MUNI

## COMMENTARY TO HABILITATION THESIS[1]

For the Habilitation Thesis, I have submitted this commentary along with an extended commentary written in thesis format. The 23 papers that are included in the habilitation thesis and as part of the extended commentary consist of reviews and original empirical reports that lay out a framework to understand the neurochemistry of sexual reward states, and how first experiences of sexual reward come to sensitize dopamine, oxytocin, and vasopressin systems toward sexual incentive cues. The reviews were written either by me as first author or by colleagues and former students with me as senior author. In the case where I was first author, colleagues and students typically made small editorial comments that I incorporated into the paper and provided figures. In the case where I was senior author, I would write assigned sections of the paper and perform a final review prior to submission.

Almost all of the original studies were written by former graduate students who used them as part of their theses and dissertations. I served as senior author on those papers and made final editorial decisions. For my students, the supervision of their theses and these papers was 100% and consisted of making comments and editorial corrections that helped them learn how to write. As their thesis supervisor, my role in the research direction was also between 80% and 100%, with extensive discussions of the experiments and methodology before any rats were ordered. I was not a micromanager, but I did have final say over student training in experimental techniques, quality control of the experiments themselves, and what was sent out for publication from my laboratory. For work with colleagues that are not students (e.g., Review paper #2), the writing was divided into relatively equal sections and then put together by the first author. In that paper, however, the review of the animal work was written by me, and represents 40% of the research direction taken.

The specific work reported in these papers is collected and summarized in more detail in the extended commentary of the habilitation thesis. Conclusions are reached regarding the contribution of this research to our understanding of how the brain is organized for sexual behavior, attraction, and bonding, and what this means for human sexual behavior and the development of preferences for both natural sexual stimuli and fetish objects.

### 1. Review papers

[1] **PFAUS, James G.**, Tod E. KIPPIN, Genaro A. CORIA-AVILA, Helene GELEZ, Veronica M. AFONSO, Nafissa ISMAIL and Mayte PARADA. Who, What, Where, When (and Maybe Even Why)? How the Experience of Sexual Reward Connects Sexual Desire, Preference, and Performance. *Archives Of Sexual Behavior* [online]. 2012, **41**(1), 31–62. ISSN 1573-2800. Available at: doi:10.1007/s10508-012-9935-5.

Although sexual behavior is controlled by hormonal and neurochemical actions in the brain, sexual experience induces a degree of plasticity that allows animals to form instrumental and Pavlovian associations that predict sexual outcomes, thereby directing the strength of sexual responding. This narrative review summarizes the results of our early studies up to that point on how experience with sexual reward strengthens the development of sexual behavior and induces sexually-conditioned place and partner preferences in rats. In both male and female rats, early sexual experience with partners scented with a neutral or even noxious odor induces a preference for scented partners in subsequent choice tests. Those preferences can also be induced by injections of morphine or oxytocin paired with a male rat's first

[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

exposure to scented females, indicating that pharmacological activation of opioid or oxytocin receptors can "stand in" for the sexual reward-related neurochemical processes normally activated by sexual stimulation. Conversely, conditioned place or partner preferences can be blocked by the opioid receptor antagonist naloxone. A somatosensory cue (a rodent jacket) paired with sexual reward comes to elicit sexual arousal in male rats, such that paired rats with the jacket off show dramatic copulatory deficits. In this review, we propose that endogenous opioid activation forms the basis of sexual reward, which also sensitizes hypothalamic and mesolimbic dopamine systems in the presence of cues that predict sexual reward. Those systems act to focus attention on, and activate goal-directed behavior toward, reward-related sexual stimuli. Thus, a critical period exists during an individual's early sexual experience that creates a "love map" or Gestalt of features, movements, feelings, and interpersonal interactions associated with sexual reward.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
|---|---|---|---|
| 80 | 100 | 90 | 100 |

[2] GEORGIADIS, Janniko R., Morten L. KRINGELBACH and **James G. PFAUS**. Sex for fun: a synthesis of human and animal neurobiology. *Nature Reviews Urology* [online]. 2012, **9**(9), 486–498. ISSN 1759-4820. Available at: doi:10.1038/nrurol.2012.151.

In this narrative review of experiments from our own and others' laboratories, we argue that sex is a fundamental pleasure that is crucial to the survival of all mammalian species (if not all vertebrate species). Though not many people would disagree with the proposition that sexual behavior depends on the brain, the neuroscientific study of human sex is still relatively taboo and much remains to be discovered. However, excellent experimental animal models (mostly in rats) are available that have uncovered major behavioral, neurochemical, and neuroanatomical characteristics of sexual behavior. Restructuring sexual behavior into broader terms reflecting behavioral states (wanting, liking, and inhibition, as has been conceived for other natural rewards, like food, and for drugs of abuse) facilitates species comparison, revealing many similarities between animal and human sexual pleasure cycles. Some of these similarities have served as fruitful avenues for new human sex research. In particular, behavioral and brain evidence clearly shows that motivational and consummatory phases are fundamentally distinct, and that genitally-induced sexual reward is a major factor in sexual learning mechanisms. My contribution was to review the animal work and write those sections, then review the work in several iterations and make editorial comments and changes until all three authors were satisfied and ready to send it out.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 80 | 25 | 33 | 40 |

[3] **PFAUS, James G.**, Tina SCARDOCHIO, Mayte PARADA, Christine GERSON, Gonzalo R. QUINTANA and Genaro A. CORIA-AVILA. Do rats have orgasms?. *Socioaffective Neuroscience & Psychology* [online]. 2016, **6**(31883, Article 31883). ISSN 2000-9011. Available at: doi:10.3402/snp.v6.31883..

This paper reviewed the available evidence from our laboratory studies and those of other laboratories that both male and female rats display orgasm-like responses (OLRs). Although humans experience orgasms with a degree of statistical regularity, they remain among the most enigmatic of sexual responses; difficult to define and even more difficult to study empirically. The question of whether animals experience orgasms is hampered by similar

lack of definition and the additional 15 necessity of making inferences from behavioral responses. In this review we define three behavioral criteria, based on dimensions of the subjective experience of human orgasms described by Mah and Binik, to infer OLRs in other species: 1) physiological criteria that include pelvic floor and anal muscle contractions that stimulate seminal emission and/or ejaculation in the male, or that stimulate uterine and cervical contractions in the female; 2) short-term behavioral changes that reflect immediate awareness of a pleasurable hedonic reward state during copulation, including vocalization patterns indicative of appetitive and consummatory reward states; and 3) long-term behavioral changes that depend on the reward state induced by the OLR, including sexual satiety, the strengthening of patterns of sexual arousal and desire in subsequent copulations, and the generation of conditioned place and partner preferences for contextual and partner-related cues associated with the reward state. We then examined whether physiological and behavioral data from observations of male and female rats during copulation, and in sexually-conditioned place- and partner-preference paradigms, are consistent with these criteria. From the available data, it become clear that both male and female rats show OLRs. Males experience this during the post-ejaculatory refractory period whereas females experience this during a similar, but shorter, refractory period induced either by paced copulation (where the female controls the initiation and rate of the copulatory stimulation they receive) or artificial clitoral stimulation that is distributed in time to match the females' own preferred intervals. The ability to infer OLRs in rats offers new possibilities to study this phenomenon in neurobiological and molecular detail, and to provide both comparative and translational perspectives that would be useful for both basic and clinical research.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
|---|---|---|---|
| 100 | 100 | 90 | 100 |

[4] QUINTANA, Gonzalo R., Conall E. MAC CIONNAITH and **James G. PFAUS**. Behavioral, Neural, and Molecular Mechanisms of Conditioned Mate Preference: The Role of Opioids and First Experiences of Sexual Reward. *International Journal Of Molecular Sciences* [online]. 2022, **23**(16, Article 8928). ISSN 1422-0067. Available at: doi:10.3390/ijms23168928.

This is also a narrative review that concentrates on the role of μ opioid receptor (MOR) activation during first experiences of sexual reward in sensitizing brain dopamine, oxytocin, and vasopressin systems toward incentive stimuli associated with that reward state. Although mechanisms of mate preference are thought to be relatively hard-wired, experience with appetitive and consummatory sexual reward has been shown to condition preferences for partner related cues and even objects that predict sexual reward. We reviewed evidence from our own work on rats, and other work using species like voles, on sexually conditioned place, partner, and ejaculatory preferences in males and females, as well as the neurochemical, molecular, and epigenetic mechanisms putatively responsible. We concluded that opioid transmission at MORs forms the basis of sexual pleasure and reward, which then sensitizes dopamine, oxytocin, and vasopressin systems responsible for attention, arousal, and bonding, leading to cortical activation that creates awareness of attraction and desire. First experiences with sexual reward states follow a pattern of sexual imprinting during which partner- and/or object-related cues become crystallized by conditioning into idiosyncratic "types" that are found sexually attractive and arousing. These mechanisms tie reward and reproduction together, blending proximate and ultimate causality in the maintenance of variability within a species. My contribution was to write a first draft of the paper with my former Ph.D. student and first author, Gonzalo Quintana, then have another former Ph.D. student, Conall Mac Cionnaith, add the most recent data from his dissertation on oxytocin

sensitization. This paper won the *Josef Hynie Prize* from the Division of Sexology, Czech Medical Association, for 2023.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 90 | 100 | 70 | 90 |

## 2. Original studies on the conditioning of sexual behavior in male rats

[5] KIPPIN, T.E., S. TALIANAKIS, L. SCHATTMANN, S. BARTHOLOMEW and **J.G. PFAUS.** Olfactory conditioning of sexual behavior in the male rat (Rattus norvegicus). *Journal Of Comparative Psychology* [online]. 1998, 112(4), 389–399. ISSN 0735-7036. Available at: doi:10.1037/0735-7036.112.4.389.

This paper replicated and extended my original finding of a conditioned preference of male rats to ejaculate with females bearing a neutral odor CS (almond extract) that had been paired previously with copulation to ejaculation (Pfaus, Jacobs, and Wong, 1986). In this study my Ph.D. student Tod Kippin and his team utilized both a paired group, where males were allowed to copulate with sexually receptive females that had a neutral odor (almond or lemon) painted on the back of their necks and anogenital region, and two control groups, an unpaired group where males were allowed to copulate with receptive females that had distilled water painted on the neck and anogenital region, or a random-paired control group where males were exposed to receptive females that had almond extract or distilled water painted on those regions randomly during different acquisition trials (making the odor or no-odour not predictive of copulation to ejaculation). Nine training trials were conducted in bilevel pacing chambers at 4-day intervals to approximate the estrous cycle of the female (see extended commentary). In the final open field test with two receptive females, one scented with almond extract and the other unscented, males in the paired group ejaculated first and more frequently with the scented females whereas males in the unpaired group ejaculated more frequently with the unscented females. Males in the random paired group did not show any preference. In the second experiment of that paper, Kippin et al. utilized an explicitly paired, unpaired, and random paired group. Explicitly paired males copulated with scented receptive females but every 48 hrs after each copulatory trial, they were placed into the bilevel chambers and received access to unscented non-receptive females. The opposite order of association was given to the explicitly unpaired males, whereas the random control group had the odor or no-odor randomly associated with receptive or non-receptive females. During the final open field test, males in the explicitly paired group ejaculated first and more frequently with the scented females, whereas males in the explicitly unpaired group not only ejaculated first and more frequently with the unscented females, they also chose them more frequently for mounts and intromissions, despite the active solicitations by both receptive females. Again, males in the random paired group did not show any preference. The preference to ejaculate with the familiar female (i.e., whose olfactory status predicted the postejaculatory sexual reward state), was termed a Conditioned Ejaculatory Preference (CEP). This paper established the procedure for conditioning and testing, and discussed potential neural and neurochemical mechanisms by which the conditioning might occur.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 90 | 100 |

[6] KIPPIN, T.E. and **J.G. PFAUS**. The development of olfactory conditioned ejaculatory preferences in the male rat I. Nature of the unconditioned stimulus. *Physiology & Behavior* [online]. 2001, 73(4), 457–469. ISSN 0031-9384. Available at: doi:10.1016/S0031-9384(01)00484-X.

This follow-up study established what the actual experience of sexual reward is that is necessary for the establishment of a CEP. Different groups of male rats were allowed during conditioning trials to copulate with scented females to 2 ejaculations, 1 ejaculation and the post-ejaculatory interval, 1 ejaculation without a post-ejaculatory interval (i.e., the female was pulled out of the bilevel chamber immediately after the male ejaculated), or 5 intromissions without ejaculation. CEP was examined on the final open-field test with two females, one scented and the other unscented. Only males that copulated to 2 ejaculations, or 1 ejaculation with post-ejaculatory exposure to the scented female, displayed CEP. Thus, ejaculation per se was necessary but not sufficient. Post-ejaculatory exposure to the scented receptive female was critical, despite the fact that males are largely asleep during this period. In fact, this period is one of consolidation of the CS as an incentive cue predictive of the UCS as a sexual reward state, consistent with Hebb's idea of "reverberation." This gave rise to the idea that dopamine sensitization might be involved, something that was studied subsequently in my laboratory using in vivo microdialysis and voltammetry to look at extracellular dopamine concentrations in the nucleus accumbens (NAc) and medial preoptic area (mPOA) in paired versus unpaired males during the presentation of the odor alone on a gauze pad. The increases in extracellular dopamine concentration in paired versus unpaired males was reported in the first review paper above.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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[7] KIPPIN, T.E., S.W. CAIN and **J.G. PFAUS**. Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. *Neuroscience* [online]. 2003, 117(4), 971–979. ISSN 0306-4522. Available at: doi:10.1016/S0306-4522(02)00972-7.

Before examining extracellular dopamine concentrations, we utilized immunohistochemistry (IHC) to examine nuclear Fos protein as a marker of neuronal activation in paired versus unpaired male brains in response to the odor alone presented on a gauze pad. Males were trained as before, given the open field choice test, and then two paired or unpaired reconditioning trials prior to being placed into a holding cage with clean bedding and presented with a gauze pad scented with the almond odor. After an hour, the animals were deeply anesthetized with sodium pentobarbital, perfused intracardially with saline and paraformaldehyde, brains removed, and prepared for IHC. 30-µm coronal sections from rostral to caudal (starting in the prefrontal cortex and ending in the central grey) were cut on a sliding microtome, quenched and washed, then exposed for 48 hr to a primary polyclonal antibody raised in rabbit to Fos, then to a biotinylated goat anti-rabbit IgG secondary 4 hr, then to a tertiary avidin-HRP complex for 4 hr, prior to 10 min of bathing in a DAB solution with 4% $NiCl_2$ (to turn the reaction product blue-black), after which the tissue were washed in Tris-buffered saline. Sections were mounted on gel-coated slides, dehydrated in a series of alcohols, and coverslipped prior to examination at the light microscopic level and the number of cells containing Fos protein in a particular region counted using NIH Image v.3. The study revealed that the paired odor activated Fos in reward-related regions of the brain, including main olfactory cortex and olfactory tubercle, NAc (more core than shell), basolateral amygdala, mPOA, and lateral hypothalamus, in paired but not unpaired males. Exposure of males in both groups to bedding from unscented estrous females activated regions of the accessory olfactory system and reward system, including the NAc core and shell, mPOA,

medial bed nucleus of the stria terminalis, medial amygdala, ventromedial hypothalamus, and ventral tegmental area. This study demonstrated that distinct neural pathways are activated by estrous odors and a sexually-conditioned odors, though there is overlap in the activation of reward-related regions, notably the NAc, mPOA and regions of the amygdala.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 70 | 100 |

[8] ISMAIL, Nafissa, Fabienne GIRARD-BERIAULT, Satoru NAKANISHI and **James G. PFAUS**. Naloxone, but Not Flupenthixol, Disrupts the Development of Conditioned Ejaculatory Preference in the Male Rat. *Behavioral Neuroscience* [online]. 2009, **123**(5), 992–999. ISSN 1939-0084. Available at: doi:10.1037/a0017096.

Another Ph.D. student, Nafissa Ismail, was interested in replicating and extending Tod Kippin's research on the CEP model. In this study, she and her team examined the neurochemical basis of the sexual reward state. As with our previous studies examining the pharmacology of partner preference in female rats (see below), this study used systemic injections of the opioid receptor antagonist naloxone, the dopamine receptor antagonist flupenthixol, or saline (separate controls for each drug) to male rats prior to 10 paired conditioning trials with almond scented, sexually receptive females in unilevel pacing chambers with a one-hole divider (see extended commentary). As always, conditioning trials were conducted at 4-day intervals. Prior to the open-field test of CEP with a scented and unscented receptive female, all males were given a systemic injection of saline so that the expectation of copulation with blunted opioid or dopamine receptor activation could be examined on partner preference. Males in the saline control groups displayed significant CEP, indicating that the injection procedure alone prior to every conditioning trial did not affect the development of CEP. Males injected with flupenthixol during conditioning also developed CEP, whereas males injected with naloxone did not, and in fact showed a preference to mount and intromit with the novel (unscented) female. Those data indicated that blockade of opioid receptors shifted the preference away from the familiar to the novel female, and suggested that the opioid receptor activation (and likely the specific activation of MORs relative to δ opioid receptors (DORs) or κ opioid receptors (KORs)), but not dopamine receptor activation, is critical for the sexual reward state induced by ejaculation. This is similar to the sexual reward state induced by paced copulation in female rats (see below and extended commentary).

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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[9] ISMAIL, Nafissa, Sherri Lee JONES, M. Dean GRAHAM, Sarah SYLVESTER and **James G. PFAUS**. Partner preference for strain of female in Long-Evans male rats. *Physiology & Behavior* [online]. 2011, **102**(3), 285–290. ISSN 0031-9384. Available at: doi:10.1016/j.physbeh.2010.09.005.

In this paper, Nafi and her team showed that CEP could be induced by the strain of female. Instead of using a neutral odor, conditioning here consisted of paired copulation in unilevel pacing chambers with a one-hole or four-hole divider with either sexually receptive Long-Evans (pigmented) or Wistar (albino) females at 4-day intervals. Males that were given 10 conditioning trials in the one-hole, but not four-hole, condition displayed significant CEP for Long-Evans females but not Wistar females, during the final open-field choice test. Those

data extended the CEP phenomenon to strain cues, some of which are visual, perhaps auditory, and possibly olfactory or pheromonal (like major histocompatibility complexes). Moreover, the significant CEP to females of the male's own pigmented strain, but not to the albino strain, is evidence of positive assortative mating (homogamy). Finally, relative to the four-hole divider, where females could pass through to the male's side at their own preferred interval, the one-hole divider induced longer contact return latencies during the early conditioning trials (as the male had to learn to move away from the hole so that the female could return; see extended commentary). This suggests that the lack of immediate reinforcement (i.e., having to wait longer) induced greater arousal in the males. In bilevel pacing chambers, males must chase the females from level to level, thus creating a similar condition of waiting, along with active pursuit, which also may increase arousal. Arousal thus becomes a necessary, but not sufficient, component of the CEP phenomenon. However, reward that rides on high arousal is well known to be more rewarding than the same reward that rides on low arousal.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 70 | 100 |

[10] **PFAUS, James G.**, Kirsten A. ERICKSON and Stella TALIANAKIS. Somatosensory conditioning of sexual arousal and copulatory behavior in the male rat: A model of fetish development. *Physiology & Behavior* [online]. 2013, **122**, 1–7. ISSN 0031-9384. Available at: doi:10.1016/j.physbeh.2013.08.005.

The experiments in this paper came from a serendipitous experience. One of my undergraduate thesis students needed good copulating males for a quick experiment. The only ones we had at the time were males that had been used as stimuli for tests of female sexual partner preference based on a neutral olfactory cue (see below and extended commentary). In those studies, stimulus males used during the open field choice tests had to be tethered in opposite corners of the open field. To do this, those males wore a Lorimer tethering jacket that fit around their upper torsos. The jacket contained a ring on the back that could be connected to a tether (in this case a 35-cm spring), allowing males ample room to copulate, but forcing the females to choose to spend time near one of the two males (e.g., scented versus unscented) to received copulatory stimulation from their preferred male. The student tried to give those males a refresher in bilevel chambers before her experiment but none of them copulated. We thought that maybe the female hormones had gone bad, so we remade them (estradiol benzoate and progesterone) and tried again. None of the males copulated despite the females showing clear and robust proceptive and receptive behaviors. Then we tried testing the males in the open field. Some made sluggish attempts to mount and intromit, but none ejaculated. As I was riding my bike home, I had an epiphany: THE JACKETS! I made an abrupt U-turn, almost getting hit by a car, and raced back to the lab. The females were still sexually receptive, so we tested the males in the bilevel chambers with the jackets on, and all copulated to ejaculation normally. This set the stage for a formal experiment to test this.

In the first experiment, two groups of sexually naïve male rats had their first 9 copulatory experiences at 4-day intervals with receptive females in bilevel chambers with or without the Lorimer tethering jacket. On the 10$^{th}$ test, half of the rats in each group were tested with the jacket on or off. Rats trained and tested without the jacket copulated normally, as did rats trained and tested with the jacket on, and rats trained without the jacket but tested with the jacket on. However, significantly fewer rats trained with the jacket on but tested with the jacket off copulated to ejaculation, and those that did made significantly fewer anticipatory level changes and had significantly longer mount, intromission, and ejaculatory latencies,

and fewer ejaculations. In the second experiment, one group of sexually naïve males was given 9 differential conditioning trials in bilevel chambers to associate the jacket on with sexual reward (copulation to ejaculation with a sexually receptive female) and the jacket off with sexual inhibition (thwarted copulatory attempts with a sexually nonreceptive female). A second group had the opposite order of association over the same 9 trials. On the 10th test, all males were tested with the jacket on. Males trained to associate the jacket with excitation displayed normal copulation whereas males trained to associate the jacket with inhibition displayed significantly fewer anticipatory level changes and ejaculations, and had significantly longer ejaculation latencies. This study showed that a somatosensory cue can signal sexual excitation or inhibition in male rats depending on the associative conditioning history. The two experiments were conducted by two undergraduate students (my co-authors) for their honors theses with 100% supervision by me. I wrote the paper and gave it to them to edit and provide figures from their theses. Once compiled, and after a final edit by the three of us, the paper was sent out for publication and is one of only a few papers I have had accepted without revisions.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 90 | 100 |

[11] QUINTANA, Gonzalo R., Andres GUIZAR, Sarah RASSI and **James G. PFAUS**. First sexual experiences determine the development of conditioned ejaculatory preference in male rats. *Learning & Memory* [online]. 2018, **25**(10), 522–532. ISSN 1549-5485. Available at: doi:10.1101/lm.048090.118.

This study by my Ph.D. student Gonzalo Quintana examined further the role of early experiences with the post-ejaculatory sexual reward state on the development of CEP. In a sister study, we reported that preexposing males to a neutral odor alone prior to its pairing with sexual reward results in latent inhibition. In this study we examined the phenomenon of unconditioned stimulus (US) preexposure, in which male rats experienced the ejaculatory reward state either one or five times with almond-scented versus unscented females prior to multiple ejaculatory trials with females in the opposite condition (e.g., males in the 1- or 5-scented reward preexposure groups received 10 subsequent ejaculatory trials at 4-day intervals with unscented females, whereas males in the 1- or 5-unscented preexposure groups received 10 subsequent ejaculatory trials at 4-day intervals with scented females). As with our previous studies, CEP was evaluated in an open field where each male had access to two receptive females, one scented and the other unscented. Males that underwent five trials of preexposure did not display a CEP for either female. Conversely, males that copulated to ejaculation once with a scented female and later trained with unscented females developed a preference for the latter, whereas males that copulated to ejaculation once with the unscented female, and then copulated to ejaculation with scented females did not show a preference for any of the females. The behavioral data show that a male rat's first sexual experience with an unscented receptive female inhibits the ability of subsequent pairings of the odor with the postejaculatory sexual reward state to become a conditioned incentive. Conversely, the male's first experience of a postejaculatory sexual reward state with scented females did not prevent the conditioning of a subsequent preference for unscented females. In contrast, five ejaculatory preexposures to either scented or unscented receptive females inhibited the development of CEP to unscented or scented receptive females, respectively. On a final test, males were exposed to the almond odor on gauze, then overdosed on sodium pentobarbital, perfused, and their brains process for Fos IHC. Males that had displayed a significant CEP for scented females had significantly greater Fos activation in the mPOA, NAc core, basolateral amygdala, and VTA., relative to males that did not develop significant CEP. These findings demonstrate the pivotal role of first sexual experiences in the establishment of future sexual partner preference in the male rat, and suggest an innate

preference for estrous odors over neutral odors that can become conditioned subsequently as predictors of sexual reward.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 80 | 100 |

[12] QUINTANA, Gonzalo R., Steve DESBIENS, Sarah MARCEAU, Narges KALANTARI, James BOWDEN and **James G. PFAUS**. Conditioned Partner Preference in Male and Female Rats for a Somatosensory Cue. *Behavioral Neuroscience* [online]. 2019, **133**(2), 188–197. ISSN 1939-0084. Available at: doi:10.1037/bne0000300.

Given the fact that male rats could form Pavlovian associations with a rodent tethering jacket and sexual reward that led to enhanced sexual arousal with the jacket on, we wondered whether males could form the same association for CEP with the jacket on a receptive female. However, in this study we examined the effect in both males and females. In the first experiment, sexually naïve Long-Evans males and females underwent 14 copulatory conditioning trials for 30 min at 4-day intervals with their opposite sex partner in unilevel pacing chambers with a one-hole divider for males and a four-hole divider for females. On the final test, each experimental male or female was placed into an open field with two sexually receptive partners, one jacketed and the other unjacketed. A trend was found for more males to ejaculate first with jacketed females relative to the unjacketed females, whereas the females had no preference. Males and females in the second experiment were exposed sequentially to jacketed, sexually receptive partners, and unjacketed, sexually nonreceptive partners prior to the final open field test. Receptive partners were ovariectomized (OVX) females given EB and P, and gonadally intact, sexually vigorous male studs. Nonreceptive partners were OVX females not given hormone replacement, and castrated sexually naïve males not given hormone replacement. Results of the second experiment showed that both males and females displayed a significant ejaculatory and copulatory preferences for the jacketed opposite-sex partner. Thus, a somatosensory cue previously used to establish sexual arousal as a contextual cue on rats can be used as a discrete, partner-based cue to establish a copulatory preference for a particular partner wearing the jacket and that stronger conditioning occurs when the jacket is explicitly paired with the sexual reward state.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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[13] QUINTANA, Gonzalo R., Morgan BIRREL, Sarah MARCEAU, Narges KALANTARI, James BOWDEN, Yvonne BACHOURA, Eric BORDUAS, Valerie LEMAY, Jason W. PAYNE, Conall MAC CIONNAITH and **James G. PFAUS**. 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* [online]. 2019, **236**(12), 3613–3623. ISSN 1432-2072. Available at: doi:10.1007/s00213-019-05334-9.

Given that systemic injections of naloxone during training block CEP for both olfactory and somatosensory (rodent jacket) cues, Gonzalo and his team examined the effect of microinfusions of naloxone or saline to the mPOA or VTA on the development of CEP for the almond odor versus the rodent jacket. Sexually naïve Long-Evans males were implanted with bilateral guide cannula aimed at either site before they underwent multi-ejaculatory conditioning trials at 4-day intervals with sexually receptive females that bore either the

almond odor or rodent jacket (see extended commentary). Infusions of NAL (1 µl/side) or SAL (1 µl/side) were made prior to each conditioning trial. All males were infused with SAL prior to the final open-field choice test with two sexually receptive females, one scented and the other unscented, or one jacketed and the other unjacketed. Males previously conditioned with SAL in either region showed significant CEP for the odor or jacket. In contrast, prior infusions of NAL to the mPOA shifted the preference towards the novel female, whereas prior infusions to the VTA abolished CEP for the odor. Subsequent detection of Fos protein induced by the odor or jacket cue showed that, relative to SAL-treated males, prior experience with NAL in the mPOA suppressed Fos in both the mPOA and VTA, whereas prior experience with NAL in to the VTA suppressed Fos in the VTA alone. These findings are reminiscent of earlier reports of the ability of naloxone to disrupt the development of conditioned place preference for the post-ejaculatory sexual reward state. So, we concluded that opioid antagonism in the mPOA produces a state of non-reward whereas in the VTA it disrupted the ability of the odor or jacket to acquire incentive properties.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 70 | 100 |

[14] MENARD, Shann, Helene GELEZ, Fabienne GIRARD-BERIAULT, Genaro CORIA-AVILA and **James G. PFAUS**. Differential role of oxytocin and vasopressin in the conditioned ejaculatory preference of the male rat. *Physiology & Behavior* [online]. 2019, **208**(112577, Article 112577). ISSN 0031-9384. Available at: doi:10.1016/j.physbeh.2019.112577.

The neuropeptides oxytocin (OT) and vasopressin (AVP) are critical in the formation of pair bonding in the vole, and potentially in other species. As part of her Masters thesis work, Shann Ménard examined the ability the almond odor cue to activate Fos within oxytocin or vasopressin neurons once CEP had developed, and then asked whether systemic injections of oxytocin or vasopressin during the male's first sexual experience could facilitate the acquisition of CEP on the second experience. In the first experiment, sexually naïve Long-Evans male rats were given 9 multi-ejaculatory conditioning trials at 4-day intervals with either almond-scented (paired) or unscented (unpaired) sexually receptive females in bilevel pacing chambers. CEP was examined in the final open field test with two receptive females, one scented with almond and the other unscented. Males in both groups were given two reconditioning trials and presented with the almond odor on gauze for 1 h prior to sacrifice. Neuronal activation was assessed by IHC detection of Fos protein within OT or AVP neurons using specific primary antibodies for nuclear Fos protein and antibodies specific to OT or AVP in the cytoplasm. Exposure to the odor induced significantly greater activation of OT neurons in parvocellular neurons of the paraventricular nucleus (PVN) and of AVP in magnocellular neurons of the supraoptic nucleus (SON) in the paired group compared to the unpaired group. The second experiment examined whether oxytocin or vasopressin could enhance the acquisition of a CEP. Sexually naïve male Long-Evans rats received a subcutaneous injection of OT, AVP, or the saline vehicle, prior to their first sexual experience with an almond-scented female. CEP was examined 4 days later in the open field. Males injected with OT, but not AVP or saline, displayed significant CEP. We concluded that the selective activation of OT neurons by the conditioned odor in the paired group, and the ability of OT injections to enhance the association of the odor and sexual reward, indicates that enhanced OT transmission is critical in the formation of CEP in male rats.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 90 | 100 |

[15] GERME, Katuschia and **James G. PFAUS**. Acute ethanol disrupts conditioned inhibition in the male rat. *Psychopharmacology* [online]. 2024, **241**(10), 2061–2071. ISSN 1432-2072. Available at: doi:10.1007/s00213-024-06618-5.

In the second experiment of Kippin's original paper from 1998, males in the explicitly unpaired group received sequential pairings of no odor on sexually receptive females (leading the ejaculatory reward state), and odor on sexually nonreceptive females (leading to a state of sexual nonreward). This state of sexual nonreward is learned, as male rats typically attempt to mount the nonreceptive females on their first trial with them and are thwarted by the females' rejection responses. Thus, those males not only displayed CEP for the unscented female on the final choice test, but also showed selective mounting and intromissions with unscented females, despite both the scented and unscented females being sexually receptive on the final choice test. This is an example of conditioned sexual inhibition induced by the odor associated with sexual nonreward. We had shown previously that a low to moderate dose of alcohol in male rats could disinhibit sexual advances toward sexually nonreceptive females, so as part of her Ph.D. dissertation, my student Katuschia Germé asked whether alcohol could do the same to conditioned sexual inhibition, and where in the brain this disinhibition might occur. Sexually-naïve Long-Evans rats received 20 alternate 30-min conditioning sessions in bilevel chambers with unscented receptive or scented (almond) non-receptive females at 2-day intervals. Following the conditioning phase, males were injected with saline, alcohol 0.5 g/kg v/v or 1 g/kg v/v, 45 min before a copulatory test with two receptive females, with one bearing the olfactory cue. Fos activation was later assessed, following exposure to alcohol and the olfactory cue alone, in several brain regions involved in the expression and regulation of male sexual behavior. While males in the saline group displayed sexual avoidance towards the scented female, those injected with alcohol before the copulatory test, regardless of the dose, copulated indiscriminately with both females. Subsequent exposure to alcohol and the olfactory cue alone induced different Fos expression between groups in several brain regions. Exposure to the inhibitory cue alone (No Odor vs Odor) or in the combination alcohol + inhibitory odor (Odor condition) induced a significant increase in Fos in the paralimbic prefrontal cortex, NAc core, mPOA and VTA and a significant decrease in the NAc shell, BLA, medial amygdala and central nucleus of the amygdala. The different patterns of Fos activation between groups, when exposed to the olfactory cue and even when under the influence of alcohol, confirm the ability of the male rats to discriminate the inhibitory olfactory cue. Accordingly, the differences in Fos expression observed between the saline and alcohol groups within the same brain area indicate that the almond odor was interpreted and processed differently. Thus, we concluded that low to moderate doses of alcohol disrupt conditioned sexual inhibition in male rats and induce a differential pattern of neuronal activation in regions involved in the expression and regulation of sexual behavior and incentive motivation. This may occur through a process of disinhibition, in which low to moderate doses of alcohol inhibit systems that are inhibitory.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 85 | 100 |

## 3. Original studies on the conditioning of sexual behavior in female rats

[16] CORIA-AVILA, G.A., A.J. OUIMET, P. PACHECO, J. MANZO and **J.G. PFAUS**. Olfactory conditioned partner preference in the female rat. *Behavioral Neuroscience* [online]. 2005, **119**(3), 716–725. ISSN 1939-0084. Available at: doi:10.1037/0735-7044.119.3.716.

Genaro Coria-Avila was another Ph.D. student in my laboratory. He came after finishing his Master's degree in neuroethology from the Instituto de Neuroetología at the Universidad Veracruzana in Xalapa, Mexico specifically to examine whether conditioned sexual partner preference might occur in female rats. We decided to train and test the females in nearly identical fashion to the males. OVX, sexually naïve females were made sexually receptive with EB and P. Paired females were given sequential access to almond-scented males (painted with the almond odor on the back of the neck and anogenital region) in unilevel pacing chambers with a four-hole divider, and unscented males (with distilled water applied to the same regions) in unilevel pacing chambers without the divider at 4-day intervals for a total of 8 conditioning trials (4 in each condition). Previous studies by my Mexican colleague Raúl Paredes had shown that such discriminative conditioning results in a conditioned place preference for the pacing condition (with the divider) relative to the unpaced condition (without the divider). Unpaired females were given the reverse order (scented males in the unpaced condition and unscented males in the paced condition), and random-paired females were given random pairing of scented and unscented males in paced and unpaced conditions. On the final open field test, females were given access to two sexually vigorous males, one scented and the other unscented. In the initial experiments, Genaro discovered that male rats were highly cooperative and would take turns copulating with the female in addition to crawling over one another, thus scenting the unscented male. To avoid this, we decided to tether the males using the Lorimer rodent tethering jacket to opposite corners of the open field. On the final test using tethered males, females in the paired group solicited the scented male significantly more frequently, chose the scented male for their 1st ejaculation, and displayed significantly more full lordosis reflexes with the scented male relative to the unscented male. Neither the unpaired or random-paired groups displayed any preference. Thus, the neutral almond odor paired with paced copulation was shown to elicit conditioned partner preference in female rats (both to solicit their preferred male and receive their first ejaculation from their preferred male). This is similar to the ability of paced copulation to elicit significant conditioned place preference in female rats.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 90 | 100 |

[17] CORIA-AVILA, Genaro A., Sherri L. JONES, Carrie E. SOLOMON, Alex M. GAVRILA, Gerald J. JORDAN and **James G. PFAUS**. Conditioned partner preference in female rats for strain of male. *Physiology & Behavior* [online]. 2006, **88**(4), 529–537. ISSN 0031-9384. Available at: doi:10.1016/j.physbeh.2006.05.001.

Genaro also performed the first strain conditioning experiment with females. In this study OVX Wistar (albino) or Long-Evans rats primed with EB and P received 10 sequential conditioning trials at 4-day intervals in the unilevel pacing chamber (5 per condition). In the Wistar-pacing group females copulated with Wistar males in the chamber bisected by a 4-hole partition that only the female could pass through. Four days later, they copulated with Long-Evans males without the partition. The Long-Evans-pacing group received the opposite association. In the final partner preference test, all females chose freely between two males tethered in opposite corners of the open field, one Wistar and the other Long-Evans. Regardless the strain of male, females displayed more solicitations toward the pacing-related male, and most of the females received their first ejaculation from that male. The preference was facilitated if the pacing-related male was of the same strain as the female, especially the choice for ejaculations made by Long-Evans females that received paced copulation with Long-Evans males. These results suggest that female rats have an unconditioned preference for males of the same strain (positive assortative mating, as observed also in males), but that this preference can be switched towards males of a different strain if those males are associated with the sexual reward induced by paced copulation and the male of their same

strain is associated with unpaced copulation. These data also indicate that strain differences in visual appearance, vocalizations, and MHCs or other olfactory stimuli, can serve as compound cues for the development of sexual partner preferences in female rats.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
|---|---|---|---|
| 100 | 100 | 85 | 100 |

[18] CORIA-AVILA, G. A. and **J. G. PFAUS**. Neuronal activation by stimuli that predict sexual reward in female rats. *Neuroscience* [online]. 2007, **148**(3), 623–632. ISSN 1873-7544. Available at: doi:10.1016/j.neuroscience.2007.05.052.

This study compared the activation of Fos protein in the brain by the almond odor or strain cues associated with paced versus unpaced copulation in female rats. The odor conditioning was conducted as before in OVX, hormone-primed Long-Evans rats, whereas the strain conditioning was conducted as before in OVX, hormone-primed Wistar and Long-Evans rats. The final open-field preference test replicated the previous findings of selective choice of the pacing-related male for solicitations and ejaculations. Subsequently, females were exposed to the CS (odor or strain) alone for 1 h prior to overdose with sodium pentobarbital, perfusion and preparation of their brains for Fos IHC. Although the odor associated with paced copulation activated Fos in more regions than the strain cues, in both experiments, the odor or strain CSs associated with paced copulation activated significantly more Fos-IR in the piriform cortex (main olfactory cortex), NAc, mPOA, and VTA, relative to the same odor or strain cues associated with nonpaced copulation. These findings provide evidence that the state associated with paced copulation can be conditioned to environmental stimuli such as neutral odors or strain cues, which earn an incentive value via Pavlovian conditioning. The regions by both cues were consistent with the activation of the mesolimbic and incertohypothalamic dopamine pathways.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
|---|---|---|---|
| 100 | 100 | 85 | 100 |

[19] CORIA-AVILA, Genaro A., Carrie E. SOLOMON, Erica Barbosa VARGAS, Ivana LEMME, Richard RYAN, Shann MENARD, Alex M. GAVRILA and **James G. PFAUS**. Neurochemical basis of conditioned partner preference in the female rat: I. Disruption by naloxone. *Behavioral Neuroscience* [online]. 2008, **122**(2), 385–395. ISSN 1939-0084. Available at: doi:10.1037/0735-7044.122.2.385.

Genaro also examined whether naloxone could disrupt the odor and strain conditioning in females. As with the study above, almond odor conditioning was conducted using OVX, hormone-primed Long-Evans rats, with the paced condition occurring in a unilevel pacing chamber bisected by a 4-hole divider and the unpaced condition occurring in the same chamber but without the divider at 4-day intervals. Strain conditioning was conducted in OVX Long-Evans or Wistar rats paired with either Long-Evans or Wistar rats in the paced condition, and the other strain with the unpaced condition at 4-day intervals. In both cases, 10 sequential conditioning trials were made (5 in each paced versus unpaced condition). Systemic injections of naloxone or saline were made prior to each conditioning trial. All females received saline prior to the final open field preference test. Females treated previously with saline developed a significant preference for the pacing related males in both the odor and strain conditioning paradigms. In contrast, females treated previously with naloxone did not develop a significant partner preference for the pacing related male, and in fact displayed fewer solicitations and lower lordosis scores overall compared to the females

that received saline prior to each conditioning trial. Females treated with naloxone prior to each conditioning trial also displayed more rejection responses during the open field preference test, and as a result, received fewer mounts, intromissions, and ejaculations overall from the males during that test. Compared to the subsequent study in males, blockade of opioid reward made fully primed females far less sexually proceptive and receptive, in addition to blocking the development of partner preference. This was also consistent with the disruptive effect of naloxone on the development of sexually conditioned pace preferences.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 80 | 100 |

[20] PARADA, Mayte, Liliane CHAMAS, Sabrina CENSI, Genaro CORIA-AVILA and **James G. PFAUS**. Clitoral stimulation induces conditioned place preference and Fos activation in the rat. *Hormones And Behavior* [online]. 2010, **57**(2), 112–118. ISSN 1095-6867. Available at: doi:10.1016/j.yhbeh.2009.05.008.

Another Ph.D. student in my laboratory, Mayte Parada, examined the specific role of clitoral stimulation (CLS) in the development of sexually conditioned place and partner preferences. In her studies, CLS was administered using a soft natural fiber #4 paintbrush with three upward strokes to mimic the kind of stimulation the female receives from the pelvic thrusting of the male during mounts. The three upward strokes were considered 1 train of stimulation, and these were presented to females in either massed (1 train every second) or distributed (1 train every 5 seconds). In this first study of her dissertation, OVX, hormone-primed sexually naïve Long-Evans rats were randomly assigned to receive either distributed CLS (1 train every 5 s for 1 min prior to being placed in one distinctive side of a nonbiased conditioned place preference (CPP) box for 2 min, after which the cycle of stimulation and CPP exposure were repeated for 4 more cycles, totaling 60 stimulations) or continuous CLS (1 stimulation per second for 1 min with 2 min in one side of the CPP box, repeated for 4 more cycles, totaling 300 stimulations). Two days later, females were placed into the other side of the CPP box without prior stimulation. CPP was tested after 5 sequential exposures each of CLS and no stimulation. Females given distributed stimulation developed a significant CPP whereas females given massed stimulation did not. In the second experiment Fos activation was examined following CLS. Fos was induced in hypothalamic and limbic structures, including the piriform cortex, NAc, mPOA, arcuate nucleus, and dorsomedial portion of the ventromedial hypothalamus, compared to no stimulation. However, distributed CLS induced more Fos in the mPOA than massed CLS or no stimulation. In contrast, massed CLS induced more Fos in the posteroventral medial amygdala compared to no stimulation. These data indicated that CLS induces a reward state in the rat and a pattern of Fos activation in regions of the brain that process genitosensory input, incentive salience, and reward. Mayte went on to show that almond odor on gauze paired with the CLS-induced reward state in sexually naïve females created a preference to solicit selectively the male bearing the odor during their first copulatory experience in an open field with two sexually vigorous tethered males, one scented and the other unscented (see extended commentary).

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 80 | 100 |

[21] HOLLEY, Amanda, Shy SHALEV, Shannon BELLEVUE and **James G. PFAUS**. Conditioned mate-guarding behavior in the female rat. *Physiology & Behavior* [online]. 2014, **131**, 136–141. ISSN 0031-9384. Available at: doi: 10.1016/j.physbeh.2014.04.034

Here the work of another Ph.D. student in my laboratory, Amanda Holley, is highlighted. In early work for her dissertation, Amanda found that presenting the same unscented Long-Evans male to the OVX, hormone-primed Long-Evans female during 10 paced (but not unpaced) conditioning trials at 4-day intervals led to the female developing a partner preference for the familiar male in the open field test relative to a novel male. Given that the male was of the same strain, we surmised that differences in MHCs or other potential pheromonal/odor cues may become the salient conditioned stimulus, just as the neutral almond odor was in Genaro's experiments.

Amanda then asked what might happen if the female in a sexually receptive state and her preferred male were confronted by another sexually receptive female in the open field. In all our previous work, female partner preference was tested with two males (FMM test), as male partner preference (CEP) was tested with two females (MFF test). We knew from the work of Martha McClintock that group mating in open fields involved much competitive behavior of females soliciting and intercepting males, with dominant females intercepting males and taking ejaculations after the subordinate females had done all the copulatory work. Thus, in this study, Amanda used an F-M/F test, in which the "M/F" was the "couple" (OVX, hormone-primed female and her preferred male) in the open field and a competitor female, also sexually receptive, was introduced. Despite rats being described as promiscuous, the paired female with her preferred male displayed mate-guarding behaviors nearly identical to female Prairie voles (see extended commentary). Three main behaviors were observed: *hovering and presenting* (H&P) where the female remained close to the male and presented her anogenital region to the male, often enticing the male to mount. If the competitor female approached the male, the paired female would move between them in what was described as *competitor blocking* (CB). If the competitor female solicited the male (making a headwise orientation to the male followed by a runaway), the paired female would jump on the back of the competitor and mount her as a *dominance display*, often pushing her head into the inner wall of the open field. Sometimes this would result in fighting, but it was always the case that the paired female would assert her dominance and force the competitor away from the male. After several trials, the competitor female learned not to approach the M/F couple (requiring us to use different females as competitors). However, if the paired female and competitor were sexually receptive and placed into the open field with a different male, they would both show normal competitive sexual solicitations and pacing, but no mate guarding. Thus, similar to monogamous Prairie voles, "promiscuous" female rats show mate-guarding behaviors, but only when the females are sexually receptive and in the presence of their preferred male. Similarly, female Prairie voles are also known to sneak copulations with novel males when not with their preferred male. Monogamy is not synonymous with sexual fidelity in any species, and being promiscuous does not imply an inability to condition a preference by experiencing a sexual reward state with a particular copulatory partner.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 80 | 100 |

[22] HOLLEY, Amanda, Shannon BELLEVUE, Daniel VOSBERG, Kerstin WENZEL, Sieger ROORDA JR. and **James G. PFAUS**. The role of oxytocin and vasopressin in conditioned mate guarding behavior in the female rat. *Physiology & Behavior* [online]. 2015, **144**, 7–14. ISSN 1873-507X. Available at: doi:10.1016/j.physbeh.2015.02.039.

Amanda and her team replicated and extend her finding of mate-guarding behavior in female rats to examine whether oxytocin and/or vasopressin played a role. Preliminary experiments had revealed that females given access to the familiar male show more Fos induction within regions of the brain that contain oxytocin and vasopressin cell bodies, notably the SON and PVN relative to females given sexual experience with different males. The first experiment of this study used double IHC to examine whether the Fos induction in these regions occurred specifically within oxytocin and/or vasopressin cell bodies in paired females exposed to their preferred male behind a screen, relative to unpaired females exposed to a male behind a screen. Female rats that display conditioned mate guarding had significantly more double-labeled Fos/oxytocin neurons in both SON and PVN, and significantly more Fos/vasopressin neurons in the PVN. The second experiment examined whether injections of oxytocin, vasopressin, or saline during their first paced sexual experience could potentiate mate guarding behaviors, similar to the experiment by Shann Ménard where peripheral oxytocin injections prior to the male's first sexual experience were found to potentiate CEP on the second test 4 days later. In this experiment, peripheral administration of oxytocin prior to the female's first sexual experience augmented the number of H&Ps and solicitations displayed in the FM-F test 4 days later, which resulted in the females receiving more intromissions by the male. In contrast, peripheral administration of AVP prior to their first sexual experience with the familiar male augmented the number of CBs the female displayed when the competitor female approached the male, but not the number of H&Ps or other aspects of mate guarding. Injections of saline prior to the first sexual experience did not alter any aspect of mate guarding or copulatory behavior on the second trial. These results indicate that female rats' first experiences with sexual reward when paired with the same male induce changes to bonding networks in the brain. Specifically, the activation of oxytocin transmission appears to consolidate affiliative behaviors leading to H&Ps and solicitations with the female's preferred male. In contrast, activation of vasopressin transmission facilitates CB behaviors aimed at the competitor female, but not affiliative behaviors with the male.

| Experimental work (%) | Supervision (%) | Manuscript (%) | Research direction (%) |
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| 100 | 100 | 75 | 100 |

[23] HOLLEY, Amanda, Lara JOULAKIAN, Kerstin WENZEL, Sieger ROORDA JR., Brunella GONZALEZ, Lindsay SPARKS and **James G. PFAUS**. Inhibition of lysine-specific demethylase enzyme disrupts sexually conditioned mate guarding in the female rat. *Physiology & Behavior* [online]. 2018, **196**, 78–83. ISSN 0031-9384. Available at: doi:10.1016/j.physbeh.2018.08.005.

Knowing that naloxone treatment prior to each sexual experience could abolish conditioned preferences in both females and males, we supposed that opioid activation of MORs to induce pleasure formed the basis of sexual reward states. Although agonist activity at MOR receptors activates a Gi/o protein to inhibit cAMP and facilitate $K^+$ efflux (thus hyperpolarizing neurons and inhibiting cAMP-dependent second messenger actions along with CREB protein activation of gene transcription), MOR receptor internalization is also critical for the sensitization of dopamine transmission in response to cues associated with drugs of abuse, and also for the sensitization of oxytocin transmission through the transcription of the CD38 protein product within oxytocin neurons that acts as an intracellular $Ca^{2+}$ mobilizer. Both effects are dependent on MOR activation of MAP kinase pathways. Amanda took this one step further to ask whether the preferences were dependent on demethylation of DNA

wrapped around histones. For this she used the drug oryzon, a lysine (K)-specific demethylase 1a (LSD-1a) inhibitor, prior to each of 10 paired or unpaired conditioning trials at 4-day intervals in unilevel pacing chambers with the 4-hole divider (paired females copulated with the same male each trial, whereas unpaired females copulated with a different male each trial). Relative to saline, oryzon inhibited the development of H&P and CB in the paired group, but had no significant effect on behavior in the unpaired group. Following the final F-M/F test, brains were removed and examined for double labelling of Fos with oxytocin or vasopressin in the paired females receiving either saline or oryzon, and the unpaired females receiving saline. Results showed that oryzon decreased the number of double-labelled cells for oxytocin or vasopressin in the SON and PVN of paired females relative to saline and females in the unpaired group. Thus, inhibition of lysine (K)-specific demethylase 1a produced effects similar to those of naloxone in disrupting both the partner preference and augmented activation of oxytocin and vasopressin transmission that in part underlies it.

| **Experimental work (%)** | **Supervision (%)** | **Manuscript (%)** | **Research direction (%)** |
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The Habilitation Thesis itself discusses the implications of these findings in more detail for our understanding of brain mechanisms of sexual behavior and reward in general, in the relationship of sexual reward to reproduction, in human sexual behavior, and in the development of paraphilias. Specific limitations of the behavioral paradigms developed in my laboratory are also discussed.

Date: 27 January 2026 Signature: