

Behavioral, and Neural Mechanisms Of Conditioned Partner Preference In The Female

Rat.

Genaro A. Coria-Avila

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

January 2007

© Genaro A. Coria-Avila, 2007



Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 978-0-494-30120-3

Our file Notre référence

ISBN: 978-0-494-30120-3

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.


Canada

ABSTRACT

Behavioral and neural mechanisms of conditioned partner preference in the female rat

Genaro A. Coria-Avila, Ph.D.
Concordia University, 2007

We assessed the development of a conditioned partner preference in female rats towards males associated with paced copulation (PC), relative to nonpaced copulation (NPC). Chapter 1 assessed the behavioral mechanisms. Ovariectomized, hormone-primed females were conditioned to associate an odor or a strain of male with PC. A partner preference test occurred in an open field with two tethered males in opposite corners, one associated with PC and the other with NPC. Paired females (relative to unpaired or random-paired groups) developed a partner preference for PC-related males. They displayed more solicitations and hops and darts, and were more likely to choose the PC-related male for their first ejaculation. Similarly, strain conditioning produced partner preference towards the PC-related male, regardless of the strain; although females were more likely to receive their first ejaculation from the PC-related male only if it was of their own strain.

The experiments in Chapter 2 assessed neuroanatomical correlates. The brains of females in both paired and unpaired conditions were processed for Fos-immunoreactivity (Fos-IR) following exposure only to the conditioned stimulus (CS) associated either with PC or with NPC. The CS paired with PC induced more Fos-IR in the piriform cortex, the medial preoptic area, and the ventral tegmental area, compared to the induction by the same CS associated with NPC.

The experiments in Chapter 3 assessed neurochemical mechanisms. In Part 1, females were treated with the general opioid antagonist naloxone (4 mg/kg) before every conditioning trial, but were drug-free during the final partner preference test. Naloxone-treated females did not develop a conditioned partner preference. In Part 2, females were treated with the dopamine antagonist flupenthixol (.25 mg/kg) before conditioning trials. Only Long-Evans females failed to develop an olfactory conditioned partner preference, just like naloxone-treated females. However, flupenthixol had no effect on conditioned preference for a strain of male in Wistar females.

The experiments in Chapter 4 depict preliminary data on the activation of oxytocin, vasopressin and gonadotrophin-releasing hormone (GnRH) neurons following exposure to the CS associated with PC. These data show trends toward a significant activation of Fos within oxytocin neurons in the paraventricular nucleus of the hypothalamus, and GnRH neurons in the anterior hypothalamus, in paired females exposed to the CS.

These results demonstrate that the sexual reward induced by PC can be associated with cues on a partner to develop a conditioned preference. When this occurs via olfactory conditioning of a neutral odor, the mechanisms depend on the interaction of opioids and dopamine in mesolimbic areas. However, the conditioning process for a strain of male appears to depend only on opioids.

ACKNOWLEDGEMENTS

To my Ph.D. mentor:

Dr. Jim Pfaus. Thank you for believing in me and for taking me under your wing.

The years I spent in your lab changed the way I think and the way I live my life.

You have been a great supervisor and friend.

To the granting agencies:

The research conducted for this thesis was supported by a postgraduate fellowship

to G.A. Coria-Avila by the Consejo Nacional de Ciencia y Tecnología

(CONACyT) of Mexico (128099) and by grants to J.G. Pfaus from the Canadian

Institutes for Health Research (MOP-74563) and the Natural Sciences and

Engineering Research Council of Canada (OGP-0138878).

To my thesis reviewers:

I want to thank Dr. Barbara Woodside and Dr. Wayne Brake of the Center for

Studies in Behavioral Neurobiology, Department of Psychology, Concordia

University, Dr. Grant Brown of the Department of Biology, Concordia University,

and my external reviewer, Dr. Larry J. Young from the Center for Behavioral

Neuroscience and Dept. of Psychiatry at Emory University, for their comments on the thesis.

To my undergrads:

I want thank to Jessica Haley, Allison Ouimet, Sherri Jones, Kathleen McNulty,

Diala Arzouni, Marina Sorochinski, Erica Barbosa, Carrie Solomon, Alex

Gavrila, Gerald Jordan, Ivana Lemme, Richard Ryan, Brigitte Boulard, Erin

Saucke-Lacelle, Natalie-Anne Charron and Geoffrey Stanley. Without your help

this project would have been difficult, if not impossible, to complete.

To those Professors who influenced my formation as a scientist.

Drs. Mary Erskine, Larry Young, Pablo Pacheco, Jorge Manzo, Peter Shizgal, Norman Segalowitz, Barbara Woodside, and Dave Mumby, thank you for training me, exposing me to new ideas, and allowing me the space to learn from my mistakes.

DEDICATORY

All the hard work, all the sleepless nights, all the effort is dedicated to the people that I love the most. Consider this thesis as a tribute for the great and positive influence that you all have had in my life.

My family:

Georgina, Charis, Lety, Any, Rosy and Mayi, the six most influential women in my life. I love you very much. Thanks for cheering me up via internet, phone and mail.

To my greatest support:

Shann Ménard. Thanks for your love and your motivation. Thanks for introducing me to so many good things during my Ph.D.. You are by far the main reason I love Canada.

To my friends:

Said Kourrick, Hélène Gelez, Alex Gavrila, Nafissa Ismail, Michaela Georgescu, and Veronica Afonso. During my years at Concordia you all contributed in so many ways to my wellbeing. Thank you.

TABLE OF CONTENTS

	Page
List of Figures	xv
List of Tables	xvii
Contributions of authors	xviii
List of Abbreviations	xxii
 General Introduction	 1
Role of learning in sexual behavior and Conditioned Partner Preference	3
Influence of Pavlovian conditioning on sexual behaviour	3
Early postnatal period	4
Postpubertal period	10
Conditioned Place Preference	10
Conditioned Partner Preference	12
Influence of Instrumental Conditioning on Sexual Behaviour	14
Role of sexual reward in learned preferences	17
Outline of the experiments	20
 1. Chapter 1. Behavioral Mechanisms of Conditioned Partner Preference ..	 22
1.1. Experiment 1. Olfactory Conditioned Partner Preference in Female Rats	22
1.1.1. Abstract	23
1.1.2. Introduction	24
1.1.3. Method	26

1.1.3.1. Animals and surgery	26
1.1.3.2. Odor conditioning	27
1.1.3.3. Place preference conditioning	28
1.1.3.4. Copulatory preference Test	29
1.1.3.5. Behavioral measures and statistical analyses	29
1.1.4. Results	
1.1.4.1. Place Preference Test	30
1.1.4.2. Copulatory Preference Test	32
1.1.4.3. Effect of single Paired Conditioning	42
1.1.5. Discussion	42
1.2. Experiment 2. Conditioned Partner Preference in Female Rats for	
Strain of Male	47
1.2.1. Abstract	48
1.2.2. Introduction	49
1.2.3. Materials and methods	52
1.2.3.1. Animals and surgery	52
1.2.3.2. Conditioning	53
1.2.3.3. Copulatory Preference Test	53
1.2.3.4. Behavioral measures and statistical analysis	54
1.2.4. Results	54
1.2.4.1. Wistar females	55
1.2.4.2. Long-Evans females	62
1.2.5. Discussion	67

1.2.6. Acknowledgements	73
1.2.7. Summary of Chapter 1	74
2. Chapter 2:	
2.1. Neuronal Activation by Stimuli that Predict Sexual Reward	
in Female Rats	75
2.1.1. Abstract	76
2.1.2. Introduction	77
2.2. Experiment 1. Brain Activation by an Odor Paired with	
Sexual Reward	80
2.2.1. Animals and surgery	80
2.2.2. Odor conditioning	81
2.2.3. Activation of Fos-IR by the odor	81
2.2.4. Histological and statistical analysis	82
2.3. Experiment 2. Brain Activation by Strain Cues Associated	
with Sexual Reward	83
2.3.1. Animals and surgery	83
2.3.2. Strain conditioning	83
2.3.3. Activation of Fos-IR by strain cues	84
2.3.4. Histological and statistical analysis	84
2.4. Results	85
2.4.1. Experiment 1. Brain Activation by an Odor Paired	
with Sexual Reward	85

2.4.2. Experiment 2. Brain Activation by Strain Cues Associated	
with Sexual Reward	89
2.5. Discussion	94
2.5.1. Role of Piriform Cortex	97
2.5.2. Role of Medial Preoptic Area	98
2.5.3. Role of Ventral Tegmental Area	99
2.5.4. Other regions	100
2.6. Acknowledgements	102
2.7. Summary of Chapter 2	103
 3. Chapter 3	
3.1. Neurochemical Basis of Conditioned Partner Preference in	
Female Rats: I. Disruption by Naloxone	104
3.1.1. Abstract	105
3.1.2. Introduction	106
3.1.3. General methods	109
3.1.3.1. Subjects and surgery	109
3.1.3.2. Drugs	110
3.1.4. Experiment 1. Effects on Olfactory Conditioning	110
3.1.4.1. Method	110
3.1.4.1.1. Odor conditioning	110
3.1.4.1.2. Copulatory preference test	111
3.1.4.1.3. Statistical analysis	112

3.1.4.2. Results	112
3.1.5. Experiment 2. Effects on Conditioning by Strain of Male	119
3.1.5.1. Method	119
3.1.5.1.1. Strain conditioning	119
3.1.5.1.2. Copulatory preference test	119
3.1.5.1.3. Statistical analysis	120
3.1.6. Results	120
3.1.7. Discussion	126
3.2. Neurochemical Basis of Conditioned Partner Preference in	
Female Rats: I. Disruption by Flupenthixol	135
3.2.1. Abstract	136
3.2.2. Introduction	137
3.2.3. General methods	140
3.2.3.1. Subjects and surgery	140
3.2.3.2. Drugs	141
3.2.4. Experiment 1. Effects on olfactory conditioning	142
3.2.4.1. Method	142
3.2.4.1.1. Odor conditioning	142
3.2.4.1.2. Copulatory preference test	143
3.2.4.1.3. Statistical analysis	143
3.2.4.2. Results	144
3.2.5. Experiment 2. Effects on conditioning by strain of male	150
3.2.5.1. Method	150

3.2.5.1.1. Strain conditioning	150
3.2.5.1.2. Copulatory preference test	150
3.2.5.1.3. Statistical analysis	151
3.2.5.2. Results	151
3.3. Discussion	158
3.4. Summary of Chapter 3	165
 4. Chapter 4	
Activation of Oxytocin, Vasopressin And Gonadotrophin Releasing Hormone (GnRH) Neurons During Conditioned Partner Preference in Female Rats	
4.1. Abstract	167
4.2. Introduction	168
4.3. General methods	171
4.3.1. Subjects and surgeries	171
4.3.2. Conditioning	172
4.3.3. Copulatory preference test	173
4.4. Exposure to strain cues on Fos activation in OT and AVP neurons	173
4.4.1. Procedure	173
4.4.2. Statistical analysis	175
4.5. Exposure to strain cues on Fos activation in GnRH neurons	176
4.5.1. Procedure	176
4.5.2. Statistical analysis	177
4.6. Results	

4.6.1. Experiment 1. Fos induction in OT and AVP neurons	177
4.6.2. Experiment 2. Fos Induction in GnRH neurons	181
4.7. Discussion	186
General Discussion	191
Solicitations as an index of sexual desire	193
Pacing, sexual reward, conditioned desire and reproductive success		194
Neuroanatomical mechanisms	199
Neurochemical mechanisms		
Dopamine	203
Oxytocin and vasopressin	208
Opioids	210
A model of conditioned partner preference	213
Conclusions	216
References	217

LIST OF FIGURES

	Page
Figure 1. Conditioned place preference induced by paced copulation.	31
Figure 2. Solicitations in olfactory conditioned female rats.	35
Figure 3. Number of hops and darts in olfactory conditioned female rats.	37
Figure 4. Choice of male for first mount in olfactory conditioned female rats.	39
Figure 5. Preference of male for first, second, or third ejaculation and frequency of lordoses in olfactory conditioned female rats.	40
Figure 6. Solicitations in Wistar female rats conditioned by strain of male.	60
Figure 7. Choice of male to receive their first, second, and third ejaculation in Wistar female rats.	61
Figure 8. Solicitations in Long-Evans female rats conditioned by strain of male.	64
Figure 9. Choice of male to receive their first, second, and third ejaculation in Long-Evans female rats.	66
Figure 10. Brain areas of Long-Evans female rats that express significant differences in Fos immunoreactivity following exposure to a conditioned odor associated with sexual reward.	86
Figure 11. Brain areas of Long-Evans female rats that do not express significant differences in Fos immunoreactivity following exposure to a conditioned odor associated with sexual reward.	87
Figure 12. Brain areas of Wistar female rats that express significant differences in Fos immunoreactivity following exposure to a male of a strain associated with sexual reward.	90
Figure 13. Brain areas of Wistar female rats that express significant differences in Fos immunoreactivity following	

	exposure to a Long-Evans male.	91
Figure 14.	Common brain areas activated by CSs associated with paced copulation	93
Figure 15.	Effect of naloxone treatment during conditioning trials on solicitations and lordosis magnitude in olfactory conditioned Long-Evans females.	115
Figure 16.	Effect of naloxone treatment during conditioning trials on solicitations and lordosis magnitude in Wistar females conditioned by strain of male.	123
Figure 17.	Effect of flupenthixol treatment during conditioning trials on solicitations and lordosis magnitude in olfactory conditioned female rats.	146
Figure 18.	Effect of flupenthixol treatment during conditioning trials on solicitations and lordosis magnitude in Wistar females conditioned by strain of male.	154
Figure 19.	Activation of OT and AVP neurons following exposure to preferred or non-preferred males.	179
Figure 20.	Effect of exposure to a CS paired with paced copulation on the Fos-IR within OT neurons in the PVN.	180
Figure 21.	Activation of GnRH neurons following exposure to the preferred or non-preferred males.	183
Figure 22.	Effect of exposure to a CS paired with paced copulation on the Fos-IR within GnRH neurons.	185
Figure 23.	Model of conditioned partner preference in females rats induced by paced copulation.	215

LIST OF TABLES

	Page
Table 1. Sexual behavior of olfactory conditioned Long-Evans female rats in a two-males one-female situation.	33
Table 2. Sexual behavior of Wistar and Long-Evans female rats in a two-males one-female situation, conditioned by strain of male.	58
Table 3. Brain areas of olfactory conditioned Long-Evans female rats that expressed Fos immunoreactivity following exposure to a conditioned odor associated with sexual reward.	88
Table 4. Brain areas of Wistar female rats that expressed Fos immunoreactivity following exposure to a male of a strain associated with sexual reward.	92
Table 5. Effects of naloxone on the sexual behavior of olfactory conditioned Long-Evans female rats in a two-males one-female situation.	118
Table 6. Effects of naloxone on the sexual behavior of Wistar female rats in a two-males one-female situation, conditioned by strain of male.	125
Table 7. Effects of flupenthixol on the sexual behavior of olfactory conditioned Long-Evans female rats in a two-males one-female situation.	149
Table 8. Effects of flupenthixol on the sexual behavior of Wistar female rats in a two-males one-female situation, conditioned by strain of male.	157
Table 9. GnRH neurons, total Fos, and GnRH neurons with Fos in females exposed to strain cues associated with paced or nonpaced copulation.	184

CONTRIBUTION OF AUTHORS

CHAPTER 1.

Experiment 1. Olfactory Conditioned Partner Preference in Female Rats.

Authors:

Genaro A. Coria-Avila: Collected most of the data, performed all the data analysis and graphing, and was responsible for writing the paper.

Allison J. Ouimet: Collected some behavioral data.

Pablo Pacheco and *Jorge Manzo* co-supervised me during the first research stay in 2000 when the project started. They also contributed by obtaining financial support for my project from Universidad Veracruzana and UNAM in Mexico.

James G. Pfaus: Author of the theoretical design and helped with the writing.

Not an author:

Jessica Haley: Collected data from some of the animals in the pilot study.

Marina Soroichinsky. Collected data from some animals in the pilot study.

Experiment 2. Conditioned Partner Preference in Females Rats for Strain of Male

Authors:

Genaro A. Coria-Avila: Author of the theoretical design, collected a most of the data, performed all the data analysis and graphing, and was responsible for writing the paper.

Sherri L. Jones: Collected data in the pilot study and coauthor of the theoretical design.

Carrie Solomon: Collected data from Wistar animals.

*Alex M. Gavril*a: Collected data from Wistar animals.

Gerald J. Jordan: Collected data from Long-Evans animals.

James G. Pfaus: Helped with the theoretical design and the writing

Not an author:

Diala Arzouni. Collected data from some animals in the pilot study.

Kathleen McNulty. Collected data from some animals in the pilot study.

CHAPTER 2

Experiment 1 and 2. Neuronal Activation by Stimuli that Predict Sexual Reward in
Female Rats

Authors:

Genaro Coria-Avila: Coauthor of the theoretical design, collected all the data,
performed all the data analysis and graphing, and was responsible for writing the
paper.

James G. Pfaus: Author of the theoretical design and helped with the writing.

CHAPTER 3

Part 1. Neurochemical basis of Conditioned Partner Preference in the Female Rat: I.
Disruption by Naloxone

Authors:

Genaro A. Coria-Avila: Author of the theoretical design, collected a most of the data, all the data analysis and graphing, and was responsible for writing the paper.

Carrie Solomon: Collected behavioral data.

Erica Barbosa-Vargas: Collected behavioral data.

Ivana Lemme: Collected behavioral data.

Richard Ryan: Collected behavioral data.

Shann Menard: Collected behavioral data.

*Alex M. Gavril*a: Collected behavioral data.

James G. Pfaus: Coauthor of the theoretical design and helped with the writing.

Part 2. Neurochemical basis of Conditioned Partner Preference in the Female Rat: II.

Disruption by Flupenthixol

Authors:

Genaro A. Coria-Avila: Author of the theoretical design, collected a most of the data, performed all the data analysis and graphing, and was responsible for writing the paper.

*Alex G. Gavril*a: Collected behavioral data.

Brigitte Boulard: Collected behavioral data.

Natalie-Anne Charron: Collected behavioral data.

Geoffrey Stanley: Collected behavioral data.

James G. Pfaus: Coauthor of the theoretical design and helped with the writing.

Not an author

Erin Saucke-Lacelle: Collected data for some animals.

CHAPTER 4

Activation of Oxytocin, Vasopressin or Gonadotrophin Releasing Hormone (GnRH)

Neurons by Strain Cues Associated with Conditioned Partner Preference in Female Rats

Authors:

Genaro A. Coria-Avila. Author of the theoretical design, collected a most of the data, performed all the data analysis and graphing.

James G. Pfaus. Coauthor of the theoretical design

LIST OF ABBREVIATIONS

6-OHDA	Hydroxidopamine
ACC	Anterior cingulate cortex
aLHA	Anterior hypothalamus, antero lateral
ANOVA	Analysis of variance
AOB	Accessory olfactory bulb
ArcN	Arcuate nucleus
AVP	Arginine vasopressin
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
BNSTpd	Bed nucleus of the stria terminalis posterodorsal
BSR	Brain stimulation reward
CEP	Conditioned ejaculatory preference
CoA	Cortical amygdala
CPA	Conditioned place aversion
CPP	Conditioned place preference
CPu	Caudate putamen
CR	Conditioned response
CS	Conditioned stimulus
DA	Dopamine
DLS	Dorsolateral striatum
DLTh	Dorsolateral thalamus
DMS	Dorsomedial striatum
DMTh	Dorsomedial thalamus
DOR	Delta opioid receptor
fMRI	Functional magnetic resonance imaging
Fos-IR	Fos immunoreactivity
GABA	Gamma amino butyric acid
GnRH	Gonadotrophin Releasing Hormone
H&D	Hops and Darts
HPG	Hypothalamic pituitary gonadal axis
i.c.v.	Intracerebro ventricular
I.M.	Intramuscular
i.p.	Intraperitoneal
ICC	Immunocytochemistry
KO	Knock out
KOR	Kappa opioid receptor
LE	Long-Evans
LH	Luteinizing hormone
Lhab	Lateral habenulla
LiCl	Lithium Chloride
LS	Lateral septum
LSD	Least significant difference

MeA	Medial amygdala
MePD	Medial amygdala posterodorsal
MHC	Major Histocompatibility Complex
MOB	Main olfactory bulb
MOR	Mu opioid receptor
mPOA	Medial preoptic area
NAc	Nucleus accumbens
NAcc	Nucleus accumbens core
NAcSh	Nucleus accumbens shell
NTS	Nucleus of the tractus solitarius
OT	Oxytocin
PAG	Periaqueductal gray matter
Pir Ctx	Piriform cortex
POA	Preoptic area
PVN	Paraventricular nucleus
s.c.	Subcutaneous
SEM	Standard error of the mean
T	testosterone
UCR	Unconditioned response
UCS	Unconditioned stimulus
VCS	Vagino cervical stimulation
VMH	Ventro medial hypothalamus
VMHvl	Ventro medial hypothalamus, ventrolateral
VTA	Ventral tegmental area
W	Wistar

GENERAL INTRODUCTION

*“That animals choose their mates, as opposed to mating indiscriminately,
is an attractive and evolutionarily important hypothesis.”*

-- Halliday, (1983) pp 28

Partner preferences are displayed by many social species, including humans. They are commonly observed as selective contacts with an individual, more time spent together and directed courtship behavior that leads to selective copulation. Species with non-exclusive preferences for a particular partner are usually referred to as polygamous. A polygamous individual may display a preference toward multiple individuals of the same kind (e.g., sexually active or receptive partners), but this preference usually lasts only for periods of mating. Moreover, the preference is not for particular individuals or features of an individual, other than the overall display of sexual receptivity. On the other hand, species that display exclusive and long-lasting preferences towards one particular partner are usually referred to as monogamous. A monogamous individual will display a very selective preference to court, copulate, nest-build, and raise offspring, with a particular partner that bears specific and recognizable features.

Partner preferences are the result of a systematic interrelationship between genetic mechanisms, hormonal effects and learning. For example, an individual can be born with the genetic information that directs brain organization and the hormonal profile that facilitates the sensitivity to respond towards a particular type of partner, which generally occurs towards a sexually mature individual of the opposite sex. However, animals can learn new preferences based on exposure to individuals of their own species since birth. This early contact facilitates phenomena such as imprinting (Bateson, 1978), in which the

first conspecific-related stimuli sensed during critical periods of development can direct future partner preferences. Consequently, the partner preference observed in a sexually naïve adult individual, may be the result of innate factors combined with early learning experiences during critical periods. In addition, all individuals can develop new preferences or aversions throughout the life span, and make new associations in order to pursue pleasure and avoid pain. Accordingly, adult partner preferences may become conditioned to stimuli that have become predictors of sexual reward. Thus, in the presence of a predictor of sexual reward, partner preference may be facilitated or more easily expressed, whereas in the presence of a negative predictor, partner preference may be disrupted. As a consequence, “innate” partner preferences (e.g., for assortative features) may become further narrowed or even changed by subsequent conditioning in adulthood by features associated specifically with sexual reward.

Partner preferences can be studied from different perspectives in many species of animal. For example, from a biological point of view it is important to study the consequences of having a partner preference on the survival and reproductive fitness of a species. From the psychological perspective, partner preferences are studied because they can lead to social attachments, referred to as pair bonds in animals, and so-called “romantic love” in humans, and disruption of established attachments can have negative effects on mental health (Insel & Young, 2001). Thus, understanding the bases of partner preference formation is necessary to understand an important part of social behavior in animals and humans.

Role of learning in sexual behavior and conditioned partner preference

Learning can affect many aspects of sexual behavior, which in turn, can affect directly or indirectly the expression of partner preferences. These aspects include the expression of sexual excitement, the capacity to locate and recognize a mate, courtship behavior, and copulatory parameters (Pfaus, Kippin, & Centeno, 2001). Learning can increase or decrease an animal's sensitivity to respond to internal and external stimuli, which trigger their own sexual desire and indicate who is a potential mate and what a sexual incentive is. This occurs as a result of two learning mechanisms, Pavlovian conditioning and instrumental (operant) conditioning.

Influence of Pavlovian conditioning on sexual behavior

Pavlovian or classical conditioning refers to an association that is formed between two stimuli (Pavlov, 1927). For example, under normal circumstances unconditioned stimuli (UCS) will elicit physiological unconditioned responses (UCRs). UCRs are those unlearned responses already present in an animal's natural repertoire. UCSs are natural stimuli that normally elicit UCRs in a presumably hardwired stimulus-response (S-R) neural connection. Neutral stimuli, however, will not trigger any UCR, but if properly paired in time with an UCS, animals can make a predictive association between the neutral stimulus and the UCS, which then triggers the UCR. When a neutral stimulus is capable of triggering a response that was not present before learning, it is referred to as a conditioned stimulus (CS), and the response is referred to as a conditioned response (CR). When this occurs it is believed that the CS elicits a representation of the UCS at a neural level.

There are different ways in which Pavlovian conditioning can affect sexual behavior, and ultimately the expression of partner preference. First, a mate can be seen as a conjunction of multiple stimuli; some of them are UCS, which trigger UCRs, but many others are not (Kippin & Pfaus, 2001a). Ineffective natural stimuli, may become associated with UCSs through sexual experience, and in turn, may be able to elicit CRs. Also, originally neutral or ineffective stimuli can become conditioned if are paired in contingency with the UCS. And finally, certain patterns in an environment or context can be conditioned to sexually relevant UCSs and in that way become able to elicit CRs. Conditioning of sex-related stimuli appears to occur during two important critical periods of development, one during an early postnatal phase, and another after puberty, during an animal's first sexual encounters.

Early postnatal period

Certain stimuli that are sensed during early critical periods of life become associated through Pavlovian conditioning with innate rewards (maternal care, nutrient intake, etc.). This type of conditioning is termed "imprinting", and can strongly affect sexual preferences in adulthood (Bateson, 1978). This is a special form of conditioning in that the brain is especially sensitive during the critical period to make the association. Imprinting usually occurs to the features of parents and species, and is considered the first step in the phenomenon of assortative mating, in which animals choose to mate selectively with members of their own strain relative to members of a different strain or species that are genetically less similar. Assortative mating is believed to maintain homozygosity in a strain, and thereby keep strains from outbreeding positive characteristics. In humans, assortative mating can be observed in partner preferences for

phenotypic (e.g., racial, facial, etc.), social (e.g., cultural/religious beliefs), and personality characteristics (e.g., introversion/extroversion) that are somewhat similar to one's own (Luo & Klohnen, 2005; Malina, Selby, Buschang, Aronson, & Little, 1983; Salces, Rebato, & Susanne, 2004).

There is evidence indicating that males of different species can develop sexual imprinting for mates that bear cues associated with the female that nursed them or cues associated with the nursing period *per se*. In one study, for example, neonatal rats were nursed by their biological mother which had a neutral odor (lemon) applied on her abdomen. At the appropriate time, the males were weaned and never exposed to the essence again, until about 100 days of age, when they were paired with scented or unscented females for copulation. The results indicated that males exposed in infancy to a lemon essence, displayed shorter ejaculation latency with lemon-scented females, relative to the ejaculation latency observed when they were exposed to non-scented females (Fillion & Blass, 1986). This experiment was one of the first to demonstrate that neutral odors that are sensed during early periods can increase sexual excitement during future sexual encounters. In this case, a stronger sexual excitement was observed as shorter ejaculation latency with a receptive female bearing the odor.

Other experiments with imprinting in male rats have been more focused on partner preference, and have demonstrated that this type of learned preference may depend on rewarding stimuli that the mother provides to the offspring during critical periods of life. For example, the positive effects of licking during the first ten days of life can be conditioned to olfactory stimuli as well. In one recent study, newborn male pups were taken away from their mothers during 15 minutes every day. During this time away,

males in a Paired group were exposed to a lemon essence sprayed on the woodchip bedding of a different cage. At the same time they received tactile reward stimulation performed artificially with a small paintbrush on their back and head, so that the strokes would mimic the dam's licking at the time that they smelled the lemon essence. Males in a control group were exposed to woodchip bedding sprayed with water alone. Both groups were weaned at 21 days of age, and never exposed to the essence again. After two months, males were placed in a large open field and allowed to copulate freely with two females at the same time, one scented and one unscented. The results of that preference test indicated that a significant proportion of the Paired males displayed a preference to ejaculate first with the scented female, whereas the control group showed no preference for scented females (Menard, Gelez, Coria-Avila, Jacobovich, & Pfaus, 2006). That study demonstrated that neutral odors paired with a maternal-like reward during early development can be learned and consequently direct partner preference during future sexual encounters. Taken together with the results of Fillion and Blass (1986), these findings indicate that olfactory stimuli sensed during early and critical periods of life can become imprinted, and therefore can increase sexual excitement and motivation to direct partner preferences in naive male rats.

Conversely, there is a study suggesting that olfactory imprinting does not impact adult sexual behavior in male rats. Specifically, Moore, Jordan & Wong (1996), showed that male rats reared by lemon-scented dams, approached lemon-scented females with the same latency than normally reared males when tested in adulthood; without any difference in copulatory behavior during successive pairings, including ejaculation latency. In addition, in experiments with a two-female one-male situation, Moore and

colleagues demonstrated that lemon-reared males performed the first mount more rapidly towards scented females, although the authors reported that normally-reared males also performed the first mount towards scented females (Moore, Jordan & Wong, 1996).

There are numerous methodological differences found between these studies that may have contributed to the disparate findings. For example, Ménard et al. (2006) assessed the proportion of males performing preferential ejaculation as evidence for conditioned partner preference, whereas Fillion & Blass (1986) and Moore et al., (1996) used frequency and latency measures of copulatory behavior as the dependent variable. It is possible that conditioned ejaculatory preferences induced by olfactory imprinting in male rats are mainly observed as a choice in mating strategy and not as a large difference in the frequency or latency of sexual behaviors. This may indicate that a presumably polygamous male rat develops an ejaculatory mating preference as a result of imprinting, although his sexual behavior overall is not directed exclusively toward the preferred partner.

Stimuli other than odors can be imprinted and direct partner preferences. Burley (2006) showed that birds can be imprinted by visual cues. For example, Zebra finches reared by parents that wore white crests with a vertical black stripe, developed sexual preference towards opposite-sexed birds wearing this type of crest, whereas birds that were reared in another colony by parents that wore crests having a horizontal black stripe developed a preference towards opposite-sexed partners with horizontal stripes (Burley, 2006).

Some experiments have demonstrated that the effects of imprinting are so powerful that it can actually induce sexual preferences towards a different species. In one

study, for example, male sheep and goats that were cross fostered developed a sexual partner preference towards females of the species of the foster mother (Kendrick, Hinton, Atkins, Haupt, & Skinner, 1998). Together, these studies indicate that animals can learn to prefer stimuli that were sensed during early critical periods of life. In some cases, such preference may be expressed as courtship behavior only. In other cases, however, the associations affect mating behaviors (i.e. ejaculatory preferences in rats).

It can be argued that constant preference for familial features in a mate should not be desirable, since it would facilitate inbreeding. Continuous inbreeding may result in the phenotypic expression of unwanted genotypic information, which is passed on as recessive genes from generation to generation without being expressed, until two parents with similar genotype reproduce. Accordingly, sexual imprinting should not be the best strategy to reproduce and animals should look for partners genetically different in order to avoid inbreeding.

Observations of the mating strategies in house mice indicate that they avoid mating with individuals that have a similar major histocompatibility complex (MHC). The MHC genes produce molecules that help the immune system distinguish organisms that are different, and that could potentially cause diseases. A MHC that is more heterogeneous will have a broader range to recognize what is familiar or different. Consequently, the more different are the genes from the two parents, the more heterogeneous will be the MHC of the offspring, which results in a more capable immune system. It has been argued that animals should have systems that evolved to recognize and prefer potential mates with different MHC. That is, partner preference should be directed towards non-related individuals, rather than towards genetically-similar partners

that are potential carriers of unwanted genotype. There is evidence indicating that the natural tendency of mice to mate with partners of a different haplotype is not innate, since sexual preference can be reversed towards a partner of the same haplotype through imprinting. In one study, for example, male mice that were reared by a foster mother of a genetically different strain, displayed a copulatory preference towards females of their own strain (Yamazaki et al., 1988), which may suggest that the relatives were not recognized as familial and therefore were preferred as mates.

Learning to recognize familial odors would indicate to an animal its family identity, and therefore would help to avoid mating with them (potentially carrying similar genes). Penn & Potts (1998) demonstrated that mice can recognize MHC of other individuals through olfactory signals, and that such olfactory recognition is learned through imprinting in early periods of life. In one study, they cross-fostered female mice pups with mothers that had different MHC genes. When the pups became adults, partner preference was tested toward individuals with similar MHC or with MHC genes of the foster family. Similar to the results of Yamazaki et al., (1988), Penn & Potts showed that females avoided mating with males carrying MHC genes similar to the foster family, which supported the hypothesis that MHC-dependent familial imprinting provides a mechanism for avoiding inbreeding (Penn & Potts, 1998).

Bateson (1978) claimed that sexual imprinting facilitates the best possible outbreeding and prevents animals from inbreeding. His statement was based on a series of mate-choice experiments with Japanese quails. He demonstrated that males showed the highest rates of approach and copulation with females whose coloration was slightly different from that of the foster mother, relative to females with the exact coloration

(Batenson, 1978). This has led to the suggestion that as a result of imprinting, mate choice is directed towards a partner that bears cues slightly unfamiliar, which is evaluated based on familiar memories consolidated during early and critical periods of life. Accordingly, imprinting facilitates preference towards an individual that is slightly different to guarantee outbreeding, and at the same time it guarantees breeding with an individual that is familiar and probably equally adapted to the environmental circumstances.

Nevertheless, the causes and consequences of sexual imprinting are not completely understood, and it may differ completely from one species to another. It is yet unknown what is the neurobiology and neurochemistry of behaviors produced by imprinting, and whether conditioned cues such as artificial odors are imprinted in the same manner that natural odors (i.e. those that result from a specific MHC), or visual and acoustic cues.

Postpubertal period

The postpubertal period is more flexible in time than the early postnatal period, and typically involves a cascade of experiences that move the animal toward copulation and sexual reward. Here, the hormonal activation of brain regions that underlie adult sexual behavior is critical, as are the actual stimuli perceived by animals during their first sexual experiences. There is evidence indicating that animals can develop preferences for places and partners that predict sexual reward.

Conditioned Place Preference: Male rats develop a conditioned place preference (CPP) for distinctive sides of a CPP box following ejaculation, but not intromissions alone, indicating that ejaculation creates a state of reward that can be associated with an environmental stimulus (Ågmo & Berenfeld, 1990). Thus, a distinctive side of a CPP box

(CS) earns incentive value through Pavlovian association with the reward state induced by ejaculation (UCS). The development of ejaculation-induced CPP can be blocked by administration of the opioid receptor antagonist naloxone, but not the dopamine receptor antagonist pimozide (Ågmo et al., 1990; Miller & Baum, 1987). This indicates that the augmentation of endogenous opioid activity following ejaculation mediates its rewarding aspects. Bilateral infusions of the opioid antagonist methylnaloxonium to the medial preoptic area (mPOA) of male rats also abolished an ejaculation-induced CPP (Ågmo & Gomez, 1993), suggesting that opioid release in this region is critical. In contrast, although extracellular dopamine concentrations in the mPOA and limbic areas such as the nucleus accumbens (NAc) increase during mounts with intromission, they fall rapidly after ejaculation (Blackburn, Pfaus, & Phillips, 1992). This makes it unlikely that dopamine release contributes to the ejaculation-induced reward state.

Female rats develop a CPP following experience with the ability to control or “pace” the initiation and rate of copulation (Paredes & Alonso, 1997). Pacing allows females to regulate the timing of copulatory contact with males (Erskine, 1989; Gilman & Hitt, 1978; Pfaus, Smith, & Coopersmith, 1999) and enforces a time interval between successive intromissions. Control over the rate of vaginocervical stimulation (VCS) received from intromissions facilitates pregnancy by activating the appropriate neuroendocrine systems [e.g., bydaily prolactin surges in rats; (Adler, 1969; Kornberg & Erskine, 1994; Terkel & Sawyer, 1978)]. In the natural world, pacing occurs simply by the female soliciting the male and running away at her preferred intervals. In the laboratory, “pacing” chambers allow females to do this by imposing a barrier that she must cross to get to the male. This is typically done using a Plexiglas partition with small

holes cut out of the bottom that are large enough to allow the female to pass from one side without a male to the other side with a male, but too small to allow the male to pass from side to side. Thus, the female regulates her sexual contact with the male by running back and forth to his side. Nonpaced copulatory conditions can be contrasted in the same apparatus without the partition. Female rats find paced copulation rewarding, and will form CPP for distinctive sides of a CPP box associated with paced copulation relative to sides associated with nonpaced copulation (Paredes et al., 1997). Indeed, applying VCS in a distributed pattern manually with a lubricated glass rod prior to CPP training, relative to a single, powerful VCS of long duration, produces a CPP for the side associated with the distributed VCS (Afonso & Pfaus, submitted). Thus the state associated with distributed VCS is a UCS that supports the development of CPP. As with males, the expression of a copulatory CPP is abolished by treatment with naloxone, but not the dopamine antagonist flupenthixol, during training (Garcia Horsman & Paredes, 2004; Paredes & Martinez, 2001).

Conditioned place preference and conditioned approach behavior are consistent with the idea that animals will approach and prefer conditioned stimuli that have been paired with reward (for review see Nader, Bechara, & van der, 1997; Tzschentke, 1998; Pfaus, Kippin, & Coria-Avila, 2003). This indicates a search strategy based on past experiences, so that animals have greater probability of coming into contact with stimuli that induce a positive affect and that, if found on partners, may represent increased fitness (Panksepp, Knutson, & Burgdorf, 2002).

Conditioned partner preference: Neutral olfactory stimuli paired with copulation to ejaculation can become CSs and alter neuroendocrine and behavioral responses. For

example, Graham and Desjardins (1980) showed that in male rats, the levels of luteinizing hormone and testosterone were increased following exposure to a conditioned odor (i.e. wintergreen) previously paired with copulation. The increases were similar to those following exposure to estrous odors in naïve males, suggesting that association with the copulatory reward state makes a neutral odor to become a CS capable of triggering a conditioned neuroendocrine response that prepares the animal for a sexual behavior.

Conditioned odors associated with copulation can also facilitate partner preference. For example, Kippin et al. (1998) trained one group of males (the Paired group) to associate an almond or lemon odor painted on the back of a female's neck and anogenital region with copulation to ejaculation. Another group (the Unpaired group) received copulatory trials with unscented females. On a final test in an open field, the males received access to two sexually receptive females, one scented with the odor and the other unscented. Males in the Paired group displayed a conditioned partner preference in which the scented females were chosen to receive the males' first ejaculation. Subsequent studies revealed that the learning of this conditioned ejaculatory preference (CEP) took place during the postejaculatory refractory period (Kippin et al., 2001a) and that the males became "choosy" the closer they were to ejaculating (Kippin & Pfau, 2001b). Thus, polygamous male rats could learn a rudiment of pair-bonding with a simple Pavlovian conditioning procedure that links a neutral olfactory stimulus to sexual reward. Such findings supported the idea that the post ejaculatory period is capable of supporting conditioned preferences, in a similar manner to the CPP that develops following ejaculation (Ågmo et al., 1990).

The neural areas involved in CEP have also been studied. Fos immunoreactivity (Fos-IR) was used to assess the activation of neural areas activated by the conditioned odor, relative to the areas activated by estrous odors, which normally produce sexual motivation in males (Kippin, Cain, & Pfaus, 2003). In that study, Kippin and colleagues showed that estrous odors induced significantly more levels of Fos-IR in the nucleus accumbens core (NAcc), medial bed nucleus of the stria terminalis (BNSTm), mPOA, ventromedial hypothalamus (VMH) and ventral tegmental area (VTA). The conditioned odor, however, induced more Fos-IR in the piriform cortex (Pir Ctx), anterior portion of the lateral hypothalamus (aLH), and basolateral amygdala (BLA). Consequently, the authors concluded that conditioned and pheromonal odors activate similar (i.e. NAcc) but also independent pathways in the limbic system and hypothalamus.

Influence of instrumental conditioning on sexual behavior

Instrumental or operant learning describes a response-reinforcer contingency in which an animal learns to operate on its environment (Skinner, 1953, 1966).

Instrumental learning occurs when an animal adapts its behavioral responses under particular schedules of reinforcement (Ferster & Skinner, 1957), circumstances that have been associated with the delivery of reward or punishment. Specifically, when an animal shows a response that is followed by a reward, the frequency of that response increases and its latency decreases. This is referred to as positive reinforcement. Conversely, when an animal's response is associated with punishment, the response is likely to diminish in frequency and increase in latency. Responses that decrease the likelihood of punishment are more likely to be engaged in through a process of negative reinforcement. On the

other hand responses that eliminate the possibility of positive reinforcement (so called “time-out” periods) are less likely to be engaged in.

Sexually experienced animals can learn to perform a variety of tasks in order to gain access to a partner, presumably because of the association with sexual reward (Pfaus et al., 2001). It is believed that the capacity to experience reward during sexual behavior evolved to facilitate the likelihood of copulation. Therefore, from a psychological perspective, sex must have rewarding properties if stimuli that predict copulation increase the probability of appetitive instrumental responses aimed at working for or approaching those stimuli. Observing the amount of work that an animal is willing to perform to gain access to another individual can be used to calculate the degree of motivation that induces a preference toward a particular conspecific. Such performance can indicate levels of sexual motivation triggered by the cues on the partner, or can be used to infer partner preference if animals are allowed to choose between several potential conspecifics to copulate with. Male rats, for example, can easily learn to bar press in order to gain access to a receptive female (Beck, 1971; Beck & Chmielewska, 1976; Schwartz, 1956), and females can also learn to bar press to gain access to a sexually active male (Beck, 1971; Bermant & Westbrook, 1966; French, Fitzpatrick, & Law, 1972). Commonly, these studies involve a rat learning to bar press in an operant chamber, in which after certain number of bar presses, a mate is delivered into the box (e.g., Everitt, Cadogan, & Robbins, 1989). Other species also learn to work for access to a mate. These include bar pressing in male and female rhesus monkeys (Michael & Keverne, 1968; Keverne, 1976) and key pecking in pigeons (Gilbertson, 1975). In rats, operant learning also includes climbing over a obstacle to have access to a receptive female (Sheffield, Wulff, & Bakker, 1951),

digging through sand or crossing shock grids (Anderson, 1938), and turning a wheel (Denniston, 1954), to gain access to sex partners.

Because gaining access to sex partners is necessary for copulation, and therefore for the experience of sexual reward, operant tasks are also preparatory behaviors that animals must perform to obtain those rewards (Blackburn et al., 1992; Pfaus, 1999). In the natural world, and to some extent in the laboratory, male rats must chase females after each solicitation in order to copulate with them, and doing so appears to enhance the female's incentive value and be a necessary antecedent of sexual reward. For example, female rats treated systemically with DA antagonists such as haloperidol or flupenthixol, display no solicitations or precopulatory (proceptive) behaviors but show increased lordosis quotients and durations (Everitt, 1990; Kato & Sakuma, 2000). Male rats do not display robust sexual behavior with those females, despite the augmentation of lordosis, and typically have longer intromission and ejaculation latencies indicative of decreased arousal. Indeed, castrated males, or males with subthreshold mesolimbic DA depletions (produced by infusions of 6-hydroxydopamine to the nucleus accumbens and striatum that do not induce motor impairment) will not even attempt copulation with those females, whereas they copulate robustly with females that display solicitations (Madlafousek, Hlinak, & Beran, 1976; Everitt, 1990). Like chasing behavior for males, pacing behavior is a necessary antecedent of sexual reward in females, as it provides them with a distributed intromission pattern from the male that facilitates both reproduction and reward. In unilevel pacing chambers bisected by a Plexiglas partition with holes cut out of the bottom, females must cross the partition to obtain intromissions

and ejaculations. Thus, pacing in these boxes is both an operant and a preparatory behavior.

Role of sexual reward in learned preferences

In males, the sexual reward state induced by ejaculation is a critical UCS that facilitates a subsequent preference for stimuli that predict it. It has been hypothesized that CEP in rats may be a rudiment of the monogamous behavior observed in other species of rodents (Pfaus et al., 2001). For example, mating facilitates pair-bonding in monogamous Prairie voles (Williams, Catania, & Carter, 1992), which has led to the hypothesis that mating-induced pair bonds are mediated by sexual reward (Young & Wang, 2004). Pair bonds are observed when a vole has the choice of two partners, one familiar, with whom copulation occurred previously, and one novel. A bonded vole usually selects the familiar one to spend more time, copulate and reproduce with. Some reports indicate that this behavior may last for life, since bonded individuals rarely mate with other partners even following permanent separation from the original partner (Pizzuto & Getz, 1998; Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993). It is possible that a bonded vole remains monogamous because of the constant positive reinforcement from the partner during social contact and recurrent mating.

The study of Kippin et al. (2003) indicates that both estrous odors and conditioned odors associated with ejaculation in male rats increase Fos-IR in some independent and some common mesolimbic and hypothalamic areas important for many motivated behaviors. Accordingly, two Prairie voles may be initially attracted given the natural effects of estrous pheromones on motivation, but this preference will be reinforced by

subsequent mating and sexual reward. As a result, the specific features of the partner (e.g. olfactory signature) may become conditionally preferred and reinforced by social stimulation and mating.

There are arousing stimuli other than those experienced during copulation which can also facilitate the formation of pair bonding. For example, in male Prairie voles, long periods of swimming are believed to be stressful. If voles are forced to swim and then allowed to cohabit for a period of six hours (which is normally not enough time to induce bonding), pair bonds are more likely to occur (Carter, 1998; DeVries, DeVries, Taymans, & Carter, 1996). This behavior is believed to be facilitated via the hormones that are released during the stress response (i.e. corticosteroids), because injections of corticosterone in males facilitate the formation of pair bonds (DeVries et al., 1996).

The previous data demonstrate that there are many natural stimuli that can facilitate the development of partner preferences. They can develop relatively slowly as a consequence of cohabitation, but arousing stimuli such as stress and reward readily facilitate and speed up its formation. Social stimulation in bonded animals is believed to be rewarding because it reduces anxiety and stress. In social species such as the monogamous Prairie voles, stressful situations might facilitate social contact because bonding is one way to counterbalance stress and anxiety.

It is still unknown whether partner preferences or pair bonds that develop slowly as a consequence of cohabitation or rapidly as a consequence of stress or reward, have a similar neural basis. It is probable that preferences that develop fast may be the result of directed attention and learning towards stimuli that represent an advantage for

reproduction or survival. Thus, stimuli that predict sexual reward will induce responses that prepare the animal to obtain it.

Some species develop compulsive behaviors or ignore threats in order to get access to a sex partner or a drug. Regardless of the fact that drug effects are more severe and long-lasting, it has been argued that reward during sex or drug-taking may play an important role in the process that leads to the formation of pair bonds or becoming addicted. In fact, Insel (2003) has argued with tongue-in-cheek that social attachment may be an “addictive disorder” given the similarity in function and neural systems that underlie it.

As mentioned throughout the general introduction, sexual reward has been reported to facilitate the formation of partner preference in various species of rodents and potentially humans. In monogamous Prairie voles, sexual reward is required to induce long-lasting and selective preferences for a particular mate. In polygamous male rats, conditioned odors direct males to ejaculate preferentially with females that bear the odor, despite the “innate” preference for different or novel females commonly assumed to occur as a natural display of the “Coolidge effect” (Bermant, Lott, & Anderson, 1968). It is not yet known if a similar preference can be established in female rats. Although paced copulation can induce a state of reward that is sufficient to induce a copulatory CPP, whether the same reward can induce conditioned partner preference is not yet known. If so, then evolutionary biologists and psychologists must take into consideration the idea that an individual’s experience with sexual reward can override presumably “innate” mate choices (e.g., assortativeness) or mate strategies (e.g., monogamy or polygamy) by means of Pavlovian and operant contingencies. In fact, it is likely as innate to learn about

the environment in ways that maximize reward and minimize aversive outcomes, making so-called “proximate” causes (e.g., pleasure) ultimately more powerful predictors of social behavior and choice than so-called “ultimate” causes (e.g., genetic or reproductive fitness). In this respect, finding common brain regions and neurochemical or endocrine systems activated in monogamous and polygamous species during their initial experiences with sexual reward should have a profound impact on our understanding of diversity in mate choice and mate strategies.

Outline of the experiments

The experiments in this thesis investigated how female rats develop a conditioned partner preference for cues associated with sexual reward. To investigate this, we used the experimental manipulation of paced copulation (PC), which has been shown to induce a reward state of sufficient intensity to be conditioned to environmental stimuli and thus form a CPP (Paredes et al., 1997; Paredes & Vazquez, 1999). Chapter 1 presents the behavioral results of two studies showing that odor or strain cues paired with PC induced a strong conditioned partner preference in Paired females towards males bearing the CS, compared to females in Unpaired or Random control groups that did not display a preference (Coria-Avila, Ouimet, Pacheco, Manzo, & Pfaus, 2005; Coria-Avila et al., 2006). Chapter 2 comprises two experiments carried out to study the brain areas involved in such preference. Those experiments assessed the general expression of the neuronal-activation marker Fos, the protein product of the immediate-early gene *c-fos*, to map brain regions activated following exposure to CSs associated with PC. Chapter 3 examined pharmacological mechanisms. The role of two neurotransmitter systems,

opioids and dopamine, were assessed in the development of conditioned partner preferences using the receptor antagonists naloxone or flupenthixol, respectively. Chapter 4 assessed preliminary results of pilot studies that used a double-labeling technique to examine the expression of Fos within oxytocin (OT), vasopressin (AVP), and Gonadotrophin Releasing Hormone (GnRH) neurons following exposure to strain cues alone in paired and unpaired animals.

All of the studies in the chapters, with the exception of those in Chapter 4, are papers that have been either published or submitted for publication to indexed, peer-reviewed scientific journals. Although each paper has its own introduction and discussion, a general discussion is included at the end of the thesis to consider overall implications of the research and future directions.

CHAPTER ONE

PART 1

Olfactory Conditioned Partner Preference in the Female Rat

Genaro A. Coria-Avila and Allison J. Ouimet
Concordia University

Pablo Pacheco and Jorge Manzo
Universidad Veracruzana

James G. Pfaus
Concordia University

RUNNING HEAD: CONDITIONED PARTNER PREFERENCE

Center for Studies in Behavioral Neurobiology, Department of Psychology,
Concordia University, Montréal, QC, Canada, and Instituto de Neuroetología,
Universidad Veracruzana, Xalapa, VER, México.

This research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada (OGP-0138878) to JGP, a postgraduate fellowship from CONACyT of Mexico (128099) to GAC, and an NSERC summer undergraduate scholarship to AJO. The authors would like to thank Drs. Tod Kippin and Raúl Paredes for useful discussions, and Jessica-Marie Haley for her help conducting the experiments. All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

Correspondence concerning this article should be addressed to Genaro A. Coria-Avila, D.V.M., M.Sc., CSBN-Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC, H4B 1R6, CANADA. E-mail: coria@csbn.concordia.ca

Citation: *Behavioral Neuroscience* (2005), 119, 716-25

© American Psychological Association, reprinted with permission

Abstract

Paced copulation induces conditioned place preference in female rats. We examined whether associating almond-scented males with paced copulation induces conditioned partner preference. The Paired group received 4 paced copulations with almond-scented males, and 4 non-paced copulations with unscented males, sequentially at 4-day intervals. The Unpaired group received the opposite order of association, whereas the Random-Paired group received random associations. A fourth group received a single pairing. On the final test, females were placed into an open field with two males, one scented and one unscented. Females in the Paired group solicited the scented male more frequently and most chose the scented male for their first ejaculation. Thus, an odor paired with paced copulation elicits conditioned partner preference in female rats.

Olfactory stimuli play an important role in the expression and modulation of sexual behavior in the rat (Cain & Paxinos, 1974; Larsson, 1971; McClintock & Adler, 1978; McClintock, 1978; McClintock, 1984b; Schank & McClintock, 1997). Pheromonal and olfactory stimuli emitted naturally from sexually receptive females (i.e. estrous odors) are attractive to males (Bressler & Baum, 1996), and produce sexual arousal (Sachs, 1997). Estrous odors also increase serum luteinizing hormone and testosterone levels in male rats (Graham & Desjardins, 1980) and increase Fos activation within a common set of brain structures in several rodent species, including the accessory olfactory bulb, nucleus accumbens core and shell, medial preoptic area, bed nucleus of the stria terminalis, medial amygdala, and the ventral tegmental area (Bialy & Kaczmarek, 1996; Kippin et al., 2003; Newman, Parfitt, & Kollack-Walker, 1997; Pfaus & Heeb, 1997; Swann & Fiber, 1997; Veening & Coolen, 1998). Likewise, female rats find the odors of male rats attractive (Bermant, 1961; Bermant et al., 1966; Eliasson & Meyerson, 1975; Pfaus et al., 1999), and exposure to male odors increases Fos activation in similar brain regions (Pfaus, Kleopoulos, Mobbs, Gibbs, & Pfaff, 1993; Wersinger, Baum, & Erskine, 1993).

Neutral odors (e.g., almond, lemon, wintergreen) associated with copulation to ejaculation in male rats elicit conditioned elevations in serum luteinizing hormone and testosterone (Graham & Desjardins, 1980), increases in sexual arousal, and a phenomenon referred to as “conditioned ejaculatory preference” in which males display a partner preference for ejaculation but not copulation per se (Kippin, Talinakis, Chattmann, Bartholomew, & Pfaus, 1998). In the Kippin et al. studies, males in the paired group copulated with sexually receptive females that had the odor smeared on the

back of their necks and anogenital regions. Several Pavlovian control groups were utilized in which males received either no copulatory experience with the odor (unpaired), random pairing of the odor with copulation, or the odor explicitly unpaired (i.e., paired with exposure to non-receptive females). The final test took place in an open field with two receptive females, one scented and the other unscented. Although all males mounted and intromitted indiscriminately with the two females, those in the paired group ejaculated preferentially with the scented females, and did so for at least the first two ejaculations. Males in the unpaired group did not show a significant preference for either female. Males in the randomly paired-group switched females for each subsequent ejaculation, reminiscent of a “Coolidge effect” (Beach & Jordan, 1956), whereas males in the explicitly unpaired group displayed an early avoidance of the scented female. Conditioned ejaculatory preferences did not develop if males did not ejaculate (Kippin et al., 2001a), and significant preferences were observed following a paired male’s first ejaculation (Kippin, Samaha, Sotiropoulos, & Pfaus, 2001c).

Currently, more is known about the role of learning in the sexual behavior of males relative to females (reviewed in Pfaus, Kippin, & Centeno, 2001). In the case of females, however, it is known that they display complex patterns of behavior that allow them to control virtually all aspects of sexual interaction with males (Pfaus et al., 2001). Females attract and solicit males (Beach, 1976; Erskine, 1989; McClintock, 1984a) and control or “pace” the rate of sexual interaction (Erskine, Kornberg, & Cherry, 1989); (Pfaus et al., 1999). Like males, females display a variety of conditioned appetitive behaviors, including a conditioned place preference (CPP) for distinctive environments associated with sexual reward (Oldenburger, Everitt, & de Jonge, 1992). As with

conditioned ejaculatory preferences, significant CPP develops in males only if they ejaculate during the conditioning trials. What constituted the precise rewarding effect of copulation for females remained elusive until Paredes and colleagues found that female rats show CPP for distinctive sides associated with paced copulation, relative to non-paced copulation (Paredes et al., 1997; Paredes et al., 1999). As with male rats, this copulatory CPP in female rats can be disrupted by injections of the opioid receptor antagonist naloxone (Paredes et al., 2001), suggesting that elevated levels of endogenous opioids induced by paced copulation form an important part of the neurochemical reward substrate, as they do in male rats following ejaculation (Miller et al., 1987; Agmo et al., 1990). Thus, paced sexual interactions induce a positive affective state of sufficient intensity and duration to induce CPP.

In the present study, we asked whether the association of almond odor with pacing would induce a preference for almond scented males, similar to the preference observed in males for females bearing an almond odor paired with ejaculation.

Method

Animals and Surgery

Long-Evans male (300-350 g) and female (200-250 g) rats were purchased from Charles River Canada (St-Constant, QC). They were housed in groups of 4 in large hanging wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Food and water were continuously available.

The females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then ovariectomized bilaterally via a lumbar incision. All females were given a week of post-surgical recovery prior to odor conditioning trials. Intact Long-Evans male rats that served as stimulus males had at least 10 tests of sexual behavior in bi-level chambers prior to the start of these experiments. These males were sexually vigorous and initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test. If scented, males received almond extract (Blue Ribbon, Etobicoke, ON) applied to the back of their necks and anogenital regions with a cotton swab. Unscented males received similar applications of distilled water to the same areas.

Odor Conditioning

Odor conditioning trials occurred at 4-day intervals during the middle third of the rat's dark circadian cycle following hormone priming. Paced copulation occurred in semicircular chambers (38² x 60 x 38 cm) bisected by a clear Plexiglas divider with 4 holes cut into the bottom (4 x 4 cm) that rested on bedding. These holes were large enough for the female to crawl through, but too small for the males to crawl through. Non-paced copulation occurred in identical chambers, but with the divider removed. Females in the Paired group copulated with almond scented males in the paced condition and unscented males in the non-paced condition. Females in the Unpaired group copulated with unscented males in the paced condition and scented males in the non-

paced condition. Females in the Randomly-Paired group copulated with scented and unscented males paired randomly with paced and non-paced copulation. Females received a total of 8 sequential conditioning trials (4 in each condition). The order of conditioning was counterbalanced for all groups, such that half in each group had their first sexual experience in the paced condition and the other half in the non-paced condition. During each test, females were allowed to copulate for 30 min, after which they were transferred to another testing room with CPP boxes and placed in one of the distinctive sides for an additional 30 min before being taken back into their home cages. All testing with scented and unscented males took place in different rooms to assure containment of the scent.

Place Preference Conditioning

Before sex testing began, females were tested in CPP boxes to determine any unconditioned preference (Oldenburger et al., 1992). The boxes were constructed of wood with three compartments (Pfaus, Kippin, & Coria-Avila, 2003). The two distinctive environments were rectangular in shape and were connected by guillotine doors to either side of a smaller start compartment. One of the goal compartments (darker) was painted with black and white stripes and had a grid floor. The other compartment (lighter) was painted exclusively in white. Most of the rats showed a significant unconditioned preference for the darker side with the stripes. For all animals, the initially non-preferred side was then paired with the paced condition whereas the preferred side was paired with the non-paced condition. Thus, any shift in preference toward the initially non-preferred side was taken as evidence of reward associated with those place cues.

The CPP test was conducted 4 days after the 8th odor-conditioning test. Females did not copulate prior to this test, but instead were placed into the start compartment for 5 min, after which the guillotine doors were opened to allow free access to both goal compartments. The amount of time rats spent in each side was recorded by computer. Females received a 9th odor-conditioning trial 4 days after the CPP test.

Copulatory Preference Test

Four days after the final odor-conditioning test, each female was placed into a large open field (123 x 123 x 46 cm) with a thin layer of woodchip bedding. At two diagonal corners of this open field were two sexually vigorous males, one scented with almond and the other unscented. Each male wore a rodent jacket connected on the back to a spring 30 cm in length. This allowed the males roam freely within the 45-cm radius, but not beyond. The females were placed in the center of the open field and allowed to copulate freely with both males for a 30-min period. These tests were videotaped and scored.

Behavioral Measures and Statistical Analyses

Place preference was determined by changes in preference ratio for the reinforced compartment (time spent in the compartment divided by total time of test) before and after conditioning, and by the difference between time spent in the non-reinforced and reinforced compartments of the CPP box. Female copulatory preference was determined by latency and frequency measures for female solicitation (defined as a head-wise orientation to the male followed by a runaway, forcing the male to chase her), hops and darts, pacing (defined as intermittent approaches and withdrawals from a male, as in (Hlinak & Madlafousek, 1977; McClintock, Anisko, & Adler, 1982), lordosis magnitude

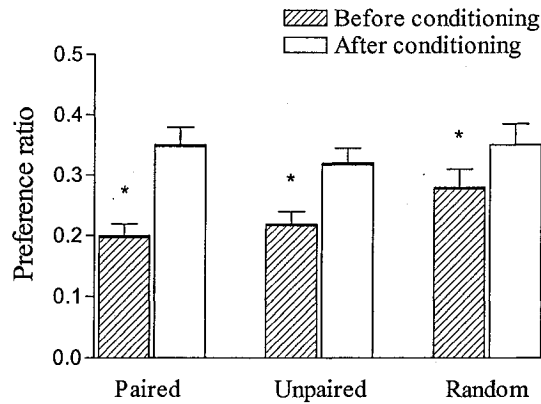
(on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in (Hardy & DeBold, 1972), and the amount of time spent in quadrants of the open field that contained each of the males. Olfactory investigation was considered as a single event from the moment a female started sniffing the male's body until she moved her head away. The male chosen for first mount, first intromission, and ejaculation for each ejaculatory series was also recorded. Chi-square analyses were used to determine differences in the proportion of females in the conditioning groups that chose the scented or unscented male for copulation. One-way Analysis of Variance (ANOVAs) were used to determine differences in the frequencies and latencies of copulatory behaviors between the conditioning groups, and to analyze the preference ratio and difference between the time spent in the two distinctive sides of the CPP apparatus. For all significant main effects, Tukey posthoc tests were conducted to assess differences between individual means. The level of significance for all comparisons was $p < .05$.

Results

Place Preference Test

The CPP test confirmed that females in all groups found the pacing condition rewarding. In the pre-conditioning test, 39 of the 60 females preferred the darker side of the CPP apparatus, $\chi^2(1,57) = 5.4, p = .02$.

A. Preference ratio for reinforced compartment



B. Difference between time spent in the non-reinforced and reinforced compartment

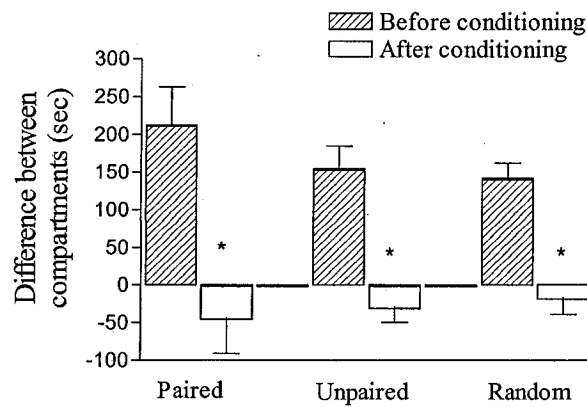


Figure 1: (A) Preference ratio for the reinforced compartment (time spent in the compartment divided by the total time of test) in three groups: Paired (n= 20), Unpaired (n= 20) and randomly-paired (n= 20). There was a significant increase in preference ratio after associating the reinforced side with paced copulation. (B) Difference between the time spent in the non-reinforced and reinforced compartment (non-reinforced minus reinforced). Positive before conditioning and negative after conditioning, indicating an increase in time spent in the reinforced compartment. Data are means + SEM. * = $p < 0.05$

In the post-conditioning test, 38 of the 60 females spent more time in the reinforced side, and 22 in the non-reinforced side $\chi^2(1,57) = 4.2, p=.03$. The ANOVA detected a significant shift in the overall preference ratio, $F(1,56) = 23.5, p<.01$. Tukey posthoc tests revealed that this shift was a significant increase for all groups after conditioning (Figure 1-A): Paired (Before: $\underline{M}=.20$; After: $\underline{M}=.35$ after); Unpaired (Before: $\underline{M}=.22$; After: $\underline{M}=.32$); and Randomly-Paired (Before: $\underline{M}=.28$; After: $\underline{M}=.35$). The ANOVA also detected an overall difference between the time spent in the non-reinforced and reinforced compartments (Figure 1-B), $F(1,56) = 70, p<.01$. Tukey posthoc tests revealed that the time spent in these compartments shifted significantly in all three groups. Before conditioning there was a positive difference in all groups: Paired ($\underline{M} = 213$ sec), Unpaired ($\underline{M} = 155$ sec) and Randomly-Paired group ($\underline{M} = 142$ sec). This difference became negative after conditioning: Paired ($\underline{M} = -46$ sec \pm 32), Unpaired ($\underline{M} = -31$ sec \pm 19), and Randomly-Paired ($\underline{M} = -19$ sec \pm 15).

Copulatory Preference Tests

Behavioral results during the copulatory preference test are shown sequentially in Figures 2 - 5. Table 1 lists means and significance levels. Figure 2 shows the latency and frequency of solicitations, and the proportions of females that solicited the scented or unscented males. The ANOVA detected a significant effect of conditioning on solicitation latency, $F(2,56) = 5, p=.01$. Posthoc Tukey tests revealed that females in the Paired group showed a shorter first-solicitation latency for scented males than for unscented males, whereas no differences were found in first solicitation latency between these males in the Unpaired or Randomly-Paired groups. In the Paired group, 75% of the females directed their first solicitation toward the scented male (Figure 2-B) compared to

Behavior	Paired group		Unpaired group		Random group		One conditioning trial	
	scented	unscented	scented	unscented	scented	unscented	scented	unscented
Solicitation latency	150 ± 33*	495 ± 98	410 ± 90	385 ± 54	378 ± 60	377 ± 56	22.5 ± 4.0	20.3 ± 4.1
Solicitation frequency	19 ± 3 *	8 ± 3	12 ± 2	14 ± 2	10 ± 4	14 ± 3	2.2 ± .22	3.7 ± .35
Choice of male for								
1 st Solicitation (n) (%)	15 (75%) *	5 (25%)	9 (45 %)	11 (55 %)	11 (55 %)	9 (45 %)	10 (50 %)	10 (50 %)
Hops and darts	27 ± 3.5 *	15.7 ± 1.18	19.4 ± 3.3	17.6 ± 3.7	18 ± 2	17.5 ± 1	12 ± 3	15 ± 2
Olfactory investigation frequency	10 ± 1.3 *	7 ± 1	6.9 ± .7 *	5.6 ± .8	9 ± .7 *	6 ± .5	2.4 ± 1	2.3 ± 1
1 st Olfactory investigation latency	104 ± 29 *	246 ± 59	140 ± 35 *	328 ± 126	148 ± 55	70 ± 30	41.9 ± 29	47.2 ± 25
Choice of male for 1 st mount (n) (%)	14 (73 %)*	5 (27 %)	11 (56 %)	8 (44 %)	11 (55 %)	9 (45 %)	7 (45 %)	13 (65 %)
Mount frequency ^a	1.9 ± .2 *	0.3 ± .2	2.8 ± .2	1.5 ± .2	1.3 ± .5	1.5 ± .5	5.1 ± 1	5.6 ± 1.8
Intromission frequency	10 ± .8	9.8 ± .8	9.2 ± .5	7.4 ± 1	9 ± .8	8 ± .5	6.2 ± 1.3	6.5 ± .6
Intromission latency	75 ± 17.9	174 ± 42	206.7 ± 84.5	183 ± 60	48 ± 8.8	92 ± 22	80 ± 20	67 ± 49
Mean Inter Intromission Interval	53.5 ± 15	77 ± 27	55 ± 19.5	61 ± 8	46 ± 2.7	42 ± 1.8	45 ± 14	48 ± 17
Ejaculation frequency	2.3 ± .19	1.9 ± .22	1.9 ± .11	1.7 ± .19	2.1 ± .24	2.4 ± .11	1.7 ± .3	1.9 ± .31
Hit Rate	.57 ± .05	.65 ± .2	.60 ± .05	.67 ± .03	.63 ± .03	.64 ± .03	.65 ± .02	.60 ± .02
Choice of male for								
1 st Ejaculation (n) (%)	14 (73 %) *	5 (26%)	8 (45%)	10 (55 %)	7 (35 %)	11 (55 %)	9 (45 %)	11 (55 %)
2 nd Ejaculation (n) (%)	9 (50 %)	9 (50 %)	10 (62 %)	6 (38 %)	8 (45 %)	10 (55 %)	9 (45%)	11 (55%)
3 rd Ejaculation (n) (%)	9 (53 %)	8 (47 %)	8 (50 %)	8 (50 %)	10 (55 %)	8 (45 %)	9 (50%)	9 (50%)
Lordosis magnitude 1	9.8 ± 1.5	7 ± 1.5	10.4 ± 1.4*	3.7 ± 1.5	10 ± 1.4	8 ± 1.5	8.5 ± 1.1	7 ± 1
Lordosis magnitude 2	5.9 ± 1	1.9 ± 1.5	7.3 ± 1.2	4.9 ± .9	6.2 ± .9	9.3 ± 1.6	10 ± 1.5	9.2 ± .9
Lordosis magnitude 3	10.4 ± 2.7 *	4.9 ± 1.5	11.9 ± 3	12.7 ± 2.1	4.1 ± 1.2	6.8 ± 1.2	4.5 ± .9	3 ± 1
Visits	40.1 ± 2.9	38.6 ± 3.1	37.2 ± 2.5	34.5 ± 2.5	27.7 ± 4.1	28.4 ± 3.4	38.7 ± 3	40 ± 3
Mean Inter Visits Interval	44.8 ± 3	46.6 ± 2.1	48.3 ± 2	52.1 ± 4	64.9 ± 5.1	63.3 ± 3	46.5 ± .9	45 ± 1.6
Time spent with:								
5 min of test	97.4 ± 8.8	96.5 ± 11.7	87.7 ± 4.7	102.8 ± 9.3	95 ± 8	100 ± 11	80 ± 25	75 ± 20
10 min of test	192 ± 10.9	159.6 ± 11	188 ± 11.3	190.6 ± 15	185 ± 11	171 ± 20	150 ± 16	140 ± 18
30 min of test	545.9 ± 50	454.4 ± 30	538.5 ± 37	511.3 ± 44	543 ± 40	470 ± 35	503 ± 50	486 ± 49

Table 1. Mean Number of Sexual Behaviors Displayed Toward Scented or Unscented Male During the Copulatory Preference Test. Four groups are shown. In the Paired group (n= 20) a scented male was associated with paced copulation. In the Unpaired group (n= 20) a scented male was associated with non-paced copulation. In the random group (n= 20) the association of paced copulation was randomly distributed between scented and unscented males. In the Paired-1 group (n= 20), females received only one exposure to a scented male in the paced condition, and an unscented male in a non-paced condition, before the partner preference test. Data are means ± SEM during the 30-min test, except where pointed out. Latencies are expressed in seconds. ^a = during first ejaculatory series; * = p < 0.05 between scented and unscented males within group.

the unscented male, $\chi^2(1, 19) = 5.0, p = .025$. No significant preferences were detected in the Unpaired or Randomly-Paired groups for first solicitation. The analysis of proportions, frequencies, and latencies throughout the 30-min test revealed more differences between groups. The ANOVA detected a significant effect of conditioning on number of solicitations, $F(2, 56) = 3.3, p < .05$, and hops and darts, $F(2, 56) = 3.8, p < .05$. Posthoc analyses revealed that females in the Paired group performed more solicitations (Figure 2-C) toward scented males than unscented males, and displayed more hops and darts (Figure 3-A) toward scented males than unscented males. Females in both Unpaired and Randomly-Paired groups did not display these preferences.

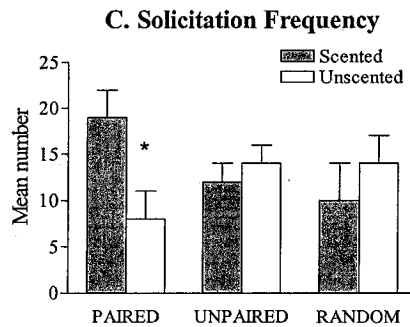
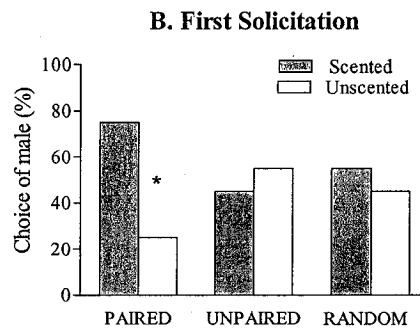
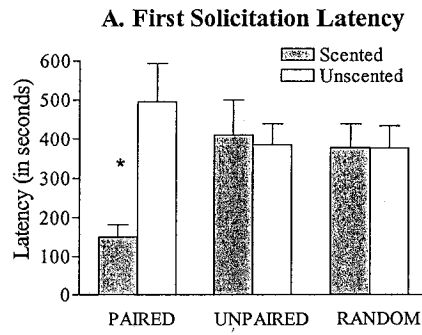


Figure 2: (A) Latency to perform the first solicitation in three groups: Paired ($n=20$, scented male associated with paced copulation), Unpaired ($n=20$, scented male associated with non-paced copulation) and Randomly-paired ($n=20$, scented males associated randomly with paced and non-paced copulation); (B) Choice of male for first solicitation; (C) Number of solicitations during 30 min test. Frequency data are means + SEM. * = $p < 0.05$.

The analysis of olfactory investigations revealed a main effect of scented males regardless the association with pacing or no pacing (Figure 3-B). In all groups, females showed higher frequency of olfactory investigations toward scented than toward unscented males $F(2, 56) = 4.6, p < .05$. The latency for first olfactory investigation in the Paired group (Figure 3-C) was shorter toward scented males than toward unscented males. This was also found for scented males in the Unpaired group relative to unscented males $F(2, 56) = 4, p < .05$. This difference, however, did not occur for the Randomly-paired group.

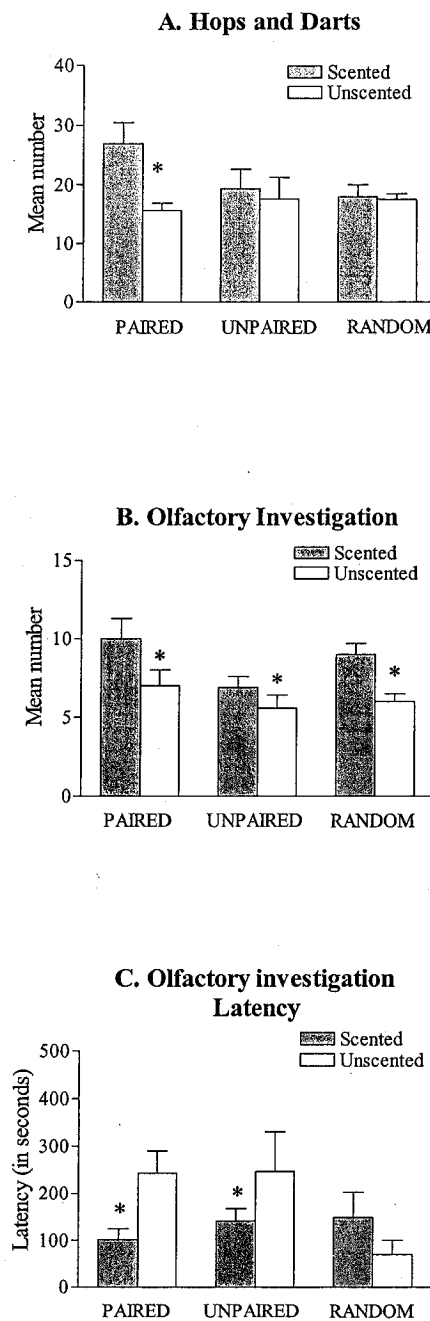


Figure 3: (A) Number of hops and darts toward scented and unscented males. Females showed a preference for scented males only in the Paired group. (B) Number of olfactory investigations. (C) Latency to first olfactory investigation. Data are means + SEM * = $p < 0.05$.

Differences in proportion and frequency of mounts (Figure 4) were also detected.

Significantly more females in the Paired group received their first mount from a scented male, $\chi^2(1, 19) = 4.2, p=.03$, whereas no significant differences in proportion were detected for either the Unpaired or Randomly-paired groups. The ANOVA detected a significant effect of conditioning on the number of mounts received from either male during the first ejaculatory series, $F(2, 56) = 3.9, p= 0.02$. Posthoc analyses revealed that females in the Paired group received significantly more mounts from the scented males relative to the unscented males, whereas these differences were not significant for females in the other groups.

Figure 5 shows females' choice of male for the first three ejaculations, and the numbers of lordoses with low, moderate, or high magnitude toward scented or unscented males. Of the 60 females, all but 5 copulated to ejaculation with one or both males on the final copulatory preference test. Of the females that did not receive an ejaculation, 1 was in the Paired group, 2 in the Unpaired group, and 2 in the Randomly-paired group. Accordingly, these females were not included in the analysis of ejaculation data. A significant proportion of females in the Paired group (75%) received their first ejaculation from the scented male relative to the unscented male, $\chi^2(1, 19) = 4.26, p=.03$.

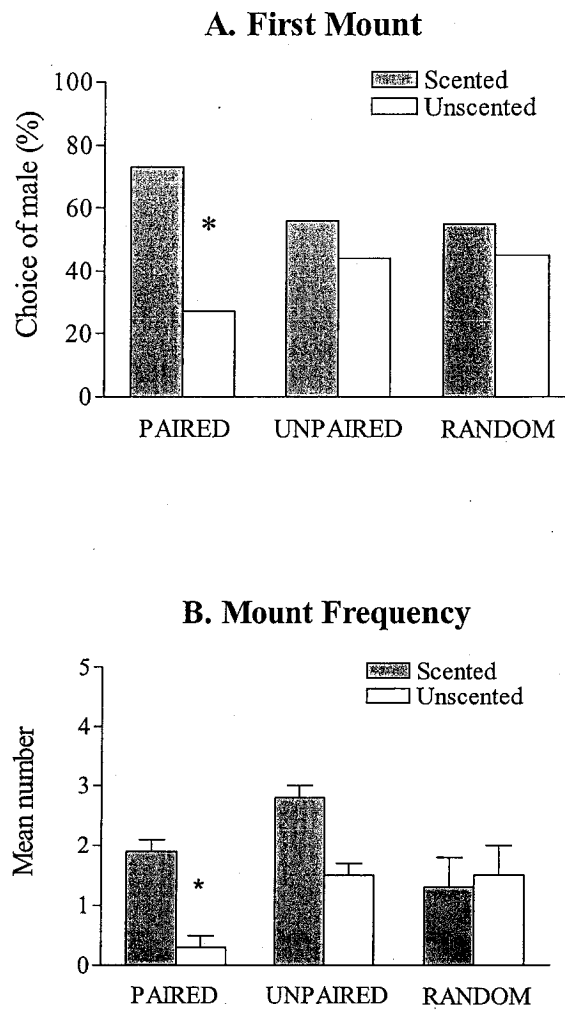


Figure 4: (A) Choice of male for first mount. (B) Number of mounts with each male. Frequency data are means + SEM. * = $p < 0.05$.

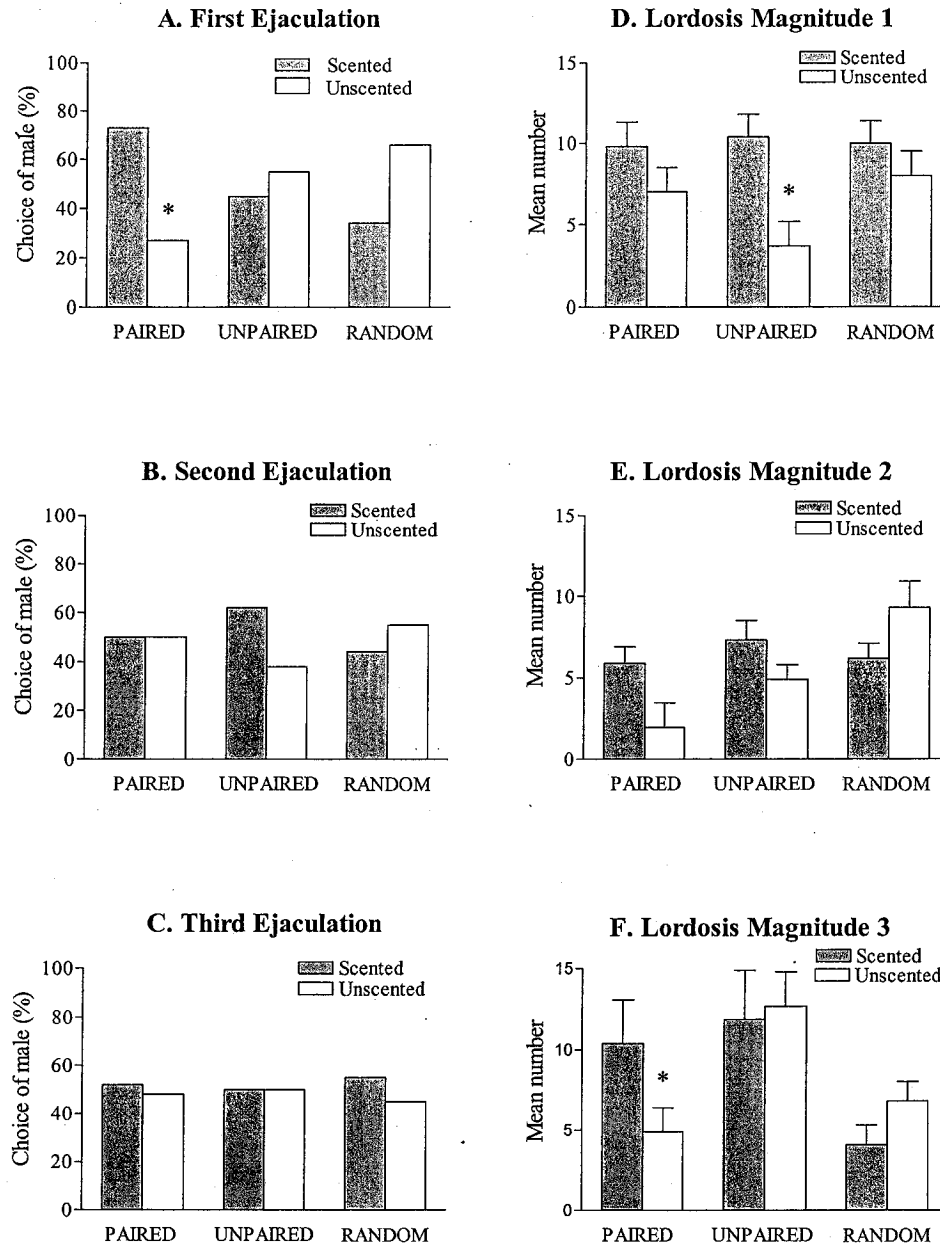


Figure 5: (A, B, C): Preference of male for first, second, or third ejaculation, respectively. (D) Number of low-magnitude lordoses (magnitude 1, flat back). (E) Number of medium-magnitude lordoses (magnitude 2, either head or tail pointing upward). (F) Number of high-magnitude lordoses (magnitude 3, head and tail pointing upward). Frequency data are means + SEM. * = $p < 0.05$.

No significant differences in the choice of male for ejaculation were found in the Unpaired, $\chi^2(1, 17) = .22, p = .63$, or Randomly-Paired, $\chi^2(1, 17) = .88, p = .30$, groups (Figure 5-A). During the second ejaculatory series, six females did not receive a second ejaculation (2 in the Paired group; 4 in the Unpaired group, and 2 in the Randomly-Paired group). No preferences were detected for either male for second ejaculation in any group. Likewise, during the third ejaculatory series, nine females did not receive a third ejaculation (3 in the Paired group, 4 in the Unpaired group, and 2 in the Randomly-Paired group (Figure 5-C). The analysis of proportions of females that switched partner for consecutive ejaculations revealed no differences. Ten of the females (55%) in the Paired group switched partner for the second ejaculation, $\chi^2(1, 17) = .22, p = 0.63$; 7 females (44%) in the Unpaired group switched partner, $\chi^2(1, 15) = .25, p = 0.61$, and 9 females in the Randomly-paired group switched partner $\chi^2(1, 17) = .2, p = 0.65$. For the third ejaculation, 11 females (68%) in the paired group switched partner $\chi^2(1, 16) = 2.2, p = 0.13$; 8 females (50%) in the unpaired group switched partner, and 10 females (55%) in the Randomly-paired group switched partner.

The ANOVA detected a significant effect of conditioning on the number of high-magnitude lordoses, $F(2, 56) = 3.59, p < 0.05$. Posthoc analyses revealed that only females in the Paired group displayed a higher number of high-magnitude lordoses with scented males than with unscented males. The ANOVA also detected a significant effect of conditioning on the number of low-magnitude lordoses, $F(2, 56) = 6.93, p < 0.01$. Posthoc analyses detected that females in the Unpaired group showed a higher number of low-magnitude lordoses with the scented male than with the unscented male. No differences in the number of low-magnitude lordoses were found in the Paired or

Randomly-paired groups between the scented and unscented males, and no group showed differences in frequency of moderate lordosis (Figure 5-D, E, F).

Effect of Single Paired Conditioning Trials

An additional group, Paired-1, was added to the experiment and analyzed independently to examine whether a single pairing of odor and paced copulation was sufficient to induce a conditioned partner preference. Females copulated in the same conditions of the Paired group, but with only one exposure to a scented male in the paced condition, and an unscented male in a non-paced condition, before the final copulatory preference test. The analysis of frequencies, latencies and proportions overall did not reveal any significant difference in the choice between scented and unscented males (see Table 1).

Discussion

As with male rats, female rats develop olfactory conditioned partner preference for cues associated with sexual reward. Relative to non-paced copulation, paced copulation induced a place preference in the Paired, Unpaired and Random-Paired groups, replicating previous findings by Paredes and colleagues (Martinez & Paredes, 2001; Paredes et al., 1997; Paredes et al., 1999), and confirming that the three groups were capable of discriminating these conditions. However, subsequent partner preferences were displayed by females in the Paired, but not Unpaired or Random-Paired groups. Paired females chose the scented male for their first solicitation, first mount, and first ejaculation, and displayed more solicitations with the scented male, shorter solicitation latencies, and more hops and darts, relative to the unscented male. In

contrast, females in the Unpaired and Random-paired groups did not display a significant preference for either male, indicating that the almond scent alone does not carry unconditional appetitive value.

Kippin et al., (1998) demonstrated that repeated pairing of a neutral almond odor with copulation to ejaculation in male rats produces a subsequent conditioned ejaculatory preference for females bearing that odor. However, in that study, males in the Paired group did not display a copulatory preference for any female with regard to mounts and intromissions prior to ejaculation. Subsequent experiments determined that males in the Paired group became “choosy” only during the mounts immediately preceding ejaculation (Kippin et al., 2001a), suggesting that they were choosing the female specifically to receive their ejaculation and that they differentiate copulation from mating. Moreover, the necessary UCS for this conditioning was exposure to the scented female during the post-ejaculatory refractory period during each conditioning trial (Kippin et al., 2001a), indicating that a post ejaculatory reward state is critical for the induction of partner preference in the male rat, as it is for the induction of CPP (Ågmo et al., 1990). In contrast, Paired females in the present study displayed both copulatory (regarded as those behaviors that are needed but not sufficient to induce pregnancy) and mate (i.e., ejaculatory) preferences, suggesting that the reward state induced by paced copulation occurs throughout the conditioning trial. Another important difference concerns the magnitude of the reward state. A single pairing of the odor and the post ejaculatory reward state was sufficient to induce significant partner preferences in male rats (Kippin et al., 2001c), although multiple trials was more effective. We and others have argued previously that early experience with sexual reward may form a flexible “critical period”

in which salient features of the first partner come to be preferred in subsequent partners (e.g., Pfaus et al., 2001; Storms, 1981). In contrast, a single set of exposures of odor with pacing, and no odor with no pacing, was not sufficient in the present study to induce any conditional preference in females. It may be that the reward state induced by pacing in females is of a smaller magnitude to that induced by ejaculation in males, such that multiple conditioning trials are necessary to induce the conditioned preference in females. Although the activation of endogenous opioid systems in both male and female rats is necessary for the induction of CPP (Miller et al., 1987; Paredes et al., 2001; Ågmo et al., 1990), it is not yet known whether similar mechanisms control partner or mate preferences in male and female rats.

Partner preferences can be viewed as a rudiment of pair bonding, in which familiar cues are preferred or chosen relative to novel cues (Wickler & Seibt, 1983; Young et al., 2004). Pair bonding occurs in putatively monogamous species, such as cichlid fish (Taylor, Morley, Rico, & Balshine, 2003), certain birds such as budgerigars (Hile, Plummer, & Striedter, 2000), and mammals such as prairie voles (Carter & Getz, 1993). In contrast, the general mating strategy of rats has been described as polygamous and opportunistic (McClintock et al., 1982). Indeed, male and female rats both display a “Coolidge effect”, in which a novel partner produces more sexual arousal and copulatory behavior relative to a familiar one (Bermant et al., 1968). However, McClintock, (1984a) and Tiefer, (1969) have shown that during group mating, female rats compete actively while copulating with a male, and that such competition is greatest when the male is about to ejaculate. McClintock (1984b) has also observed sexual choice strategies between dominant and subordinate female rats in group-mating conditions in which the

dominant females are more likely than the subordinates to intercept males and take their early ejaculations. Although rats are not observed to form pair bonds in such group mating conditions, our data suggest that they are capable of doing so if the conditions are different. This suggests a degree of experience-dependent plasticity in the brains of both male and female rats regarding mate strategies. It may be the case that similar neural mechanisms that underlie the formation of partner preferences in other species, for example, oxytocin or vasopressin release (Bales, Kim, Lewis-Reese, & Sue, 2004; Insel, Winslow, Wang, & Young, 1998; Insel, Winslow, Wang, Young, & Hulihan, 1995; Carter, DeVries, & Getz, 1995; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993), may also be necessary for the formation of conditioned partner preference in rats.

In addition to facilitative effects on sexual arousal and partner preference, an odor paired with sexual reward can also enhance reproductive function in male rats. Graham and Desjardins (1980) reported a conditioned increase in serum testosterone and luteinizing hormone in male rats following access to a wintergreen odor previously paired with copulation to ejaculation. Males in an unpaired control group did not show any increase in hormone levels following exposure to the odor. However, males in both groups exposed to estrous vaginal secretions showed identical increases in these hormones, suggesting that unconditioned hormone release is mimicked reliably following pairing of odor with sexual reward. In female rats, pacing serves an important reproductive function, allowing females to control the rate of vaginocervical stimulation (VCS). Such control serves to distribute VCSs in time, which in turn enhances both sperm transport and the induction of nightly prolactin surges that support the maintenance of progesterone release by the corpora lutea (Erskine, 1989; Matthews, Jr. & Adler,

1978). Accordingly, paced copulation is more effective than non-paced copulation in inducing luteal function, a faster termination of estrus, and pregnancy (Bermant et al., 1966; Erskine, 1989; Lodder & Zeilmaker, 1976). Moreover, paced copulation increases litter size compared to non-paced copulation (Coopersmith & Erskine, 1994), and females are more likely to become pseudopregnant following exposure to non-paced relative to paced copulation (Frye & Erskine, 1990). Thus, copulating with a male in a paced situation is more rewarding and has positive effects on reproduction. It is not yet known whether odors paired with paced copulation could induce luteal function, a faster termination of estrus, or enhance pregnancy.

In summary, female rats can learn to associate a neutral odor with sexual reward. An odor paired with the ability of females to pace copulation becomes a sexual incentive, such that males bearing the odor will be preferred when females are given a choice between scented and unscented males. Thus, just like male rats, female rats can learn to modify their sexual behavior and partner preferences based on experience with sexual reward. This confirms that early sexual experiences have particularly powerful influences on subsequent sexual preferences, and that the development of sexual preferences is influenced by interactions between CS-UCS pairings and motivational variables.

CHAPTER ONE

PART 2

Conditioned partner preference in female rats for strain of male

Genaro A. Coria-Avila*, Sherri L. Jones, Carrie E. Solomon, Alex M. Gavrilu, Gerald J. Jordan,
James G. Pfaus

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

RUNNING HEAD: PARTNER PREFERENCE FOR STRAIN OF MALE

*Corresponding author: Center for Studies in Behavioral Neurobiology,
Department of Psychology, Concordia University, 7141 Sherbrooke W.,
Montréal, QC, H4B 1R6 CANADA. Fax: 514-848-2817.
E-mail: coria75@yahoo.com

Citation: *Physiology & Behavior* (2006), 88,529-37

© Elsevier Inc., reprinted with permission

Abstract

CORIA-AVILA, G.A., S.L. JONES, C.E. SOLOMON, A.M. GAVRILA, G.J. JORDAN AND J.G. PFAUS *Conditioned partner preference in female rats for strain of male.* PHYSIOL BEHAV. Female rats show conditioned place preference following paced copulation, and we have recently demonstrated that pairing almond odor with paced copulation induces a conditioned partner preference for almond-scented males. The present study examined whether cues of two different strains of male (albino and pigmented) induce a conditioned partner preference for the strain of male associated with paced copulation. Ovariectomized, hormone-primed Wistar (W) or Long-Evans (LE) female rats received 10 conditioning trials at 4-day intervals. In the Wistar-Pacing group females copulated with W males in a chamber bisected by a 4-hole partition that only the female could pass through. Four days later, they copulated with LE males without the partition. The Long-Evans-Pacing group received the opposite association. In the final preference test all females chose freely between two males tethered in opposite corners of an open field, one W and one LE. 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. These results suggest that female rats have an unconditioned preference for males of the same strain, but this preference can be switched towards males of a different strain if that male is associated with the sexual reward induced by paced copulation.

Keywords: Pacing, Appetitive, Sexual reward, Conditioning, Sexual behavior, Wistar, Long-Evans.

1. Introduction

Conditioned stimuli (CSs) can activate appetitive sexual behaviors and neuroendocrine functions (Graham et al., 1980; Kippin et al., 1998; Kippin et al., 2001b; Pfaus et al., 2001; Pfaus et al., 2003; Zamble, Hadad, Mitchell, & Cutmore, 1985), or suppress them (Ågmo, 2002), depending on their association with rewarding or aversive experiences, respectively. For example, male rats perform anticipatory behaviors in the presence of stimuli that predict copulation (Pfaus, 1996; Pfaus et al., 1999), but will display avoidance responses toward sex-related stimuli such as estrous odors associated with malaise (e.g. paired with the gastrointestinal distress induced by injections of LiCl) (Ågmo, 2002). In male rats, ejaculation induces a state of reward sufficient to induce a conditioned place preference (CPP) (Ågmo et al., 1990). Males spend more time in sides of a CPP chamber associated with the ejaculation induced reward state. This can occur even if males are placed into the CPP box after they have ejaculated in another chamber. Systemic administration of the opioid receptor antagonist naloxone during conditioning blocks ejaculation-induced CPP (Ågmo et al., 1990), indicating that activation of endogenous opioids is an important component of the reward state, and of the conditioning mechanism that links the contextual CS to the reward. The post-ejaculatory reward state is also critical for conditioned partner preference. For example, Kippin et al. (2001a) found that male rats develop a preference to ejaculate with females bearing and odor (almond), but only if they had experienced the odor during the postejaculatory refractory period.

Females display complex sexual behaviors that allow them to control or “pace” their sexual interaction with males (Pfaus et al., 2001). They solicit males Beach, 1976;

Erskine, 1989; McClintock, 1984a), and also pace the rate of sexual interaction with approaches or withdrawals if they are given the opportunity (Erskine et al., 1989; Pfaus et al., 1999). Female rats develop CPP for distinctive sides of a chamber associated with paced copulation, relative to non-paced copulation (Paredes et al., 1997; Paredes et al., 1999). This copulatory CPP in female rats is blocked by injections of naloxone (Paredes et al., 2001), suggesting that increased levels of endogenous opioids induced by paced copulation form an significant part of the neurochemical reward substrate, as they do in male rats following ejaculation. Accordingly, paced sexual interactions induce a positive affective state of sufficient intensity and duration to induce conditioning.

We have recently reported that females given the ability to pace copulations with almond-scented males develop a conditioned partner preference for almond-scented males (Coria-Avila et al., 2005). In our Paired group, female rats copulated with almond-scented males in chambers bisected by a Plexiglas divider with four small holes that allowed the female to run back and forth from the side with the male, but that were too small for the male to pass through. In the unpaced condition, females copulated in the same chamber with unscented males, but without the divider. After 5 sequential pairings of paced and unpaced copulation (with and without the odor, respectively), females were tested in a large open field with two sexually vigorous copulating males, one scented and the other unscented. Paired females showed a significant preference for the almond-scented male for their first solicitation, first mount, and first ejaculation, and displayed shorter first solicitation latencies and a higher frequency of solicitations and hops and darts throughout the test with those males compared to females in an Unpaired group (that had the odor on males presented in the unpaced condition, and no odor on males

presented in the paced condition) or in a Random-Paired group (that had the odor presented equally in paced and unpaced conditions). This study demonstrated that neutral odors can acquire sexual incentive value and modulate partner preference when paired with the rewarding effects of paced copulation.

Erskine (2005) has argued that conditioned place and partner preference paradigms offer important glimpses into female sexual motivation and mate choice, but raised the question of how such preferences are exhibited in natural conditions (i.e., without the addition of almond or other foreign odors) (Erskine, 2005). Female rats are polyandrous, and descriptions of group mating in the wild (Calhoun, 1962) suggest that females copulate indiscriminately with many males. However, laboratory modeling of group mating in large open fields has revealed that females show serial preferences throughout the mating sequence (McClintock et al., 1982). It is not clear what mate strategies or choices may underlie these shifts in preference, and individual differences in mate strategies exist, especially between dominant and subordinate females.

Albino and pigmented strains of rats have been used in experiments of sexual competition, and females in particular appear to react differently toward males of their same or other strain (Austin & Dewsbury, 1986; Coria-Avila, Pfaus, Hernandez, Manzo, & Pacheco, 2004; Moore & Wong, 1992). This suggests that female rats can differentiate different strains of male rat, although the exact cues the females attend to have not been determined (e.g., olfactory, visual, and/or auditory). The present study examined whether pairing a particular strain of male (albino or pigmented) with paced copulation would condition subsequent appetitive sexual behaviors and partner preferences in female rats. Based on our previous findings (Coria-Avila et al., 2005) we hypothesized that regardless

of the strain of male, female rats would show a partner preference for those males paired with paced copulation.

2. Materials and Methods

2.1. Animals and surgery

Wistar (W) and Long-Evans (LE) female rats (200-250 g) and W and LE male rats (350-400 g) were purchased from Charles River Canada (St-Constant, QC). Males were housed in groups of four in Plexiglas cages and females were housed in groups of 4 in large wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Commercial food and water were continuously available.

The females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then ovariectomized bilaterally via a lumbar incision. Post-surgical treatment included single subcutaneous injections of Flunixin meglumine 2.5 mg/kg (Banamine®) for analgesia, and Enrofloxacin 5 mg/kg (Baytril®) to prevent post surgical bacterial infections. All females were given a week of post-surgical recovery prior to conditioning trials. Gonadally intact W and LE male rats that served as stimuli had at least 4 tests of sexual behavior in the same conditioning chambers prior to the start of these experiments. These males initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all

ovariectomized females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test.

2.2. Conditioning

The experiment included two counterbalanced groups. Females in the Wistar-pacing (W-P) group copulated with Wistar males in the paced condition and Long-Evans males in the non-paced condition. Females in the Long-Evans-pacing (LE-P) group copulated with Long-Evans males in the paced condition and Wistar males in the non-paced condition. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark circadian cycle following hormone priming. Paced copulation occurred in semicircular chambers (38² x 60 x 38 cm) bisected by a clear Plexiglas divider with 4 holes cut into the bottom (4 x 4 cm) that rested on bedding (Pfaus et al., 2003). These holes were large enough for the female to crawl through, but too small for the males to pass through. Non-paced copulation occurred in the same chambers, but with the divider removed. Females received a total of 10 sequential experiences of 30-min each in paced and non-paced conditions at 4-day intervals (5 each). The order of condition for the first conditioning trial was counterbalanced so that half of each group started with paced condition and the other half with non-paced copulation. Animals were allowed to copulate for 30 min before being taken back into their home cages.

2.3. Copulatory preference test

Four days after the 10th conditioning trial, each female was placed into a large open field (123 x 123 x 46 cm) with a thin layer of woodchip bedding. At two diagonal corners of this open field were two males, one W and the other LE. Both males had received their last copulatory test 4 days before the open field test. Each male wore a

rodent jacket connected on the back to a spring wire 30 cm in length. This allowed the males roam freely within a 45 cm radius. The females were placed in one neutral corner of the open field and allowed to copulate freely with both males for a 30-min period. These tests were videotaped and scored.

2.4. Behavioral measures and statistical analyses

Female copulatory preference was assessed as in previous olfactory conditioned experiments (Coria-Avila et al., 2005) and determined by latency and frequency measures for female solicitations, hops and darts (H&D), visits to the males, lordosis magnitude (on a scale of 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in Hardy et al., (1972), and the amount of time spent in quadrants that contained either male. A 2 X 2 (group X strain) mixed design ANOVA was used to determine differences in the frequencies and latencies of copulatory behaviors towards the two males. All significant main effects and interactions were followed by a Tukey Least Significant Difference (LSD) post hoc test to detect specific differences between individual means. The level of significance for all comparisons was $p < .05$. The proportion of ejaculations received from either male was assessed with a Chi-square test. The level of significance for all comparisons was $p < .05$.

3. Results

Some females were eliminated from statistical analysis because one or both of the males became free from their tethered jackets during the final preference test. Of a total of 60 W females this left 57 (n=30 in the W-P group; n=27 in the LE-P group), and of a total of 24 LE females this left 22 (n=10 in the W-P group; 12 in the LE-P group). Table

2 shows the means + standard errors for all the behaviors assessed during the first ejaculatory series and throughout the 30-min test.

3.1. Wistar females

3.1.1. Olfactory investigations and visits

The analysis of olfactory investigations during the first ejaculatory series showed a main effect of strain of male ($F(1, 55) = 6.06, p = 0.01$), and an interaction between group and strain of male ($F(1, 55) = 7.1, p = 0.009$). Posthoc analyses indicated significant differences between W ($\underline{M} = 8.6$) and LE males ($\underline{M} = 12.8$) in the LE-P group, but not in the W-P group. Throughout the 30-min test there was also a main effect of strain of male ($F(1, 55) = 8.09, p = 0.006$). Posthoc analyses indicated significant differences between W ($\underline{M} = 21.1$) and LE males ($\underline{M} = 27.1$) in the LE-P group, but not in the W-P group, indicating that overall females displayed more olfactory investigations towards LE males from the beginning of the final preference test, and this effect was facilitated when LE males were associated with paced copulation. The ANOVA did not detect any main effects or interactions for olfactory investigation latency, indicating that females performed their first olfactory investigation towards W and LE males with the same latency. Likewise, the ANOVA did not detect any significant effects regarding the number of visits during the first ejaculatory series, or during the 30-min test; as similar lack of effect was found for the first visit latency. This indicates that the females in both conditioning groups visited the W and LE males equally throughout the 30-min test.

3.1.2. Appetitive sexual behaviors

The analysis of appetitive behaviors revealed significant differences between the conditioning groups (Fig. 6). With regard to solicitations during the first ejaculatory

series, the ANOVA detected an interaction between group and strain of male ($F(1, 55) = 6.07, p = 0.01$). However, regardless of this significance, the LSD post hoc test failed to reveal significant differences between W and LE males in the W-P ($p = 0.10$) or LE-P groups ($p = 0.07$). The analysis of solicitations during the 30-min test revealed a significant interaction between group and strain of male ($F(1, 55) = 13.7, p = 0.0004$), and the post hoc test indicated a significant difference between W ($\underline{M} = 13.6$) and LE males ($\underline{M} = 9.3$) in the W-P group, and between W ($\underline{M} = 10.4$) and LE males ($\underline{M} = 17.5$) in the LE-P group, indicating that, overall, females displayed more solicitations towards the pacing-related male. With regard to the first solicitation latency there was a significant interaction between group and strain of male ($F(1, 47) = 5.5, p = 0.02$). However, the post hoc test did not reveal significant differences between W ($\underline{M} = 162$ sec) and LE males ($\underline{M} = 223$ sec) in the W-P group ($p = 0.15$) or between W ($\underline{M} = 271$ sec) and LE males ($\underline{M} = 121$ sec) in the LE-P group ($p = 0.06$). This suggests that there was a trend for females to perform their first solicitation with a shorter latency towards the pacing-related male.

With regard to H&D, although no significant main effects or interaction were detected during the first ejaculatory series, the analysis during the 30-min test indicated a main effect of strain of male ($F(1, 55) = 6.2, p = 0.01$). Posthoc analyses revealed a significant difference between W ($\underline{M} = 28.4$) and LE males ($\underline{M} = 43.1$) in the LE-P group ($p = 0.007$), but not between W ($\underline{M} = 35$) and LE males ($\underline{M} = 38.4$) in the W-P group ($p = 0.49$), indicating that females displayed more H&D towards LE exclusively in the LE-P group. No significant main effects or interaction were found for first H&D latency. Together, these results indicate that, regardless of the strain of male, females displayed

more appetitive behaviors towards the pacing-related males, relative to non-pacing-related males.

3.1.3. Lordosis

With regard to the different magnitudes of lordosis during the first ejaculatory series, the ANOVA found a significant interaction of between group and strain of male for lordosis magnitude 3 ($F(1, 55) = 4.3, p = 0.04$). The post hoc test showed that females in the W-P group displayed higher number of lordosis magnitude 3 towards W males ($\underline{M} = 4.1$) than towards LE males ($\underline{M} = 1.8$). In the LE-P group however, no significant differences were detected between W ($\underline{M} = 3$) and LE ($\underline{M} = 3.7$) males. No significant main effects or interaction were found for the other lordosis magnitudes, nor did the analysis of lordosis magnitude 1, 2 and 3 during the 30-min test reveal any main effects or interactions.

Behavior	Wistar females		Long-Evans females					
	Wistar-Pacing group (n=30)		Long-Evans-Pacing group (n=27)		Wistar-Pacing group (n=10)		Long-Evans-Pacing group (n=12)	
	Wistar male	Long-Evans male	Wistar male	Long-Evans male	Wistar male	Long-Evans male	Wistar male	Long-Evans male
1 st visit latency	19.2 ± 3.9	16 ± 7.9	61.9 ± 44	10.8 ± 1.5	43.4 ± 18	26.2 ± 9.5	15.9 ± 5.1	18.3 ± 4.9
Visits frequency								
First ejaculatory series	22.1 ± 2.2	23.1 ± 2.5	22.6 ± 2.9	25 ± 2.5	14.4 ± 2.3	14.3 ± 2.6	17.6 ± 2.4	20.2 ± 2.9
30 minutes of test	56.5 ± 6.1	58.9 ± 6.1	67.7 ± 8.7	71.5 ± 8.4	63.6 ± 6.3	59.2 ± 7.2	85.5 ± 8.9	88.6 ± 4.9
1 st Olfactory investigation latency	32.5 ± 5.2	29.0 ± 8.7	81.9 ± 47.6	14.0 ± 1.8	56.7 ± 19	75.8 ± 35	102.8 ± 33	104.6 ± 4.5
Olfactory investigation frequency								
First ejaculatory series	11.2 ± 1.1	11.0 ± 1.0	8.6 ± 1.1 *	12.8 ± 1.7	1.8 ± .41	2.9 ± .73	1.7 ± .39	2.2 ± .60
30 minutes of test	22.5 ± 1.2	24.2 ± 1.9	21.1 ± 2.0 *	27.1 ± 2.0	11.9 ± 1.5	11.1 ± .2	9.2 ± 1.3	12.5 ± 1.5
1 st hop and dart latency	117.7 ± 62	83.1 ± 36	146.04 ± 51	52.0 ± 9.4	74 ± 16	77.6 ± 22	76.8 ± 27	79.4 ± 26
Hops and darts								
First ejaculatory series	15.2 ± 2.9	13.8 ± 2.3	10.1 ± 1.5	14.9 ± 2.7	7.8 ± 1.8	10.5 ± 2.3	8.9 ± 1.8	9.9 ± 1.6
30 minutes of test	35.0 ± 4.7	38.4 ± 4.3	28.4 ± 3.4 *	43.1 ± 5.4	46.6 ± 4.9	34.8 ± 6.9	40.5 ± 7.2	37.3 ± 6.9
1 st solicitation latency	162.2 ± 41	223.3 ± 52	271.4 ± 81	121.7 ± 20	138.7 ± 17.3	133.6 ± 32	155.9 ± 28	119.3 ± 23
Solicitation frequency								
First ejaculatory series	5.7 ± 1.63	3.6 ± .72	3.2 ± .68	5.7 ± 1.2	3.4 ± .92	3.2 ± .80	2.08 ± .52 *	5 ± 1.1
30 minutes of test	13.6 ± 2.4 *	9.3 ± 1.7	10.4 ± 1.8 *	17.5 ± 2.8	14.1 ± 2.5	9.8 ± 1.6	13.7 ± 2.3 *	21 ± 2.6
1 st Mount latency	236.8 ± 55	178.0 ± 44	450 ± 122	211.4 ± 97	128.2 ± 33	188.1 ± 75	373.4 ± 152	108 ± 24
Mount frequency								
First ejaculatory series	4.3 ± 1.2	1.7 ± .50	2.5 ± .78	3.5 ± 1.0	2.3 ± .84	2.4 ± .85	1.5 ± .33	2.5 ± .54
30 minutes of test	9.5 ± 1.7	7.1 ± 1.6	7.5 ± 1.3	9.0 ± 1.7	11 ± 1.5	8.5 ± 2.2	9.2 ± 1.6	8.6 ± 1.3
1 st Intromission latency	193.7 ± 44	217.6 ± 85	274.8 ± 83	187.3 ± 72	136.3 ± 40	57.3 ± 16	94.7 ± 18.6	94.1 ± 16
Intromission frequency								
First ejaculatory series	4.1 ± .65	2.3 ± .55	2.9 ± .65	3.4 ± .56	3.7 ± 1.08	5.5 ± 1.1	3.5 ± .59	4.2 ± .82
30 minutes of test	8.5 ± 1.1	6.3 ± 1.2	7.9 ± 1.1	6.8 ± 1.0	18.2 ± 1.6	14.9 ± 3	16 ± 2.1	14.2 ± 1.4
1 st Ejaculation latency	592.0 ± 84	618.0 ± 103	575.0 ± 96	485.0 ± 99	429 ± 93	230 ± 54	564 ± 85 *	315 ± 34
Ejaculation frequency								
30 minutes of test	1.3 ± .21	1.2 ± .23	1.3 ± .22	1.1 ± .23	2.4 ± .42	2.4 ± .45	1.7 ± .27	3.08 ± .25
Choice of male for:								
First ejaculation (n)	83 % (20) *	16 % (4)	50 % (12)	50 % (12)	30 % (3)	70 % (7)	8.3 % (1) *	91.6 % (11)
Second ejaculation (n)	60.8 % (14)	39.2 % (9)	56.5 % (13)	43.5 % (10)	40 % (4)	60 % (6)	16.6 % (2) *	83.3 % (10)
Third ejaculation (n)	25 % (4) *	75 % (12)	63 % (12)	37 % (7)	33.3 % (3)	66.6 % (6)	20 % (2) *	80 % (8)
Lordosis magnitude 1								
First ejaculatory series	1.3 ± .38	1.03 ± .25	1.0 ± .33	1.6 ± .54	3 ± .15	1.1 ± .1	1.6 ± .11	.08 ± .08
30 minutes of test	3.7 ± .68	4.5 ± 1.0	3.8 ± .84	4.4 ± 1.0	3 ± .30	.6 ± .33	1.9 ± .63	.66 ± .22
Lordosis magnitude 2								
First ejaculatory series	2.7 ± .94	1.1 ± .28	1.3 ± .46	1.6 ± .58	2.5 ± 1.3	5 ± 1.05	2.6 ± .51	4 ± .69
30 minutes of test	5.3 ± 1.1	3.6 ± .84	3.6 ± .78	4.1 ± 1.0	15 ± 3	16.7 ± 3.9	14.8 ± 1.9	13.3 ± 1.6
Lordosis magnitude 3								
First ejaculatory series	4.1 ± .81 *	1.8 ± .50	3.0 ± .72	3.7 ± .71	2.6 ± .89	2.7 ± .65	2.08 ± .46	2.08 ± .71
30 minutes of test	10.2 ± 1.6	6.1 ± 1.2	8.8 ± 1.3	8.2 ± 1.5	14.3 ± 2.2 *	7.6 ± .2	9.2 ± 1.7	10.3 ± 1.9

Table 2. Sexual behaviors displayed by Wistar or Long-Evans females during the final partner preference test with two males, one Wistar and one Long-Evans. In Wistar-Pacing groups, females were trained to associate Wistar males with paced copulation and Long-Evans males with nonpaced copulation. In Long-Evans-Pacing groups, females received the opposite association. * = $p < 0.05$ within groups.

3.1.4. Choice of male for ejaculation and male responses

Chi-square analyses for proportions of ejaculations indicated significant differences between males (Fig. 7). With regard to the choice of male to receive the first ejaculation, 20 (83%) of the 24 females in the W-P group that received an ejaculation received their first from the W male and 4 (16%) from the LE male ($\chi^2 (1, N=24) = 10.6, p = 0.001$). However, in the LE-P group 12 (50%) of 24 females that received an ejaculation received their first from the W male and 12 (50%) from the LE male ($\chi^2 (1, N=24) = 0.0001, p = 1.0$). For the choice of male to receive the second ejaculation, in the W-P group, 14 (60.8%) of 23 females received the second ejaculation from the W male and only 9 (39.2%) from the LE male ($\chi^2 (1, N=23) = 1.08, p = 0.29$). In the LE-P group 13 (56.5%) of 23 females received the second ejaculation from the W male and 10 (43.5%) from the LE male ($\chi^2 (1, N=23) = 0.39, p = 0.53$). For the choice of male to receive a third ejaculation, in the W-P group, 4 (25%) of 16 females, received a third ejaculation from the W male and 12 (75%) from the LE male ($\chi^2 (1, N=16) = 4, p = 0.04$). In the LE-P group, 12 (63%) of 19 females, received their ejaculation from the W male and 7 (37%) from the LE male ($\chi^2 (1, N=19) = 1.3, p = 0.25$).

No significant main effects or interactions were found for the number of mounts, intromissions, or ejaculations, received by the females from the W or LE males, nor any main effects or interactions for the mount, intromission, or ejaculation latencies during the first ejaculatory series. These results indicate that females allowed both strains of male to initiate copulation at comparable times, and that they received a comparable number of ejaculations from each male.

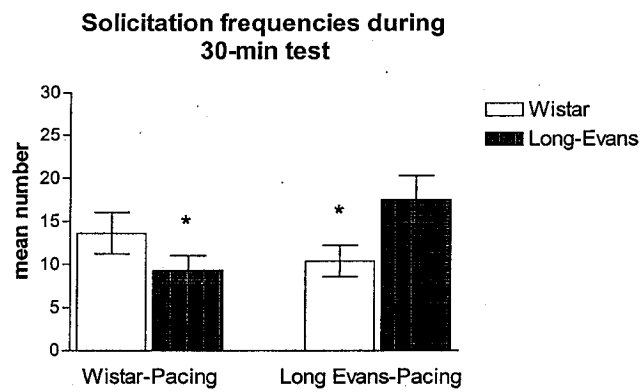
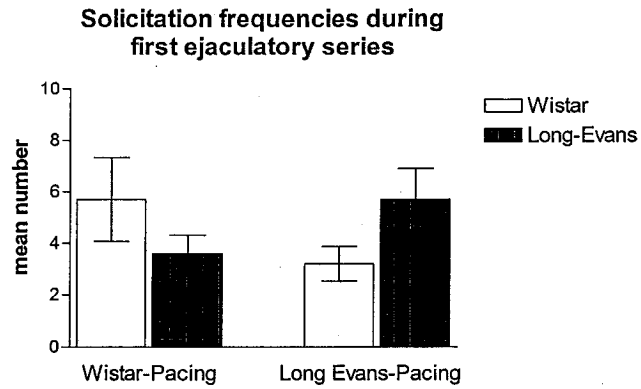


Figure 6: Solicitation frequencies of Wistar females during first ejaculatory series (top) and during the 30-min partner-preference test (bottom). In the Wistar-pacing group females were conditioned to associate Wistar males with paced copulation, and Long-Evans males with nonpaced copulation. In the Long-Evans-pacing group, females received the opposite associations. Data are means \pm SEM. * = $p < 0.05$ for differences within group for Wistar and Long-Evans males.

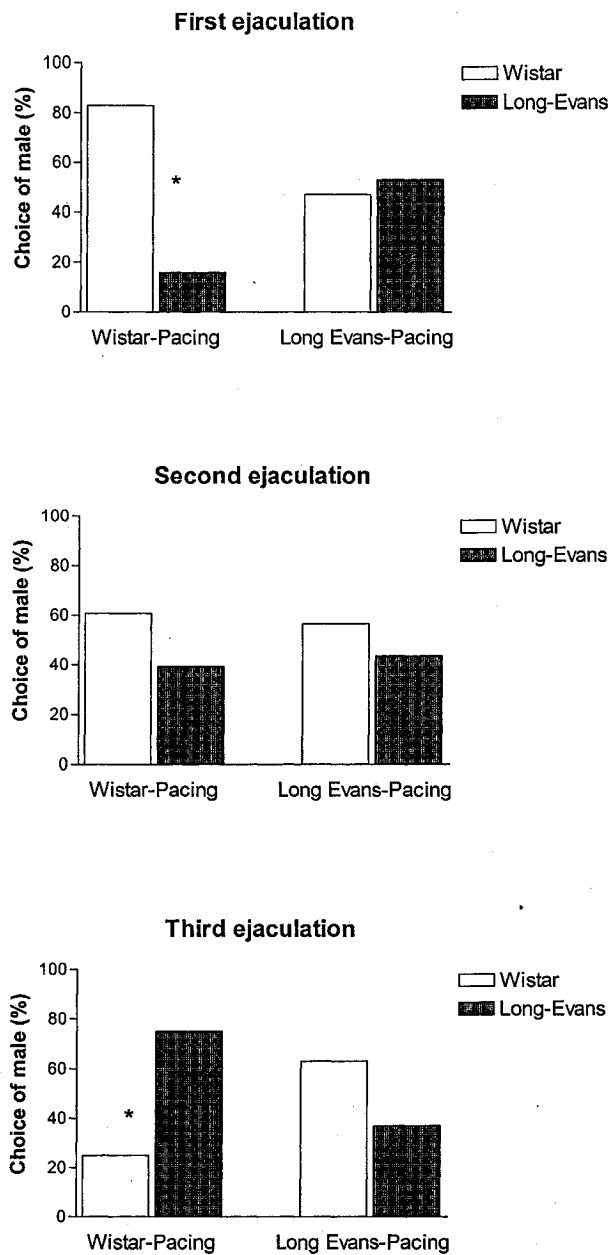


Figure 7: Wistar females' choice of male to receive their first (top), second (middle), and third (bottom) ejaculation during the 30-min partner preference test. * = $p < 0.05$ for differences within groups for Wistar and Long-Evans males.

3.2. Long-Evans females

3.2.1. Visits and olfactory investigations

The analysis of visit frequencies during the 30-min test revealed a significant main effect of group ($F(1, 20) = 8.05, p = 0.01$). The post hoc test indicated that females in the LE-P group visited both males with a higher frequency overall than did females in the W-P group. With regard to the first visit latency, there was a trend for a main effect of group ($F(1, 20) = 3.9, p = 0.059$), but no trends were detected for strain of male or the interaction of group and strain of male. With regard to number of visits during the first ejaculatory series, the ANOVA failed to detect any main effect or interaction. No significant main effects or interactions were found for the analysis of olfactory investigations. This suggests that females investigated both strains of male equally.

3.2.2. Appetitive sexual behaviors

With regard to the number of solicitations during the first ejaculatory series (Fig. 8), the ANOVA detected an interaction between group and strain of male ($F(1, 20) = 5.9, p = 0.02$). Posthoc analyses indicated that in the LE-P group, females displayed a higher number of solicitations towards LE males ($\underline{M} = 5$) than towards W males ($\underline{M} = 2.08$), but no differences between W and LE males were detected in the W-P group. The analysis of solicitations frequency during the 30-min test detected a main effect of group ($F(1, 20) = 4.4, p = 0.04$) and an interaction between group and strain of male ($F(1, 20) = 7.7, p = 0.01$). Posthoc analyses indicated that females displayed more solicitations towards LE males in the LE-P group ($\underline{M} = 21$) than towards W males ($\underline{M} = 13.7$) of the same group, and also more than W ($\underline{M} = 3.4$) and LE ($\underline{M} = 3.2$) males in the W-P group. This indicates that, overall, females displayed more solicitations towards the pacing-related

males, but this effect was facilitated significantly if the pacing males was LE. No main effects or interaction were found for first solicitation latency, nor were significant effects found for H&D frequencies or latencies.

3.2.3. *Lordosis*

The ANOVA detected a main effect of strain of male ($F(1, 20) = 5.3, p = 0.03$), and an interaction between group and strain of male ($F(1, 20) = 10.3, p = 0.004$) for the frequency of lordosis magnitude 3 for the entire 30-min test. Posthoc analyses indicated that in the W-P group, females displayed higher numbers towards W males ($\underline{M} = 14.3$) than towards LE males ($\underline{M} = 7.6$). In the LE-P group no differences were detected in the frequency of lordosis magnitudes 1, 2, or 3. No differences were detected for the frequency of lordosis magnitudes during the first ejaculatory series.

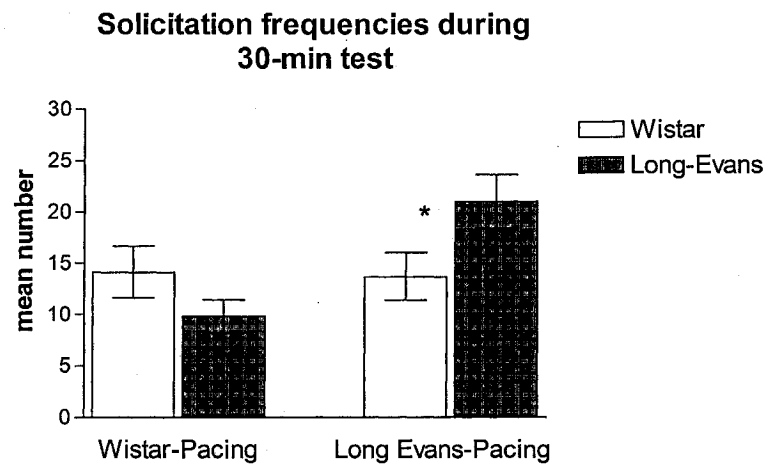
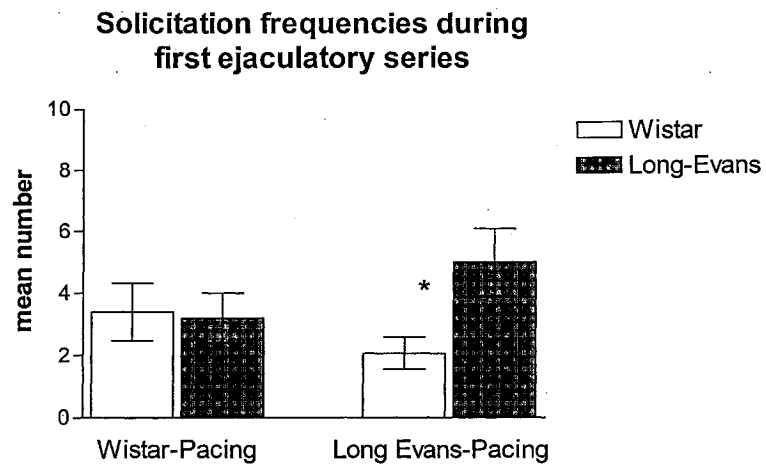


Figure 8: Solicitation frequencies of Long-Evans females during first ejaculatory series (top) and during the 30-min partner preference test (bottom). Data are means \pm SEM. * = $p < 0.05$ for differences within group for Wistar and Long-Evans males.

3.2.4. Choice of male for ejaculation and male responses

Chi-square analyses for proportions of ejaculations indicated significant differences between males (Fig. 9). Regarding the choice of male to receive the first ejaculation, 11 (91.6%) of the 12 females in the LE-P group that received an ejaculation received their first from the LE male and 1 (8.3%) from the W male ($\chi^2 (1, N=12) = 8.3, p = 0.04$). In the W-P group, the choice of male was not significantly different from chance ($\chi^2 (1, N=10) = 1.6, p = 0.20$). With regard to the choice of male to receive the second ejaculation, 10 (83.3%) of 12 females in the LE-P group received their second ejaculation from the LE male, and 2 (16.6%) from the W male ($\chi^2 (1, N=12) = 5.3, p = 0.02$). In the W-P group the proportion was not different from chance ($\chi^2 (1, N=10) = .4, p = 0.52$). For the choice of male to receive the third ejaculation, 8 (80%) of 10 females in the LE-P group receive their third ejaculation from the LE male, and 2 (20%) from the W male ($\chi^2 (1, N=10) = 5.3, p = 0.02$). In the W-P group the proportion of ejaculations from either male was not different from chance ($\chi^2 (1, N=10) = 1, p = 0.3$). Overall, the analysis of ejaculations indicated that in the LE-P group, most of the females chose LE males to receive the first three ejaculations. In the W-P group however, there was no preference for either male.

No significant main effects or interactions were found for the number of mounts, intromissions, or ejaculations, received by the females from the W or LE males, nor any main effects or interactions for the mount, intromission, or ejaculation latencies during the first ejaculatory series. These results indicate that, like the W females, the LE females allowed both strains of male to initiate copulation at comparable times, and that they received a comparable number of ejaculations from each male.

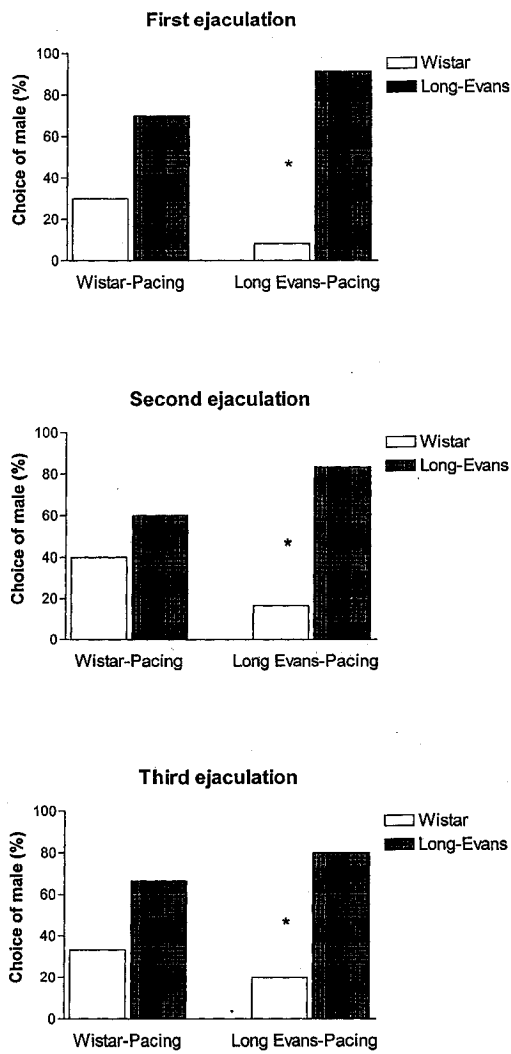


Figure 9: Long-Evans females' choice of male to receive their first (top), second (middle), and third (bottom) ejaculation during the 30-min partner preference test. * = $p < 0.05$ for differences within groups for Wistar and Long-Evans males.

4. Discussion

The present study demonstrates that W and LE female rats can develop a conditioned partner preference for a strain of male previously associated with paced copulation. This preference was expressed most strongly and consistently by an increase in solicitations toward the pacing-related male, regardless of strain. W, but not LE, females also displayed a higher frequency of lordosis magnitude 3 toward the pacing-related male. Thus, natural cues of different strains of male can acquire learned significance as predictors of sexual reward for female rats. However, this study also showed that the conditioned partner preference was more robust when the pacing-related male was of the same strain as the female. This facilitation of the partner preference was evident in both appetitive and consummatory behaviors, and in particular with the choice of male for first ejaculation. This suggests that both W and LE females have an unconditioned preference for males of the same strain that can be enhanced by an association with paced copulation. However, when the cues of the dissimilar male were paired with pacing, both W and LE females altered their preference with regard to solicitation. These results suggest that solicitations (and possibly also hops and darts) are the most sensitive appetitive measures of female sexual motivation and choice, and reflect the reward characteristics of paced copulation.

The choice of male for the second and third ejaculation indicated a difference in copulatory strategies between W and LE females. Although W females displayed a significant preference for the pacing-related male of the same strain for their first ejaculation, this preference was not displayed for second ejaculation, and for the third ejaculation, the preference was switched for the originally-nonpreferred male. This

phenomenon resembles a Coolidge effect, in which animals switch their preference from a familiar partner to a novel one. In this case, the preferred male was chosen for the first ejaculation and the non-preferred male for the third ejaculation. However, a Coolidge effect implies increased sexual desire for a novel partner relative to a familiar one, and females continued to solicit the pacing-related male more throughout the 30-min test, indicating a willingness to copulate more with that male. In contrast, LE females did not show any shift in preference of male for the second or third ejaculation. This preference however, was not evident when LE males were associated with nonpaced copulation, indicating further that innate preferences can be devaluated or bypassed by the development of a conditioned preference.

In our previous study using an almond odor paired with paced copulation (Coria-Avila et al., 2005), LE females in the Paired group displayed significant preferences for scented LE males over unscented LE males in the same open field test, including increased solicitations, hops and darts, higher magnitude lordoses, and also a clear mate preference in obtaining their first ejaculation from the scented male. Taken together with the present study, these results suggest that neutral cues such as almond odor paired with sexual reward, in the presence of naturally-preferred cues from a male of the female's own strain, induce a robust conditioning that is reflected in appetitive behaviors such as solicitations and hops and darts, consummatory behaviors such as high magnitude lordoses, and in the choice to receive that male's ejaculations. Interestingly, although the LE females in that study chose the scented male for their first ejaculation, they switched males for their second and third ejaculations, similar to the W females in the present study. It may be that when strain cues of the males are held constant for LE females, the

odor has a greater conditioned effect on solicitations relative to the choice of male for ejaculations.

This study also shows that the relationship and expression of innate and conditioned partner preferences are of different magnitude in W and LE females. Regarding the distribution of ejaculations during the 30-min test, W females expressed a choice for the same strain of male only for the first ejaculation, whereas LE females expressed it for three ejaculations. In the case of solicitations, W females clearly displayed a higher frequency of solicitations for the pacing-related males, regardless of the strain of male. However, in the case of LE females, the significance was reached exclusively when the pacing-related male was LE. Interestingly, the mean number of solicitations made toward W males during the 30-min test was nearly identical for W and LE females in the W-P condition, and the lower mean number of solicitations made toward the LE males was also nearly identical in this conditioning group (Figs. 6 and 8). It is likely that the lower number of LE relative to W females contributed to the lack of significance on this measure. Therefore, we suggest that this difference may be more apparent than real. However, taken together, those data suggest that the synergy between innate and conditioned preferences was stronger in LE females than in W females.

One possible explanation for the magnitude differences in conditioning between W and LE females concerns the inherent differences between the two strains with regard to their sensory abilities. Females might have distinguished the males visually, although differences in natural odors or inherent vocalizations by each strain of male cannot be ruled out. Albino rats have diminished visual acuity (Prusky, Harker, Douglas, & Whishaw, 2002), but no auditory disadvantage (Syka, Rybalko, Brozek, & Jilek, 1996)

relative to pigmented rats. If visual differences between W and LE males were the most salient cues, conditioning may have been weaker in W females relative to LE females. Other physiological differences between albino and pigmented rats could account for differences in sensitivity to the partner cues. For example, it has been shown that pigmented male rats exhibit noncontact erections in response to remote odor cues from sexually receptive females (Sachs, Akasofu, Citron, Daniels, & Natoli, 1994; Sachs, 1997), whereas albino males do not (Sachs, 1996) unless treated with the serotonin synthesis inhibitor p-chlorophenylalanine (Matsumoto, Kondo, Sachs, & Yamanouchi, 1997). Those data indicate that albino rats have higher circulating serotonin levels compared to pigmented rats, and it is conceivable that high serotonin transmission in the hypothalamus or limbic system may lead to greater sexual inhibition (Pfaus et al., 2003). Albino and pigmented females may also be differentially affected by the environmental circumstances that allow them to pace copulatory contact. In a previous pilot study (Coria-Avila, Jones, & Pfaus, 2003) we reported that Wistar females under the same conditioning protocol, but tested in a smaller, three-compartmented chamber (rather than a large open field), received more mounts, intromissions, and ejaculations from the pacing-related males, regardless of strain. The ability to express these additional copulatory choices may depend on certain features of the testing chamber that allow females greater control over the copulatory responses of the males, in addition to the strength of the conditioned cue (e.g. olfactory vs. visual).

The present results add to a growing body of evidence that paced copulation induces both conditioned place and partner preferences in female rats (Martinez et al., 2001; Paredes et al., 1997; Paredes et al., 1999; Coria-Avila et al., 2005). At present, it is

not clear what component of paced copulation produces the reward necessary to support these conditioned associations. The positive effect of paced copulation may depend on a cascade of events that start with the ability of the female to control the rate of vaginocervical stimulation (VCS) she receives from male intromissions. In female rats, pacing serves a significant reproductive purpose, allowing females to control VCS at their preferred intervals. Such preferred intervals may not only be species-specific, but individual-specific, and serve to distribute VCSs in time, which in sequence enhances both sperm transport and the induction of prolactin surges that support the maintenance of progesterone release by the corpora lutea (Erskine et al., 1989; Matthews, Jr. et al., 1978; McClintock et al., 1978). Accordingly, paced copulation is more efficient than nonpaced copulation for inducing luteal function, a faster termination of estrus, and pregnancy or pseudopregnancy (Bermant et al., 1966; Erskine et al., 1989; Lodder et al., 1976), and increases litter size compared to nonpaced copulation (Coopersmith et al., 1994).

Finally, the present study confirms previous findings that early experiences, and in particular early sexual experiences, have powerful influences on subsequent sexual preferences (Kippin et al., 1998; Kippin et al., 2001a; Kippin et al., 2001b; Pfaus et al., 2001; Coria-Avila et al., 2005). Such influences may begin when animals are young and learn to identify their own strain. For example, early postnatal experiences induce social preferences for an animal's own strain, and lead to assortative mating (D'Udine & Alleva, 1983), in which animals choose to mate selectively with members of their own strain relative to members of a different strain of a species that are genetically less similar. Assortative mating is believed to maintain homozygosity in a strain, and thereby keep

strains from outbreeding positive characteristics. However, cross-fostering studies have revealed a degree of plasticity in this phenomenon. For example, mice raised by rat mothers engage in more sexual contact with small rats and less with mice, relative to mice raised by mouse mothers (Lagerspetz & Heino, 1970). Early postnatal experience of male rats with a neutral odor (citral) smeared on the teats and anogenital area of female rat mothers increased sexual arousal in those males as adults: more Paired males ejaculated with citral-scented estrous females compared to Paired males that copulated with unscented females (Fillion et al., 1986). Taken together with our previous studies, the current results suggest that an animal's initial sexual experiences represent a second "critical period" in which place and strain characteristics become preferred, and in which the effects of early postnatal experience can be modified. These sexual preferences depend strongly on the contingency between rewarding or aversive unconditioned stimuli and the cues available during those initial sexual experiences. In the case of females, it is not yet known how the spinal cord and brain integrate the VCS during paced copulation and the available sensory cues to induce place or partner preferences. However, both are disrupted by the administration of the opioid antagonist naloxone during conditioning (Coria-Avila, Solomon, & Pfaus, 2005; Paredes et al., 2001), suggesting the involvement of endogenous opioids as a primary substrate of sexual reward in both males and females.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP and a postgraduate fellowship from CONACyT of Mexico (128099) to GAC. The authors would like to thank Diala Arzouni and Kathleen M. McNulty for their help conducting the pilot experiments, and Dr. Mary Erskine for her insightful comments on an earlier paper that helped frame the experiments reported here. All animal procedures conformed to the guidelines of the Canadian Council for Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Summary of Chapter 1

Chapter 1 presented the behavioral data of conditioned partner preference in female rats induced by sexual reward. The first paper demonstrated that an originally-neutral almond odor paired with PC induced a conditioned partner preference towards almond-scented males. Such partner preference was inferred from shorter first solicitation latencies, higher frequency of solicitations, more hops & darts, higher frequency of lordosis magnitude 3, receiving the first mount, and the first ejaculation from the preferred male. In contrast, when the odor was paired with nonpaced copulation, partner preference did not develop, nor did it develop when the contingency between paced and nonpaced copulation was random. This indicates that only the association with paced copulation was sufficient to induce a conditioned partner preference. The second paper demonstrated that female Long-Evans and Wistar rats also develop a conditioned partner preference toward a particular strain of male (Long-Evans or Wistar) paired with PC, indicating that the natural strain cues can become preferred. However, the effect was not as robust as observed in the odor-conditioning study: Females displayed more solicitations toward, and receiving the first ejaculation from, the preferred male. Although the frequency of solicitations was always greater toward the reward-related male, the ejaculatory preference for this male was always greater if the reward-related male was of the same strain as the female. This suggests the presence of assortative mating preferences that can be augmented or counterbalanced by an animal's first sexual experiences.

CHAPTER TWO

Neuronal Activation by Stimuli that Predict Sexual Reward in Female Rats

Genaro A. Coria-Avila*, James G. Pfaus

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

Running Head: Fos activation by cues for sexual reward

*Corresponding author: Center for Studies in Behavioral Neurobiology,
Department of Psychology, Concordia University, 7141 Sherbrooke W.,
Montréal, QC, H4B 1R6 CANADA. Fax: 514-848-2817.
E-mail: coria75@yahoo.com

For submission to *Neuroscience*

Abstract

Conditioned stimuli (CSs) associated with paced copulation (PC) induce a conditioned partner preference for males bearing the CS. Here we examined the activation of Fos immunoreactivity (Fos-IR) following exposure to a CS previously paired with either PC or nonpaced copulation (NPC). Ovariectomized, hormone-primed rats received 10 sequential conditioning trials at 4-day intervals. In Experiment 1, females in the Paired group learned to associate an almond odor on a male with PC and an unscented male with NPC. In the Unpaired group, females received the opposite association. In Experiment 2, females associated two different strains of male, Long-Evans or Wistar, with PC or NPC respectively. A preference test indicated that females in both experiments developed a conditioned preference for the pacing-related males, as indicated by significantly more solicitations toward the male and a preference to copulate with the pacing-related male. Subsequently, females were exposed to the CS (odor or strain) alone for 1 hr prior to sacrifice and preparation of their brains for Fos immunocytochemistry. In both experiments, the CS associated with PC induced significantly more Fos-IR in the Piriform cortex, medial preoptic area, and ventral tegmental area, relative to the same odor or strain cues associated with NPC. These findings provide evidence that PC can be conditioned to environmental stimuli such as neutral odors or strain cues, which earn an incentive value via classical conditioning. The significance of the brain areas activated is discussed with regard to their role in sexual and other motivated behaviors.

Keywords: Fos, partner preference, Pavlovian conditioning, estradiol, progesterone, odor, strain, hypothalamic, limbic, priming stimuli

Learning can affect many aspects of sexual behavior in animals (For review see Pfaus et al., 2001). In rodents, for example, these include changes in the expression of sexual excitement (Pfaus, 1996; Pfaus, 1999; Mendelson & Pfaus, 1989), in the capacity to locate and recognize a place associated with mating (Everitt, 1990), in courtship behaviors and mate-directed vocalizations (Nyby, Bigelow, Kerchner, & Barbehenn, 1983), in copulatory parameters (Larsson, 1956; Dewsbury, 1969), and in partner preference (Kippin et al., 2001a; Coria-Avila et al., 2005; Coria-Avila et al., 2006).

The effects of learning on conditioned partner preference can be observed when an individual has the opportunity to choose between two or more potential partners. Typically, the conditioned preference is displayed as more time spent with, or more sexual behavior directed towards, an individual that bears recognizable features that have been associated with sexual reward. For instance, olfactory cues (e.g., almond odor) paired with the reward state induced by ejaculation in males can induce males to ejaculate preferentially with almond-scented females when males are given a choice to copulate freely with scented and unscented, sexually receptive females in an open field (Kippin et al., 2001c; Kippin et al., 2001a; Kippin et al., 2001b). In the case of females, almond odor paired with their ability to “pace” or control the rate of copulation induces females to solicit and receive ejaculations preferentially from an almond-scented male when they are given a choice to copulate freely with scented and unscented, sexually vigorous males in an open field. This preference is not displayed if the odor is paired with nonpaced copulation (Coria-Avila et al., 2005). Similarly, female rats can learn to associate a strain of male (pigmented Long-Evans (LE) versus albino Wistar (W) rats) with pacing, and

display a conditioned preference to solicit males of the pacing-related strain when given a free choice between the two different strains in an open field (Coria-Avila et al., 2006).

Like ejaculation in males, paced copulation induces a reward state of sufficient intensity to induce conditioning in females (Paredes et al., 1997; Paredes et al., 1999). The rewarding effects of paced copulation are believed to be the result of the appropriate vaginocervical stimulation (VCS) distributed over time at the female's preferred interval. This is supported by the fact that female rats will develop CPP when they are actively pacing, but also when the males are removed by the researcher from the copulatory arena at the female's preferred interval (Jenkins & Becker, 2003) or when females are given manual VCS with a lubricated glass rod distributed in clusters at preferred intervals (Afonso & Pfaus, submitted).

Brain regions sensitive to sexual stimulation have been identified using Fos immunoreactivity (IR) as a marker of neuronal activation in females (Pfaus et al., 1997). A number of hypothalamic and limbic structures are sensitive to VCS, either from multiple intromissions by the male or following manual application with a lubricated glass rod (Pfaus et al., 1993; Pfaus, Marcangione, Smith, Manitt, & Abillamaa, 1996). These regions include the piriform cortex (PirCtx), anterior cingulate cortex (ACC), nucleus accumbens (NAc), lateral septum (LS), medial nucleus of the preoptic area (mPOA), posteriomedial region of the bed nucleus of the stria terminalis (BNSTpm), the paraventricular nucleus of the hypothalamus (PVN), ventrolateral portion of the ventromedial hypothalamus (VMHvl), the arcuate nucleus (ArcN), posteriodorsal region of the medial amygdala (MeApd), and the ventral tegmental area (VTA). Females that receive intromissions express a higher number of Fos-labelled cells in these regions

compared to females that receive mounts only, indicating that the expression of Fos in these areas during copulation is not due to olfactory input from the male or cutaneous somatosensory input received from flank stimulation during mounts only (Erskine, 1993). However, Erskine & Hanrahan, (1997) found that when females were allowed to pace either 5 or 15 intromissions, there was more Fos-IR in the MeApd relative to females that received 5 or 15 nonpaced intromissions. In addition, females that received 5 paced intromissions had as much Fos-IR as those that receive 15 nonpaced intromissions, indicating that the MeApd is particularly sensitive to the type and frequency of VCS. These differences were not detected in the mPOA. Furthermore, they reported that the BNSTpm and the VMHvl expressed more Fos-positive neurons following paced copulation relative to females that received mounts alone, or to those that remained in their home cages. Such differences were not observed following nonpaced copulation.

Kippin et al., (2003) trained males to associate an almond odor on females with the postejaculatory reward state, or to have the almond odor unpaired with that state. Subsequently, males in the paired and unpaired groups were presented with the almond odor alone in bedding. The odor activated Fos selectively in the PirCtx, NAcc core, basolateral amygdala (BLA), and the anterior region of the lateral hypothalamic area, in paired relative to unpaired males. Conversely, estrous odors alone in bedding activated Fos within the accessory olfactory bulb, NAcc core and shell, mPOA, VMH, MeApd, and VTA of males in either the paired or unpaired groups, indicating that the conditioned odor activated main olfactory terminals whereas unconditionally appetitive estrous odors activated accessory olfactory terminals. Given that a neutral odor such as almond, or the particular strain of a male, can gain conditioned incentive value by being associated with

paced copulation, and thus direct the copulatory preferences of female rats, this study examined the pattern of Fos induction following exposure to a conditioned odor alone, or a pacing-related male behind a wire-mesh screen, who had previously been associated with the ability to pace copulation.

EXPERIMENTAL PROCEDURES

Experiment 1: brain activation by an odor paired with sexual reward

Animals and surgery. Long-Evans male (300-350 g) and female (200-250 g) rats were purchased from Charles River Canada (St-Constant, QC). They were housed in groups of 4 in large hanging wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Food and water were continuously available.

Females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Females were then ovariectomized (OVX) bilaterally via a lumbar incision. All females were given a week of post-surgical recovery prior to odor conditioning trials. Intact Long-Evans male rats that served as stimulus males had at least 10 tests of sexual behavior in bi-level chambers prior to the start of these experiments. These males were sexually vigorous and initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test. If scented, males received almond extract (Blue Ribbon, Etobicoke, ON) applied to the

back of their necks and anogenital regions with a cotton swab. If unscented, males received distilled water to the same areas.

Odor conditioning. The training procedures used in each experiment were similar to those in (Coria-Avila et al., 2005). Odor conditioning trials occurred at 4-day intervals during the middle third of the dark phase of the circadian cycle following hormone priming. Paced copulation occurred in semicircular chambers ($38^2 \times 60 \times 38$ cm) bisected by a clear Plexiglas divider with 4 holes cut into the bottom (4×4 cm) that rested on bedding. These holes were large enough for the female to crawl through, but too small for the males to crawl through. Nonpaced copulation occurred in identical chambers, but with the divider removed. Females in the Paired group copulated with almond scented males in the paced condition and unscented males in the nonpaced condition. Females in the Unpaired group copulated with unscented males in the paced condition and scented males in the nonpaced condition. Females received 9 sequential experiences in paced and non-paced conditions at 4-day intervals. The order of presentation of paced and non-paced conditions was counterbalanced for the Paired and Unpaired groups. During each test, females were allowed to copulate freely for 30 min with the male, after which they were transferred to their home cages. All testing with scented and unscented males took place in different rooms to assure containment of the scent.

Activation of Fos-IR by the odor. Four days after the final training session, each female was placed in a Plexiglas chamber containing wood shavings as bedding and a cotton gauze pad saturated with almond odor for 60 min. Females were then injected with an overdose of sodium pentobarbital (120 mg/kg, i.p.) and perfused intracardially with 350 ml of phosphate-buffered saline followed by 350 ml of 4% paraformaldehyde. The

brains were removed and post fixed in 4% paraformaldehyde for 4 h and stored overnight in 30% sucrose solution. The brains were then covered in aluminum foil and stored at -80 °C.

Coronal brain sections of 30 µm, were incubated sequentially with 30% hydrogen peroxide (H₂O₂) in Tris-buffered saline (TBS) for 30 min at room temperature, with 3% normal goat serum (NGS) in 0.05% Triton TBS for 90 min at 4 °C, with rabbit polyclonal anti-Fos (Oncogene Science, Boston, MA, USA; diluted 1:40,000) in 0.05% Triton TBS with 3% NGS for 72 h at 4 °C, with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA; 1:200) in 0.05% Triton TBS with 3% NGS for 1 h at 4 °C, and avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; diluted 1:55) for 2 h at 4 °C. Sections were washed in TBS (3×5 min) between each incubation. Immunoreactions were stained by sequential treatments at room temperature with 50-mM Tris for 10 min, 3,3'-diaminobenzidine (DAB) in 50-mM Tris (0.1 ml of DAB/Tris buffer, pH 7.8) for 10 min, DAB/3% H₂O₂ in 50-mM Tris for 10 min, and 8% nickel chloride (400 µl per 100 ml of DAB/Tris buffer+H₂O₂). Sections were mounted on gel-coated slides and allowed to dry, then dehydrated in alcohol 70%, 90% and 100% 10 min each respectively, cleared in Xylene (2 hrs), coverslipped, and examined under a light microscope.

Histological and statistical analysis. Tissue sections were examined at 40x and the number of Fos-positive cells was counted bilaterally for each region from six sections per rat using NIH Image connected to a Leitz Laborlux microscope. The regions were defined by using the atlas of Paxinos & Watson, (1998): NAc Core (NAcc, plates 13 and 14), NAc Shell (NAcSh, plates 13 and 14), olfactory tubercle (Tu, plates 13 and 14), Pir

Ctx (plates 13 and 14), ACC (plates 13 and 14), caudate-putamen (CPu; plates 13 and 14), mPOA (plates 20 and 21), Lateral septum (LS, plates 13 and 14), BNSTpm (plates 21 and 22), PVN (plates 22-25), aLHA (plates 23 and 24), VMHvl (plates 27 and 28), ArcN (plates 27-30), MePD (plates 28 and 29), basolateral amygdala (BLA; plates 28 and 29), cortical amygdala (CoA; plates 28 and 29), and VTA (plates 40 and 41). A mean was calculated for each area for each rat and statistical analyses were conducted for rats in each group (N=6/condition) using Students *t*-tests for independent samples with a Bonferroni correction to compare the mean number of Fos-positive neurons in the Paired vs. Unpaired conditions. The level of significance for all comparisons was $p < 0.05$.

Experiment 2: brain activation by strain cues associated with sexual reward

Animals and surgery. Stimulus males were of the Long-Evans and Wistar strains (300-350 g) and the females were Wistars (200-250 g) purchased from Charles River Canada. Females were OVX, and sexual receptivity was induced by EB and P priming, as in Experiment 1. Long-Evans and Wistar males were sexually vigorous and had at least 4 sexual experiences before the start of the conditioning trials.

Strain conditioning. The training procedures used in each experiment were similar to those in Coria-Avila et al. (2006) and in Experiment 1. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark circadian cycle following hormone priming. Paced copulation occurred in the same semicircular chambers as in Experiment 1. There were two counterbalanced groups. In the Wistar-Pacing (W-P) group females copulated with Wistar males in the paced condition and with Long-Evans males in the nonpaced condition. In the Long-Evans-Pacing (LE-P) group females

copulated in pacing chambers with LE males and in non-pacing chambers with W males. Females received 10 sequential experiences in paced and non-paced conditions at 4-day intervals. The order of presentation of paced and non-paced conditions was counterbalanced for the both groups, so that half of the group had the first sexual experience in a pacing condition and the other half in non-pacing condition. During each test, females were allowed to copulate for 30 min, after which they were transferred back to their home cages.

Activation of Fos-IR by strain cues. Four days after the final training session, each receptive female was paired with a male of the strain associated with either paced or nonpaced copulation. The pairing occurred in a chamber divided by a wire mesh screen that allowed olfactory, visual, and auditory cues to be detected, but not physical contact, between the male and the female (e.g., Pfaus et al., 1990). In all conditions, females were allowed to remain undisturbed in the chamber for 60 min. Females were then sacrificed as in Experiment 1, and their brains removed and processed for Fos-IR using the same antibody and immunocytochemical technique as in Experiment 1.

Histological and statistical analysis. Tissue sections were examined exactly as in Experiment 1. For the statistical analysis, a two-way (pacing X strain of male) between subjects Analysis of Variance (ANOVA) was performed to assess differences in Fos induction between females exposed randomly to 4 conditions: Fos-IR following exposure to 1) Wistar male associated with paced copulation, 2) Long-Evans male associated with nonpaced copulation, 3) Wistar male associated with nonpaced copulation, and 4) Long-Evans male associated with paced copulation (N=5 females/condition). For each

significant ANOVA, posthoc analysis of mean differences were made using the Least Significant Difference (LSD) method, $P < 0.05$.

RESULTS

Experiment 1: brain activation by an odor paired with sexual reward

More Fos-positive neurons were found in the Tu, PirCtx, ACC, NAcc, mPOA, LS, PVN, VTA, CPu and ArcN of Paired relative to Unpaired females (Figures 10, 14, and Table 3). For the mPOA, the statistical analysis revealed that females in the Paired group there was more Fos-IR cells relative to the Unpaired group, $t(10)=2.22$, $P < 0.05$. For the NAcc, more Fos-IR cells were found in the Paired relative to the Unpaired group, $t(10)=2.5$, $P < 0.02$. For the Pir Ctx, more Fos-IR cells were found in the Paired relative to the Unpaired group, $t(10)=2.7$, $P < 0.02$. For the ACC more Fos cells were observed in the Paired relative to the Unpaired group, $t(10)=2.7$, $P < 0.02$. For the PVN more Fos cells were observed in the Paired relative to the Unpaired group, $t(9)=2.68$, $P < 0.02$. For the VTA more Fos cells were found in the Paired relative to the Unpaired group, $t(10)=2.2$, $P=0.049$. The statistical analysis detected a trend for significance in the analysis of the CPu, with more Fos-IR cells in the Paired relative to the Unpaired group, $t(10)=2.04$, $P=0.06$. A trend toward significance was also detected for the ArcN, with more Fos-IR cells in the Paired relative to the Unpaired group, $t(10)=1.9$, $P=0.08$. There were no significant differences in the NAcSh, MePD, CoA, BLA, Lateral Hypothalamus, BNST, and VMH (Figure 11, and Table 3).

Neural Activation by Conditioned Odors

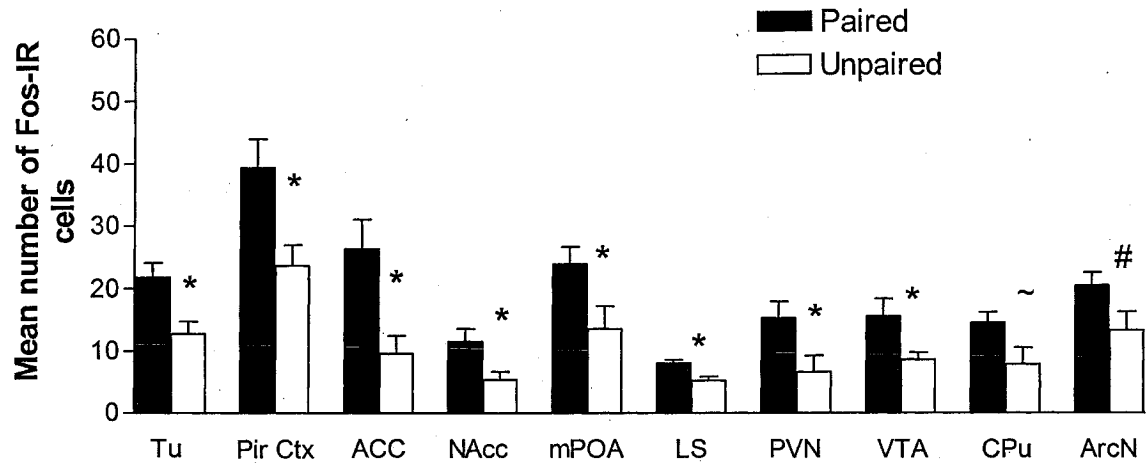


Figure 10. Brain areas of female rats that expressed significant differences in Fos-IR following exposure to a conditioned odor alone. For females in the Paired group, the odor (almond extract) was associated with paced copulation during previous conditioning trials. In the Unpaired group, the odor was associated with nonpaced copulation. mPOA, medial preoptic area; NAcc, nucleus accumbens core; PirCtx, piriform cortex; ACC, anterior cingulate cortex; PVN, paraventricular nucleus of the hypothalamus; VTA, ventral tegmental area; CPu, caudate putamen. The bars represent the mean number + SEM of Fos-IR cells within a sample area of the same dimensions for all the regions. * $P < 0.05$; ~ $P < 0.06$, # $P < 0.08$, between Paired and Unpaired groups.

Areas with no differences

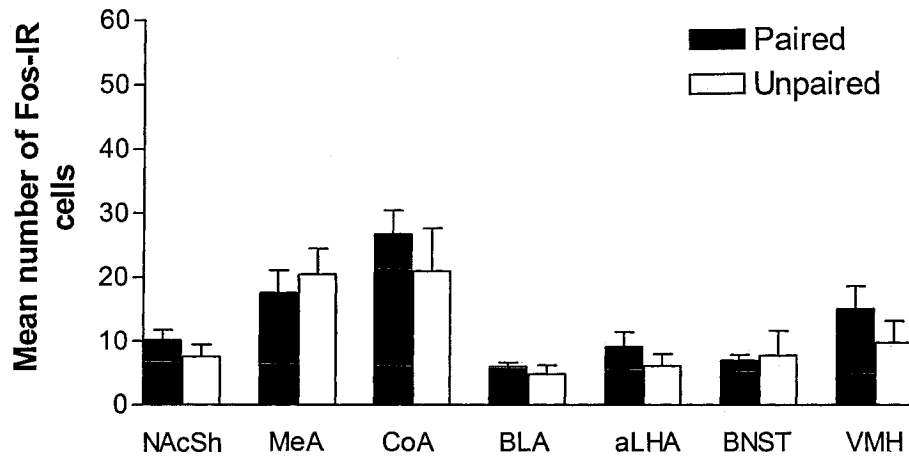


Figure 11. Brain areas of female rats that did not express significant differences in Fos-IR following exposure to a conditioned odor alone. In the Paired group, the odor (almond extract) was associated with paced copulation during previous conditioning trials. In the Unpaired group, the odor was associated with nonpaced copulation. NAcSh, nucleus accumbens shell; ArcN, arcuate nucleus; MeA, medial amygdala; CoA, cortical amygdala; BLA, basolateral amygdala; aLHA, lateral hypothalamus; BNST, bed nucleus of the stria terminalis; VMH, ventromedial hypothalamus. The bars represent the mean number + SEM of Fos-IR cells within a sample area of the same dimensions for all the regions.

Area	Paired	Unpaired
Amygdala (Basolateral)	6.10 ± .60	4.90 ± 1.35
Amygdala (Cortical)	26.8 ± 3.70	21.0 ± 6.79
Amygdala (Medial)	17.6 ± 3.54	20.5 ± 4.07
Arcuate Nucleus	20.5 ± 2.15 #	13.4 ± 2.90
Bed Nucleus of the Stria Terminalis	7.09 ± .85	7.81 ± 3.91
Caudate Putamen	14.6 ± 1.71 ~	7.92 ± 2.78
Cingulate Cortex (anterior)	26.4 ± 4.75 **	9.60 ± 2.99
Lateral Hypothalamus (anterior portion)	9.23 ± 2.33	6.25 ± 1.88
Lateral septum	8.14 ± .55 **	5.35 ± .67
Medial Preoptic Area	24.0 ± 2.72 *	13.6 ± 3.78
Nucleus Accumbens Core	11.6 ± 2.00 **	5.45 ± 1.26
Nucleus Accumbens Shell	10.2 ± 1.63	7.60 ± 1.87
Olfactory tubercle	21.8 ± 2.35 **	12.8 ± 2.04
Paraventricular Nucleus	15.5 ± 2.66 **	7.3 ± 1.5
Piriform Cortex	39.4 ± 4.60 **	23.7 ± 3.27
Ventral Tegmental Area	15.6 ± 2.80 *	8.70 ± 1.1
Ventromedial Hypothalamus	15.2 ± 3.51	9.92 ± 3.44

Table 3. Brain areas of female rats that expressed Fos-IR following exposure to a conditioned odor alone. In the Paired group, the odor (almond extract) was associated with paced copulation during previous conditioning trials. In the Unpaired group, the odor was associated with nonpaced copulation. The data are expressed in mean + SEM. The mean number of Fos-IR cells were taken from a sample area of the same dimensions for all the regions * $P < 0.05$, ** $P < 0.02$, ~ $P = 0.06$, # $P < 0.08$, between Paired and Unpaired.

Experiment 2: brain activation by strain cues associated with sexual reward

Regardless of strain, males associated with paced copulation induced greater Fos-IR in PirCtx, mPOA, VMH, and VTA (Figures 12, 14, and , Table 4). For the PirCtx, the statistical analysis revealed a main effect of pacing $F(1, 16)= 4.57, P=0.04$, but no main effect of strain of male or interaction. For the mPOA, the ANOVA revealed a main effect of pacing $F(1, 16)= 4.1, P=0.05$, but no main effect of strain or interaction. For the VMH there was a main effect of pacing $F(1, 16)= 4.1, P=0.05$, but no main effect of strain or interaction. Finally, for the VTA there was a main effect of pacing $F(1, 16)= 6.2, P=0.02$, but no main effect of strain or interaction.

In the PVM, MeApd, CoA, aLHA, and BNST, exposure to LE males induced more Fos compared to exposure to W males (Figure 13 and Table 4). For the PVN the ANOVA confirmed a main effect of strain of male, $F(1, 16)= .4.9, P=0.04$, but no main effect of pacing or interaction. For the MeApd there was a main effect of strain of male, $F(1, 16)= 6.25, P=0.02$, and a trend for significant main effect of pacing $F(1, 16)= 3.99, P=0.06$, but no interaction. For the CoA there was a main effect of strain of male $F(1, 16)= 4.6, P=0.04$, but no effect of pacing or interaction. For the aLHA there was a main effect of strain of male $F(1, 16)= 6.33, P=0.02$, but no effect of pacing or interaction. For the BNST there was a main effect of strain of male $F(1, 16)= 6.51, p=0.02$, but no main effect of pacing or interaction.

Finally, no differences were detected between the groups in Fos induction in the ACC, NAcc, NAcSh, CPu, ArcN, and BLA.

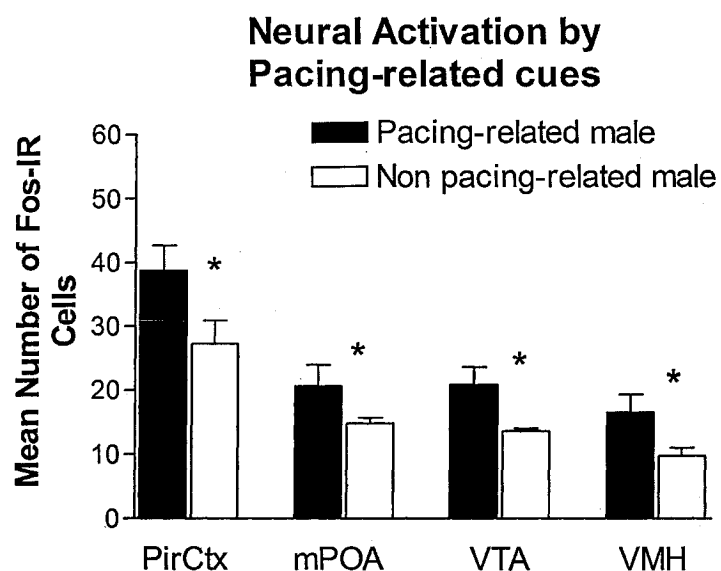


Figure 12. Brain areas of Wistar female rats that expressed significant differences in Fos-IR following exposure to a strain of male associated with paced- or nonpaced-copulation respectively. Regardless of the strain, males associated with paced copulation induced more Fos-IR than males associated with nonpaced copulation. PirCtx, Piriform cortex; mPOA, medial preoptic area; VTA, ventral tegmental area; VMH, ventromedial hypothalamus. The bars represent the mean number + SEM of Fos-IR cells within a sample area of the same dimensions for all the regions. * $P < 0.05$.

Neural Activation by Strain-related cues

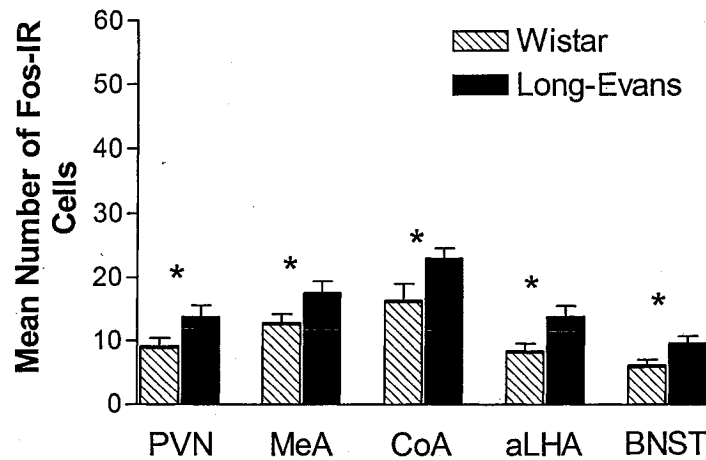


Figure 13. Brain areas of Wistar female rats that expressed significant differences in Fos-IR following exposure to a Wistar or Long-Evans male associated with paced- or nonpaced copulation. Overall, exposure to Long-Evans males induced more Fos-IR than exposure to Wistar males. PVN, paraventricular nucleus; MeA, medial amygdala; CoA, cortical amygdala; aLHA, anterior lateral hypothalamus; BNST, bed nucleus of the stria terminalis. The bars represent the mean number + SEM of Fos-IR cells within a sample area of the same dimensions for all the regions. * $P < 0.05$.

Area		Wistar-Pacing		Long-Evans-Pacing	
		Wistar	Long-Evans	Wistar	Long-Evans
Amygdala (Basolateral)		5.8 ± .1	6.2 ± 1.3	4.8 ± 1.7	6.4 ± 2
Amygdala (Cortical)	*	19.8 ± 3.4	22.6 ± 2.4	12.2 ± 3.5	23.7 ± 1.8
Amygdala (Medial)	*	15.8 ± 1.5	16.9 ± 2.22	9.3 ± 1.4	19.1 ± 3.79
Arcuate Nucleus		28.6 ± 8	18.8 ± 3.5	18.6 ± 5.8	31 ± .31
Bed Nucleus of the Stria Terminalis	*	5.8 ± .82	8.9 ± .84	6.7 ± 1.6	11.2 ± 3.2
Caudate Putamen		10.1 ± 3	8.01 ± 1.2	5.7 ± .95	6.4 ± 1.5
Cingulate Cortex (anterior)		15 ± 2.9	10.17 ± 2.22	8.5 ± 1.37	10.92 ± 3.5
Lateral Hypothalamus (anterior portion)	*	9.2 ± 1.8	14.7 ± 2.2	7.4 ± .96	12.2 ± 2.1
Lateral septum		8.09 ± 1	9.6 ± 2.08	6.81 ± .51	6.45 ± .47
Medial Preoptic area	#	18.7 ± 3.9	15.3 ± 1.2	14.2 ± 1.4	24.5 ± 6.8
Nucleus Accumbens Core		13.7 ± 3.9	7.77 ± .87	4.9 ± .65	7.4 ± .7
Nucleus Accumbens Shell		8.9 ± 2.7	7.67 ± 1.4	4.6 ± .64	5.7 ± 1.03
Olfactory tubercle		26.3 ± 3.3	26.0 ± 3.06	24.2 ± 2.7	21.6 ± 3.5
Paraventricular Nucleus	*	9.9 ± 1.9	12.8 ± 1.84	8.2 ± 1.9	15.9 ± 4.7
Piriform Cortex	#	40.6 ± 5.7	34.8 ± 5.3	20 ± 4.8	35.1 ± 4.3
Ventral Tegmental Area	#	21.7 ± 4.1	14.2 ± .43	13.2 ± .57	19.2 ± 1.19
Ventromedial Hypothalamus	#	17.8 ± 4.15	11.68 ± 1.92	7.6 ± .99	13.9 ± 2.9

Table 4. Brain areas of female rats that expressed Fos-IR following exposure to a Wistar or Long-Evans male, associated with paced- or nonpaced copulation. In the Wistar-Pacing group, females were trained to associate a Wistar male with paced copulation and a Long-Evans male with nonpaced copulation. In the Long-Evans-Pacing group, females received the opposite training. Table shows mean number + SEM. The mean number of Fos-IR cells were taken from a sample area of the same dimensions for all the regions * = main effect of strain of male. # = main effect of pacing. $P < 0.05$

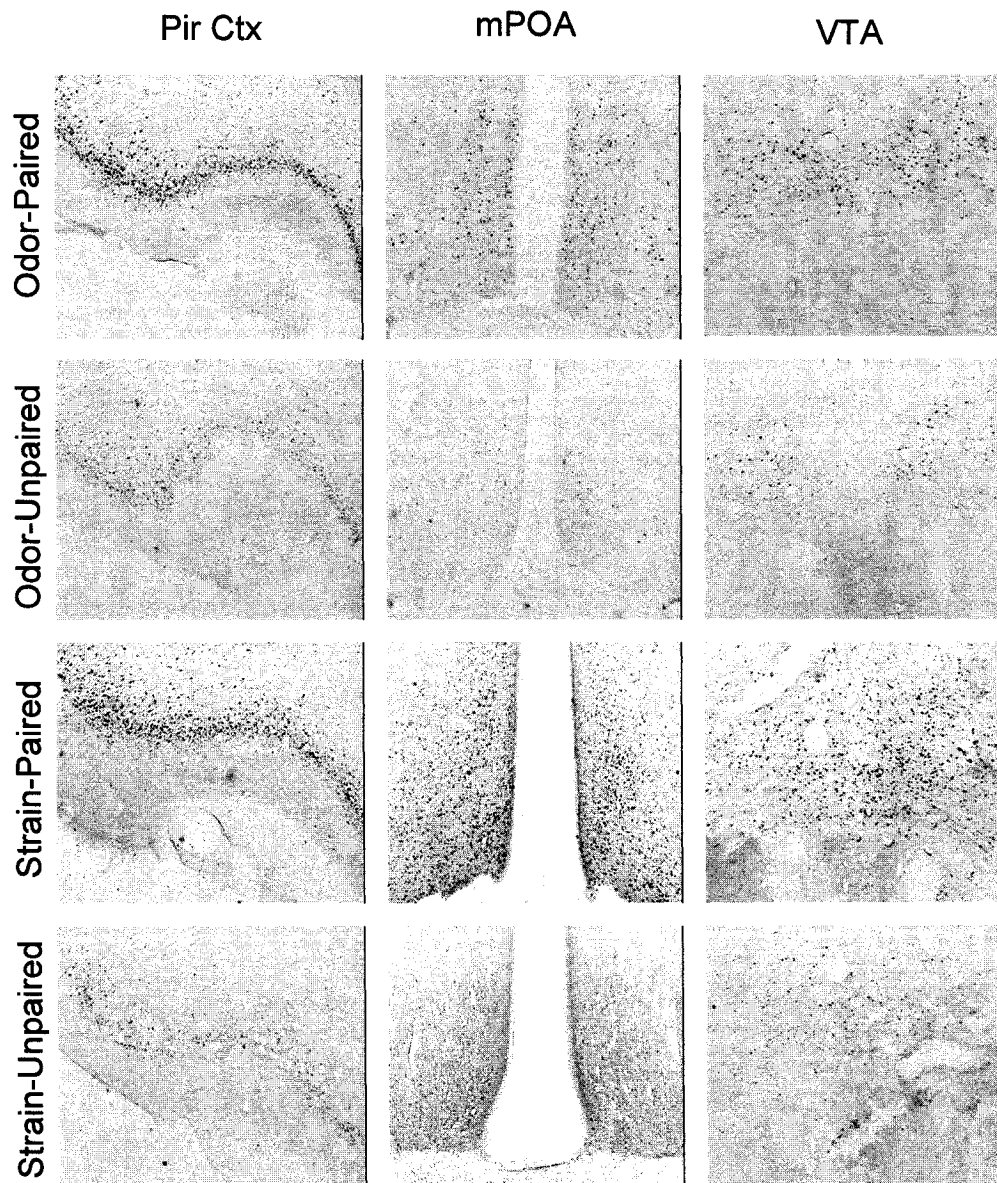


Figure14. Common brain areas that express significantly more Fos-IR in female rats exposed to conditioned odors or strains of male associated with paced (Paired) or nonpaced (Unpaired) copulation. Pir Ctx, piriform cortex; mPOA, medial preoptic area; VTA, ventral tegmental area.

DISCUSSION

We have reported previously that neutral odors or strain cues paired with paced copulation can become conditioned stimuli (CSs) that predict sexual reward in female rats, and that lead to the development of a conditioned partner preference for males possessing those characteristics (Coria-Avila et al., 2005; Coria-Avila et al., 2006). The present study shows that exposure to the CS generates greater Fos-IR in brain areas associated with olfactory processing, appetitive motivation, and reward-related learning, in Paired versus Unpaired females. In Experiment 1, an almond odor associated with pacing induced more Fos-IR in the Tu, Pir Ctx, ACC, NAc core, mPOA, LS, PVN, and VTA, and there was a trend for significance in the CPu and ArcN. These data suggest that those areas are involved in the development and/or expression of olfactory conditioned partner preference. In Experiment 2, the strain of male associated with paced copulation induced more Fos-IR in the Pir Ctx, the mPOA, the VTA and the VMH. The results of Experiment 1 and 2 indicate that there at least three common neural areas that express Fos-IR as a main effect of exposure to stimuli associated with pacing: Pir Ctx, mPOA, and the VTA. In addition to these regions, the activation of the VMH, and lack of activation in the NAc and LS, by strain cues associated with pacing is consistent with the Fos activation patterns in monogamous Prairie vole females during their first copulatory contact with a male (Curtis & Wang, 2003; Cushing, Yamamoto, Hoffman, & Carter, 2003). Thus the differential effects of olfactory and strain conditioning noted above may result from a different pattern of activation in main and accessory olfactory pathways.

In Experiment 2, Fos was induced in the BNST, PVN, MePD, CoA, and aLHA of W females as a function of exposure to LE males relative to W males. We have shown

previously that if the strain of male associated with sexual reward is the same as the female subject, stronger conditioning emerges (Coria-Avila et al., 2006). For example, W females that associate W males with paced copulation not only solicit the W male more than the LE male, but also are more likely to choose the W male to receive their first ejaculation. Although W females solicit the LE male significantly more than the W male if LE is paired with paced copulation, they do not display a significant ejaculatory preference toward the LE male. However, W females display significantly more anogenital investigation of LE males in general, suggesting that elements of the accessory, rather than main, olfactory pathway are being activated. This is consistent with the activation of Fos in secondary and tertiary terminals of the accessory olfactory pathway, including the BNST and MePD, by exposure to LE males regardless of conditioning history in the present study.

Both olfactory and strain cues associated with paced copulation induced similar activation of the PirCtx, mPOA, and VTA. These three regions form an interconnected circuit that is well-suited to translate olfactory and genitosensory information into motivated behavioral responses by way of outputs through the VTA and ventral pallidum. All three regions are activated by VCS (Pfaus et al., 1993; Pfaus et al., 1996), and receive inputs from both main and accessory olfactory bulbs (Coolen & Wood, 1998; Rosin, Datiche, & Cattarelli, 1999; Swanson, 1976; Wilson, 2001). The differential effect of olfactory versus strain conditioning may be determined in part by multiple outputs to the VTA, especially from the mPOA in the case of olfactory conditioning (Brackett & Edwards, 1984; Swanson, 1976), and additional outputs from the BNST that may modulate those of the mPOA in the case of strain conditioning (Holstege, Meiners, &

Tan, 1985). Given the relatively equivalent activation of the PirCtx, mPOA, and VTA in the two experiments, it is unlikely that the differences observed in some brain regions between the two conditions, such as ACC, NAc core, VMH, PVN, and ArcN, were due to the use of different strains of female in Experiment 1 (LE), and Experiment 2 (W). In our previous studies, no differences were observed between LE and W females in the number of solicitations or time spent with the pacing-related male as a function of olfactory or strain conditioning (Coria-Avila et al., 2005; Coria-Avila et al., 2006), suggesting that females of both strains process CSs that predict sexual reward in a similar manner.

Systemic administration of the dopamine antagonist flupenthixol during training blocked olfactory conditioning but not conditioning by strain (Coria-Avila et al., in preparation, Chapter 3, Part 2). Similarly, in the present study the odor induced significantly more Fos in the NAc core of Paired versus Unpaired rats, but no differences were detected in Fos induction in this region following exposure to strain cues. This suggests that mesolimbic dopamine transmission may be critical for olfactory conditioning, which involves a truly Pavlovian contingency between a neutral stimulus and a rewarding UCS, relative to an already biased CS (strain cues that naturally activate the accessory olfactory pathway) and the rewarding UCS. Previous studies have shown a modest increase in extracellular concentrations of NAc dopamine when female rats are presented with a sexually vigorous male behind a screen (Pfaus, Damsma, Wenkstern, & Fibiger, 1995) whereas much higher concentrations are reported during copulation in those studies, and especially in situations where females are allowed to pace their copulatory interactions (Mermelstein & Becker, 1995). In the latter study, significant extracellular dopamine concentrations were also reported in the CPu, and we note

significant effects of the conditioned odor on Fos induction in the CPu in the present study. In contrast, systemic administration of the opioid antagonist naloxone to female rats during training blocks the development a conditioned partner preference for both odor or strain (Coria-Avila et al., submitted, Chapter 3, part 1). Although it is not known where the inhibition of opioid receptors may inhibit the development of conditioned partner preference, there is evidence to suggest it may occur in the mPOA.

Role of the PirCtx

The PirCtx constitutes primary olfactory cortex, and its activation may be critical for learning the odor- and strain-conditioned partner preference. In addition to receiving direct inputs from the main olfactory bulb, the PirCtx is reciprocally connected to other brain regions, including the entorhinal cortex, amygdala, thalamus, and NAc (Kowianski, Lipowska, & Morys, 1999), and thus can be activated indirectly by the accessory olfactory system. Accordingly, the information that represents the incentive value of a conditioned odor or strain cue may be transmitted to limbic areas that will mediate sexual motivation. The PirCtx has been heavily investigated for its role in olfactory discrimination, and several studies have observed synaptic and neuronal alterations in PirCtx associated with learning olfactory discrimination tasks, or tasks in which electrical stimulation of the olfactory inputs is used as a discriminative stimulus for positive reward (Datiche, Roullet, & Cattarelli, 2001; Roman, Staubli, & Lynch, 1987; Roman, Chaillan, & Soumireu-Mourat, 1993; Saar, Grossman, & Barkai, 1999). Piriform cell firing in an 8-odor discrimination task is also influenced by whether odors were associated with reward (Schoenbaum & Eichenbaum, 1995). Fos is elevated in the PirCtx of male rats exposed to almond-scented bedding previously paired with copulation to ejaculation, but not by

unconditionally rewarding estrus odors (Kippin et al., 2003) or in response to the almond odor unpaired with copulation. The PirCtx may therefore be part of a distinct neural pathway for the learning of contingencies between neutral odors and sexual reward, and sends that information to the rest of the limbic system. Although it is unclear what olfactory or other stimuli the females were responding to during strain conditioning, it is clear that they can easily distinguish pigmented LE males from albino W males at a distance (Coria-Avila et al., 2006), suggesting that olfactory and/or pheromonal differences between the strains of male were salient.

Role of the mPOA

The mPOA receives inputs from both the primary and accessory olfactory systems (Shipley & Ennis, 1996). The number of neurons expressing Fos, along with extracellular dopamine concentrations, are increased in the mPOA of male or female rats by sex-related olfactory stimuli, along with genitosensory stimuli that may travel along the spinohypothalamic pathway in females via the pelvic and hypogastric nerves (Baum & Everitt, 1992; Blackburn et al., 1992; Cliffer, Burstein, & Giesler, Jr., 1991; Hull & Dominguez, 2006; Paredes, Lopez, & Baum, 1998; Pfaus, Manitt, & Coopersmith, 2006; Wersinger et al., 1993; Xiao, Kondo, & Sakuma, 2005). Lesions of the mPOA abolish appetitive solicitations and hops and darts (Hoshina, Takeo, Nakano, Sato, & Sakuma, 1994; Whitney, 1986), but can increase lordosis in OVX, steroid-primed rats during paced or nonpaced copulation (Whitney, 1986; Xiao et al., 2005). The mPOA also receives inputs from the ArcN (Zaborszky & Makara, 1979), a significant proportion of which contain opioids or melanocortins (Chronwall, 1985; Eskay, Giraud, Oliver, & Brown-Stein, 1979; Mills, Sohn, & Micevych, 2004; Piekut & Knigge, 1984; Roselli-

Rehfuss et al., 1993). Dopamine release in the mPOA increases during copulation (Matuszewich, Lorrain, & Hull, 2000), and the mPOA contains populations of neurons that increase their firing rate when female rats solicit sex; these neurons are inhibited by dopamine antagonists such as pimozide, which also inhibit solicitations (Kato et al., 2000). Indeed, the mPOA is an important site of action for melanocortin and dopamine stimulation of solicitation (Gelez, et al., submitted). Given that treatment with the opioid antagonist naloxone during training also reduces solicitations and hops and darts significantly (Coria-Avila et al., submitted; Chapter 3 Part 1), and that melanocortin actions stimulate solicitations, the mPOA could be a critical site that integrates the vaginocervical and olfactory stimulation received during paced copulation with opioid reward and melanocortin actions that trigger solicitations toward the preferred male on subsequent trials. In the present study, the conditioned odor and the strain cues activated a cluster of Fos in and around the median nucleus of the mPOA. A nearly identical distribution of Fos-positive neurons was activated by systemic administration of the melanocortin agonist bremelanotide (Gelez, et al. submitted), suggesting that a common population of melanocortin-sensitive neurons in the mPOA may be critical for solicitation. Efferent projections of the mPOA to the cingulate and PirCtx (Gaykema, Luiten, Nyakas, & Traber, 1990), and to the VTA (Brackett et al., 1984), may be critical for the expression of conditioned solicitation.

Role of the VTA

The medial VTA is the site of dopamine cell bodies that project throughout the limbic system (Fallon & Moore, 1978; Phillipson, 1979), including regions of the NAc, Tu, and ACC. The mesolimbic dopamine system is thought to mediate attention toward

reward-related stimuli, and in particular the incentive salience of stimuli (Berridge, 2006; Robinson et al., 1993), whether they relate to reward, punishment, or general arousal (Horvitz, 2000; Insel, 2003). Although the dopamine system that projects to the mPOA arises from a different population of cell bodies in the zona incerta (Bjorklund, Lindvall, & Nobin, 1975), nearly identical patterns of dopamine release during copulation have been found in the mPOA and NAc of male rats (Blackburn et al., 1992) and female rats (Matuszewich et al., 2000; Mermelstein et al., 1995; Pfaus et al., 1995). Outputs from the mPOA to the VTA may link incertohypothalamic and mesolimbic dopamine turnover to coordinate goal-directed behaviors with the hypothalamic control of sympathetic and parasympathetic activation during sexual behavior (Dominguez & Hull, 2005). In Prairie voles, blockade of oxytocin or dopamine D2 receptors in the NAc blocks copulation-induced partner preference (Liu & Wang, 2003; Young, Lim, Gingrich, & Insel, 2001). However, activation of D1 dopamine receptors in the NAc blocks partner preferences, and these receptors are upregulated in male Prairie voles that bond after their initial copulatory experiences (Aragona et al., 2006), suggesting a mechanism for turning off plasticity once the bond has been established.

Other regions

Odor conditioning in Experiment 1 induced Fos in several cortical, limbic, motor, and hypothalamic regions. Some of those regions, such as the NAc, PVN and LS, have also been implicated in pair bonding in monogamous voles (Young et al., 2004; Young, Murphy Young, & Hammock, 2005), whereas others, such as the ACC, are involved in discrimination of conditioned stimuli and tagging their emotional meaning (Bussey, Everitt, & Robbins, 1997; Cardinal, Parkinson, Hall, & Everitt, 2002). Interestingly, these

regions did not display significant Fos induction in Experiment 2 following exposure to strain cues associated with paced copulation. It is possible that activation of accessory olfactory inputs from the strain cues alone are sufficient to stimulate motor and autonomic outputs without significant processing, and that their association with sexual reward in regions such as the mPOA is prepotent. It may well be that a truly neutral stimulus requires more activation of attentional mechanisms, such as mesolimbic dopamine release, to solidify the CS-UCS relationship between stimulation and reward. Indeed, Hernandez et al., (2006) have shown that low trains of electrical stimulation of the medial forebrain bundle in rats result in sustained dopamine release in the NAc, whereas dopamine release is transient during higher trains of stimulation. The rewarding impact of the stimulation was reduced if high trains were administered prior to testing, relative to low trains, suggesting that stimuli of high unconditional incentive value diminish the need for sustained dopamine release. Thus, in Experiment 2 strain cues may have been of sufficient intensity to bypass the activation of associative mechanisms required to link neutral olfactory stimulation to sexual reward. Such a “dual system” has been found previously in male rats exposed to estrous vaginal secretions relative to the same almond odor associated with copulation to ejaculation (Kippin et al., 2003). Although the pattern of Fos activation throughout the forebrain and midbrain was different following exposure to vaginal secretions or the conditioned odor, comparable Fos activation was found in the NAc regardless of the stimuli. However, in that study, odors and vaginal secretions were presented in bedding rather than on gauze as in the present study. It is not clear whether the odor or pheromonal intensity was similar between the two presentation methods, or whether the different activation patterns

between our former and present studies reflect sex differences in sexual reward intensity or the way odor or strain cues are linked to sexual reward.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP and a postgraduate fellowship from CONACyT of Mexico (128099) to GAC. The authors would like to thank Drs. Brandon Aragona, Mary Erskine, Tod Kippin, Zuo-Xin Wang, and Larry Young, for valuable discussions.

Summary of Chapter 2

Chapter 2 examined the neuroanatomy of conditioned partner preference in female rats induced by odor or strain cues associated with paced copulation. Fos was activated in cortical, limbic, motor, and hypothalamic regions by the conditioned odor, whereas it was activated largely in terminal fields of the accessory olfactory pathway following exposure to strain cues. However, three regions, the PirCtx, mPOA, and VTA, were activated commonly by odor or strain cues, suggesting a preferential involvement in associating salient external cues with sexual reward. Given the nature of these areas, it was suggested that part of the neurochemical mechanisms responsible for sexual reward and conditioned partner preference may depend on its neurotransmitters. The involvement of two important neurotransmitter systems, opioid and dopamine, in the establishment of conditioned partner preference by odor or strain cues were examined in Chapter 3.

CHAPTER THREE

PART 1

Neurochemical Basis of Conditioned Partner Preference in the Female Rat:

I. Disruption by Naloxone

Genaro A. Coria-Avila, Carrie E. Solomon, Erica Barbosa-Vargas, Ivana Lemme, Richard Ryan,

Shann Ménard, Alex M. Gavrilu, James G. Pfaus

Concordia University

RUNNING HEAD: OPIOIDS AND CONDITIONED PARTNER PREFERENCE

Center for Studies in Behavioral Neurobiology, Department of Psychology,
Concordia University, Montréal, QC.

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP and a postgraduate fellowship from CONACyT of Mexico (128099) to GAC. The authors would like to thank Drs. Mary Erskine, Tod Kippin and Raúl Paredes for useful discussions. All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

Correspondence concerning this article should be addressed to Genaro A. Coria-Avila, D.V.M., M.Sc., CSBN-Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC, H4B 1R6, CANADA. E-mail: coria75@yahoo.com.

For submission to *Behavioral Neuroscience*

Abstract

The effects of the opioid antagonist naloxone were examined on the development of conditioned partner preference induced by paced copulation in female rats. In Experiment 1, ovariectomized, hormone-primed rats were conditioned to associate scented and unscented males with paced and nonpaced copulation respectively. Females in Experiment 2 associated albino or pigmented males with paced or nonpaced copulation. Naloxone or saline was administered prior to each conditioning trial. During a final drug-free preference test, females could choose to copulate with either a pacing related or unrelated male. Saline-trained females in the paired group copulated preferentially with the pacing-related male whereas naloxone-trained females did not show a preference. We conclude that opioids mediate the conditioned partner preference induced by paced copulation.

Keywords: Paced copulation, Odors, Sexual reward, Conditioning, Sexual behavior, Wistar, Long-Evans, Opioids

Introduction

Different types of environmental stimuli can become sexually conditioned stimuli (CSs) if they are associated in contingency with rewarding sexual experiences. Accordingly, CSs can become predictors of reward during sexual encounters and increase appetitive sexual behaviors, indicative of sexual desire (Pfaus et al., 2001; Pfaus et al., 2003). In male rats for example, it has been shown that the state of reward that follows ejaculation, is of sufficient intensity to induce conditioned place preference (CPP) (Ågmo et al., 1990). Specifically, males learn to prefer one distinctive side of a CPP box if it was previously associated with the post-ejaculatory reward state. The underlying process of this conditioning is poorly understood, but pharmacological studies have shed light on the role of different substances involved. For example, systemic injections of the opioids antagonist naloxone effectively block such CPP (Ågmo et al., 1990), demonstrating that opioids form part of the neurochemical substrate that mediates the conditioning process that occurs following sexual reward induced by ejaculation.

In female rats, a similar conditioning process occurs following paced copulation. Specifically, when females control or “pace” the rate of sexual interaction with approaches or withdrawals from the males (Erskine et al., 1989; Pfaus et al., 1999) females develop CPP for distinctive sides associated with paced copulation (Paredes et al., 1997; Paredes et al., 1999). This copulatory CPP in females is also disrupted by administration of naloxone (Paredes et al., 2001), indicating that the rewarding properties induced by paced copulation depend on endogenous opioid turnover, similar to the rewarding effects of ejaculation in males.

The rewarding effects of copulation can also induce conditioned partner preferences towards individuals that bear CSs predictive of sexual reward. For example, male rats can display a conditioned ejaculatory preference (CEP) towards almond-scented females when the almond odor predicts sexual receptivity; but it does not occur when the odor predicts no receptivity (Kippin et al., 2001c; Kippin et al., 2001a; Kippin et al., 2001b). In the case of females, we have demonstrated that they can display a conditioned partner preference towards males that bear an odor associated with paced copulation relative to males associated with nonpaced copulation (Coria-Avila et al., 2005). Females in the paired group copulated with an almond-scented male in a pacing chamber, which was divided by a Plexiglas partition that had 4 small holes in the bottom to allow the females exclusively to pass freely from one side to the other. Four days later, the females copulated with an unscented male in the same chambers but with the partitions removed (nonpaced). These two conditions were alternated for a total of 8 conditioning trials (4 in each condition). Four days after the final conditioning trial, females were tested for a conditioned partner preference in an open field with two tethered males, one scented and the other unscented. This procedure allowed the females to choose freely between the two males. Females in the paired group displayed significantly more solicitations, hops and darts, and high magnitude lordosis with the scented male, from whom they also chose to receive more mounts and their first ejaculation. In contrast, the preference toward scented males was not observed in the unpaired group, in which the scented males were associated with nonpaced copulation, or in a randomly paired group, in which scented and unscented males were randomly paired with paced copulation.

Female rats can also develop a conditioned partner preference for strain cues that differentiate an albino male from a pigmented male (Coria-Avila et al., 2006). In that study, Wistar (W) and Long-Evans (LE) females were conditioned to associate one of these two strains of males with paced copulation and the other with nonpaced copulation. Following 10 conditioning trials (5 in each condition), females were tested for conditioned partner preference with one W and one LE male tethered in opposite corners of an open field. Regardless of the strain, females displayed more solicitations towards the pacing-related male and chose him to receive the first ejaculation. Thus, despite the fact that the mating strategy of rats has been described as polygamous, (McClintock et al., 1982; McClintock, 1984a), both male and female rats show conditioned partner preferences based on CSs associated with sexual reward. This conditioned partner preference may be considered a rudiment of the pair bonding behavior observed in other species of rodents and in humans.

Given evidence that opioids are part of the underlying reward mechanism that facilitates CPP in female rats following paced copulation; the present study attempted to clarify the role of opioids in the development of conditioned partner preference. We hypothesized that blockade of opioid receptors with the antagonist naloxone during the conditioning process would disrupt the rewarding properties of paced copulation. Thus, in the absence of sexual reward, the development of conditioned partner preference would not occur.

General Methods

Subjects and Surgery

Long-Evans male (300-350 g) and female (200-250 g) rats were purchased from Charles River Canada (St-Constant, QC). They were housed in groups of 4 in large hanging wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Food and water were continuously available. The females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then ovariectomized bilaterally via a lumbar incision. Following ovariectomy females were given daily subcutaneous injections of Flunixin meglumine 2.5 mg/kg (Banamine ®) for analgesia, and Enrofloxacin 5 mg/kg (Baytril ®) during three days to prevent post surgical bacterial infections. All females were given a week of post-surgical recovery prior to conditioning trials.

Gonadally intact Long-Evans male rats that served as stimulus males had at least 10 tests of sexual behavior in bi-level chambers prior to the start of these experiments. These males were sexually vigorous and initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test. If scented, males received almond extract (Blue Ribbon, Etobicoke, ON) applied to the back of their necks and anogenital regions with a cotton swab. Unscented males received similar applications of distilled water to the same areas.

Drugs

Naloxone hydrochloride (Sigma, St. Louis, MO, USA), a general opioid receptor antagonist, was dissolved in 0.9 % physiological saline and injected intraperitoneally in a dose of 4 mg/kg in a volume of 1 ml/kg, 1 min before every conditioning trial (as in (Paredes et al., 2001). Control animals were injected with physiological saline 1 min before testing.

Experiment 1

The first experiment tested the effect of injections of naloxone vs. saline on the development of olfactory conditioned partner preference induced by paced copulation with scented males, as in the paired group reported by Coria-Avila et al. (2005). In this experiment only Long-Evans females and males were used.

Method

Odor conditioning

Conditioning trials occurred every 4 days in semicircular chambers (38² cm X 60 cm X 38 cm). For paced copulation, the chambers were divided by a middle Plexiglas partition with four equidistant holes cut into the bottom (4 cm X 4 cm) resting on bedding. The holes were large enough for the female to pass through, but too small for the males. Nonpaced copulation occurred in the same chambers but without the partition. Females copulated with scented males in the pacing condition, and four days later with unscented males in the nonpacing condition. If scented, almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) was applied on the back of the neck and anogenital area of

the males with a cotton swab. Females received a total of 10 conditioning trials (5 paced and 5 nonpaced). The order of conditioning was counterbalanced, such that half of the rats in every condition had their first sexual experience in a paced situation and the other half nonpaced. During each conditioning trial, experimental females were injected with naloxone 1 min before the start of the session, and females in the control group were injected with saline. They were allowed to copulate for 30 min before being returned to their home cages. All tests with scented and unscented males took place in different rooms to prevent noncontingent odor associations.

Copulatory preference test

Four days after the final conditioning trial, females were placed into a large open field (123 cm X 123 cm X 46 cm) with a thin layer of wood-chip bedding. At two diagonal corners there were two males unfamiliar to the female, one scented and one unscented. Each male wore a rodent jacket connected on the back to a spring wire 30 cm in length, which allowed the males to roam freely in a radius of approximately 45 cm. For this final partner preference test females in the experimental and control group were injected exclusively with saline, so that all behavioral differences between naloxone- and saline-trained rats were the result of the conditioning process and not because of any drug effect during the final test. Females were allowed to copulate freely with both males during the 30-min test.

Conditioned partner preference was determined as in Coria-Avila et al. (2005, 2006) by latency and frequency measures of female visits, olfactory investigations, solicitations (described as a headwise orientation of the female to the male, followed by a runaway), hops and darts (H&D), and lordosis magnitudes (on a scale of 1 to 3, with 1

representing low magnitude and 3 high magnitude) during interactions with each male. In addition we assessed the male chosen for the first ejaculation, as well as the frequency of mounts, intromissions, and ejaculations during the final preference test.

Statistical analysis

A 2 X 2 (odor X drug) mixed design ANOVA was used to determine differences in frequencies and latencies. Chi-square analyses were used to determine the differences in proportion of males chosen for first mount, intromission, and ejaculation. All significant main effects in the ANOVAs were followed by a Tukey Least Significant Difference (LSD) post hoc test to assess differences between individual means. The level of significance for all tests was $p < 0.05$.

Results

Of the total of 39 females originally conditioned, 8 were eliminated from the statistical analysis because one or both males became free from their tethered jackets during the final preference test. This left 31 females ($n=17$ in the control group; $n=14$ in the naloxone-treated group). Table 1 shows means + standard errors for all the behaviors assessed during the 30-min test for saline- vs. naloxone-treated females.

Visits and olfactory investigations. With regard to the first visit latency, the ANOVA detected no main effects of drug $F(1, 29) = .69, p = 0.41$, odor $F(1, 29) = .65, p = 0.42$, or interaction of drug and odor $F(1, 29) = .28, p = 0.59$, indicating that females in both groups performed their first visit with approximately the same latency towards scented or unscented males. The analysis of visit frequencies did not reveal any main effect of odor $F(1, 29) = 0.0002, p = 0.98$, or drug $F(1, 29) = .48, p = 0.49$, indicating

than the odor or the naloxone alone did not affect the number of visits towards both males. However, an interaction of odor and drug was found, $F(1, 29) = 6.90, p = 0.01$. The post hoc analysis revealed differences only within the naloxone-treated females, indicating that females in this group visited the scented males fewer times than the unscented males. With regard to the number of olfactory investigations, the ANOVA failed to detect a main effect of odor $F(1, 29) = .04, p = 0.83$, or drug $F(1, 29) = 1.2, p = 0.26$, but detected an interaction between the two $F(1, 29) = 5.05, p = 0.03$. The post hoc analysis revealed that saline-treated females displayed more olfactory investigation towards scented males than towards unscented males. There were no main effects of drug or odor on first olfactory investigation latency. Together, these results indicate that naloxone-treated females visited fewer times the pacing-related male, and that saline-treated females performed more olfactory investigations of pacing-related males.

Solicitations and hops & darts. With regard to solicitation frequencies there was a main effect of drug $F(1, 29) = 5.8, p = 0.02$, and an interaction between drug and odor $F(1, 29) = 4.7, p = 0.03$, but there were no effects of odor alone $F(1, 29) = .000, p = 0.99$. The post hoc analysis revealed a significant difference in the number of solicitations between scented males in the saline and naloxone group, but failed to detect differences within groups. With regard to the latency for the first solicitation, the ANOVA detected a main effect of drug $F(1, 29) = 9.4, p = 0.004$. The post hoc analysis revealed that saline-treated females performed their first solicitation significantly faster than naloxone-treated females. There was no main effect of odor or interaction between odor and drug with regard to first solicitation latency.

With regard to frequencies of H&D, the ANOVA detected a main effect of drug $F(1, 29) = 4.6, p = 0.03$, and an interaction between drug and odor $F(1, 29) = 7.9, p = 0.008$, but no main effect of odor alone $F(1, 29) = 3.5, p = 0.07$. The post hoc analysis revealed that saline-treated females performed more H&D towards scented males than towards unscented males. This difference was not detected in the naloxone-treated females. The analysis of the first H&D latency did not show main effects of drug $F(1, 29) = .57, p = 0.45$, odor $F(1, 29) = .31, p = 0.58$, or interaction between the two $F(1, 29) = 1.07, p = 0.30$, indicating that the first H&D occurred at approximately the same time for all females, regardless of the group or the status of the male. Overall, the analysis of solicitations and H&D indicated that the saline-trained females performed more of these proceptive behaviors towards pacing-related males, whereas naloxone-trained females did not.

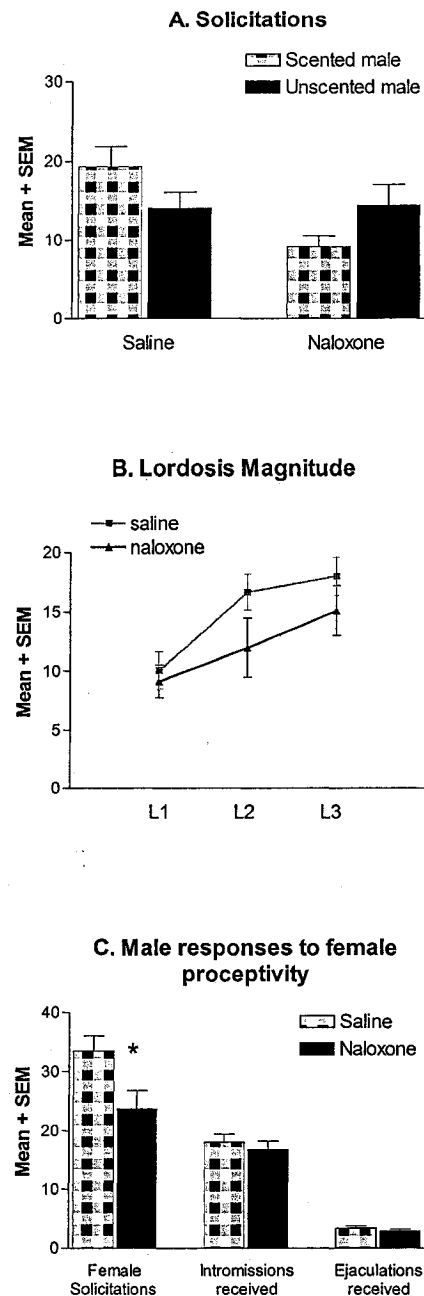


Figure 15 A: Number of solicitations during 30-min test in two groups: saline ($n = 17$) and naloxone ($n = 14$). Scented males associated with paced copulation, and unscented males associated with nonpaced copulation. B: Lordosis magnitude of saline- and naloxone-treated females. C: Total number of solicitations of saline- and naloxone-treated females and the number of intromissions and ejaculations received. See table 1 for details of behavior. * $p < .05$

Lordosis. The analysis of frequencies for lordosis magnitude 1 indicated a main effect of odor $F(1, 29) = 5.9, p = 0.02$, but there were no main effects of drug $F(1, 29) = .18, p = 0.67$ or interaction between drug and odor $F(1, 29) = .01, p = 0.90$. The post hoc analysis failed to show significant differences within or between groups. The analysis of lordosis magnitude 2 showed a significant interaction between drug and odor $F(1, 29) = 7.6, p = 0.009$, but there were no main effects of drug or odor alone. The post hoc analysis indicated that saline-treated females displayed more lordosis magnitude 2 towards scented males relative to unscented males within group, and relative to scented in the naloxone group. The analysis of lordosis magnitude 3 indicated a main effect of odor $F(1, 29) = 5.4, p = 0.02$, and a trend for an interaction between drug and odor $F(1, 29) = 3.13, p = 0.08$. The post hoc analysis revealed that naloxone-treated females displayed significantly fewer lordosis magnitude 3 towards scented males than towards unscented males, and also relative to scented and unscented males in the saline-trained group.

Choice of male for ejaculation and male responses. Of the 17 females in the saline group, 5 (30 %) received the first ejaculation from the scented male, and 12 females (70 %) from the unscented male. Chi-square analyses detected a trend toward significance in these proportions $\chi^2(1, N=17) = 2.8, p = 0.09$. Of the total of 14 females in the naloxone-treated group, 6 (43 %) received their first ejaculation from scented males, and 8 (57 %) from unscented males. Chi square analysis failed to detect any significant difference in these proportions $\chi^2(1, N=14) = .28, p = 0.59$. There were significant differences in frequencies and latencies of male sexual behavior. With regard to mount frequencies, the ANOVA detected an interaction between drug and odor $F(1, 29) = 5.2, p = 0.02$. The post hoc analysis indicated that naloxone-treated females

received fewer mounts from scented males than from unscented males, and fewer compared to scented males in the saline group. The analysis of the latency to the first mount showed a main effect of drug $F(1, 23) = 6.1, p = 0.02$, odor $F(1, 23) = 9.7, p = 0.004$, and an interaction of drug and odor $F(1, 23) = 6.7, p = 0.01$. The post hoc test indicated scented males in the naloxone treated group had a higher latency relative to unscented males in the same group, and toward scented and unscented males in the saline group. There were no significant differences in the latency or frequency for intromissions and ejaculations.

Conditioned Partner Preference by Olfactory Cues	Odor-Pacing group SALINE		Odor-Pacing group NALOXONE	
	Scented	Unscented	Scented	Unscented
1 st visit latency (sec)	11.8 ± 2.7	16.7 ± 3.0	18.9 ± 5.7	19.9 ± 8.2
Visits frequency	46 ± 2.7	41.5 ± 2.3	40 ± 2.5 *	47 ± 3.8
1 st Olfactory investigation latency (sec)	35 ± 9.3	35 ± 6.4	23.6 ± 6.5	30.9 ± 9
Olfactory investigation frequency	32.8 ± 2.6 *	25 ± 1.4	28.2 ± 2.4	30.8 ± 4.2
1 st hop and dart latency (sec)	34 ± 6.3	74 ± 35	85 ± 34	73 ± 31
Hops and darts	42.9 ± 5.3 *	24.5 ± 2.5	24.3 ± 1.9 #	28 ± 3.4
1 st solicitation latency (sec)	88.4 ± 21	122 ± 27	369 ± 76 #	301 ± 115 #
Solicitation frequency	19.4 ± 2.5	14.1 ± 2	9.2 ± 1.4 #	14.5 ± 2.6
1 st Mount latency (sec)	68 ± 22	47.5 ± 11.4	239 ± 72 * #	63.9 ± 26
Mount frequency	13.1 ± 2	10.6 ± 1.9	5 ± 1.2 * #	12 ± 2.5
1 st Intromission latency (sec)	333 ± 149	82 ± 20	95 ± 30	191 ± 97
Intromission frequency	8.5 ± 1.2	9.5 ± .81	6.1 ± 1.4	10.7 ± 1.2
1 st Ejaculation latency (sec)	528 ± 110	406 ± 64	422 ± 115	519 ± 95
Ejaculation frequency	1.3 ± .3	2.05 ± .23	1.2 ± .33	1.7 ± .24
Choice of male for First ejaculation	5 (30 %)	12 (70 %)	6 (43 %)	8 (57 %)
Lordosis magnitude 1	3.8 ± .63	6.3 ± 1.4	3.2 ± .83	6 ± 1.3
Lordosis magnitude 2	10.2 ± 1.6 *	6.4 ± .9	4 ± 1.1 #	7.9 ± 1.8
Lordosis magnitude 3	8.6 ± 1.2	9.3 ± 1.1	5 ± 1.4 * #	10.1 ± 1.2

Table 5. Female sexual behavior during an olfactory conditioned partner preference test with two males, one scented and one unscented. Scented males were associated with paced copulation and unscented with nonpaced copulation. Two groups are shown, saline-treated (n= 17) and naloxone-treated (n= 14). Data are means (± SEM) during the 30-min preference test. Latencies are expressed in seconds. * $p < .05$ between scented and unscented males within groups. # $p < .05$ compared to control.

Experiment 2

This experiment examined the effects of naloxone on the development of conditioned partner preference for strain of male associated with paced copulation, as in Coria-Avila et al. (2006).

Method

Strain Conditioning

There were two counterbalanced conditioning groups. Females in the Wistar-pacing (W-P) group ($n=30$) copulated with Wistar males in the same paced condition as in Experiment 1 and with Long-Evans males in the same nonpaced condition as in Experiment 1. Females in the Long-Evans-pacing (LE-P) group ($n=30$) copulated with Long-Evans males in the paced condition and Wistar males in the nonpaced condition. Half of each group received saline as control and the other half received naloxone 1 min before every conditioning trial. This procedure led to 4 subgroups W-P + saline ($n=16$), LE-P + saline ($n=14$), W-P + naloxone ($n=16$) and LE-P + naloxone ($n=14$). Females received a total of 10 conditioning trials (5 paced and 5 nonpaced) of 30 min each before being taken back into their home cages.

Copulatory Preference Test

As in Experiment 1, four days after the 10th conditioning trial, a final test was carried out for each female into a large open field. At two opposite corners of this open field were two males, one Wistar and the other Long-Evans. All the females were injected exclusively with saline intraperitoneally one minute before being placed in one

neutral corner of the open field and were allowed to copulate freely with both males for a 30-min period. The same behavioral measures were assessed as in Experiment 1.

Statistical Analyses. A 2 X 2 X 2 (group X drug X strain of male) mixed design ANOVA was used to analyze differences in the frequencies and latencies of copulatory behaviors toward the two males. All significant main effects and interactions were followed by a Tukey Least Significant Difference (LSD) post hoc test to detect specific differences between group means.

Results

Visits and olfactory investigations. With regard to the first visit latency, the ANOVA detected an interaction between group, drug, and strain of male $F(3, 56) = 7.45$, $p = 0.008$. Posthoc analyses revealed that in the W-P + saline group, W males received their first visit with a longer latency than LE males within group, but no other group showed differences within group. The analysis of visits frequency detected a main effect of strain of male $F(3, 56) = 24.9$, $p = 0.000006$, and an interaction between strain of male and group $F(3, 56) = 12.47$, $p = 0.0008$. Posthoc analyses indicated that in the W-P + saline and W-P + naloxone groups, females visited both males with the same frequency. In the LE-P + saline and LE-P + naloxone females visited more the LE males, indicating that overall, females preferred to visit LE males exclusively when they were associated with paced copulation, but this preference in visits did not occur when W males were associated with pacing. With regard to the first olfactory investigation latency, the ANOVA showed no effects of drug, strain of male or interactions. The analysis of olfactory investigation frequencies detected a main effect of drug $F(3, 56) = 8.22$, $p =$

0.005, a main effect of strain of male $F(3, 56) = 6.3, p = 0.01$, and an interaction between group and strain of male $F(3, 56) = 4.3, p = 0.04$. Posthoc analyses indicated that naloxone-treated females displayed more olfactory investigations than saline-treated females, and that this effect was more robust towards LE males.

Solicitations and hops & darts. The ANOVA detected significant differences between the saline- and naloxone-treated females. With regard to solicitation frequencies there was a main effect of drug $F(3, 56) = 10.3, p = 0.002$, an interaction between group and strain of male $F(3, 56) = 15.7, p = 0.0002$, and an interaction between group, strain of male and drug $F(3, 56) = 5.6, p = 0.02$. Post hoc analyses revealed that in the W-P + saline group, females displayed more solicitations toward W males than LE males. In the LE-P + saline group, females displayed more solicitations toward LE than W males, indicating that overall females solicited more the pacing-related males. In the naloxone-treated females there were no differences in the amount of solicitations towards either strain of male. In addition, the amount of solicitations in all naloxone-treated females was not different from the amount of solicitations displayed towards no pacing males. With regard to the latency for the first solicitation, the ANOVA detected a main effect of drug $F(3, 56) = 4.3, p = 0.04$. The post hoc analysis indicated that females from the W-P + saline group displayed their first solicitation towards W males with a shorter latency than towards W males in the W-P + naloxone and than towards W males in the LE-P + naloxone group. This difference was not significant in the between LE males between groups.

The frequency of H&Ds differed among the groups. The ANOVA detected a main effect of strain of male $F(3, 56) = 10.3, p = 0.002$, and an interaction between drug

and strain of male $F(3, 56) = 4.3, p = 0.04$. The direction of the data indicated that overall, naloxone-treated females display more H&D relative to saline-treated females, but this effect was particularly more robust towards the LE males. The post hoc analysis however, only showed differences within groups in the LE-P + naloxone but did not in any other group. For the first H&D latency the ANOVA detected a main effect of strain of male $F(3, 56) = 7.03, p = 0.01$. The post hoc test however, only detected that the latency towards W males in the LE-P + naloxone group was significantly longer compared to the LE males. These results indicate that females preferred the pacing-related males and that this effect was disrupted by naloxone treatment. Together, the results of solicitations and H&D also showed dissociation between these two proceptive behaviors and the effects of the drug treatment on them.

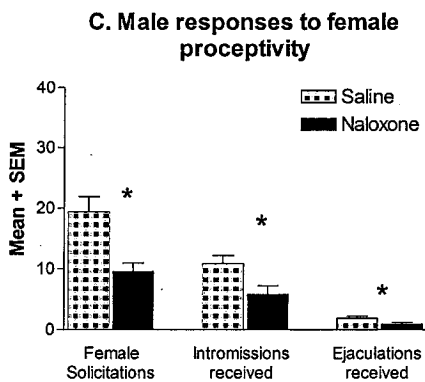
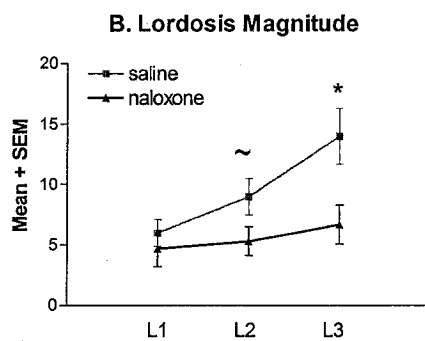
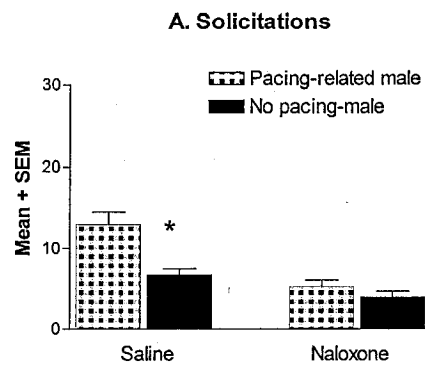


Figure 16. A: Number of solicitations during 30-min test in two groups: saline and naloxone. Pacing-related were of a strain associated with paced copulation, and no pacing-related males were of a different strain associated with nonpaced copulation. B: Lordosis magnitude of saline- and naloxone-treated females. C: Total number of solicitations of saline- and naloxone-treated females and the number of intromissions and ejaculations received. See table 2 for details of behaviors. * $p < .05$

Lordosis. The analysis of frequencies for lordosis magnitude 1 and 2 failed to detect any significant effect of drug, strain of male or interaction. However, the ANOVA indicated a main effect of drug for lordosis magnitude 3, $F(3, 56) = 6.27, p = 0.01$, indicating that overall, saline-treated females performed more lordosis of high magnitude than naloxone-treated females.

Choice of male for ejaculation and male responses. From a total of 60 females conditioned, 25 did not receive an ejaculation from either male during the final partner preference test. Most of these females ($n=17$) belonged to the naloxone group. Of a total of 12 females in the W-P + saline group that received an ejaculation, 5 (42 %) received the first ejaculation from the W male, and 7 females (58 %) from the LE male. Chi-square analyses failed to detect any significant difference in these proportions $\chi^2(1, N=12) = .33, p = 0.56$. Of the total of 10 females in the LE-P saline group that received an ejaculation, 4 (40 %) received their first ejaculation from the W male and 6 (60 %) from the LE male $\chi^2(1, N=10) = .40, p = 0.52$. Of the total of 7 females in the W-P + naloxone group that received an ejaculation, 5 (71 %) received their first ejaculation from W male, and 2 (29 %) from LE males $\chi^2(1, N=7) = 1.2, p = 0.25$. Of the total of 6 females in the LE-P + naloxone group that received an ejaculation, 3 (50 %) received their first ejaculation from the W male and 3 from the LE male. There were no significant differences for the proportion of males chosen for the second and third ejaculation.

There were no significant differences in the frequency or latency for mounts, but the analysis of intromission frequencies detected a main effect of drug $F(3, 56) = 5.54, p = 0.02$, indicating that overall, saline-treated females received more intromissions than naloxone-treated females. The post hoc analysis showed significant mean differences

Conditioned Partner Preference by Strain of Male	Wistar-Pacing group SALINE		Long-Evans-Pacing group SALINE		Wistar-Pacing group NALOXONE		Long-Evans-Pacing group NALOXONE	
	Wistar	Long-Evans	Wistar	Long-Evans	Wistar	Long-Evans	Wistar	Long-Evans
1 st visit latency (sec)	16.2 ± 3.6 *	5.6 ± .52	10.8 ± 1.8	11.1 ± 2.5	9.7 ± 1.7	16.6 ± 6.8	19.8 ± 4.5	9.7 ± 1.3
Visits frequency	40.7 ± 4.3	43.1 ± 4.3	38.6 ± 5 *	43.7 ± 5.3	38.2 ± 3.9	38.1 ± 3.3	35.4 ± 3.5 *	43.4 ± 3.3
1 st Olfactory investigation latency (sec)	23.3 ± 5.7	22.4 ± 7.3	36.8 ± 17.4	14.3 ± 2.9	19.6 ± 5.9	26.8 ± 10.7	24.5 ± 4.9	17.7 ± 4.6
Olfactory investigation frequency	22.8 ± 1.7	22.6 ± 2	19.1 ± 2.7	23.7 ± 2.9	29.6 ± 2.8	32.1 ± 3.4	21.3 ± 1.7 *	30.5 ± 2.5
1 st hop and dart latency (sec)	61 ± 13.4	91.1 ± 61	104 ± 38	63.1 ± 14.4	106.7 ± 48	80.6 ± 42	229 ± 94 *	45 ± 21
Hops and darts	36.8 ± 6.7	31.8 ± 5	26.8 ± 5	39.2 ± 7	31.4 ± 8 *	44.6 ± 8.2	30.7 ± 8 *	52.4 ± 8.6
1 st solicitation latency (sec)	155.7 ± 63	224 ± 85	304 ± 115	135 ± 33	347 ± 165 #	368 ± 107	351 ± 88	385 ± 143
Solicitation frequency	11.5 ± 2.4 *	7.2 ± 1.5	6.1 ± 1.4 *	14.1 ± 2.9	4.9 ± 1.6 #	4.5 ± .95	3.4 ± 1.2	6.2 ± 1.4 #
1 st Mount latency (sec)	246.3 ± 93	155 ± 68	295 ± 153	322 ± 180	219 ± 70	415 ± 266	207 ± 102	61.9 ± 12.6
Mount frequency	10.2 ± 2.2	6 ± 2.1	8.1 ± 2.2	9.1 ± 2.6	7 ± 2.4	3.2 ± 1.8	5.6 ± 2.6	5.3 ± 2.4
1 st Intromission latency (sec)	214 ± 74	177 ± 143	301 ± 124	236.8 ± 122	333 ± 112	526 ± 402	229 ± 67	242 ± 160
Intromission frequency	7 ± 1.4	5.4 ± 1.5	4.2 ± 1	4.9 ± 1.4	4.1 ± 1.6	2.2 ± 1.1	2.6 ± 1.3	2.6 ± 1.2
1 st Ejaculation latency (sec)	781 ± 161	494 ± 162	766 ± 207	639 ± 219	732 ± 82	489 ± 132	758 ± 118	353 ± 41
Ejaculation frequency	.81 ± .24	1.3 ± .36	.78 ± .26	.85 ± .34	.62 ± .23	.31 ± .21	.57 ± .3	.42 ± .25
Choice of male for First ejaculation	5 (42 %)	7 (58 %)	4 (40 %)	6 (60 %)	5 (71 %)	2 (29 %)	3 (50 %)	3 (50 %)
Lordosis magnitude 1	3.1 ± .74	3.1 ± 1.1	2.7 ± 1.1	2.9 ± 1.1	1.6 ± .75	2.2 ± 1.4	2.5 ± 1.3	3 ± 1.5
Lordosis magnitude 2	6 ± 1.7	3.3 ± 1	4 ± 1.4	4.7 ± 1.6	4.1 ± 1.6	1.2 ± .62	2.8 ± 1.1	2.5 ± 1.1
Lordosis magnitude 3	8.8 ± 2.1	5.6 ± 1.7	6.2 ± 1.7	7.2 ± 2.5	5.7 ± 2.1	2.1 ± .99	3 ± 1.6	2.4 ± 1 #

Table 6. Conditioned partner preference test with two males, one Wistar and one Long-Evans. In Wistar-pacing groups. Females were trained to associate Wistar males with paced copulation. In Long-Evans-pacing groups females received the opposite association. Two subgroups are shown, saline- and naloxone-treated female. Data are means (± SEM) during the 30-min preference test. Latencies are expressed in seconds. * $p < .05$ between Wistar and Long-Evans males within groups. # $p < .05$ compared to control.

between W males in the W-P + saline group compared to LE males in the W-P + saline, and to W and LE males in the LE-P + naloxone group.

With regard to ejaculation frequency, the ANOVA detected a main effect of drug $F(3, 56) = 6.42, p = 0.01$, indicating that overall, naloxone-treated females received fewer ejaculations than saline-treated females. The post hoc test only demonstrated significant differences between LE males in the W-P + saline group and LE males copulating with naloxone-treated females. There were no significant differences for first intromission or first ejaculation latencies.

General Discussion

The present study shows that the development of a conditioned partner preference is disrupted by treatment with the opioid receptor antagonist naloxone (4 mg/kg) injected before conditioning trials. In agreement with our previous findings (Coria-Avila et al., 2005, 2006), saline-treated females displayed more solicitations towards the pacing-related males, indicating that CSs (odors, strain cues) associated with paced copulation become predictors of sexual reward. Naloxone-treated females did not show a similar increase in solicitations, and in fact showed decreases in solicitations overall on the final partner preference test. This suggests strongly that opioids are a necessary substrate of reward in the formation of conditioned partner preference induced by paced copulation, similar to the findings of Paredes and Martinez (2001), in which naloxone blocked the formation of pacing-induced CPP.

In our experimental paradigm, solicitations were the most reliable measure of partner preference, and the measure most sensitive to the effects of naloxone.

Solicitations represent the female's desire for sexual interaction (Pfaus et al., 2003). In Experiments 1 and 2, females trained with naloxone performed fewer solicitations on the final test, comparable to the number of solicitations that saline-treated females performed toward the nonpacing males in the saline groups. Fewer solicitations represent a decrease in the motivation to copulate. In addition to soliciting less, naloxone-treated females in Experiment 2 received fewer intromissions and ejaculations, and displayed lordoses of lower magnitude compared to saline-treated females. Thus, females appeared less motivated to copulate overall, despite the fact that they were primed fully with estradiol benzoate and progesterone. This suggests that the absence of opioid transmission during a female's early sexual experiences induces an expectation of nonreward that decreases sexual motivation.

Expectation can be inferred from responses made in anticipation of reinforcement or punishment (Toates, 1986), thus anticipatory or appetitive sexual responses, including conditioned excitation and preference behaviors that depend on sexual reward, can be taken as measures of an animal's expectancy of sexual reward (Pfaus et al., 2003). For example, male rats display anticipatory locomotion in bilevel chambers during a 5-min period prior to the introduction of a sexually receptive female rat and copulation to ejaculation (Mendelson et al., 1989; Pfaus, Mendelson, & Phillips, 1990). This behavior is dependent on the presence of olfactory cues in the chamber (van Furth & Van Ree, 1996a), does not develop if male rats are given access to sexually nonreceptive females or other males (Mendelson et al., 1989), it is inhibited if males are given systemic

injections of naloxone, or infusions of naloxone directly to the ventral tegmental area (VTA), prior to copulatory trials (van Furth & Van Ree, 1996b; van Furth et al., 1996a). This suggests that exposure to nonreceptive partners, or antagonism of opioid receptors in the VTA, lead to an expectation of sexual nonreward as inferred from the lack of anticipatory locomotion.

Endogenous opioid actions at μ opioid receptors in the VTA appear to disinhibit dopamine (DA) neurons by hyperpolarizing inhibitory GABA interneurons (Ford, Mark, & Williams, 2006; Gysling & Wang, 1983; Kalivas, Duffy, & Eberhardt, 1990; Sotomayor, Forray, & Gysling, 2005). Indeed, infusions of morphine to the VTA induce CPP (Olmstead & Franklin, 1997), and stimulate sexually sluggish male rats to mount receptive females (Mitchell & Stewart, 1990). Repeated intermittent exposure of sexually naïve males to estrous vaginal secretions sensitizes DA release in the nucleus accumbens (Mitchell & Gratton, 1992), and this effect is abolished by naloxone treatment prior to exposure. Release of DA in mesolimbic terminals drives goal-directed locomotion in response to incentive cues (Berridge & Robinson, 1998), although antagonism of DA receptors in either the nucleus accumbens or medial preoptic area (which receives DA inputs from the incertohypothalamic DA system) abolish conditioned level changing in anticipation of sexual reward (Pfaus & Phillips, 1991).

In female rats, expectation of sexual reward appears to be important when they desire sex, but not when they are not in heat. Pfaus, Barbosa, and d'Ostie-Racine (submitted) trained OVX female rats primed with EB and P to associate one side of a CPP box with paced copulation in a racetrack chamber, and the other side with nonpaced copulation (with the racetrack insert removed). Females were then primed with EB +P,

EB alone, or no steroids, and tested for CPP. Females in all three hormone-priming groups displayed significant CPP, as has been observed by previously Paredes and colleagues who test for CPP a day after hormone priming (Paredes et al., 1997; Paredes et al., 1999; Paredes et al., 2001). The authors then put the females on a partial extinction schedule, in which they were placed in the side of the CPP box associated previously with paced copulation but without antecedent copulatory contact. Females also remained in the hormone priming condition that CPP was originally tested in: EB+P, EB alone, or no steroids. After 5 extinction trials, only the females primed with EB+P showed significant extinction of CPP. Females in the EB-alone, and no-steroid conditions maintained their preference for the side previously associated with paced copulation. Because females primed with EB+P display high rates of solicitations and lordosis, and low rates of pacing, whereas females primed with EB alone or no steroids display low numbers of solicitations, low lordosis, and high rates of pacing (Pfaus et al., 1999), the authors concluded that the lack of sexual reward produced partial extinction in only the fully-primed females who anticipated sexual reward based on their previous experience.

Although paced copulation reliably induces CPP in female rats when contrasted with nonpaced copulation, it can be induced under certain circumstances by nonpaced copulation when contrasted with no copulation (Meerts, Christensen, & Clark, 2005; Meerts & Clark, 2006; Yamada, Horibe, & Kondo, 2006), or by manual vaginocervical stimulation with a lubricated glass rod that is distributed in time when contrasted with no stimulation (Afonso & Pfaus, submitted). Interestingly, systemic naloxone treatment does not alter the CPP induced by nonpaced copulation (Yamada et al., 2006). This suggests that the rewarding effects of paced copulation are opioid-dependant, whereas the

rewarding effects of nonpaced copulation may involve the action of other neurotransmitter systems. Sexual reward may well be a continuum, in which chemosensory signals from a potential mate induce small increments of arousal and reward sufficient to trigger motivation whereas paced copulation (or ejaculation in male rats) induces a maximal level of reward. However, the rewarding properties of nonpaced copulation are not sufficient to induce conditioned partner preference. Thus, learning to prefer a place seems to be neurochemically distinct from learning to prefer a partner. When augmentation of endogenous opioids is achieved via paced copulation, it may produce a stronger state of reward that can be conditioned to both environmental (i.e. place) and partner cues (i.e. odors, strains), but when augmentation of opioids does not occur, sexual reward appears to be sufficient for conditioning of place cues.

Paced copulation does not induce conditioning in every female. Using CPP as an example, we have reported previously that approximately 60% of females develop CPP for an originally-non preferred place if associated with paced copulation relative to nonpaced copulation (Coria-Avila et al., 2005). This is also noticeable with regard to conditioned partner preference, because among all the females that undergo 9 conditioning trials pairing odor or strain with paced copulation, not all develop a preference for the pacing-related male. There are at least three reasons why this may be so.

First, paced copulation may simply increase the probability of experiencing sexual reward relative to nonpaced copulation given that females can control the initiation and rate of copulatory contact. In a previous factor analysis of sexual behaviors displayed by female rats in bilevel chambers, three pacing-related factors were detected

that determined 1) the chasing behavior of the male, 2) control of the number of mounts and intromissions, and 3) control over the number of ejaculations (Pfaus et al., 1999). Clearly, females adjust their behavioral responses depending on the stimulation they receive from males. Females that are given access to sexually naïve or sluggish males typically mount those males as a supersolicital behavior (Afonso & Pfaus, 2006; Beach, 1968), which in many cases prompts males to mount the females in return. Likewise, females that copulate in small chambers that do not allow them to escape the male, or with males that do not chase them, are typically observed to solicit copulation using hops and darts in the immediate vicinity of the male rather than soliciting as they would in a bilevel chamber or open field, with a headwise orientation to the male followed by a runaway (McClintock, 1984a; Pfaus et al., 2003). Thus, control over the copulatory contact may be a cognitive and emotional mechanism that activates endogenous opioids fully within reward pathways. Females given the opportunity to pace copulation efficiently at their preferred rate may therefore be more likely to experience sexual reward. Indeed, male rats that do not have control over their copulatory interactions do not form a sexual CPP, despite ejaculating on each conditioning trial (Camacho, Sandoval, & Paredes, 2004).

Second, paced copulation facilitates reproductive outcome. For example, females that pace a moderate number of intromissions (e.g., 10-15) before ejaculation are significantly more likely to get pregnant than females that are not allowed to pace the intromissions (e.g., Coopersmith et al., 1994; Erskine et al., 1989). Indeed, to the extent that sexual reward and satiety result in sexual inhibition, it is noteworthy that paced copulation (Erskine et al., 1989) or distributed vaginocervical stimulation (Pfaus, Smith,

Byrne, & Stephens, 2000) also result in a faster termination of estrus. Intromissions spaced in time facilitate reproductive events such as sperm transport and prolactin surges that support the maintenance of progesterone release by the corpora lutea (Erskine et al., 1989; Matthews, Jr. et al., 1978; McClintock et al., 1978). However, it is not known to what extent mechanisms of sexual reward are related to mechanisms of reproduction, although it is likely that the sequential actions of estrogen and progesterone set both into motion at a genomic and functional level (Pfaff, 1999).

Third, individual differences may exist in the propensity of paced copulation to activate reward or bonding mechanisms, such as oxytocin or vasopressin release or binding in the hypothalamus, septum, or ventral forebrain (Carter, Witt, Thompson, & Carlstead, 1988; Insel, Preston, & Winslow, 1995; Young et al., 2005). For example, monogamous Prairie vole males have greater vasopressin 1a (V1a) receptor density in the ventral pallidum compared to polygamous Meadow voles (Young et al., 2004). Increasing the number of V1a receptors in this region of Meadow voles following viral transfection renders them far more likely to bond after their first copulatory experience (Lim et al., 2004). It is therefore possible that individual differences in this or other mechanisms of bonding may result in differences in propensity to form place or partner preferences.

Some studies indicate that naloxone administration can produce aversive effects. This is supported by the fact that naloxone induces conditioned place aversion (CPA) in mice (Skoubis, Matthes, Walwyn, Kieffer, & Maidment, 2001) and in rats (Azar, Jones, & Schulteis, 2003). The aversive effects are believed to occur due to a disruption in the tonic effect on hedonic homeostasis exerted by opioids (Bals-Kubik, Herz, &

Shippenberg, 1989). It is unlikely that the behaviors observed in the naloxone-treated females in the present study were the consequence of a CPA because the final test did not occur under the influence of naloxone, nor did it occur in the same chambers as conditioning occurred (e.g., semicircular pacing chambers versus the open field). It is also unlikely that females developed an aversion towards the males, since the amount of visits and olfactory investigations were not affected by the drug, but only sexual behaviors. In fact, females did not stop displaying solicitations altogether, which would be more suggestive of an aversion. Instead, they showed a reduction in frequency similar to the number of solicitations displayed by saline-trained females toward nonpacing-related males. This suggests a selective disruption of conditioned preference, and in context with the decrease in high-level lordosis, a diminished sexual desire.

In summary, the present study confirms that opioids are an important neurochemical substrate for the development of pacing-induced conditioned partner preference in female rats. This type of preference depends on the contingency between rewarding stimuli (UCSs) and the available cues (CSs). The nature of these cues can be olfactory alone (Coria-Avila et al., 2005) or a mixture of olfactory, auditory, and visual, as might differentiate two strains of males (Coria-Avila et al., 2006). In the monogamous female Prairie vole, the development of pair bonding depends on DA and oxytocin. It is unknown what the effects of DA and oxytocin are on the conditioned partner preference observed in female rats. It is possible that sexual reward induced by paced copulation (the UCS) may be accompanied by an augmentation of endogenous opioid activity that leads in sequence to increased DA and oxytocin activity. It may be that opioid activation underlies both pacing-related CPP and conditioned partner preference, but that DA and

oxytocin are necessary for the development of conditioned partner preference, but not for CPP (given that administration of the DA receptor antagonist flupenthixol does not disrupt CPP; Garcia Horsman et al., 2004). The latter systems may be required to facilitate the contingency of reward and social recognition during exposure to male cues.

CHAPTER THREE

PART 2

Neurochemical Basis of Conditioned Partner Preference in the Female Rat:

II. Disruption by Flupenthixol

Genaro A. Coria-Avila, Alex M. Gavrilu, Brigitte Boulard, Natalie Charron,

Geoffrey Stanley, James G. Pfaus

Concordia University

RUNNING HEAD: DOPAMINE AND CONDITIONED PARTNER PREFERENCE

Center for Studies in Behavioral Neurobiology, Department of Psychology,
Concordia University, Montréal, QC.

This research was supported by a grant from the Canadian Institutes of Health Research (MOP-74563) to JGP and a postgraduate fellowship from CONACyT of Mexico (128099) to GAC. The authors would like to thank Drs. Mary Erskine, Tod Kippin and Raúl Paredes for useful discussions. All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

Correspondence concerning this article should be addressed to Genaro A. Coria-Avila, D.V.M., M.Sc., CSBN-Psychology, Concordia University, 7141 Sherbrooke W., Montréal, QC, H4B 1R6, CANADA. E-mail: coria75@yahoo.com.

For submission to *Behavioral Neuroscience*

Abstract

The effects of the dopamine receptor antagonist flupenthixol were examined on the development of conditioned partner preference induced by paced copulation in female rats. In Experiment 1, ovariectomized, hormone-primed rats were conditioned to associate scented and unscented males with paced and nonpaced copulation respectively. Females in Experiment 2 associated albino or pigmented males with paced or nonpaced copulation. Flupenthixol or saline was administered prior to each conditioning trial. During a final drug-free preference test, females could choose to copulate with either a pacing related or unrelated male. Saline-trained females copulated preferentially with the pacing-related male whereas flupenthixol disrupted odor, but not strain conditioning. The role of dopamine in conditioned partner preference depends on the type of stimuli to be learned.

Keywords: Paced copulation, Odors, Sexual reward, Conditioning, Sexual behavior, Wistar, Long-Evans, Dopamine

Introduction

For successful sexual interactions to occur, animals must be able to respond to internal stimuli that signal their own sexual desire and to external stimuli that indicate where to find a mate. Once potential mates are found, animals must be able to distinguish those that are receptive and fecund from those that are not; and when more than one potential mate is found, animals may display partner preferences based on the incentive value that each partner represents (Pfaus et al., 2001). The perception of sexual incentive value depends on an interaction between the animal's own internal state (e.g., hormonal priming and level of arousal) and the accurate prediction of successful sexual outcomes with a particular partner (Whalen, 1966).

There is evidence indicating that rats can develop conditioned partner preferences based on the association that is formed between a conditioned stimulus (CS) and a sexually rewarding unconditioned stimulus (UCS). For example, male rats develop a conditioned ejaculatory preference for females bearing an odor, such as almond or lemon, that was previously associated with the sexual reward state induced by ejaculation (Kippin et al., 1998; Kippin et al., 2001a). Female rats also develop conditioned partner preference for males bearing cues associated with sexual reward (Coria-Avila et al., 2005; Coria-Avila et al., 2006). In this case, sexual reward is induced by the ability of females to control the initiation and rate of copulation (called "pacing"), and is paired with either almond odor or a particular strain of male (albino versus pigmented). Preferences are tested in a large open field with two vigorous copulating males tethered in two opposite corners, one scented with the odor and the other unscented, or one albino and the other pigmented. Females in which the paced copulation was paired with a

particular cue show increased solicitations toward the males bearing the cue, higher magnitude lordosis, and in certain circumstances, also receive more ejaculations from pacing-related males. As with males, females for whom the cue is not paired with sexual reward do not show any preference for a male bearing the cue.

In the laboratory settings, paced copulation commonly occurs in a chamber divided by a wall with few holes cut into the bottom, which allow exclusively the female to crawl through and get away from the male. This allows her to control the amount of vagino-cervical stimulation (VCS) received from the male, which is believed to induce sufficient reward to facilitate conditioning. The neurochemical substrate of such reward seems to depend on an augmentation of endogenous opioids during pacing, which is based on evidence that the conditioned place preference (CPP) (Paredes et al., 2001) and the conditioned partner preference (Coria-Avila et al., 2007b) induced by paced copulation can be readily disrupted by injections of the opioid antagonist naloxone given before each conditioning trial.

During copulation in females, levels of dopamine (DA) increase in brain areas such as the nucleus accumbens (NAc) and striatum (Pfaus et al., 1995). Interestingly, females that pace copulation show greater DA release in NAc and striatum relative to females that cannot pace copulation (Mermelstein et al., 1995). Such differences are observed even when females in both conditions (i.e. paced and nonpaced) receive the same number of intromissions and ejaculations during an hour of testing (Becker, Rudick, & Jenkins, 2001), indicating that the increase of DA does not depend on the amount of VCS received from the male *per se*, but on the amount of paced VCS that is suitable for every female.

There is a close relationship between opioid and DA activation during paced copulation. The rewarding effects of drugs that activate μ or δ opioid receptors appear to depend on the activity of the ventral tegmental area (VTA) of the midbrain through its mesolimbic dopaminergic (DA) projections to the NAc (Wise & Bozarth, 1982; Balfour, Yu, & Coolen, 2004). For example, destruction of VTA DA neurons by infusions of the DA neurotoxin 6-hydroxidopamine (6-OHDA) to the NAc, or infusions of the DA D1 receptor antagonist SCH 23390 to the NAc, disrupt the CPP associated with the rewarding effects of systemic opioids (Shippenberg, Bals-Kubik, & Herz, 1993). Furthermore, DA D2 knockout mice fail to display opioid-induced CPP (Maldonado et al., 1997). Injections of the μ opioid agonists morphine or DAMGO to the lateral ventricles induce CPP and increase DA release within the NAc (DiChiara & Imperato, 1988). Conversely, injections of κ opioid agonists, such as U 50466 produce aversive effects (Mucha & Herz, 1985) and i.c.v. injections of U 50466 decreases extracellular concentrations of DA within the NAc (DiChiara et al., 1988). Accordingly, it has been hypothesized that the rewarding or aversive effects of opioids depend on increases or decreases of NAc DA, respectively (Herz, 1998; Balfour et al., 2004).

Conditioned responses made toward cues that predict reward may be mediated by mesolimbic DA release (Robinson et al., 1993). Indeed, our previous reports of conditioned partner preference, taken together with the microdialysis findings of Becker et al. (2001) and Mermelstein and Becker (1995), suggest that increased DA release in NAc and/or striatum during paced copulation are mechanisms that link sexual reward to conditioned motor responses made preferentially toward a male bearing a cue associated with paced copulation. However, systemic administration of the DA receptor antagonist

flupenthixol or raclopride do not block the CPP induced by paced copulation, despite the fact that the same doses of those drugs successfully blocked the CPP induced by amphetamine (Garcia Horsman et al., 2004). This has been taken as evidence to suggest that the rewarding properties of sex require opioids but not DA (Paredes & Ågmo, 2004). However, it is not yet known whether the place cues are too diffuse and distributed to induce DA release, or whether they produce a large-enough release of DA that competes successfully with an antagonist for occupancy of DA receptors. Given a role of mesolimbic DA in attention to conditioned stimuli (CSs) (Young, Moran, & Joseph, 2005), it may be the case that DA release occurs most strongly toward cues that are specific and discrete. In our previous conditioned partner preference studies, either a discrete odor or an obvious difference in the strain of the partner was used as the CS. Stimuli on a potential partner may be more likely to induce DA release than more diffuse place cues. Therefore, in the present study we examined the effect of the DA receptor antagonist flupenthixol on the conditioned preference for olfactory or strain-of-male cues paired with paced copulation.

General Method

Subjects and Surgery

Long-Evans male (300-350 g) and female (200-250 g) rats were purchased from Charles River Canada (St-Constant, QC). They were housed in groups of 4 in large hanging wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Food and water were continuously available. The females were anesthetized with a mixture of ketamine hydrochloride (50

mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then ovariectomized bilaterally via a lumbar incision. Following ovariectomy females were given daily subcutaneous injections of Flunixin meglumine 2.5 mg/kg (Banamine®) for analgesia, and Enrofloxacin 5 mg/kg (Baytril®) during three days to prevent post surgical bacterial infections. All females were given a week of post-surgical recovery prior to conditioning trials.

Gonadally intact Long-Evans male rats that served as stimulus males had at least 10 tests of sexual behavior in bi-level chambers prior to the start of these experiments. These males were sexually vigorous and initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test. If scented, males received almond extract (Blue Ribbon, Etobicoke, ON) applied to the back of their necks and anogenital regions with a cotton swab. Unscented males received similar applications of distilled water to the same areas.

Drugs

Cis (Z) flupenthixol (Sigma, St. Louis, MO, USA), a D1/D2 DA receptor antagonist, was dissolved in 0.9 % physiological saline and injected intraperitoneally in a dose of 0.25 mg/kg in a volume of 1 ml/kg, 30 min before every conditioning trial (as in Garcia Horsmann & Paredes, 2004). Animals that served as the control group were injected with physiological saline 30 min before testing.

Experiment 1

The first experiment tested the effect of injections of flupenthixol vs. saline on the development of olfactory conditioned partner preference induced by paced copulation with scented males, as in the paired group reported by Coria-Avila et al. (2005). In this experiment only Long-Evans females and males were used.

Method

Odor conditioning

Conditioning trials occurred every 4 days in semicircular chambers (38² cm X 60 cm X 38 cm). For paced copulation, the chambers were divided by a middle Plexiglas partition with four equidistant holes cut into the bottom (4 cm X 4 cm) resting on bedding. The holes were large enough for the female to pass through, but too small for the males. Nonpaced copulation occurred in the same chambers but without the partition. Females copulated with scented males in the pacing condition, and four days later with unscented males in the nonpacing condition. If scented, almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) was applied on the back of the neck and anogenital area of the males with a cotton swab. Females received a total of 10 conditioning trials (5 paced and 5 nonpaced). The order of conditioning was counterbalanced, such that half of the rats in every condition had their first sexual experience in a paced situation and the other half nonpaced. During each conditioning trial, experimental females were injected with naloxone 1 min before the start of the session, and females in the control group were injected with saline. They were allowed to copulate for 30 min before being returned to

their home cages. All tests with scented and unscented males took place in different rooms to prevent noncontingent odor associations.

Copulatory preference test

Four days after the final conditioning trial, females were placed into a large open field (123 cm X 123 cm X 46 cm) with a thin layer of wood-chip bedding. At two diagonal corners there were two males unfamiliar to the female, one scented and one unscented. Each male wore a rodent jacket connected on the back to a spring wire 30 cm in length, which allowed the males to roam freely in a radius of approximately 45 cm. For this final partner preference test females in the experimental and control group were injected exclusively with saline, so that all behavioral differences between naloxone- and saline-trained rats were the result of the conditioning process and not because of any drug effect during the final test. Females were allowed to copulate freely with both males during the 30-min test.

Conditioned partner preference was determined as in Coria-Avila et al. (2005, 2006) by latency and frequency measures of female visits, olfactory investigations, solicitations (described as a headwise orientation of the female to the male, followed by a runaway), hops and darts (H&D), and lordosis magnitudes (on a scale of 1 to 3, with 1 representing low magnitude and 3 high magnitude) during interactions with each male. In addition we assessed the male chosen for the first ejaculation, as well as the frequency of mounts, intromissions, and ejaculations during the final preference test.

Statistical analysis

A 2 X 2 (odor X drug) mixed design ANOVA was used to determine differences in frequencies and latencies. Chi-square analyses were used to determine the differences

in proportion of males chosen for first mount, intromission, and ejaculation. All significant main effects in the ANOVAs were followed by a Tukey Least Significant Difference (LSD) post hoc test to assess differences between individual means. The level of significance for all tests was $p < 0.05$.

Results

Of the total of 40 females originally conditioned, 6 were eliminated from the statistical analysis either because of sickness or because one or both males became free from their tethered jackets during the final preference test. This left 34 females ($n=18$ in the control group; $n=16$ in the flupenthixol-treated group). Table 1 shows the means + standard errors for all the behaviors assessed during the 30-min test for saline- vs. flupenthixol-treated females.

Visits and olfactory investigations. With regard to the first visit latency, the ANOVA did not detect a main effect of drug $F(1, 32) = .96, p = 0.33$, odor $F(1, 32) = 1.07, p = 0.30$, or interaction between drug and odor $F(1, 32) = 2.2, p = 0.14$, indicating that females in both groups performed their first visit with approximately the same latency towards scented or unscented males. The analysis of visits frequency showed a main effect of odor $F(1, 32) = 9.84, p = 0.003$. The post hoc analysis revealed that saline-treated females visited the scented male more times than the unscented male. In the flupenthixol-treated females however, there were no differences in visit frequencies between scented and unscented males. With regard to the number of olfactory investigations, the ANOVA did not detect a main effect of odor $F(1, 32) = 1.2, p = 0.26$, drug $F(1, 32) = .30, p = 0.58$, or interactions between odor and drug $F(1, 32) = 0.26, p =$

.58. There were no main effects of drug $F(1, 32) = .006, p = 0.93$ odor $F(1, 32) = .04, p = 0.82$ or interactions between odor and drug $F(1, 32) = .53, p = .47$ on first olfactory investigation latency.

Solicitations and hops & darts. The ANOVA detected significant differences between the saline- and flupenthixol-trained females. With regard to solicitation frequencies there was main effect of drug $F(1, 32) = 4.3, p = 0.045$, a main effect of odor $F(1, 32) = 7.8, p = 0.009$, and an interaction between drug and odor $F(1, 32) = 5.46, p = 0.02$. The post hoc analysis revealed that saline-trained females displayed significantly more solicitations toward the scented versus unscented males ($p < 0.05$). In contrast, flupenthixol-trained females made significantly fewer solicitations overall, and did not distinguish between the scented and unscented males. With regard to the latency for the first solicitation, the ANOVA did not detect any main effect of drug $F(1, 27) = .10, p = 0.75$, odor $F(1, 27) = 1.23, p = 0.27$ or interactions. The analysis of frequencies for H&D did not show any main effect of drug $F(1, 32) = 1.4, p = 0.23$, odor $F(1, 32) = 1.8, p = 0.18$, but a trend toward a significant interaction of drug and odor $F(1, 32) = 3.15, p = 0.08$. The analysis of the first H&D latency showed a trend toward a significant effect of odor, $F(1, 29) = 3.4, p = 0.07$, but no main effect of drug $F(1, 29) = .22, p = 0.63$, or interaction between drug and odor $F(1, 29) = 1.7, p = 0.19$, indicating that the first H&D occurred at approximately the same time in all groups.

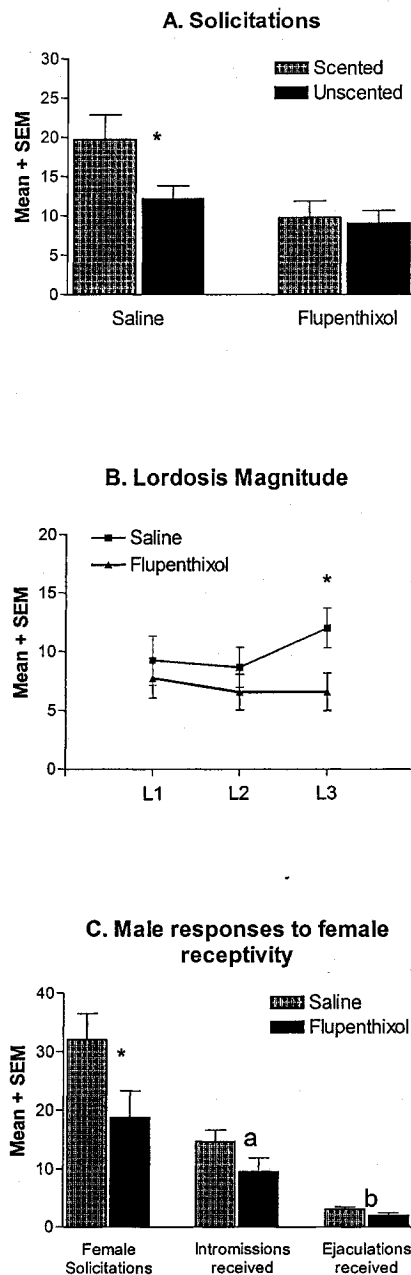


Figure 17. A: Number of solicitations during 30-min test in two groups: saline (n= 18) and Flupenthixol (n= 16). Scented males were associated with paced copulation, and unscented males associated with nonpaced copulation. B: Lordosis magnitude of saline- and flupenthixol-treated females. C: Total number of solicitations of saline- and flupenthixol-treated females and the number of intromissions and ejaculations received. See table 7 for details of behavior. * $p < .05$, ^a $p=0.09$, ^b $p=0.06$.

Lordosis. The analysis of lordosis magnitude 3 indicated a main effect of drug $F(1, 32) = 5.01, p = 0.03$. The post hoc analysis revealed that overall, flupenthixol-trained females displayed fewer lordosis magnitude 3 relative to saline-trained females. The analysis of lordosis magnitude 2 showed no main effects of drug $F(1, 32) = .80, p = 0.37$, odor $F(1, 32) = 1.23, p = 0.27$, or interaction between drug and odor $F(1, 32) = .44, p = 0.50$. Likewise, the analysis of frequency for lordosis magnitude 1 showed no main effects of drug $F(1, 32) = .30, p = 0.58$, odor $F(1, 32) = .84, p = 0.36$, or interaction between drug and odor $F(1, 32) = .99, p = 0.32$.

Choice of male for ejaculation and male responses. Of a total of 18 females in the saline group, 17 received ejaculations. Of those 17 females 9 (52%) received the first ejaculation from the scented male, and 8 females (47%) from the unscented male. Chi-square analyses failed to detect any significant difference in these proportions $\chi^2(1, N=17) = .059, p = 0.80$. Of the total of 16 females in the flupenthixol-treated group, 10 received ejaculations during the final test. Of those females 3 (30%) received their first ejaculation from scented males, and 7 (70%) from unscented males. Chi square analysis failed to detect any significant difference in these proportions $\chi^2(1, N=10) = 1.6, p = 0.20$. Only 15 females in the saline-treated group received a second ejaculation, 11 (73%) from the scented male, and 4 (26%) from the unscented male. Chi square analysis revealed a trend toward significance in these proportions $\chi^2(1, N=10) = 3.2, p = 0.07$. In the flupenthixol group, 10 females received a second ejaculation, 6 (60%) from the scented male and 4 (40%) from the unscented male $\chi^2(1, N=10) = .40, p = 0.52$. Only 11 females in the saline-treated group received a third ejaculation, 4 (36%) from the scented male, and 7 (63%) from the unscented male. Chi square analysis failed to detect any

significant difference in these proportions $\chi^2 (1, N=10) = .81, p = 0.36$. In the flupenthixol group, 7 females received a third ejaculation, 5 (71%) from the scented male and 2 (29%) from the unscented male $\chi^2 (1, N=10) = 1.2, p = 0.25$.

With regard to ejaculation frequencies, the ANOVA detected a trend for effect of drug $F(1, 32) = 3.7, p = 0.06$, but no main effect of odor $F(1, 32) = .48, p = 0.48$, or interaction between drug and odor $F(1, 32) = .03, p = 0.8$. With regard to mount frequencies, the ANOVA failed to detect any main effect of drug $F(1, 32) = .27, p = 0.60$, odor $F(1, 32) = .61, p = 0.43$ or interaction $F(1, 32) = .27, p = 0.60$. The analysis of the first mount latency showed no main effects or interactions. With regard to intromission frequencies, the ANOVA did not detect any effect of drug $F(1, 32) = 2.88, p = 0.09$, odor $F(1, 32) = .025, p = 0.87$, or interaction between drug and odor $F(1, 32) = .15, p = 0.69$.

Conditioned Partner Preference by Olfactory Cues	Odor-Pacing group SALINE		Odor-Pacing group FLUPENTHIXOL	
	Scented	Unscented	Scented	Unscented
1 st visit latency (sec)	20.2 ± 3.1	28.3 ± 8.2	64 ± 36	19 ± 2.8
Visits frequency	42 ± 3 *	37 ± 2.7	38 ± 3.5	35.5 ± 3.5
1 st Olfactory investigation latency (sec)	37 ± 9.2	50 ± 18	46 ± 11	39.7 ± 9.9
Olfactory investigation frequency	28.4 ± 1.9	25.5 ± 1.9	29.4 ± 1.8	28.4 ± 1.9
1 st hop and dart latency (sec)	175 ± 51	150 ± 52	283 ± 127	245 ± 92
Hops and darts	31 ± 5.8	21 ± 3.16	18.8 ± 3.8	20.1 ± 4.2
1 st solicitation latency (sec)	300 ± 110	318 ± 112	555 ± 162	258 ± 66
Solicitation frequency	19.8 ± 3.1 * #	12.2 ± 1.6	9.8 ± 2.1	9.1 ± 1.6
1 st Mount latency (sec)	284 ± 122	215 ± 84	414 ± 212	375 ± 147
Mount frequency	6.6 ± 2.1	6.5 ± 1.29	4.5 ± 1.09	6.18 ± 1.58
1 st Intromission latency (sec)	242 ± 73	217 ± 109	286 ± 87	211 ± 63
Intromission frequency	7.1 ± 1.07	7.6 ± 1.2	4.9 ± 1.3	4.7 ± 1.1
1 st Ejaculation latency (sec)	655 ± 95	423 ± 85	729 ± 153	443 ± 53
Ejaculation frequency	1.6 ± .24	1.4 ± .31	1.06 ± .26	.93 ± .23
Choice of male for First ejaculation	9 (52 %)	8 (47 %)	3 (30 %)	7 (70 %)
Lordosis magnitude 1	3.9 ± 1.3	5.4 ± 1.07	3.9 ± .97	3.8 ± 1.1
Lordosis magnitude 2	5.11 ± 1.4	3.6 ± .65	3.5 ± .86	3.1 ± .93
Lordosis magnitude 3	5.8 ± .98 #	6.2 ± 1.1	2.9 ± .91	3.7 ± .95

Table 7. Female sexual behavior during an olfactory conditioned partner preference test with two males, one scented and one unscented. Scented males were associated with paced copulation and unscented with nonpaced copulation. Two groups are shown, saline-treated (n= 18) and flupenthixol-treated (n= 16). Data are means (± SEM) during the 30-min preference test. Latencies are expressed in seconds. * $p < .05$ within groups. # $p < .05$ between groups.

Experiment 2

This experiment examined the effects of flupenthixol on the development of conditioned partner preference for strain of male associated with paced copulation, as in Coria-Avila et al. (2006).

Method

Strain Conditioning

Female rats were randomly assigned into two counterbalanced groups. Females in the Wistar-pacing (W-P) group ($n=30$) copulated with Wistar males in the paced condition and Long-Evans males in the nonpaced condition. Females in the Long-Evans-pacing (LE-P) group ($n=30$) copulated with Long-Evans males in the paced condition and Wistar males in the nonpaced condition. Half of each group was used as control and received exclusively I.P. saline and the other half received I.P. flupenthixol 30 min before every conditioning trial as in Experiment 1. This procedure led to 4 subgroups meant to have 15 females each. However, three females died due to reasons other than the experimental procedure, and the data of 10 more females were lost due to technical problems. This led to a reduced number of animals in each group: W-P + saline ($n=13$), LE-P + saline ($n=12$), W-P + flupenthixol ($n=10$) and LE-P + flupenthixol ($n=12$). Females received a total of 10 conditioning trials (5 paced and 5 nonpaced) of 30 min each before being taken back into their home cages.

Copulatory Preference Test

As in Experiment 1, four days after the 10th conditioning trial, a final test was carried out for each female into a large open field. At two opposite corners of this open

field were two males, one Wistar and the other Long-Evans. All the females were injected exclusively with saline intraperitoneally 30 min before being placed in one neutral corner of the open field and were allowed to copulate freely with both males for a 30-min period. The same behavioral measures were assessed as in Experiment 1.

Statistical Analyses. A 2 X 2 X 2 (group X drug X strain of male) mixed design ANOVA was used to analyze differences in the frequencies and latencies of copulatory behaviors toward the two males. In some cases where significant results were expected we used a 2 X 2 (pacing condition X drug). All significant main effects and interactions were followed by a Tukey Least Significant Difference (LSD) post hoc test to detect specific differences between group means.

Results

Visits and olfactory investigations. With regard to the first visit latency, the ANOVA failed to indicate any main effect of drug $F(3, 43) = 2.01, p = 0.16$, group $F(3, 43) = .18, p = 0.89$ or strain of male $F(3, 43) = .28, p = 0.59$. The analysis of visit frequencies showed a trend toward a main effect of drug $F(3, 43) = 3.7, p = 0.06$, but no main effect of group $F(3, 43) = .02, p = 0.87$ or strain of male $F(3, 43) = .37, p = 0.54$. With regard to the first olfactory investigation latency, the ANOVA showed no effects of drug $F(3, 43) = 2.6, p = 0.11$, group $F(3, 43) = .60, p = 0.44$, or strain of male $F(3, 43) = .02, p = 0.88$. The analysis of olfactory investigation frequencies showed a trend toward a main effect of drug, $F(3, 43) = 2.9, p = 0.09$, but no main effect of group $F(3, 43) = .004, p = 0.94$ or strain of male $F(3, 43) = .84, p = 0.36$. These results indicate that

females in all groups visited both types of male with the same latency and frequency. They also performed the same number of olfactory investigations.

Solicitations and hops & darts. With regard to solicitation frequencies the ANOVA detected a significant interaction between group and strain of male $F(3, 43) = 4.9, p = 0.03$. The posthoc analyses, however, failed to reveal any significant difference between W and LE males in any group. Accordingly, a second 2 X 2 (pacing-condition X drug) ANOVA was carried out. This second analysis allowed us to compare the effects of pacing-related males vs. nonpacing-related males and the interaction with saline and flupenthixol treatments. The results indicated that regardless of the strain of male, females displayed more solicitations towards pacing-related males ($\bar{M} = 15.7$) than towards nonpacing-related males ($\bar{M} = 11.5$) $F(1, 45) = 5.1, p = 0.02$ (Figure 18-A). The results also indicated that there was no effect of drug treatment $F(1, 45) = .008, p = 0.93$, nor an interaction between pacing condition and drug treatment $F(1, 45) = .000, p = 0.98$. With regard to the latency for the first solicitation, the ANOVA detected only a trend to significance in main effect of drug $F(3, 38) = 3.17, p = 0.06$, and a trend for interaction between group and drug $F(3, 38) = 3.7, p = 0.058$ but no main effect of group $F(3, 38) = 0.01, p = 0.89$ or strain of male $F(3, 38) = .84, p = 0.36$. A second analysis using pacing-related vs nonpacing-related males failed to demonstrate any significant effect of pacing $F(1, 45) = .57, p = 0.45$ or drug $F(1, 45) = 2.6, p = 0.11$.

The analysis of frequencies for H&D showed no significant differences. There was no main effect of drug $F(3, 43) = .007, p = 0.93$, group $F(3, 43) = .01, p = 0.90$ or strain of male $F(3, 43) = .96, p = 0.33$. For the first H&D latency the ANOVA detected a trend for main effect of group $F(3, 43) = 3.04, p = 0.08$, drug $F(3, 43) = 3.46, p = 0.06$

and strain of male $F(3, 43) = 3.77, p = 0.059$, and an interaction group and drug treatment $F(3, 43) = 9.44, p = 0.003$, and an interaction between group, drug treatment and strain of male $F(3, 43) = 5.2, p = 0.02$. Posthoc analyses revealed that in the W-P flupenthixol-treated groups, females displayed a longer latency for the first H&D towards LE relative to any other group ($p < 0.0001$).

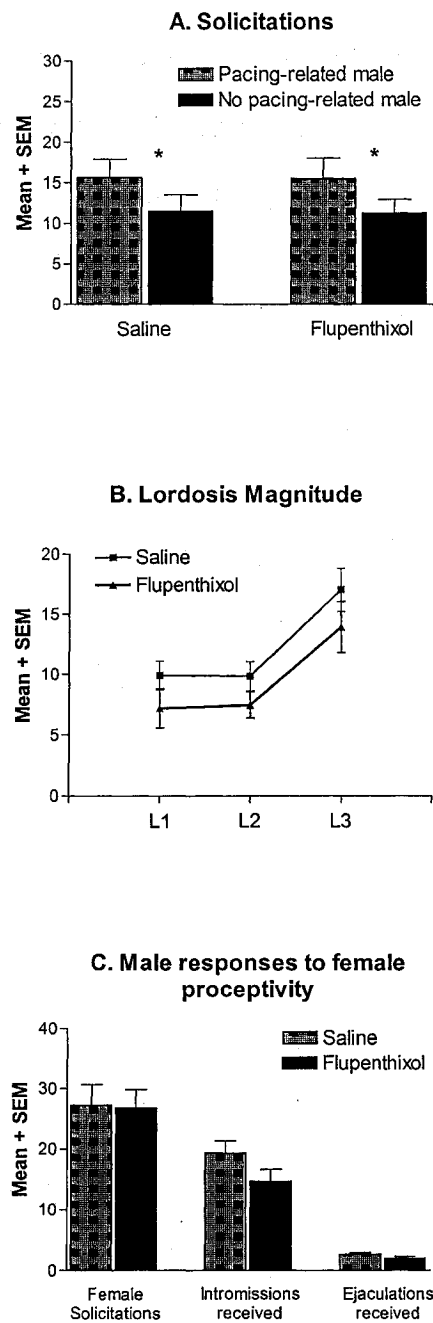


Figure 18. A: Number of solicitations during 30-min test in two groups: saline and flupenthixol. Pacing-related were of a strain associated with paced copulation, and no pacing-related males were of a different strain associated with nonpaced copulation. B: Lordosis magnitude of saline- and flupenthixol-treated females. C: Total number of solicitations of saline- and flupenthixol-treated females and the number of intromissions and ejaculations received. See table 8 for details of behaviors. * $p < 0.05$.

Lordosis. For lordosis magnitude 3 there was no main effect of drug $F(3, 43) = 1.6, p = 0.21$, group $F(3, 43) = .82, p = 0.36$ or strain of male $F(3, 43) = 1.4, p = 0.24$. For lordosis magnitude 2 there was no main effect of drug $F(3, 43) = 1.6, p = 0.20$, group $F(3, 43) = .13, p = 0.71$ or strain of male $F(3, 43) = .01, p = 0.89$. Likewise, for lordosis magnitude 1 there was no main effect of drug $F(3, 43) = 1.6, p = 0.20$, group $F(3, 43) = .11, p = 0.72$ or strain of male $F(3, 43) = .35, p = 0.55$. This indicates that saline- and flupenthixol-trained females displayed similar frequencies of lordosis magnitudes 1, 2, and 3.

Choice of male for ejaculation and male responses. From a total of 47 females conditioned, 5 did not receive an ejaculation from either male during the final partner preference test. Of the total of 12 females in the W-P + saline group that received an ejaculation, 8 (66%) received the first ejaculation from the W male and 4 females (33%) from the LE male. Chi-square analyses failed to detect any significant difference in these proportions $\chi^2(1, N=12) = 1.3, p = 0.24$. Of the total of 11 females in the LE-P saline group that received an ejaculation, 3 (27.2%) received their first ejaculation from the W male and 8 (72.7%) from the LE male $\chi^2(1, N=11) = 2.2, p = 0.13$. Of the total of 9 females in the W-P + flupenthixol group that received an ejaculation, 2 (23%) received their first ejaculation from W male, and 7 (77%) from LE males $\chi^2(1, N=9) = 2.7, p = 0.09$. Of the total of 10 females in the LE-P + flupenthixol group that received an ejaculation, 4 (40 %) received their first ejaculation from the W male and 6 (60%) from the LE male $\chi^2(1, N=10) = .4, p = 0.52$. There were no significant differences for the proportion of males chosen for the second and third ejaculation.

With regard to first ejaculation latency there was a main effect of drug $F(3, 43) = 5.3, p = 0.03$, and a trend for main effect of strain of male $F(3, 43) = 4.4, p = 0.053$. However, with regard to ejaculation frequency there was no main effect of drug $F(3, 43) = 1.6, p = 0.20$, group $F(3, 43) = .37, p = 0.54$ or strain of male $F(3, 43) = .24, p = 0.62$. With regard to mount frequency there was no main effect of drug $F(3, 43) = 1.3, p = 0.24$, group $F(3, 43) = .86, p = 0.35$ or strain of male $F(3, 43) = .08, p = 0.77$. For first mount latency there was no main effect of drug $F(3, 43) = .91, p = 0.76$, group $F(3, 43) = .91, p = 0.34$ or strain of male $F(3, 43) = .21, p = 0.64$. For intromission frequencies there was no main effect of drug $F(3, 43) = 2.3, p = 0.13$, group $F(3, 43) = .32, p = 0.57$ or strain of male $F(3, 43) = 1.3, p = 0.24$. For first intromission latency there was no main effect of drug $F(3, 43) = .78, p = 0.38$, group $F(3, 43) = .09, p = 0.75$ or strain of male $F(3, 43) = .003, p = 0.95$.

Conditioned Partner Preference by Strain of Male	Wistar-Pacing group SALINE		Long-Evans-Pacing group SALINE		Wistar-Pacing group FLUPENTHIXOL		Long-Evans-Pacing group FLUPENTHIXOL	
	Wistar	Long-Evans	Wistar	Long-Evans	Wistar	Long-Evans	Wistar	Long-Evans
1 st visit latency (sec)	19.7 ± 6.8	8.6 ± 2.6	11.4 ± 2.7	13 ± 3.2	16.6 ± 3.5	14.6 ± 2.5	16.2 ± 5.8	20.3 ± 4.9
Visits frequency	43.1 ± 3.5	42.6 ± 4	42.5 ± 5.2	41.6 ± 4.8	52 ± 6.22	48.1 ± 5.9	51.6 ± 3.9	53 ± 4.7
1 st Olfactory investigation latency (sec)	27.8 ± 8.4	23.2 ± 11.2	13.1 ± 3.1	17.5 ± 3.5	26.1 ± 7.2	33 ± 11.8	35.5 ± 9.8	25.7 ± 8.2
Olfactory investigation frequency	30.1 ± 2.1	25.6 ± 2.6	23.9 ± 2.4	24.6 ± 3.2	28.4 ± 2.2	27.9 ± 3.07	32.4 ± 3.1	30.5 ± 2.6
1 st hop and dart latency (sec)	74 ± 24 *	45 ± 12	77 ± 17	130 ± 58	148 ± 35	297 ± 95	57 ± 19	70.4 ± 21
Hops and darts	37.1 ± 5.6	35.5 ± 4.5	31.4 ± 5.4	30.9 ± 5.2	37.6 ± 8.6	24.4 ± 5.4	36.1 ± 5.8	38.5 ± 7.1
1 st solicitation latency (sec)	161 ± 32	167 ± 43	433 ± 98	239 ± 109	517 ± 181	496 ± 165	362 ± 129	285 ± 110
Solicitation frequency (§)	14.6 ± 2.9	11.1 ± 2.5	12 ± 3.1	16.8 ± 3.3	15.1 ± 2.6	11.2 ± 2.4	11.4 ± 2.4	15.9 ± 4.3
1 st Mount latency (sec)	407 ± 145	420 ± 159	271 ± 77	328 ± 78	494 ± 137	195 ± 76	369 ± 104	319 ± 82
Mount frequency	8.1 ± 2.5	8.5 ± 2.07	9 ± 2.03	6.08 ± 1.7	6.8 ± 1.98	7.6 ± 2.2	3.6 ± 1.2	7.2 ± 2.4
1 st Intromission latency (sec)	190 ± 78	378 ± 157	145 ± 31	220 ± 49	351 ± 125	233 ± 129	448 ± 178	355 ± 169
Intromission frequency	13.5 ± 2.8	7.6 ± 2.13	9.6 ± 1.8	7.8 ± 1.5	7.7 ± 1.4	6.9 ± 1.9	6.7 ± 2.2	8.1 ± 2.05
1 st Ejaculation latency (sec)	740 ± 147	536 ± 126	691 ± 116	363 ± 54	1093 ± 183 #	523 ± 139	990 ± 175 #	678 ± 178
Ejaculation frequency	1.4 ± 31	1.07 ± .30	1.41 ± .25	1.5 ± .35	.80 ± .29	1.3 ± .33	1 ± .30	1.25 ± .30
Choice of male for First ejaculation	8 (66 %)	4 (33 %)	3 (27.2 %)	8 (72.7 %)	2 (23 %)	7 (77 %)	4 (40 %)	6 (60 %)
Lordosis magnitude 1	5.07 ± 1.7	5.3 ± 1.4	5.6 ± 1.29	4 ± 1.12	3.2 ± .89	4.6 ± 1.8	2.3 ± .85	4.6 ± 1.7
Lordosis magnitude 2	5.8 ± 1.8	4.6 ± 1.2	5.5 ± 1.31	3.8 ± 1.19	3.6 ± 1.01	4.1 ± 1.3	2.8 ± .89	4.6 ± 1.4
Lordosis magnitude 3	11.9 ± 2.4	7.3 ± 2.1	8.7 ± 1.5	7.5 ± 1.5	8.4 ± 2.3	6.8 ± 2.17	6 ± 2.03	6.8 ± 1.6

Table 8. Conditioned partner preference test with two males, one Wistar and one Long-Evans. In Wistar-pacing groups. Females were trained to associate Wistar males with paced copulation. In Long-Evans-pacing groups females received the opposite association. Two subgroups are shown, saline- and flupenthixol-treated female. Data are means (± SEM) during the 30-min preference test. Latencies are expressed in seconds. * $p < .05$ between Wistar and Long-Evans males within groups. # $p < 0.05$ relative to its control group. § $p < 0.05$ between pacing-related males vs nonpacing-related males.

General Discussion

The results of Experiment 1 demonstrated that flupenthixol (0.25 mg/kg) disrupts olfactory conditioned partner preference towards a scented male that results from association of the odor with paced copulation. Saline-trained females displayed more solicitations towards the scented male, indicating an increase of sexual desire towards the male paired with paced copulation, as we have observed previously (Coria-Avila et al., 2007b). In contrast, flupenthixol-trained females failed to develop a preference, suggesting that the odor paired with paced copulation did not become an incentive that increased sexual desire. In fact, the number of solicitations and hops & darts toward scented males was reduced in frequency overall to a level comparable to those displayed by saline-trained females toward unscented males that had been paired with nonpaced copulation. This observation indicates that the blockade of DA transmission with flupenthixol during a female's initial experiences with paced copulation blocks either reward or incentive learning about reward-related cues, in this case, neutral odors. However, in Experiment 2, the same dose and route of administration of flupenthixol failed to disrupt the development of a conditioned partner preference for a strain of male associated with paced copulation in Wistar females. Both saline- and flupenthixol-trained females expressed more solicitations towards the strain of male paired with paced copulation, indicating that both groups formed a partner preference. This suggests either that DA transmission is not necessary for the conditioning of strain cues to sexual reward, or that extracellular concentrations of DA are so high during strain conditioning that they compete favorably against the dose of flupenthixol used in this study for occupancy at DA receptors.

Female rats show a different pattern of neural activation between odors and strain cues paired with paced copulation (Coria-Avila & Pfaus, 2007a) submitted; Chapter 2). In that study, exposing olfactory-conditioned females to the conditioned odor alone on cotton gauze for 1 hr induced significantly more Fos immunoreactivity (Fos-IR) in a variety of hypothalamic and limbic regions, including the olfactory tubercle, piriform cortex, anterior cingulate cortex, NAc core, medial preoptic area (mPOA), lateral septum, paraventricular nucleus, and VTA, with trends toward significance in the striatum and arcuate nucleus, relative to females in an unpaired group. In contrast, exposing strain-conditioned females to the strain of male associated with paced copulation behind a wire-mesh screen induced significantly more Fos-IR in the piriform cortex, mPOA, ventromedial hypothalamus, and VTA compared to the male associated with nonpaced copulation. Interestingly, no differences in Fos-IR were observed in mesolimbic DA terminal regions of the olfactory tubercle, lateral septum, anterior cingulate, or NAc, nor in the striatum, between females exposed to the strain of male associated with paced versus nonpaced copulation. Because Fos activation in the NAc can be induced by DA release, for example, following a behavioral sensitizing regimen of amphetamine infusions to the VTA (Hu et al., 2002), it seems reasonable to argue that strain cues may possess biased, or unconditionally arousing, properties (e.g., male pheromones, odors, auditory cues, visual cues, etc.) that do not require mesolimbic or striatal DA turnover for conditioning.

Those results are not easy to reconcile with the fact that a male behind a screen associated with paced or nonpaced copulation induces significant DA release in the NAc (Mermelstein et al., 1995; Pfaus et al., 1995). Moreover, it is difficult to hypothesize what

the role of the VTA might be, given that it was activated similarly in both odor and strain conditioning (Coria-Avila et al., 2007a, Chapter 2). However, there are at least two differences in procedure that could account for this discrepancy. First, the females used in studies of DA release were of the pigmented Long-Evans strain, whereas albino Wistar females were used in the present strain-conditioning experiment. It is not known whether differences in strain could account for potential DA-dependent versus independent processes, although differences in sexual arousability between pigmented and albino strains have been noted previously in the ability of Long-Evans male rats to show noncontact erections in response to odor cues from females, relative to the inability of Wistar males to show this response unless treated with a serotonin synthesis inhibitor (Matsumoto et al., 1997; Sachs et al., 1994). However, we note that differences were not observed in the number of solicitations and other appetitive sexual behaviors displayed by Long-Evans and Wistar females toward pacing-related males in the present and previous studies, especially in terms of strain conditioning (Coria-Avila et al., 2006). Indeed, treatment with the opioid antagonist naloxone disrupted both odor and strain conditioning in our previous study in Long-Evans and Wistar rats, respectively (Coria-Avila et al., 2007b, Chapter 3, Part 1). This suggests that opioid activation serves as a necessary neurochemical substrate of sexual reward in both strains, further arguing against strain differences in neurochemical activation as a cause of the discrepancy.

Second, the females used in the studies of DA release during paced and nonpaced copulation did not have a sexual history in which the two types of copulation had been differentiated and confounded intentionally with a different strain of male. It may be easier to condition strain cues to sexual reward because some of the cues may be

“prepotent” in terms of activating mesolimbic DA unconditionally (e.g., pheromonal cues). In males, for example, estrous vaginal secretions activate mesolimbic DA release immediately upon their presentation in bedding or on glass slides, even in sexually naïve males (Mitchell et al., 1992; Phillips, Pfaus, & Blaha, 1991; Wenkstern, Pfaus, & Fibiger, 1993). It is possible, therefore, that the DA release necessary to induce a significant increase in Fos in the NAc is simply not activated once strain cues have been conditioned. But if strain cues are not differentiated, their prepotency in terms of activating DA release remains, making the discrepancy an interaction between reward predictability and innate DA activation by accessory olfactory cues. Indeed, Hernandez et al., (2006) demonstrated that NAc dopamine levels affect the threshold of electrical stimulation that induces brain stimulation reward. If NAc DA levels were low, then animals required a higher stimulation frequency to support instrumental lever-pressing. However, when NAc DA levels were high, lower stimulation frequencies were sufficient to induce instrumental lever pressing. To the extent that this kind of phenomenon plays a role in the generation of conditioned responses to preferred sex partners, it could be that strain cues alone induce more DA release than neutral olfactory cues prior to their association with sexual reward. This should make the association easier to learn, and perhaps less susceptible to disruption by DA antagonists.

Dopamine release in the NAc is critical for pair bonding in monogamous prairie voles. Systemic injections of the DA antagonist haloperidol, or infusions directly into the NAc, disrupt the formation of pair bonds during copulation, whereas low doses of DA agonists (i.e. apomorphine) facilitate pair bonding in the absence of copulation (Aragona, Liu, Curtis, Stephan, & Wang, 2003; Gingrich, Liu, Cascio, Wang, & Insel, 2000). DA

D2 receptors appear to be critical for pair bonding. In one study, the blockade of NAc DA with the D2 receptor antagonist eticlopride prevented the formation of pair bonding after mating, or after 24 hrs of cohabitation (Gingrich et al., 2000). In the same study, females infused with the D2 receptor agonist quinpirone, developed a pair bond towards a familiar male even during short periods of cohabitation, or without mating.

Recently, it has been shown that, D1 and D2 receptors have opposite roles in the formation of pair bonds (Aragona et al., 2006). Activation of D1 receptors within the rostral shell of the NAc of male voles prevents the formation of pair bonds with a familiar partner, whereas activation of D2 receptors facilitates it. In fact, pair-bonded males show significant upregulation of D1 receptors within the rostral shell. Aragona et al. (2006) suggest that D1 receptors may function to prevent the formation of more than one pair bond in monogamous species. Indeed, that study showed that polygamous meadow voles (which do not pair bond) express more D1 receptors in the NAc relative to monogamous voles.

Rats are traditionally regarded as polygamous (McClintock et al., 1982; McClintock, 1984a). Their relative inability to display long-lasting and selective pair bonds may depend on the same D1-D2 interactions as in polygamous meadow voles, although conditioning as we have examined it here may alter this interaction. It is possible that the increase of DA during paced copulation in a naïve female activates both D1 and D2 receptors, which could decrease the likelihood of female rats acquiring an immediate conditioned partner preference. Indeed, a single conditioning trial is not sufficient to induce olfactory conditioned partner preference in female rats (Coria-Avila et al., 2005). However, the stimulation of D2 receptors during repeated trials may well

result in an increased likelihood of partner preference. In a polygamous species like rats, the development of immediate partner preference or pair bonds in one individual may represent a disadvantage to its reproductive fitness, relative to the other members of the species who will mate with multiple partners. Consequently, in putative polygamous species, the formation of partner preferences may take longer to develop (i.e., more than one conditioning trial). This hypothesis could be tested by examining whether infusions of a D2 agonist facilitate the development of conditioned partner preference in rats.

Finally, as in our previous studies, solicitations were the most reliable measure of partner preference (Coria-Avila et al., 2005, 2006), and the measure most sensitive to the disruptive effects of flupenthixol in Experiment 1. Solicitations represent the female's desire for sexual interaction (Pfaus et al., 2003). Solicitations and other measures of appetitive sexual behavior (e.g., hops and darts) are inhibited by lesions to the mPOA (Hoshina et al., 1994). Indeed, nose-poking through a wire-mesh in response to stud male odors is also inhibited following lesions to the mPOA (Xiao et al., 2005). The mPOA is also the terminal region of the incertohypothalamic DA system (Bjorklund et al., 1975). DA release patterns in the mPOA of males during appetitive and consummatory phases of copulation are nearly identical to those of the NAc (Blackburn et al., 1992), and the two may be linked by lateralized outputs from the mPOA to the VTA (Brackett et al., 1984). We have found recently that infusions of flupenthixol to the mPOA block the facilitation of solicitations induced by systemic administration of the melanocortin agonist bremelanotide (Gelez, Jacobovich, & Pfaus, submitted), indicating that DA transmission in the mPOA may play a much larger role in the control of appetitive female sexual behaviors than previously considered. Opioids regulate the sexual behavior of

male rats in the mPOA (Band & Hull, 1990; van Furth, van Emst, & Van Ree, 1995), and infusions of the opioid antagonist naloxone to the mPOA blocks the induction of ejaculation-induced conditioned place preference (Ågmo et al., 1993). If the same neurochemical systems exist in the female, the mPOA may well serve as a site in which appetitive sexual responses are linked to opioid-induced sexual reward, ultimately activating DA release to initiate sexual responses toward sexual incentives.

Summary of Chapter 3

Chapter 3 examined the effects of opioids and dopamine blockade on the development of conditioned partner preference. Both, olfactory conditioned and strain conditioned females treated with the opioid antagonist naloxone before every conditioning trial failed to develop a conditioned partner preference, indicating that opioids are required for this conditioning. The dopamine antagonist flupenthixol given before every conditioning trial blocked the olfactory but not the strain conditioning, suggesting that dopamine is required only when the conditioned cue is originally neutral (i.e. almond odor), relative to strain cues that may be naturally more attractive to females.

CHAPTER 4

Activation of Oxytocin, Vasopressin or Gonadotrophin-Releasing Hormone (GnRH) Neurons by Strain Cues Associated with Conditioned Partner Preference in Female Rats

Genaro A. Coria-Avila and James G. Pfaus

Preliminary data

Abstract

The present study examined whether strain cues associated with paced copulation activate oxytocin (OT), arginine vasopressin (AVP), or gonadotrophin-releasing hormone (GnRH) neurons. Two groups of ovariectomized, hormone-primed Wistar (W) rats were trained to associate W or Long-Evans (LE) males with paced or nonpaced copulation in a counterbalanced fashion. A final preference test indicated that females developed a preference for pacing-related males. Females were then exposed for 1 hr to either pacing-related or non pacing-related males behind a screen that allowed visual, acoustic, and olfactory stimulation, but prevented tactile stimulation, after which their brains were processed for double immunocytochemical labeling of Fos within OT, AVP, or GnRH neurons. Results indicated trends for greater Fos-IR within OT in the PVN and greater Fos-IR in GnRH neurons in the anterior POA of females exposed to strain cues associated with paced relative to nonpaced copulation. Activation of OT neurons may be a mechanism by which cues associated with paced copulation come to direct partner preferences, whereas activation of GnRH neurons could be an additional mechanism by which sexual behavior is facilitated, one that may also indicate a greater potential for reproductive success with preferred males.

Keywords: Pavlovian conditioning, Sexual behavior, Pacing, Hormones, Reproduction

Introduction

There is evidence indicating that conditioned stimuli (CSs) associated with sexual reward can affect many aspects of sexual behavior by inducing neural activity and endocrine responses similar to those that would occur during exposure to unconditioned stimuli (UCSs) such as odors or pheromones from potential mates (e.g., Graham & Desjardins, 1980; Kippin, Cain, & Pfaus, 2003). The ability of a neutral stimulus, like an odor, to activate a neural representation of sexual reward involves mechanisms of Pavlovian conditioning, in which the neutral CS is paired in time and in neural activation with the rewarding UCS (Pavlov, 1927). Conditioned responses that occur to the CS are typically in the same direction as those induced by the UCS. Thus the CS acts as a “priming” stimulus that predicts the onset of the UCS, and accordingly, activates an identical behavioral outflow, or behaviors that are engaged in anticipation of the reward.

Conditioned partner preference is a type of conditioned response, in which animals choose a mate depending on CSs that help them to predict a potential sexual outcome based on previous sexual encounters. For example, male rats that are trained to associate a neutral odor (i.e. almond) on a female during copulation can develop a conditioned ejaculatory preference (CEP) for scented females during subsequent partner preference tests, but this does not occur if the odor was paired with unreceptive females during training (Kippin et al., 1998; Kippin et al., 2001a; Kippin et al., 2001b).

Furthermore, exposure to sexually-conditioned cues can induce neural activation that did not occur before learning as revealed by the expression of the immediate early-gene *c-Fos*. Specifically, Kippin, Cain, & Pfaus, (2003) demonstrated that the same olfactory CS that facilitated CEP in males of a Paired group, induced more neural activation as

assessed by Fos immunoreactivity (IR) in the piriform cortex (PirCtx), basolateral amygdala, anterior lateral hypothalamus, and nucleus accumbens (NAc) core, compared to males in the Unpaired group.

In females, similar mechanisms of Pavlovian conditioning have been reported. For example, CSs such as almond odor (Coria-Avila et al., 2005) or the features of a strain of male (Coria-Avila et al., 2006) paired with paced copulation can induce conditioned partner preference for males that bear the CS, similar to the CEP that occurs in males towards scented females. Furthermore, females exposed to CSs (odor or strain cues) alone express greater Fos-IR in three common areas that are the ventral tegmental area (VTA), the PirCtx and the medial preoptic area (mPOA) compared to expression of Fos-IR in the same areas following exposure to CSs paired with nonpaced copulation (Coria-Avila & Pfaus, submitted; Chapter 2). Despite these similarities, different patterns of Fos induction occur following exposure to conditioned olfactory versus strain cues. Although initially, neutral odors have less incentive value compared to strain cues of a male, once conditioned they induce Fos throughout the hypothalamus and limbic system, including the olfactory tubercle, NAc core, anterior cingulate cortex, paraventricular nucleus (PVN), and lateral septum, compared to the Fos-IR induced by the same odors associated with nonpaced copulation or by strain cues. In contrast, conditioned strain cues activate the ventromedial hypothalamus (VMH) compared to strain cues associated with nonpaced copulation or with conditioned olfactory cues.

Putative monogamous rodents, such as the prairie vole, develop pair bonds following mating (Williams et al., 1992). Such pair bonds can be considered a form of conditioned partner preference because its formation depends on associating cues of the

partner with the reward induced by the first copulation. The neurochemical substrate of pair bonding depends on a neurochemical cascade that includes the activation of opioids, dopamine, oxytocin (OT), and arginine vasopressin (AVP) (Young et al., 2001; Young et al., 2004). The latter two appear to be critical in the formation of partner preferences in female and male prairie voles, respectively, and are produced mainly in neurons in the PVN and supraoptic nucleus (SON) (Swaab, Pool, & Nijveldt, 1975). OT mediates pair bonding behavior via its receptors in the nucleus accumbens and prefrontal cortex, whereas AVP appears to activate receptors in the ventral pallidum and LS (Young et al., 2004). OT in particular has been extensively reported as critical for social recognition of mate and of the offspring (for review see Bielsky & Young, 2004).

Olfactory conditioned partner preference in female rats involves an increase in solicitations, hops and darts, and lordosis with the preferred male, when females are given a choice between two sexually vigorous males, one scented and one unscented (Coria-Avila, et al., 2005; Chapter 1, Part 1). Conditioned partner preference by strain also involves an increase in solicitations and lordosis made toward the strain of male associated with paced copulation (Coria-Avila et al., 2006; Chapter 2, Part 2). Among the neurochemical systems involved in an increase in sexually proceptive and receptive behavior, gonadotrophin releasing hormone (GnRH) is known to increase sexual receptivity by central actions (Moss & McCann, 1975; Sakuma & Pfaff, 1983), 1983) while inducing ovulation by its signaling of luteinizing hormone release from the pituitary (Greep, 1973; McCann et al., 1973). However, GnRH administration in estrogen-primed females does not facilitate their preference for a sexually active male over a castrated male (Dudley & Moss, 1985), suggesting that sexual preference and

solicitation behaviors, sexually receptive behaviors such as lordosis, and ovulation, are separate processes that require at least the coordinated activation of OT, AVP, and GnRH.

Given the role of OT and AVP in mating-induced partner preference and mate recognition in prairie voles, and the increase in sexual behavior of a variety of species by GnRH, two experiments were conducted to examine whether strain cues paired with paced copulation would conditionally activate OT, AVP, and/or GnRH neurons. Experiment 1 examined whether strain cues paired with paced copulation could activate more Fos-IR within OT or AVP neurons of the PVN and SON. Experiment 2 examined the same phenomenon in GnRH neurons.

General Methods

Subjects and surgery

Wistar (W) and Long-Evans (LE) males (300-350 g) and W female rats (200-250 g) were purchased from Charles River Canada (St-Constant, QC). Males were housed in groups of four in Plexiglas cages and females were housed in groups of 4 in large wire mesh cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21°C. Commercial food and water were continuously available.

Females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then ovariectomized (OVX) bilaterally via a lumbar incision. Post-surgical treatment included

single subcutaneous injections of Flunixin meglumine 2.5 mg/kg (Banamine®) for analgesia, and Enrofloxacin 5 mg/kg (Baytril®) to prevent post surgical bacterial infections. All females were given a week of post-surgical recovery prior to conditioning trials. Male rats were sexually experienced. They initiated copulatory activity with females within 15 sec of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all ovariectomized females by subcutaneous injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test.

Conditioning

The experiment required females to associate one strain of male with paced copulation and the other with nonpaced copulation. Two groups were formed, in the Wistar-pacing (W-P) group females copulated with W males in the paced condition and LE males in the nonpaced condition. Females in the LE-pacing (LE-P) group copulated with LE males in the paced condition and W males in the non-paced condition. Conditioning trials occurred at 4-day intervals during the middle third of the rat's dark circadian cycle following hormone priming. Paced copulation occurred in semicircular chambers (38² x 60 x 38 cm) bisected by a clear Plexiglas divider with 4 holes cut into the bottom (4 x 4 cm) that rested on bedding (Pfaus, Kippin, & Coria-Avila, 2003). These holes were large enough for the female to crawl through, but too small for the males to pass through. Nonpaced copulation occurred in the same chambers, but without the divider. Females received a total of 10 sequential experiences of 30-min each in paced and nonpaced conditions at 4-day intervals (5 each). The order of condition for the first conditioning trial was counterbalanced so that half of each group started with paced

condition and the other half with nonpaced copulation. Animals were allowed to copulate for 30 min before being taken back into their home cages.

Copulatory preference test

Four days after the 10th conditioning trial, each female was placed into a large open field (123 x 123 x 46 cm) with a thin layer of woodchip bedding. At two diagonal corners of this open field were two males, one W and the other LE. Each male wore a rodent jacket connected on the back to a spring wire 30 cm in length. This allowed the males to roam freely within a 45 cm radius. The females were placed in one neutral corner of the open field and allowed to copulate freely with both males for a 30-min period. These tests were videotaped and scored. The results of the behavioral test were reported in Chapter 1, Part 2 (Coria-Avila et al., 2006), and confirmed that regardless of the strain, females developed a conditioned partner preference for pacing-related males.

Experiment 1: Exposure to strain cues on Fos activation in OT and AVP neurons

Procedure

Four days after the behavioural test, 20 W females were hormone-primed with estradiol benzoate (10 µg) and progesterone (500 µg) S.C. 48 and 4 hrs, respectively, before the copulatory session. According to conditioning schedule, females were placed in one side of a two-compartment chamber, divided by a wire mesh that allowed visual, olfactory and acoustic stimulation from a male placed in the other side, but not tactile stimulation nor copulation. Some females were exposed to the strain of male associated with paced copulation and other females were exposed to the male associated with nonpaced copulation. This left four groups, the W-P (n=6) in which females were trained

to associated a W male with paced copulation; the LE-NP (n=6) in which LE males were associated with nonpaced copulation; the W-NP (n=5) in which females were trained to associate a W male with nonpaced copulation; and LE-P (n=3) in which LE males were associated with paced copulation. After 1 hr females were overdosed with sodium pentobarbital (120 mg/kg I.P.) and perfused intracardially with 350 ml of phosphate-buffered saline followed by 350 ml of 4% paraformaldehyde. The brains were removed and post fixed in 4% paraformaldehyde for 4 h and stored overnight in 30% sucrose solution. The brains were then covered in aluminum foil and stored at -80 °C.

Serial frozen coronal brain sections (30µm) that correspond to plates 22 to 25 of Paxinos & Watson, (1998) were cut from each brain on a sliding microtome. They were incubated sequentially with 30% w/w hydrogen peroxide (H₂O₂) in Tris-buffered saline (TBS) for 30 min at room temperature, then in 3% normal goat serum (NGS) in 0.05% Triton TBS for 90 min at 4 °C, then in rabbit polyclonal anti-Fos Ab5 (Oncogene Science, Boston, MA, USA; diluted 1:75,000) in 0.05% Triton TBS with 3% NGS for 72 h at 4 °C, then in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA; diluted 1:200) in 0.05% Triton TBS with 3% NGS for 1 h at 4°C, and then in avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; diluted 1:55) for 2 h at 4 °C. Sections were washed in TBS (3×5 min) between incubations. The immunoreactions were stained by sequential treatments at room temperature with 50-mM Tris for 10 min, 3,3'-diaminobenzidine (DAB) in 50-mM Tris (0.1 ml of DAB/Tris buffer, pH 7.8) for 10 min, DAB/3% H₂O₂ in 50-mM Tris for 10 min, and 8% NiCl₂ (400 µl per 100 ml of DAB/Tris buffer+ H₂O₂). The sections were, then, rinsed (3x10 min in TBS) and transferred again to 3% normal goat serum (NGS) in

0.05% Triton TBS for 90 min at 4 °C. Half of the sections were incubated with rabbit polyclonal anti-body raised against OT (Chemicon International, Temecula, CA, USA; diluted 1:10,000) and half – with rabbit polyclonal anti-body raised against AVP (Chemicon International, Temecula, CA, USA; diluted 1:10,000) in 0.05% Triton TBS with 3% NGS for 18 h at 4 °C. Secondary and tertiary phases, as well as the immunoreactions were equivalent to the ones described above; however, no NiCl_2 was added in the last phase of the immunoreactions in order to generate a reddish-brown cytoplasmic reaction product (Figure 18). Sections were mounted onto gelatin-coated slides, dehydrated with ethanols and cleared in xylenes, and coverslipped. The sections were analyzed under a light microscope at 10 X magnification and counted as OT neurons with Fos and AVP neurons with Fos. OT and AVP neurons were identified in the entire area of the PVN and SON by their reddish-brown cytoplasmic color and Fos was identified as a black nucleus. The counting was blind since the observer did not know the classification of each female.

Statistical analysis

There were 20 females in total: W-P (n=6), LE-NP (n=6), W-NP (n=5), LE-P (n=3). A one-way analysis of variance (ANOVA) was used to assess the differences in Fos-IR within OT and AVP neurons between the four groups. The level of significance was set at $p < 0.05$. All significant results were followed by a Least Significant Difference (LSD) post hoc test. In addition, a partial correlation was performed between the solicitations, and double-labeled cells, controlling for group. The level of significance was $p < 0.05$.

Experiment 2: Exposure to strain cues on Fos activation in GnRH neurons

Procedure

The brains of some females of Chapter 1 Part 2 of this thesis (Coria-Avila et al., 2006), and some females of Chapter 2 (Coria-Avila & Pfaus, submitted), were used. All the females were handled, OVX, trained, sacrificed and perfused as females in Experiment 1 of this paper. Approximately 40 serial frozen sections (30 μ m) from the anterior septum, to the POA, corresponding to plates 14-19 of Paxinos & Watson (1998) were cut from each brain on a sliding microtome.

Sections were washed in Tris-buffered saline (TBS) and incubated with an affinity-purified monoclonal antibody raised against Fos protein (diluted 1:40,000) at 4° C for 48 h in 0.05% triton X-100 with 1% normal goat serum (NGS). The sections were rinsed in TBS and incubated at 4° C for 1 h with a biotinylated antirabbit IgG made in goat in 0.05% Triton X-100 with 1% NGS. The sections were rinsed and incubated at 4° C for 2 h in an avidin-horseradish peroxidase complex (vectastain Elite ABC Kit, Vector Laboratories). The sections were rinsed in 50 mM Tris buffer (pH 7.6), and rinsed for 10 min in 0.05% 3,3'-diaminobenzidine (DAB) in 50mM Tris. The sections were then incubated on an orbit shaker for 10 min in DAB/Tris buffer with 0.01% H₂O₂ added to catalyzed the DAB and 8% NiCl₂ to color the DAB chromagen product blue-black. The sections were rinsed in TBS to stop the reaction, then rinsed in TBS 0.03% H₂O₂ to react any unreacted peroxidase. The sections were then rinsed again in TBS and incubated and incubated with an affinity-purified rabbit polyclonal antibody raised against GnRH (LR1-II, diluted 1:10,000; a generous gift from Dr. R. Benoit of the Montreal General Hospital), at 4° C for 48 h in 0.05% Triton X-100 with 1% NGS. The sections were

rinsed in TBS and incubated at 4° C for 1 h with a biotinylated antirabbit IgG made in goat (diluted 1:200) in 0.05% Triton with 1% NGS. The section were rinsed in TBS, incubated as above in the vectastain Elite avidin-horseradish complex, rinsed in TBS, rinsed in 50mM tris buffer (pH 7.6) and rinsed as above in DAB. NiCl₂ was not added in order to generate a reddish-brown cytoplasmic reaction product (Figure 20). Sections were rinsed in TBS, mounted on gelatin-coated slides, dihydrated with ethanols, cleared in xylene, coverslipped, and examined under a microscope (Figure 20). In every slice all the GnRH alone and GnRH with Fos found were counted. The analysis was blind since the observer did not know the classification of each female.

Statistical analysis

There were 15 females in total. W-P (n=4), LE-NP (n=4), W-NP (n=4), LE-P (n=3). A mixed design (4 groups X 2 anterior/posterior) analysis of variance (ANOVA) was performed to assess the differences in the number of GnRH neurons, Fos alone, and GnRH neurons with Fos. The level of significance was set at $p < 0.05$. All significant results were followed by a Least Significant Difference (LSD) post hoc test. In addition, partial correlations controlling for group and bivariate correlations were carried out between solicitation frequency and GnRH cells, GnRH with Fos, and ratio. The level of significance was $p < 0.05$.

Results

Experiment 1: Fos induction in OT and AVP neurons

There was no distinction made between parvocellular and magnocellular neurons within the PVN, neither anterior nor posterior. Within this nucleus, however, most of the

double labeled cells were found in the lateral parts. Figure 20 shows the mean number + SEM of double labeled neurons for Fos within OT or Fos within AVP neurons of the PVN and SON. For the number of double-labeled Fos/OT neurons in the PVN, the ANOVA failed to detect significant differences between the groups $F(3,16)= 1.38, p=.28$. For the amount of Fos-IR cells within AVP neurons within the PVN, the ANOVA failed to detect significant differences between the groups $F(3,16)= .45, p=.71$. For the amount of Fos-IR cells within OT neurons within the SON, the ANOVA failed to detect significant differences between the groups $F(3,16)= .97, p=.42$. For the amount of Fos-IR cells within AVP neurons within the SON, the ANOVA failed to detect significant differences between the groups $F(3,16)= .79, p=.51$.

The results of partial correlations revealed significant differences in the amount of solicitation and double-labeled cells by area (OT-PVN, OT-SON, AVP-PVN, AVP-SON), controlling for group (W-P, LE-NP, W-NP, LE-P). There was a positive correlation between solicitations and OT in the PVN controlling for group, $r(17)= .46, p=0.044$. There was a positive correlation between solicitations and OT in the SON controlling for group, $r(17)= .68, p=0.001$. There was no correlation between solicitations and AVP in the PVN controlling for group, $r(17)= .26, p=0.27$. There was a positive correlation between solicitations and AVP in the SON controlling for group, $r(17)= .66, p=0.002$. The analysis did not reveal significant differences between olfactory investigations and OT in the PVN, $r(N=20)= .10, p=0.67$; OT in the SON, $r(N=20)= .12, p=0.60$; AVP in the PVN, $r(N=20)= .02, p=0.92$; and AVP in the SON, $r(N=20)= .15, p=0.51$.

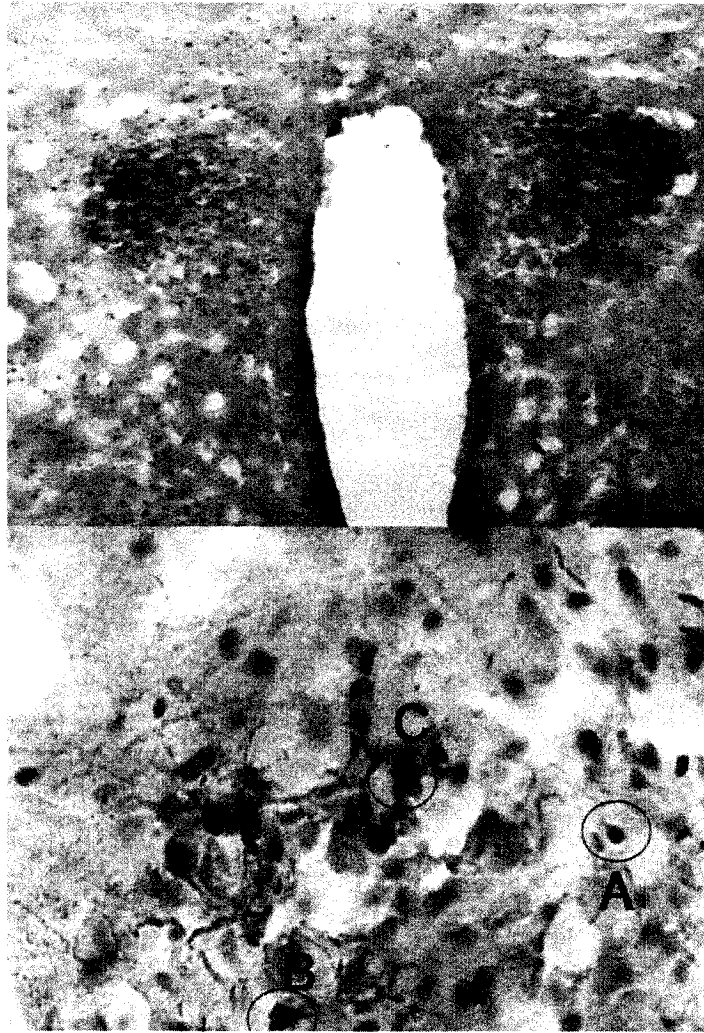


Figure 19. Upper panel: Distribution of Fos-IR and OT neurons within the PVN. Lower: Identification of neurons as (A) Fos alone; (B) OT alone, or (C) double-labeled Fos within OT neurons. OT neurons are observed with a reddish-brown cytoplasm, whereas the Fos labeling is a darker stain in the nucleus.

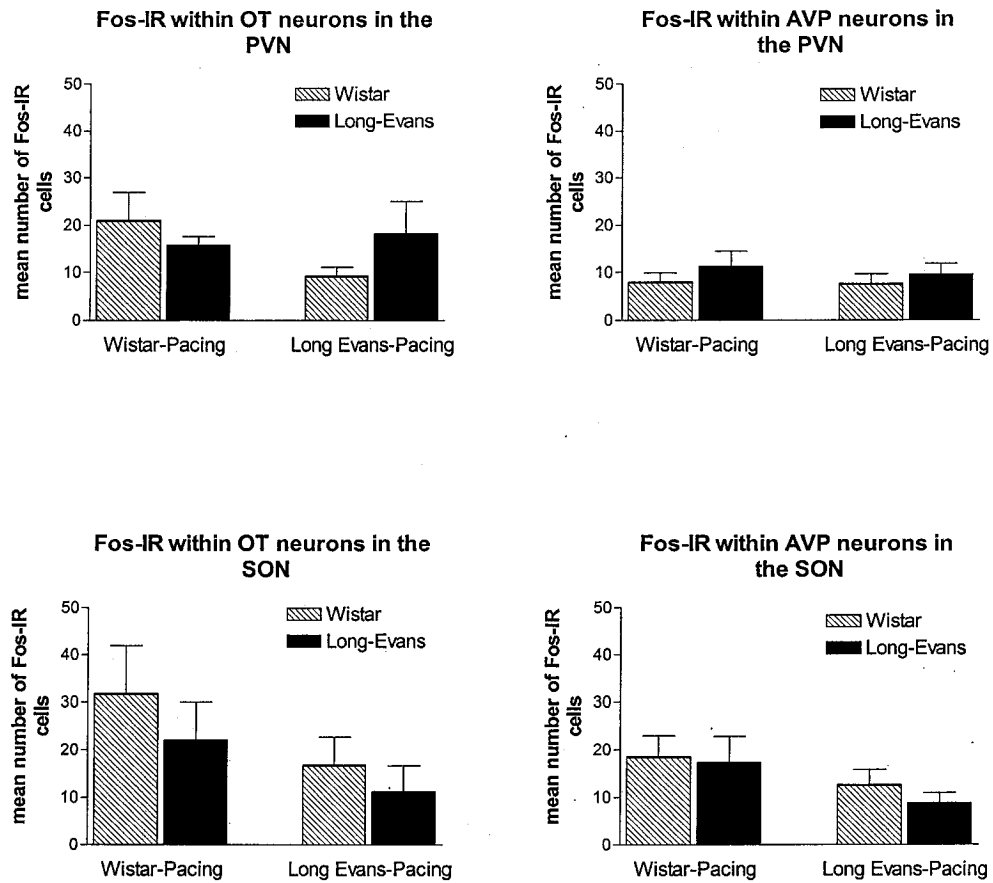


Figure 20. Mean number + SEM of Fos-IR cells within oxytocin (OT) and vasopressin (AVP) neurons of the paraventricular nucleus of the hypothalamus (PVN) or supraoptic nucleus (SON). Females in the Wistar-Pacing group were trained to associate a Wistar male with paced copulation and a Long-Evans with nonpaced copulation. In the Long-Evans-Pacing group females received the opposite association.

Experiment 2: Fos induction in GnRH neurons

A distinction was made between anterior and posterior GnRH neurons. Those found between plates 14 and 17 were classified as anterior; whereas those found between plate 18 and 19 were classified as posterior. Accordingly, the number GnRH neurons, Fos alone and GnRH neurons with Fos within anterior and posterior areas were counted (Table 10), as well as the total count of GnRH, Fos alone and GnRH with Fos, regardless of the anterior or posterior location (Figure 21).

The analysis of labeled GnRH neurons failed to demonstrate any significant difference between the groups $F(3,11) = .43, p = .73$, but indicated a main effect of area $F(1,11) = 13.3, p = .003$, indicating that overall there were more GnRH labeled neurons in the anterior area relative to the posterior. The post hoc analysis revealed that these differences were significant only within the LE-NP and within the LE-P groups. There were no interactions between group and area $F(3,11) = 1.4, p = .27$. The analysis of Fos alone failed to demonstrate any main effect of group $F(3,11) = 1.5, p = .25$, area $F(1,11) = .03, p = .84$ or interaction between group and area $F(3,11) = .12, p = .94$. The analysis of GnRH neurons with Fos indicated a trend for a main effect of group $F(3,11) = 2.9, p = .07$, and a main effect of area $F(1,11) = 13.7, p = .003$. The direction of the data indicated that there were more GnRH neurons with Fos in the posterior area than in the anterior. The post hoc analysis indicated that these differences were significant only in the LE-NP and LE-P groups.

A second analysis was made without distinction between anterior and posterior neurons. The statistical analysis failed to demonstrate significant differences in any variable, but showed interesting trends for significance (Figure 22). With regard to the

total number of GnRH neurons labeled, the ANOVA did not detect significant differences between the groups $F(3,11) = .73, p = .55$. With regard to the total number of GnRH cells that expressed Fos the ANOVA did not detect differences between groups $F(3,11) = 2.47, p = .11$. However, the ANOVA detected a trend for significance in the proportion of GnRH neurons that expressed Fos-IR between the groups $F(3,11) = 3.5, p = .053$, which suggested that GnRH neurons in Wistar females are more likely to be activated by the presence of a Long-Evans male associated with paced copulation.

There was a significant correlation between solicitations frequency directed towards the pacing male and the number of GnRH-Fos neurons in the anterior hypothalamus, $r(15) = .54, p = 0.03$. There were no significant correlations between the total solicitations frequency and total number GnRH-labeled cells controlling for group, $r(12) = -.32, p = 0.25$; for total solicitations and total number of GnRH cells with Fos, $r(12) = -.02, p = 0.93$ and for total solicitations and total ratio of GnRH cells with Fos, $r(12) = .17, p = 0.55$. In addition, the analysis did not reveal significant correlations between solicitations and ratio GnRH-Fos in the posterior hypothalamus, $r(n=15) = -.40, p = 0.61$; between solicitations and total GnRH cells with Fos in the anterior $r(n=15) = .22, p = 0.41$ or posterior hypothalamus, $r(n=15) = .04, p = 0.88$; between solicitations and total GnRH cells in the anterior $r(n=15) = -.40, p = 0.13$ or posterior hypothalamus, $r(n=15) = .05, p = 0.84$.

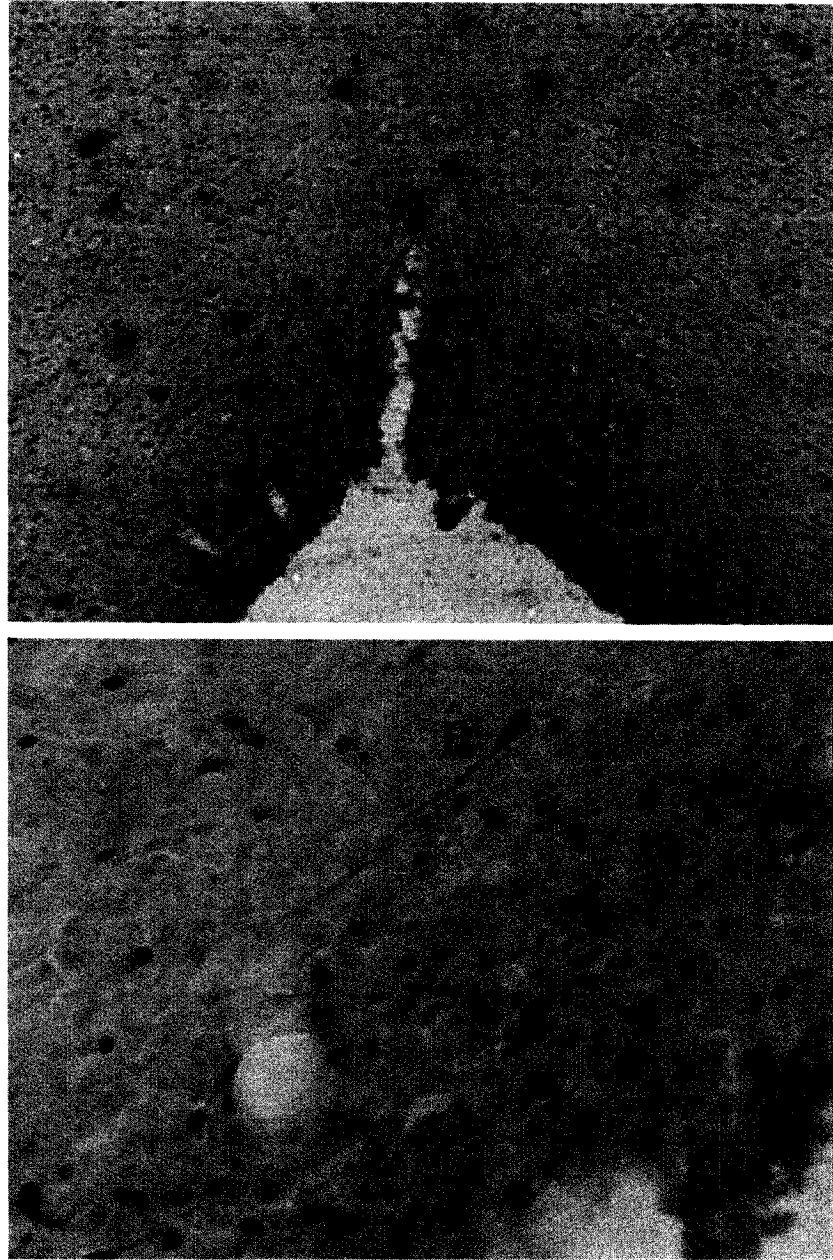


Figure 21. Fos-IR within GnRH neurons of the organum vasculosum of the lamina terminalis (OVLT) in the anterior preoptic area. Upper panel: Distribution of Fos-IR and GnRH neurons within the OVLT. Lower: Identification of neurons as (A) Fos alone; (B) GnRH alone, or (C) double-labeled Fos within GnRH. GnRH neurons are observed with a reddish-brown cytoplasm, whereas the Fos labeling is a darker stain in the nucleus.

Group	GnRH anterior	GnRH posterior	Fos alone anterior	Fos alone posterior	Ratio GnRH/Fos anterior	Ratio GnRH/Fos posterior
W-P	16.42 ± 1.6	12.76 ± 2.7	4.19 ± 1.1	3.94 ± .37	.26 ± .06	.33 ± .07
LE-NP	17.93 ± 4.1*	8.28 ± 1.1	3.06 ± .94	3.41 ± .47	.19 ± .04*	.43 ± .06
W-NP	11.76 ± 3.1	10.15 ± 2.0	2.46 ± .67	2.36 ± .52	.17 ± .04	.24 ± .01
LE-P	15.80 ± 4.4*	8.40 ± 1.9	3.76 ± .61	4.12 ± .98	.24 ± .03*	.45 ± .02

Table 9. Mean number + SEM of GnRH neurons, Fos alone and GnRH neurons with Fos from the anterior septum to the preoptic area. Anterior refers to those neurons counted between plates 14 and 17, and posterior refers to neurons counted between plates 18 and 19 of the Rat Brain Atlas (Paxinos et al., 1998). W-P, females exposed to a Wistar male associated with paced copulation; LE-NP, females exposed to a Long-Evans male associated with nonpaced copulation; W-NP, females exposed to Wistar male associated with nonpaced copulation; LE-P, females exposed to Long-Evans male associated with paced copulation. * $p < 0.05$ anterior vs posterior within groups.

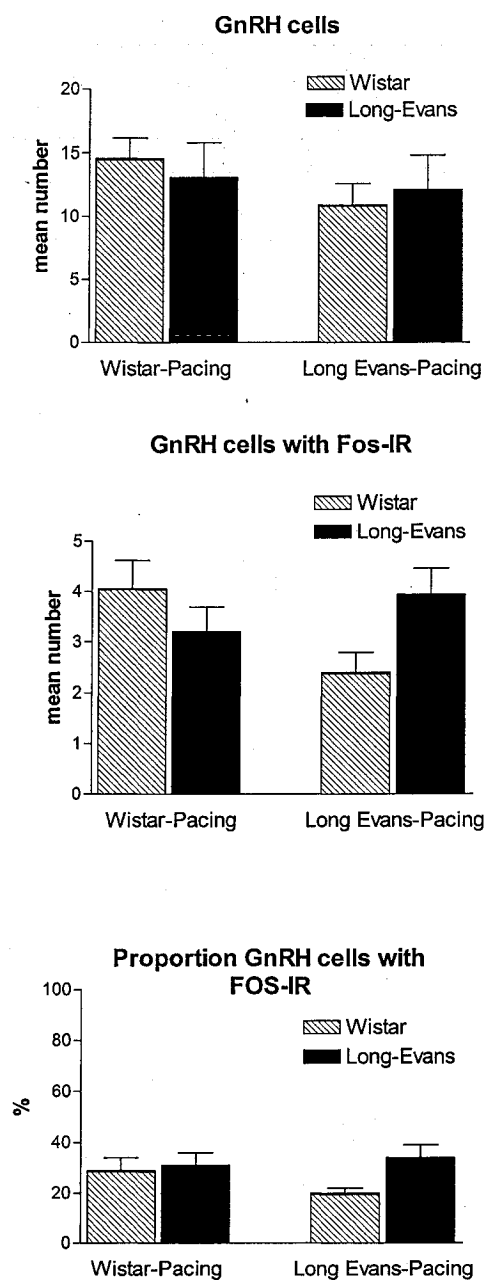


Figure 22. Mean number + SEM of Fos-IR cells within Gonadotrophin-Releasing Hormone neurons of the organum vasculosum of the lamina terminalis, through the anterior septum, to the mPOA. Females in the Wistar-Pacing group were trained to associate a Wistar male with paced copulation and a Long-Evans with nonpaced copulation. In the Long-Evans-Pacing group females received the opposite association.

Discussion

Exposure of female rats to strain cues associated with paced copulation produced significant correlations between Fos induction in OT neurons in the PVN and SON with prior numbers of solicitations made toward the pacing-related male. A similar correlation was found between Fos induction in GnRH neurons in the anterior preoptic area with prior solicitations. No correlations were found with Fos induction in AVP neurons and prior solicitations. Although differences in the actual number of double labeled Fos-OT neurons between groups in the PVN and SON did not reach statistical significance, according to the direction of the data, it is possible that a larger group size might have revealed significant differences in Fos/OT neurons between groups in the PVN. A trend toward significance was observed in total numbers of double-labeled Fos-GnRH neurons, suggesting a more pronounced effect of conditioned activation of these neurons by strain cues associated with paced copulation.

Females from the Wistar-pacing group exposed during 1 hr to Wistar males appeared to express more Fos-IR in OT neurons than females of the same group exposed to a Long-Evans male associated with nonpaced copulation (Fig. 20). In contrast, females from the Long-Evans-pacing group exposed to a Wistar male appeared to express less Fos-IR than females of the same group exposed to a Long-Evans male paired with paced copulation. The direction of the bars also resembles the amount of solicitations that conditioned females commonly perform towards preferred males associated with paced copulation. This was confirmed by a positive correlation between the solicitations frequency and the amount of OT+Fos cells in the PVN, OT+Fos cells in the SON and AVP+Fos cells in the SON, but not between solicitations and AVP+Fos cells in the PVN.

A large body of research shows that OT facilitates the formation of pair bonds following mating in monogamous female prairie voles (Carter, Williams, Witt, & Insel, 1992; Insel, Winslow, Wang, Young, & Hulihan, 1995; Williams, Insel, Harbaugh, & Carter, 1994). Females given an OT antagonist fail to develop mating-induced pair bonding, whereas OT agonists induce pair bonding even in the absence of mating (Insel & Hulihan, 1995).

In female rats copulation activates Fos within OT neurons in the PVN (Flanagan, Pfaus, Pfaff, & McEwen, 1993), suggesting that OT levels are elevated during copulation. In fact, there is evidence indicating that in humans the levels of plasma OT increase during sexual stimulation (Carmichael et al., 1987). It is possible that such increase is part of the neurochemical substrate of sexual reward, since administration of OT in rats appears to induce sufficient reward to induce CPP (Liberzon, Trujillo, Akil, & Young, 1997). Accordingly, it is possible that OT within the PVN mediates the formation of conditioned partner preference in female rats towards a pairing-related male following sexual reward.

Another possibility is that OT mediates social recognition. For example, both OT (Ferguson et al., 2000) and AVP knockout mice (Bielsky, Hu, Szegda, Westphal, & Young, 2004) fail to develop social memory compared to wild type mice, although they have no impairment in normal olfaction. OT treatment fully restores social recognition in the OT knockout mice, and when they are exposed to a conspecific they express much less Fos-IR in the medial amygdala, and its projections, compared to wild type mice (Ferguson, Aldag, Insel, & Young, 2001). Accordingly, we had the alternate hypothesis that Fos-IR within OT neurons of the PVN reflected a neuroendocrine response during

social recognition. However, not significant correlations were detected between the frequency of olfactory investigations and OT and AVP in neither the PVN nor in the SON. Therefore, it is possible that the observed trends revealed sexual motivation and not olfactory recognition.

In our data, the low number of animals in the LE-P group ($n=3$) may have contributed to the large differences observed between individuals. Thus, it is possible that an increase in the number of animals in that group may clarify whether or not OT neurons of the PVN are differentially activated by cues associated with paced vs nonpaced copulation. Nevertheless, given the general role of OT and the direction of our data, we are cautiously optimistic that OT in the PVN might play a role in the conditioned partner preference of female rats. Specifically, paced copulation may induce the activation of OT neurons in the PVN, which in turn, may be needed for both experiencing sexual reward and for the formation of social memory during future encounters. Subsequently, CSs associated with paced copulation may facilitate social recognition and sexual behavior through the activation of OT neurons and OT release in the PVN, mPOA, and VMH (Arletti & Bertolini, 1985; Schulze & Gorzalka, 1991).

We have previously reported that exposure to a neutral almond odor paired with the postejaculatory reward state in male rats induces Fos within parvocellular OT neurons in the PVN (Ménard et al., 2005). OT neurons in the PVN of male rats are activated by dopamine (Argiolas & Melis, 2004), and the activation of dopamine by neutral stimuli paired with reward may drive OT release in turn. It will be important to examine whether OT neurons in the PVN are activated similarly by conditioned olfactory cues in female

rats, and in both genders whether such activation is driven by a dopaminergic mechanism.

In Experiment 2, exposure to strain cues associated with paced copulation produced a trend toward more Fos induction in GnRH neurons across the four groups ($p=0.053$), with more double-labeling detected in the posterior preoptic area relative to the anterior regions. However, there was a positive correlation between the solicitations frequency towards the pacing-related male and the proportion of GnRH+Fos neurons in the anterior hypothalamus. The activation of GnRH neurons by strain cues may reflect a conditioned neuroendocrine response that could facilitate reproduction with a preferred male, similar to the results of Graham & Desjardins (1980), in which exposure to a conditioned odor paired with copulation to ejaculation was sufficient to increase the levels of LH and T in male rats, but not if the odor was unpaired. Paced copulation in bilevel chambers, and manual distributed VCS both activate Fos in GnRH neurons of female rats (Pfaus, Jakob, Kleopoulos, Gibbs, & Pfaff, 1994; Schulze et al., 1991). Future experiments should assess the effect of conditioned odors that predict paced copulation on the expression of Fos-IR within GnRH neurons, as well as plasma levels of LH and P, or even pregnancy rates following exposure to the CS. Such comparison will shed light on the effect of CSs associated with sexual reward on reproductive events.

Taken together, the preliminary data of Chapter 4 contributes to the idea that CSs associated with paced copulation induce conditioned neuroendocrine responses that can increase the sexual motivation towards males that bear the CSs, and at the same time may facilitate neuroendocrine responses that to aid reproduction with the preferred male. It is clear that behavioral mechanisms such as pair bonding or mate guarding facilitate sexual

exclusivity between partners. However, it is also possible that CSs that predict a positive sexual experience may facilitate reproduction. There is already evidence indicating that female rats experience paced copulation as more rewarding (Paredes & Alonso, 1997; Paredes & Vazquez, 1999), and that paced copulation produces bigger litter sizes compared to those of females that did not have the opportunity to pace their copulatory contact (Coopersmith & Erskine, 1994). These findings suggest that the neural mechanisms that mediate sexual reward during paced copulation may also enhance reproductive success.

GENERAL DISCUSSION

The research reported in this thesis was undertaken to examine whether female rats develop a conditioned partner preference for males bearing cues associated with sexual reward. Because rat mating strategies are generally described as polygamous, the display of rudiments of pair bonding, such as partner preference, indicates that mating strategies are not fixed but rather are flexible depending on what rats learn in their early sexual experience. Indeed, male rats learn to associate neutral olfactory stimuli, such as almond or lemon odor, with the postejaculatory reward state, and when given a choice between two receptive females, one scented with the conditioned odor and one unscented, conditioned males choose to ejaculate preferentially with the scented female, whereas males in unpaired or random paired groups do not (Kippin et al., 1998). Knowing that female rats find paced copulation rewarding (Paredes et al., 1997; Paredes et al., 1999), it was straightforward to ask whether olfactory or strain cues associated with paced copulation would alter appetitive and consummatory sexual behavior and partner preference in the female.

Chapter 1 demonstrated that the association of a neutral olfactory stimulus (almond odor) on a male, or strain cues (pigmented versus albino) of a male, with the sexual reward induced by paced copulation induces the development of conditioned partner preference in female rats for that male. Such partner preference was commonly expressed with a higher frequency of solicitations toward the preferred male, relative to females in unpaired or random paired groups that did not develop a preference. However, in some studies, the conditioned preference was also expressed with shorter latency during first solicitation, more hops & darts, lordosis of higher magnitude towards the

preferred male, as well as the female choosing to receive her first ejaculation from the preferred male.

Chapter 2 revealed that exposure to the conditioned odor alone activated Fos in hypothalamic, limbic, and cortical brain regions, whereas exposure to the strain cues alone activated Fos in a subset of these regions. This suggests that, once conditioned, the cues act as “priming stimuli” to activate regions of the brain that are activated by olfactory and tactile stimuli (e.g., VCS) during paced copulation. Of these regions, the PirCtx, mPOA, and VTA were common to both odor and strain conditioning. PirCtx receives mesocortical DA inputs from the VTA, along with main olfactory and genitosensory stimuli. The mPOA receives DA inputs from the zona incerta, and in turn projects to the VTA. All three regions are innervated by opioid neurons or interneurons, suggesting the involvement of both opioids and dopamine in conditioned partner preference.

Chapter 3 examined the role of opioid and dopamine transmission in the development of conditioned odor and strain preferences. Blockade of opioid receptors by naloxone during training abolished both types of conditioned partner preference, and resulted in female rats that displayed fewer solicitations and lower lordosis magnitudes overall on the final choice test (without naloxone), suggesting that sexual activity without concomitant opioid reward results in a lack of desire for sexual interaction despite being primed fully with EB and P. In contrast, blockade of DA receptors with flupenthixol during training abolished odor conditioned partner preference, but not strain conditioned partner preference. Taken together with the results of Chapter 2, these data suggest that mesolimbic DA turnover is important for associating a truly neutral stimulus like almond

odor with sexual reward, but that strain cues which possess potentially more innately arousing properties, do not require DA turnover for conditioning.

Finally, Chapter 4 described preliminary results of a double-labeling study that found a trend toward significance in the number of Fos-labeled OT neurons in the PVN, and GnRH neurons in the caudal POA, in females exposed to strain cues associated with paced copulation relative to the same cues associated with nonpaced copulation. This suggests that the activation of OT neurons may be a mechanism by which cues associated with paced copulation come to direct partner preferences, whereas activation of GnRH neurons could be an additional mechanism by which sexual behavior is facilitated, one that may also indicate a greater potential for reproductive success with preferred males.

Solicitations as an index of sexual desire

In general, solicitation behaviors have been considered as those “species-typical behaviors displayed by the estrous female which encourage the male to mate and which regulate the pattern of copulation” (Erskine, 1989). In female rats, however, solicitations have a very specific behavioral pattern, not to be confused with other proceptive behaviors such as hops & darts (H&D), or ear wiggling (McClintock, 1984a). Thus, in spite of the fact that all of these behaviors can be referred to as “proceptive” (Beach, 1976), only solicitations were consistently increased toward males with cues associated with sexual reward in the present experiments. Solicitations are an important means by which female rats express their sexual desire and preference for a male (Pfaus et al., 2003). This may be supported by the fact that in sexually-receptive females manual flank stimulation will induce the display of lordosis, and even other proceptive behaviors such as H&Ds, ear wiggling towards the researcher’s hand. Such responses may indicate

sexual arousal and motivation, but not partner preference. Solicitations, however, are displayed exclusively within the context of copulation with a male rat. The finding that female rats that learn to associate a CS with paced copulation display higher frequencies of solicitations toward a male bearing the CS, indicates that partner preferences are based on the level of sexual desire that a male triggers in a female. In turn, levels of sexual desire reflect an expectation of sexual reward. Although female rats do not display solicitations or any other sexual behavior unless in the proper hormonal state (following the sequential actions of estrogen and progesterone), the direction of their behavior when they are in the proper hormonal state reflects their desire.

Pacing, sexual reward, conditioned desire, and reproductive success

Learning to prefer a partner based on CSs that have been paired with paced copulation may have reproductive advantages. For example, it is believed that the capacity to pace VCS facilitates sexual reward and triggers a cascade of neuroendocrine changes. In a series of studies it was demonstrated that the induction of pregnancy or pseudopregnancy in the golden hamster was related to the number and rate of intromissions that the females received (Diamond & Yanagimachi, 1968). The authors hypothesized that a proper combination of the number and rate of sexual stimulation would be crucial for successful reproduction in other species. Diamond (1970) also found that different combinations of intromission frequency, duration of each insertion, and the interval between insertions, lead to different percentages of success to induce pseudopregnancy in mice. He hypothesized that this so-called “vaginal code” revealed the reproductive needs of the female that would lead to adaptive advantages on the part of males that stimulated the females correctly. The notion of a vaginal code is important

because it suggests that female reproductive needs play themselves out in the female's behavior with individual males (as individual pacing rates or displays), behavior that is largely under the female's control. If females are allowed to initiate and control the rate of copulation, then the reproductive needs are satisfied. If not, then it is far more difficult for the females to get pregnant.

As with other rodents, pacing in rats serves a significant reproductive purpose, allowing females to control VCS at their preferred intervals. Such preferred intervals may not be only species-specific, but individual-specific, and almost always serve to distribute VCS in time. Doing so facilitates the induction of nightly prolactin surges in rats, which support the maintenance of progesterone release by the corpus luteum (Erskine et al., 1989; Matthews, Jr. et al., 1978; McClintock et al., 1978). Consequently, paced copulation is believed to be more efficient than nonpaced copulation for inducing luteal function and pregnancy (Bermant et al., 1966; Erskine et al., 1989; Lodder et al., 1976). Indeed, gonadally intact females that are allowed to pace have larger litters compared to females that do not pace (Coopersmith et al., 1994).

It is possible that the positive effects of paced copulation have an analogy in species other than rodents. For example, in a preliminary study, Paredes and Vazquez (1999) argued that women's sexual satisfaction was negatively correlated with the male's sexual control (in addition to other factors, such as economic and psychological control). This suggests that sexual gratification in women depends on the perception of being free to choose the type and amount of stimulation, contrary to the more traditional notion of "being controlled". This statement, however, applies to many species and many circumstances, since overstimulation or lack of control may induce aversion. There is no

evidence of any positive or negative neuroendocrine effect of female-controlled (paced) vs. uncontrolled (nonpaced) intercourse in humans. However, “pacing” is difficult to define in humans, and anecdotal information suggest that a vaginal code may exist in women as well, since the same type or amount of sexual stimulation will not culminate in orgasm in all women. Indeed, a woman that receives preferred sexual stimulation may be more likely to experience orgasm(s), and there is evidence that experiencing orgasm and sexual pleasure with a male partner increases the desire for pregnancy with that partner, especially if her orgasms are experienced both before and after the male’s ejaculation (Baker & Bellis, 1995; Singh, Meyer, Zambarano, & Hurlbert, 1998). Thus, the positive effects of paced copulation in female rats may be due to the combination of perceived freedom to choose, the actual type and distribution of VCSs received from the male partner, and the degree of reward experienced.

The data in rats suggest that stimuli that induce sexual reward also have positive effects on reproduction. Although there is no evidence at present that female rats experience anything similar to orgasm as it is known in humans, there are clear reproductive advantages to paced intromissions, including the induction of nightly prolactin surges (Terkel et al., 1978) that maintain progesterone secretion from the corpus luteum, which in turn facilitate the support of pregnancy until the placenta is fully formed (Freeman, 1994). However, women do not depend on sexual stimulation to ovulate and have a functional corpus luteum. In addition, the observation of bigger litter size in rats following paced copulation indicates more eggs that were released or less uterine reabsorption of blastocytes *post-implantation*. Orgasm in women has not been directly

correlated with the number of eggs that are ovulated, or with the likelihood of becoming pregnant, despite increases in the *desire* to become pregnant with a preferred partner.

It has been suggested that sexual arousal and orgasm in women accelerates sperm transport (Matteo & Rissman, 1984). This statement has been supported by studies showing the rapid presence of spermatozooids in the uterus following coitus in humans (Settlage, Motoshima, & Tredway, 1973). However, in a critical review of the evidence, Levin (2002) argued that these studies had errors in interpretation due to the methodology that was used. For example, the volunteers in the Settlage et al., study were anaesthetized, therefore could not be aroused or experience orgasm. Furthermore, the intrauterine spermatozooids were only stained, which does not indicate whether they were actually fertile. Nevertheless, Levin suggested that some of the physiological responses during arousal in women (and perhaps during orgasm as well) could aid reproduction. In particular, he has suggested an effect of vaginal “tenting”, which is observed when the inner third of the anterior wall becomes elevated, away from the posterior wall together with the uterus and cervix. According to Levin, vaginal tenting may facilitate the delay of spermatozooids transport rather than facilitate intrauterine transport. Accordingly, the delay probably occurs so that the spermatozoa have time to decoagulate from the seminal fluids, freeing large numbers at the same time. Levin also suggests that vaginal tenting keeps the cervix away from the semen pool during orgasmic contractions of the uterus, which would reduce the rapid uptake of the spermatozooids by the contentious “uterine insuck” mechanism (Levin, 2002).

Regardless of whether orgasm or other sexual rewards facilitate reproduction, they likely increase the chances that copulation will occur again. This indicates that some

aspects of sexuality including orgasms, may not be necessary for reproduction, and in fact may exist exclusively for pleasure and the consequences on learning. Levin (2002) has argued that:

“To arbitrarily refuse to recognize the duarchy of sexual arousal, and that some genital mechanisms can be more rationally explained in terms of reproduction rather than sexual pleasure, restricts rather than enhances our knowledge about female genital physiology, suggests a doctrine of censorship of thought that has no place in academic study, and implies that reproductive mechanisms (and their study) are somehow inferior to those generating sexual pleasure” (pp. 405).

It is probable that sexual reward plays a more important role in the development of preferences and social attachments, rather than a direct role in reproduction, which can occur without reward. However, conditioning can clearly affect neuroendocrine mechanisms that control both sexual behavior and reproduction. In one study, for example, Graham & Desjardins (1980) demonstrated that in male rats, an olfactory CS paired with copulation to ejaculation with a receptive female for 14 trials, elicited the same augmentation of luteinizing hormone (LH) and testosterone (T) as did exposure to a receptive female alone. LH and T are necessary for spermatogenesis and both appetitive and consummatory sexual motivation and behavior. The preliminary results in Chapter 4 of this thesis also suggest that CSs pair with sexual reward can increase the activation of GnRH neurons, and in a way that is linearly related to the number of solicitations the females display with their partners (in particular males of the strain associated with paced copulation). Rats are capable of secreting more than one egg during ovulation (Freeman, 1994), and the data of Chapter 4 suggest that they could be more likely to do this in the

presence of a preferred male. Whether the increased Fos in GnRH neurons results in increased blood titers of LH remains to be determined.

Neuroanatomical mechanisms

As mentioned above, Diamond (1970) proposed that during copulation a “comparator” or “integrator” is involved in the integration of strength, frequency, and length of intromissions, along with the interval between them. This potential integrator determines the vaginal code, and thus sets the “correct” number and intervals of VCS that result in enhanced reward and reproduction. This integrator may be formed by one or several neural structures in the spinal cord and/or brain.

Sexual stimulation before and during copulation activates at least four afferent genitosensory pathways in the female rat. Anogenital investigation and mounting by the male produces tactile stimulation of the flanks, posterior rump, perineum, and tail base, which in turn activate the pudendal nerve (Kow & Pfaff, 1973; Pfaff, 1980). As the male intromits, pressure receptors in the vagina, cervix, and uterus are stimulated, which in turn activate the hypogastric, pelvic, and vagus nerves, along with lower lumbar and sacral regions of the spinal cord (Berkley, Robbins, & Sato, 1993; Carlson & De, V, 1965; Chinapen, Swann, Steinman, & Komisaruk, 1992; Komisaruk et al., 1996). Flank and perineal stimulation trigger the lordosis reflex, a dorsiflexion of the back which raises the rump and exposes the vagina, allowing the male to gain intromission. However, VCS during intromission or ejaculation produces an immediate inhibition of lordosis and stimulation of pacing behavior, in which the female leaves the vicinity of the male, forcing him to chase her.

Neurons of segment L6 in the spinal cord of female rats show a decrease in Fos-IR following 5 paced intromissions, relative to 5 nonpaced intromissions, but this decrease is lost if females receive more intromissions, regardless of being paced or nonpaced (Lee & Erskine, 2000). This pattern of data suggests that VCS activates an inhibitory system in the lower lumbar region of the spinal cord that is itself inhibited with low amounts of paced relative to nonpaced VCS. It is not known whether this decrease allows other information to pass into the spinal cord and alter the perception of VCS. It also has not yet been demonstrated how spinal cord and brain integrate VCS following paced copulation. For conditioned partner preference to develop, paced stimulation has to be sensed as more rewarding relative to nonpaced. The sexual reward has to occur in contingency with the awareness of stimuli that are predictors of it. For example, spinal cord and brain have to integrate the copulatory stimulation and social information (e.g., chemosensory, auditory, visual, tactile) from the partner to connect the UCS with the CS, so that the CS becomes capable of triggering a conditioned partner preference.

Activation of Fos in the LS, BNST, mPOA, VMH, and MeApd, by VCS is blocked by transections of the pelvic, but not hypogastric or pudendal nerves (Pfaus, Mannit & Coopersmith, 2006). The information from VCS in rats can be sent almost directly to these brain regions via the spinohypothalamic pathway (Cliffer et al., 1991) and alter ongoing sexual behavior and neuroendocrine processes. For example, transection of both pelvic and hypogastric nerves abolishes the facilitation of lordosis that occurs with small amounts of VCS (Gomora, Beyer, Gonzalez-Mariscal, & Komisaruk, 1994). Transection of the pelvic nerve alone abolishes the induction of pseudopregnancy that occurs after moderate levels of VCS (Carlson et al., 1965), along with the

abbreviation of behavioral estrus that occurs after higher levels of VCS (Lodder et al., 1976). Bilateral infusions of lidocaine in or around the MeApd, but not the mPOA, during copulation significantly reduces the proportion of rats that become pseudopregnant (Coopersmith, Gans, Rowe, & Erskine, 1996). Bilateral infusions of the sodium channel blocker tetrodotoxin to the VMH of female rats immediately before a large number of distributed manual VCSs lengthens the time that females display lordosis and allow males to gain vaginal intromission during late phases of estrus termination, but does not affect other measures of sexual or aggressive behavior during estrus termination (Pfaus, Dobbek, & Lavoie, in preparation). A similar effect was reported following transection of the pelvic nerve alone (Emery & Whitney, 1985). Bilateral infusions of the protein synthesis inhibitor anisomycin to the mPOA of female hamsters prior to copulation with a male lengthens the lordosis durations during estrus termination relative to females that receive a control infusion of cholesterol (Ramos & DeBold, 1999). Taken together, these data indicate that stimulation of the pelvic nerve by VCS results in the activation of different hypothalamic and limbic sites that mediate different sexual and neuroendocrine responses to VCS. Although manual distributed VCS also activates the PirCtx and VTA (Pfaus et al., 1993), the pathways by which VCS activates these regions have not yet been determined.

The experiments of Chapter 2 revealed three main areas that responded significantly to odor and strain CSs paired with paced copulation. These areas are the PirCtx, the mPOA, and the VTA, which expressed more Fos-IR following exposure to odor or strain CSs that were paired with paced copulation, but not following exposure to the CS that was paired with nonpaced copulation. In a conditioned female the PirCtx may

supply information about the incentive value of odors to the mPOA, which may play a role in linking solicitations to opioid activation during sexual reward. The VTA may be activated to provide DA to areas important for motivation and decision making (e.g. NAcc, prefrontal cortex, ACC), especially when the stimulus is truly neutral (e.g., odor) compared to composite strain cues that may be prepotent. These three areas may act as a “common core” to link sexual reward to stimuli, that subsequently serve as CSs or primes that activate forward-directed, and sex-related, locomotion, although clearly other regions may participate depending on the nature of the CS.

Although the MeA overall did not express differences in Fos-IR following exposure to CSs associated with paced copulation, Erskine et al., (1997) showed that in female rats the MeApd expressed higher levels of Fos-IR during 5 or 15 paced intromissions relative to the same number of nonpaced intromissions. In addition, artificial control of the interintromission intervals (III) resulted in more expression of Fos-IR within the MeApd. The MeA may be important for detecting the differences in VCS during paced or nonpaced copulation, and perhaps plays a role in sexual reward. The MeA may interact with the mPOA to integrate the input from olfactory and sensory cortices during copulation, which results in a conditioned preference. To clarify this, future experiments will have to examine the effect of lesions in the MeA and the consequences on CPP or conditioned partner preference.

The previous findings contribute to the idea that the expression of Fos-IR following exposure to a CS is not necessarily the same expression observed following exposure to the UCS. There is more evidence of this in the research of drugs of abuse. For example, following systemic administration of drugs such as heroin (Pontieri, Calo,

Di, Orzi, & Passarelli, 1997), and cocaine or amphetamines (Graybiel, Moratalla, & Robertson, 1990), rats express more Fos-IR in areas such as the CPu, NAc, ACC, Pir Ctx, PVN, and amygdala among others. Such Fos-IR may be regarded as the effect of the UCS (drug) on those brain areas. Interestingly, exposure to a CS that has been paired with the administration of cocaine, increases expression of Fos-IR within the ACC, claustrum, LS, PVN, lateral habenula, and the amygdala, but no conditional activation is observed within the Pir Ctx, NAc, or CPu (Brown, Robertson, & Fibiger, 1992). In a different study, rats exposed to an auditory CS paired with the administration of cocaine, expressed Fos-IR in the BLA and in the mPFC, but no activation was found either in the dorsal or ventral striatum (Ciccocioppo, Sanna, & Weiss, 2001) as it was with the consumption of the drug. Thus, in spite of the fact that UCS and CS not always activate similar brain areas, there are common behavioral responses that occur during exposure to both. For example, a CS paired with cocaine can induce conditioned locomotor activity, craving and relapse. Similarly, a CS associated with paced copulation will induce sensitized behavioral responses towards a male, such as solicitations, and partner preference.

Neurochemical Mechanisms

Chapter 3 demonstrated a role of opioids and DA in the development of a conditioned partner preference. Blockade of opioid receptors readily disrupted the development of both olfactory and strain conditioned preferences, indicating that opioids are part of the reward system activated during paced copulation. In the case of DA receptor blockade, only the olfactory conditioned preference was disrupted. Those results were in agreement with the results of Chapter 2, in which exposure to the olfactory CS

increased the expression of Fos-IR cells in the VTA but also in the NAcc, which is one of several important mesolimbic DA terminal regions. However, blockade of DA receptors did not have an effect on conditioned partner preference for strain. This finding is also in agreement with the lack of significant differences in Fos-IR in the NAcc between females exposed to strain cues associated with paced versus nonpaced copulation. A further neurochemical assessment was undertaken in Chapter 4 to examine whether strain cues could activate Fos in OT, AVP, GnRH neurons. Although preliminary, the results suggested that the number of solicitations expressed toward the pacing-related male was associated with a significant induction of Fos in OT neurons of the PVN and SON, and GnRH neurons in the anterior preoptic area. No significant correlation was found for solicitations and the activation of Fos in AVP neurons.

Dopamine. Studies using microdialysis and voltametry have demonstrated that sexual behavior enhances DA release in the NAc of male (Mas, Gonzalez-Mora, Louilot, Sole, & Guadalupe, 1990; Pfaus et al., 1990) and female rats (Mermelstein et al., 1995; Pfaus et al., 1995). Becker et al. (2001) demonstrated that levels DA in the NAc of females were higher if they were allowed to pace the copulatory contact, or if the males were withdrawn from the copulatory arena by the researcher at the females' preferred intervals, compared to nonpaced copulation. During the paced copulation or preferred interval situation, the levels of NAc DA increased about 50% above the baseline 15 min after the male was introduced, and continued to increase until the end of the test, reaching approximately 250% above baseline. In the case of females that were not allowed to pace copulation, or females that paced but had a vaginal mask that prevented intromission, the levels of NAc DA never increased significantly above baseline, indicating that paced

copulatory intervals are more efficient in increasing NAc DA only when they are associated with intromissive stimulation.

In males, the NAc expresses Fos-IR following exposure to estrous odors, and both males (Kippin et al., 2003) and females (Coria-Avila & Pfaus, Chapter 2, Part 1) express Fos-IR following exposure to sexually-conditioned odors. When the NAc is pharmacologically lesioned, male rats fail to display non-contact erections and ejaculation (Kippin, Sotiropoulos, Badih, & Pfaus, 2004) and female rats are more likely to avoid any kind of sexual interaction (Jenkins & Becker, 2001). Thus, NAc activity responds to both copulatory stimulation in male and female rats, and to stimuli associated with ejaculation or paced copulation.

With the evidence of enhanced mesolimbic DA activity during copulation in rats, it was hypothesized that DA mediates the formation of mating-induced pair bonds in voles (Wang et al., 1999). Wang and colleagues demonstrated that systemic administration of a DA agonist (i.e. apomorphine) facilitated pair bond formation in female voles in the absence of mating, whereas a DA antagonist (i.e. haloperidol) disrupted the formation of pair bonds even following mating. In addition, they demonstrated that systemic injections of a D2- but not D1-receptor antagonist disrupted pair bond formation following mating, whereas a D2- but not a D1-receptor agonist facilitated pair bond formation without mating. Similar to what occurs in females, the general DA agonist apomorphine facilitates pair-bonding in male prairie voles, whereas the general antagonist haloperidol disrupts pair-bonding (Aragona et al., 2003). In addition, Aragona and colleagues (2003) demonstrated that injections of haloperidol in the NAc disrupted the facilitation of pair-bonding induced by mating or apomorphine,

whereas intra-NAc apomorphine facilitated pair-bonding in the absence of mating. Taken together, the data on olfactory conditioned partner preferences in rats and pair bonds in prairie voles suggest that NAc DA plays a critical role in the motivational process that leads to this type of affiliative behavior. Ejaculation alone does not activate the NAc in rats, and in fact decreases extracellular concentrations of DA (Blackburn et al., 1992) by a serotonin-driven mechanism in the lateral hypothalamus (Lorrain, Riolo, Matuszewich, & Hull, 1999). Therefore, the rewarding properties of ejaculation and paced copulation are not exclusively associated with NAc DA activity.

The research on the neurochemical actions of drugs of abuse suggests some intriguing similarities between pair-bonding and drug-taking. For example, studies with voltammetry (Ng, Hubert, & Justice, Jr., 1991) and microdialysis (Hernandez & Hoebel, 1988) indicate that systemic administration of psychomotor stimulant drugs such as cocaine enhance DA activity within the NAc of rats. However, intra-NAc cocaine does not induce CPP (Hemby, Jones, Justice, Jr., & Neill, 1992), although it induces conditioned locomotor activity. Other drugs of abuse such as amphetamines also enhance DA activity in the NAc (Hernandez, Lee, & Hoebel, 1987), and unlike intra-NAc cocaine, injections of amphetamines directly into the NAc induce CPP (Hemby et al., 1992). Furthermore, the amphetamine-induced DA increases in the NAc have been correlated with reports of euphoria in humans (Drevets et al., 2001), indicating that DA release within this area is correlated with a state of reward that can be conditioned to environmental stimuli. Furthermore, studies on conditioned reinforcement have demonstrated that intra-NAc injections of D1- and D2-receptor antagonists completely block the reinforcing effects of amphetamine (Wolterink et al., 1993) and systemic

injections of D2 agonists induce CPP, whereas D1 agonists induce place aversion (Hoffman & Beninger, 1988). Thus, if intra-NAc injections of amphetamine can induce CPP, indicative of reward, it is also possible that the intra-NAc injections with apomorphine or D2-like agonists also induce a state of reward of sufficient intensity for male and female prairie voles to display a conditioned pair-bonding in the absence of mating (Liu et al., 2003; Wang et al., 1999; Aragona et al., 2003). It is not yet known whether administration of DA agonists, systemically or to the NAc, facilitate the development of conditioned partner preference in female rats in the absence of paced copulation. Although DA release patterns in the striatum of female rats are greater during paced compared to nonpaced copulation (Mermelstein et al., 1995), virtually nothing is known about DA receptor activation in the striatum and the sexual behavior of female rats.

The direct association of DA and reward is still a debated question. Some researchers have suggested that DA mediates craving during addiction by enhancing how much an individual “wants” the drug, without affecting the hedonic properties of drugs, or how much an individual “likes” the drug effect (Berridge et al., 1998). Accordingly, drugs of abuse will render an individual addicted by sensitizing mesolimbic DA release, and thus induce abnormal levels of “wanting” the drug (craving) without increasing the actual drug reward or “liking”. In fact, there is evidence in humans indicating that drugs of abuse such as morphine will increase craving and drug-seeking without a noticeable hedonic effect of the drug (Lamb et al., 1991). Given this evidence, it is also possible that the formation of pair bonds that follow apomorphine or D2 agonists in the absence of mating occur without a noticeable effect on reward in prairie voles.

Another brain area in which DA may be important for sexual behavior is the mPOA. The mPOA is a terminal region of the incertohypothalamic DA system (Bjorklund et al., 1975). The pattern of DA release in the mPOA of male rats during appetitive and consummatory phases of copulation are nearly identical to those of the NAc (Blackburn et al., 1992), and the two may be linked by lateralized outputs from the mPOA to the VTA (Brackett et al., 1984). D1 and D2 receptors in the mPOA play opposing roles in males, with D2 receptor activation facilitating erections, and D1 activation facilitating ejaculation (Hull et al., 1992). Copulation in female rats also increases DA release in the mPOA (Matuszewich et al., 2000), and infusions of the DA receptor antagonist flupenthixol to the mPOA abolishes the facilitation of solicitations by the melanocortin agonist bremelanotide (Gelez, Jacobowitz, & Pfaus, 2006; in preparation). Lesions of the mPOA abolish solicitations and other measures of appetitive sexual behavior such as hops and darts, along with attention to stud male odors (Hoshina et al., 1994; Xiao et al., 2005). Although the role of DA in the mPOA of females has not been examined in detail, it is possible that it mediates appetitive sexual behaviors linked to sexual reward.

Oxytocin and vasopressin. There is abundant evidence in prairie voles that OT and AVP facilitate the formation of pair bonds in females and males, respectively. Comparison of the distribution of OT receptors in the brain of monogamous vs. polygamous voles has revealed important differences in receptor populations for these neuropeptides (Insel & Shapiro, 1992). For example, the monogamous prairie voles express more OT receptors within the NAc. When female prairie voles are given intra-NAc injections of OT they develop pair bonds even in the absence of mating (Young et

al., 2001), and an OT receptor antagonist injected directly in the NAc disrupt the formation of pair bonds induced by mating. In the case of AVP, it has been demonstrated that monogamous and polygamous voles also differ in the distribution of AVP receptors in the brain (Wang & Young, 1997), and systemic injections of AVP facilitate pair bond formation in males voles (Cushing, Martin, Young, & Carter, 2001). Indeed, viral transfection of the V1a receptor into the ventral pallidum of polygamous meadow vole males results in the development of pair bonding during their first copulation identical to that observed in prairie vole males (Lim et al., 2004).

In rats, copulation increases Fos-IR within OT neurons in the PVN (Flanagan et al., 1993; Pfaus et al., 1997; Witt & Insel, 1994), and Chapter 4 of this thesis showed a significant correlation between the number of solicitations made toward the pacing related male and the amount of Fos-positive OT neurons in the PVN of females exposed to the CS alone. Interestingly, an almond odor paired with copulation to ejaculation in males increases the number of Fos-positive OT neurons in the PVN, compared to males in an unpaired group (Ménard et al., 2005). It has been reported that following ejaculation, the number of magnocellular cells in the PVN that express Fos increase about 50%, whereas Fos in parvocellular cells increases more than 120% (Witt et al., 1994). In male rats, injection of OT prior to their first sexual experience with almond-scented females increases the number of males that display a conditioned partner preference (Gelez et al., unpublished observations). Thus the increases in OT neuron activation in the brain may reflect a general mechanism by which conditioned cues on a partner come to elicit rudiments of pair bonding. Increases in magnocellular OT release in males may

also link peripheral actions of OT, such as facilitation of erection and ejaculation, with conditioned cues.

Oxytocin has been also associated with drug addiction. For example, in a review by Kovacs, Sarnyai, & Szabo, (1998) the effects of OT on different types of drugs of abuse were reported. Among the findings they indicated that OT blocks various symptoms of morphine withdrawal in mice. Furthermore, OT in rats decreases intravenous self-administration of heroin, and the cocaine-induced hyperlocomotion and stereotyped grooming behavior (Kovacs et al., 1998). In addition, subcutaneous injections of OT induce CPP (Liberzon et al., 1997), which indicates that can induce a state of reward to be conditioned with environmental stimuli. Again, this evidence may indicate that the conditioned ejaculatory preference in rats, and conditioned partner preference in female rats and the formation of pair bonds in female prairie voles may be the result of the rewarding properties of OT during copulation. This may help to explain how intra-NAc injections of OT facilitate pair-bonding in voles.

Systemic administration of AVP facilitates pair-bonding in male (Insel et al., 1995) but not female prairie voles. This is in agreement with our findings in Chapter 4, in which no evidence was found linking Fos induction in AVP neurons in either the PVN or SON to solicitation or any other response to the pacing-related male. Accordingly, we suggest that in the case of female rats (but perhaps true in females of other species) OT produced by parvocellular neurons of the PVN mediates conditioned partner preference induced by sexual reward.

Opioids. Opioid systems play a critical role in reward (Wise & Bozarth, 1981) and sexual function (Pfaus & Gorzalka, 1987). They can cause euphoria in humans and

are self-administered in virtually all animals. Systemic or intracerebroventricular injections of μ and δ opioid receptor agonists induce CPP, whereas κ receptor agonists induce conditioned place aversion (Mucha & Herz, 1985). Injections of morphine dose-dependently inhibit the sexual behavior of both male and female rats (Pfaus et al., 1987). Infusions of the μ agonist morphine to the mPOA reduce ejaculation latencies (Band et al., 1990), whereas infusions of the endogenous μ/δ agonist β -endorphin to the mPOA inhibits mounts, intromissions, and ejaculations in male rats (Hughes, Everitt, & Herbert, 1987; Hughes, Everitt, & Herbert, 1990). Intracerebroventricular infusions of δ opioid agonists to female rats facilitates lordosis and proceptive hops and darts (Pfaus & Pfaff, 1992), whereas infusion of μ agonists inhibits these behaviors. The effect of the δ agonist appears to occur by diffusion to the VMH (Acosta-Martinez & Etgen, 2002). In males, infusion of morphine or the κ agonist dynorphin 1-13 to the VTA facilitates mounting behavior in a dose-dependent manner (Mitchell et al., 1990), although subsequent work showed a facilitation of male sexual behavior following infusions of the κ antagonist norbinaltorphamine to the VTA (Leyton & Stewart, 1992). As mentioned in Chapter 3, Part 1, endogenous opioid actions at μ receptors in the VTA appear to disinhibit DA neurons by hyperpolarizing inhibitory GABA interneurons (Ford et al., 2006; Gysling et al., 1983; Kalivas et al., 1990; Sotomayor et al., 2005). Indeed, infusions of morphine to the VTA induce CPP (Olmstead et al., 1997), and repeated intermittent exposure of sexually naïve males to estrous vaginal secretions sensitizes DA release in the nucleus accumbens (Mitchell & Gratton, 1991), an effect that is abolished by naloxone treatment prior to exposure.

It has been demonstrated that the rewarding or aversive effects of opioids (as observed with CPP) are mediated in the VTA by facilitating or inhibiting DA release in the NAc. This is believed because the injections of the neurotoxin 6-OHDA in the NAc abolishes both, the rewarding and the aversive properties of opioids. In addition, injections of μ or δ agonist within the VTA enhance DA activity within the NAc, whereas injections of a κ agonist decrease NAc DA (DiChiara et al., 1988). Conversely, injections of μ antagonist decrease DA activity within the NAc, whereas injections of a κ antagonist increase NAc DA. The disinhibition of DA neurons by μ or δ opioids arriving from the arcuate nucleus to the VTA inhibit GABA neurons (Mucha et al., 1985a) which results in activation of VTA DA neurons which project and release DA in the NAc (Herz, 1998). Furthermore, DA neurons from the VTA to the NAc have been demonstrated to contain κ receptors in the NAc terminals, which inhibit DA release (Heijna et al., 1990).

It has been shown that the rewarding properties of ejaculation in rats is disrupted by systemic injections of the general opioid antagonist naloxone (Miller et al., 1987; Ågmo et al., 1990). Furthermore, the rewarding properties of paced copulation that induce CPP in female rats are also disrupted by naloxone (Paredes et al., 2001), as well as the conditioned partner preference that follows paced copulation in females (Chapter 3, Part 1). Interestingly, the DA antagonist flupenthixol does not disrupt the pacing-induced CPP (Garcia Horsman et al., 2004), or the pacing-induced conditioned partner preference in Wistar females (Coria-Avila et al., Chapter 3, Part 2) although it disrupts the pacing-induced partner preference in Long-Evans females (Chapter 3, Part 2). It appears that both the mPOA and VTA are regions in which endogenous opioids can modulate sexual reward, and especially drive appetitive systems related to cues associated with sexual

reward. It is noteworthy that these two regions are interconnected and were two of three that showed significant Fos activation in response to both odor and strain cues associated with paced copulation. It is possible that the increases in Fos reflect a conditioned activation of opioid release in these regions that may drive DA or other neurochemical systems downstream to initiate conditioned appetitive responses that form the basis of partner preference.

A model of conditioned partner preference

Taken as a whole, the data from the present thesis suggests a preliminary model of conditioned partner preference in the female rat (Figure 23). During the process of olfactory conditioning receptive female rats display solicitations, which indicate their desire to copulate. If they receive the appropriate stimulation (i.e. sexual reward during paced copulation) UCS-detector centers become activated [green circle]. The MeApd is sensitive to paced copulation and projects information about it to the mPOA which serves as a main integrator area. The VTA is activated to provide DA to areas important for motivation and decision making (e.g. NAcc, ACC) and motor activity (CPu, VP). ArcN release opioids to hyperpolarize inhibitory GABAergic neurons (not shown), and therefore increase DA release by the VTA. ArcN also projects α -MSH and opioids to the mPOA to facilitate solicitations and reward, respectively, and perhaps concurrently. The PVN may release OT into the mPOA to facilitate reward and bonding. Concurrently the Tu [blue circle] and PirCtx sense the CS. PirCtx projects olfactory information to the mPOA and to the NAcc to strengthen the incentive value of the CS. Exposure to the conditioned odor alone will activate the common core areas (gray boxes) which trigger motivation and integrate information [yellow circle] about males that bear the CS.

In contrast, although exposure to strain cues activates a similar set of core areas as conditioned odors (PirCtx, mPOA, VTA), but neither the NAcc nor the other mesolimbic, hypothalamic, or cortical regions are activated significantly. The DA antagonist flupenthixol does not disrupt conditioned partner preference, indicating that long-term or sustained DA release is not necessary for strain conditioning.

Taken together with other data in the literature cited in this thesis, it becomes apparent that opioids set the basic neurochemistry for sexual reward; DA and other monoamines set arousal, attention, and appetitive/consummatory behaviors; melanocortins and OT set contact, consummatory behaviors, and bonding. Paced copulation can induce a state of reward of sufficient intensity to be conditioned to cues that become predictors of sexual reward, and in turn facilitate partner preference towards males. Sexual reward during paced copulation depends on an augmentation of endogenous opioids which are required for both olfactory and strain conditioning. DA activity appear to be more required for olfactory, than for strain conditioning. Accordingly, neutral stimuli with no incentive value require DA to become predictors and modify behaviors. Strain cues may be prepotent stimuli that are naturally more relevant to females, and therefore may need less DA to become predictors.

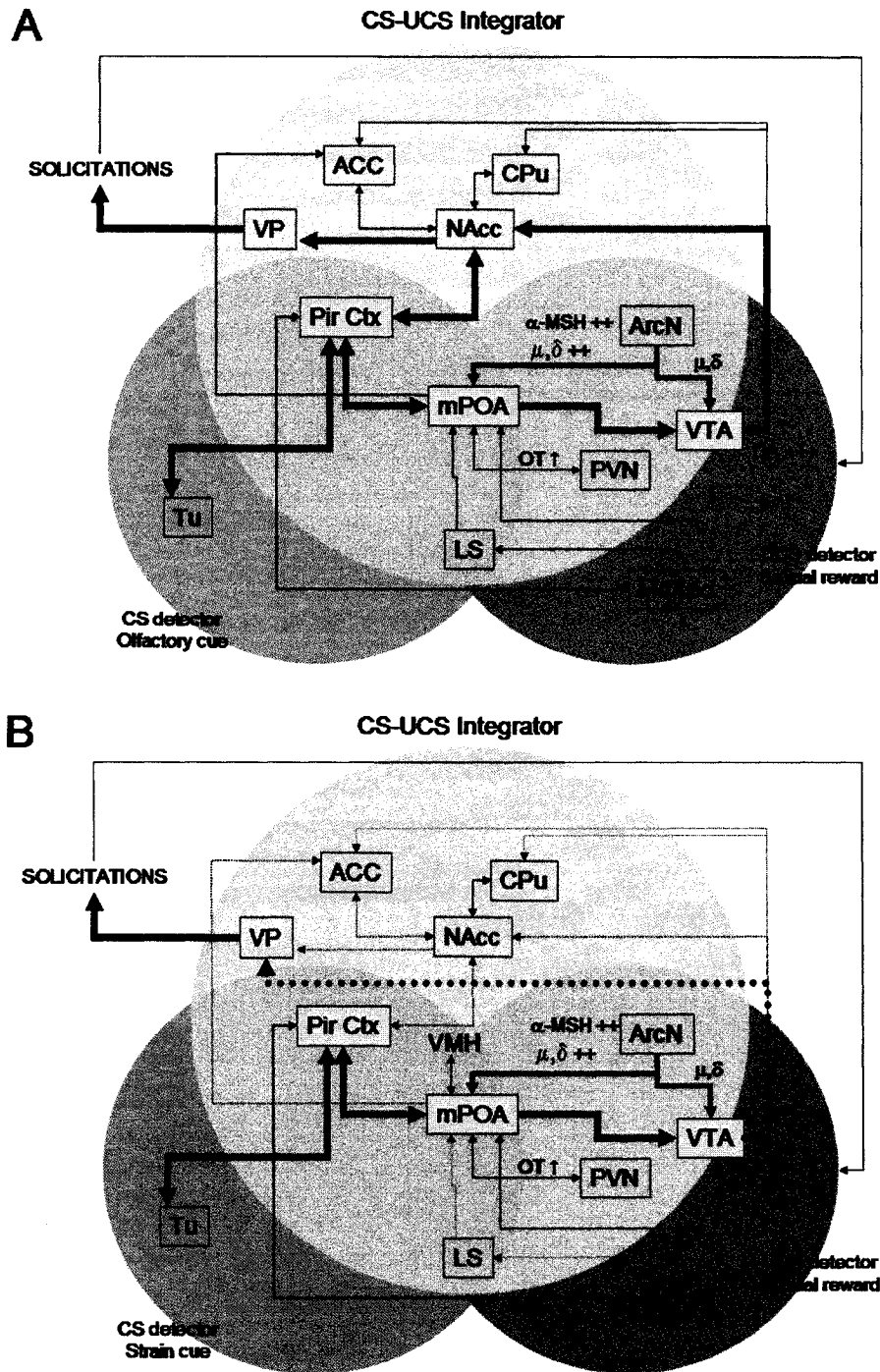


Figure 23. A model for conditioned partner preference. A. Process of olfactory conditioning. UCS sexual reward detection centers are found in the green circle. CS detection centers are found in the blue circle. Their integration comprises the yellow circle. Heavy lines represent important connections. Dotted lines represent inferred projections. B. Process of strain conditioning.

The development of partner preferences may involve the activation of mechanisms of bonding along a continuum, such that some species like rats form short-lasting preferences, whereas other species like prairie voles form long-lasting preferences. Interestingly, during orgasm in men and women, concentrations of arousing neurotransmitters, like noradrenaline, and potential bonding neurotransmitters, like OT and prolactin, increase in the cerebrospinal fluid and bloodstream (Carmichael et al., 1987; Exton et al., 1999; Kruger et al., 1998). This suggests a link between the experience of orgasm, sexual reward, and the neurochemical mechanisms of pair bonding, something alluded to in Stendhal's Principle concerning how love develops in couples that experience physical closeness after orgasm Stendahl (1821/1959), and later in John Money's concept of how "love maps" develop (Money, 1997). Partner-related cues experienced in the presence of sexual reward come to elicit a representation of that reward, and thereby become desired features that identify the partner as the beloved, or that are chosen to the exclusion of other features in subsequent partners. This process is especially potent during an animal's first sexual experiences, but may be additive throughout the lifespan, such that several love maps develop, all of which are attractive and preferred (Money, 1997). That such a phenomenon occurs in the putatively polygamous rat suggests that mating strategies are fluid and opportunistic, highly sensitive to the situations in which they are first experienced, and perhaps experienced differently by individuals that vary according to the degree of reward, attention, and bonding capacity they are born with.

Reference List

- Acosta-Martinez, M. & Etgen, A. M. (2002). The role of delta-opioid receptors in estrogen facilitation of lordosis behavior. *Behav Brain Res.*, 136, 93-102.
- Adler, N. T. (1969). Effects of the male's copulatory behavior on successful pregnancy of the female rat. *J Comp Physiol Psychol*, 69, 613-622.
- Afonso, V. M. & Pfaus, J. G. (2006). Hormonal and experiential control of female-male mounting in the female rat. *Horm.Behav*, 49, 30-37.
- Ågmo, A. (2002). Copulation-contingent aversive conditioning and sexual incentive motivation in male rats: evidence for a two-stage process of sexual behavior. *Physiol Behav.*, 77, 425-435.
- Ågmo, A. & Berenfeld, R. (1990). Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. *Behav.Neurosci.*, 104, 177-182.
- Ågmo, A. & Gomez, M. (1993). Sexual reinforcement is blocked by infusion of naloxone into the medial preoptic area. *Behav Neurosci*, 107, 812-818.
- Anderson, E. E. (1938). The interrelationship of drives in the albino rat. *Comparative Psychology Monographs*, 14.
- Aragona, B. J., Liu, Y., Curtis, J. T., Stephan, F. K., & Wang, Z. (2003). A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. *J Neurosci.*, 23, 3483-3490.

- Aragona, B. J., Liu, Y., Yu, Y. J., Curtis, J. T., Detwiler, J. M., Insel, T. R. et al. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.*, 9, 133-139.
- Argiolas, A. & Melis, M. R. (2004). The role of oxytocin and the paraventricular nucleus in the sexual behaviour of male mammals. *Physiol Behav*, 83, 309-317.
- Arletti, R. & Bertolini, A. (1985). Oxytocin stimulates lordosis behavior in female rats. *Neuropeptides*, 6, 247-253.
- Austin, D. & Dewsbury, D. A. (1986). Possible influence of strain differences on pregnancy initiation in laboratory rats. *Physiol Behav.*, 37, 621-625.
- Azar, M. R., Jones, B. C., & Schulteis, G. (2003). Conditioned place aversion is a highly sensitive index of acute opioid dependence and withdrawal. *Psychopharmacology (Berl)*, 170, 42-50.
- Baker, R. R. & Bellis, M. A. (1995). *Human sperm competition*. London: Chapman and Hall.
- Bales, K. L., Kim, A. J., Lewis-Reese, A. D., & Sue, C. C. (2004). Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. *Horm. Behav.*, 45, 354-361.
- Balfour, M. E., Yu, L., & Coolen, L. M. (2004). Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology*, 29, 718-730.

- Bals-Kubik, R., Herz, A., & Shippenberg, T. S. (1989). Evidence that the aversive effects of opioid antagonists and kappa-agonists are centrally mediated. *Psychopharmacology (Berl)*, 98, 203-206.
- Band, L. C. & Hull, E. M. (1990). Morphine and dynorphin(1-13) microinjected into the medial preoptic area and nucleus accumbens: effects on sexual behavior in male rats. *Brain Res.*, 524, 77-84.
- Bardo, M. T., Miller, J. S., & Neisewander, J. L. (1984). Conditioned place preference with morphine: the effect of extinction training on the reinforcing CR. *Pharmacol.Biochem.Behav*, 21, 545-549.
- Batenson, P. (1978). Early experience and sexual preferences. In J.B.Hutchinson (Ed.), *Biological determinants of sexual behavior* (pp. 29-53). Chichester John Wiley & Sons.
- Baum, M. J. & Everitt, B. J. (1992). Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field. *Neuroscience*, 50, 627-646.
- Beach, F. A. (1968). Factors involved in the control of mounting behavior by female mammals. In E.Diamond (Ed.), *Perspectives in Reproduction and Sexual Behavior: A Memorial to William C. Young* (pp. 83-131). Bloomington, IN: Indiana University Press.
- Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm.Behav.*, 7, 105-138.

- Beach, F. A. & Jordan, L. (1956). Effects of sexual reinforcement upon the performance of male rats in a straight runway. *J.Comp Physiol Psychol.*, 49, 105-110.
- Beck, J. (1971). Instrumental conditioned reflexes with sexual reinforcement in rats. *Acta Neurobiol.Exp (Wars.)*, 31, 251-252.
- Beck, J. & Chmielewska, J. (1976). Contact with estrus female as a reward for instrumental response in a growing male rat from the 3rd up to the 14th week of life. *Acta Neurobiol.Exp (Wars.)*, 36, 535-543.
- 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. *J Neurosci*, 21, 3236-3241.
- Berkley, K. J., Robbins, A., & Sato, Y. (1993). Functional differences between afferent fibers in the hypogastric and pelvic nerves innervating female reproductive organs in the rat. *J.Neurophysiol.*, 69, 533-544.
- Bermant, G. (1961). Response latencies of female rats during sexual intercourse. *Science*, 133, 1771-1773.
- Bermant, G., Lott, D. F., & Anderson, L. (1968). Temporal characteristics of the Coolidge effect in male rat copulatory behavior. *J.Comp Physiol Psychol.*, 65, 447-452.
- Bermant, G. & Westbrook, W. H. (1966). Peripheral factors in the regulation of sexual contact by female rats. *J.Comp Physiol Psychol.*, 61, 244-250.

- Berridge, K. C. (2006). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)*.
- Berridge, K. C. & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.*, 28, 309-369.
- Bialy, M. & Kaczmarek, L. (1996). c-Fos expression as a tool to search for the neurobiological base of the sexual behaviour of males. *Acta Neurobiol. Exp (Wars.)*, 56, 567-577.
- Bielsky, I. F., Hu, S. B., Szegda, K. L., Westphal, H., & Young, L. J. (2004). Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology*, 29, 483-493.
- Bjorklund, A., Lindvall, O., & Nobin, A. (1975). Evidence of an incerto-hypothalamic dopamine neurone system in the rat. *Brain Res.*, 89, 29-42.
- Blackburn, J. R., Pfaus, J. G., & Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. *Prog. Neurobiol.*, 39, 247-279.
- Brackett, N. L. & Edwards, D. A. (1984). Medial preoptic connections with the midbrain tegmentum are essential for male sexual behavior. *Physiol Behav*, 32, 79-84.

- Bressler, S. C. & Baum, M. J. (1996). Sex comparison of neuronal Fos immunoreactivity in the rat vomeronasal projection circuit after chemosensory stimulation. *Neuroscience*, 71, 1063-1072.
- Brown, E. E., Robertson, G. S., & Fibiger, H. C. (1992). Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *J Neurosci*, 12, 4112-4121.
- Burley, N. T. (2006). An eye for detail: selective sexual imprinting in zebra finches. *Evolution Int.J Org Evolution*, 60, 1076-1085.
- Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1997). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav.Neurosci.*, 111, 908-919.
- Cain, D. P. & Paxinos, G. (1974). Olfactory bulbectomy and mucosal damage: effects on copulation, irritability, and interspecific aggression in male rats. *J.Comp Physiol Psychol.*, 86, 202-212.
- Calhoun, J. B. (1962). The ecology and sociology of the norway rat. [U.S.Public Health Service Publication No.1008].
- Camacho, F., Sandoval, C., & Paredes, R. G. (2004). Sexual experience and conditioned place preference in male rats. *Pharmacol.Biochem.Behav*, 78, 419-425.

- Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci.Biobehav.Rev.*, 26, 321-352.
- Carlson, R. R. & De, F., V (1965). Role of the pelvic nerve vs. the abdominal sympathetic nerves in the reproductive function of the female rat. *Endocrinology*, 77, 1014-1022.
- Carmichael, M. S., Humbert, R., Dixen, J., Palmisano, G., Greenleaf, W., & Davidson, J. M. (1987). Plasma oxytocin increases in the human sexual response. *J Clin.Endocrinol.Metab*, 64, 27-31.
- Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*, 23, 779-818.
- Carter, C. S., DeVries, A. C., & Getz, L. L. (1995). Physiological substrates of mammalian monogamy: the prairie vole model. *Neurosci.Biobehav.Rev.*, 19, 303-314.
- Carter, C. S. & Getz, L. L. (1993). Monogamy and the prairie vole. *Sci.Am.*, 268, 100-106.
- Carter, C. S., Witt, D. M., Thompson, E. G., & Carlstead, K. (1988). Effects of hormonal, sexual, and social history on mating and pair bonding in prairie voles. *Physiol Behav*, 44, 691-697.

- Chinapen, S., Swann, J. M., Steinman, J. L., & Komisaruk, B. R. (1992). Expression of c-fos protein in lumbosacral spinal cord in response to vaginocervical stimulation in rats. *Neurosci Lett.*, 145, 93-96.
- Chronwall, B. M. (1985). Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides*, 6 Suppl 2, 1-11.
- Ciccocioppo, R., Sanna, P. P., & Weiss, F. (2001). Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. *Proc.Natl.Acad.Sci.U.S.A*, 98, 1976-1981.
- Cliffer, K. D., Burstein, R., & Giesler, G. J., Jr. (1991). Distributions of spinothalamic, spinohypothalamic, and spinothalamic fibers revealed by anterograde transport of PHA-L in rats. *J Neurosci*, 11, 852-868.
- Coolen, L. M. & Wood, R. I. (1998). Bidirectional connections of the medial amygdaloid nucleus in the Syrian hamster brain: simultaneous anterograde and retrograde tract tracing. *J Comp Neurol*, 399, 189-209.
- Coopersmith, C. & Erskine, M. S. (1994). Influence of paced mating and number of intromissions on fertility in the laboratory rat. *J.Reprod.Fertil.*, 102, 451-458.
- Coopersmith, C., Gans, S. E., Rowe, D. W., & Erskine, M. S. (1996). Infusions of lidocaine into the amygdala, but not the preoptic area, block pseudopregnancy in the rat. *J.Neuroendocrinol.*, 8, 259-266.

- Coria-Avila, G. A., Jones, S. L., and Pfaus, J. G.,(2003). Conditioned partner preference by strain in female rats. *Abstract viewer/Itinerary planner.Washington, DC: Society for Neurosciences Online Abstract.*
- Coria-Avila, G. A., Jones, S. L., Solomon, C. E., Gavrilu, A. M., Jordan, G. J., & Pfaus, J. G. (2006). Conditioned partner preference in female rats for strain of male. *Physiol Behav, 88, 529-537.*
- Coria-Avila, G. A., Ouimet, A. J., Pacheco, P., Manzo, J., & Pfaus, J. G. (2005). Olfactory conditioned partner preference in the female rat. *Behav Neurosci, 119, 716-725.*
- Coria-Avila, G. A. & Pfaus, J. G. (2007a). Neural activation by stimuli that predict sexual reward in female rats. *Neuroscience, submitted.*
- Coria-Avila, G. A., Pfaus, J. G., Hernandez, M. E., Manzo, J., & Pacheco, P. (2004). Timing between ejaculations changes paternity success. *Physiol Behav., 80, 733-737.*
- Coria-Avila, G. A., Solomon, C., Barbosa-Vargas, E., Lemme, I., Ryan, R., Menard, S. et al. (2007b). Disruption of conditioned partner preference in female rats by Naloxone. *Behav Neurosci., submitted.*
- Coria-Avila, G. A., Solomon, C., and Pfaus, J. G.,(2005). Disruption of conditioned partner preference in female rats by naloxone. *Abstract viewer/Itinerary planner.Washington, DC: Society for Neurosciences Online Abstract.*

- Curtis, J. T. & Wang, Z. (2003). Forebrain c-fos expression under conditions conducive to pair bonding in female prairie voles (*Microtus ochrogaster*). *Physiol Behav*, 80, 95-101.
- Cushing, B. S., Martin, J. O., Young, L. J., & Carter, C. S. (2001). The effects of peptides on partner preference formation are predicted by habitat in prairie voles. *Horm.Behav.*, 39, 48-58.
- Cushing, B. S., Yamamoto, Y., Hoffman, G. E., & Carter, C. S. (2003). Central expression of c-Fos in neonatal male and female prairie voles in response to treatment with oxytocin. *Brain Res.Dev.Brain Res.*, 143, 129-136.
- D'Udine, B. & Alleva, E. (1983). Early experience and sexual preferences in rodents. In P.Batenson (Ed.), *Mate choice* (pp. 311-327). Cambridge, UK: Cambridge University Press.
- Datiche, F., Roullet, F., & Cattarelli, M. (2001). Expression of Fos in the piriform cortex after acquisition of olfactory learning: an immunohistochemical study in the rat. *Brain Res.Bull.*, 55, 95-99.
- Denniston, R. H. (1954). Quantification and comparison of sex drives under various conditions in terms of a learned response. *J Comp Physiol Psychol*, 47, 437-440.
- DeVries, A. C., DeVries, M. B., Taymans, S. E., & Carter, C. S. (1996). The effects of stress on social preferences are sexually dimorphic in prairie voles. In *Proceedings of the National Academy of Science* (pp. 11980-11984). U.S.A..

- Dewsbury, D. A. (1969). Copulatory behaviour of rats (*Rattus norvegicus*) as a function of prior copulatory experience. *Anim Behav*, 17, 217-223.
- Diamond, M. & Yanagimachi, R. (1968). Induction of pseudopregnancy in the golden hamster. *J.Reprod.Fertil.*, 17, 165-168.
- DiChiara, G. & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc.Natl.Acad.Sci.U.S.A*, 85, 5274-5278.
- Dominguez, J. M. & Hull, E. M. (2005). Dopamine, the medial preoptic area, and male sexual behavior. *Physiol Behav*, 86, 356-368.
- Drevets, W. C., Gautier, C., Price, J. C., Kupfer, D. J., Kinahan, P. E., Grace, A. A. et al. (2001). Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol.Psychiatry*, 49, 81-96.
- Dudley, C. A. & Moss, R. L. (1985). LHRH and mating behavior: sexual receptivity versus sexual preference. *Pharmacol.Biochem.Behav*, 22, 967-972.
- Eliasson, M. & Meyerson, B. J. (1975). Sexual preference in female rats during estrous cycle, pregnancy and lactation. *Physiol Behav*, 14, 705-710.
- Emery, D. E. & Whitney, J. F. (1985). Effects of vagino-cervical stimulation upon sociosexual behaviors in female rats. *Behav Neural Biol.*, 43, 199-205.
- Erskine, M. S. (1989). Solicitation behavior in the estrous female rat: a review. *Horm.Behav.*, 23, 473-502.

- Erskine, M. S. (2005). Learning about sex: conditioning of partner preference: theoretical comment on coria-avila et Al. (2005). *Behav Neurosci.*, 119, 1136-1139.
- Erskine, M. S. (1993). Mating-induced increases in FOS protein in preoptic area and medial amygdala of cycling female rats. *Brain Res.Bull.*, 32, 447-451.
- Erskine, M. S. & Hanrahan, S. B. (1997). Effects of paced mating on c-fos gene expression in the female rat brain. *J.Neuroendocrinol.*, 9, 903-912.
- Erskine, M. S., Kornberg, E., & Cherry, J. A. (1989). Paced copulation in rats: effects of intromission frequency and duration on luteal activation and estrus length. *Physiol Behav.*, 45, 33-39.
- Eskay, R. L., Giraud, P., Oliver, C., & Brown-Stein, M. J. (1979). Distribution of alpha-melanocyte-stimulating hormone in the rat brain: evidence that alpha-MSH-containing cells in the arcuate region send projections to extrahypothalamic areas. *Brain Res.*, 178, 55-67.
- Everitt, B. J. (1990). Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neurosci Biobehav.Rev.*, 14, 217-232.
- Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. *Neuroscience*, 30, 63-75.

- Exton, M. S., Bindert, A., Kruger, T., Scheller, F., Hartmann, U., & Schedlowski, M. (1999). Cardiovascular and endocrine alterations after masturbation-induced orgasm in women. *Psychosom.Med.*, 61, 280-289.
- Fallon, J. H. & Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol*, 180, 545-580.
- Ferguson, J. N., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J.Neurosci.*, 21, 8278-8285.
- Ferguson, J. N., Young, L. J., Hearn, E. F., Matzuk, M. M., Insel, T. R., & Winslow, J. T. (2000). Social amnesia in mice lacking the oxytocin gene. *Nat.Genet.*, 25, 284-288.
- Ferster, C. B. & Skinner, B. F. (1957). *Schedules of reinforcement*. New York: Appleton-Century-Crofts.
- Fillion, T. J. & Blass, E. M. (1986). Infantile experience with suckling odors determines adult sexual behavior in male rats. *Science*, 231, 729-731.
- Fisher, H., Aron, A., & Brown, L. L. (2005). Romantic love: an fMRI study of a neural mechanism for mate choice. *J Comp Neurol*, 493, 58-62.

- 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, 352-358.
- Ford, C. P., Mark, G. P., & Williams, J. T. (2006). Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. *J Neurosci*, 26, 2788-2797.
- Freeman, M. E. (1994). The neuroendocrine control of the ovarian cycle of the rat. In E. Knobil & J. D. Neill (Eds.), *The physiology of reproduction* (Second ed., pp. 613-658). New York: Raven Press.
- French, D., Fitzpatrick, D., & Law, O. T. (1972). Operant investigation of mating preference in female rats. *J Comp Physiol Psychol*, 81, 226-232.
- Frye, C. A. & Erskine, M. S. (1990). Influence of time of mating and paced copulation on induction of pseudopregnancy in cyclic female rats. *J.Reprod.Fertil.*, 90, 375-385.
- Garcia Horsman, P. & Paredes, R. G. (2004). Dopamine antagonists do not block conditioned place preference induced by paced mating behavior in female rats. *Behav Neurosci.*, 118, 356-364.
- Gaykema, R. P., Luiten, P. G., Nyakas, C., & Traber, J. (1990). Cortical projection patterns of the medial septum-diagonal band complex. *J Comp Neurol*, 293, 103-124.

- Getz, L. L., McGuire, B., Pizzuto, T., Hofmann, J., & Frase, B. (1993). Social organization of the prairie vole, *Microtus ochrogaster*. *J.Mammal.*, 74, 44-58.
- Gilbertson, D. W. (1975). Courtship as a reinforcement for key pecking in the pigeon, *Columbia livia*. *Anim Behav.*, 23, 735-744.
- Gilman, D. P. & Hitt, J. C. (1978). Effects of gonadal hormones on pacing of sexual contacts by female rats. *Behav Biol.*, 24, 77-87.
- Gingrich, B., Liu, Y., Cascio, C., Wang, Z., & Insel, T. R. (2000). Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). *Behav.Neurosci.*, 114, 173-183.
- Gomora, P., Beyer, C., Gonzalez-Mariscal, G., & Komisaruk, B. R. (1994). Momentary analgesia produced by copulation in female rats. *Brain Res.*, 656, 52-58.
- Graham, J. M. & Desjardins, C. (1980). Classical conditioning: induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. *Science*, 210, 1039-1041.
- Graybiel, A. M., Moratalla, R., & Robertson, H. A. (1990). Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc.Natl.Acad.Sci.U.S.A*, 87, 6912-6916.
- Greep, R. O. (1973). The gonadotrophins and their releasing factors. *J Reprod.Fertil.Suppl*, 20, 1-9.

- Gysling, K. & Wang, R. Y. (1983). Morphine-induced activation of A10 dopamine neurons in the rat. *Brain Res.*, 277, 119-127.
- Halliday, T. R. (1983). The study of mate choice. In P. Batenson (Ed.), *Mate choice* (pp. 3-28). Cambridge: Cambridge University Press.
- Hardy, D. F. & DeBold, J. F. (1972). Effects of coital stimulation upon behavior of the female rat. *J. Comp Physiol Psychol.*, 78, 400-408.
- Hatfield, E. & Sprecher, S. (1986). Measuring passionate love in intimate relationships. *J Adolesc.*, 9, 383-410.
- Heijna, M. H., Padt, M., Hogenboom, F., Portoghese, P. S., Mulder, A. H., & Schoffelmier, A. N. (1990). Opioid receptor-mediated inhibition of dopamine and acetylcholine release from slices of rat nucleus accumbens, olfactory tubercle and frontal cortex. *Eur. J Pharmacol.*, 181, 267-278.
- Hemby, S. E., Jones, G. H., Justice, J. B., Jr., & Neill, D. B. (1992). Conditioned locomotor activity but not conditioned place preference following intra-accumbens infusions of cocaine. *Psychopharmacology (Berl)*, 106, 330-336.
- Hernandez, G., Hamdani, S., Rajabi, H., Conover, K., Stewart, J., Arvanitogiannis, A. et al. (2006). Prolonged rewarding stimulation of the rat medial forebrain bundle: neurochemical and behavioral consequences. *Behav Neurosci*, 120, 888-904.

- Hernandez, L. & Hoebel, B. G. (1988). Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci.*, 42, 1705-1712.
- Hernandez, L., Lee, F., & Hoebel, B. G. (1987). Simultaneous microdialysis and amphetamine infusion in the nucleus accumbens and striatum of freely moving rats: increase in extracellular dopamine and serotonin. *Brain Res. Bull.*, 19, 623-628.
- Herz, A. (1998). Opioid reward mechanisms: a key role in drug abuse? *Can. J. Physiol. Pharmacol.*, 76, 252-258.
- Hile, A. G., Plummer, T. K., & Striedter, G. F. (2000). Male vocal imitation produces call convergence during pair bonding in budgerigars, *Melopsittacus undulatus*. *Anim. Behav.*, 59, 1209-1218.
- Hlinak, Z. & Madlafousek, J. (1977). Female precopulatory behavior as a determinant of sexual activity in male rats. *Activitas Nervosa Superior*, 19, 242-243.
- Hoffman, D. C. & Beninger, R. J. (1988). Selective D1 and D2 dopamine agonists produce opposing effects in place conditioning but not in conditioned taste aversion learning. *Pharmacol. Biochem. Behav.*, 31, 1-8.
- Holstege, G., Meiners, L., & Tan, K. (1985). Projections of the bed nucleus of the stria terminalis to the mesencephalon, pons, and medulla oblongata in the cat. *Exp. Brain Res.*, 58, 379-391.

- Horvitz, J. C. (2000). Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience*, 96, 651-656.
- Hoshina, Y., Takeo, T., Nakano, K., Sato, T., & Sakuma, Y. (1994). Axon-sparing lesion of the preoptic area enhances receptivity and diminishes proceptivity among components of female rat sexual behavior. *Behav Brain Res.*, 61, 197-204.
- Hu, X. T., Koeltzow, T. E., Cooper, D. C., Robertson, G. S., White, F. J., & Vezina, P. (2002). Repeated ventral tegmental area amphetamine administration alters dopamine D1 receptor signaling in the nucleus accumbens. *Synapse*, 45, 159-170.
- Hughes, A. M., Everitt, B. J., & Herbert, J. (1987). Selective effects of beta-endorphin infused into the hypothalamus, preoptic area and bed nucleus of the stria terminalis on the sexual and ingestive behaviour of male rats. *Neuroscience*, 23, 1063-1073.
- Hughes, A. M., Everitt, B. J., & Herbert, J. (1990). Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behaviour in male rats: studies of instrumental behaviour, conditioned place preference and partner preference. *Psychopharmacology (Berl)*, 102, 243-256.
- Hull, E. M. & Dominguez, J. M. (2006). Getting his act together: Roles of glutamate, nitric oxide, and dopamine in the medial preoptic area. *Brain Res.*
- Hull, E. M., Eaton, R. C., Markowski, V. P., Moses, J., Lumley, L. A., & Loucks, J. A. (1992). Opposite influence of medial preoptic D1 and D2 receptors on genital reflexes: implications for copulation. *Life Sci.*, 51, 1705-1713.

Insel, T. R. (2003). Is social attachment an addictive disorder? *Physiol Behav.*, 79, 351-357.

Insel, T. R. & Hulihan, T. J. (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behav Neurosci*, 109, 782-789.

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: behavioral consequences. *Physiol Behav*, 57, 615-627.

Insel, T. R. & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc.Natl.Acad.Sci.U.S.A*, 89, 5981-5985.

Insel, T. R., Winslow, J. T., Wang, Z., & Young, L. J. (1998). Oxytocin, vasopressin, and the neuroendocrine basis of pair bond formation. *Adv.Exp.Med.Biol.*, 449, 215-224.

Insel, T. R., Winslow, J. T., Wang, Z. X., Young, L., & Hulihan, T. J. (1995). Oxytocin and the molecular basis of monogamy. *Adv.Exp.Med.Biol.*, 395, 227-234.

Insel, T. R. & Young, L. J. (2001). The neurobiology of attachment. *Nat.Rev.Neurosci.*, 2, 129-136.

Jenkins, W. J. & Becker, J. B. (2001). Role of the striatum and nucleus accumbens in paced copulatory behavior in the female rat. *Behav Brain Res.*, 121, 119-128.

- Jenkins, W. J. & Becker, J. B. (2003). Female rats develop conditioned place preferences for sex at their preferred interval. *Horm.Behav.* 43, 503-507.
- Kalivas, P. W., Duffy, P., & Eberhardt, H. (1990). Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J Pharmacol.Exp Ther.*, 253, 858-866.
- Kato, A. & Sakuma, Y. (2000). Neuronal activity in female rat preoptic area associated with sexually motivated behavior. *Brain Res.*, 862, 90-102.
- Kaye, S. & Darke, S. (2004). Non-fatal cocaine overdose among injecting and non-injecting cocaine users in Sydney, Australia. *Addiction*, 99, 1315-1322.
- Kendrick, K. M., Hinton, M. R., Atkins, K., Haupt, M. A., & Skinner, J. D. (1998). Mothers determine sexual preferences. *Nature*, 395, 229-230.
- Keverne, E. B. (1976). Sexual receptivity and attractiveness in the female rhesus monkey. In J.S.Rosenblatt, R. A. Hinde, E. Shaw, & C. Beer (Eds.), *Advances in the study of behavior* (pp. 155-200). New York: Academy Press.
- 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, 971-979.
- Kippin, T. E. & Pfaus, J. G. (2001a). The development of olfactory conditioned ejaculatory preferences in the male rat. I. Nature of the unconditioned stimulus. *Physiol Behav.*, 73, 457-469.

- Kippin, T. E. & Pfaus, J. G. (2001b). The nature of the conditioned response mediating olfactory conditioned ejaculatory preference in the male rat. *Behav. Brain Res.*, 122, 11-24.
- Kippin, T. E., Samaha, A. N., Sotiropoulos, V., & Pfaus, J. G. (2001c). The development of olfactory conditioned ejaculatory preferences in the male rat. II. Parametric manipulation of conditioning session number and duration. *Physiol Behav.*, 73, 471-485.
- Kippin, T. E., Sotiropoulos, V., Badih, J., & Pfaus, J. G. (2004). Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behaviour in the male rat. *Eur. J Neurosci*, 19, 698-704.
- Kippin, T. E., Talinakis, E., Chattmann, L., Bartholomew, S., & Pfaus, J. G. (1998). Olfactory Conditioning of Sexual Behavior in the Male Rat (*Rattus norvegicus*). *Journal of Comparative Psychology*, 112, 389-399.
- Komisaruk, B. R., Bianca, R., Sansone, G., Gomez, L. E., Cueva-Rolon, R., Beyer, C. et al. (1996). Brain-mediated responses to vaginocervical stimulation in spinal cord-transected rats: role of the vagus nerves. *Brain Res.*, 708, 128-134.
- Kornberg, E. & Erskine, M. S. (1994). Effects of differential mating stimulation on the onset of prolactin surges in pseudopregnant rats. *Psychoneuroendocrinology*, 19, 357-371.
- Kovacs, G. L., Sarnyai, Z., & Szabo, G. (1998). Oxytocin and addiction: a review. *Psychoneuroendocrinology*, 23, 945-962.

- Kow, L. M. & Pfaff, D. W. (1973). Effects of estrogen treatment on the size of receptive field and response threshold of pudendal nerve in the female rat. *Neuroendocrinology*, 13, 299-313.
- Kowianski, P., Lipowska, M., & Morys, J. (1999). The piriform cortex and the endopiriform nucleus in the rat reveal generally similar pattern of connections. *Folia Morphol.(Warsz.)*, 58, 9-19.
- Kruger, T., Exton, M. S., Pawlak, C., von zur Muhlen, A., Hartmann, U., & Schedlowski, M. (1998). Neuroendocrine and cardiovascular response to sexual arousal and orgasm in men. *Psychoneuroendocrinology*, 23, 401-411.
- Lagerspetz, K. & Heino, T. (1970). Changes in social reactions resulting in early experience with another species. *Psychol Rep*, 27, 255-262.
- Lamb, R. J., Preston, K. L., Schindler, C. W., Meisch, R. A., Davis, F., Katz, J. L. et al. (1991). The reinforcing and subjective effects of morphine in post-addicts: a dose-response study. *J Pharmacol.Exp Ther.*, 259, 1165-1173.
- Larsson, K. (1971). Impaired mating performances in male rats after anosmia induced peripherally or centrally. *Brain Behav.Evol.*, 4, 463-471.
- Larsson, K. (1956). *Conditioning and sexual behavior in the male albino rat*. Stockholm: Almqvist.

- Lee, J. W. & Erskine, M. S. (2000). Changes in pain threshold and lumbar spinal cord immediate-early gene expression induced by paced and nonpaced mating in female rats. *Brain Res.*, 861, 26-36.
- Lett, B. T. (1989). Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berl)*, 98, 357-362.
- Levin, R. J. (2002). The physiology of sexual arousal in the human female: a recreational and procreational synthesis. *Arch.Sex Behav*, 31, 405-411.
- Leyton, M. & Stewart, J. (1992). The stimulation of central kappa opioid receptors decreases male sexual behavior and locomotor activity. *Brain Res.*, 594, 56-74.
- Liberzon, I., Trujillo, K. A., Akil, H., & Young, E. A. (1997). Motivational properties of oxytocin in the conditioned place preference paradigm. *Neuropsychopharmacology*, 17, 353-359.
- Lim, M. M., Wang, Z., Olazabal, D. E., Ren, X., Terwilliger, E. F., & Young, L. J. (2004). Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature*, 429, 754-757.
- Liu, Y. & Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience*, 121, 537-544.

- Lodder, J. & Zeilmaker, G. H. (1976). Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. *J.Comp Physiol Psychol.*, 90, 925-929.
- Lorrain, D. S., Riolo, J. V., Matuszewich, L., & Hull, E. M. (1999). Lateral hypothalamic serotonin inhibits nucleus accumbens dopamine: implications for sexual satiety. *J Neurosci*, 19, 7648-7652.
- Luo, S. & Klohnen, E. C. (2005). Assortative mating and marital quality in newlyweds: a couple-centered approach. *J Pers.Soc Psychol*, 88, 304-326.
- Madlafousek, J., Hlinak, Z., & Beran, J. (1976). Decline of sexual behavior in castrated male rats: effects of female precopulatory behavior. *Horm.Behav*, 7, 245-252.
- Maldonado, R., Saiardi, A., Valverde, O., Samad, T. A., Roques, B. P., & Borrelli, E. (1997). Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature*, 388, 586-589.
- Malina, R. M., Selby, H. A., Buschang, P. H., Aronson, W. L., & Little, B. B. (1983). Assortative mating for phenotypic characteristics in a Zapotec community in Oaxaca, Mexico. *J Biosoc.Sci.*, 15, 273-280.
- Martinez, I. & Paredes, R. G. (2001). Only self-paced mating is rewarding in rats of both sexes. *Horm.Behav.*, 40, 510-517.

- Mas, M., Gonzalez-Mora, J. L., Louilot, A., Sole, C., & Guadalupe, T. (1990). Increased dopamine release in the nucleus accumbens of copulating male rats as evidenced by in vivo voltammetry. *Neurosci Lett.*, 110, 303-308.
- Matsumoto, T., Kondo, Y., Sachs, B. D., & Yamanouchi, K. (1997). Effects of p-chlorophenylalanine on reflexive and noncontact penile erections in male rats. *Physiol Behav*, 61, 165-168.
- Matteo, S. & Rissman, E. F. (1984). Increased sexual activity during the midcycle portion of the human menstrual cycle. *Horm.Behav*, 18, 249-255.
- Matthews, M. K., Jr. & Adler, N. T. (1978). Systematic interrelationship of mating, vaginal plug position, and sperm transport in the rat. *Physiol Behav.*, 20, 303-309.
- Matuszewich, L., Lorrain, D. S., & Hull, E. M. (2000). Dopamine release in the medial preoptic area of female rats in response to hormonal manipulation and sexual activity. *Behav Neurosci*, 114, 772-782.
- McCann, S. M., Krulich, L., Cooper, K. J., Kalra, P. S., Kalra, S. P., Libertun, C. et al. (1973). Hypothalamic control of gonadotrophin and prolactin secretion, implications for fertility control. *J Reprod.Fertil.Suppl*, 20, 43-59.
- McClintock, M. K. (1978). Estrous synchrony and its mediation by airborne chemical communication (*Rattus norvegicus*). *Horm.Behav.*, 10, 264-275.
- McClintock, M. K. (1984b). Estrous synchrony: modulation of ovarian cycle length by female pheromones. *Physiol Behav.*, 32, 701-705.

- McClintock, M. K. (1984a). Group mating in the domestic rat as a context for sexual selection: Consequences for analysis of sexual behavior and neuroendocrine responses. *Advances in the Study of Behavior. New York: Academic Press, 14*, 1-50.
- McClintock, M. K. & Adler, N. T. (1978). Induction of persistent estrus by airborne chemical communication among female rats. *Horm.Behav., 11*, 414-418.
- 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. *Anim Behav., 30*, 410-425.
- Meerts, S. H., Christensen, A. E., and Clark, A. S.,(2005). Parameters of mating-induced conditioned place preference in female rats. *Abstract viewer/Itinerary planner.Washington, DC: Society for Neurosciences Online 35th annual meeting): Abstract.*
- Meerts, S. H. and Clark, A. S.,(2006). Induction of a conditioned place preference by nonpaced mating behavior in female rats. *Abstract viewer.Pittsburgh, PA: Annual meeting Society for Behavioral Neuroendocrinology Abstract.*
- Ménard, S., Coria-Avila, G. A., Gelez, H., Godfrey, J., Soroichinsky, M., and Pfaus, J. G.,(2005). Activation of oxytocin and vasopressin neurons following the presentation of an odour associated with sexual reward in the male rat. *Horm.Behav. 48 (1): 115-Abstract.*

Menard, S., Gelez, H., Coria-Avila, G. A., Jacobovich, M., and Pfaus, J. G., (2006).

Impact of neonatal olfactory conditioning on later sexual partner preference in the male rat. *Abstract viewer. Pittsburgh, PA: Annual meeting Society for Behavioral Neuroendocrinology Abstract.*

Mendelson, S. D. & Pfaus, J. G. (1989). Level searching: a new assay of sexual motivation in the male rat. *Physiol Behav.*, 45, 337-341.

Mermelstein, P. G. & Becker, J. B. (1995). Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. *Behav Neurosci.*, 109, 354-365.

Michael, R. P. & Keverne, E. B. (1968). Pheromones in the communication of sexual status in primates. *Nature*, 218, 746-749.

Miller, R. L. & Baum, M. J. (1987). Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. *Pharmacol. Biochem. Behav.*, 26, 781-789.

Mills, R. H., Sohn, R. K., & Micevych, P. E. (2004). Estrogen-induced mu-opioid receptor internalization in the medial preoptic nucleus is mediated via neuropeptide Y-Y1 receptor activation in the arcuate nucleus of female rats. *J Neurosci*, 24, 947-955.

Mitchell, J. B. & Gratton, A. (1991). Opioid modulation and sensitization of dopamine release elicited by sexually relevant stimuli: a high speed chronoamperometric study in freely behaving rats. *Brain Res.*, 551, 20-27.

- Mitchell, J. B. & Gratton, A. (1992). Mesolimbic dopamine release elicited by activation of the accessory olfactory system: a high speed chronoamperometric study. *Neurosci Lett.*, 140, 81-84.
- Mitchell, J. B. & Stewart, J. (1990). Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. *Pharmacol.Biochem.Behav.*, 35, 643-650.
- Money, J. (1997). Evolutionary sexology: the hypothesis of song and sex. *Med Hypotheses*, 48, 399-402.
- Moore, C. L., Jordan, L., & Wong, L. (1996). Early olfactory experience, novelty, and choice of sexual partner by male rats. *Physiol Behav.*, 60, 1361-1367.
- Moore, C. L. & Wong, L. (1992). Copulatory behavior, reproduction, and sperm competition in two strains of male rats. *Physiol Behav.*, 51, 569-573.
- Moss, R. L. & McCann, S. M. (1975). Action of luteinizing hormone-releasing factor (lrf) in the initiation of lordosis behavior in the estrone-primed ovariectomized female rat. *Neuroendocrinology*, 17, 309-318.
- Mucha, R. F. & Herz, A. (1985). Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology (Berl)*, 86, 274-280.
- Nader, K., Bechara, A., & van der, K. D. (1997). Neurobiological constraints on behavioral models of motivation. *Annu.Rev.Psychol*, 48, 85-114.

- Newman, S. W., Parfitt, D. B., & Kollack-Walker, S. (1997). Mating-induced c-fos expression patterns complement and supplement observations after lesions of the male syrian hamster brain. In C. S. Carter & I. I. Lederhendler (Eds.), *The integrative neurobiology of affiliation* (pp. 239-259). New York: New York Academy of Sciences.
- Ng, J. P., Hubert, G. W., & Justice, J. B., Jr. (1991). Increased stimulated release and uptake of dopamine in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. *J Neurochem.*, 56, 1485-1492.
- Nyby, J., Bigelow, J., Kerchner, M., & Barbehenn, F. (1983). Male mouse (*Mus musculus*) ultrasonic vocalizations to female urine: why is heterosexual experience necessary? *Behav Neural Biol.*, 38, 32-46.
- Oldenburger, W. P., Everitt, B. J., & de Jonge, F. H. (1992). Conditioned place preference induced by sexual interaction in female rats. *Horm.Behav.*, 26, 214-228.
- Olmstead, M. C. & Franklin, K. B. (1997). The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. *Behav Neurosci*, 111, 1324-1334.
- Panksepp, J., Knutson, B., & Burgdorf, J. (2002). The role of brain emotional systems in addictions: a neuro-evolutionary perspective and new 'self-report' animal model. *Addiction*, 97, 459-469.

- Paredes, R. G. & Ågmo, A. (2004). Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. *Prog.Neurobiol.*, 73, 179-226.
- Paredes, R. G. & Alonso, A. (1997). Sexual behavior regulated (paced) by the female induces conditioned place preference. *Behav.Neurosci.*, 111, 123-128.
- Paredes, R. G., Lopez, M. E., & Baum, M. J. (1998). Testosterone augments neuronal Fos responses to estrous odors throughout the vomeronasal projection pathway of gonadectomized male and female rats. *Horm.Behav.*, 33, 48-57.
- Paredes, R. G. & Martinez, I. (2001). Naloxone blocks place preference conditioning after paced mating in female rats. *Behav.Neurosci.*, 115, 1363-1367.
- Paredes, R. G. & Vazquez, B. (1999). What do female rats like about sex? Paced mating. *Behav.Brain Res.*, 105, 117-127.
- Pavlov, I. (1927). *Conditioned reflexes*. Oxford: University Press.
- Paxinos, G. & Watson, C. (1998). *The Rat Brain: In stereotaxic coordinates*. (Fourth Edition ed.) Academic Press.
- Penn, D. & Potts, W. (1998). MHC-disassortative mating preferences reversed by cross-fostering. *Proc.Biol.Sci.*, 265, 1299-1306.
- Pfaff, D. W. (1999). *Drive: Neural and Molecular Mechanisms for Sexual Motivation*. Cambridge, MA: MIT Press.
- Pfaff, D. W. (1980). A neural analysis of a hormone controlled mammalian reproductive behavior. In *Estrogens and brain function* (New York: Springer.

Pfaus, J. G. (1999). Revisiting the concept of sexual motivation. *Annu.Rev.Sex Res.*, 10, 120-156.

Pfaus, J. G. (1996). Frank A. Beach award. Homologies of animal and human sexual behaviors. *Horm.Behav.*, 30, 187-200.

Pfaus, J. G., Damsma, G., Nomikos, G. G., Wenkstern, D. G., Blaha, C. D., Phillips, A. G. et al. (1990). Sexual behavior enhances central dopamine transmission in the male rat. *Brain Res.*, 530, 345-348.

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, 21-30.

Pfaus, J. G. & Gorzalka, B. B. (1987). Selective activation of opioid receptors differentially affects lordosis behavior in female rats. *Peptides*, 8, 309-317.

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, 397-407.

Pfaus, J. G., Jakob, A., Kleopoulos, S. P., Gibbs, R. B., & Pfaff, D. W. (1994). Sexual stimulation induces Fos immunoreactivity within GnRH neurons of the female rat preoptic area: interaction with steroid hormones. *Neuroendocrinology*, 60, 283-290.

- Pfaus, J. G., Kippin, T. E., & Centeno, S. (2001). Conditioning and sexual behavior: a review. *Horm.Behav.*, 40, 291-321.
- Pfaus, J. G., Kippin, T. E., & Coria-Avila, G. (2003). What can animal models tell us about human sexual response? *Annu.Rev.Sex Res.*, 14, 1-63.
- Pfaus, J. G., Kleopoulos, S. P., Mobbs, C. V., Gibbs, R. B., & Pfaff, D. W. (1993). Sexual stimulation activates c-fos within estrogen-concentrating regions of the female rat forebrain. *Brain Res.*, 624, 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. *Physiol Behav.*
- 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 Res.*, 741, 314-330.
- Pfaus, J. G., Mendelson, S. D., & Phillips, A. G. (1990). A correlational and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat. *Psychoneuroendocrinology*, 15, 329-340.
- Pfaus, J. G. & Pfaff, D. W. (1992). Mu-, delta-, and kappa-opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone. *Horm.Behav.*, 26, 457-473.

- 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, 727-743.
- Pfaus, J. G., Smith, W. J., Byrne, N., & Stephens, G. (2000). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. II. Patterns of estrus termination following vaginocervical stimulation. *Horm. Behav.*, 37, 96-107.
- 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. *Horm. Behav.*, 35, 224-240.
- Phillips, A. G., Pfaus, J. G., & Blaha, C. D. (1991). Dopamine and motivated behavior: Insights provided by *In Vivo* analyses. In P. Willner & J. Schleele-Krüger (Eds.), *The mesolimbic dopamine system: from motivation to action* (pp. 199-224). Chichester, England: John Wiley & Sons Ltd.
- Phillipson, O. T. (1979). The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. *J Comp Neurol*, 187, 85-98.
- Piekut, D. T. & Knigge, K. M. (1984). Relationship of alpha MSH-specific neurons to the arcuate opiocortin neuronal system as determined by dual antigen immunocytochemical procedures. *Peptides*, 5, 1089-1095.
- Pizzuto, T. & Getz, L. L. (1998). Female prairie voles (*Microtus ochrogaster*) fail to form a new pair after loss of mate. *Behav. Processes*, 43, 79-86.

- Pontieri, F. E., Calo, L., Di, G. R., Orzi, F., & Passarelli, F. (1997). Functional correlates of heroin sensitization in the rat brain. *Eur.J Pharmacol.*, 335, 133-137.
- Prusky, G. T., Harker, K. T., Douglas, R. M., & Whishaw, I. Q. (2002). Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behav.Brain Res.*, 136, 339-348.
- Ramos, S. M. & DeBold, J. F. (1999). Protein synthesis in the medial preoptic area is important for the mating-induced decrease in estrus duration in hamsters. *Horm.Behav*, 35, 177-185.
- Robinson, T. E. & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res.Brain Res.Rev.*, 18, 247-291.
- Roman, F., Staubli, U., & Lynch, G. (1987). Evidence for synaptic potentiation in a cortical network during learning. *Brain Res.*, 418, 221-226.
- Roman, F. S., Chaillan, F. A., & Soumireu-Mourat, B. (1993). Long-term potentiation in rat piriform cortex following discrimination learning. *Brain Res.*, 601, 265-272.
- Roselli-Rehfuss, L., Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., Low, M. J., Tatrow, J. B. et al. (1993). Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc.Natl.Acad.Sci.U.S.A*, 90, 8856-8860.

- Rosin, J. F., Datiche, F., & Cattarelli, M. (1999). Modulation of the piriform cortex activity by the basal forebrain: an optical recording study in the rat. *Brain Res.*, 820, 105-111.
- Saar, D., Grossman, Y., & Barkai, E. (1999). Reduced synaptic facilitation between pyramidal neurons in the piriform cortex after odor learning. *J Neurosci*, 19, 8616-8622.
- Sachs, B. D. (1996). Penile erection in response to remote cues from females: albino rats severely impaired relative to pigmented strains. *Physiol Behav*, 60, 803-808.
- Sachs, B. D. (1997). Erection evoked in male rats by airborne scent from estrous females. *Physiol Behav.*, 62, 921-924.
- Sachs, B. D., Akasofu, K., Citron, J. H., Daniels, S. B., & Natoli, J. H. (1994). Noncontact stimulation from estrous females evokes penile erection in rats. *Physiol Behav*, 55, 1073-1079.
- Sakuma, Y. & Pfaff, D. W. (1983). Modulation of the lordosis reflex of female rats by LHRH, its antiserum and analogs in the mesencephalic central gray. *Neuroendocrinology*, 36, 218-224.
- Salces, I., Rebato, E., & Susanne, C. (2004). Evidence of phenotypic and social assortative mating for anthropometric and physiological traits in couples from the Basque country (Spain). *J Biosoc.Sci.*, 36, 235-250.

- Schank, J. C. & McClintock, M. K. (1997). Ovulatory pheromone shortens ovarian cycles of female rats living in olfactory isolation. *Physiol Behav.*, 62, 899-904.
- Schoenbaum, G. & Eichenbaum, H. (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *J Neurophysiol.*, 74, 733-750.
- Schulze, H. G. & Gorzalka, B. B. (1991). Oxytocin effects on lordosis frequency and lordosis duration following infusion into the medial pre-optic area and ventromedial hypothalamus of female rats. *Neuropeptides*, 18, 99-106.
- Schwartz, M. (1956). Instrumental and consummatory measures of sexual capacity in the male rat. *J Comp Physiol Psychol*, 49, 328-333.
- Settlage, D. S., Motoshima, M., & Tredway, D. R. (1973). Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitation study. *Fertil.Steril.*, 24, 655-661.
- Sheffield, F. D., Wulff, J. J., & Bakker, R. (1951). Reward value of copulation without sex drive reduction. *J Comp Physiol Psychol*, 44, 3-8.
- Shiple, M. T. & Ennis, M. (1996). Functional organization of olfactory system. *J Neurobiol.*, 30, 123-176.
- Shippenberg, T. S., Bals-Kubik, R., & Herz, A. (1993). Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the

- mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J Pharmacol.Exp Ther.*, 265, 53-59.
- Singh, D., Meyer, W., Zambarano, R. J., & Hurlbert, D. F. (1998). Frequency and timing of coital orgasm in women desirous of becoming pregnant. *Arch.Sex Behav*, 27, 15-29.
- Skinner, B. F. (1953). Some contributions of an experimental analysis of behavior to psychology as a whole. *American Psychologist*, 8, 69-78.
- Skinner, B. F. (1966). What is the experimental analysis of behavior? *Journal of Experimental Analysis of Behavior*, 9, 213-218.
- Skoubis, P. D., Matthes, H. W., Walwyn, W. M., Kieffer, B. L., & Maidment, N. T. (2001). Naloxone fails to produce conditioned place aversion in mu-opioid receptor knock-out mice. *Neuroscience*, 106, 757-763.
- Sotomayor, R., Forray, M. I., & Gysling, K. (2005). Acute morphine administration increases extracellular DA levels in the rat lateral septum by decreasing the GABAergic inhibitory tone in the ventral tegmental area. *J Neurosci Res.*, 81, 132-139.
- Spyraki, C., Nomikos, G. G., & Varonos, D. D. (1987). Intravenous cocaine-induced place preference: attenuation by haloperidol. *Behav Brain Res.*, 26, 57-62.
- Stendahl (1821). *De l'amour*. New York: Schoenhof's Foreign Books.

- Storms, M. (1981). A theory of erotic orientation development. *Psychological Review*, 91, 340-353.
- Swaab, D. F., Pool, C. W., & Nijveldt, F. (1975). Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. *J Neural Transm.*, 36, 195-215.
- Swann, J. & Fiber, J. M. (1997). Sex differences in function of a pheromonally stimulated pathway: role of steroids and the main olfactory system. *Brain Res.Bull.*, 44, 409-413.
- Swanson, L. W. (1976). An autoradiographic study of the efferent connections of the preoptic region in the rat. *J Comp Neurol*, 167, 227-256.
- Syka, J., Rybalko, N., Brozek, G., & Jilek, M. (1996). Auditory frequency and intensity discrimination in pigmented rats. *Hear.Res.*, 100, 107-113.
- Taylor, M. I., Morley, J. I., Rico, C., & Balshine, S. (2003). Evidence for genetic monogamy and female-biased dispersal in the biparental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. *Mol.Ecol.*, 12, 3173-3177.
- Tennov, D. (1979). Love and limerence: the experience of being in love. New York. Stein and Day.
- Ref Type: Generic
- Terkel, J. & Sawyer, C. H. (1978). Male copulatory behavior triggers nightly prolactin surges resulting in successful pregnancy in rats. *Horm.Behav*, 11, 304-309.

- Tiefer, L. (1969). Copulatory behaviour of male *Rattus norvegicus* in a multiple-female exhaustion test. *Anim Behav.*, 17, 718-721.
- Toates, F. (1986). *Motivational Systems*. Cambridge: Cambridge University Press.
- Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog.Neurobiol.*, 56, 613-672.
- van Furth, W. R., van Emst, M. G., & Van Ree, J. M. (1995). Opioids and sexual behavior of male rats: involvement of the medial preoptic area. *Behav Neurosci*, 109, 123-134.
- van Furth, W. R. & Van Ree, J. M. (1996a). Appetitive sexual behavior in male rats: 1. The role of olfaction in level-changing behavior. *Physiol Behav*, 60, 999-1005.
- van Furth, W. R. & Van Ree, J. M. (1996b). Appetitive sexual behavior in male rats: 2. sexual reward and level-changing behavior. *Physiol Behav*, 60, 1007-1012.
- Veening, J. G. & Coolen, L. M. (1998). Neural activation following sexual behavior in the male and female rat brain. *Behav Brain Res.*, 92, 181-193.
- Wang, Z. & Young, L. J. (1997). Ontogeny of oxytocin and vasopressin receptor binding in the lateral septum in prairie and montane voles. *Brain Res.Dev.Brain Res.*, 104, 191-195.
- Wang, Z., Yu, G., Cascio, C., Liu, Y., Gingrich, B., & Insel, T. R. (1999). Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles

- (*Microtus ochrogaster*): a mechanism for pair bonding? *Behav Neurosci*, 113, 602-611.
- Wenkstern, D., Pfaus, J. G., & Fibiger, H. C. (1993). Dopamine transmission increases in the nucleus accumbens of male rats during their first exposure to sexually receptive female rats. *Brain Res.*, 618, 41-46.
- Wersinger, S. R., Baum, M. J., & Erskine, M. S. (1993). Mating-induced FOS-like immunoreactivity in the rat forebrain: A sex comparison and dimorphic effects of pelvic nerve transection. *Journal of Neuroendocrinology*, 5, 557-568.
- Whalen, R. E. (1966). Sexual motivation. *Psychol Rev.*, 73, 151-163.
- Whitney, J. F. (1986). Effect of medial preoptic lesions on sexual behavior of female rats is determined by test situation. *Behav Neurosci*, 100, 230-235.
- Wickler, W. & Seibt, U. (1983). Monogamy: An ambiguous concept. In P. Batenson (Ed.), *Mate choice* (pp. 33-50). Cambridge, England: Cambridge University Press.
- 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. *Horm. Behav.*, 26, 339-349.
- Wilson, D. A. (2001). Receptive fields in the rat piriform cortex. *Chem. Senses*, 26, 577-584.

- Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R., & Insel, T. R. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, 365, 545-548.
- Wise, R. A. & Bozarth, M. A. (1982). Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. *Pharmacol.Biochem.Behav*, 17, 239-243.
- Witt, D. M. & Insel, T. R. (1994). Increased Fos expression in oxytocin neurons following masculine sexual behavior. *J.Neuroendocrinol.*, 6, 13-18.
- Wolterink, G., Phillips, G., Cador, M., Donselaar-Wolterink, I., Robbins, T. W., & Everitt, B. J. (1993). Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacology (Berl)*, 110, 355-364.
- Xiao, K., Kondo, Y., & Sakuma, Y. (2005). Differential regulation of female rat olfactory preference and copulatory pacing by the lateral septum and medial preoptic area. *Neuroendocrinology*, 81, 56-62.
- Yamada, K., Horibe, S, and Kondo, Y,(2006). Effects of opiate antagonist on mating-induced conditioned place preference in female rats. *Abstract viewer.Pittsburgh, PA: Annual meeting Society for Behavioral Neuroendocrinology Abstract.*
- Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L., & Boyse, E. A. (1988). Familial imprinting determines H-2 selective mating preferences. *Science*, 240, 1331-1332.

- Young, A. M., Moran, P. M., & Joseph, M. H. (2005). The role of dopamine in conditioning and latent inhibition: what, when, where and how? *Neurosci Biobehav.Rev.*, 29, 963-976.
- Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Horm.Behav.*, 40, 133-138.
- Young, L. J., Murphy Young, A. Z., & Hammock, E. A. (2005). Anatomy and neurochemistry of the pair bond. *J Comp Neurol*, 493, 51-57.
- Young, L. J. & Wang, Z. (2004). The neurobiology of pair bonding. *Nat.Neurosci.*, 7, 1048-1054.
- Zaborszky, L. & Makara, G. B. (1979). Intrahypothalamic connections: an electron microscopic study in the rat. *Exp Brain Res.*, 34, 201-215.
- Zamble, E., Hadad, G. M., Mitchell, J. B., & Cutmore, T. R. (1985). Pavlovian conditioning of sexual arousal: first- and second-order effects. *J.Exp.Psychol.Anim Behav.Process*, 11, 598-610.