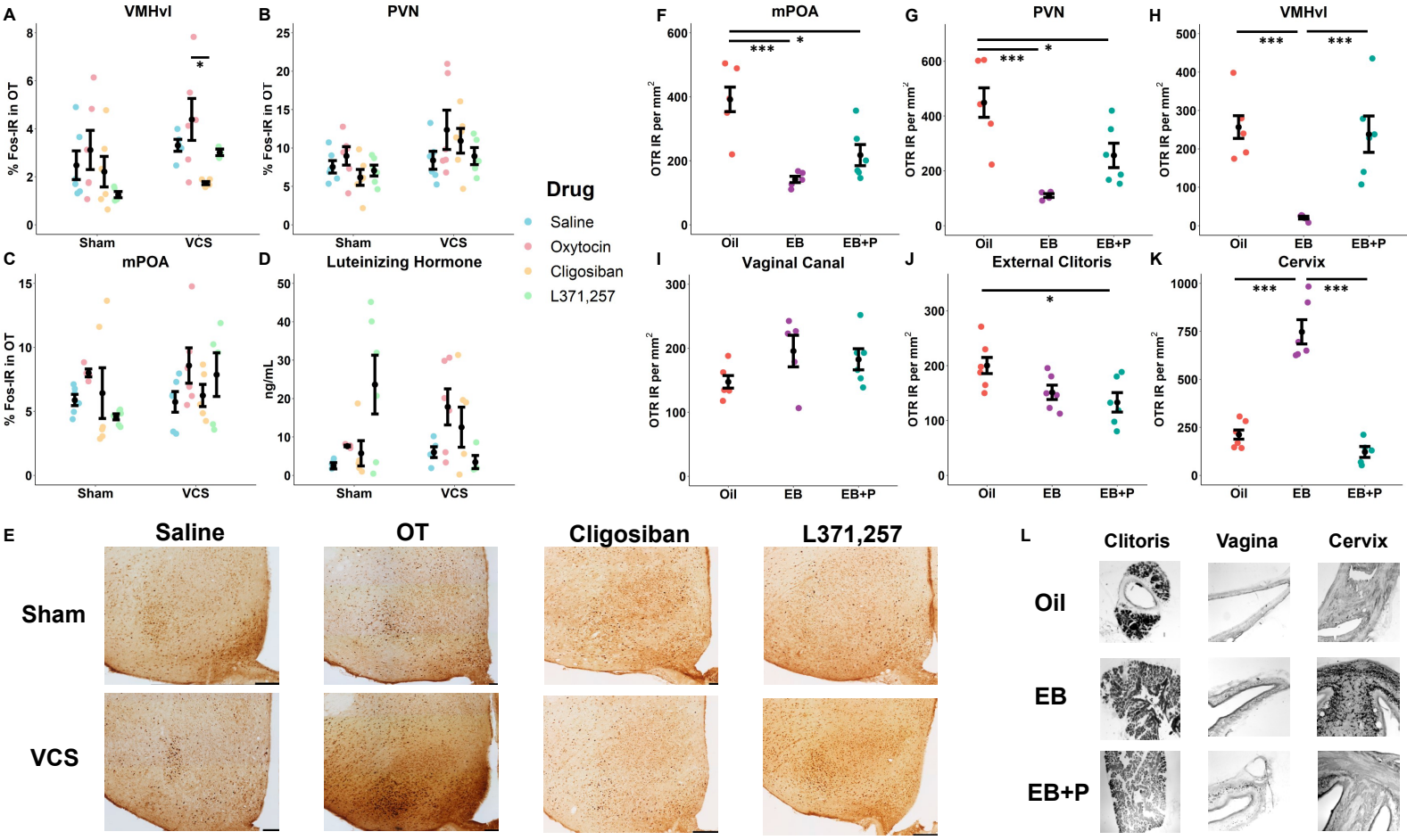


Figure 1. The effect of OT and OTR antagonists, cligosiban or L371,257, on the percentage of Fos IR in OT-IR positive neurons in the VMHvl (1A), PVN (1B), mPOA (1C), and LH in serum (1D) following either VCS or sham stimulation. Representative photomicrographs of the VMH per group shown in 1E. Also shown are the effect of exogenous steroid hormone priming on OTR IR in the mPOA (1F), PVN (1G), VMHvl (1H), vaginal canal (1I), external glans of the clitoris (1J), and cervix (1K) with representative photomicrographs of vaginal, clitoral, and cervical tissues (1L). Data shown are means ± SEM following removal of outliers (beyond 1st/3rd quartile ± 1.5 x IQR). 1A) Females injected with OT and given VCS showed significantly increased Fos IR in OT-IR cells compared to those injected with cligosiban. 1F) OTR IR was significantly higher in females injected with vehicle compared to EB+P and to EB alone. 1G) In the PVN, OTR IR was significantly greater in vehicle controls compared to EB+P females and EB alone. 1H) Females injected with EB had significantly less OTR IR in the VMHvl relative to vehicle controls and to females injected with EB+P. 1J) Females injected with EB+P had significantly less OTR IR than vehicle controls.1K) Females injected with EB had significantly greater OTR IR in the cervix than those injected with EB+P and vehicle controls. (n = 4-6 per group). * < .05; *** <.005

findings of others showing that while estradiol increases the abundance of OTR mRNA, additional priming with P decreases OTR mRNA and receptor binding (Larcher et al., 1995; Tribollet et al., 1990). In the VMHvl the increase we report in OTR IR is also a well-established finding (Schumacher et al., 1989). EB+P increases the binding ability of OTR in the VMHvl, relative to EB alone, where OTR activation facilitates lordosis (Schumacher et al., 1990).

Next, we tested whether peripheral OTRs are necessary for neuronal and neuroendocrine activation following VCS by peripherally injecting females with either OT, an OTR antagonist that can cross the blood brain barrier, cligosiban, or one that does not, L371,257. This allowed us to pharmacologically distinguish some aspects of peripheral and central OT signaling. VCS increases Fos-protein IR in the mPOA, PVN, and VMHvl and increases serum LH (Flanagan et al., 1993; Pfaus et al., 2006; Spies & Niswender, 1971). Therefore, we examined Fos protein expression, an indirect marker of neuronal activation (Pfaus & Heeb, 1997) within OT-containing neurons in the mPOA, VMH, and the PVN. We also examined LH and prolactin in serum following VCS combined with an injection of OT or the OTR antagonists.

OT alone without VCS induces a two-fold (Figure 1D), yet not statistically significant, increase in serum LH compared to vehicle, and also induces a greater increase in LH with VCS. Prolactin in serum was undetectable in all females but one, regardless of VCS or drug (Supp Fig 2H). This finding was not surprising as the surge in prolactin is circadian period dependent and occurs several hours after VCS (Gunnet & Freeman, 1983). While VCS increased overall Fos IR in OT-IR cells in the PVN, this was not affected by OTR agonist or antagonist administration. We found no differences in Fos IR in OT-IR cells in the VMHdm (Supp Fig 2I). When females received VCS, Fos IR in OT-IR cells in the VMHvl are most abundant in females injected with OT, and are less abundant following administration of saline, L371,257, or cligosiban (Figure 1A). This begs the question of how an antagonist that only acts peripherally would have a similar effect as one that acts both peripherally and centrally. While OTR activation in the VMHvl is required for progestational responses following copulation (Northrop & Erskine, 2008), these findings also suggest an important role for peripheral OTRs in initiating neuronal responses linked to progestation. If so, peripheral OTR activation should be necessary for responses associated with progestation following VCS.

When a female rat becomes sexually receptive, the duration of receptivity is determined not only by whether a female copulates, but also by the amount of and the duration of the interval between VCSs (Pfaus et al., 2000). Sexual stimulation inhibits later sexual receptivity through neuroendocrine changes and specific patterns of neuronal activation, such as nocturnal surges in prolactin and progesterone (Erskine, 1995). Behaviorally, female rats given VCS in a paced manner are significantly less likely to be sexually receptive 12-hours later compared to unstimulated females(Pfaus et al., 2000). Paced artificial VCS applied in this manner induces pseudopregnancy and the prolactin surges associated with progestational states (Erskine, 1995). Therefore, estrous termination brought on by VCS is a behavioral measure of the progestational effects of copulation.

We show that systemic OT mimics the behavioral effects of VCS as one injection is sufficient to induce estrous termination without any VCS. With no VCS, i.e., sham stimulation, females injected with OT 12 hours before an estrous termination test, are no longer sexually receptive. Specifically, they receive fewer intromissions (Figure 2C), show lower lordosis quotients (Figure 2E), and lower reflex magnitudes of lordosis (Figure 2F), and solicit significantly less (Figure 2D). Estrous termination occurs regardless of stimulation in females injected with OT, indicating that OT has effects similar to that of VCS, if not greater.

Estrous termination requires OTR activation as these effects are reversed by both the OTR antagonists, cligosiban and L371,257. Females injected with cligosiban or L371,257 and given VCS were as sexually proceptive and receptive compared to when they were given sham stimulation (Figure 2C-2F). These females did not terminate estrous after VCS. Females given VCS and injected with L371,257, were significantly more receptive and proceptive (Figures 2C-2F) relative to controls given VCS. Whereas females injected with cligosiban were similar to controls after VCS. This suggests that, while both cligosiban and L371,257 block the effects of VCS, L371,257 has a larger inhibitory effect on estrous termination. Though cligosiban enters the brain, we cannot exclude the possibility that its inhibition of estrous termination after VCS is primarily through peripheral receptors.

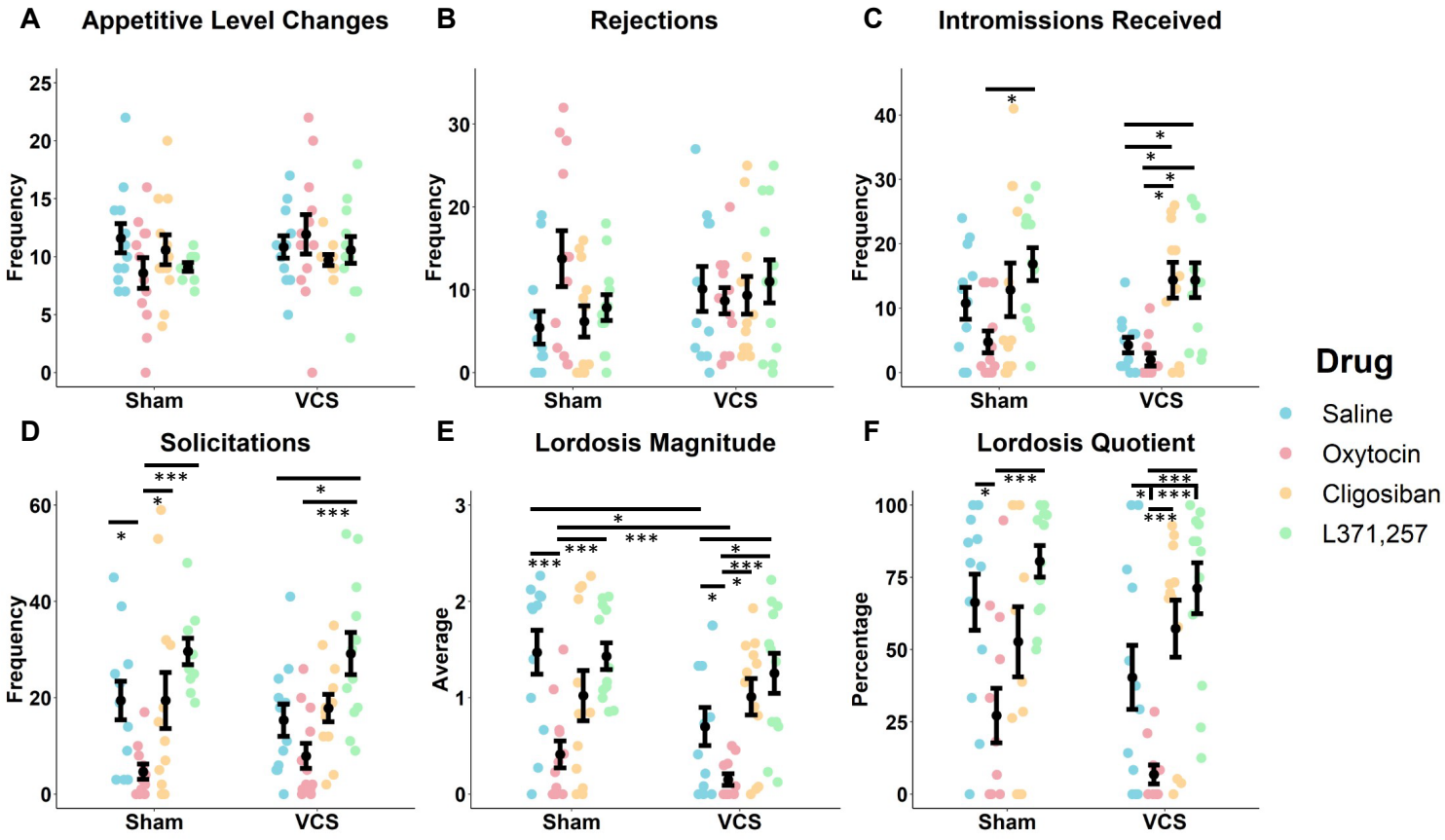


Figure 2. Proceptive (2A and 2D) and receptive sexual behaviours (2B, 2C, 2E, and 2F) during estrous termination tests. Both OTR antagonists, cligosiban and L371,257, attenuate estrous termination following VCS, compared to when females were given sham. Data shown are means ± SEM, after outliers (beyond 1st/3rd quartile ± 1.5 x IQR) were removed. Estimate marginal means were generated by a mixed ANOVA with drug and stimulation (VCS/sham) as factors in order to conduct four planned comparisons, and several Holm-adjusted post-hoc comparisons of interest. 1C) Females given VCS and injected with OT or saline, receive significantly fewer intromissions than those injected with the OTR antagonists. 1D) Relative to saline, females injected with OT solicited significantly less, despite only receiving sham stimulation. Relative to females given VCS and injected with saline, L371,257 solicited significantly more indicating the effects of VCS were reversed. 1E) Females injected with saline, have significantly lower lordosis magnitudes after VCS. The VCS-induced decrease in lordosis magnitude was reversed by L371,257. OT alone, without any VCS, significantly reduced lordosis magnitudes relative to saline. 1F) Lordosis quotients were significantly reduced in females injected with OT with no VCS. The effects of VCS on reducing lordosis quotients were reversed by L371,257. (n = 12). * $p < .05$; *** $p < .005$

Therefore, we then examined whether OT acts centrally, peripherally or both, to terminate estrous following VCS. We demonstrate that peripheral OTR activation is not required when OT levels are artificially increased in the brain via intracerebroventricular infusion. An infusion of OT into the lateral ventricle induces estrous termination in females also injected with the peripheral OTR antagonist, L371,257. Females injected with L371,257, infused with OT, and given VCS, were significantly less receptive and proceptive than saline-infused controls (Figure 3C-3F). Once OT is increased in the brain, peripheral OTR antagonism no longer inhibits estrous termination. However, under natural circumstances, VCS precedes and induces an increase in central OT retaining an important role for these peripheral OTRs in estrous termination.

We also replicated our previous findings demonstrating that L371,257 attenuates estrous termination in females given VCS. Females infused with saline and injected with L371,257 were significantly more sexually receptive and proceptive, compared to vehicle controls (Figures 3C-3F). Surprisingly, a centrally-infused water-soluble OTR antagonist, OTA, does not attenuate estrous termination following VCS (Figure 3C-3F). In females infused with OTA prior to VCS, the peripheral OTR antagonist L371, 257 did not attenuate estrous termination either. This effect is opposite to the attenuation we have shown with L371,257 alone. Though this effect is counter intuitive, OTR antagonism may inhibit or facilitate estrous termination based on the site of infusion. For example, β-endorphin (β-END) infused into the lateral ventricle facilitates lordosis, but lordosis is inhibited when β-END is infused into the third ventricle (Gorzalka et al., 1997). As such antagonism of peripheral OTRs may inhibit estrous termination, whereas central antagonism may facilitate estrous termination. Taken together with the previous experiment, these results suggest that estrous termination is mediated by peripheral OTR activation during VCS by increasing central OT signaling. OT whether centrally infused or peripherally injected is thus sufficient to induce estrous termination.

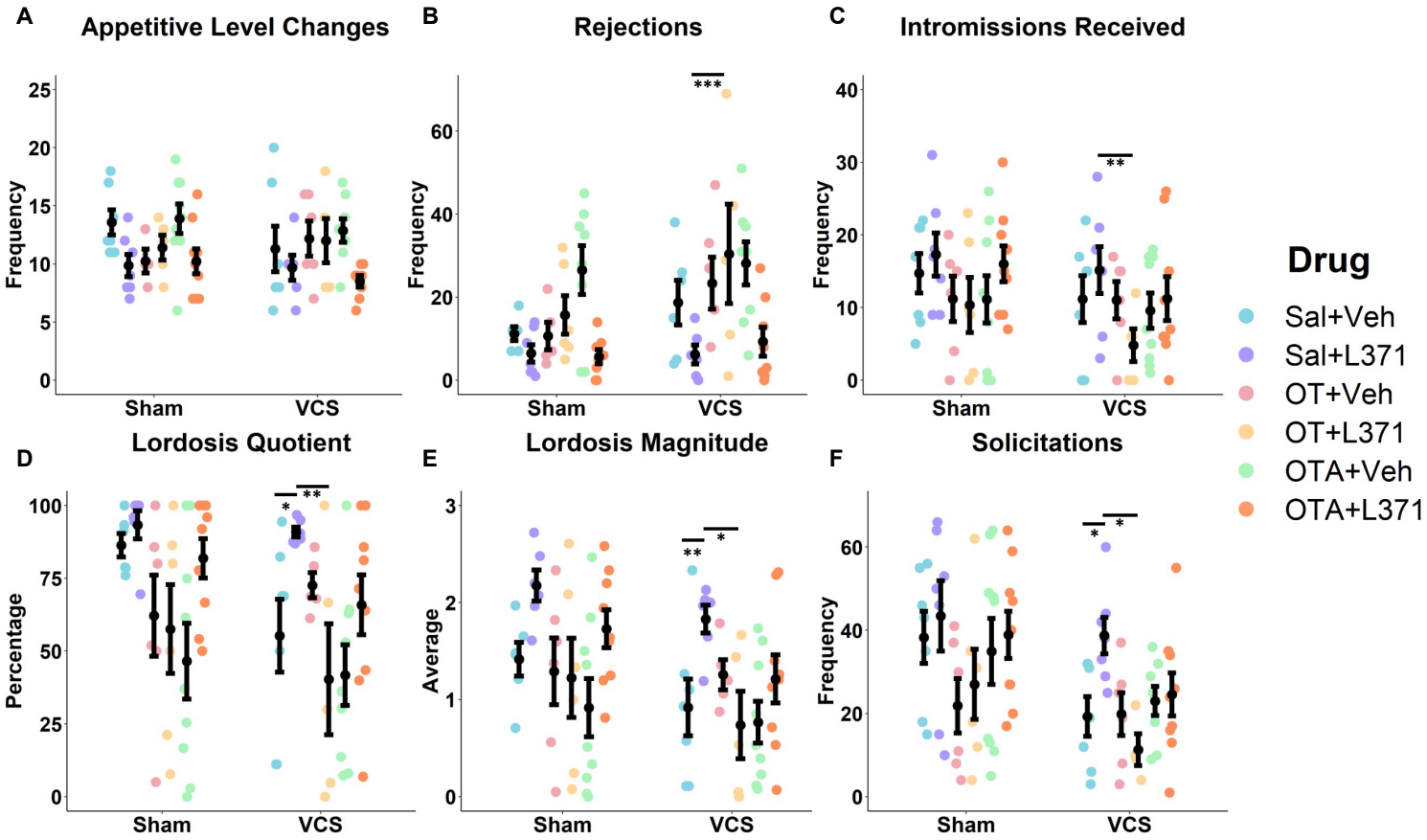


Figure 3. The effect of manipulating central and peripheral OT signalling during VCS on proceptive (3A and 3F) and receptive sexual behaviors (3B, 3C, 3D, and 3E) in estrous termination tests. Data shown are means ± SEM, after outliers (beyond 1st/3rd quartile ± 1.5 x IQR) were removed. To compared specific groups, estimated marginal means were generated using a linear mixed effects model with stimulation (VCS/Sham), infusion (saline/OT/OTA), and peripheral injection (L371,257/vehicle) as fixed factors. Subject was entered into the model as a random effect (random intercepts only). 3B) Females given VCS, injected with L371,257, and infused with OT made significantly more aggressive rejections of a male than those infused with saline. 3C) Females infused with OT, injected with L371,257 and given VCS received significantly fewer intromissions than females infused with saline. 3D) Lordosis quotients were significantly higher in saline-infused females injected with L371,257 compared to vehicle following VCS, and significantly reduced in females infused with OT. 3E) Lordosis magnitudes were significantly higher in saline-infused females injected with L371,257 following VCS. Females infused with OT and injected with L371,257 had significantly lower magnitudes. 3F) Solicitations were increased in females infused with saline and injected with L371,257 following VCS. An infusion of OT blocked the effect of L371,257 as females solicited significantly less after VCS. (n = 4-7) * $p < .05$; ** $p < .01$; *** $p < .005$

We then hypothesized that experientially-mediated mate preference is also driven by OT through its estrous terminating effects. If OTR signaling is required for the progestational behaviors after copulation, blocking peripheral OTRs should also inhibit the formation of a conditioned mate preference. Conversely, OT should facilitate the formation of a conditioned mate preference. In prairie voles, partner preference formation is facilitated by both central infusions or systemic injections of OT (Cushing & Carter, 2000; Williams et al., 1994) and is blocked by intracranial infusions of an OTR antagonist (Williams et al., 1994). Because mate preference is determined by a female's sexual motivation directed toward a specific partner previously associated with paced copulation, we consider conditioned mate preferences to be a measure of prior sexual reward. Under drug-free conditions, female rats do not form sexual preferences for familiar males after only one sexual experience (Coria-Avila et al., 2005). However, if systemic OT facilitates partner preference in voles, mate guarding in female rats (Holley et al., 2015), and terminates estrous in rats, we expected OT-injected females to display a conditioned mate preference after their one and only experience with paced copulation.

Indeed, we show here that a single, systemic injection of OT facilitates the formation of a conditioned mate preference after one paced copulation with an artificially scented male (Figure 4A and 4D). This shows that systemic OT facilitates the formation of mate preference in rats, similar to the induction of mate recognition and mate guarding by OT in female rats (Holley et al., 2015). A mate preference was evident in females given two different doses of OT, 20μg and 50μg. We also show that intraperitoneal injections of either OTR antagonist, cligosiban or L371,257, results in a preference to solicit a novel male (Figure 4B and 4E); in other words, an avoidance of a male previously associated with paced copulation under the influence of an OTR antagonist. This finding indicates that peripheral OT signaling is necessary and sufficient for the formation of conditioned mate preferences.

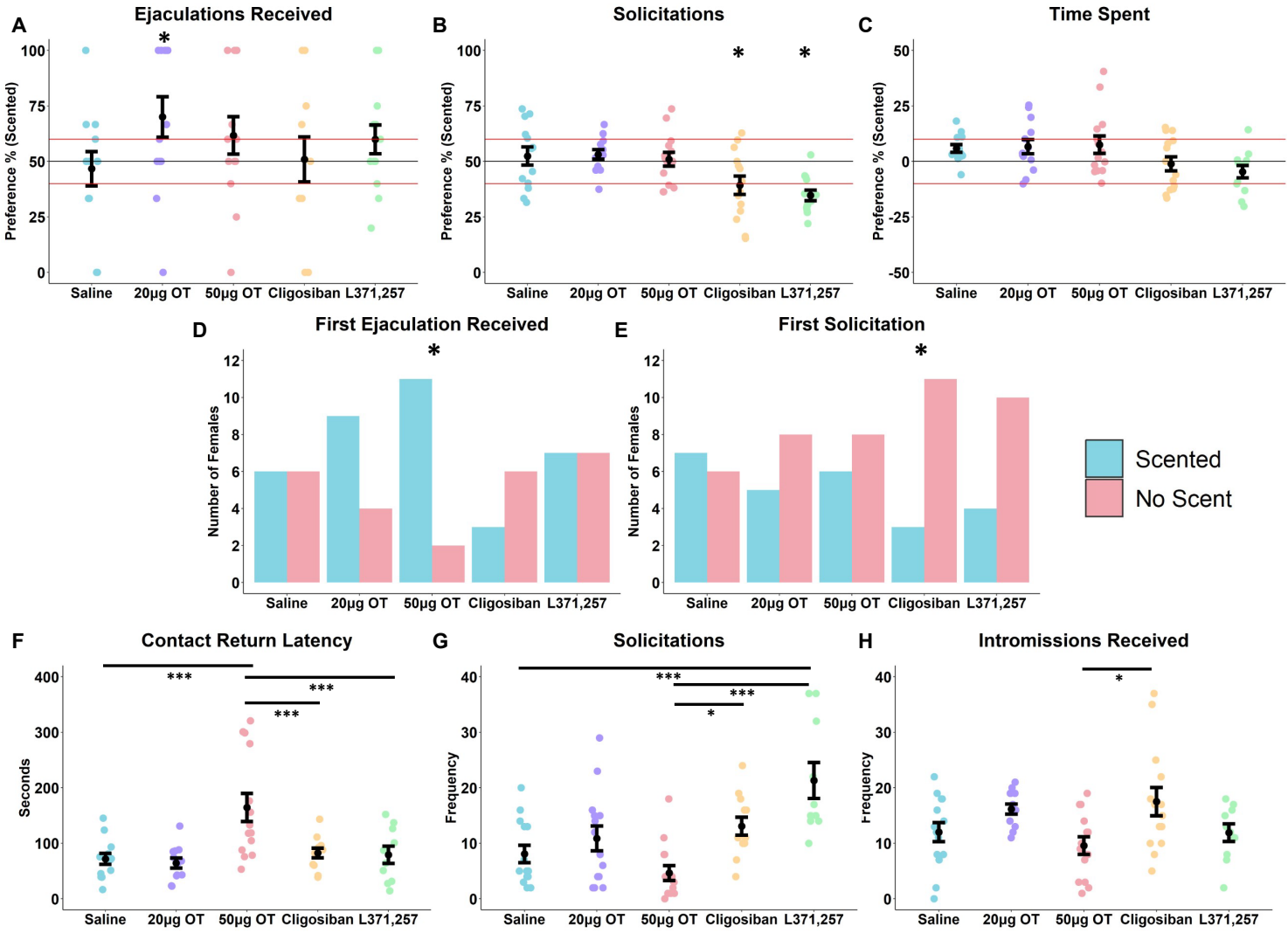


Figure 4. The effect of OT and OTR antagonists on the formation of conditioned mate preference (4A-4E; n = 13-14). The acute effects of OT and OTR antagonists on sexual behavior during preference formation conditions were tested with one-way ANOVAs followed by Holm-adjusted mean comparisons (4F-4H; n = 10-14). Mate preference ratios were calculated by dividing the number of ejaculations received or solicitations made toward a scented male by the total number of ejaculations received or solicitations made in a mate preference test (4A and 4B). Time preference was calculated was by dividing the amount of time a female spent in the roaming range of a scented male by the total duration of the trial (~1850s). Equivalence tests were used to determine whether a female displayed a significant preference toward a scented or unscented male. Equivalence bounds were set to ±10% around a score of no preference (see red lines). A two-tailed one-sample t-test tested whether there was a significant preference, and two one-tailed t-tests were conducted against the equivalence bounds were used to determine whether the mean preference ratio was equivalent to no preference. 4A) Females injected with 20µg OT significantly received proportionally more ejaculations from a scented male. 4B) Females injected with cligosiban and L371,257 proportionally solicited an unscented male significantly more often. 4C) Though females injected with saline spent significantly more time with a scented male their preference score was statistically equivalent to no preference based on the equivalence bounds. 4D) Females injected with 50µg OT preferentially received the first ejaculation of the trial from a scented male. 4E) Females injected with cligosiban were solicited an unscented male first significantly more often. 4F) In a unilevel pacing chamber, females injected with 50µg OT imposed significantly longer intervals before returning to a male after receiving an intromission, i.e., contact return latency. 4G) Females injected with L371,257 solicited significantly more than controls, whereas both L371,257 and cligosiban injected females solicited significantly more than females injected with 50µg OT. 4H) Females injected with cligosiban received significantly more intromissions than those injected with 50µg OT. * $p < .05$; *** $p < .005$

As the preference test was a drug free trial, we sought to examine whether the drugs acutely augment sexual behaviors during preference acquisition conditions. Females were injected systemically with either saline, 20µg OT, 50µg OT, cligosiban, or L371,257. They were then allowed to copulate in identical conditions as the paced conditioning trial used to induce a mate preference. During copulation, males provide both CLS and VCS via mounts with pelvic thrusts and vaginal intromissions (Pfaff et al., 1977),which readily induce mate and conditioned place preferences (Coria-Avila et al., 2005; Mac Cionnaith et al., 2020; Paredes & Alonso, 1997). Females acutely given 50µg OT received fewer intromissions than cligosiban-injected females. One would predict that more intromissions would positively predict a future mate preference. We found the opposite, that females injected with both the OTR antagonists avoided soliciting a male previously associated with paced copulation, suggesting that peripheral OTR activation is also necessary for sexual reward.

Females given 50µg OT pace with significantly longer intervals between intromissions (Figure 4F). During estrous termination females also impose longer pacing intervals (Pfaus et al., 2000). When females can pace, fewer intromissions are required to induce a progestational state (Erskine, 1995). Females solicit significantly more when given the OTR antagonists cligosiban or L371,257 (Figure 4G). Solicitations are typically positively associated with female sexual motivation (Pfaus et al., 2015). Given that females injected with OT solicited less, and imposed longer pacing intervals, yet demonstrated a mate preference, it may be that OT acutely regulates sexual satiety. This is evident on a longer time scale as OT terminates estrous, reducing the motivation to copulate, and here we show that OT acutely decreases sexual motivation but also induces a mate preference.

Discussion

Data from the present study show that OT facilitates behavioral progestational responses together with the formation of a mate preference in female rats. Because progestational responses do not occur with an injection of OT following pelvic nerve transection (Helena et al., 2011), we reasoned that much of the post-copulatory proreproductive responses and the formation of a mate preference depend on activation of OTRs at the level of the vagina, cervix, and clitoris. We demonstrated that the expression of OTRs is affected by hormone priming in a region-dependent manner and that peripheral OTRs in the cervix, vagina, and clitoris are expressed but less abundantly during hormonally-primed sexual receptivity.

We also showed that OT signaling has important behavioral progestational effects, as evidenced by the facilitation of estrous termination with one injection of systemic OT alone. We propose that these progestation-related behaviors facilitate acquired mate preferences and consequently proximal mate choice. Systemic OT alone induces estrous termination more potently than VCS. During copulation, systemic OT also increases pacing intervals facilitating the likelihood of pregnancy (Erskine, 1995), while also signaling estrous termination and the cessation of copulation. We have shown here that systemic OT facilitates a preference to receive ejaculations from a male associated with pacing. Consequently, longer pacing intervals induced by OT may facilitate sperm transfer when a female receives an ejaculation from a preferred male.

Based on the mate preference findings, we propose the OTR antagonist-induced increase in solicitations is because sexual satiety, via VCS, was blunted by the antagonists. As such, this increase in solicitations is a means to receive more intromissions. Both topical anesthetic applied to the cervix and pelvic nerve transection result in females imposing significantly shorter pacing intervals between intromissions (Meerts et al., 2015; Meerts & Clark, 2009). These findings, in conjunction with our observation that systemic OT increases pacing intervals, which is also observed during estrous termination (Pfaus et al., 2000), leads us to propose that OT plays an important role in sexual satiety and consequently reward, as others have suggested (Witt, 1995).

Consistent with this idea, females injected with an OTR antagonist, cligosiban or L371,257, avoided soliciting a male associated with paced copulation, potentially due a sexually conditioned avoidance. States of sexual frustration resulting from a lack of satiety or reward induce sexual avoidance of a familiar partner (Parada et al., 2013). Our results indicate females experienced a lack of sexual reward and/or a lack of satiety during their only sexual conditioning trial because of peripheral OTR antagonism. Both OTR antagonists also inhibit estrous termination following VCS. Thus, it may be that sexual satiety, mediated by the likelihood of estrous termination and progestational responses are an important component of sexual reward in the female rat. Though it is not possible to verify the subjective state of a rat, paced copulation induces changes in measures of sexual motivation, i.e., conditioned mate and place preferences (Coria-Avila et al., 2005; Mac Cionnaith et al., 2020; Paredes & Alonso, 1997). Conditioned approaches to cues, places, or partners, i.e., wanting, are theorized to be related to hedonic effects, i.e., liking, during the consumption of a reward (Berridge & Robinson, 2016).

The changes we observed in OTR expression are important for sexual function in general. During states of sexual arousal, clitoral and vaginal smooth muscle tissue relax, blood flow increases, and the clitoris and vaginal epithelium become engorged (Munarriz et al., 2003). The area of vaginal epithelium important for lubrication is increased with estradiol and progesterone (Pessina et al., 2006). Nitric oxide synthase (NOS) and vasoactive intestinal polypeptide are important drivers of this vasodilation and smooth muscle relaxation (Munarriz et al., 2003) The transcription of NOS is estrogen dependent. OT also facilitates smooth muscle relaxation via the release of intracellular Ca^{2+} (Duridanova et al., 1997) which is critical for the ability of NOS to exert its effects during sexual arousal. Additionally, OTR activation itself increases NOS activity (Melis et al., 1997) and this would then enhance OT's effects on sexual arousal.

The significant increase of OTR IR in the VMHvl shown here is important for the acute display of lordosis (Vincent & Etgen, 1993). OT signaling in the VMHvl is also required for the nocturnal prolactin surge that follows VCS (Northrop & Erskine, 2008). Here we show that females injected with the OTR antagonists have less activation of OT neurons in the VMHvl following VCS. Within the VMHvl, Fos in OT neurons was most abundant in females given systemic OT combined with VCS. This finding indicates that OT neurons are sensitive to increases in OT, whether by central infusion or because of VCS. This in conjunction with our findings showing that a centrally-infused OTR antagonist does not attenuate estrous termination after VCS suggests peripheral OTRs are important for the facilitation of reproductive responses and consequently, acquired mate preferences. As the progestational effects of injected OT are disrupted following pelvic nerve transection (Helena et al., 2011), the effects of cligosiban are likely due to its actions in the periphery.

Here, we also show the reproductive importance of mate preferences that have a proximate experientially-mediated cause. While much is known about rat's copulatory patterns, there is little evidence that rats choose mates based on traits associated with an evolutionary ultimate causality. Some have even suggested that mate choice may be random in female rats (Le Moëne & Snoeren, 2018). Calhoun (1963) noted that dominant males can form a harem and monopolize access to females resident in its warren. McClintock (1984) showed that female rats imposed longer intervals before resuming copulation after receiving a dominant male's ejaculation which increases the likelihood of sperm transport. Dominant males come and go, and so a female likely benefits from flexibly changing mate preferences over time. Many copulations in naturalistic environments do not result in surviving litters (Calhoun, 1963). As such, a female also benefits

evolutionarily by copulating preferentially with a male that increases its likelihood of pregnancy. McClintock (1984) provided evidence of a dominant female displaying a preference to receive ejaculations selectively from one male in particular in a group mating context. This is similar to the conditioned mate preference observed for males bearing a scent cue (Coria-Avila et al., 2005), or natural scents/pheromones (Holley et al., 2014, 2015; Mac Cionnaith et al., 2020) associated with paced copulation.

We have previously shown that conditionally preferred mates activate OT neurons in the PVN, mPOA, and the VMH (Mac Cionnaith et al., 2020). Given this pattern of OT activation, when a female recognizes a familiar preferred mate, there is likely an increase in central and peripheral OT which may have important functions for mate choice, genital arousal, and estrous termination after copulation. Conditioned preferences may form links between cues of a preferred partner with patterns of activation that facilitate successful pregnancy. Conditioned release of OT in the presence of familiar males may underpin proximal experientially-mediated mate choice. Consequently, conditional OT-release in the presence of preferred male may have at least four effects on female reproductive capability: 1) to potentiate lordosis responses; 2) to facilitate physiological sexual arousal (e.g., clitoral engorgement and stimulation); 3) to augment peripheral release of OT which acts on vaginal smooth muscle and cervical myometrial tissue to facilitate sperm transport from a preferred male (Dittrich et al., 2009); and 4) to increase satiety and reward, and facilitate estrous termination, which decreases the likelihood of a female copulating with another male.

Another finding of note relates to systemic OT administration in general. The debate over whether systemic OT enters the central nervous system remains controversial. Our experiments do not test this question, but we do show that peripheral OTRs play a necessary role in relaying reproductively-salient information to the CNS. As mentioned previously, there is emerging evidence of OT transport into the brain via RAGE proteins (Yamamoto et al., 2019). While we cannot ensure that the effects of systemic OT on inducing estrous termination and mate preference were not due to central transport, we show both estrous termination and mate preference required peripheral OTR activation. Our findings, taken together with Helena et al. (Helena et al., 2011), suggest much of the effects reported here can be attributed to peripheral OT signaling.

Materials and Methods

Experimental Subjects. Long-Evans female rats (200-250g) were purchased from Charles River (Kingston, NJ, USA). Females were housed in pairs in Plexiglass shoebox cages at the Animal Care Facility, Concordia University. Females used in the infusion study were housed individually after cannulation surgery. Females had unrestricted access to food (standard laboratory chow, Charles River #5075, Montreal) and water. Females were maintained on a 12h:12h reverse light cycle (lights on 8:00pm; lights off 8:00am) and housed in the same room as male Long-Evans rats.

Stimulus Animals. Male Long-Evans (300-350g) rats were used as sexual stimuli in the behavioral experiments and were purchased from Charles River (Kingston, NJ, USA) and from Envigo (Lachine, QC, Canada). Males were housed in Plexiglass gang cages in groups of two or four and kept in the same conditions as females. Males copulated at least five times prior to serving as sexual stimuli.

Surgeries. One week after all female rats arrived their ovaries were removed to allow for the control of sexual receptivity with exogenous steroid hormones. Female rats were anaesthetized with Isoflurane gas (4% for anaesthesia induction, and 2% for maintenance) at a flowrate of 0.8L/min. Ovaries were removed through an incision in lower lumber region of the back which was then sutured with surgical thread.

For cannulation surgeries, rats were anaesthetized with Isoflurane and secured onto a stereotax apparatus. A 3mm stainless steel cannula (23G, Plastics-One, Ronaoke, VA, USA) was unilaterally implanted, targeting the lateral ventricle. The coordinates, relative to Bregma, were antero-posterior (AP) = -0.72mm, medio-lateral (ML) = ± 1.8mm, dorso-ventral (VP) = 3mm. The guide cannula was fixed with dental cement and jeweller's screws affixed to the skull. Cannula placements were counterbalanced across hemispheres. An obturator (26G, Plastics-One, Roanoke, VA, USA) plush cut to 3.5mm was inserted into the cannula. Placements were later verified with an infusion of cresyl violet directly prior to perfusion.

Females were injected s.c. with an anti-inflammatory analgesic, Ketoprofen (Anafen 100 mg/ml, Boehringer Ingelheim) and the antibiotic Penicillin G (PenG, 30,000IU) during surgery. Females were administered 0.03mL of Ketoprofen (s.c., 100mg/mL) each day for the next five days and given approximately one week to recover.

Copulation chambers. Estrous termination tests occurred in bilevel pacing chambers. The chamber is made of Plexiglass (Height 51cm x Width 70cm x Depth 15cm). The chamber contains an upper and lower platform connected by stairs on each side. Females pace by changing levels.

Mate preference conditioning and acute sexual behaviour trials took place in a unilevel pacing chamber, made of Plexiglass (Height 38 cm × Width 60 cm × Length 38 cm) that contains a wire grid with woodchip bedding. The chamber contains a divider with a hole (4cm x 4cm) large enough for the female to cross but too small for the male to cross, allowing a female to pace. VCS was given to females in a modified unilevel chamber with two small doors and no pacing divider.

Drugs and exogenous steroid hormones. Oxytocin acetate was purchased from Bachem (#4016373) and dissolved in 0.2mL sterile physiological 0.9% saline at two different concentrations, 20 versus 50 µg. Cligosiban was acquired from MedChemExpress (#HY-15023). Cligosiban (also known as PF-3274167) is a selective oxytocin receptor antagonist that crosses the blood brain barrier. Cligosiban was dissolved in a solution of 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, #276855), 40% polyethylene glycol 300 (Sigma-Aldrich, 8.07484), 5% Tween-80 (Sigma-Aldrich, #P4780), and 45% saline. Cligosiban was prepared at a dosage of 5mg/mL and injected at volume of 1mL/kg. L371,257 was purchased from Tochris (#2410), dissolved in DMSO (10% of final solution) and sonicated for 75s at 45°C before Tween-80 and saline (10% and 80% of final solution, respectively) were added. L371,257 was used because it does not enter the central nervous system. Oxytocin, saline, L371,257, and Cligosiban were all injected intraperitoneally (i.p.). There were no studies, to our knowledge, on the effects of Cligosiban or L371,257 on female rat sexual behaviors. Thus, a preliminary dose response study on the effects of Cligosiban and L371,257 on female sexual behavior was conducted to select a dose for the current studies and can be found in supplementary (S1).

For the infusions, oxytocin acetate (Bachem, #4016373) was dissolved in sterile saline to achieve a concentration of 2µg/1µL. (d(CH_2)$_5^1$,Tyr(Me)^2,Thr^4,Orn^8,des-Gly-NH_2^9)-Vasotocin (OTA) was purchased from Bachem (#4031339) and dissolved in sterile saline for a concentration of .25µg/1µL.

Females were primed with exogenous hormones to induce sexual receptivity after ovariectomies. Estradiol benzoate (EB) and progesterone (P) were acquired from Sterloids INC (Newport, RI, USA). Reagent grade sesame oil (Sigma Aldrich, Canada, #S3547) was used as a vehicle for the exogenous hormones, and

served as a vehicle control. Females were injected with EB (10 μg in 0.1 ml sesame oil, s.c.) 48 hours prior to testing and P (500 μg in 0.1 ml sesame oil, S.C.) four hours before testing. The same hormone doses were used in the estrous termination experiments, but the timing of administration differed.

Perfusions and immunohistochemistry. Females were intracardially perfused with approximately 300mL of phosphate buffer solution and 4%v/v paraformaldehyde. Brains, clitorises, and vaginal canals (from the vaginal opening to the uterus) were excised and post-fixed in for 4-hours and transferred to a 30%v/v sucrose solution for a minimum of 60-hours before being stored at -80°C. Brains were sectioned coronally in 30μm slices using a Leica cryostat. Clitoral, vaginal, and cervical sections were sectioned into 60 μm slices. Double-labelling immunohistochemistry was performed as described in Mac Cionnaith et al. (Mac Cionnaith et al., 2020). Staining for OTR was performed similarly, but only one primary antibody incubation occurred followed by staining with 3,3′-diaminobenzidine (DAB), H_20_2, and nickel chloride (NiCl). Briefly, sections were quenched in 3% H_20_2 and pre-blocked with normal goat serum before a primary antibody incubation for 72 hours (4°c). We used an anti-mouse polyclonal OTR antibody made in rabbit (1:500, AbCam, ab217212), an anti-rat Fos antibody made in rabbit (1:20,000, Synaptic Systems, 226 003), and a polyclonal anti-rat Oxytocin made in rabbit antibody (Abcam, AB911). Sections were then incubated with a biotinylated goat anti-rabbit secondary antibody (1:200, Vector Laboratories). After an incubation with avidin-biotinylated-peroxidase complex (1:55, Vectastain ELITE® ABC KIT, Vector Laboratories), sections were stained with DAB+H_20_2+NiCl, or DAB+H_20_2. Sections were mounted on Permafrost glass slides. dehydrated in ethanol baths, cleared with Xylenes, and coverslipped.

Fos induction and quantification of Fos IR in OT-IR neurons and ELISAs. Females were given five 30min sexual experiences with stimulus males in bilevel chambers. Sexual receptivity was induced with EB and P, 48- and 4- hours prior to each trial and Fos induction. To induce Fos, females were i.p. injected with either saline, OT 50μg, Cligosiban (5mg/kg), or L371,257 (1mg/kg) 1-min prior to VCS or sham. VCS (i.e., five stimulations applied every 6 mins with a lubricated glass rod) or sham stimulation (i.e., lifting the base of the tail for 5secs every 6 mins) was given over the course of an hour. Females then rested in the chamber for approximately 20 mins before perfusion. Directly prior to a perfusion, 5mL of blood was collected from the

heart. Luteinizing hormone solid phase ELISA kits were purchased from Abnova (KA2332) and prolactin sandwich ELISA kits were purchased from Novus Bio (NBP3-06974).

Photomicrographs were taken on a Nikon TI microscope at 20x magnification. QuPath (Bankhead et al., 2017) was used to quantify Fos IR within OT-IR cells. QuPath allowed us to deconvolute images based on the stain colors generated by the DAB+NiCl+H_20_2 black/dark brown stain and the DAB+H_20_2 reddish-brown stain. Oxytocin-IR positive cells were identified by this deconvolution and further filtered with an optical density threshold of 0.05, with a watershedding algorithm and a minimum size filter of 5µm applied. We then used QuPath's subcellular detection module to quantify Fos IR positive nuclei within OT-IR positive cells. Fos IR threshold was set to 0.4.

OTR hormonal induction and quantification. Females were randomly assigned (n = 6 per group) to be hormonally primed with either oil + oil, EB + oil, or EB + P to characterize the effect of exogenous hormones on OTR IR. Females were hormonally primed as described above. Subjects underwent four hormonal priming cycles (i.e., 16 days) to allow for a washout period after use in a previous experiment. Sections from the cervix of three females in the oil + oil group were unobtainable and therefore, three additional females were added to the oil + oil group. Sections were stained for OTR IR as described above. Photomicrographs were taken on a Leica DM6000B microscope. Using FIJI, images were thresholded using the MaxEntropy algorithm, segmented using the default segmentation algorithm, and IR puncta were counted provided they were greater than 5µm in size and above .6 in circularity. Immunoreactivity counts were then divided by the area of the sample/brain regions to give a density count per mm^2.

Estrous termination and infusions. Ovariectomized females were given five sexual experiences prior to the estrous termination trials. Females used in the infusion study were cannulated after their fourth copulatory experience, which allowed us to ensure sexual behaviors on the fifth trial were not affected by the surgery.

Two days before VCS, females were injected with EB at 2.30pm. On the day of the VCS trial, females were injected with progesterone at 5:00pm. One minute before receiving VCS or sham stimulation, females were injected with either saline, OT 50µg, cligosiban (5mg/kg), or L371,257 (1mg/kg). VCS and sham stimulation were given as described above..

For infusions, 5-mins before VCS or sham, hormonally primed females were infused with either saline, OT (4µg), or OTA (1µg), at a rate of 2µL/min for 2mins. An infuser tip plush cut to extend 1.5mm below the cannula guide was attached to medical grade polyethylene microtubing (0.58mm x 0.99mm) and connected to a 25µL Hamilton syringe on an infusion pump. Females were i.p. injected with either vehicle or L371,257 1-min before VCS or sham.

Twelve-hours after the start of the trial estrous termination tests occurred. Females were placed alone into a bilevel chamber for 5mins. A male was then placed in the chamber for 10mins and the pair were allowed to copulate. The male was then removed, and after 5mins another male was placed into the chamber. Trials occurred every 8 days.

Mate preference procedure and acute effects of OTR agonist and antagonists. Females were hormonally primed with EB+P and injected with either saline, OT 50µg, cligosiban (5mg/kg), or L371,257 (1mg/kg) 1-min before being placed into a unilevel chamber for 30mins with a male scented with 2.5mL of food grade almond extract (Clubhouse, Canada, #066200013271).

Four days later, hormonally primed females were placed into an open-field mating arena (123cm x 123cm x 46xcm) with woodchip bedding on the floor. A scented male and an unscented male were tethered to a Velcro rat jacket (Lomir, #RJ03) attached to a 30 cm spring on opposing corners of the arena. The female was placed into the arena and allowed to copulate for 30mins. The preference tests were recorded on a GoPro camera. Data for two animals were removed because a male escaped the jacket.

The acute trial was identical to the conditioning trial; however, four subjects were removed because the video files become corrupted.

Data Analysis and Statistical Packages. All statistical analyses were conducted using R software Version 4.0.3. All ANOVAs and mixed effects models were conducted with afex: Analysis of Factorial Experiments (Singmann et al., 2022) and any subsequent means comparisons (i.e., planned and post-hoc) were conducted using emmeans: Estimated Marginal Means, aka Least- Squares Means (Lenth et al., 2022). Equivalence tests were conducted with the TOSTER package (Lakens et al., 2018). All graphs were made with the package ggplot2: Elegant Graphics for Data Analysis (Wickham et al., 2022).

Supplementary Materials.

Preliminary Dose Response for Cligosiban and L371,257.

Subjects. Sixteen Long Evans females were used in this dose response experiment. Females were ovariectomized as described above. Sexually experienced Long Evans males from Charles River were used as sexual stimuli. Prior to the start of the dose response trials, females copulated five times for 30 min in a bilevel pacing chamber. Two groups of eight females (n = 8) received differing doses of either Cligosiban or L371,257. As such, this study used a within-subjects' design for each drug.

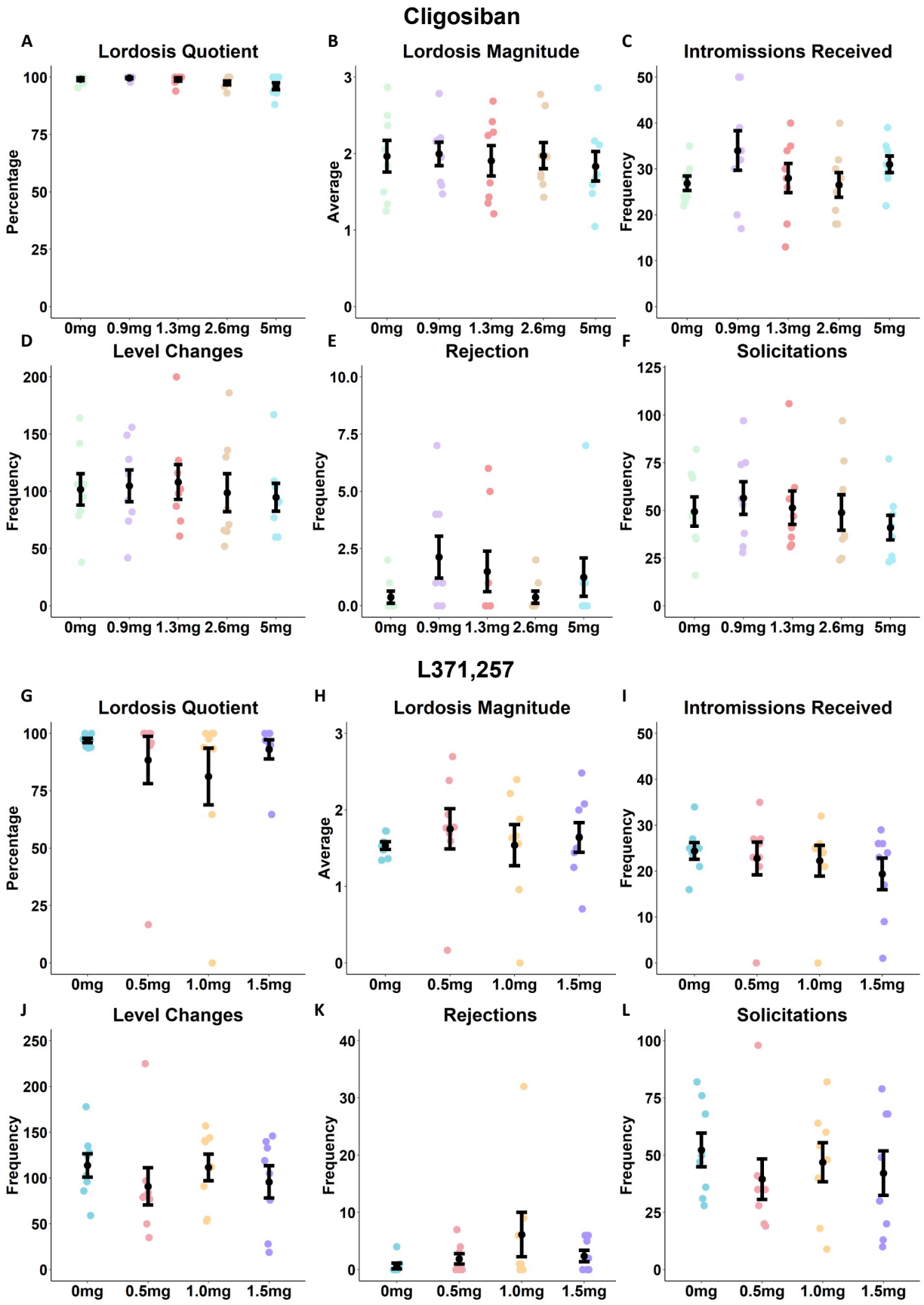
Drugs. Different doses of cligosiban were prepared as described above. In this dose response study, females were administered: vehicle, 0.9 mg/kg, 1.3 mg/kg, 2.6 mg/kg, and 5 mg/kg. Different doses of L371,257 were also prepared as described in the general methodology. Females were administered: vehicle, 0.5mg/kg, 1mg/kg, and 1.5mg/kg.

Procedure. Prior to each sexual trial, females were OVX and hormonally primed with EB and P before each sexual trial, as described in the general methodology. Females were given five copulatory sessions in the bilevel chamber before the start of the dose response study. Each trial, both the copulatory exposure and drug trials, lasted 30 min. On the first drug trial females were I.P. injected with vehicle. On each subsequent trial the administered doses of cligosiban and L371,257 were increased. This allowed us to ensure the drug did not have noticeable side-effects as the dose was increased. Stimuli males were placed in the bilevel chamber and given five minutes to habituate to the chamber before the trial began. Females were injected with either vehicle or either antagonist, one minute prior to being placed in the bilevel chamber and allowed to freely copulate. At the end of the 30 min trial the male and female rats were removed from the bilevel chamber. Each female's sexual behavior during the drug administration trials was recorded and scored by a researcher blinded to the drug dose (including vehicle trials). Trials were scored for proceptive and receptive sexual behaviours.

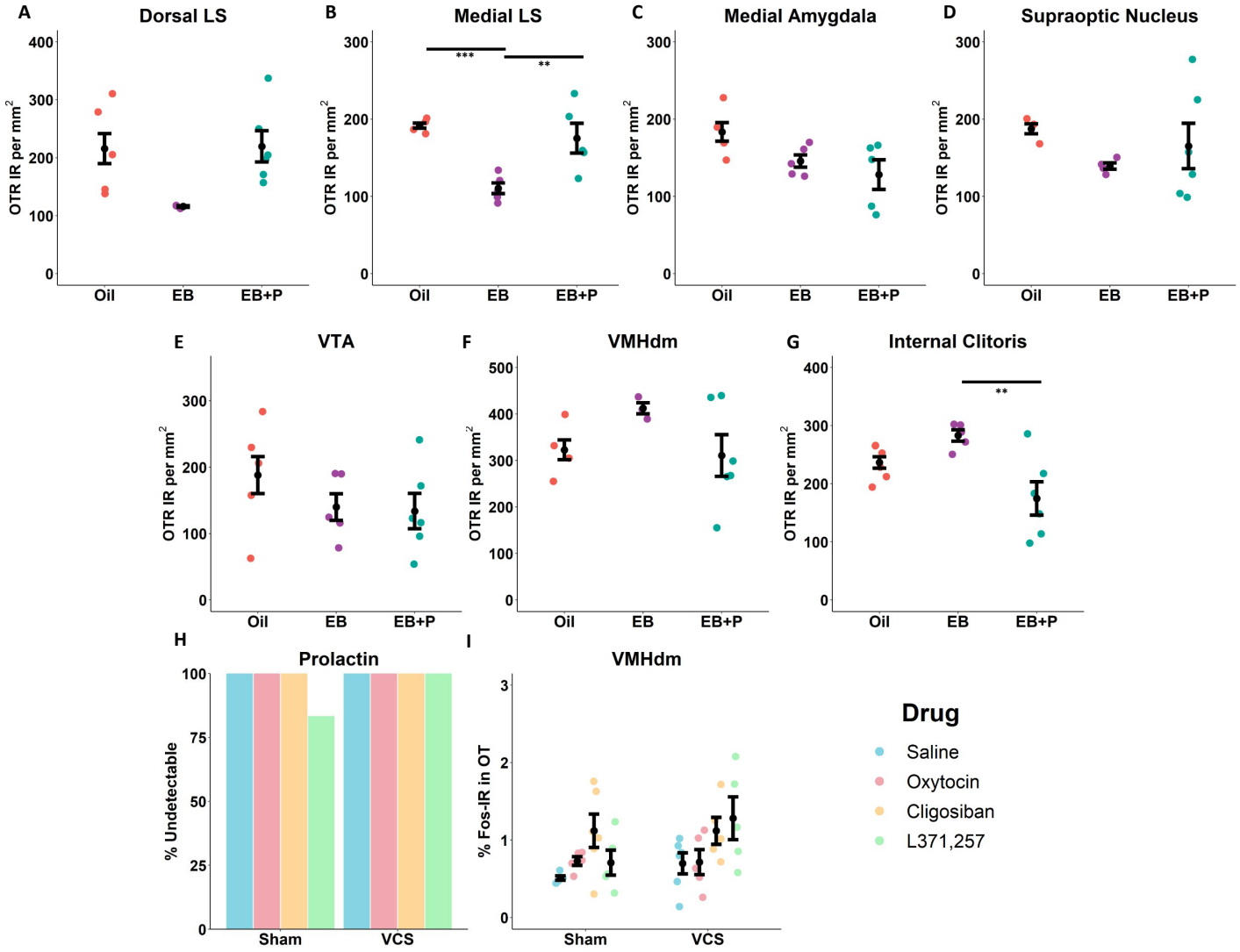
Results. The repeated measures ANOVAs revealed that there was no effect of cligosiban or L371,257 drug dose on solicitations, LQ, LM, rejections, level changes, or intromissions received. Based on these findings, we chose to use 5mg/kg as the dose for cligosiban, and 1mg/kg as the dose for L371,257. Though 1mg/kg was

not the largest dose tested, the cost and solubility of a higher dose led us to choose 1mg/kg. Please see Supp Fig 1.

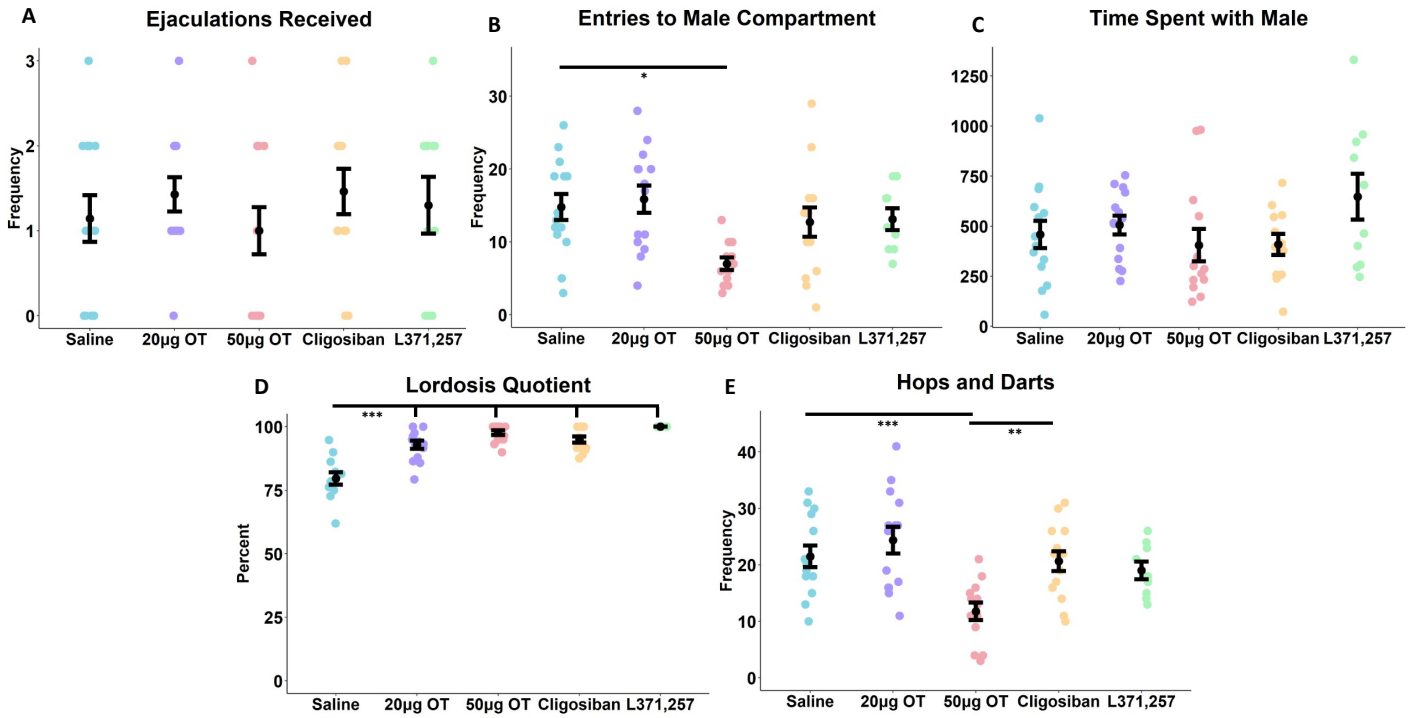
.



Supplementary Figure 1. Displays the dose response raw data on sexual behaviours for cligosiban (A-F) and L371,257 (G-L). The means ± SEM are also shown. There was no significant effect of drug dose on sexual behaviours for both cligosiban and L371,257



Supplementary Figure 2. The effects of exogenous hormone priming on OTR IR in the dorsal lateral septum (2A), medial lateral septum (2B), medial amygdala (2C), supraoptic nucleus (2D), ventral tegmental nucleus (2E), VMHdm (2F), and internal portion of the clitoris (2G). Also shown are the percentage of subjects with undetectable serum prolactin (2H) and Fos IR in OT-IR (2I) neurons following VCS or sham combined with an injection of OT or OTR antagonists. Means ± SEM are also shown and means comparison are based on estimated marginal means generated by ANOVA models. 2B) Females primed with EB alone showed significantly less OTR IR than those injected with either EB+P or vehicle. 2G) Females injected with EB+P showed significantly less OTR IR in the internal clitoris compared to those injected with EB alone. (n = 4-6). Outliers ((beyond 1st/3rd quartile ± 1.5 x IQR) were removed. * $p < .05$; ** $p < .01$; *** $p < .005$



Supplementary Figure 3. The acute effects of OT and OTR antagonists on the number of ejaculations a female receives (3A), the number of entries to a male's compartment (3B), the time a female spent in a male's compartment (3C), lordosis quotients (3D), and proceptive hops and darts (3E) in a unilevel pacing chambers (i.e., identical conditions to mate conditioning). Outliers (i.e., (beyond 1st/3rd quartile ± 1.5 x IQR) were removed. Mean differences were detected with one-way ANOVAs, followed by specific group comparisons based on estimated marginal means. 3B) Relative to controls, females injected with 50µg OT made significantly fewer entries to a male's compartment. 3D) Controls showed significantly lower lordosis quotients relative to females injected with OT and the OTR antagonists. 3E) Females injected with 50µg OT made significantly fewer hops and darts relative to controls and females injected with cligosiban. (n = 10-14)

* $p < .05$; ** $p < .01$; $p < .005$.

General Discussion

The aim of this thesis was to examine the role of OT in the formation of conditioned mate preferences in female rats. Because of OT's known roles in the formation of partner preference in the prairie vole, and conditioned mate guarding in the female rat, it was hypothesized that OT regulates the formation of sexual preferences too. Additionally, the experiments were carried out to clarify the links between the rewarding and reproductive consequences of paced VCS and conditioned sexual preferences.

In the first chapter, we built on the work of Coria-Avila et al. (2005, 2006) and Holley et al. (2014). Coria-Avila et al. (2005) demonstrated that females can form a preference to solicit and receive the first ejaculation from a male previously associated with paced copulation. Such a preference can be formed for males artificially scented with almond extract and also for a male of a particular strain, a more naturalistic cue (Erskine, 2005). Holley et al. (2014) showed that females given ten paced copulation trials with the same male displayed mate guarding behaviours in a subsequent copulatory trial with a competitor female present. This thesis adds to previous work, showing that pacing conditions associated with elongated pacing intervals via the one-hole divider (Ismail et al., 2009), relative to the four-hole pacing divider, results in a selective preference to receive the first and more overall ejaculations from an individual male paired with paced copulation. It was also demonstrated that exposure to preferred males significantly increases activation, as measured indirectly by Fos protein IR, in OT neurons in the PVN, mPOA, and VMH.

The findings of the first chapter indicate that the type of paced copulation is a significant determinant of the type of sexual preference displayed by a female. In contrast to Coria-Avila et al. (2005, 2006) who used a discriminatory conditioning design and allowed females to pace with a four-hole divider, no solicitation preference was found here. Pacing with the one-hole divider, relative to the four-hole, results in significantly long pacing intervals (Ismail et al., 2009). When the number of intromissions is held constant, longer pacing intervals are more effective at inducing a progestational state (Edmonds et al., 1972), and consequently, pregnancy or pseudopregnancy (Adler, 1969; Chester & Zucker, 1970; Kornberg & Erskine, 1994). The ejaculatory preference shown by females in the one-hole pacing condition was surprisingly strong, to the extent that 11 of the 16 females only received ejaculations from a familiar male. Such a preference would bestow a strong reproductive advantage on a preferred male as only the preferred male could sire offspring following this mating test. Given the reproductive significance of elongated pacing intervals, and the heavily

biased ejaculatory preferences, these behavioural data indicate that mate preferences are amenable to conditioning. These data show that female rats can recognize individual males with which they previously copulated and selectively receive ejaculations from a male in a way that increases its likelihood of siring a female's offspring.

The experiments of Chapter Two expanded on these findings by examining the effects of OT transmission during the formation of a sexually conditioned mate preference. The methodology of Chapter One allowed us to examine the contributions of OT neuronal activation during the conditions that allow for the display of a preference, i.e., Fos protein induction after exposure to a preferred mate. The findings of chapter two show that OT transmission has important reproductive functions, specifically regulating the induction of estrous termination, a measure of sexual satiety, and an early component of progestational responses. It was found that the formation of a mate preference was facilitated by systemic OT, whereas systemic OTR antagonists resulted in a preference for a novel male, i.e., conditioned avoidance. The effect of the OTR antagonists on the avoidance of a pacing-associated male may be because they inhibited sexual satiety. This indicates that sexual satiety is an important component of sexual reward.

An important finding of these experiments is the necessary role of peripheral OTRs in the induction of estrous termination and conditioned mate preference. These data offer behavioural corroboration of the findings of Helena et al. (2011) who showed that the progestation induced by a systemic injection of OT alone was blocked by the transection of the pelvic nerve. The findings of Chapter Two and that of Helena and colleagues (2011) show that the activation of and signalling from peripheral OTRs, via the pelvic nerve, are necessary for sexually conditioned preferences, sexual satiety and reward, and progestation. Altogether these findings indicate an OT-mediated coordination of sexual incentive motivation and arousal, satiety and reward, and reproductive responses to VCS. Altogether, these interacting behavioural and neurobiological systems give rise to an emergent mate preference that may be one means by which proximally guided mate choice occurs.

A behavioural and neurobiological model of conditioned mate preference.

Conditioned mate preferences cab be conceptualized as three independent, but interacting, behavioural and neurobiological systems; comprising arousal, motivation, and satiety and reward (see Figure

1). The display of a preference requires sexual motivation for selective sexual behaviours to be directed towards a conspecific male. For a preference to be formed, a female's previous sexual experience with a male must induce a state of satiety and reward. The findings from Chapter Two suggest that sexual satiety, shown by estrous termination, is a necessary component of sexual reward, or at least co-occurs with a subjective state of reward. There is consequently an inseparable link between satiety, reward, and future directed incentive motivation. This link may be a general feature of acquired incentive motivation, as a link between satiety and the rewarding properties of feeding leading to future food preferences has also been posited (see Sclafani, 1995)). Sexual arousal is necessary for a female to copulate and receive VCS and facilitates sexual motivation and the satiating effects of copulation. Arousal and motivation interact in a bidirectional facilitative manner, both components are necessary for satiety and subsequent sexual reward from paced VCS. Satiety and subjective reward inhibit sexual arousal and motivation as shown by the termination of estrous following VCS. Following sufficient paced VCS, an amount of stimulation sufficient to induce a conditioned place preference (Meerts & Clark, 2009; Paredes & Alonso, 1997), there is a reduction in precopulatory anticipation (Pfaus et al., 2000), and consequently sexual arousal. Indeed, experience with reward can control future arousal and motivation. At least in males, when a somatosensory cue is repeatedly paired with copulation to ejaculation, the presence of the cue is required for male arousal and motivation (Pfaus et al., 2013).

The data presented in this thesis indicate that OT facilitates the formation of a conditioned mate preference. OT containing neurons in hypothalamic regions important for sexual motivation, arousal, and satiety are activated by preferred partners. Multiple lines of evidence suggest that the formation of sexually conditioned preferences in the female rat and partner preference in the prairie vole is regulated by OT, DA, and endogenous opioids, i.e., β-End transmission (Aragona et al., 2006; Burkett et al., 2011; Coria-Avila, Gavrila, et al., 2008; Coria-Avila, Solomon, et al., 2008; Cushing & Carter, 2000; Insel & Shapiro, 1992). The facilitation of conditioned mate preference by OT may be both through its direct actions and indirect effects of DA and β-End transmission (Quintana et al., 2022).

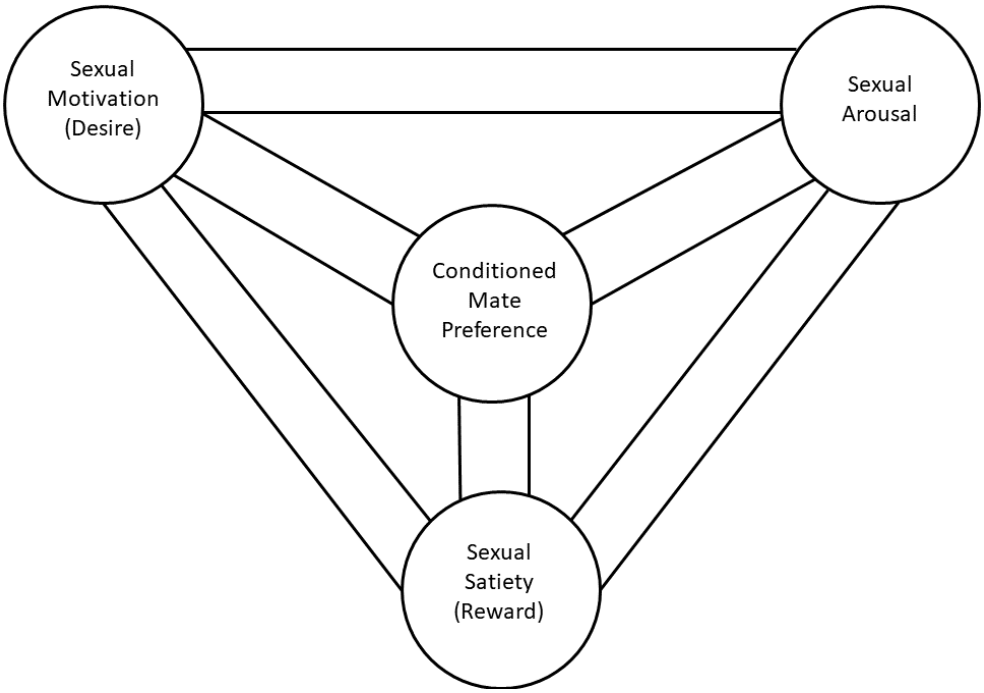


Figure 1. The trinitarian model of sexually conditioned preferences. Incentive motivation, arousal, and satiety/reward processes interact during the formation of a mate preference. Preferred mates acquire incentive properties by their association with subjective states of reward and sexual satiety during periods of sexual arousal

The foundational assumption of this model is that the neurobiological systems that underpin Pavlovian associations between a previously neutral partner and unconditioned states of sexual incentive motivation, arousal, satiety and reward, becomes a neural representation of the unconditioned state (Pavlov, 1927). A partner associated with prior rewarding copulation thus activates neurobiological systems that potentiate incentive motivation, arousal, and reproductive outcomes because of its association with sexual satiety and reward.

Sexual motivation

Sexual motivation, often called desire in humans, is a somewhat theoretically fuzzy and often circular concept. Multiple models have been proposed that attempt to link behavioural data from humans and animals (Beach, 1976; Hardy, 1964; Toates, 2009; Whalen, 1966). Neurobiological studies in male and female animals from mammalian to avian species indicate that there is DA-driven phylogenetically common system that guides incentive sexual motivation across species (Graham & Pfaus, 2010; Kleitz-Nelson et al., 2010; Pfaus, 2010; Pfaus et al., 2001). Incentive models of motivation, in which the attractivity of an external cue (e.g., food, water, sexual partner) depends on physiological internal states (e.g., hunger, thirst, desire/lust), to elicit an approach/appetite to or for a stimulus, and consummatory behaviours. For example, a female's motivation to approach and copulate with a male is dependent on an internal hormonal state induced by endogenous or exogenous ovarian hormones (Beach et al., 1942; Boling & Blandau, 1939; Clark et al., 2004; Tennent et al., 1980). Incentive motivation is not a homeostatic corrective drive. No animal has ever died from a lack of sex, nor do food-deprived rats bar-press for the intravenous or intragastric administration of nutrients (Toates, 1981). Neutral cues that predict the consummation of a reward acquire incentive motivational properties (i.e., incentive salience; Berridge, 2012; Berridge & Robinson, 1998). Stimuli that elicit incentive motivation are unconditioned stimuli that are inherently rewarding when consummated given the appropriate physiological state. This relates to the analogy of proximate and ultimate causes underpinning sexual behaviours and preferences. Evolution has set the stage in which animals innately respond to unconditioned sexual incentives, and proximate experience with reward assigns incentive salience to cues, mates, and places that have previously predicted rewarding copulation.

The mesolimbic DA system is known to be involved in incentive motivation toward appetitive stimuli. These stimuli range from primary reinforcers such as pacing in female rats (Cummings & Becker,

2012) to secondary reinforcers that become associated with a primary reinforcer through consummatory experience, such as scent or strain cues or individual conspecifics (Coria-Avila et al., 2005, 2006; Mac Cionnaith et al., 2020). Pavlovian associations between external cues and rewarding internal states are the foundation of conditioned mate preferences (for a review see Pfaus et al., 2012; Quintana et al., 2022). Dopaminergic neurons in the VTA are activated by incentive stimuli (Berridge, 2012; Berridge & Robinson, 1998; Ferguson et al., 2020). The VTA projects to the NAcc core and shell (Ferguson et al., 2020; Saddoris et al., 2015; Saunders et al., 2018), the BNST (Georges & Aston-Jones, 2001; Soden et al., 2022), and the MeA via the BNST (DiBenedictis et al., 2014). The VTA also has DAergic efferents to the mPOA and in turns receives reciprocal GABA, glutamatergic, and neurotensin inputs from the mPOA which can modulate DA release (McHenry et al., 2017; Miller & Lonstein, 2009; Tobiansky et al., 2013, 2016). When sexually receptive females are exposed to an inaccessible male behind a screen, extracellular DA in the NAcc increases (Pfaus et al., 1995). This shows that a sexually incentive stimulus drives DA transmission within the mesolimbic DA system. Lesions of the mPOA ablate a sexually receptive female's preference to spend time with a male instead of a female (Guarraci & Clark, 2006), showing an important role for the mPOA in the instigation of sexual motivation. Indeed, estradiol sensitive neurotensin neurons that project from the mPOA to the VTA, excite VTA DA neurons and increase extracellular DA in the NAcc shell initiating sexual motivation (McHenry et al., 2017). The DAergic input from the VTA back to the mPOA may act to coordinate incentive motivation with hypothalamic circuits required for stereotyped sexual behaviours, i.e., solicitations and lordosis. In sexually receptive females primed with EB+P, the binding affinity of DA D2-like receptors (D2Rs) decreases, biasing the mPOA toward increased DA D1-like receptor (D1R) to D2R ratio (Graham et al., 2015). Under the influence of EB+P and an increased D1R/D2R ratio, D1R agonists increase solicitations (Graham & Pfaus, 2012). Thus, as the mPOA integrates information about arousal, internal physiological hormonal states, sexual incentive cues, and sexual stimulation, it can influence incentive motivation by its actions on the VTA to NAcc DA circuit.

Consummation of a sexual reward, i.e., paced copulation, increases extracellular levels of DA in the NAcc and dorsal striatum (Becker et al., 2001; Meisel et al., 1993; Mermelstein & Becker, 1995; Pfaus et al., 1995). Dopamine transmission during paced conditioning trials is necessary for the formation of a sexually conditioned partner preference for a scented male (Coria-Avila, Gavrila, et al., 2008). The systemic injection

of a non-selective DA receptor antagonist, flupenthixol, during conditioning blocks a later preference to solicit and receive the first ejaculation from a scented male in a drug free preference test (Coria-Avila, Gavrila, et al., 2008). However, preferences for the strain of a male are not disrupted significantly with the systemic administration of flupenthixol. The disruption of sexual preference formation by flupenthixol is likely due the type of conditioned cue, i.e., pheromonal/MHC based vs olfactory. These inputs recruit different neural pathways, i.e., accessory vs main olfactory systems, and possess different unconditioned stimulus qualities. For example, scents from females in estrous induce DA release and Fos IR in the NAcc of male rats (Kippin et al., 2003; Pfaus & Phillips, 1991). In female rats, the number of MUPs in a male's urine markings is also associated with increased Fos IR in the MePD of the MeA (Kumar et al., 2014). The MeApd, a subregion of the MeA, provides input to the PVN, mPOA, and VMH, and to the VTA but indirectly via the BNST (DiBenedictis et al., 2014). Therefore, pheromonal and MHC cues likely result in stronger conditioned preferences because of its increased unconditioned incentive properties through increased DA release (Coria-Avila & Pfaus, 2007).

Based on the findings from Chapter Two, the ability to pair cues with sexual reward is also OT dependent. The main and accessory olfactory bulbs show stark differences in the number of OTRs, with the AOB containing significantly greater numbers of OTRs. The AOB receives direct OT neuronal innervation from the PVN (Knobloch et al., 2012; Tribollet et al., 1988). Because of OT's role in social memory and recognition, the additional recruitment of the OT system for strain-based cues may facilitate a female's association of a male with paced copulation. The increase in Fos IR in OT neurons found in Chapter One, suggests that individual scent cues, likely signalled through pheromones and MHCs increase activation in the PVN, which may facilitate mate recognition via its inputs to the AOB.

Partner preference formation in the prairie vole requires activation of D2Rs in the NAcc shell, whereas, the activation of D1R maintains a preference (Aragona et al., 2006). One of OT's central roles may be to gate the activity of DA during the formation of a preference. Within the NAcc shell, D2Rs and OTRs form complementary heterocomplexes in which D2R activation potentiates the response of OTR to OT, and vice-versa (Romero-Fernandez et al., 2013). It has also been shown that the direct OT neuronal projections from the PVN to the VTA gate social reward. Optogenetic activation of this pathway promotes social behaviour in mice and increases the excitation of VTA DA neurons and the release of DA into the NAcc shell

(Hung et al., 2017). Optogenetic inhibition of OT PVN neurons blocks the formation of a conditioned place preference for social contact in mice (Hung et al., 2017). Xiao et al. (2018) demonstrated that OT neurons arising from the PVN directly stimulate DA release and also inhibit GABAergic interneurons in the VTA that tonically inhibit VTA DA cells. Consequently, conditional activation by preferred partners and cues may increase DA release from the VTA into the NAcc, driving sexual motivation toward a preferred partner. Interestingly, we did not find increased Fos IR in VTA OT IR neurons following non-contact exposure to a partner. This is in contrast to Coria-Avila and Pfaus (2007) who showed increased Fos IR to both preferred males of a given strain and scented males associated with paced copulation. Thus, this difference may exist but was missed due to counting Fos and OT IR colabelled cells and not Fos IR cells alone.

Conditional release of OT from the PVN by preferred partners may thus drive incentive motivation to copulate with a preferred male over others. The data presented in this thesis suggest that this mate preference is not exerted through a solicitation preference, but by the selective receipt of, and overall tendency, to receive more ejaculations from a male associated with past paced copulation. Of course, such an ejaculatory preference still requires solicitations to be directed toward a male directly prior to the intromission that precedes ejaculation. Females may selectively solicit as a male approaches ejaculation and as a male makes a characteristic 22kH ultrasonic call (White et al., 1990). Interestingly, the application of OT onto mPOA excised neurons significantly increases the resting membrane potential from approximately -60mV to -55mV, and increases the amplitude of depolarizations (Sharma et al., 2019). Within the mPOA, OT signalling facilitates lordosis (Caldwell et al., 1989; Gorzalka & Lester, 1987). An auditory cue associated with a preferred male may conditionally release OT to facilitate lordosis responses in order to receive that male's ejaculation.

Sexual arousal

As discussed in the introduction, sexual motivation and arousal are closely linked and can act bidirectionally to facilitate or inhibit each other. For example, amphetamine, a drug that increases DA release and blocks its reuptake, readily enhances the incentive salience of conditioned cues (Pecina, 2005) and also increases vaginal lubrication, a measure of sexual arousal (Mott & Goeders, 2022). The integration of sexual motivation and arousal is coordinated primarily by the mPOA which receives DAergic input from the VTA and zona incerta (ZI). This is in addition to the mPOA's control of autonomic arousal and sensitivity to

somatic stimulation of the genitalia. During the precopulatory phase, males engage in olfactory investigation and anogenital licking that stimulates the clitoris, vulva, and perineum. As these genital tissues have direct projections to the PVN, early precopulatory licking and subsequent VCS stimulate the release of OT from PVN parvocellular projections to hindbrain and spinal sites. This results in an increase in spinal OT (Sansone et al., 2002). This activation of hindbrain and spinal sites is known to facilitate sympathetic arousal of the genitals. While both sympathetic and parasympathetic activation are necessary for sexual arousal, both OT and VCS increase sympathetic arousal (Komisaruk & Sansone, 2003). Oxytocin neuronal signalling in hindbrain and spinal sites increase sympathetic arousal while also facilitating parasympathetic tone, as demonstrated by an increase in heart rate variability (Grippo et al., 2009; Quintana et al., 2013). Taken in conjunction with the findings of Sansone et al. (2002) that hormonally-released peripheral OT and spinal OT do not correlate, this suggests that the actions of systemic OT are not at spinal sites. Consequently, this endocrine effect must occur through receptors accessible to peripheral blood flow. The hormonal release of OT from the posterior pituitary via magnocellular cells during and following VCS may act on OTRs located in the cervix, vagina, and clitoris which facilitate vasodilation and arousal through OT's intracellular actions to increase NOS expression and signalling (Japundzic-Zigon, 2013; Munarriz et al., 2003).

Increased spinal OT from central neuronal projections may act at sites of dorsal roots that receive sensory information from the flanks and perineum, further potentiating arousal, sexual motivation, and satiety. It has been shown that TRPV1 and Piezo2 receptors, important for converting mechanical stimulation into neural signalling, are colocalized with OTRs in spinal dorsal root ganglion neurons (Noguri et al., 2022). One consequence of OTR activation on dorsal root sensory neurons may be to facilitate the lordosis reflex in response to flank stimulation. Thus, the increase in intracellular Ca^{2+} caused by OTR activation (Gimpl & Fahrenholz, 2001) may increase spinal excitation. This may act to facilitate lordosis and potentiate sensory information from the pelvic, pudendal, and hypogastric nerves that carry sensory information.

The peripheral release of OT during VCS may also act to gate the excitability of mechanoreceptors in the clitoris, vaginal canal, and cervix. The staining patterns of OTR IR, presented in Chapter Two are of relevance here. Leaving aside the effects of hormonal treatment on the abundance of OTR IR, the photomicrographs of clitoral tissue show staining patterns that appear in clusters that look like mechano-corpuscles (see Figure 2).

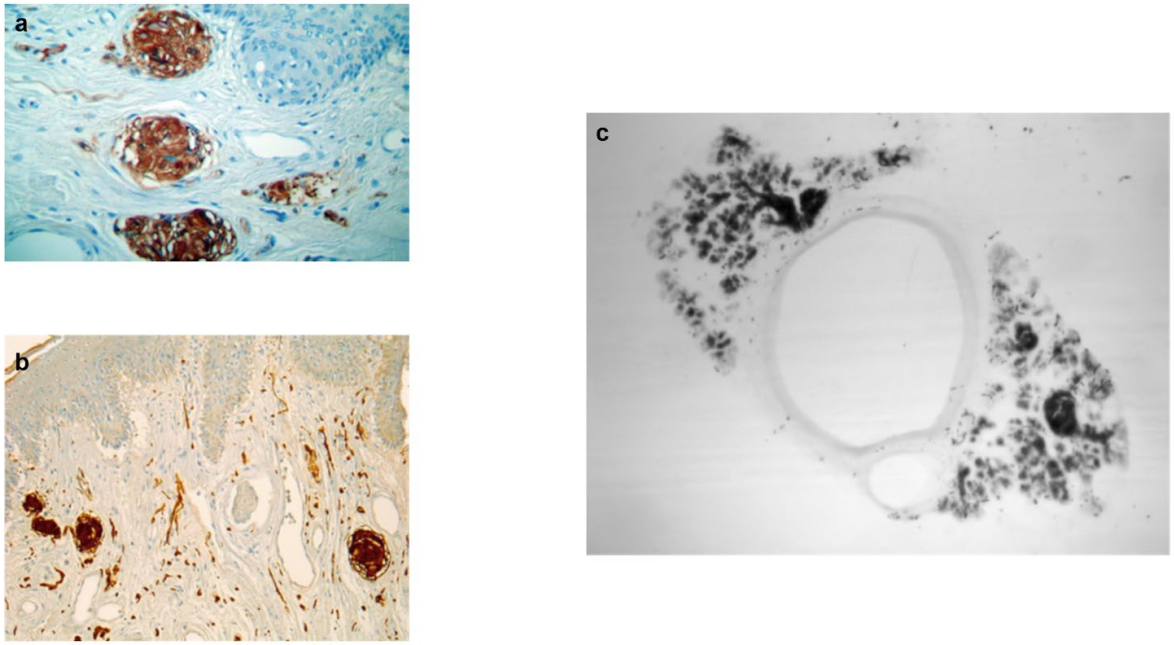


Figure 2. Commonalities between patterns of OTR staining in tissue from the glans of the clitoris and the well-established sensory corpuscles of the clitoral glans. a) PS100 staining showing corpuscles de volupté/genital corpuscles. From Di Marino and Lepidi (2014) b) Immunostaining of bulbous corpuscles and free nerve endings. From Di Marino and Lepidi (2014). c) Rat clitoral glans section stained with 3,3-DAB for OTR, with some immunoreactivity clumping in a circular/spherical manner similar to the corpuscles highlighted in 2a and 2b.

As OTRs are known to colocalize with TRPV1 and Piezo2 receptors (Noguri et al., 2022), in other sensory nerves, OT may facilitate signal transduction of mechanical stimulation of the genitalia. Whether such colocalization between OTR and mechanoreceptors occurs in the mechanosensory corpuscles of the clitoris, vagina, and cervix is not yet known. However, such a mechanism by which mechanosensory receptors are either activated or gated by OT may suggest a potential mechanism by which the systemic injection of OT mimics the effects of VCS to induce estrous termination. One consequence of increased sensitivity to CLS and VCS may be to facilitate the hedonic rewarding effects of copulation, potentiating sexual satiety.

Sexual satiety and reward

For a partner to acquire conditioned incentive properties it needs to induce a subject state of reward (for a review see; Quintana, Mac Cionnaith & Pfaus, 2022). The findings of Chapter Two suggest that sexual satiety caused by the receipt of sufficient VCS is a primary component of sexual reward. The administration of OTR antagonists that attenuate satiety following VCS also results in an avoidance of a male associated with copulation. Females can be conditioned to form preference for a scented male, when they repeatedly receive CLS in the presence of a scented gauze (Parada et al., 2011). However, when CLS is given in the presence of an inaccessible scented male, females display a preference for a novel male. This effect may be due to a lack of sexual satiety and/or a state of sexual frustration. This is reminiscent of the avoidance shown by females conditioned with a scented male under the influence of the OTR antagonists. Endogenous opioids are thought to signal the hedonic state induced by the consummation of a reward (Berridge & Robinson, 2016; Castro & Berridge, 2014). β-End is a primary endogenous ligand for the μOR (Desjardins et al., 1990). It is a neuropeptide that is cleaved from the large prepropeptide proopiomelanocortin (Solomon, 1999). A primary source of POMC-containing neurons arises in the arcuate nucleus of the hypothalamus and has projections to many of the areas that regulate sexual motivation and behaviour viz. the VMH, the mPOA, and PVN (Joseph et al., 1983; Wang et al., 2015). In male rats, whole brain β-End concentrations are increased following ejaculation (Szechtman et al., 1981), indicating that β-End transmission also occurs via diffusion to distal sites. Additionally, copulation to ejaculation in male rats results in the internalization of μOR, a marker of receptor activation (Coolen et al., 2004). Arcuate POMC neurons show increased Fos IR following VCS (Yang et al., 2000). When females are systemically injected with a broad-spectrum opioid receptor antagonist, naloxone, they do not form a preference for a scented male associated with paced copulation (Coria-Avila,

Solomon, et al., 2008). Instead, they demonstrate a preference to solicit a novel male during a sexual preference test (Coria-Avila, Solomon, et al., 2008). In combination with the findings from Chapter Two, these findings suggest that OTR and μOR antagonists have convergent effects on preference formation. Both block preference formation and facilitate a preference for a novel male.

In male rats, activation of μORs in the mPOA and the VTA are necessary for the formation of conditioned sexual preferences (Quintana, Birrel, et al., 2019). Internalization of μOR following ejaculation has been shown to occur in the mPOA (Coolen et al., 2004). Activation of μORs in the VTA facilitates DA transmission from the VTA into the NAcc shell through a disinhibitory mechanism. Transmission of DA from the VTA into the NAcc is tonically inhibited by GABAergic interneurons that contain μORs. Their activation by β-End increases DA transmission by disinhibiting the tonic inhibition of the GABA interneurons. Indeed, μOR activation in both the VTA and NAcc shell are required for conditioned stimuli to acquire incentive salience (Panksepp et al., 1980; Peciña & Berridge, 2013; Quintana, Birrel, et al., 2019). Mating-induced partner preference formation in the prairie vole is also blocked by the systemic injections of the longer acting opioid receptor antagonist naltrexone (Burkett et al. 2011) and by infusions of a specific μOR antagonist into the NAcc shell (Resendez et al., 2013). Paced VCS that induces the release of β-End is a likely mechanism that results in a partner and its cues acquiring sexual incentive properties (see Figure 3).

Paced copulation induces satiety and subjective states of reward, and both OT and β-End transmission are necessary. Paced VCS and systemic injections of OT induce a progestational state, signalled by diurnal spikes in serum prolactin. This progestational state is preceded by the termination of estrous, a state of satiety. Estrous termination requires an increase in excitatory glutamate signalling in the VMHvl (Georgescu et al., 2012, 2014) and concurrently, OTR activation is also necessary for estrous termination (Northrop & Erskine, 2008). Tracing studies using conventional tracing methods in rats and genetically encoded markers of OT fibres from the PVN show no direct projections to the VMHvl (Canteras et al., 1994; Liao et al., 2020). The VMHvl contains many OTRs and OT fibres (Coirini et al., 1991; Griffin et al., 2010; Schumacher et al., 1989, 1990). Hormonal priming with EB+P, dramatically increases the number of OTRs in the VMHvl, and structurally reorganizes the VMH increasing the number of OTR lateral to the VMHvl. These newly transcribed OTRs are adjacent to PVN projecting OT axons (Daniels & Flanagan-Cato, 2000; Schumacher et al., 1990). OT transmission to the VMHvl also likely occurs through diffusion from the PVN (Ludwig, 2003;

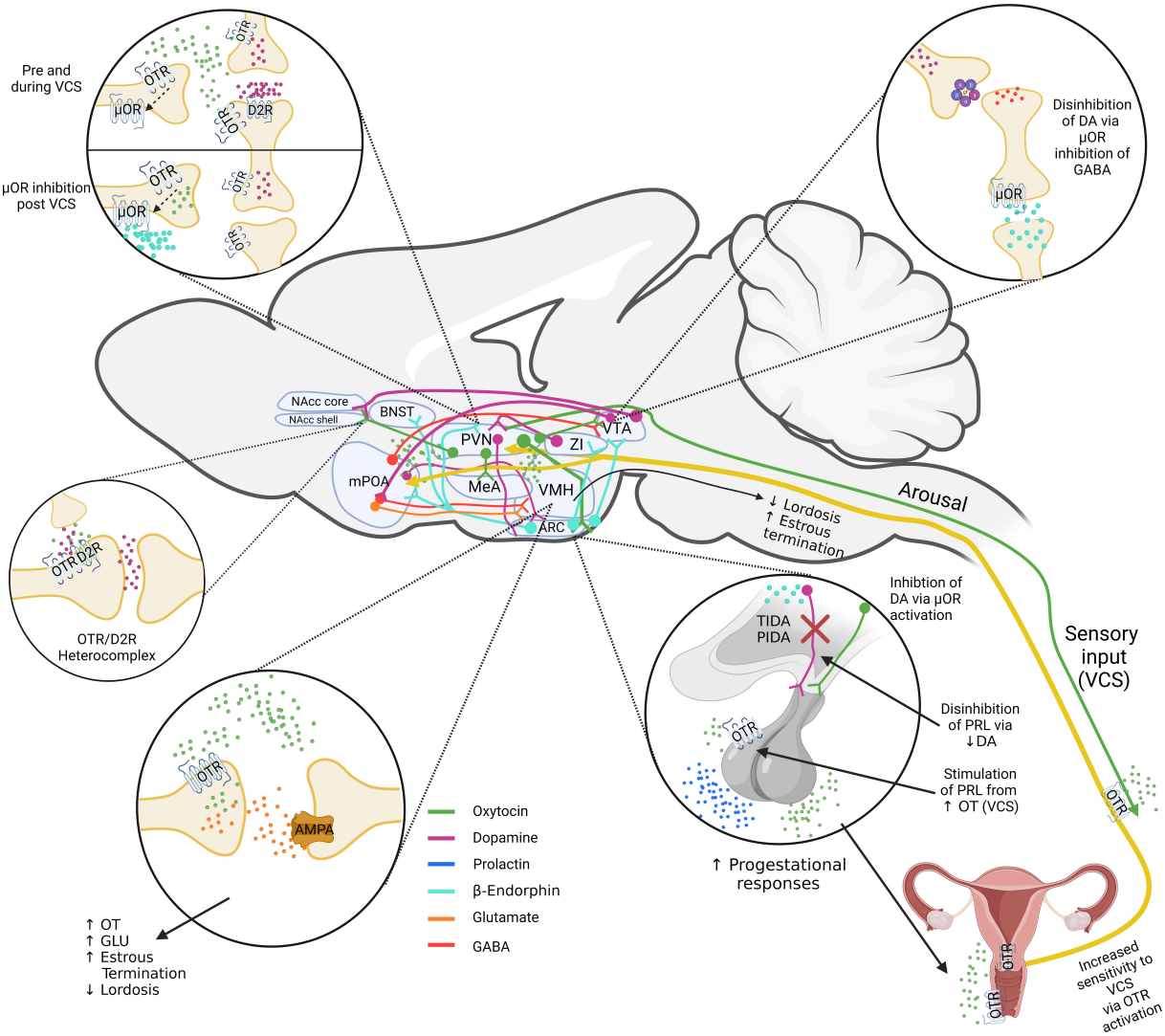


Figure 3: A hypothetical mechanism of mate preference formation in the female rat. Paced copulation induces the central, peripheral, and spinal release of OT from the PVN. As a female paces, the VTA releases DA onto the NAcc shell, facilitating the acquisition of incentive salience. The PVN releases OT onto the NAcc shell, where OTRs and D2R-like receptors are known to form heterocomplexes, potentiating the effects of D2R and OTR binding. The peripheral and spinal release of OT increases sexual arousal which positively affects sexual motivation. Peripherally released OT acts on OTRs in the vaginal and cervix to facilitate sexual satiety and induce satiety. The release of OT in the VMH stimulates the release of glutamate, which induces estrous termination. As pacing continues, POMC neurons in the arcuate are activated resulting in axonal release or somatic diffusion of β-End onto distal cites. Acting in the VTA and mPOA, β-End activation of μOR dishibits DA release and is necessary for sexual reward. Incertohypothalamic DA projections to the median eminence arising in the ZI tonically inhibits prolactin release from the pituitary. Some of these neurons pass through the mPOA and PVN, and are typically excited by local OT release. The central release of OT, act as a positive allosteric modulator of μORs, which inhibits further OT release as β-End signalling increases. Concurrently, μOR activation on incertohypothalamic DA neurons inhibit DA release, allowing for prolactin release and progestation.

Pow & Morris, 1989). Initially, OT in the VMHvl facilitates the lordosis reflex (Caldwell et al., 1989; Gorzalka & Lester, 1987), but as glutamate neurons in the VMHvl become activated by VCS this excitation is inhibitory to lordosis (Georgescu et al., 2009; Georgescu & Pfaus, 2006a). This co-occurs with estrous termination. Within the VMHvl, local OT neurons also corelease glutamate (Griffin et al., 2010), and OT signalling is known to increase the excitability of VMHvl neurons (Inenaga et al., 1991). One potential reason for this may be the increase in intracellular Ca^{2+} caused by OTR activation which would increase the excitability of glutamate neurons and the release of glutamate to induce estrous termination and sexual satiety.

A relation between satiety and reward and reproductive outcomes?

The work in this thesis has demonstrated that paced VCS supports the formation of a conditioned mate preference, further confirming prior observations that paced copulation/VCS is a potent reward (Coria-Avila et al., 2005, 2006; Holley et al., 2014, 2015; Paredes & Alonso, 1997). Systemic OT also facilitates the formation of a mate preference after only one conditioning trial. Because one conditioning trial alone is not sufficient to support the formation of a preference (Coria-Avila et al., 2005), it supports the hypothesis that OT induces a state of satiety, necessary for reward. The satiety and reward induced by pacing may thus be related to the significantly greater release of OT from the PVN relative to unpaced copulation (Nyuyki et al., 2011). Interestingly, systemic OT can support the formation of a conditioned place preference, suggesting that it alone can induce a state of reward (Liberzon et al., 1997). However, both OT and VCS also induce a progestational state required for pregnancy (Helena et al., 2011; Kornberg & Erskine, 1994). It could thus be hypothesized that sexual satiety and hedonic reward regulate aspects of reproductive success. Given μOR's role in hedonic responses to the consummation of a reward (Peciña & Berridge, 2013) and its role in partner preference acquisition (Coria-Avila, Solomon, et al., 2008), it would then follow that β-End or other μOR specific transmission also regulates progestational responses. Indeed, a systemic injection of naloxone given prior to the receipt of VCS decreases progestational responses, i.e., diurnal prolactin surges, for up to four days after copulation (Sirinathsinghji & Audsley, 1985). Altogether, converging lines of evidence suggest that the signalling of satiety and reward through OT and β-End, are important regulators of pregnancy following VCS. Moreover, successful reproduction requires the initiation of diurnal prolactin surges following VCS(Arey & Freeman, 1990; Erskine et al., 1989). Both OT and β-End play regulatory roles in prolactin secretion (Arey & Freeman, 1990, 1992; Tilders et al., 1985; Voogt et al., 2001)

Dopamine transmission in the median eminence exerts direct inhibitory control over prolactin secretion (MacLeod et al., 1970). The incertohypothalamic DA system provides the primary DA innervation of hypothalamic structures (Björklund et al., 1975; Moore & Lookingland, 1995). In addition to its projections to the mPOA, the incertohypothalamic DA system converges on the tuberoinfudibular dopamine (TIDA) system beginning in the dorsomedial arcuate nucleus (Moore & Lookingland, 1995). The TIDA system receives DA inputs via the mPOA, and the medial portion of the PVN via periventricular DA (PIDA) neuronal projections. Some of these projections arise from DAergic cell bodies in the Zona Incerta (ZI; Cheung et al., 1998; Goudreau et al., 1995; Wagner et al., 1995). Because the release of OT onto DA PIDA and TIDA neurons is stimulatory to DA release (Arey & Freeman, 1990, 1992), OT release following copulation needs to be inhibited after the initial surge and increase OT induced by VCS. Within the PVN, OT is a positive allosteric modulator of the μOR (Meguro et al., 2018), and consequently, local OT release in the PVN during may potentiate the effects of μOR activation also caused by VCS. Activation of μORs in the PVN inhibits OT release (Ortiz-Miranda et al., 2003), inhibiting OT's stimulatory effects on hypothalamic DA release. Indeed, β-End released from POMC arcuate neurons resulting in the inhibition of OT release may be a mechanism by which this occurs, suggesting a potential mechanism by which satiety, reward, and reproduction are integrated.

Conclusion

What can be summarized about the formation of conditioned mate preference in the female rat is that OT, DA, and β-End interact over different timescales and in different brain regions to facilitate sexual behaviour and learning, followed by the inhibition of sexual behaviour arising from a state of sexual satiety. Indeed, a primary function of these two neuropeptides is the direct and indirection modulation of DA release, assigning incentive salience to a mate associated with pacing. Oxytocin initially has facilitative effects on some aspects of sexual behaviour, such as lordosis, and the initial state of sexual receptivity. Its facilitation of preference formation occurs by inhibitory effect on sexual behaviours by inducing sexual satiety and progestational responses. The data from the present thesis indicate that estrous termination and preference formation are facilitated by OT signalling, be it centrally or peripherally, and that both estrous termination and preference formation are blocked by OTR antagonists. A relatively simple test of this hypothesis would be to

test whether the intensity of estrous termination following paced copulation predicts future mate preference behaviours in the same female rats.

The data from Chapter One demonstrates OT neurons in the PVN, mPOA, and VMH are activated by preferred mates. Based on the findings in Chapter Two, it was suggested that conditioned mate preferences facilitate the ability of a female to selectively direct sexual behaviours toward a male in a manner that increases the likelihood of a female being impregnate by a preferred mate (see Figure 4). Given the conditional activation of OT neurons found in Chapter One, this may result in central and peripheral release of OT that facilitates incentive motivation toward a mate, arousal, lordotic responses, sperm transport by OT's actions on cervical myometrial tissues (Duridanova et al., 1997), earlier estrous termination, and progestational responses. Conditional release of OT in the presence of a preferred mate may have additional effects that reinforce preference behaviours. By positively gating the excitability of OTRs in the clitoris, vagina and cervix, this may result in a greater perceived magnitude of CLS and VCS, increasing the reward from stimulation. Greater sensitivity of the cervix to VCS would lead to earlier estrous termination following VCS and receipt of ejaculations. Consequently, quickened estrous termination may be a potential mechanism that biases the likelihood of a preferred male siring proportionally more offspring after a copulatory bout as the likelihood of a female copulating with other males decreases. Altogether, OT coordinates the initiation, consummation, and cessation of sexual behaviour.

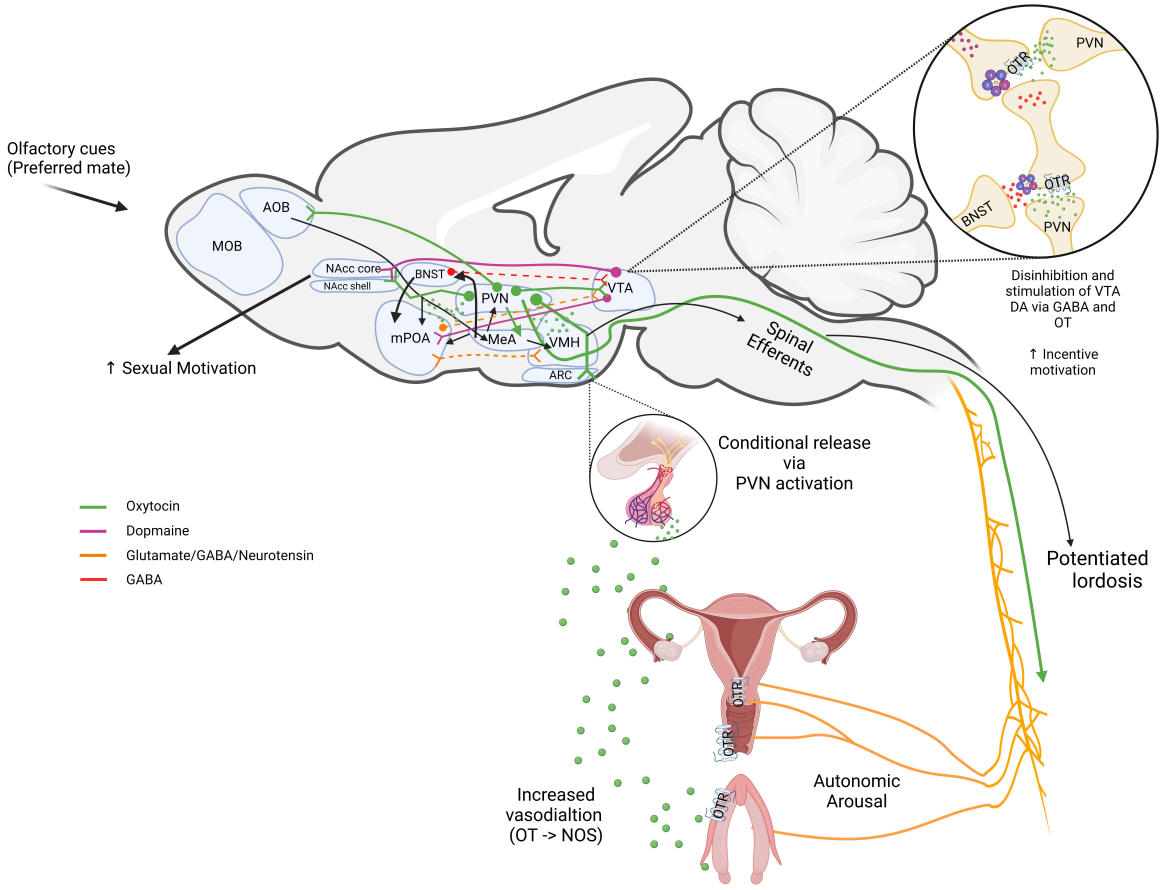


Figure 4: The role of conditional release of OT by preferred mates in the display of a mate preference. Olfactory cues (pheromonal and MHC) processed by the AOB activate the medial amygdala and mPOA. Several systems interact to modulate DA release from the VTA on the NAcc shell inducing sexual incentive motivation. Neurotensin neurons in the mPOA sensitive to male cues directly stimulate the release of DA from the VTA onto the NAcc shell. Conditional release of OT from the PVN facilitates the recognition of a conspecific via activation in the MeA. Activation of the MeA by an appetitive stimulus induces the release of BNST GABA projections onto GABAergic interneurons in VTA. The diffusion of OT from the PVN onto the mPOA may further facilitate VTA DA release and conditional sexual motivation. The OT projections of the PVN directly stimulate VTA DA release, and also inhibit GABAergic interneurons. The DAergic input back to the mPOA regulates autonomic arousal, and the behavioural switch between solicitations and lordosis. Conditional activation of OT neurons in the VMH are expected to potentiate lordosis responses. This occurs in tandem with increases in spinal OT which can potentiate the excitability of sensory dorsal route nerves, further facilitating lordosis. Spinal OT also facilitates sympathetic and parasympathetic tone independent, necessary for sexual arousal. Peripherally released OT binds to OTRs in the clitoris, vaginal canal, and cervix. These peripheral actions increase vasodilation, furthering sexual arousal, and may facilitate sperm transport via cervical contractions.

References

Addessi, E., Crescimbene, L., & Visalberghi, E. (2007). Do capuchin monkeys (Cebus apella) use tokens as symbols? *Proceedings of the Royal Society B: Biological Sciences*, *274*(1625), 2579–2585.

Adler, N. T. (1969). Effects of the male's copulatory behavior on successful pregnancy of the female rat. *Journal of Comparative and Physiological Psychology*, *69*(4, Pt.1), 613–622. https://doi.org/10.1037/h0028244

Ågmo, A. (2011). On the intricate relationship between sexual motivation and arousal. *Hormones and Behavior*, *59*(5), 681–688.

Akesson, T. R., Simerly, R. B., & Micevych, P. E. (1988). Estrogen-concentrating hypothalamic and limbic neurons project to the medial preoptic nucleus. *Brain Research*, *451*(1–2), 381–385.

Althammer, F., & Grinevich, V. (2018). Diversity of oxytocin neurones: Beyond magno- and parvocellular cell types? *Journal of Neuroendocrinology*, *30*(8), e12549. https://doi.org/10.1111/jne.12549

Anderson, E. E. (1937). Interrelationship of drives in the male albino rat. I. Intercorrelations of measures of drives. *Journal of Comparative Psychology*, *24*(1), 73–118. https://doi.org/10.1037/h0062846

Andersson, M. (1994). *Sexual Selection* (Vol. 72). Princeton University Press; JSTOR. https://doi.org/10.2307/j.ctvs32s1x

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, *21*(6), 296–302. https://doi.org/10.1016/j.tree.2006.03.015

Aragona, B. J., Liu, Y., Yu, Y. J., Curtis, J. T., Detwiler, J. M., Insel, T. R., & Wang, Z. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nature Neuroscience*, *9*(1), 133–139. https://doi.org/10.1038/nn1613

Arakawa, H., Cruz, S., & Deak, T. (2011). From models to mechanisms: Odorant communication as a key determinant of social behavior in rodents during illness-associated states. *Neuroscience & Biobehavioral Reviews*, *35*(9), 1916–1928.

Arey, B. J., & Freeman, M. E. (1990). Oxytocin, Vasoactive-Intestinal Peptide, and Serotonin Regulate the Mating-Induced Surges of Prolactin Secretion in the Rat*. *Endocrinology*, *126*(1), 279–284. https://doi.org/10.1210/endo-126-1-279

Arey, B. J., & Freeman, M. E. (1992). Activity of oxytocinergic neurons in the paraventricular nucleus mirrors the periodicity of the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology*, *130*(1), 126–132. https://doi.org/10.1210/endo.130.1.1727695

Arnold, S. J. (1983). Sexual selection: The interface of theory and. *Mate Choice*, 67.

Asdell, S. A. (1946). Patterns of mammalian reproduction. *Patterns of Mammalian Reproduction.*

Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P. D., McQuaid, S., Gray, R. T., Murray, L. J., Coleman, H. G., James, J. A., Salto-Tellez, M., & Hamilton, P. W. (2017). QuPath: Open source software for digital pathology image analysis. *Scientific Reports*, *7*(1), Article 1. https://doi.org/10.1038/s41598-017-17204-5

Barnett, S. A. (1958). An Analysis of Social Behaviour in Wild Rats. *Proceedings of the Zoological Society of London*, *130*(1), 107–152. https://doi.org/10.1111/j.1096-3642.1958.tb00565.x

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity*, *2*(3), 349–368.

Bateson, P. (1983). *Mate Choice*. Cambridge University Press. https://books.google.cl/books?id=HY-onXFuTcoC

Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Hormones and Behavior*, *7*(1), 105–138.

Beach, F. A., Etkin, W., & Rasquin, P. (1942). Importance of Progesterone to Induction of Sexual Receptivity in Spayed Female Rats. *Proceedings of the Society for Experimental Biology and Medicine*, *51*(3), 369–371. https://doi.org/10.3181/00379727-51-13979

Becker, J. B., Rudick, C. N., & Jenkins, W. J. (2001). The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. *Journal of Neuroscience*, *21*(9), 3236–3241.

Berridge, K. C. (2012). From prediction error to incentive salience: Mesolimbic computation of reward motivation: From prediction error to incentive salience. *European Journal of Neuroscience*, *35*(7), 1124–1143. https://doi.org/10.1111/j.1460-9568.2012.07990.x

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

Berridge, K. C., & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist*, *71*(8), 670.

Beynon, R. J., & Hurst, J. L. (2004). Urinary proteins and the modulation of chemical scents in mice and rats. *Peptides*, *25*(9), 1553–1563.

Bindra, D. (1974). A motivational view of learning, performance, and behavior modification. *Psychological Review*, *81*(3), 199.

Björklund, A., Lindvall, O., & Nobin, A. (1975). Evidence of an incerto-hypothalamic dopamine neurone system in the rat. *Brain Research*, *89*(1), 29–42.

Blandau, R. J., Boling, J. L., & Young, W. C. (1941). The length of heat in the albino rat as determined by the copulatory response. *The Anatomical Record*, *79*(4), 453–463. https://doi.org/10.1002/ar.1090790405

Boling, J. L., & Blandau, R. J. (1939). The estrogen-progesterone induction of mating responses in the spayed female rat. *Endocrinology*, *25*(3), 359–364.

Bredt, D. S., Hwang, P. M., & Snyder, S. H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, *347*(6295), 768–770.

Bressler, S., & Baum, M. (1996). Sex comparison of neuronal Fos immunoreactivity in the rat vomeronasal projection circuit after chemosensory stimulation. *Neuroscience*, *71*(4), 1063–1072.

Bronson, F. H. (1989). *Mammalian reproductive biology* (pp. ix, 325). University of Chicago Press.

Brown, R. E. (1977). Odor preference and urine-marking scales in male and female rats: Effects of gonadectomy and sexual experience on responses to conspecific odors. *Journal of Comparative and Physiological Psychology*, *91*(5), 1190.

Brown, R. E., & Moger, W. H. (1983). Hormonal correlates of parental behavior in male rats. *Hormones and Behavior*, *17*(4), 356–365.

Brownstein, M. J., Russell, J. T., & Gainer, H. (1980). Synthesis, transport, and release of posterior pituitary hormones. *Science*, *207*(4429), 373–378.

Burkett, J. P., Spiegel, L. L., Inoue, K., Murphy, A. Z., & Young, L. J. (2011). Activation of μ-Opioid Receptors in the Dorsal Striatum is Necessary for Adult Social Attachment in Monogamous Prairie Voles. *Neuropsychopharmacology*, *36*(11), 2200–2210. https://doi.org/10.1038/npp.2011.117

Busnelli, M., Saulière, A., Manning, M., Bouvier, M., Galés, C., & Chini, B. (2012). Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes. *Journal of Biological Chemistry*, *287*(6), 3617–3629.

Buss, D. M. (1987). Sex differences in human mate selection criteria: An evolutionary perspective. *Sociobiology and Psychology: Ideas, Issues, and Applications*, 335–352.

Butcher, R. L., Collins, W. E., & Fugo, N. W. (1974). *Plasma Concentration of LH, FSH, Prolactin, Progesterone and Estradiol-17/3 Throughout the 4-Day Estrous Cycle of the Rat*. *94*(6), 5.

Caldwell, J. D., Greer, E. R., & Pedersen, C. A. (n.d.). *Medial Preoptic Area Oxytocin and Female Sexual Receptivity*. 8.

Caldwell, J. D., Jirikowski, G., Greer, E., & Pedersen, C. (1989). Medial preoptic area oxytocin and female sexual receptivity. *Behavioral Neuroscience*, *103*(3), 655.

Caldwell, J. D., Johns, J., Faggin, B., Senger, M., & Pendersen, C. (1994). Infusion of an oxytocin antagonist into the medial preoptic area prior to progesterone inhibits sexual receptivity and increases rejection in female rats. *Hormones and Behavior*, *28*(3), 288–302.

Caldwell, J. D., Walker, C. H., Pedersen, C. A., Baraka, A. S., & Mason, G. A. (1994). Estrogen increases affinity of oxytocin receptors in the medial preoptic area-anterior hypothalamus. *Peptides*, *15*(6), 1079–1084.

Calhoun, J. B. (1963). *The ecology and sociology of the Norway rat* (Issue 1008). US Department of Health, Education, and Welfare, Public Health Service.

Canteras, N., Simerly, R., & Swanson, L. (1994). Organization of projections from the ventromedial nucleus of the hypothalamus: A Phaseolus vulgaris-leucoagglutinin study in the rat. *Journal of Comparative Neurology*, *348*(1), 41–79.

Carlson, R., & De Feo, V. (1965). Role of the pelvic nerve vs. The abdominal sympathetic nerves in the reproductive function of the female rat. *Endocrinology*, *77*(6), 1014–1022.

Carr, W. J., Loeb, L. S., & Dissinger, M. L. (1965). Responses of rats to sex odors. *Journal of Comparative and Physiological Psychology*, *59*(3), 370–377. https://doi.org/10.1037/h0022036

Carter, C. S., Devries, A. C., & Getz, L. L. (1995). Physiological substrates of mammalian monogamy: The prairie vole model. *Neuroscience & Biobehavioral Reviews*, *19*(2), 303–314.

Castro, D. C., & Berridge, K. C. (2014). Opioid hedonic hotspot in nucleus accumbens shell: Mu, delta, and kappa maps for enhancement of sweetness “liking” and “wanting.” *Journal of Neuroscience*, *34*(12), 4239–4250.

Chen, K.-K., Chan, S. H., Chang, L. S., & Chan, J. Y. (1997). Participation of paraventricular nucleus of hypothalamus in central regulation of penile erection in the rat. *The Journal of Urology*, *158*(1), 238–244.

Chester, R. V., & Zucker, I. (1970). Influence of male copulatory behavior on sperm transport, pregnancy and pseudopregnancy in female rats. *Physiology & Behavior*, *5*(1), 35–43. https://doi.org/10.1016/0031-9384(70)90010-7

Cheung, S., Ballew, J. R., Moore, K. E., & Lookingland, K. J. (1998). Contribution of dopamine neurons in the medial zona incerta to the innervation of the central nucleus of the amygdala, horizontal diagonal band of Broca and hypothalamic paraventricular nucleus. *Brain Research*, *808*(2), 174–181.

Cho, M. M., DeVries, A. C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). *Behavioral Neuroscience*, *113*(5), 1071.

Cibrian-Llanderal, T., Tecamachaltzi-Silvaran, M., Triana-Del Rio, R., Pfaus, J. G., Manzo, J., & Coria-Avila, G. A. (2010). Clitoral stimulation modulates appetitive sexual behavior and facilitates reproduction in rats. *Physiology & Behavior*, *100*(2), 148–153.

Clark, A. S., Kelton, M. C., Guarraci, F. A., & Clyons, E. Q. (2004). Hormonal status and test condition, but not sexual experience, modulate partner preference in female rats. *Hormones and Behavior*, *45*(5), 314–323.

Clutton-Brock, T. (2017). Reproductive competition and sexual selection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1729), 20160310.

Coirini, H., Schumacher, M., Flanagan, L., & McEwen, B. (1991). Transport of estrogen-induced oxytocin receptors in the ventromedial hypothalamus. *Journal of Neuroscience*, *11*(11), 3317–3324.

Conn, P. M., & Crowley, W. F. (1994). Gonadotropin-releasing hormone and its analogs. *Annual Review of Medicine*, *45*, 391–405. https://doi.org/10.1146/annurev.med.45.1.391

Coolen, L. M., Fitzgerald, M. E., Yu, L., & Lehman, M. (2004). Activation of μ opioid receptors in the medial preoptic area following copulation in male rats. *Neuroscience*, *124*(1), 11–21.

Coolen, L. M., Peters, H. J., & Veening, J. G. (1996). Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: A sex comparison. *Brain Research*, *738*(1), 67–82.

Coopersmith, C., Candurra, C., & Erskine, M. S. (1996). Effects of paced mating and intromissive stimulation on feminine sexual behavior and estrus termination in the cycling rat. *Journal of Comparative Psychology*, *110*(2), 176–186. https://doi.org/10.1037/0735-7036.110.2.176

Coria-Avila, G. A., Gavrila, A. M., Boulard, B., Charron, N., Stanley, G., & Pfaus, J. G. (2008). Neurochemical basis of conditioned partner preference in the female rat: II. Disruption by flupenthixol. *Behavioral Neuroscience*, *122*(2), 396–406. https://doi.org/10.1037/0735-7044.122.2.396

Coria-Avila, G. A., Jones, S. L., Solomon, C. E., Gavrila, A. M., Jordan, G. J., & Pfaus, J. G. (2006). Conditioned partner preference in female rats for strain of male. *Physiol Behav*, *88*(4–5), 529–537. https://doi.org/10.1016/j.physbeh.2006.05.001

Coria-Avila, G. A., Ouimet, A. J., Pacheco, P., Manzo, J., & Pfaus, J. G. (2005). Olfactory Conditioned Partner Preference in the Female Rat. *Behavioral Neuroscience*, *119*(3), 716–725. https://doi.org/10.1037/0735-7044.119.3.716

Coria-Avila, G. A., & Pfaus, J. G. (2007). Neuronal activation by stimuli that predict sexual reward in female rats. *Neuroscience*, *148*(3), 623–632. https://doi.org/10.1016/j.neuroscience.2007.05.052

Coria-Avila, G. A., Pfaus, J. G., Hernandez, M. E., Manzo, J., & Pacheco, P. (2004). Timing between ejaculations changes paternity success. *Physiol Behav*, *80*(5), 733–737. https://doi.org/10.1016/j.physbeh.2003.12.008

Coria-Avila, G. A., Solomon, C. E., Vargas, E. B., Lemme, I., Ryan, R., Ménard, S., Gavrila, A. M., & Pfaus, J. G. (2008). Neurochemical basis of conditioned partner preference in the female rat: I. Disruption by naloxone. *Behavioral Neuroscience*, *122*(2), 385–395. https://doi.org/10.1037/0735-7044.122.2.385

Cummings, J. A., & Becker, J. B. (2012). Quantitative assessment of female sexual motivation in the rat: Hormonal control of motivation. *Journal of Neuroscience Methods*, *204*(2), 227–233.

Curtis, J. T., & Wang, Z. (2003). Forebrain c-fos expression under conditions conducive to pair bonding in female prairie voles (Microtus ochrogaster). *Physiology & Behavior*, *80*(1), 95–101.

Cushing, B. S., & Carter, C. S. (2000). Peripheral pulses of oxytocin increase partner preferences in female, but not male, prairie voles. *Hormones and Behavior*, *37*(1), 49–56.

Dale, H. H. (1906). On some physiological actions of ergot. *The Journal of Physiology*, *34*(3), 163.

Dale, H. H. (1909). The action of extracts of the pituitary body. *Biochemical Journal*, *4*(9), 427.

Daniels, D., & Flanagan-Cato, L. M. (2000). Functionally-defined compartments of the lordosis neural circuit in the ventromedial hypothalamus in female rats. *Journal of Neurobiology*, *45*(1), 1–13.

Darwin, C. (1859). *On the Origin of Species, 1859*. Routledge. https://doi.org/10.4324/9780203509104

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. John Murray.

Dass, S. A. H., Vasudevan, A., Dutta, D., Soh, L. J. T., Sapolsky, R. M., & Vyas, A. (2011). Protozoan Parasite Toxoplasma gondii Manipulates Mate Choice in Rats by Enhancing Attractiveness of Males. *PLOS ONE*, *6*(11), e27229. https://doi.org/10.1371/journal.pone.0027229

de Boer, R. A., Vega-Trejo, R., Kotrschal, A., & Fitzpatrick, J. L. (2021). Meta-analytic evidence that animals rarely avoid inbreeding. *Nature Ecology & Evolution*, *5*(7), 949–964.

Deaner, R. O., Khera, A. V., & Platt, M. L. (2005). Monkeys pay per view: Adaptive valuation of social images by rhesus macaques. *Current Biology: CB*, *15*(6), 543–548. https://doi.org/10.1016/j.cub.2005.01.044

Desjardins, G. C., Brawer, J. R., & Beaudet, A. (1990). Distribution of μ, δ and κ opioid receptors in the hypothalamus of the rat. *Brain Research*, *536*(1–2), 114–123.

Dewsbury, D. A., & Hartung, T. G. (1980). Copulatory behaviour and differential reproduction of laboratory rats in a two-male, one-female competitive situation. *Animal Behaviour*, *28*(1), 95–102.

Di Marino, V., & Lepidi, H. (2014). *Anatomic study of the clitoris and the bulbo-clitoral organ* (Vol. 91). Springer.

Diakow, C. (1975). Motion picture analysis of rat mating behavior. *Journal of Comparative and Physiological Psychology*, *88*(2), 704.

Diamond, M. (1970). Intromission Pattern and Species Vaginal Code in Relation to Induction of Pseudopregnancy. *Science*, *169*(3949), 995–997. https://doi.org/10.1126/science.169.3949.995

DiBenedictis, B. T., Helfand, A. I., Baum, M. J., & Cherry, J. A. (2014). A quantitative comparison of the efferent projections of the anterior and posterior subdivisions of the medial amygdala in female mice. *Brain Research*, *1543*, 101–108.

Dittrich, R., Mueller, A., Oppelt, P. G., Hoffmann, I., Beckmann, M. W., & Maltaris, T. (2009). Differences in muscarinic-receptor agonist–, oxytocin-, and prostaglandin-induced uterine contractions. *Fertility and Sterility*, *92*(5), 1694–1700. https://doi.org/10.1016/j.fertnstert.2008.08.117

Dölen, G., Darvishzadeh, A., Huang, K. W., & Malenka, R. C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature*, *501*(7466), 179–184.

Dunbar, R. I. M. (1982). Intraspecific Variations in Mating Strategy. In P. P. G. Bateson & P. H. Klopfer (Eds.), *Ontogeny* (pp. 385–431). Springer US. https://doi.org/10.1007/978-1-4615-7578-8_9

Duridanova, D. B., Nedelcheva, M. D., & Gagov, H. S. (1997). Oxytocin-induced changes in single cell K+ currents and smooth muscle contraction of guinea-pig gastric antrum. *European Journal of Endocrinology*, *136*(5), 531–538. https://doi.org/10.1530/eje.0.1360531

Dutertre, S., Croker, D., Daly, N. L., Andersson, Å., Muttenthaler, M., Lumsden, N. G., Craik, D. J., Alewood, P. F., Guillon, G., & Lewis, R. J. (2008). Conopressin-T from Conus tulipa Reveals an Antagonist Switch in Vasopressin-like Peptides *. *Journal of Biological Chemistry*, *283*(11), 7100–7108. https://doi.org/10.1074/jbc.M706477200

Edmonds, S., Zoloth, S., & Adler, N. (1972). Storage of copulatory stimulation in the female rat. *Physiology & Behavior*, *8*(2), 161–164.

Egli, M., Bertram, R., Toporikova, N., Sellix, M. T., Blanco, W., & Freeman, M. E. (2006). Prolactin secretory rhythm of mated rats induced by a single injection of oxytocin. *American Journal of Physiology-Endocrinology and Metabolism*, *290*(3), E566–E572. https://doi.org/10.1152/ajpendo.00427.2005

Eklund, A. (1997). The major histocompatibility complex and mating preferences in wild house mice (*mus domesticus*). *Behavioral Ecology*, *8*(6), 630–634. https://doi.org/10.1093/beheco/8.6.630

Eliava, M., Melchior, M., Knobloch-Bollmann, H. S., Wahis, J., da Silva Gouveia, M., Tang, Y., Ciobanu, A. C., Triana del Rio, R., Roth, L. C., Althammer, F., Chavant, V., Goumon, Y., Gruber, T., Petit-Demoulière, N., Busnelli, M., Chini, B., Tan, L. L., Mitre, M., Froemke, R. C., … Grinevich, V. (2016). A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron*, *89*(6), 1291–1304. https://doi.org/10.1016/j.neuron.2016.01.041

Ermisch, A., Rühle, H.-J., Landgraf, R., & Hess, J. (1985). Blood—Brain Barrier and Peptides. *Journal of Cerebral Blood Flow & Metabolism*, *5*(3), 350–357. https://doi.org/10.1038/jcbfm.1985.49

Erskine, M. S. (1985). Effects of paced coital stimulation on estrus duration in intact cycling rats and ovariectomized and ovariectomized-adrenalectomized hormone-primed rats. *Behavioral Neuroscience*, *99*(1), 151.

Erskine, M. S. (1995). Prolactin release after mating and genitosensory stimulation in females. *Endocrine Reviews*, *16*(4), 508–528.

Erskine, M. S. (2005). *Learning about sex: Conditioning of partner preference: Theoretical comment on Coria-Avila et al.(2005).*

Erskine, M. S., & Baum, M. (1982). Effects of paced coital stimulation on termination of estrus and brain indoleamine levels in female rats. *Pharmacology Biochemistry and Behavior*, *17*(4), 857–861.

Erskine, M. S., Kornberg, E., & Cherry, J. A. (1989). Paced copulation in rats: Effects of intromission frequency and duration on luteal activation and estrous length. *Physiology & Behavior*, *45*(1), 33–39.

Faivre, B., Grégoire, A., Préault, M., Cézilly, F., & Sorci, G. (2003). Immune activation rapidly mirrored in a secondary sexual trait. *Science*, *300*(5616), 103–103.

Fang, L.-Y., Quan, R.-D., & Kaba, H. (2008). Oxytocin facilitates the induction of long-term potentiation in the accessory olfactory bulb. *Neuroscience Letters*, *438*(2), 133–137.

Ferguson, L. M., Ahrens, A. M., Longyear, L. G., & Aldridge, J. W. (2020). Neurons of the ventral tegmental area encode individual differences in motivational "wanting" for reward cues. *Journal of Neuroscience*, *40*(46), 8951–8963.

Ferkin, M. (2018). Odor Communication and Mate Choice in Rodents. *Biology*, *7*(1), 13. https://doi.org/10.3390/biology7010013

Fisher, R. A. (1930). *The genetical theory of natural selection* (pp. xiv, 272). Clarendon Press. https://doi.org/10.5962/bhl.title.27468

Fisher, R. A. (1958). *The genetical theory of natural selection*. Dover Publications.

Flanagan, L. M., Pfaus, J. G., Pfaff, D. W., & McEwen, B. S. (1993). Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. *Neuroendocrinology*, *58*(3), 352–358.

Fox, J., & Weisberg, S. (2019). *An {R} Companion to Applied Regression*.

Freeman, M. E., & Neill, J. D. (1972). The pattern of prolactin secretion during pseudopregnancy in the rat: A daily nocturnal surge. *Endocrinology*, *90*(5), 1292–1294. https://doi.org/10.1210/endo-90-5-1292

Freeman, M. E., Smith, M. S., Nazian, S. J., & Neill, J. D. (1974). Ovarian and hypothalamic control of the daily surges of prolactin secretion during pseudopregnancy in the rat. *Endocrinology*, *94*(3), 875–882. https://doi.org/10.1210/endo-94-3-875

French, D., Fitzpatrick, D., & Law, O. T. (1972). Operant investigation of mating preference in female rats. *Journal of Comparative and Physiological Psychology*, *81*(2), 226.

Fuchs, A.-R., Fuchs, F., Husslein, P., Soloff, M. S., & Fernström, M. J. (1982). Oxytocin receptors and human parturition: A dual role for oxytocin in the initiation of labor. *Science*, *215*(4538), 1396–1398.

Fusco, G., & Minelli, A. (2019). *The Biology of Reproduction*. Cambridge University Press; Cambridge Core. https://doi.org/10.1017/9781108758970

Gainer, H., Sarne, Y., & Brownstein, M. J. (1977). Biosynthesis and axonal transport of rat neurohypophysial proteins and peptides. *The Journal of Cell Biology*, *73*(2), 366–381.

Galef, B. G., Lim, T. C., & Gilbert, G. S. (2008). Evidence of mate choice copying in Norway rats, Rattus norvegicus. *Animal Behaviour*, *75*(3), 1117–1123.

Gawienowski, A. M., DeNicola, D. B., & Stacewicz-Sapuntzakis, M. (1976). Androgen dependence of a marking pheromone in rat urine. *Hormones and Behavior*, *7*(4), 401–405.

Gelez, H., Poirier, S., Facchinetti, P., Allers, K. A., Wayman, C., Alexandre, L., & Giuliano, F. (2010). Neuroanatomical Evidence for a Role of Central Melanocortin-4 Receptors and Oxytocin in the Efferent Control of the Rodent Clitoris and Vagina. *The Journal of Sexual Medicine*, *7*(6), 2056–2067. https://doi.org/10.1111/j.1743-6109.2010.01760.x

Georges, F., & Aston-Jones, G. (2001). Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. *Journal of Neuroscience*, *21*(16), RC160–RC160.

Georgescu, M., Afonso, V., Graham, M., & Pfaus, J. (2014). Glutamate release in the ventromedial hypothalamus of the female rat during copulation: Modulation by estradiol. *Hormones and Behavior*, *65*(2), 119–126.

Georgescu, M., Cyr, D., & Pfaus, J. G. (2012). AMPA/kainate receptors in the ventromedial hypothalamus mediate the effects of glutamate on estrus termination in the rat. *Pharmacology Biochemistry and Behavior*, *102*(1), 146–150.

Georgescu, M., & Pfaus, J. G. (2006a). Role of glutamate receptors in the ventromedial hypothalamus in the regulation of female rat sexual behaviors: I. Behavioral effects of glutamate and its selective receptor agonists AMPA, NMDA and kainate. *Pharmacology Biochemistry and Behavior*, *83*(2), 322–332.

Georgescu, M., & Pfaus, J. G. (2006b). Role of glutamate receptors in the ventromedial hypothalamus in the regulation of female rat sexual behaviors: II. Behavioral effects of selective glutamate receptor antagonists AP-5, CNQX, and DNQX. *Pharmacology Biochemistry and Behavior*, *83*(2), 333–341. https://doi.org/10.1016/j.pbb.2006.02.019

Georgescu, M., Sabongui, C., Del Corpo, A., Marsan, L., & Pfaus, J. G. (2009). Vaginocervical stimulation induces Fos in glutamate neurons in the ventromedial hypothalamus: Attenuation by estrogen and progesterone. *Hormones and Behavior*, *56*(4), 450–456.

Georgiadis, J. R., Kringelbach, M. L., & Pfaus, J. G. (2012). Sex for fun: A synthesis of human and animal neurobiology. *Nature Reviews. Urology*, *9*(9), 486–498. https://doi.org/10.1038/nrurol.2012.151

Gerall, A. A., & Dunlap, J. L. (1973). The Effect of Experience and Hormones on the Initial Receptivity in Female and Male Rats. *Physiology & Behavior*, *10*(5), 851–854. https://doi.org/10.1016/0031-9384(73)90053-x

Getz, L. L., Carter, C. S., & Gavish, L. (1981). The Mating System of the Prairie Vole, Microtus ochrogaster: Field and Laboratory Evidence for Pair-Bonding. *Behavioral Ecology and Sociobiology*, *8*(3), 189–194.

Gimpl, G., & Fahrenholz, F. (2001). The Oxytocin Receptor System: Structure, Function, and Regulation. *Physiological Reviews*, *81*(2), 629–683. https://doi.org/10.1152/physrev.2001.81.2.629

Giuliano, F., & Julia-Guilloteau, V. (2006). Neurophysiology of female genital response. In *Women's Sexual Function and Dysfunction: Study, Diagnosis, and Treatment*. London: Taylor & Francis.

Giuliano, F., Rampin, O., & Allard, J. (2002). Neurophysiology and pharmacology of female genital sexual response. *Journal of Sex & Marital Therapy*, *28*(sup1), 101–121.

Giuliano, F., Rampin, O., Brown, K., Courtois, F., Benoit, G., & Jardin, A. (1996). Stimulation of the medial preoptic area of the hypothalamus in the rat elicits increases in intracavernous pressure. *Neuroscience Letters*, *209*(1), 1–4.

Gorzalka, B. B., Heddema, G. M., Lester, G. L., & Hanson, L. A. (1997). Beta-endorphin inhibits and facilitates lordosis behaviour in rats depending on ventricular site of administration. *Neuropeptides*, *31*(5), 517–521. https://doi.org/10.1016/s0143-4179(97)90048-2

Gorzalka, B. B., & Lester, G. L. (1987). Oxytocin-induced facilitation of lordosis behaviour in rats is progesterone-dependent. *Neuropeptides*, *10*(1), 55–65.

Goudreau, J. L., Falls, W. M., Lookingland, K. J., & Moore, K. E. (1995). Periventricular-hypophysial dopaminergic neurons innervate the intermediate but not the neural lobe of the rat pituitary gland. *Neuroendocrinology*, *62*(2), 147–154.

Goyeneche, A. A., Deis, R. P., Gibori, G., & Telleria, C. M. (2003). Progesterone Promotes Survival of the Rat Corpus Luteum in the Absence of Cognate Receptors1. *Biology of Reproduction*, *68*(1), 151–158. https://doi.org/10.1095/biolreprod.102.007898

Graham, J. M., & Desjardins, C. (1980). Classical conditioning: Induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. *Science*, *210*(4473), 1039–1041. https://doi.org/10.1126/science.7434016

Graham, M. D., Gardner Gregory, J., Hussain, D., Brake, W. G., & Pfaus, J. G. (2015). Ovarian steroids alter dopamine receptor populations in the medial preoptic area of female rats: Implications for sexual motivation, desire, and behaviour. *European Journal of Neuroscience*, *42*(12), 3138–3148. https://doi.org/10.1111/ejn.13121

Graham, M. D., & Pfaus, J. G. (2010). Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. *Pharmacology Biochemistry and Behavior*, *97*(2), 284–292. https://doi.org/10.1016/j.pbb.2010.08.012

Graham, M. D., & Pfaus, J. G. (2012). Differential effects of dopamine antagonists infused to the medial preoptic area on the sexual behavior of female rats primed with estrogen and progesterone. *Pharmacology Biochemistry and Behavior*, *102*(4), 532–539.

Green, R., Luttge, W. G., & Whalen, R. E. (1970). Induction of receptivity in ovariectomized female rats by a single intravenous injection of estradiol-17β. *Physiology & Behavior*, *5*(2), 137–141. https://doi.org/10.1016/0031-9384(70)90056-9

Griffin, G. D., Ferri-Kolwicz, S. L., Reyes, B. A., Van Bockstaele, E. J., & Flanagan-Cato, L. M. (2010). Ovarian hormone-induced reorganization of oxytocin-labeled dendrites and synapses lateral to the

hypothalamic ventromedial nucleus in female rats. *Journal of Comparative Neurology*, *518*(22), 4531–4545.

Grinevich, V., Knobloch-Bollmann, H. S., Eliava, M., Busnelli, M., & Chini, B. (2016). Assembling the Puzzle: Pathways of Oxytocin Signaling in the Brain. *Biological Psychiatry*, *79*(3), 155–164. https://doi.org/10.1016/j.biopsych.2015.04.013

Grinevich, V., & Neumann, I. D. (2021). Brain oxytocin: How puzzle stones from animal studies translate into psychiatry. *Molecular Psychiatry*, *26*(1), 265–279. https://doi.org/10.1038/s41380-020-0802-9

Grippo, A. J., Trahanas, D. M., Zimmerman II, R. R., Porges, S. W., & Carter, C. S. (2009). Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. *Psychoneuroendocrinology*, *34*(10), 1542–1553.

Guarraci, F. A., & Clark, A. S. (2006). Ibotenic acid lesions of the medial preoptic area disrupt the expression of partner preference in sexually receptive female rats. *Brain Research*, *1076*(1), 163–170.

Gunnet, J. W., & Freeman, M. E. (1983). The Mating-induced Release of Prolactin: A Unique Neuroendrocine Response*. *Endocrine Reviews*, *4*(1), 44–61. https://doi.org/10.1210/edrv-4-1-44

Halliday, T. (1983). The study of mate choice. *Mate Choice*, *1*, 462.

Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: A role for parasites? *Science*, *218*(4570), 384–387.

Hancock, M. (1976). Cells of origin of hypothalamo-spinal projections in the rat. *Neuroscience Letters*, *3*(4), 179–184.

Hardy, D. F., & Debold, J. F. (1971). Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. *Physiology & Behavior*, *7*(4), 643–645. https://doi.org/10.1016/0031-9384(71)90120-X

Hardy, D. F., & DeBold, J. F. (1972). Effects of coital stimulation upon behavior of the female rat. *Journal of Comparative and Physiological Psychology*, *78*(3), 400–408. https://doi.org/10.1037/h0032536

Hardy, K. R. (1964). An appetitional theory of sexual motivation. *Psychological Review*, *71*(1), 1.

Helena, C. V., Cristancho-Gordo, R., Gonzalez-Iglesias, A. E., Tabak, J., Bertram, R., & Freeman, M. E. (2011). *Systemic oxytocin induces a prolactin secretory rhythm via the pelvic nerve in ovariectomized rats*. *301*, 6.

Hicks, C., Ramos, L., Dampney, B., Baracz, S. J., McGregor, I. S., & Hunt, G. E. (2016). Regional c-Fos expression induced by peripheral oxytocin administration is prevented by the vasopressin 1A receptor antagonist SR49059. *Brain Research Bulletin*, *127*, 208–218. https://doi.org/10.1016/j.brainresbull.2016.10.005

Holley, A., Bellevue, S., Vosberg, D., Wenzel, K., Roorda, S., & Pfaus, J. G. (2015). The role of oxytocin and vasopressin in conditioned mate guarding behavior in the female rat. *Physiology & Behavior*, *144*, 7–14. https://doi.org/10.1016/j.physbeh.2015.02.039

Holley, A., Shalev, S., Bellevue, S., & Pfaus, J. G. (2014). Conditioned mate-guarding behavior in the female rat. *Physiology & Behavior*, *131*, 136–141. https://doi.org/10.1016/j.physbeh.2014.04.034

Hollis, K. L. (1984). The biological function of Pavlovian conditioning: The best defense is a good offense. *Journal of Experimental Psychology: Animal Behavior Processes*, *10*(4), 413.

Hung, L. W., Neuner, S., Polepalli, J. S., Beier, K. T., Wright, M., Walsh, J. J., Lewis, E. M., Luo, L., Deisseroth, K., & Dölen, G. (2017). Gating of social reward by oxytocin in the ventral tegmental area. *Science*, *357*(6358), 1406–1411.

Huxley, J. S. (1938). Darwin's theory of sexual selection and the data subsumed by it, in the light of recent research. *The American Naturalist*, *72*(742), 416–433.

Inenaga, K., Karman, H., Yamashita, H., Tribollet, E., Raggenbass, M., & Dreifuss, J.-J. (1991). Oxytocin excites neurons located in the ventromedial nucleus of the guinea-pig hypothalamus. *Journal of Neuroendocrinology*, *3*(5), 569–573.

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences*, *89*(13), 5981–5985.

Ismail, N., Gelez, H., Lachapelle, I., & Pfaus, J. G. (2009). Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat. *Physiology & Behavior*, *96*(2), 201–208. https://doi.org/10.1016/j.physbeh.2008.09.013

Janeway Jr, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). The major histocompatibility complex and its functions. In *Immunobiology: The Immune System in Health and Disease. 5th edition*. Garland Science.

Japundzic-Zigon, N. (2013). Vasopressin and Oxytocin in Control of the Cardiovascular System. *Current Neuropharmacology*, *11*(2), 218–230. https://doi.org/10.2174/1570159X11311020008

Jenkins, W. J., & Becker, J. B. (2003). Dynamic increases in dopamine during paced copulation in the female rat. *European Journal of Neuroscience*, *18*(7), 1997–2001.

Jin, D., Liu, H.-X., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., Shnayder, N. A., Yamada, K., Noda, M., Seike, T., Fujita, K., Takasawa, S., Yokoyama, S., Koizumi, K., Shiraishi, Y., Tanaka, S., Hashii, M., Yoshihara, T., Higashida, K., … Higashida, H. (2007). CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature*, *446*(7131), 41–45. https://doi.org/10.1038/nature05526

Johnston, C. A., Lopez, F., Samson, W. K., & Negro-Vilar, A. (1990). Physiologically important role for central oxytocin in the preovulatory release of luteinizing hormone. *Neuroscience Letters*, *120*(2), 256–258.

Johnston, C. A., & Negro-Vilar, A. (1988). Role of oxytocin on prolactin secretion during proestrus and in different physiological or pharmacological paradigms. *Endocrinology*, *122*(1), 341–350.

Jones, S. L., Farrell, S., Gregory, J. G., & Pfaus, J. G. (2013). Sensitization of sexual behavior in ovariectomized rats by chronic estradiol treatment. *Hormones and Behavior*, *64*(1), 8–18.

Joseph, S. A., Pilcher, W. H., & Bennett-Clarke, C. (1983). Immunocytochemical localization of ACTH perikarya in nucleus tractus solitarius: Evidence for a second opiocortin neuronal system. *Neuroscience Letters*, *38*(3), 221–225.

Kavaliers, M., & Choleris, E. (2017). Social Cognition and the Neurobiology of Rodent Mate Choice. *Integrative and Comparative Biology*, *57*(4), 846–856. https://doi.org/10.1093/icb/icx042

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241.

Keller, L., & Reeve, H. K. (1995). Why do females mate with multiple males? The sexually selected sperm hypothesis. *Advances in the Study of Behaviour*, *24*, 291–316.

Kennett, J. E., & McKee, D. T. (2012). Oxytocin: An Emerging Regulator of Prolactin Secretion in the Female Rat: Oxytocin stimulates prolactin secretion. *Journal of Neuroendocrinology*, *24*(3), 403–412. https://doi.org/10.1111/j.1365-2826.2011.02263.x

Keverne, E. B. (2004). Importance of olfactory and vomeronasal systems for male sexual function. *Physiology & Behavior*, *83*(2), 177–187. https://doi.org/10.1016/j.physbeh.2004.08.013

Kippin, T. E., Cain, S. W., & Pfaus, J. G. (2003). Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. *Neuroscience*, *117*(4), 971–979. https://doi.org/10.1016/S0306-4522(02)00972-7

Kleiman, D. G. (1977). Monogamy in Mammals. *The Quarterly Review of Biology*, *52*(1), 39–69. https://doi.org/10.1086/409721

Kleitz-Nelson, H. K., Dominguez, J. M., & Ball, G. F. (2010). Dopamine release in the medial preoptic area is related to hormonal action and sexual motivation. *Behavioral Neuroscience*, *124*(6), 773.

Knobloch, H. S., Charlet, A., Hoffmann, L. C., Eliava, M., Khrulev, S., Cetin, A. H., Osten, P., Schwarz, M. K., Seeburg, P. H., Stoop, R., & Grinevich, V. (2012). Evoked Axonal Oxytocin Release in the Central Amygdala Attenuates Fear Response. *Neuron*, *73*(3), 553–566. https://doi.org/10.1016/j.neuron.2011.11.030

Knobloch, H. S., & Grinevich, V. (2014). Evolution of oxytocin pathways in the brain of vertebrates. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00031

Kokko, H., Brooks, R., McNamara, J. M., & Houston, A. I. (2002). The sexual selection continuum. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1498), 1331–1340. https://doi.org/10.1098/rspb.2002.2020

Kokko, H., Jennions, M. D., & Brooks, R. (2006). Unifying and Testing Models of Sexual Selection. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 43–66. https://doi.org/10.1146/annurev.ecolsys.37.091305.110259

Komisaruk, B. R., Adler, N. T., & Hutchison, J. (1972). Genital sensory field: Enlargement by estrogen treatment in female rats. *Science*, *178*(4067), 1295–1298.

Komisaruk, B. R., & Diakow, C. (1973). Lordosis Reflex Intensity in Rats in Relation to the Estrous Cycle, Ovariectomy, Estrogen Administration and Mating Behavior. *Endocrinology*, *93*(3), 548–557. https://doi.org/10.1210/endo-93-3-548

Komisaruk, B. R., & Sansone, G. (2003). Neural pathways mediating vaginal function: The vagus nerves and spinal cord oxytocin. *Scandinavian Journal of Psychology*, *44*(3), 241–250.

Kornberg, E., & Erskine, M. S. (1994). Effects of differential mating stimulation on the onset of prolactin surges in pseudopregnant rats. *Psychoneuroendocrinology*, *19*(4), 357–371. https://doi.org/10.1016/0306-4530(94)90016-7

Kow, L.-M., Harlan, R. E., Shivers, B. D., & Pfaff, D. W. (1985). Inhibition of the lordosis reflex in rats by intrahypothalamic infusion of neural excitatory agents: Evidence that the hypothalamus contains separate inhibitory and facilitatory elements. *Brain Research*, *341*(1), 26–34.

Kuehn, R. E., & Beach, F. A. (1963). Quantitative measurement of sexual receptivity in female rats. *Behaviour*, *21*(3–4), 282–299. https://doi.org/10.1163/156853963X00202

Kumar, V., Vasudevan, A., Soh, L. J. T., Le Min, C., Vyas, A., Zewail-Foote, M., & Guarraci, F. A. (2014). Sexual attractiveness in male rats is associated with greater concentration of major urinary proteins. *Biology of Reproduction*, *91*(6), 150–151.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 863.

Lakens, D., Scheel, A. M., & Isager, P. M. (2018). Equivalence Testing for Psychological Research: A Tutorial. *Advances in Methods and Practices in Psychological Science*, *1*(2), 259–269. https://doi.org/10.1177/2515245918770963

Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences*, *78*(6), 3721–3725. https://doi.org/10.1073/pnas.78.6.3721

Larcher, A., Neculcea, J., Breton, C., Arslan, A., Rozen, F., Russo, C., & Zingg, H. (1995). Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment. *Endocrinology*, *136*(12), 5350–5356.

Le Moëne, O., & Snoeren, E. M. (2018). Mate choice could be random in female rats (Rattus norvegicus). *Physiology & Behavior*, *184*, 1–5. https://doi.org/10.1016/j.physbeh.2017.10.031

Lehman, M. N., Coolen, L. M., & Goodman, R. L. (2010). Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology*, *151*(8), 3479–3489.

Lehmann, M. L., & Erskine, M. S. (2004). Induction of pseudopregnancy using artificial VCS: Importance of lordosis intensity and prestimulus estrous cycle length. *Hormones and Behavior*, *45*(2), 75–83. https://doi.org/10.1016/j.yhbeh.2003.09.011

Leng, G., & Sabatier, N. (2014). Electrophysiology of Magnocellular Neurons *in Vivo*. In W. E. Armstrong & J. G. Tasker (Eds.), *Neurophysiology of Neuroendocrine Neurons* (pp. 1–28). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118606803.ch1

Lenth, R. V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.7.5). https://CRAN.R-project.org/package=emmeans

Lenth, R. V., Love, J., & Herve, M. (2018). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.7.5). https://CRAN.R-project.org/package=emmeans

Liao, P.-Y., Chiu, Y.-M., Yu, J.-H., & Chen, S.-K. (2020). Mapping central projection of oxytocin neurons in unmated mice using cre and alkaline phosphatase reporter. *Frontiers in Neuroanatomy*, *14*, 559402.

Liberzon, I., Trujillo, K. A., Akil, H., & Young, E. A. (1997). Motivational properties of oxytocin in the conditioned place preference paradigm. *Neuropsychopharmacology*, *17*(6), 353–359.

Lim, M. M., Bielsky, I. F., & Young, L. J. (2005). Neuropeptides and the social brain: Potential rodent models of autism. *International Journal of Developmental Neuroscience*, *23*(2–3), 235–243.

Liu, Y., & Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience*, *121*(3), 537–544. https://doi.org/10.1016/S0306-4522(03)00555-4

Lodder, J., & Zeilmaker, G. (1976). Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. *Journal of Comparative and Physiological Psychology*, *90*(10), 925.

Lorenz, T. A., Harte, C. B., Hamilton, L. D., & Meston, C. M. (2012). Evidence for a curvilinear relationship between sympathetic nervous system activation and women's physiological sexual arousal. *Psychophysiology*, *49*(1), 111–117. https://doi.org/10.1111/j.1469-8986.2011.01285.x

Ludwig, M., Apps, D., Menzies, J., Patel, J. C., & Rice, M. E. (2016). Dendritic release of neurotransmitters. *Comprehensive Physiology*, *7*(1), 235–252. https://doi.org/10.1002/cphy.c160007

Luiten, P., Ter Horst, G., & Steffens, A. (1987). The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Progress in Neurobiology*, *28*(1), 1–54.

Lukas, M., Toth, I., Veenema, A. H., & Neumann, I. D. (2013). Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. *Psychoneuroendocrinology*, *38*(6), 916–926.

Mac Cionnaith, C. E., Lemay, A., Gomez-Perales, E. L., Robert, G., Cernik, R., Brake, W. G., & Pfaus, J. G. (2020). Fos expression is increased in oxytocin neurons of female rats with a sexually conditioned

mate preference for an individual male rat. *Hormones and Behavior*, *117*, 104612. https://doi.org/10.1016/j.yhbeh.2019.104612

Macdonald, D. W., Berdoy, M., & Smith, P. (1995). Stability of social status in wild rats: Age and the role of settled dominance. *Behaviour*, *132*(3–4), 193–212.

MacLeod, R. M., Fontham, E. H., & Lehmeyer, J. E. (1970). Prolactin and growth hormone production as influenced by catecholamines and agents that affect brain catecholamines. *Neuroendocrinology*, *6*(5–6), 283–294.

Marson, L., & Foley, K. (2004). Identification of neural pathways involved in genital reflexes in the female: A combined anterograde and retrograde tracing study. *Neuroscience*, *127*(3), 723–736.

Matthews, M., & Adler, N. T. (1977). Facilitative and inhibitory influences of reproductive behavior on sperm transport in rats. *Journal of Comparative and Physiological Psychology*, *91*(4), 727–741. https://doi.org/10.1037/h0077364

McCann, S. M., & Rettori, V. (1996). The role of nitric oxide in reproduction. *Proceedings of the Society for Experimental Biology and Medicine*, *211*(1), 7–15.

McCarthy, M. M., Malik, K. F., & Feder, H. H. (1990). Increased GABAergic transmission in medial hypothalamus facilitates lordosis but has the opposite effect in preoptic area. *Brain Research*, *507*(1), 40–44.

McCarthy, M. M., Masters, D. B., Fiber, J. M., López-Colomé, A.-M., Beyer, C., Komisaruk, B. R., & Feder, H. H. (1991). GABAergic control of receptivity in the female rat. *Neuroendocrinology*, *53*(5), 473–479.

McClintock, M. K. (1978). Estrous synchrony and its mediation by airborne chemical communication (Rattus norvegicus). *Hormones and Behavior*, *10*(3), 264–275. https://doi.org/10.1016/0018-506x(78)90071-5

McClintock, M. K. (1984). Group Mating in the Domestic Rat as a Context for Sexual Selection: Consequences for the Analysis of Sexual Behavior and Neuroendocrine Responses. In *Advances in the Study of Behavior* (Vol. 14, pp. 1–50). Elsevier. https://doi.org/10.1016/S0065-3454(08)60298-3

McClintock, M. K., & Adler, N. T. (1978). The Role of the Female During Copulation in Wild and Domestic Norway Rats (Rattus Norvegicus). *Behaviour*, *67*(1–2), 67–95. https://doi.org/10.1163/156853978X00260

McClintock, M. K., & Anisko, J. J. (1982). Group mating among Norway rats I. Sex differences in the pattern and neuroendocrine consequences of copulation. *Animal Behaviour*, *30*(2), 398–409. https://doi.org/10.1016/S0003-3472(82)80051-1

McClintock, M. K., Anisko, J. J., & Adler, N. T. (1982). Group mating among Norway rats: II. The social dynamics of copulation: Competition, cooperation, and mate choice. *Animal Behaviour*, *30*(2), 410–425. https://doi.org/10.1016/S0003-3472(82)80052-3

McCracken, J. A., Custer, E. E., & Lamsa, J. C. (1999). Luteolysis: A neuroendocrine-mediated event. *Physiological Reviews*, *79*(2), 263–323.

McGraw, L. A., & Young, L. J. (2010). The prairie vole: An emerging model organism for understanding the social brain. *Trends in Neurosciences*, *33*(2), 103–109. https://doi.org/10.1016/j.tins.2009.11.006

McHenry, J. A., Otis, J. M., Rossi, M. A., Robinson, J. E., Kosyk, O., Miller, N. W., McElligott, Z. A., Budygin, E. A., Rubinow, D. R., & Stuber, G. D. (2017). Hormonal gain control of a medial preoptic area social reward circuit. *Nature Neuroscience*, *20*(3), 449–458. https://doi.org/10.1038/nn.4487

McKenna, K. E., Chung, S. K., & McVary, K. T. (1991). A model for the study of sexual function in anesthetized male and female rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *261*(5), R1276–R1285. https://doi.org/10.1152/ajpregu.1991.261.5.R1276

Meddle, S. L., Bishop, V. R., Gkoumassi, E., van Leeuwen, F. W., & Douglas, A. J. (2007). Dynamic changes in oxytocin receptor expression and activation at parturition in the rat brain. *Endocrinology*, *148*(10), 5095–5104.

Meerts, S. H., & Clark, A. S. (2007). Female rats exhibit a conditioned place preference for nonpaced mating. *Hormones and Behavior*, *51*(1), 89–94.

Meerts, S. H., & Clark, A. S. (2009). Artificial vaginocervical stimulation induces a conditioned place preference in female rats. *Hormones and Behavior*, *55*(1), 128–132.

Meerts, S. H., Schairer, R. S., Farry-Thorn, M. E., Johnson, E. G., & Strnad, H. K. (2014). Previous sexual experience alters the display of paced mating behavior in female rats. *Hormones and Behavior*, *65*(5), 497–504.

Meerts, S. H., Strnad, H. K., & Schairer, R. S. (2015). Paced mating behavior is affected by clitoral-vaginocervical lidocaine application in combination with sexual experience. *Physiology & Behavior*, *140*, 222–229.

Meguro, Y., Miyano, K., Hirayama, S., Yoshida, Y., Ishibashi, N., Ogino, T., Fujii, Y., Manabe, S., Eto, M., & Nonaka, M. (2018). Neuropeptide oxytocin enhances μ opioid receptor signaling as a positive allosteric modulator. *Journal of Pharmacological Sciences*, *137*(1), 67–75.

Meisel, R. L., Camp, D. M., & Robinson, T. E. (1993). A microdialysis study of ventral striatal dopamine during sexual behavior in female Syrian hamsters. *Behavioural Brain Research*, *55*(2), 151–157.

Melis, M. R., Succu, S., Iannucci, U., & Argiolas, A. (1997). Oxytocin increases nitric oxide production in the paraventricular nucleus of the hypothalamus of male rats: Correlation with penile erection and yawning. *Regulatory Peptides*, *69*(2), 105–111. https://doi.org/10.1016/S0167-0115(97)00002-5

Meredith, M., & Westberry, J. M. (2004). Distinctive responses in the medial amygdala to same-species and different-species pheromones. *Journal of Neuroscience*, *24*(25), 5719–5725.

Mermelstein, P. G., & Becker, J. B. (1995). Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. *Behavioral Neuroscience*, *109*(2), 354.

Meston, C. M., Gorzalka, B. B., & Wright, J. M. (1997). Inhibition of subjective and physiological sexual arousal in women by clonidine. *Psychosomatic Medicine*, *59*(4), 399–407.

Meston, C. M., & Heiman, J. R. (1998). Ephedrine-activated physiological sexual arousal in women. *Archives of General Psychiatry*, *55*(7), 652–656.

Meston, C. M., Moe, I. V., & Gorzalka, B. B. (1996). Effects of sympathetic inhibition on receptive, proceptive, and rejection behaviors in the female rat. *Physiology & Behavior*, *59*(3), 537–542.

Micevych, P. E., & Meisel, R. L. (2017). Integrating Neural Circuits Controlling Female Sexual Behavior. *Frontiers in Systems Neuroscience*, *11*. https://www.frontiersin.org/articles/10.3389/fnsys.2017.00042

Miller, S. M., & Lonstein, J. S. (2009). Dopaminergic projections to the medial preoptic area of postpartum rats. *Neuroscience*, *159*(4), 1384–1396.

Moore, C. L., & Wong, L. (1992). Copulatory behavior, reproduction, and sperm competition in two strains of male rats. *Physiology & Behavior*, *51*(3), 569–573. https://doi.org/10.1016/0031-9384(92)90181-Z

Moore, K., & Lookingland, K. (1995). *Dopaminergic neuronal systems in the hypothalamus, Psychopharmacology: The Fourth Generation of Progress. Edited by Bloom FE, Kupfer DJ*.

Mott, M., & Goeders, N. (2022). Methamphetamine-Induced Vaginal Lubrication & Associated Hormonal Changes. *The Journal of Sexual Medicine*, *19*(4), S29.

Munarriz, R., Kim, S. W., Kim, N. N., Traish, A., & Goldstein, I. (2003). A Review of the Physiology and Pharmacology of Peripheral (Vaginal and Clitoral) Female Genital Arousal in the Animal Model. *Journal of Urology*, *170*(2S). https://doi.org/10.1097/01.ju.0000075352.03144.15

Neumann, I. D., Maloumby, R., Beiderbeck, D. I., Lukas, M., & Landgraf, R. (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology*, *38*(10), 1985–1993. https://doi.org/10.1016/j.psyneuen.2013.03.003

Noguri, T., Hatakeyama, D., Kitahashi, T., Oka, K., & Ito, E. (2022). Profile of dorsal root ganglion neurons: Study of oxytocin expression. *Molecular Brain*, *15*(1), 1–8.

Normandin, J. J., & Murphy, A. Z. (2008). Nucleus paragigantocellularis afferents in male and female rats: Organization, gonadal steroid receptor expression, and activation during sexual behavior. *The Journal of Comparative Neurology*, *508*(5), 771–794. https://doi.org/10.1002/cne.21704

Normandin, J. J., & Murphy, A. Z. (2011). Somatic genital reflexes in rats with a nod to humans: Anatomy, physiology, and the role of the social neuropeptides. *Hormones and Behavior*, *59*(5), 656–665. https://doi.org/10.1016/j.yhbeh.2011.02.006

Northrop, L. E., & Erskine, M. S. (2008). Selective oxytocin receptor activation in the ventrolateral portion of the ventromedial hypothalamus is required for mating-induced pseudopregnancy in the female rat. *Endocrinology*, *149*(2), 836–842. https://doi.org/10.1210/en.2007-1040

Nyuyki, K. D., Waldherr, M., Baeuml, S., & Neumann, I. D. (2011). Yes, I am ready now: Differential effects of paced versus unpaced mating on anxiety and central oxytocin release in female rats. *PLoS One*, *6*(8), e23599.

O'Donald, P. (1980). *Genetic models of sexual selection*. Cambridge University Press.

Ortiz-Miranda, S., Dayanithi, G., Coccia, V., Custer, E., Alphandery, S., Mazuc, E., Treistman, S., & Lemos, J. (2003). M-Opioid Receptor Modulates Peptide Release From Rat Neurohypophysial Terminals By Inhibiting Ca2+ Influx. *Journal of Neuroendocrinology*, *15*(9), 888–894.

Panksepp, J., Herman, B., Vilberg, T., Bishop, P., & DeEskinazi, F. (1980). Endogenous opioids and social behavior. *Neuroscience & Biobehavioral Reviews*, *4*(4), 473–487.

Parada, M., Abdul-Ahad, F., Censi, S., Sparks, L., & Pfaus, J. G. (2011). Context alters the ability of clitoral stimulation to induce a sexually-conditioned partner preference in the rat. *Horm Behav*, *59*(4), 520–527. https://doi.org/10.1016/j.yhbeh.2011.02.001

Parada, M., Chamas, L., Censi, S., Coria-Avila, G., & Pfaus, J. G. (2010). Clitoral stimulation induces conditioned place preference and Fos activation in the rat. *Horm Behav*, *57*(2), 112–118. https://doi.org/10.1016/j.yhbeh.2009.05.008

Parada, M., Jafari, N., & Pfaus, J. G. (2013). Sexual experience blocks the ability of clitoral stimulation to induce a conditioned place preference in the rat. *Physiol Behav*, *119*, 97–102. https://doi.org/10.1016/j.physbeh.2013.06.005

Paredes, R. G., & Alonso, A. (1997). Sexual behavior regulated (paced) by the female induces conditioned place preference. *Behavioral Neuroscience*, *111*(1), 123.

Paredes, R. G., & Vazquez, B. (1999). What do female rats like about sex? Paced mating. *Behavioural Brain Research*, *105*(1), 117–127. https://doi.org/10.1016/S0166-4328(99)00087-X

Parsons, B., MacLusky, N. J., Krey, L., Pfaff, D. W., & McEwen, B. S. (1980). The Temporal Relationship between Estrogen-Inducible Progestin Receptors in the Female Rat Brain and the Time Course of Estrogen Activation of Mating Behavior*. *Endocrinology*, *107*(3), 774–779. https://doi.org/10.1210/endo-107-3-774

Pavlov, I. (1927). *Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex*. Oxford Univ. Press; /z-wcorg/.

Paxinos, G., & Watson, C. (2006). *The Rat Brain in Stereotaxic Coordinates*. Academic Press.

Pecina, S. (2005). Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do -Opioids Cause Increased Hedonic Impact of Sweetness? *Journal of Neuroscience*, *25*(50), 11777–11786. https://doi.org/10.1523/JNEUROSCI.2329-05.2005

Peciña, S., & Berridge, K. C. (2013). Dopamine or opioid stimulation of nucleus accumbens similarly amplify cue-triggered 'wanting'for reward: Entire core and medial shell mapped as substrates for PIT enhancement. *European Journal of Neuroscience*, *37*(9), 1529–1540.

Pessina, M. A., Hoyt, R. F., Jr., Goldstein, I., & Traish, A. M. (2006). Differential Effects of Estradiol, Progesterone, and Testosterone on Vaginal Structural Integrity. *Endocrinology*, *147*(1), 61–69. https://doi.org/10.1210/en.2005-0870

Peters, L. C., Kristal, M. B., & Komisaruk, B. R. (1987). Sensory innervation of the external and internal genitalia of the female rat. *Brain Research*, *408*(1–2), 199–204.

Petraglia, F., Florio, P., Nappi, C., & Genazzani, A. R. (1996). Peptide signaling in human placenta and membranes: Autocrine, paracrine, and endocrine mechanisms. *Endocrine Reviews*, *17*(2), 156–186.

Pfaff, D., Montgomery, M., & Lewis, C. (1977). Somatosensory determinants of lordosis in female rats: Behavioral definition of the estrogen effect. *Journal of Comparative and Physiological Psychology*, *91*(1), 134–145. https://doi.org/10.1037/h0077305

Pfaff, D. W. (1980). *Estrogens and brain function*. Springer.

Pfaff, D. W., Kow, L.-M., Loose, M. D., & Flanagan-Cato, L. M. (2008). Reverse engineering the lordosis behavior circuit. *Hormones and Behavior*, *54*(3), 347–354. https://doi.org/10.1016/j.yhbeh.2008.03.012

Pfaus, J. G. (2010). *Dopamine: Helping males copulate for at least 200 million years: Theoretical comment on Kleitz-Nelson et al.(2010).*

Pfaus, J. G., Damsma, G., Wenkstern, D., & Fibiger, H. C. (1995). Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. *Brain Res*, *693*(1–2), 21–30. https://doi.org/10.1016/0006-8993(95)00679-k

Pfaus, J. G., Erickson, K. A., & Talianakis, S. (2013). Somatosensory conditioning of sexual arousal and copulatory behavior in the male rat: A model of fetish development. *Physiol Behav*, *122*, 1–7. https://doi.org/10.1016/j.physbeh.2013.08.005

Pfaus, J. G., & Heeb, M. M. (1997). Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Res Bull*, *44*(4), 397–407. https://doi.org/10.1016/s0361-9230(97)00219-0

Pfaus, J. G., Jones, S. L., Flanagan-Cato, L. M., & Blaustein, J. D. (2015). Female sexual behavior. *Knobil and Neill's Physiology of Reproduction*, *2*, 2287–2370.

Pfaus, J. G., Kippin, T. E., & Centeno, S. (2001). Conditioning and Sexual Behavior: A Review. *Hormones and Behavior*, *40*(2), 291–321. https://doi.org/10.1006/hbeh.2001.1686

Pfaus, J. G., Kippin, T. E., Coria-Avila, G. A., Gelez, H., Afonso, V. M., Ismail, N., & Parada, M. (2012). Who, what, where, when (and maybe even why)? How the experience of sexual reward connects sexual desire, preference, and performance. *Arch Sex Behav*, *41*(1), 31–62. https://doi.org/10.1007/s10508-012-9935-5

Pfaus, J. G., Kleopoulos, S., Mobbs, C., Gibbs, R., & Pfaff, D. (1993). Sexual stimulation activates c-fos within estrogen-concentrating regions of the female rat forebrain. *Brain Research*, *624*(1–2), 253–267.

Pfaus, J. G., Manitt, C., & Coopersmith, C. B. (2006). Effects of pelvic, pudendal, or hypogastric nerve cuts on Fos induction in the rat brain following vaginocervical stimulation. *Physiology & Behavior*, *89*(5), 627–636. https://doi.org/10.1016/j.physbeh.2006.07.022

Pfaus, J. G., Marcangione, C., Smith, W. J., Manitt, C., & Abillamaa, H. (1996). Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: Modulation by steroid hormones. *Brain Research*, *741*(1–2), 314–330. https://doi.org/10.1016/S0006-8993(96)00985-7

Pfaus, J. G., & Phillips, A. G. (1991). Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. *Behav Neurosci*, *105*(5), 727–743. https://doi.org/10.1037//0735-7044.105.5.727

Pfaus, J. G., Quintana, G. R., Mac Cionnaith, C. E., Gerson, C. A., Dubé, S., & Coria-Avila, G. A. (2020). Conditioning of Sexual Interests and Paraphilias in Humans Is Difficult to See, Virtually Impossible to Test, and Probably Exactly How It Happens: A Comment on Hsu and Bailey (2020). *Archives of Sexual Behavior*. https://doi.org/10.1007/s10508-020-01739-2

Pfaus, J. G., Scardochio, T., Parada, M., Gerson, C., Quintana, G. R., & Coria-Avila, G. A. (2016). Do rats have orgasms? *Socioaffect Neurosci Psychol*, *6*, 31883. https://doi.org/10.3402/snp.v6.31883

Pfaus, J. G., Smith, W. J., Byrne, N., & Stephens, G. (2000). Appetitive and Consummatory Sexual Behaviors of Female Rats in Bilevel Chambers. *Hormones and Behavior*, *37*(1), 96–107. https://doi.org/10.1006/hbeh.1999.1562

Pfaus, J. G., Smith, W. J., & Coopersmith, C. B. (1999). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. I. A correlational and factor analysis and the effects of ovarian hormones. *Hormones and Behavior*, *35*(3), 224–240. https://doi.org/10.1006/hbeh.1999.1516

Pizzari, T., & Birkhead, T. (2002). The sexually-selected sperm hypothesis: Sex-biased inheritance and sexual antagonism. *Biological Reviews*, *77*(2), 183–209.

Polston, E. K., & Erskine, M. S. (1995). Patterns of induction of the immediate-early genes c-fos and egr-1 in the female rat brain following differential amounts of mating stimulation. *Neuroendocrinology*, *62*(4), 370–384. https://doi.org/10.1159/000127027

Potts, W. K., Manning, C. J., & Wakeland, E. K. (1991). Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, *352*(6336), 619–621.

Poulin, R. (1996). Richness, nestedness, and randomness in parasite infracommunity structure. *Oecologia*, *105*(4), 545–551.

Prokop, Z. M., Michalczyk, Ł., Drobniak, S. M., Herdegen, M., & Radwan, J. (2012). Meta-analysis suggests choosy females get sexy sons more than "good genes." *Evolution: International Journal of Organic Evolution*, *66*(9), 2665–2673.

Quintana, D. S., Kemp, A. H., Alvares, G. A., & Guastella, A. J. (2013). A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness. *Frontiers in Neuroscience*, *7*. https://doi.org/10.3389/fnins.2013.00048

Quintana, G. R., Birrel, M., Marceau, S., Kalantari, N., Bowden, J., Bachoura, Y., Borduas, E., Lemay, V., Payne, J. W., Cionnaith, C. M., & Pfaus, J. G. (2019). Differential disruption of conditioned ejaculatory preference in the male rat based on different sensory modalities by micro-infusions of naloxone to the medial preoptic area or ventral tegmental area. *Psychopharmacology (Berl)*, *236*(12), 3613–3623. https://doi.org/10.1007/s00213-019-05334-9

Quintana, G. R., Desbiens, S., Marceau, S., Kalantari, N., Bowden, J., & Pfaus, J. G. (2019). Conditioned partner preference in male and female rats for a somatosensory cue. *Behav Neurosci*, *133*(2), 188–197. https://doi.org/10.1037/bne0000300

Quintana, G. R., Mac Cionnaith, C. E., & Pfaus, J. G. (2022). Behavioral, neural, and molecular mechanisms of conditioned mate preference: The role of opioids and first experiences of sexual reward. *International Journal of Molecular Sciences*, *23*(16), 8928.

R Development Core Team. (2018). *R: A Language and Environment for Statistical Computing.* https://www.r-project.org/

Resendez, S. L., Dome, M., Gormley, G., Franco, D., Nevarez, N., Hamid, A. A., & Aragona, B. J. (2013). -Opioid Receptors within Subregions of the Striatum Mediate Pair Bond Formation through Parallel

Yet Distinct Reward Mechanisms. *Journal of Neuroscience*, *33*(21), 9140–9149. https://doi.org/10.1523/JNEUROSCI.4123-12.2013

Rettori, V., Canteros, G., Renoso, R., Gimeno, M., & McCann, S. M. (1997). Oxytocin stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamic explants by releasing nitric oxide. *Proceedings of the National Academy of Sciences*, *94*(6), 2741–2744. https://doi.org/10.1073/pnas.94.6.2741

Roberts, S. C., & Gosling, L. M. (2003). Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, *35*(1), 103–106.

Robinson, G., & Evans, J. J. (1990). Oxytocin has a role in gonadotrophin regulation in rats. *Journal of Endocrinology*, *125*(3), 425–432. https://doi.org/10.1677/joe.0.1250425

Romero-Fernandez, W., Borroto-Escuela, D. O., Agnati, L. F., & Fuxe, K. (2013). Evidence for the existence of dopamine d2-oxytocin receptor heteromers in the ventral and dorsal striatum with facilitatory receptor–receptor interactions. *Molecular Psychiatry*, *18*(8), 849–850. https://doi.org/10.1038/mp.2012.103

Ross, H. E., Cole, C. D., Smith, Y., Neumann, I. D., Landgraf, R., Murphy, A. Z., & Young, L. J. (2009). Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. *Neuroscience*, *162*(4), 892–903. https://doi.org/10.1016/j.neuroscience.2009.05.055

Ross, H. E., Freeman, S. M., Spiegel, L. L., Ren, X., Terwilliger, E. F., & Young, L. J. (2009). Variation in Oxytocin Receptor Density in the Nucleus Accumbens Has Differential Effects on Affiliative Behaviors in Monogamous and Polygamous Voles. *Journal of Neuroscience*, *29*(5), 1312–1318. https://doi.org/10.1523/JNEUROSCI.5039-08.2009

RStudio Team. (2016). *RStudio: Integrated Development for R.* http://www.rstudio.com/

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential dopamine release dynamics in the nucleus accumbens core and shell reveal complementary signals for error prediction and incentive motivation. *Journal of Neuroscience*, *35*(33), 11572–11582.

Sairam, M. (1980). Inhibition of LH-induced ovulation in the rat by a hormonal antagonist. *Contraception*, *21*(6), 651–657.

Salo, A. L., & Dewsbury, D. A. (1995). Three experiments on mate choice in meadow voles (Microtus pennsylvanicus). *Journal of Comparative Psychology*, *109*(1), 42.

Sansone, G. R., Gerdes, C. A., Steinman, J. L., Winslow, J. T., Ottenweller, J. E., Komisaruk, B. R., & Insel, T. R. (2002). Vaginocervical Stimulation Releases Oxytocin within the Spinal Cord in Rats. *Neuroendocrinology*, *75*(5), 306–315. https://doi.org/10.1159/000057340

Sansone, G. R., & Komisaruk, B. R. (2001). Evidence that oxytocin is an endogenous stimulator of autonomic sympathetic preganglionics: The pupillary dilatation response to vaginocervical stimulation in the rat. *Brain Research*, *898*(2), 265–271. https://doi.org/10.1016/S0006-8993(01)02192-8

Saper, C., Loewy, A., Swanson, L., & Cowan, W. (1976). Direct hypothalamo-autonomic connections. *Brain Research*, *117*(2), 305–312.

Saunders, B. T., Richard, J. M., Margolis, E. B., & Janak, P. H. (2018). Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. *Nature Neuroscience*, *21*(8), 1072–1083.

Sawchenko, P., & Swanson, L. (1983). The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. *Journal of Comparative Neurology*, *218*(2), 121–144.

Schank, J. C. (2001). Menstrual-cycle synchrony: Problems and new directions for research. *Journal of Comparative Psychology*, *115*(1), 3–15. https://doi.org/10.1037/0735-7036.115.1.3

Schober, J., Weil, Z., & Pfaff, D. (2011). How generalized CNS arousal strengthens sexual arousal (and vice versa). *Hormones and Behavior*, *59*(5), 689–695.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, *36*(2), 241–263. https://doi.org/10.1016/s0896-6273(02)00967-4

Schumacher, M., Coirini, H., Frankfurt, M., & McEwen, B. S. (1989). Localized actions of progesterone in hypothalamus involve oxytocin. *Proceedings of the National Academy of Sciences*, *86*(17), 6798–6801. https://doi.org/10.1073/pnas.86.17.6798

Schumacher, M., Coirini, H., Pfaff, D. W., & McEwen, B. S. (1990). Behavioral Effects of Progesterone Associated with Rapid Modulation of Oxytocin Receptors. *Science*, *250*(4981), 691–694. https://doi.org/10.1126/science.2173139

Schweinfurth, M. K. (2020). The social life of Norway rats (Rattus norvegicus). *ELife*, *9*, e54020. https://doi.org/10.7554/eLife.54020

Sclafani, A. (1995). How food preferences are learned: Laboratory animal models. *Proceedings of the Nutrition Society*, *54*(2), 419–427.

Segovia, S., & Guillamón, A. (1993). Sexual dimorphism in the vomeronasal pathway and sex differences in reproductive behaviors. *Brain Research Reviews*, *18*(1), 51–74. https://doi.org/10.1016/0165-0173(93)90007-M

Sharma, K., LeBlanc, R., Haque, M., Nishimori, K., Reid, M. M., & Teruyama, R. (2019). Sexually dimorphic oxytocin receptor-expressing neurons in the preoptic area of the mouse brain. *PLoS One*, *14*(7), e0219784.

Silverman, A., Hoffman, D., & Zimmerman, E. (1981). The descending afferent connections of the paraventricular nucleus of the hypothalamus (PVN). *Brain Research Bulletin*, *6*(1), 47–61.

Silverman, A.-J., & Zimmerman, E. A. (1983). Magnocellular neurosecretory system. *Annual Review of Neuroscience*, *6*(1), 357–380.

Singmann, H., Bolker, B., Westfall, J., & Aust, F. (2018). *afex: Analysis of Factorial Experiments* (1.1-1). https://CRAN.R-project.org/package=afex

Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M. S., Højsgaard, S., Fox, J., Lawrence, M. A., Mertens, U., Love, J., Lenth, R., & Christensen, R. H. B. (2022). *afex: Analysis of Factorial Experiments* (1.1-1). https://CRAN.R-project.org/package=afex

Sirinathsinghji, D. J., & Audsley, A. R. (1985). Endogenous opioid peptides participate in the modulation of prolactin release in response to cervicovaginal stimulation in the female rat. *Endocrinology*, *117*(2), 549–556.

Soden, M. E., Yee, J. X., Cuevas, B., Rastani, A., Elum, J., & Zweifel, L. S. (2022). Distinct Encoding of Reward and Aversion by Peptidergic BNST Inputs to the VTA. *Frontiers in Neural Circuits*, *16*.

Sofroniew, M. (1980). Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. *Journal of Histochemistry & Cytochemistry*, *28*(5), 475–478.

Solomon, N. G., Keane, B., Knoch, L. R., & Hogan, P. J. (2004). Multiple paternity in socially monogamous prairie voles (*Microtus ochrogaster*). *Canadian Journal of Zoology*, *82*(10), 1667–1671. https://doi.org/10.1139/z04-142

Solomon, S. (1999). POMC-derived peptides and their biological action. *Annals of the New York Academy of Sciences*, *885*(1), 22–40.

Son, S. J., Filosa, J. A., Potapenko, E. S., Biancardi, V. C., Zheng, H., Patel, K. P., Tobin, V. A., Ludwig, M., & Stern, J. E. (2013). Dendritic peptide release mediates interpopulation crosstalk between neurosecretory and preautonomic networks. *Neuron*, *78*(6), 1036–1049.

Spies, H. G., & Niswender, G. D. (1971). Levels of prolactin, LH and FSH in the serum of intact and pelvic-neurectomized rats. *Endocrinology*, *88*(4), 937–943.

Swanson, L., & Sawchenko, P. (1980). Paraventricular nucleus: A site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology*, *31*(6), 410–417.

Swanson, L., & Sawchenko, P. (1983). Hypothalamic Integration: Organization of the Paraventricular and Supraoptic Nuclei. *Annual Review of Neuroscience*, *6*(1), 269–324. https://doi.org/10.1146/annurev.ne.06.030183.001413

Swingle, W., Seay, P., Perlmutt, J., Collins, E. J., Barlow JR, G., & Fedor, E. J. (1951). An experimental study of pseudopregnancy in rat. *American Journal of Physiology-Legacy Content*, *167*(3), 586–592.

Szechtman, H., Hershkowitz, M., & Simantov, R. (1981). Sexual behavior decreases pain sensitivity and stimulated endogenous opioids in male rats. *Eur J Pharmacol*, *70*(3), 279–285. https://doi.org/10.1016/0014-2999(81)90161-8

Tamarin, R. H. (1985). *Biology of new world Microtus*. American Society of Mammalogists.

Taylor, G. T., Haller, J., & Regan, D. (1982). Female rats prefer an area vacated by a high testosterone male. *Physiology & Behavior*, *28*(6), 953–958.

Taylor, G. T., Weiss, J., & Rupich, R. (1987). Male rat behavior, endocrinology and reproductive physiology in a mixed-sex, socially stressful colony. *Physiology & Behavior*, *39*(4), 429–433.

Tennent, B. J., Smith, E. R., & Davidson, J. M. (1980). The effects of estrogen and progesterone on female rat proceptive behavior. *Hormones and Behavior*, *14*(1), 65–75.

Terkel, J., Witcher, J. A., & Adler, N. T. (1990). Evidence for "memory" of cervical stimulation for the promotion of pregnancy in rats. *Hormones and Behavior*, *24*(1), 40–49. https://doi.org/10.1016/0018-506x(90)90025-s

Thorndike, E. L. (1911). *Animal intelligence: Experimental studies*. Macmillan Press; /z-wcorg/.

Tilders, F., Berkenbosch, F., Vermes, I., Linton, E., & Smelik, P. (1985). *Role of epinephrine and vasopressin in the control of the pituitary-adrenal response to stress. 44*(1 Pt 2), 155–160.

Toates, F. (2009). An integrative theoretical framework for understanding sexual motivation, arousal, and behavior. *Journal of Sex Research*, *46*(2–3), 168–193.

Toates, F. M. (1981). The control of ingestive behaviour by internal and external stimuli—A theoretical review. *Appetite*, *2*(1), 35–50.

Toates, F. M. (1986). *Motivational systems* (Issue 4). Cambridge University Press.

Tobiansky, D. J., Roma, P. G., Hattori, T., Will, R. G., Nutsch, V. L., & Dominguez, J. M. (2013). The medial preoptic area modulates cocaine-induced activity in female rats. *Behavioral Neuroscience*, *127*(2), 293.

Tobiansky, D. J., Will, R. G., Lominac, K. D., Turner, J. M., Hattori, T., Krishnan, K., Martz, J. R., Nutsch, V. L., & Dominguez, J. M. (2016). Estradiol in the Preoptic Area Regulates the Dopaminergic Response to Cocaine in the Nucleus Accumbens. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *41*(7), 1897–1906. https://doi.org/10.1038/npp.2015.360

Tribollet, E., Audigier, S., Dubois-Dauphin, M., & Dreifuss, J. J. (1990). Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study. *Brain Research*, *511*(1), 129–140. https://doi.org/10.1016/0006-8993(90)90232-Z

Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M., & Dreifuss, J. J. (1988). Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. *Brain Research*, *442*(1), 105–118. https://doi.org/10.1016/0006-8993(88)91437-0

Tribollet, E., & Dreifuss, J. (1981). Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat: A horseradish peroxidase study. *Neuroscience*, *6*(7), 1315–1328.

Trivers, R. L. (Ed.). (1972). Parental Investment and Sexual Selection. In *Sexual Selection and the Descent of Man*. Routledge.

Van Den Pol, A. N. (1982). The magnocellular and parvocellular paraventricular nucleus of rat: Intrinsic organization. *Journal of Comparative Neurology*, *206*(4), 317–345.

Ventura-Aquino, E., Baños-Araujo, J., Fernández-Guasti, A., & Paredes, R. G. (2016). An unknown male increases sexual incentive motivation and partner preference: Further evidence for the Coolidge effect in female rats. *Physiology & Behavior*, *158*, 54–59. https://doi.org/10.1016/j.physbeh.2016.02.026

Vincent, P. A., & Etgen, A. M. (1993). Steroid priming promotes oxytocin-induced norepinephrine release in the ventromedial hypothalamus of female rats. *Brain Research*, *620*(2), 189–194. https://doi.org/10.1016/0006-8993(93)90155-G

Voogt, J. L., Lee, Y., Yang, S., & Arbogast, L. (2001). Regulation of prolactin secretion during pregnancy and lactation. *Progress in Brain Research*, *133*, 173–185.

Wagenaar, D. A., Hamilton, M. S., Huang, T., Kristan, W. B., & French, K. A. (2010). A hormone-activated central pattern generator for courtship. *Current Biology*, *20*(6), 487–495.

Wagner, C. K., Eaton, M. J., Moore, K. E., & Lookingland, K. J. (1995). Efferent projections from the region of the medial zona incerta containing A13 dopaminergic neurons: A PHA-L anterograde tract-tracing study in the rat. *Brain Research*, *677*(2), 229–237. https://doi.org/10.1016/0006-8993(95)00128-D

Wang, D., He, X., Zhao, Z., Feng, Q., Lin, R., Sun, Y., Ding, T., Xu, F., Luo, M., & Zhan, C. (2015). Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons. *Frontiers in Neuroanatomy*, *9*, 40.

Weismann, A. (1889). The significance of sexual reproduction in the theory of natural selection. *Essays upon Heredity and Kindred Biological Problems*, *1*, 255–332.

Wersinger, S., Baum, M., & Erskine, M. (1993). Mating-lnduced FOS-Like Immunoreactivity in the Rat Forebrain: A Sex Comparison and a Dimorphic Effect of Pelvic Nerve Transection. *Journal of Neuroendocrinology*, *5*(5), 557–568.

Whalen, R. E. (1966). Sexual motivation. *Psychological Review*, *73*(2), 151.

White, N. R., Cagiano, R., Moises, A. U., & Barfield, R. J. (1990). Changes in mating vocalizations over the ejaculatory series in rats (Rattus norvegicus). *Journal of Comparative Psychology*, *104*(3), 255–262. https://doi.org/10.1037/0735-7036.104.3.255

White, P. J., Fischer, R. B., & Meunier, G. F. (1986). Female discrimination of male dominance by urine odor cues in hamsters. *Physiology & Behavior*, *37*(2), 273–277.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., & RStudio. (2022). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (3.3.6). https://CRAN.R-project.org/package=ggplot2

Williams, J. R., Catania, K. C., & Carter, C. S. (1992). Development of partner preferences in female prairie voles (Microtus ochrogaster): The role of social and sexual experience. *Hormones and Behavior*, *26*(3), 339–349.

Williams, J. R., Insel, T. R., Harbaugh, C. R., & Carter, C. S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). *Journal of Neuroendocrinology*, *6*(3), 247–250.

Winland, C., Bolton, J. L., Ford, B., Jampana, S., Tinker, J., Frohardt, R. J., Guarraci, F. A., & Zewail-Foote, M. (2012). "Nice guys finish last": Influence of mate choice on reproductive success in Long–Evans rats. *Physiology & Behavior*, *105*(3), 868–876. https://doi.org/10.1016/j.physbeh.2011.10.022

Witt, D. M. (1995). Oxytocin and rodent sociosexual responses: From behavior to gene expression. *Neuroscience & Biobehavioral Reviews*, *19*(2), 315–324. https://doi.org/10.1016/0149-7634(95)00006-Z

Xiao, L., Priest, M. F., & Kozorovitskiy, Y. (2018). Oxytocin functions as a spatiotemporal filter for excitatory synaptic inputs to VTA dopamine neurons. *Elife*, *7*.

Xiao, L., Priest, M. F., Nasenbeny, J., Lu, T., & Kozorovitskiy, Y. (2017). Biased oxytocinergic modulation of midbrain dopamine systems. *Neuron*, *95*(2), 368–384.

Yamamoto, Y., Liang, M., Munesue, S., Deguchi, K., Harashima, A., Furuhara, K., Yuhi, T., Zhong, J., Akther, S., Goto, H., Eguchi, Y., Kitao, Y., Hori, O., Shiraishi, Y., Ozaki, N., Shimizu, Y., Kamide, T., Yoshikawa, A., Hayashi, Y., … Higashida, H. (2019). Vascular RAGE transports oxytocin into the brain to elicit its maternal bonding behaviour in mice. *Communications Biology*, *2*(1), 76. https://doi.org/10.1038/s42003-019-0325-6

Yamashita, K., & Kitano, T. (2013). Molecular evolution of the oxytocin–oxytocin receptor system in eutherians. *Molecular Phylogenetics and Evolution*, *67*(2), 520–528.

Yang, S.-P., Lee, Y., & Voogt, J. L. (2000). Involvement of endogenous opioidergic neurons in modulation of prolactin secretion in response to mating in the female rat. *Neuroendocrinology*, *72*(1), 20–28.

Yao, S., Bergan, J., Lanjuin, A., & Dulac, C. (2017). Oxytocin signaling in the medial amygdala is required for sex discrimination of social cues. *Elife*, *6*.

Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Hormones and Behavior*, *40*(2), 133–138.

Young, L. J., Murphy Young, A. Z., & Hammock, E. A. D. (2005). Anatomy and neurochemistry of the pair bond. *The Journal of Comparative Neurology*, *493*(1), 51–57. https://doi.org/10.1002/cne.20771

Young, L. J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nature Neuroscience*, *7*(10), 1048–1054. https://doi.org/10.1038/nn1327

Zahavi, A. (1975). Mate selection—A selection for a handicap. *Journal of Theoretical Biology*, *53*(1), 205–214.

Zewail-Foote, M., Diehl, A., Benson, A., Lee, K. H., & Guarraci, F. A. (2009). Reproductive success and mate choice in Long–Evans rats. *Physiology & Behavior*, *96*(1), 98–103. https://doi.org/10.1016/j.physbeh.2008.09.007