Brain mechanisms of sexual disinhibition in the male rat by alcohol and amphetamine

Katuschia Germé

A Thesis

In the Department

of

Biology

And Center for Studies in Behavioral Neurobiology

Presented in Partial Fulfillment of the Requirements
For the Degree of
Doctor of Philosophy (Biology) at
Concordia University
Montréal, Québec, Canada

August 2021

CONCORDIA UNIVERSITY
School of Graduate Studies

This is to certify that the thesis prepared

By: Katuschia Germé

Entitled: Brain mechanisms of sexual disinhibition in the male rat by alcohol and amphetamine

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

Doctor of Philosophy (Biology)

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

Signed by the final Examining Committee:

_____ Chair
Dr. Y. Gélinas

_____ External Examiner
Dr. S. Kourrich

_____ External to Program
Dr. A. Chapman

_____ Examiner
Dr. C. Brett

_____ Examiner
Dr. E. Despland

_____ Supervisor
Dr. S. Amir

Approved by _____
Dr. Robert Bertand Weladji, Graduate Program Director

August 2021 _____
Dr. Pascale Sicotte, Dean
Faculty of Arts and Science

ABSTRACT

Brain mechanisms of sexual disinhibition in the male rat by alcohol and amphetamine
Katuschia Germé, PhD
Concordia University, 2021

The expression of sexual behavior requires a proper balance between inhibition and excitation at cognitive, hormonal and neurochemical levels. Inhibition of sexual behavior is considered to be important and even crucial to maintain proper social cohesion and adaptation (i.e. in response to threat, aversion, culture, fertility). In humans, spreading of sexually transmitted diseases is often due to inappropriate and risky sexual behavior (e.g. multiple partners, no use of condoms) or to a loosening of inhibitions under the influence of drugs. It is therefore important to understand where these drugs (recreational, or prescription) act to induce disinhibited sexual behavior. In this thesis, the mechanisms of action of two drugs associated famously with sexual disinhibition but possessing two largely opposite mechanisms of action, alcohol and d-amphetamine, are investigated. This is done using a conditioned partner avoidance paradigm in which male rats are trained to avoid copulation with females bearing a conditioned inhibitory olfactory cue associated with non reward. This paradigm has been used previously to reveal the disinhibitory properties of low doses of alcohol. In the first chapter, it is established that d-amphetamine disrupts conditioned partner avoidance as d-amphetamine-treated males still copulate with females bearing the conditioned inhibitory cue. Furthermore, similar to alcohol, exposure to the inhibitory cue alone under the influence of d-amphetamine activates brain regions associated with sexual behavior and sexual reward. As alcohol is known to induce disinhibition of behavior in general and of sexual behavior in particular, the focus of the second and third chapters is to examine the potential neurotransmitters involved in the disinhibitory effect of alcohol. Using microdialysis, it is found that levels of dopamine in the nucleus accumbens are increased when alcohol-treated male rats are exposed to the sexually conditioned inhibitory cue. This increase is due to activation of dopaminergic neurons from the ventral tegmental area. On the other hand, neither alcohol nor the conditioned cue has an effect on glutamate or GABA levels in the nucleus accumbens. Together these data demonstrate that dopamine release in the nucleus accumbens via activation of dopaminergic neurons in the ventral tegmental area plays a permissive role in the disinhibitory effects of alcohol (and likely amphetamine) on the sexual behavior of male rats.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my former and current supervisors Dr Jim Pfaus and Dr Shimon Amir. Thank you so much for your support and your incredible patience. Over the past years, you taught me how to write, how to rewrite, how to think inside and outside the box. You gave me the confidence I needed to finish this work. It was really really long but we made it... Thank you!

I also want to thank my committee members, Dr Christopher Brett and Dr Emma Despland for taking the time to read my thesis and my previous drafts, for listening to all my presentations and for giving me great feedback to move forward and become a better scientist. To Dr Andrew Chapman and Dr Saïd Kourrich, thank you so much for being on board and accepting to read, to review and to discuss my PhD thesis. Your comments, expertise and input are more than valuable.

I would like to thank the former members (students and lab technicians) of what was once the Pfaus lab for all the support, scientific talks, laughter, inconsiderable help, support and most importantly friendship: Dr. Mayte Parada, Dr. Dean Graham, Dr. Sherri Jones, Dr. Amanda Holley, Dr. Gonzalo Quintana-Zunino, Jonathan Greggain, Conall MacConnaith, Christine Gerson, Alex Radu Antonie, and Dr. Ziada Ayorech. You were all amazing allies in grad school and outside.

I would not have been able to manage all these experiments without the wonderful and brilliant undergraduates that helped me collect these data: Dhillon, Justine, Hannah, Leah, Julian, Cassandra, Katerina, Jean Philippe and particularly Lindsey who was, without any doubt, one of the most brilliant and devoted students I had the pleasure and the honor to work with.

To all my fellow students from the CSBN, thank you for the discussions in the grad lounge about life, grad school, statistics, food. A special thought for Dr Emily Cole and Zarish Abbas, you both are wonderful friends to talk to. Thank you so much for your support, your help and the wonderful discussions that helped me go through so many struggles. Dr Tracey D'Cunha, you were an amazing partner in the HPLC room and one of the best office mate ever. You have this particular way of motivating people maybe even without knowing it. You rock! Thank you for everything.

I could not have done this without Concordia University, the Biology department and all the members of the staff: Heshmat, thank you so so much for your help in the HPLC room, for allowing me to keep my sanity in this room that I so often called the Mordor... We made it out! Isabelle, Aileen, Jason, Jean-François, Kelly... thank you all for your time, your help and all you have done to make sure that all students including me had everything needed for their experiments, conferences and classes. None of this would have been possible without you all. Thank you to the CIHR and NSERC for providing all of the funding that made this work possible.

To Aneil Mehta, Rosslon Ollivierre, Mary Marciniak, at the very end, your help allowed me to submit this thesis with some peace of mind and made it all possible. From the bottom of my heart, thank you.

I would not have been able to go through this without my Pineapples, soon-to-be Dr Lindsay Sparks and Dr Lucy Farisello. You were my rocks, my saviors, my partners on the Titanic. I can never thank you enough for all these beautiful moments. You are both the best that ever happened to me at Concordia.

My life in Canada would not have been the same without my amazing friends, who became my family. To my Unicorns, Camille, Claire, Leïla, Julie, Marilyne, Paule, Karine (Jade), Adeline: you are the girl friends, the sisters anyone would hope for and I feel extremely lucky to have you in my life. Thank you so much for all those beautiful moments, for your amazing support throughout those years, for never doubting or judging me even when I was in doubt because of how long it took me to complete and write this PhD thesis...that helped me tremendously. When life gave me reasons to cry, you were always there to give me reasons to smile. To the men in my friends-family, Arnaud, Franck, Benoit, Nico, Charles, Romain, Michel and the newbies Gaïan and Augustin, thank you so much for all these awesome times together! Aside from being amazing partners in crimes, you are all wonderful men (plus, Gaïan and Augustin, you have the cutest little faces). Your support, your friendship, your humour, have helped me so many times to move forward and be a better person.

To my “Québec/Outaouais friends”, Mathieu, Elodie, Agnieszka, Annick, Judith, Sarah, Kévin, Dominique, Marie-Andrée, Dominique, Marc-André, Stéphanie and all the others... you

have this particular way of making people feel at home, feel loved and supported no matter what and that was really helpful in some of my doubtful times. Thank you for these amazing moments.

To my friends in Europe, Yolaine, Dr Matthieu Hammer, Dr Jennifer François, Joachim, Alice, François, Mathilde, Nino, Dr Nabila Kadiri... despite not being there physically, you were always on my mind. Our 10 to 20+ years of friendship are always with me, no matter what.

To my family, Mom, brother aunts and uncle... you are the best. Even when I decided to cross the Atlantic Ocean to pursue my dream without knowing how long it would take and how hard it would be, you never stopped to believe in me and you kept on encouraging me and being supportive. Merci pour tout!

I dedicate this thesis to Max and our baby Maïlo. Max, you are without any doubt my better half. Thank you for being so supportive and so patient, for your love every single day, even when I thought about giving up. You never ceased to remind me that I could do it... and I finally did. You made me a better person. Maïlo, I want to thank you for allowing me to write this thesis without giving me too many pregnancy symptoms at first, and then for reminding me to take breaks, for my own sanity, with your beautiful little face and your smiles. I hope that one day you will be proud of me, your mom, for finishing this thesis.

“Alone we go faster, together we go further”
I will never thank fate (and science) enough for bringing you all into my life.

CONTRIBUTION OF AUTHORS

Chapter 1: General introduction

Katuschia Germé: Manuscript writing

James G. Pfaus: Manuscript writing and editing

Shimon Amir: Manuscript editing

Chapter 2: Disruptive effects of d-amphetamine on conditioned sexual inhibition in male rats

Katuschia Germé: Study design, data collection and analyses, manuscript writing

Dhillon Persad and Justine Petit-Robinson: data collection

James G. Pfaus: Study design, manuscript writing and editing

Shimon Amir: Manuscript editing

Chapter 3: Influence of nucleus accumbens DA on the disruption of conditioned sexual inhibition by alcohol in male rats.

Katuschia Germé: Study design, data collection and analyses, manuscript writing

Lindsey R. Gossip: data collection and analyses

Julian Alvarez-Barkham, Cassandra Poirier: data collection

Tracey D'Cunha, Heshmat Rajabi: microdialysis data collection

James G. Pfaus: Study design, manuscript writing and editing

Shimon Amir: Manuscript editing

Chapter 4: Nucleus accumbens glutamate and GABA levels do not mediate the disruptive effects of alcohol on conditioned sexual inhibition in male rats.

Katuschia Germé: Study design, data collection and analyses, manuscript writing

Julian Alvarez-Barkam, Leah Cusack, Lindsey R. Gossip, Hannah S. Poupel: data collection

Heshmat Rajabi : microdialysis data collection

James G. Pfaus: Study design, manuscript writing and editing

Shimon Amir: Manuscript editing

Chapter 5: General discussion

Katuschia Germé: Manuscript writing

James G. Pfaus: Manuscript writing and editing

Shimon Amir: Manuscript editing

Table of contents

List of Figures

Chapter 1. Introduction

Chapter 2. Disruptive effects of d-amphetamine on conditioned sexual inhibition in male rats

List of Tables

Chapter 2. Disruptive effects of d-amphetamine on conditioned sexual inhibition in male rats

List of abbreviations

5-HT	serotonin
AC	anterior cingulate cortex
aCSF	artificial cerebrospinal fluid
ADHD	attention deficit/hyperactivity disorder
BLA	basolateral amygdala
BS	blocking solution
CA	catecholamine
CeA	central amygdala
CNS	central nervous system
CPP	conditioned place preference
CSI	conditioned sexual inhibition
DA	dopamine
DAB	3,3'-diaminobenzidine
d-amp	d-amphetamine/ dextroamphetamine
EtOH	ethyl alcohol
FG	Fluoro-Gold
FIHC	fluorescence immunohistochemistry
Fos	Fos protein
Fos IR	Fos immunoreactivity

GABA	γ-aminobutyric acid
Glu	glutamate
H_2O_2	hydrogen peroxide
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IL	infralimbic cortex
LiCl	lithium chloride
MeA	medial amygdala
Meth	Methamphetamine
MORs	μ opioids receptors
mPFC	medial prefrontal cortex
mPOA	medial preoptic area
MSNs	medium spiny neurons
NAc	nucleus accumbens
NAcCore	nucleus accumbens core
NAcShell	nucleus accumbens shell
NGS	normal goat serum
NMDA	*N*-methyl-D- aspartate
PB	phosphate buffered
PBS	phosphate buffer saline
PE	polyethylene

PET	positron emission tomography
PFA	paraformaldehyde
PFC	prefrontal cortex
PirCx	piriform cortex
PL	prelimbic cortex
SN	substantia nigra
TBS	tris-buffered saline
TH	tyrosine hydroxylase
VTA	ventral tegmental area

General introduction

« I mean, it didn't matter to me that there were people, it didn't matter that I was shy. Just the sound was so captivating that it helped me to get rid of those inhibitions. »

-- Eric Clapton, Clapton: The Autobiography (2007)

From inhibition to disinhibition…

Consciously or unconsciously, individuals are constantly inhibited. Inhibition is omnipresent and encompasses the control of motor and voluntary movements with reciprocal inhibition (Crone, 1993) to sharpening sensory perception with lateral inhibition (Walley and Weiden, 1973). From behavioral to motor to cognitive to neural, inhibition is what allows us to function (i.e. walk, talk, see, feel, etc...) appropriately. The ability to inhibit certain behaviors and to adjust behavioral responses appropriately to the environment is critical for adaptation, group cohesion, and ultimately survival (Jerison, 1973), and is one of several "executive functions" controlled by the prefrontal cortex in mammals (Kolb & Whishaw, 2021). By adjusting how external and internal distracters are processed and attended to, inhibitory processes are essential to allow individuals to focus their attention on the most relevant information of the environment at any given moment, in order to adapt behavior appropriately to the situation and to their own goals (Bjorklund and Kipp, 1995). Disinhibition, or the inhibition of inhibition, occurs typically with an abrupt influx of new stimuli that produce an orienting reflex in animals (Pavlov, 1927), or by opposing hormonal or neurochemical processes that release attention and behavior from the inhibition of one system or regulatory process, allowing for their excitation by another (e.g., Braitenberg, 1986; Toates, 1986).

… of sexual behavior

Sexual behavior is no exception to this type of control. Of particular importance in everyday life, inhibition of sexual behavior is an adaptive, social and regulatory mechanism that allows sexual interaction to be expressed at the right time, in the right place, and under the right social, hormonal, and reproductive circumstances (Bancroft, 1999; Pfaus, 2009). Multiple

inhibitory mechanisms, both general and specific, are present in the brain and periphery to allow the proper timing and expression of sexual behavior (Baird et al., 2007; Rodriguez-Nieto et al., 2019). Controlling and inhibiting sexual stimuli and behaviors is ultimately essential in humans for appropriate social encounters. Early theories mention the necessity of sexual inhibition to prevent conflicts within primate social groups, especially in case of dominance hierarchy (Chance, 1962). As in humans, appropriate sexual inhibition is crucial in other mammals, and perhaps in all species. Non human animal literature reports that natural inhibition of sexual behavior is also present in male rats, for example, and can occur in response to threat or aversion, or to ensure that sexual activity occurs with the proper timing to maximize fertility (e.g., as in the refractory period that marks an endpoint of sexual interaction; given that repeated ejaculations over short periods lead to lower sperm stores; reviewed in Bancroft, 1999).

Despite being ever present and essential for the regulation of appropriate behaviors, as mentioned by Eric Clapton in his quote above, one's inhibitions can be themselves inhibited. This can be done by external or internal cues of various natures that can be so distracting and salient they cause disinhibition. Although the concept of disinhibition seems straightforward, it is not nearly as well defined or understood as inhibition. It is described as a "lack of restraint or of inhibition" in psychology, and an "imbalance between excitatory and inhibitory inputs" at the neural level (Letzkus et al., 2015). The lack of a comprehensive definition of disinhibition might be due to the fact that it includes a wide range of behaviors and processes, and depends largely on the situation. At a more clinical level, disinhibition often refers to "impulsive" or "inappropriate" behaviors in a particular context, and has been studied and measured in neurological and psychopathological disorders such as dementia, attention deficit/hyperactivity disorder (ADHD), along with substance use and abuse (review in Huey, 2020).

Observed in clinical and/or non-clinical contexts, and typically reported anecdotally, the behavior of particular interest for this thesis is the disinhibition of sexual behavior. It is defined as the expression of sexual behaviors considered socially or contextually inappropriate, including thoughts or comments and/or expressions of hypersexuality (Chapman and Spitznagel, 2019).

As with other processes and behaviors, the expression of appropriate sexual behavior requires a proper balance between inhibition and excitation at cognitive, hormonal and neurochemical levels. A theoretical model of a dual control of male sexual response in humans

by both excitatory and inhibitory processes has been proposed (Bancroft and Janssen, 2000; Janssen and Bancroft, 2007). This model posits that individuals may vary in their susceptibility for inhibition as well as for excitation of sexual responses, and that these variations would result in the differential expression of sexual excitation or inhibition. The notion of dual control of male sexual response has been further expanded by Perelman (2006). With his etiological model of the Sexual Tipping Point ® (Figure 1), Perelman (2006) postulates the existence of a threshold for the expression of sexual responses for any individual person. Sexual responses may be excited or inhibited as a result of a dynamic fluctuation between excitatory and inhibitory influences of cognitive (i.e. cultural, learned) and biological (i.e. steroid hormones, neurotransmitters) factors (Bancroft, 1999). This threshold may vary within and between sexual experience(s).

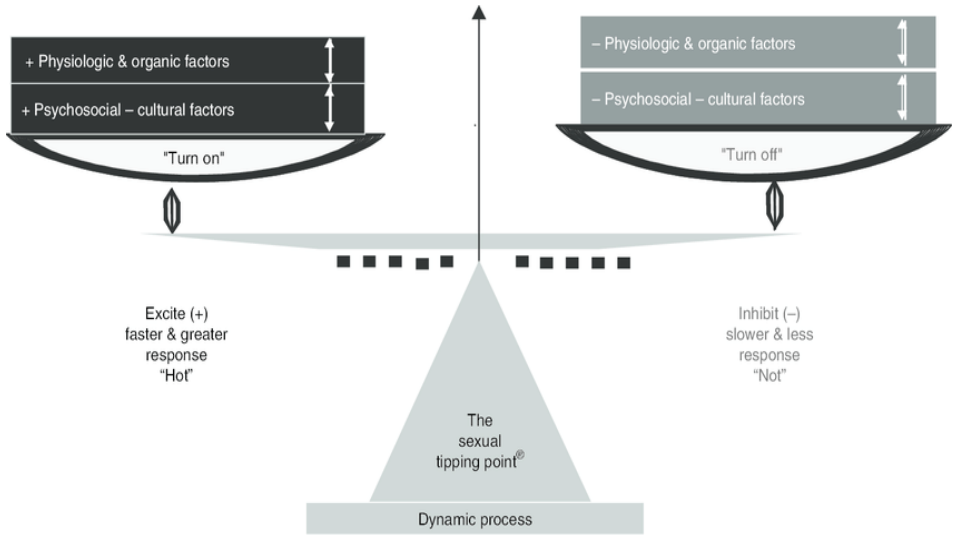


Figure 1. Sexual Tipping Point® from Perelman (2006)

Accordingly, sexual disinhibition can occur if the propensity toward sexual excitation is higher or if the propensity to sexual inhibition is decreased (Janssen and Bancroft, 2007). This imbalance between excitation and inhibition could lead to hypersexuality, or inappropriate or risky sexual behavior. Again, what causes this imbalance depends on the context. A decrease in sexual inhibition can be associated with other inhibition-related disorders such as dementia (for review, Cipriani et al., 2016), ADHD (Reid et al., 2013), and substance abuse (Buffum, 1988). In non-clinical populations, this change in balance between excitation and inhibition of sexual

behavior leading to disinhibition can be triggered by the use of drugs (Buffum, 1988; Pfaus, 2009; Pfaus et al., 2010).

Drug-induced disinhibition

Drug-induced disinhibition has been reported following the use of various prescription medications and illicit drugs (reviewed in Bond, 1998). However, the link between drugs and disinhibition is particularly complex due to factors such as the type of drug (e.g., depressants vs. stimulants), the route of administration, the dosage, and the individual drug history (e.g., acute vs. chronic) and the neural mechanisms underlying drug-induced changes in behavior remain to be fully elucidated.

As mentioned earlier, disinhibition can be induced by internal (e.g. mood, feelings, culture...) and/or external (e.g. peers, ambience...) cues that inhibit or release one's normal inhibition. As recreational drugs are often consumed in a particularly arousing context, they can mimic cues and stimuli from those contexts and induce disinhibited behaviors in the same way as those cues. For example, in a Pavlovian context, physiological effects of a drug on the brain or body can act as unconditioned stimuli. On subsequent occasions, when paired with a conditioned stimulus or cue, the cue itself that is predictive of the drug effect can induce a drug-like response. This response is dependent on the intoxicated state and subsequent exposures to the drug effect results again in the drug-induced response even if the context is not appropriate. As such, depending on the context, the drug-induced response can be a loss of restraint and/or inhibitory control leading to inappropriate – or disinhibited -- behaviors that are state dependent. One of the main problems in our understanding of this phenomenon is the wide range of drug-induced disinhibited behaviors that vary from mild disturbances to increased and extreme aggression, violence, and other crimes (Bond, 1998; Lebeau, 2013; Miczek et al., 1993).

Alcohol. The one drug commonly associated with disinhibition of a variety of behaviors is alcohol (EtOH: ethyl alcohol). As one of the most used and abused drugs worldwide, several studies have looked at the relation between alcohol and disinhibition, and a few hypotheses have emerged (reviewed in Källmén and Gustafson, 1998). The "disinhibition hypothesis" of Källmén and Gustafson (1998) postulates that alcohol has an inhibiting effect on cortical regions responsible for the inhibitory control of behavior. Accordingly, the inhibiting action of alcohol on

these inhibitory regions would lead to a general disinhibition of behavior. However, as a general anesthetic and non-specific drug, the action of alcohol is not restricted to the cortex and extends to the whole brain and body. Therefore, the disinhibitory effects of alcohol cannot be limited to its inhibitory action on cortical regions alone. Other hypotheses mention the arousal state caused by alcohol leading to a more aroused and excited state, or to cultural beliefs and expectations associated with alcohol intoxication that lead to disinhibited behaviors. In the latter hypothesis, social norms are believed to be more "relaxed" during intoxication, and society is more lenient when individuals behave inappropriately under intoxication (an important exception to this is drinking and driving). Early reports have also proposed that the disinhibitory effects of alcohol might be more due to the cultural beliefs or expectations of those effects than to the actual pharmacological effects in the brain (e.g., Room et al., 1983). Another explanation of the disinhibitory effects of alcohol is the "alcohol myopia" theory of Steele and Josephs (1990). In this theory, alcohol has a "myopic" effect on cues to which individuals are exposed: the theory states that alcohol induces a decrease in cognitive functions that limits attention to the most salient cues in the environment. They argue that the determinants of the behavior during alcohol intoxication are the internal state (e.g., learning, culture) and external releasing cues (e.g., peer pressure, context). When these cues are in conflict (e.g., inhibitory versus excitatory), alcohol restricts the attention to the most provoking cues that then guide the behavior induced by alcohol. For example, it is typically against social norms to throw rocks through the windows of people's homes. If someone has a rock in hand, and peers are pressuring the person to throw that rock through the window of someone they dislike, the myopia theory suggests that the presence of alcohol intoxication will inhibit the inhibiting learned social norms in favor of the immediate releasing stimuli (peer pressure). Despite several theories on the effects of alcohol on behavior, all agree that those effects are disinhibitory. Therefore, when individuals are intoxicated with alcohol there is a decrease in their ability to control and/or inhibit behaviors that are normally inhibited. And those effects might not be solely due to the pharmacological effects of alcohol but also to the context, to the cues present, and even to the belief that alcohol intoxication makes it "ok" to be disinhibited.

Other drugs. Reports of behavioral disinhibition by acute effects of drugs other than alcohol are less common. Nonetheless, when examined, it seems that both depressant and stimulant drugs can induce disinhibition. For example, central nervous system (CNS) depressant

drugs such as benzodiazepines (e.g., alprazolam, clonazepam, triazolam) have been reported to cause behavioral disinhibition (Bond, 1998; reviewed in Paton, 2002). Benzodiazepines induce a calm state in most individuals; however, in other individuals they can have disinhibitory effects, also referred to as "paradoxical" reactions, such as hyperactivity, sexual disinhibition, etc. As with depressants, stimulant drugs such as d-amphetamine (d-amp) and cocaine can induce behavioral disinhibition. D-amp can cause a dose dependent reduction of behavioral inhibition, meaning that under the influence of this drug, participants failed to inhibit their response to a cue when required to do so (Fillmore et al., 2003). Cocaine has also been shown to have disinhibitory effects by reducing the ability to inhibit a response (Fillmore and Rush, 2002). Both drugs can impair the inhibition of behavioral responses without affecting the ability to understand and execute the task.

Reports of drug-induced disinhibition vary largely in the literature. It mainly depends on the type of drugs, the dosage, and the context in which the drug is used. As the use of chemical intoxicants often occurs in social situations where sexual interactions are possible, if not expected, there is a perception that drug use is associated with sexual behavior, including risky sex (Buffum, 1982; Rawson et al., 2002). Therefore, a closer look at the effects of illicit drugs on sexual behavior and sexual function is warranted.

Drug-induced disinhibition of sexual behavior

Over the past few decades the relation between sexual behavior and substance use and abuse has been given increasing attention due to reports of drug-induced sexual dysfunctions, drug-induced improvement of sexual arousal and desire disorders (Pfaus, 2009; Volkow et al., 2007), and the induction of risky sexual activities and/or unwanted sexual advances indicative of sexual "disinhibition" (Baldwin, 2009; Pfaus et al., 2010). This latter effect is of particular concern because it sometimes results in rape and often increases the spread of sexually transmitted infections including HIV (e.g., Buffum, 1988; Calsyn et al., 2010; Ross & Williams, 2001; Shafer et al., 1994). It is therefore important to gain knowledge on how drugs affect sexual inhibition.

There is a wide range of behaviors affected by drugs but those that appear to be common to several drugs are inappropriate sexual behaviors and violence/aggression. Because of many

factors such as the variation in drugs use, individual reactions to drugs, and sexual context among others, the link between drugs and their specific impacts on sexual behavior, particularly drug-induced disinhibition is not always clear. However, reviews on the relation between sexual behavior and drugs in humans identify the potential of some drugs like alcohol, cannabis, cocaine, amphetamine, and methamphetamine to reduce or even eliminate sexual inhibition (Palha and Esteves, 2008; Rhodes and Stimson, 1994; Ross and Williams, 2001).

Alcohol. One of the first drugs to be examined for its effects on sexual behavior and reproductive function was alcohol (Abel, 1980; Crowe and George, 1989; Gantt, 1940, 1952, 1957; Hart, 1968; Teitelbaum and Gantt, 1958). Both preclinical studies in canines and rodents, and clinical findings in humans, generally agree on the acute dose-dependent effects of alcohol on sexual behavior. Low doses induce mild euphoria, good feelings and increased sexual arousal, whereas higher doses induce major motor impairment and inhibit penile tumescence, erection, and ejaculation. Chronic alcohol use results in sexual impotence (reviewed in Abel, 1980; George, 2019; Koob, 2014). Early reports assumed that low doses of alcohol could produce a release of inhibitions and this could lead to an increase in sexual arousal (Kaplan 1974). Rubin and Henson (1976) hypothesized that sexual disinhibition could be induced in men by alcohol. In an inhibitory setting, men were instructed to actively inhibit their sexual arousal by doing everything they can to avoid becoming sexually aroused. Despite the instructions, when given a moderate dose of alcohol (1.0 or 1.2 ml/kg) there was an increase in mean erections compared to men who did not receive alcohol, suggesting that alcohol had an effect on the ability to actively inhibit sexual arousal (Rubin and Henson, 1976). The same results were observed in a later study by Wilson and Niaura (1984). In their experiment, they also asked men to actively suppress their sexual arousal while listening to sexually explicit stories and penile tumescence was measured. Again, the consumption of a moderate dose of alcohol decreased the ability of men to restrain their sexual response and participants demonstrated shorter latencies to the onset of en erection and to peak penile tumescence (Wilson and Niaura, 1984).

Other drugs. As with alcohol, most drugs studied have a dual acute effect on sexual behavior, with low to moderate doses having little effects or increasing sexual desire while higher doses impair sexual function (for review, Frohmader et al., 2010a; Peugh and Belenko, 2001; Pfaus et al., 2010). Despite the increasing research on drugs and sexual behavior, few studies have looked

at drug-induced disinhibition of sexual behavior. The general consensus is that stimulants increase sexual desire and sexual pleasure directly (Bosma-Bleeker and Blaauw, 2018; Rawson et al., 2002) whereas depressants like alcohol disinhibits desire or motivation under inhibitory control. For example, some stimulants like methylphenidate have the ability to stimulate sexual desire and even to increase it in a non sexual context (Volkow et al., 2007). This is also the case for cocaine and amphetamine users who report increased sexual desire following ingestion of the drugs (Bell and Trethowan, 1961; Melis and Argiolas, 1995). CNS depressants other than alcohol such as barbiturates, benzodiazepines have been used in sexual context and their primary effects also seem to be disinhibitory (Buffum et al., 1988) as they decrease social inhibition (Calsyn et al, 2010). Whether individuals are using methamphetamine (Meth), cocaine or another drug it seems that each drug gives the user some sort of disinhibition and unique sexual effects ranging from increased sexual desire to increased sexual satisfaction (reviewed in Palha and Esteves, 2008).

It can be concluded then that several drugs of abuse (CNS depressants like alcohol or stimulants like amphetamine and cocaine) are acting on sexual behavior either to reduce inhibition and/or enhance sexual desire, both of which can lead to disinhibition of sexual behavior. This raises the question whether these disinhibitory drugs, despite their opposing effects on CNS (depressant vs. stimulants), use the same final mechanism(s) to disinhibit sexual behavior.

Neural mechanisms involved in drug-induced disinhibition

While interest in drug-induced disinhibition is growing, little is known about the neural mechanisms involved. Regarding sexual disinhibition, if one refers to the dual control of sexual behavior, the question arises whether drug-induced sexual disinhibition is due to an imbalance between the inhibitory and excitatory sexual systems: Does it occur through activation of excitatory brain regions and neurotransmitters or through the inhibition of inhibitory regions and neurotransmitters? Perhaps actions on both?

Despite their distinct pharmacological effects in the brain, drugs of abuse act on a common neural circuit: the mesocorticolimbic dopamine (DA) pathway. This pathway is largely recognized for its key role in processing of attention, and in particular attention to stimuli

associated with reward (Berridge and Robinson, 1998), and reward-related stimuli associated with drugs (Wise, 2008). This system encodes reward prediction signals (reviewed in Volkow and Morales, 2015) and is especially driven when errors in reward prediction occur. Originating from the ventral tegmental area (VTA) in the brainstem, DA neurons send projections to a variety of limbic brain structures such as the nucleus accumbens (NAc), amygdala, lateral septum, cortical regions like the medial prefrontal cortex (mPFC), and hypothalamic structures (Hökfelt et al., 1976). Most drugs of abuse act on the mesolimbic pathway by increasing DA in the NAc which results in their reinforcing effects (Di Chiara, 2002; reviewed Volkow and Morales, 2015). And it is through its role in reinforcing the pairing between rewards and an otherwise neutral stimulus that DA is important for motivation (reviewed in Arias-Carrión et al., 2010).

One common trait in the various reports of drugs effects on sexual behavior is an increase in sexual motivation. Methamphetamines, amphetamines, cocaine, methylphenidate reportedly increase sexual desire and arousal (Buffum, 1982; Melis and Argiolas, 1995). Accordingly, because there is a common conception that DA regulates sexual arousal and motivation (Pfaus and Phillips, 1991), this increase in sexual desire might partly reflect the increase in DA levels induced directly by drugs in the mesolimbic pathway (Melis and Argiolas 1995). Reports of cocaine and amphetamine users who experienced high sexual desire after taking their drugs also suggest an excitatory role of DA on sexual behavior (Melis and Argiolas 1995). A study by Volkow and colleagues (2007) reporting the effects of methylphenidate on sexual desire further support the role of DA transmission as methylphenidate only increase sexual desire when administered intravenously as opposed to orally possibly due to the higher DA increase evoked by the former (Volkow et al., 2007). Drugs reinforce the incentive value of the reward by increasing sexual motivation and this increased motivation could lead to disinhibited behaviors. In fact, the greater the sexual motivation, the greater the likelihood that individuals will behave to obtain the reward (i.e., sexual intercourse, orgasm, sexual pleasure) regardless of the context and/or the presence of other cues (reviewed in Arias-Carrión et al., 2010).

Despite the numerous studies reporting the effects of drugs on sexual behavior, few have focused on their disinhibitory effects and the neurobiological mechanisms behind those effects. With the increasing consumption of drugs worldwide, a better understanding of their effects on sexual behavior, their potential to disinhibit sexual behavior and the neural mechanisms

underlying these phenomena seems necessary. But investigating such mechanisms in humans is often complex because many factors such as individual drug use history (i.e., use of one drug or more, chronic use), self report measures subjectivity, and sexual behavior history before drug use cannot be fully controlled in human studies. To examine drugs effects on sexual function and their neural mechanisms, animal models have been used to alleviate those factors.

Modeling drug-induced sexual disinhibition in male rats

Although male human and male rat sexual behaviors cannot be fully compared, they nevertheless share some common sequences that can be paralleled (Figure 2). These common sequences of sexual behavior can be divided into appetitive (precopulatory) and consummatory (copulatory) phases (Ball and Balthazart, 2008; Beach, 1956; Pfaus, 1996, 1999; Sachs, 2007). During the appetitive phase, male rats can exhibit anticipatory behaviors such as increase in locomotion, investigation of the environment or investigation of the females and their anogenital area when accessible. The consummatory part consists in mounts and intromissions to reach ejaculation (Beyer et al., 1981). After the ejaculation, male rats enter a refractory period during which they are usually unable to resume sexual activity for a few minutes.

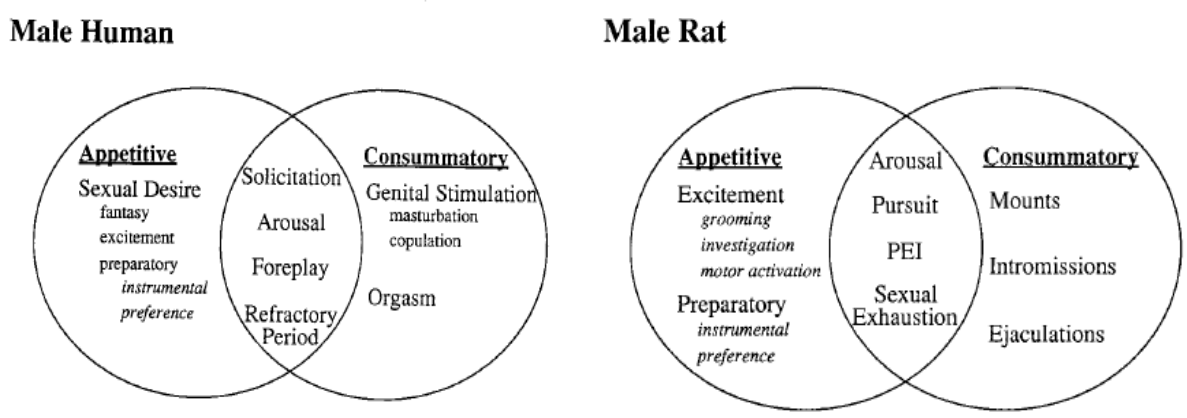


Figure 2. Incentive model for male human and male rat sexual behavior, modified from Pfaus, 1996.

Precopulatory and copulatory as well as the refractory period include behaviors that are measurable in terms of frequencies and latencies. These measures allow the study of the potential effects of drugs, conditioning, lesions of brain regions or neural changes, on the initiation (i.e. sexual motivation) and the expression (i.e. sexual performances) of male sexual behavior.

Sexual inhibition and disinhibition in male rats

In male rats, natural inhibition of sexual behavior is also adaptive and occurs in response to threat or aversion and is also important for fertility (e.g., as in the refractory period that marks an endpoint of sexual interaction), given that repeated ejaculations over short periods lead to lower sperm stores (reviewed in Bancroft, 1999). Following an ejaculation, male rats enter a refractory period that lasts several minutes, during which they cannot resume sexual activity (Beach, 1956). When it occurs after several ejaculatory series, this inhibitory period can last up to 15 days (Beach and Jordan, 1956). During this phase of sexual exhaustion (or sexual satiety), some male rats are unable to fully resume sexual activity. If some can initiate sexual behavior with attempted mounts or intromissions, they do not reach ejaculation until the longer-term effects of inhibition wear off. When examining the neural mechanisms of sexual satiety, studies have demonstrated its regulation by neurotransmitters such as serotonin (5-HT) and endogenous opioids (for review, Hull & Rodriguez-Manzo, 2009; Pfaus, 2009). This is in accordance with the dual control of sexual behavior as natural sexual inhibition is mediated by neurotransmitters that are inhibitory for sexual behavior.

Besides being observable in natural conditions, inhibition of sexual behavior can also be induced using classical conditioning. Kippin and his colleagues (1998) reported conditioned sexual inhibition (CSI) in male rats that were exposed sequentially to sexually nonreceptive females bearing a neutral almond odor and to unscented receptive females. Following this paired conditioning, males were given a final test in an open field with two receptive females, one scented with the almond odor and the other unscented. During the final test, males preferentially chose the unscented females over the scented females for their first mount, intromission, and ejaculation, and ejaculated more frequently with the unscented females. Males displayed fewer copulatory behaviors toward the scented females without any impairment of overall copulatory performance. In contrast, males in a randomly paired group (i.e., in which the almond odor was not paired in a predictable way with sexually nonreceptive females) did not display any avoidance of copulatory behavior with the scented females. This indicates that males in the paired group had developed a CSI towards the scented females (Kippin et al., 1998). According to Pavlov's definition (1927), and in Rescorla's studies (1969; 1976), the state induced by the almond odor following its association with thwarted sexual advances was one of internal

inhibition that reduced the overall sexual interest and activity with females bearing the almond scent.

As in humans, studies have shown that sexual behavior can be disinhibited in male rats. In fact, sexual inhibition, natural (i.e. sexual satiety) and conditioned (CSI) can be reversed or disrupted in male rats, naturally or by the use of drugs. Natural sexual inhibition in rats can be reversed by the introduction of a new receptive female (e.g., as in the "Coolidge effect"; Brown, 1974; McClintock, 1984; Wilson et al., 1963). To relate to the dual control of sexual behavior, the introduction of a novel receptive female would have the balance lean towards excitation which would override the inhibition and lead to the expression of sexual behavior despite the inhibitory context (e.g., refraction or sexual exhaustion as an inhibitory internal state).

Drug-induced sexual disinhibition in male rats

Some studies have demonstrated disinhibitory effects of psychostimulant drugs such as yohimbine, methamphetamine, and cocaine on male rat sexual behavior. An acute treatment of yohimbine, a α2 adrenergic receptor antagonist that prevents autoreceptors on presynaptic terminals from inhibiting noradrenaline release, can reverse sexual inhibition present at sexual exhaustion in sexually experienced males by increasing the percentage of males that mount and show penile intromission following sexual exhaustion (Rodríguez-Manzo and Fernández-Guasti, 1995a). Similarly, this drug can also disrupt conditioned inhibition in an aversive conditioning paradigm. In a copulation-illness association paradigm where lithium chloride (LiCl) is associated with the presentation of receptive females, male rats treated with yohimbine are more likely to engage in copulatory behaviors with receptive females than control rats (Peters et al., 1988). Comparable results have been observed with methamphetamine. Not only does Meth pre-treatment increase the number of conditioning sessions necessary to establish conditioned sexual aversion with LiCl, but Meth treatment also following conditioning increases the proportion of males displaying copulatory behaviors resulting in a loss of expression of inhibited sexual behavior (Frohmader et al., 2010b). The disruption of conditioned inhibition can also be observed following an acute treatment of cocaine. When male rats are trained to inhibit their sexual behavior towards females bearing an odor, cocaine disrupts the display of this second order conditioning. While males injected with saline show avoidance of the female bearing the odor associated with sexual nonreward, males treated with cocaine prior to a copulatory test do not

avoid these scented females and copulated randomly with the scented and unscented females (Pfaus et al., 2010).

Some central nervous system (CNS) depressant drugs that include barbiturates, opioids, anxiolytic drugs and alcohol have also been reported to act on sexual behavior (Pfaus et al., 2010). As observed in men, alcohol can disrupt sexual inhibition in male rats. The first study to look at the potential disinhibitory effects of alcohol on sexual behavior in rats was done by Pfaus and Pinel (1989). In their experiment, male rats were trained to inhibit their sexual behavior towards sexually non receptive females. To observe the effects of alcohol on sexual behavior, two tests were performed: a non inhibitory test where males had access to a sexually receptive female and an inhibitory test with access to a non receptive female. The non inhibitory test revealed the dose dependent effects of alcohol on sexual behavior as a lower proportion of male rats injected with the higher doses of alcohol were able to engage in copulatory behavior. The inhibitory test exposed the disinhibitory effects of a low dose of alcohol. In fact, when injected with a low dose of alcohol (0.5g/kg), the proportion of males intromitting and ejaculating with the non receptive females was significantly higher compared to saline treated male rats, demonstrating that a low dose of alcohol was able to suppress learned sexual inhibition (Pfaus and Pinel, 1989). A study from our laboratory also established the disruptive effects of alcohol on CSI (Germé et al., in preparation). In this study, male rats were exposed sequentially to unscented, sexually receptive females and to scented nonreceptive females during the conditioning phase, which resulted in them suppressing their sexual advances toward the scented, nonreceptive females by the 10^{th} conditioning trial. Males were injected with either saline or a low dose of alcohol (0.5 g/kg w/v) prior to a final open field test, where the males were presented with two receptive females, one scented and the other unscented. Males injected with saline copulated and ejaculated preferentially with the unscented females, indicating that they had formed a CSI toward the scent on a female (and despite the female on the final test being sexually receptive). In contrast, males treated with the low dose of alcohol copulated and ejaculated with both females, indicating that the CSI shown in the saline control group had been disinhibited. Those results add to the previous study by Pfaus and Pinel (1989), showing that inhibition can accrue to the sexually nonreceptive state of the female as well as to an odor associated with that state. In both studies, this dose of 0.5g/kg of alcohol did not impair the ability of males to copulate to ejaculation. Although the ejaculation latencies were slightly higher in the alcohol

group compared to the saline group, the total number of ejaculations did not differ significantly between those groups.

Potential neural mechanisms involved in drug-induced disinhibition in rats

To date, very few studies have examined the neural mechanisms of drug-induced sexual disinhibition. However, due to its role in motivated behaviors (Berridge and Robinson, 1998), sexual behavior (Damsma et al., 1992; Hull and Dominguez, 2007; Pfaus and Philips, 1991; Pfaus, 2009) and drug-related behaviors (reviewed in Adinoff, 2004), studies have mentioned the potential involvement of the mesolimbic DA system.

As previously mentioned above, natural sexual inhibition can be disrupted in rats by drugs acting on the dopaminergic system or by the presentation of a novel sexually receptive female. A study by Fiorino and colleagues revealed that the introduction of a novel receptive female increased DA levels significantly in the NAc compared to the introduction of the familiar female the males had been copulating with. This suggests that the increase in DA transmission is necessary for the reinstatement of sexual behavior in male rats (Fiorino et al., 1997). Other studies also have also mentioned DA transmission in the reinstatement of sexual behavior in male rats following sexual exhaustion. Systemic injections (Mas et al., 1995b) or infusions within the NAc (Guadarrama-Bazante and Rodríguez-Manzo, 2019) of apomorphine, a DA receptor agonist, can reverse natural sexual inhibition. When reviewing literature regarding the reversal of natural sexual inhibition, it appears that all the drugs used to disrupt this state interact with DA neurotransmission, suggesting that the DA pathway could be mediating the disruption of natural sexual inhibition (Fernandez-Guasti and Rodríguez-Manzo, 2003; Rodríguez-Manzo, 1999).

Using Fos-IR, a previous study looking at the disruptive effects of alcohol on CSI also examined the brain regions activated under the influence of alcohol in an inhibitory context (Germé et al., in preparation). Under conditions leading to the disruption of CSI (i.e. alcohol + conditioned inhibitory cue) alcohol-treated males showed higher neuronal activation in the mPFC, NAc and VTA compared to the saline-treated males but also compared to alcohol groups not exposed to the inhibitory cue (Germé et al., in preparation). In addition to the behavioral results, these findings suggest a mediating role of these regions, part of the mesolimbic pathway, in the disruptive effects of alcohol on sexual inhibition. To our knowledge, no other study

examined brain activation in a context of drug-induced disinhibition of sexual behavior. Therefore, these results can only be restricted to alcohol and a mediating role of these brain regions in the disinhibitory effects of other drugs on sexual behavior can only be suggested.

The present thesis

As in humans, disinhibition of sexual behavior can be observed in male rats. While studies on the reversal of natural sexual inhibition strongly suggest the involvement of the mesolimbic DA system, no studies on the disruption of CSI examined the potential neurotransmitters involved. Furthermore, despite the body of evidence on drug-induced changes in sexual behavior, the brain regions targeted and neurotransmitters particularly involved in their disinhibitory effects on sexual behavior remain unclear. Therefore, the experiments reported in the present thesis sought to elucidate some of the neurochemical mechanisms underlying the disruptive effects of drugs on CSI.

For all studies in this thesis, CSI was induced using second-order conditioning. During a conditioning phase, male rats were exposed to and allowed to copulate with unscented receptive females or scented non receptive females. Male rats learned to inhibit their sexual behavior towards females bearing the olfactory inhibitory cue. The potential effects of drugs on this learned inhibition were observed in a copulatory test during which males had access to two receptive females with one scented with the inhibitory olfactory cue. All drugs were administered acutely right before the copulatory test.

First, it was investigated whether a) d-amphetamine (d-amp) could induce a disruption of CSI in male rats and b) the brain regions involved in the effects of d-amp on sexual inhibition. It was hypothesized that d-amp would disrupt CSI. The study of the effects of d-amp was based on its known effects on sexual behavior (Soulairac and Soulairac, 1957) but also in increasing DA transmission (Carboni et al., 1989; Di Chiara et al., 1993). Using Fos expression as a measure of neural activation, brain regions were examined under the influence of d-amp alone and/or following exposure to the inhibitory cue or to an unscented environment. This was performed to assess the brain regions involved in the potential disinhibitory effects of d-amp and determine whether the pattern of Fos expression in certain brain regions could be similar to those observed in our previous experiment with alcohol (Germé et al., in preparation).

The second study used microdialysis to assess potential neurotransmitters involved in the disruptive effects of alcohol on CSI in male rats. Because of the known action of alcohol on the DA neurotransmission system, the extracellular levels of DA were measured in the NAc. The NAc was chosen due to its role in sexual behavior, in drug-related behavior and its pattern of neural activation following exposure to alcohol and the inhibitory cue. Following the establishment of CSI and the observed disruption of conditioned inhibition by alcohol, microdialysis probes were implanted into the NAc of male rats. Extracellular levels of DA were measured in the NAc when male rats were exposed to saline or alcohol and to the inhibitory cue or a novel odor. Triple labelling fluorescent immunohistochemistry (FIHC) assessing DA neurons using tyrosine hydroxylase (TH), neural activation using Fos expression and the retrograde tracer Fluoro-Gold (FG) was used to determine the DA neurons projecting to the NAc activated under the same conditions as were measured NAc DA levels (alcohol + olfactory cue). This follow up experiment was done to potentially reveal the origin of the extracellular levels of DA observed in the NAc.

The third study examined the potential role of glutamate (Glu) and γ-aminobutyric acid (GABA) in the NAc in the disruptive effects of alcohol on sexual inhibition. This experiment was performed because of the recognized involvement of these neurotransmitters in alcohol's effects, in male sexual behavior and their mediating role in NAc DA levels changes. This was accomplished by measuring the extracellular levels of Glu and GABA in the NAc using microdialysis. Once CSI was established and disrupted during the copulatory test by a single dose of alcohol, microdialysis probes were implanted in the NAc to determine extracellular levels of Glu and GABA in the NAc when male rats were exposed to saline or alcohol and to the inhibitory odor or a novel odor.

Chapter 2

Disruptive effects of d-amphetamine on conditioned sexual inhibition in male rats

Katuschia Germé[1], Dhillon Persad[1], Justine Petit-Robinson[1], Shimon Amir[1], and James G. Pfaus[1,2]

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

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

Running Head: Amphetamine and sexual disinhibition

For publication in *Psychopharmacology*

Abstract

Rationale: Male rats trained to associate a neutral odor (almond) with nonreceptive females during their initial sexual experiences develop a conditioned sexual inhibition (CSI) toward the female bearing the olfactory cue when given a choice in a final copulatory preference test between two receptive females in an open field, one scented and the other unscented. We have previously shown that this CSI can be abolished by acute alcohol before the copulatory test.

Objective: To examine whether acute treatment with d-amphetamine could also disrupt a CSI.

Methods: Male rats received 20 alternating conditioning sessions with an unscented receptive female or an almond-scented non-receptive female. Following the conditioning phase, males were injected with saline or one of three doses of d-amphetamine (d-amp; 0.5, 1.0, or 2.0 mg/kg) 40 min before a copulatory preference test with two receptive females one unscented and the other bearing the almond odor.

Results: The proportion of saline-treated males choosing the unscented females for their first mount, and first ejaculation was higher compared to the scented female. The frequency of ejaculation of those males with the unscented female was also higher compared to the scented female, showing the inhibition of copulatory behavior directed toward the scented female. In contrast, and regardless of the dose, males treated with d-amp prior to the final test copulated with both scented and unscented females, indicating that d-amp disrupted the CSI. Following reconditioning, males were injected with saline or d-amp and exposed to a gauze pad doused with saline or almond odor. Exposure to the odor increased the number of Fos cells detected in the nucleus accumbens shell and core relative to saline controls, suggesting a role of these subregions in the disinhibitory effects of d-amp. Although d-amp groups showed higher number of Fos cells in the medial preoptic area and ventral tegmental area, males treated with saline had significantly fewer Fos cells in these regions but more Fos cells in the piriform cortex, suggesting an active inhibition by the odor.

Conclusions: Like alcohol, a low dose of d-amphetamine disrupts the display of a CSI by acting on brain regions mediating sexual behaviour.
Key words: sexual behavior, conditioned sexual inhibition, d-amphetamine, Fos immunoreactivity

Introduction

Many drugs of abuse are associated with enhanced sexual function. For example, use of psychomotor stimulants like amphetamine, caffeine, cocaine or depressants such as alcohol or heroin, are believed to increase sexual arousal or desire, or to enhance the intensity of sexual stimulation during intercourse (reviewed in Pfaus et al., 2010). Those effects may be direct (e.g. facilitation of erection or increased sensory awareness) or indirect resulting from a cognitive disinhibition that leads individuals to engage in promiscuous, risky, marathon, or even violent sexual activity.

Despite its reputation as a "prosexual drug", findings appear to diverge on the effects of d-amphetamine (d-amp) on sexual behavior. This discrepancy has been revealed in human research where some amphetamine users reported increased sexual desire with loosening of inhibition, whereas for others the experience might not be as pleasant due to what is referred to as "mechanical sex" and a lack of connection between partners (Skårner and Svensson, 2013), or to an outright inhibition of sexual arousal and desire with higher doses in chronic users (Chou et al., 2015; Pfaus et al., 2010). Still other users report no impact on their sexual behavior and sexual function, or use stimulants like methamphetamine in conjunction with phosphodiesterase 5-inhibitors like sildenafil (Viagra®) to maintain erection for lengthy periods (Chan et al., 2015; Fisher et al., 2011). A finding of interest in all studies was that the effects of amphetamine and its derivatives were associated with dose and the frequency. These results correlate with some of the earliest pharmacological findings in nonhuman animals showing that d-amp and its derivatives can facilitate copulation and ejaculation in sexually sluggish or naïve male rats (e.g., Soulairac and Soulairac, 1957). Another early study by Bignami (1966) reported that at low doses (below 1mg/kg), male rats had a greater number of intromissions and shorter interintromission intervals prior to ejaculation; whereas at doses higher than 2mg/kg, opposite effects were observed, suggesting a potential dual effect of d-amp on male sexual behavior. Conversely, other studies reported no effects on sexual behaviour of sexually experienced male rats (Butcher et al., 1969; Ågmo and Fernández, 1989; Ågmo and Villalpando, 1995). However, the doses used in these experiments were between 0.4 and 1.5mg/kg, which enhanced sexual behaviour in sexually sluggish rats. Nonetheless, as d-amp acts on the dopaminergic system, these effects mimic the

dose-dependent effects of other dopaminergic agonists on sexual behavior with facilitation at low doses and inhibiting high doses (reviewed in Hull and Rodríguez-Manzo, 2009).

Although the effects of d-amp differ depending on the dose and level of sexual experience in sexually functional rats, the findings are more coherent in rats that have had their sexual behavior disrupted by other drugs. For example an early study by Butcher et al., (1969) mentioned the ability of d-amp to restore sexual behavior in male rats previously treated with tetrabenazine. Tetrabenazine, acting as a reducer of monoamine levels in the brain, completely abolished sexual behavior in male rats. But d-amp 0.8mg/kg reversed this effect and even restored sexual behavior to the levels comparable to untreated control animals. Similarly, male rats with lesions of the medial prefrontal cortex show increased latencies of mounts and intromissions (Ågmo and Villalpando, 1995). Although 1mg/kg of d-amp did not affect the sexual behavior of nonlesioned rats, it reduced mount and intromission latencies in the lesioned rats. They concluded that d-amp had a facilitatory effect on the initiation of sexual behaviour in sexually sluggish rats, but not on its expression once it had been initiated. This was in agreement with their previous findings that d-amp facilitates copulation by increasing arousal or sexual motivation (Ågmo and Fernandez, 1989).

Despite research done on the effects of d-amp on normal or disrupted sexual behavior, to our knowledge no study has looked at the effect of d-amp on active sexual inhibition. However, Frohmader et al. (2010b) examined the effects of methamphetamine on a form of conditioned inhibition of sexual responding induced by gastrointestinal distress. In this paradigm, males were injected with lithium chloride (LiCl) after copulating with a sexually receptive female scented with almond extract, thus making them associate sexual stimulation and the odor with the gastrointestinal malaise induced by LiCl and inducing a copulatory aversion to almond-scented females. Prior to each conditioning trial, males were also injected with either saline or methamphetamine (1 mg/kg). Males that had received saline, developed avoidance behaviors toward the almond scented female and had significant reductions in their sexual responding whereas males that received methamphetamine copulated successfully throughout the conditioning procedure. This led Frohmader et al. to conclude that methamphetamine could lead to a maladaptive seeking of sexual behavior, in this case with partners that are associated with visceral illness.

Another method to induce active and specific sexual inhibition is to use the avoidance behavior of sexually non-receptive females as a means of inducing sexual nonreward in males attempting to copulate with them. Males learn quickly not to copulate with sexually non-receptive females (Pfaus & Pinel, 1989), and associating a neutral odor like almond with sexually non-receptive females will allow the nonreward state to be predicted by the odor as a conditioned stimulus (Kippin et al., 1998). In the explicitly unpaired paradigm developed by Kippin et al., the sexual inhibition was induced in males given sequential pairing with unscented sexually receptive females and non receptive females scented with an almond odor, thus having the males associate the odor with sexual non-receptivity and therefore sexual nonreward. On a final test, males were paired with two sexually receptive females in an open field, one scented and the other unscented, and their mounts, intromissions, and ejaculations directed at both females recorded. Despite the sexual receptivity of both females, males mounted and ejaculated preferentially with the unscented females, indicating that sexual inhibition had accrued to the almond odor. Recently, we reported that low-to-moderate doses of alcohol (0.5 or 1 g/kg) could disinhibit this effect (Germé et al., in preparation). Males were trained as above, but prior to the final open field test they were injected with 0 (saline). 0.5 or 1 g/kg of 95% ethanol in saline. Males given the saline control copulated and ejaculated preferentially with the unscented female whereas males in both alcohol groups copulated and ejaculated with both unscented and scented females, thus indicating that acute alcohol had disrupted the inhibitory conditioning to the odor. After four reconditioning trials, the males from the three groups were injected again with saline or the two alcohol doses and exposed to a cotton gauze pad doused with either the almond odor or distilled water to examine subsequent nuclear Fos induction in the brain as a measure of neuronal activation. When males are exposed to the odor, alcohol induced changes in mesolimbic brain areas such as more Fos overall in the prelimbic cortex, nucleus accumbens core, and ventral tegmental area, and less Fos in the basolateral amygdala, compared to the saline controls, suggesting the activation of the first three regions, and deactivation of the fourth, contributed to the disinhibitory effects of the alcohol.

The present study asked whether d-amp (0. 0.5, 1.0 or 2.0 mg/kg) can disrupt the conditioned inhibition of male sexual behavior using the same paradigm of second order conditioned inhibition as Kippin et al. (1998) and Germé et al (in preparation). A second goal was to examine Fos induction to the odor alone under the influence of saline or effective doses of

d-amp to determine whether a similar disinhibition of neuronal activation as we observed previously with alcohol might be associated with the effect.

Materials and methods

Animals, surgery, and steroids. 48 sexually naive male Long-Evans rats were obtained from Charles River, Canada (St. Constant, QC). The males weighed approximately 250g at the start of the experiment and were housed in groups of 4 in Plexiglas cages with ad libitum access to food (Purina Rat Chow) and water. All rats were maintained on a reverse 12-hour dark-light cycle (lights on at 8:00pm) at 20 ± 1°C. Behavioral tests were performed during the middle third of their dark cycle.

Female Long-Evans rats were received from the same supplier. They were housed in pairs in rooms using the same lighting and temperature conditions as the males. At least ten days prior to their first sexual experience, females were bilaterally ovariectomized via lumbar incisions using intraperitoneal (i.p.) injections (1ml/kg body weight) of ketamine hydrochloride (50mg/ml; Ketaset©, Wyeth Canada) and xylazine hydrochloride (4mg/ml; Rompun©, Bayer Healthcare) anesthetic mixed at a ratio of 4:3, respectively. Sexual receptivity was induced by subcutaneous injections of estradiol benzoate (10µg/0.1 ml; Steraloids, Newport, RI) 48 hours and progesterone (500µg/0.1 ml; Steraolids, Newport, RI) 4 hours prior to each conditioning trials and copulatory test.

Apparatus. Each conditioning trial took place in bilevel chambers made of Plexiglas (18cm x 25cm x 65cm) with a platform (40cm in length) dividing the chamber into two levels (described in Mendelson and Gorzalka, 1987; Mendelson and Pfaus, 1989). The copulatory preference test took place in a large open field (123cm x 123cm x 96cm) with bedding covering the floor (described in Kippin et al., 1998). All conditioning trials and preference test were recorded on a video camera and scored later using a PC-based behavioral observation program (Cabilio 1996).

Drug administration. D-amphetamine sulfate (Sigma Aldrich) was diluted in 0.9% saline in order to obtain doses of 0.5, 1 and 2mg/kg in a solution of 1ml/kg. Each male was injected with their appropriate dose, 40 minutes prior to testing and placed in a shoebox until the testing began. Doses and time of injections were chosen based on previous literature showing d-amp effects on rodents' sexual behavior (Ågmo & Villalpando, 1995).

Procedure. *Conditioning phase*. Procedures to induce conditioned sexual inhibition in male rats and for the copulatory test were the same as those reported previously (Germé et al., in preparation; Kippin et al., 1998). Males were preexposed to the bilevel chambers for 15 minutes each day for seven days before the beginning of the experiment. Males were then given alternating conditioning sessions with an unscented receptive female or a scented non receptive female. The scent used was pure almond extract (Blue Ribbon, Etobicoke, ON), Approximately 1ml of pure almond extract was applied on the back of the neck and the anogenital region of the scented females. Males were placed in a bilevel chamber, alone for five minutes of habituation, followed by a 30-minute conditioning trial with a female in the appropriate sexual condition. Conditioning trials with the scented or unscented females occurred in different rooms with bilevel chambers in each (same light and temperature) to avoid any remaining odor in the chambers during trials in the unscented condition. Criteria for sexual behaviors were those described by Sachs and Barfield (1976), Meisel and Sachs (1994), and Pfaus et al., (1990b).

Copulatory preference test. To habituate the animals to i.p. injections and ensure that this manipulation on the test day would not stress the animals, each male received an injection of 1ml/kg of 0.9% saline 40 minutes prior to the last two conditioning trials. Male rats were randomly assigned to one of the four d-amp treatment groups: 0 mg/kg d-amp (saline), 0.5 mg/kg (d-amp 0.5), 1 mg/kg (d-amp 1) or 2 mg/kg (d-amp 2). Four days after the last conditioning trial with a receptive female, each male rat was placed in the open field for a 5-min habituation period, after which two females were placed simultaneously into the open field at approximately equal distance from the male. Both females were sexually receptive but one was scented with almond extract and the other one was left unscented. All male copulatory behaviors, and the females to which they were directed, were recorded. The preference tests ended 30 min after the introduction of the females.

Behavioral and statistical analyses. All behaviors were scored for each animal individually. Frequencies and latencies of mounts, intromissions and ejaculations were recorded and scored. All statistical analyses were performed using SPSS Statistics 25.0 for PC. A 4x2 (d-amp dose x female) mixed design ANOVA was used to determine differences in the frequencies and latencies of mounts, intromissions and ejaculations during the copulatory preference test followed by Tukey's post-hoc comparisons. Partial eta squared (η^2_p) was reported as a measure of effect size.

Chi-square analyses were also used to assess males' choices of females for their first mount, intromission and ejaculation on the final copulatory preference test. For all analyses, the level of significance was set to $p<.05$.

Olfactory stimulation test. Following the copulatory preference test, males were given four reconditioning sessions using the same procedure as during the conditioning phase. Four days after the last reconditioning trial with a receptive female, each male was injected with their previously assigned d-amp dose and exposed 40 minutes later to 1ml of almond extract or to 1ml of distilled water on cotton gauze in the exposure chamber (individual shoe box cage). Males remained undisturbed in the exposure room for one hour after which they were injected with a lethal dose of sodium pentobarbital (120 mg/kg, i.p.) and perfused intracardially with 300 ml of phosphate buffered saline (PBS) followed by 300 ml of 4% paraformaldehyde (4% PFA). Brains were extracted and post-fixed in 4% PFA for 4 hours then switched into a solution of 30% sucrose for 24 to 48hours. Brains were then stored at -80°C until slicing.

Fos immunoreactivity. Using a cryostat, each brain was sliced in 30 μm coronal sections from the olfactory bulbs to the VTA, corresponding to plates 4-45 (bregma 5.50mm to bregma -6.30mm) of Paxinos and Watson (1998) and collected in a solution of 0.9% tris-buffered saline (TBS). During the process of immunohistochemistry, brain sections were always rinsed in three 5-minute periods in 0.9% TBS between incubations. Sections were incubated in 30% hydrogen peroxide (H_2O_2) in TBS for 30 minutes at room temperature to react endogenous peroxidases followed by a 2-hour incubation in a pre-blocking solution of 3% normal goat serum (NGS) in 0.2% Triton TBS at room temperature. Sections were then incubated in a primary antibody solution made of 3% NGS and rabbit polyclonal anti-fos (1:40,000; Fos ab5, Calbiochem, Mississauga, ON) in 0.05% Triton TBS for 72 hours at 4°C. Following this incubation, brain sections were incubated for one hour in a secondary antibody mixture of 3% NGS and biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories Canada, Burlington, ON) in 0.05% triton TBS then in a solution of 3% NGS and avidin-biotinylated peroxidase complex (1:55; Vectastain *ELITE* ABC KIT, Vector Laboratories, Canada) at 4°C for 2 hours. Immunoreactions were stained by incubation, at room temperature, in 50mM tris buffer (Tris) for 10 minutes, then in a solution of 3,3'-diaminobenzidine (DAB) in 50mM Tris for 10 minutes and finally in a mixture of 8% nickel chloride, 3% H_2O_2 and DAB for 10 minutes. Finally, brain sections were

mounted on gel-coated slides and allowed to dry, then dehydrated in increasing concentrations of alcohol, cleared in xylene, cover-slipped and examined under a light microscope.

Histological and statistical analyses. Brain sections were examined at 40x magnification. Images of Fos immunoreactive (IR) cells were captured using Q-capture Pro, and were counted bilaterally from 3 to 5 sections per region per rat using Image J. Using the rat brain atlas of Paxinos and Watson (1998) as a guide, Fos-IR cells were counted, in the prelimbic (PL) and infralimbic (IL) cortices, piriform cortex (PirCx), nucleus accumbens core (NAcCore) and shell (NAcShell), medial preoptic area (mPOA), medial amygdala (MeA), central amygdala (CeA), basolateral amygdala (BLA), and ventral tegmental area (VTA).

The mean number of Fos-IR cells was calculated for each area per rat for each group. A one-way ANOVA was performed to compare Fos activation between treatments when rats were not exposed to the odor (No Odor condition) followed by planned contrasts. Another one-way ANOVA was performed to compare Fos activation between groups when the male rats were exposed to the almond extract (Odor condition) followed by planned contrasts. Partial eta squared (η^2_p) was reported as a measure of effect size for the ANOVAs. Unpaired *t*-tests were used to assess differences between No Odor and Odor conditions for each treatment. *Cohen's d* was reported as a measure of effect size. For all analyses, the level of significance was set to $p<.05$.

Results

Copulatory test. Of the 48 male rats, two (n=2) from the saline group and four (n=4) from the d-amp 1 group and two (n=2) from the d-amp 2 group did not ejaculate during the 30 minute copulatory test. They were therefore excluded from the analyses.

The mean number of mounts, intromissions and ejaculations are shown in Figure 1.

There were no significant differences in the frequencies of mounts and intromissions regardless the dose of d-amp or the female that the behaviors were addressed to.

Regarding the mean number of ejaculations, there were no main effects of d-amp dose or female type. However, there was a statistically significant interaction of d-amp dose x female, $F(3,36)=2.88$, $p=.049$, $\eta^2_p = 0.19$. As shown in Figure 1, males in the saline group displayed

significantly more ejaculations with the unscented female (No Odor) compared to the scented one (Odor). This difference was not observed for the d-amp dose groups.

The latencies to the first mount and first intromission were not significantly different between females and between groups. There were also no main effects of group or female type for the latency to the first ejaculation. However, as shown in Figure 2, there was a significant interaction of d-amp dose x female, $F(3,36) = 4.39$, $p=.010$, $\eta^2_p = 0.26$, revealing that the latency to their first ejaculation with the unscented female (No Odor) was significantly shorter in the saline group compared to the latency to the first ejaculation with the scented female (Odor).

Finally, the percentages of males that chose the scented or the unscented female for their first mount, intromission, or ejaculation are shown in Figure 3. Regardless of the dose of d-amp administered, males in the d-amp groups did not show a preference in their choice of female for their first mount, intromission, or ejaculation. However, males in the saline group chose significantly more frequently the unscented female (No odor) for their first mount compared to the scented one as confirmed by chi square analysis χ^2 (1, N=10) = 6.40, p=.011 and showed a trend to choose the unscented female for their first ejaculation, χ^2 (1, N=10) = 3.60, $p=.058$.

Fos immunoreactivity. All results for the Fos immunoreactivity are depicted in Figures 4 to 7 and in Table 1.

No Odor condition. When male rats were not exposed to the olfactory cue, significant differences in the mean number of Fos-IR cells between the saline and the d-amp groups were observed in the NAcCore, $F(3,21)=9.10$, $p=.002$, $\eta^2_p = 0.67$ and the VTA, $F(3,21)=14.25$, $p<.001$, $\eta^2_p = 0.72$. Planned contrasts revealed that injections of d-amp alone overall increased the mean number of Fos IR cells in these two regions: NAcCore, $t(13)=-5.02$, $p<.001$; VTA, $t(16)=-6.21$, $p<.001$ compared to the saline group. There were no differences in Fos immunoreactivity in the other brain regions observed.

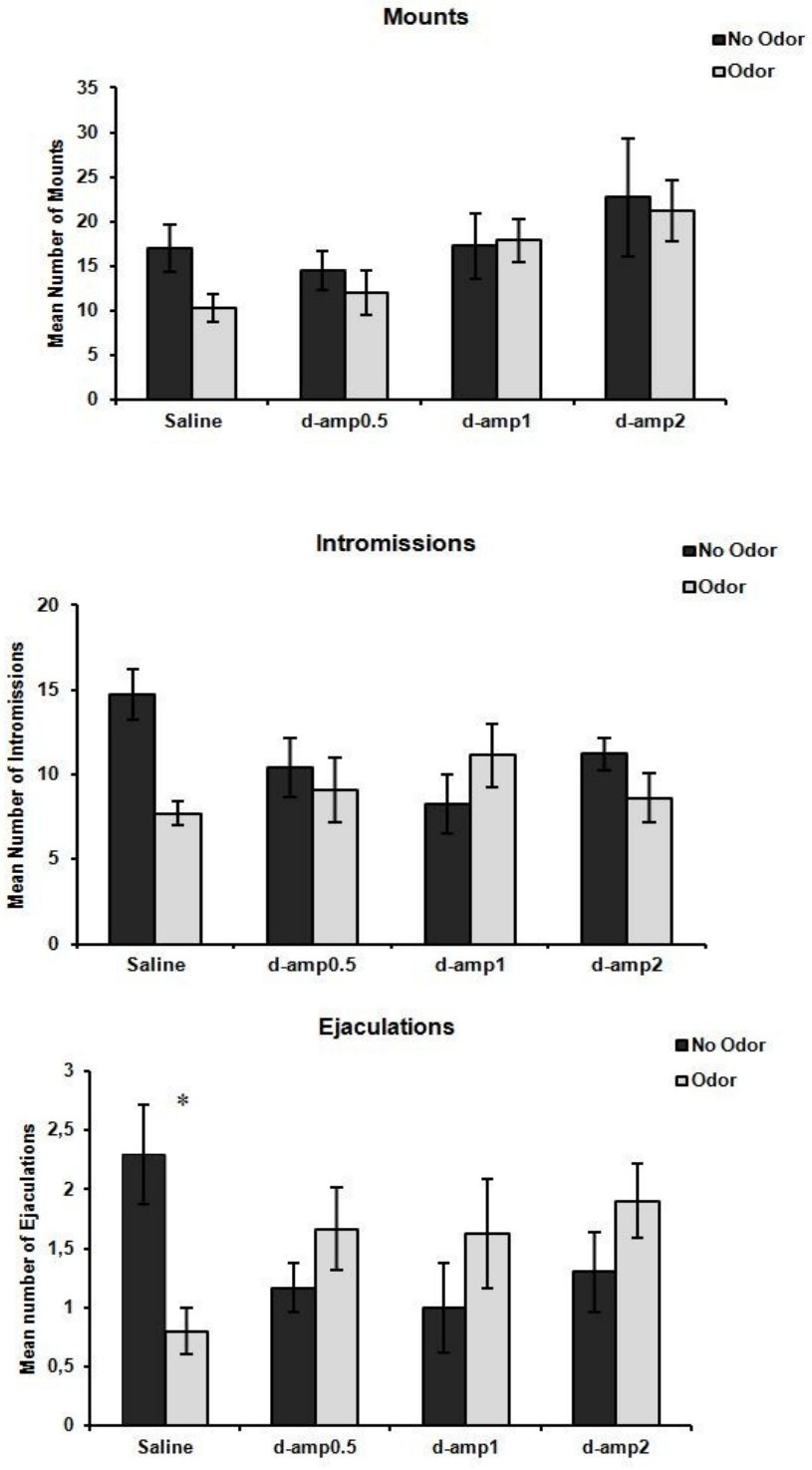


Figure 1. Mean of copulatory behaviors (± S.E.M) per group during the 30-minute copulatory test. * $p<.05$

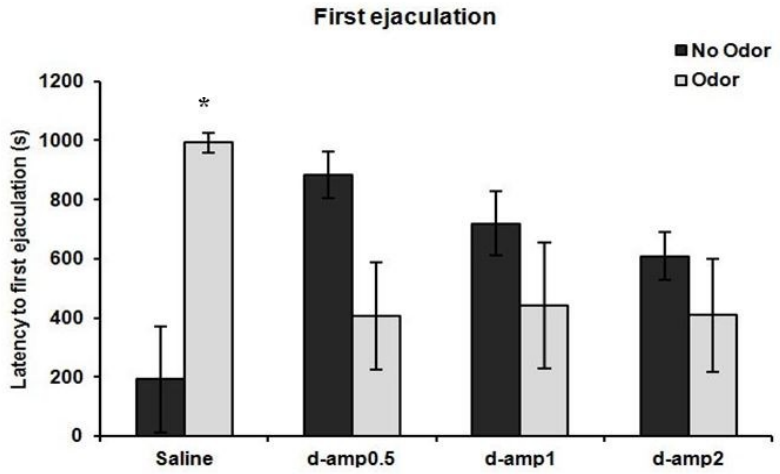


Figure 2. Mean latency to the first ejaculation (± S.E.M) for each group. * $p<.05$

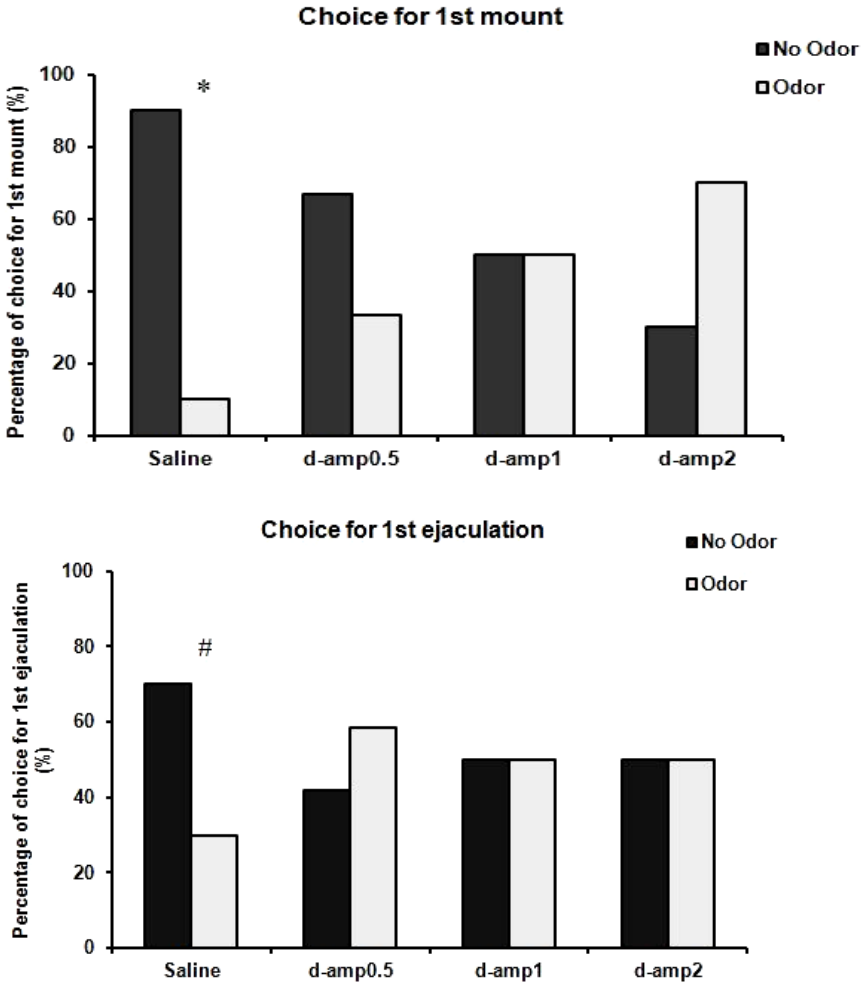


Figure 3. Percentage of choice for 1st mount and 1st ejaculation. * $p<.05$, trend # $p<.1$ ($p=.058$)

Odor condition. Exposure to the olfactory cue (almond extract) for one hour induced a significant difference between saline and d-amp groups in the mean number of Fos IR cells. This significant difference was observed in the NAcCore, $F(3,20)=24.42$, $p<.001$, $\eta^2_P= 0.81$, NAcShell, $F(3,20)=4.31$, $p=.02$, $\eta^2_p =0.43$, PirCx, $F(3,20)= 4.23$, p=.021, $\eta^2_p =0.42$, mPOA, $F(3,15)=4.30$, p=.028, $\eta^2_p =0.52$ and VTA, $F(3,20)= 9.14$, p=.001, $\eta^2_p =0.62$. Planned contrasts revealed that when exposed to the almond odor, males in the d-amp groups had significantly greater number of cells expressing Fos IR in NAcCore, $t(17)=-7.49$, $p<.001$, NAcShell, $t(17)=-3.01$, $p=.004$, mPOA, $t(17)=-3.322$, $p=.006$, and VTA, $t(17)=-4.94$, $p<.001$ but significantly fewer Fos IR cells in the PirCx, $t(17)=3.29$, $p=.004$ compared to males in the saline group.

No Odor vs Odor. Males in the saline group exposed to an unscented environment (No Odor condition) showed significantly less Fos IR cells in PirCx, $t(7)=-6.69$, $p<.001$, *Cohen's d* =4.34, but significantly more Fos IR cells in the mPOA, $t(5)=5.480$, p=.003, *Cohen's d*=4.23 compared to males in the saline group exposed to the odor (Odor condition). No significant differences were observed in the other brain regions analyzed.

As shown in Figure 4 and Figure 7, males injected with 0.5mg/kg d-amp prior to the exposure to the olfactory cue showed a higher number of Fos IR cells in the nucleus accumbens (NAcCore and NAcShell). No other statistically significant differences were found in the other brain areas observed. These observations were confirmed by independent t-test and Cohen's d effect size. Males in the d-amp 0.5 group exposed to the olfactory cue had a significantly higher number of Fos IR cells in the NAcCore $t(6.83)=-4.93$, $p=.002$, *Cohen's d*= 2.88 and in the NAcShell $t(9)=-3.31$, $p=.009$, *Cohen's d*= 2.08 compared to those in the same treatment group not exposed to the almond odor.

Male rats in the d-amp 1 group exposed to the almond extract for one hour showed significantly more Fos immunoreactivity in the NAcCore $t(7)=-6.77$, $p<.001$, *Cohen's d*=4.64 and NAcShell $t(4.427)=-3.21$, $p=.028$, *Cohen's d*= 2.05, compared males in d-amp 1 group not exposed to the odor.

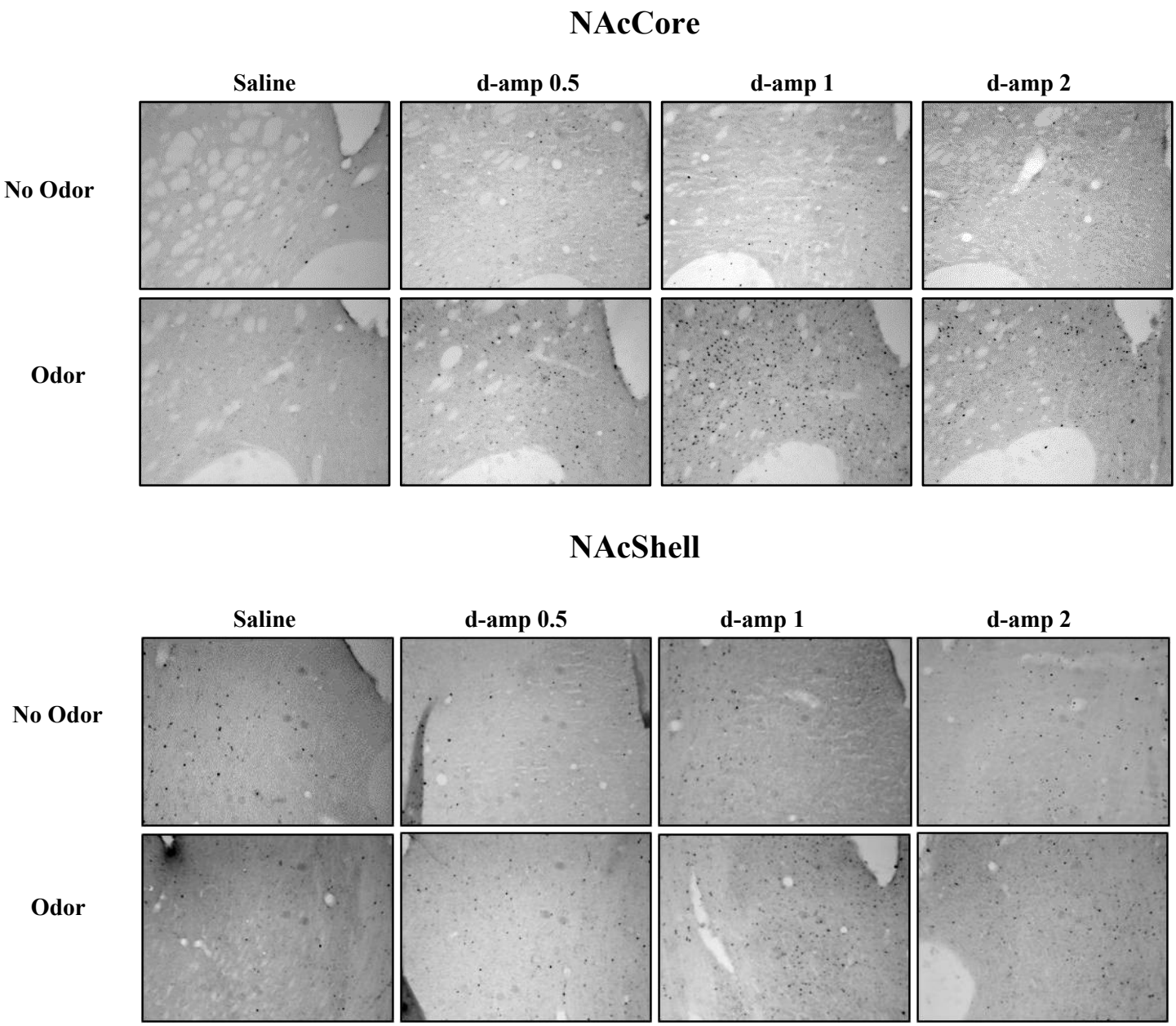


Figure 4. Photomicrographs (40x) showing Fos-IR cells (black dots) in the NAcCore and NAcShell, following a one-hour exposure an unscented gauze pad (No Odor) or to a gauze pad scented with almond extract (Odor). Males in the d-amp groups showed significantly more Fos expression following exposure to the Odor, compared to saline and to No Odor. Brain regions include NAcCore: nucleus accumbens core; NAc Shell: nucleus accumbens shell.

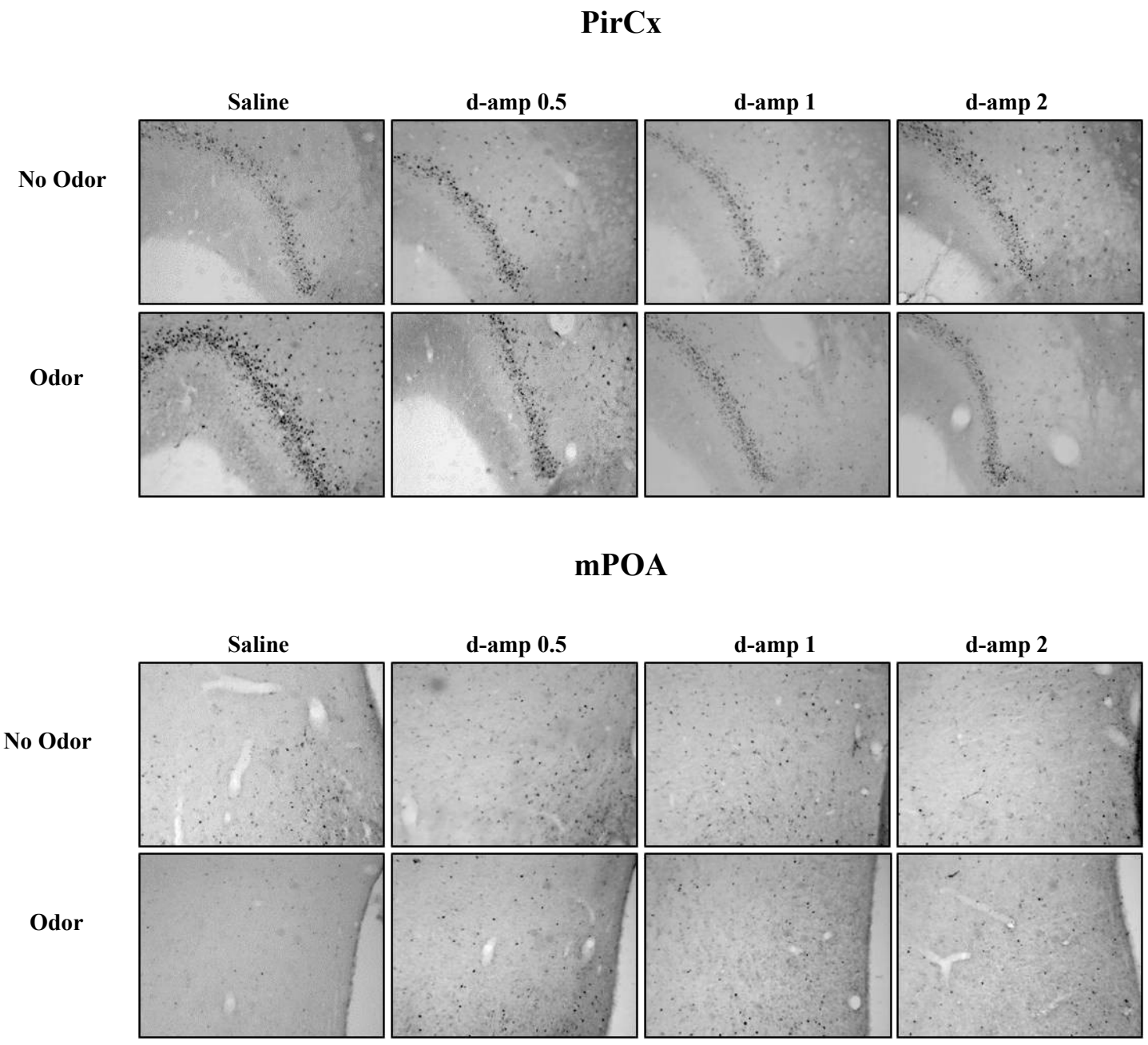


Figure 5. Photomicrographs (40x) showing Fos immunoreactivity (Fos IR cells: black dots) in PirCx and mPOA, following a one-hour exposure an unscented gauze pad (No Odor) or to a gauze pad scented with almond extract (Odor). Males in the d-amp groups showed significantly less Fos expression in the PirCx but more in the mPOA compared to the saline –treated males when exposed to the odor. Brain regions include PirCx: piriform cortex; mPOA: medial preoptic area.

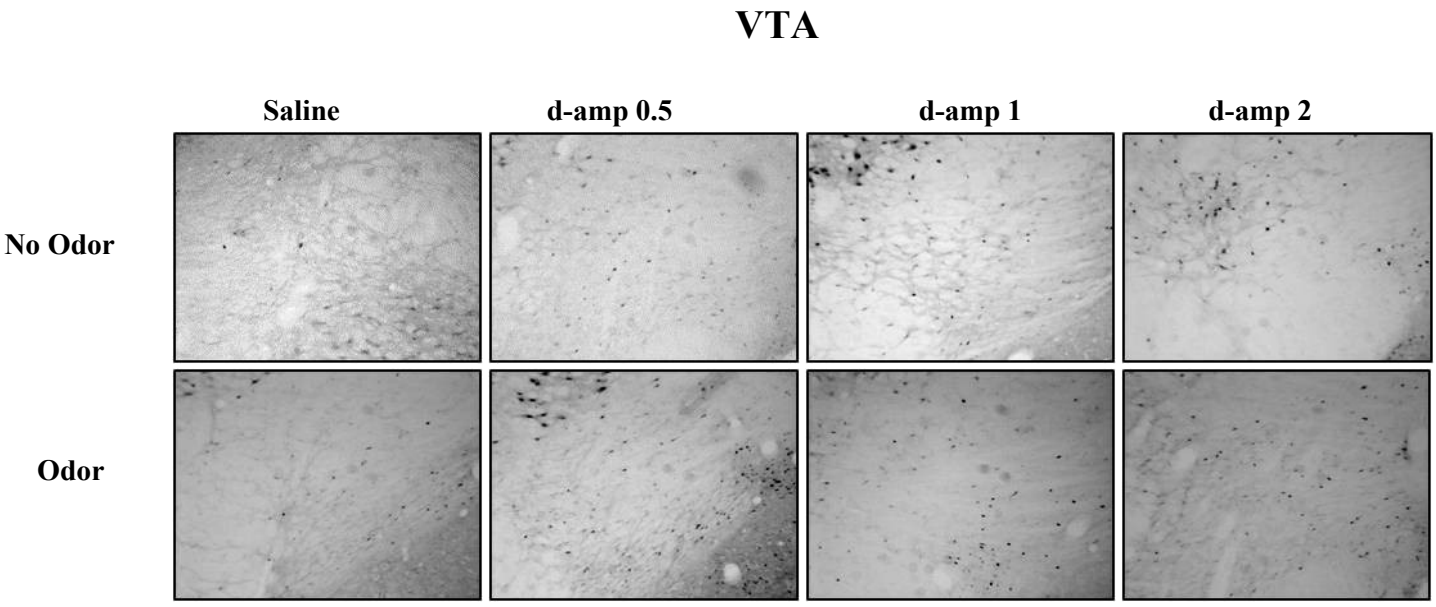


Figure 6. Photomicrographs (40x) showing Fos immunoreactivity (Fos IR cells: black dots) in VTA, following a one-hour exposure to an unscented gauze pad (No Odor condition) or to a gauze pad scented with almond extract (Odor condition). Males treated with d-amp showed significantly more Fos IR cells compared to males in the saline group in both conditions. VTA: ventral tegmental area

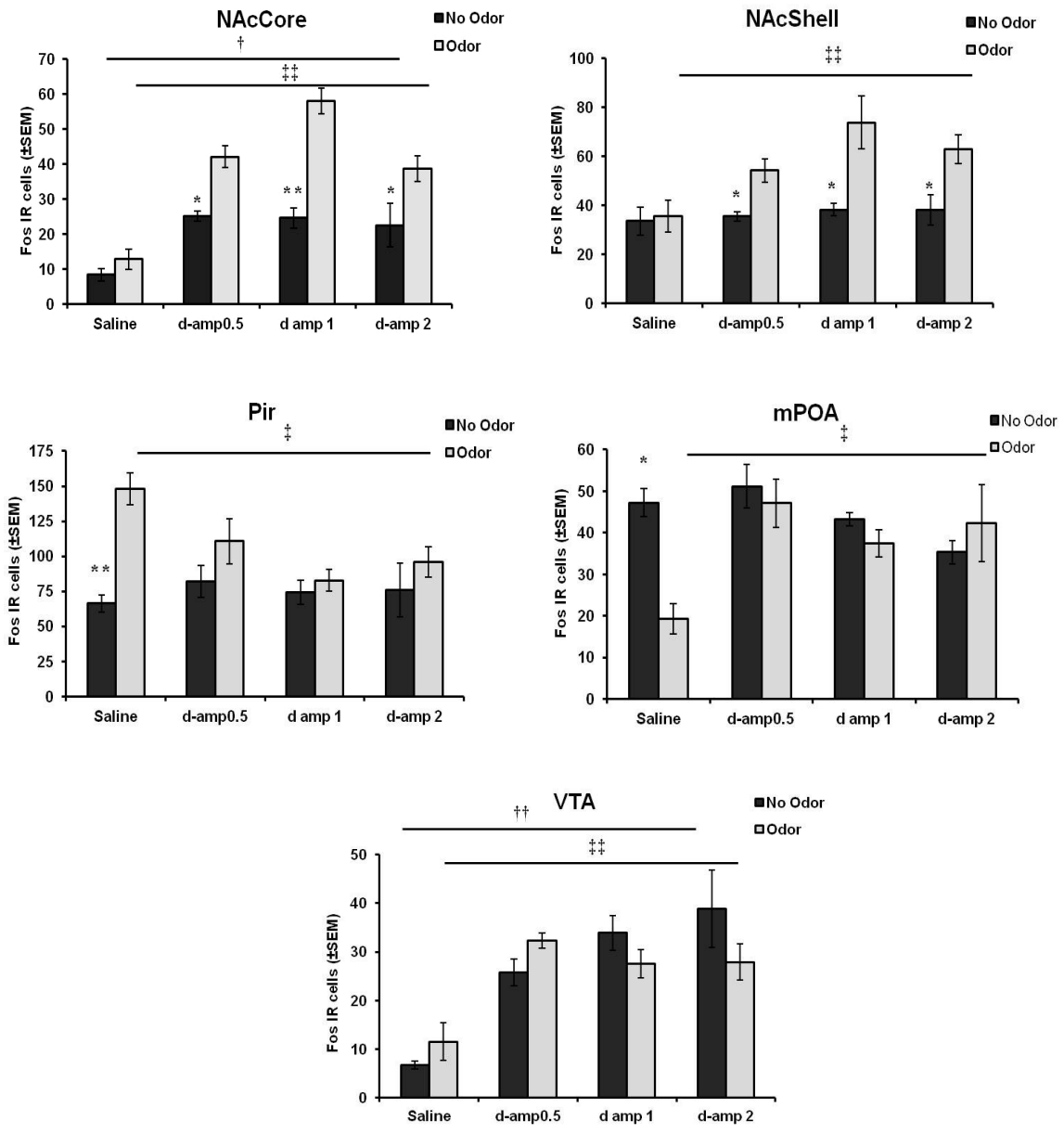


Figure 7. Mean number of Fos-IR cells in different brain regions in the No Odor (black bars) and Odor (grey bars) conditions. Comparison between saline and d- amp group in both conditions (No Odor and Odor) or between conditions (No odor vs. Odor). Data represent means ± SEM.

No Odor condition † p<.05, †† p<.001; Odor condition ‡ p<.05, ‡‡ p<.001; No odor vs. Odor * p<.05, ** p<.001

Brain regions include NAcCore: nucleus accumbens core; NAc Shell: nucleus accumbens shell; PirCx: piriform cortex; MPOA: medial preoptic area; VTA: ventral tegmental area. Error bars represent standard error of the mean.

Table 1. Mean number of Fos IR cells (± SEM) for each group and each environment condition in the brain regions of interest

Group/Condition	PL	IL	PirCx	NAcCore	NAcShell	mPOA	MeA	CeA	BLA	VTA
Saline/No Odor	76 ± 10.4	50.2 ± 14.7	66.2 ± 6.2	8.3 ± 1.7	33.5 ± 5.7	47.3 ± 3.5	56.6 ± 11.7	21.7 ± 3.9	10.2 ± 1.3	6.8 ± 0.8
Saline/ Odor	107.6 ± 16.2	75.6 ± 14.2	148.3 ± 11.4	12.8 ± 2.8	35.6 ± 6.5	19.3 ± 3.6	42.9 ± 5.8	21.9 ± 2.3	6.9 ± 1.4	11.5 ± 3.8
d-amp 0.5/No Odor	78.2 ± 23.1	41.0 ± 7.1	81.8 ± 11.3	25.1 ± 1.4	35.4 ± 2.0	51.2 ± 5.2	49.9 ± 5.2	25.0 ± 6.7	13.74 ± 3.9	25.8 ± 2.8
d-amp 0.5/Odor	112.7 ± 4.4	67.0 ± 5.1	110.6 ± 16.2	42.0 ± 3.1	54.2 ± 4.8	47.1 ± 5.8	38.0 ± 5.4	20.4 ± 5.5	12.3 ± 0.9	32.3 ± 1.5
d-amp 1/No Odor	69.6 ± 22.7	38.9 ± 5.1	74.4 ± 8.7	24.6 ± 2.9	38.3 ± 2.5	43.3 ± 1.6	53.3 ± 4.0	22.7 ± 3.1	11.5 ± 2.7	33.9 ± 3.6
d-amp 1/Odor	93.8 ± 16.4	63.7 ± 7.5	82.7 ±7.7	58.0 ± 3.7	73.7 ± 10.8	37.4 ± 3.3	61.6 ± 10.4	27.3 ± 8.1	13.8 ± 4.5	27.5 ± 2.9
d-amp 2/No Odor	57.5 ± 16.6	39.3 ± 4.4	76.0 ± 19.2	22.5 ± 6.3	38.0 ± 6.2	35.3 ± 2.8	51.5 ± 6.8	20.3 ± 3.3	11.5 ± 3.0	38.9 ± 7.9
d-amp 2/Odor	74.3 ± 4.3	61.2 ± 6.5	95.8 ± 10.9	38.7 ± 3.7	62.9 ± 5.8	42.3 ± 9.2	52.7 ± 5.1	25.7 ± 1.7	18.7 ± 2.1	27.9 ± 3.7

Brain regions include : PL : prelimbic cortex; IL : infralimbic cortex; PirCx : piriform cortex; NAcCore : nucleus accumbens core; NAcShell : nucleus accumbens shell; mPOA : medial preoptic area; MeA; medial amygdala; CeA; central nucleus of the amygdala; BLA; basolateral amygdala; VTA : ventral tegmental area.

Finally, when males in the d-amp 2 group were exposed to the olfactory cue for one hour, the number of Fos IR cells was significantly higher in NAcCore $t(7)=-2.39$, $p=.048$, *Cohen's d*= 1.62 and NAcShell $t(7)=-2.63$, $p=.034$, *Cohen's d* =1.96, compared to d-amp 2 male rats not exposed to the almond odor.

Discussion

The present experiment evaluated the effects of different doses of acute d-amp on conditioned sexual inhibition (CSI) in male rats. Males were given 20 alternating conditioning trials with an unscented, sexually receptive female or a sexually nonreceptive female scented with almond extract. Males were injected with saline or 3 doses of d-amp prior to a final open field choice test with two receptive females, one scented and the other unscented. Males injected with saline showed a preference to copulate and ejaculate with the unscented female and conditioned sexual inhibition toward the scented female. In contrast, males injected with d-amp, regardless the dose, did not display this inhibition and copulated indiscriminately with both females. Analyses of Fos IR in selected brain regions revealed a differential pattern of neural activation depending on the exposure environment (No Odor or Odor) but also on the dose of d-amp received prior to exposure. Compared to the saline group, when male rats were exposed to the unscented gauze pad (No Odor condition), d-amp induced an increase in neural activation in the NAcCore and VTA. In contrast, exposure to the odor on gauze (Odor condition) and to d-amp led to higher Fos immunoreactivity in NAcCore, NAcShell, mPOA, and VTA, but also to lower activation in PirCx compared to males injected with saline. Furthermore, when comparing the results of the two environments within groups, the same brain areas were affected. A lower number of Fos IR cells in PirCx and a higher number in mPOA were observed in the saline group in the No Odor condition as opposed to the Odor condition. Interestingly, males in the d-amp groups, regardless the dose, showed higher Fos activation in NAcCore and NAcShell when exposed to the olfactory cue compared to the unscented environment. These results show that d-amp, in the dose range tested, disrupted the conditioned sexual inhibition induced by an odor paired with sexual nonreward, and that this disinhibition also induced changes in the activation of brain regions associated with sexual motivation and copulatory behavior such as the NAc, the mPOA and the VTA.

The current results contribute to a growing body of evidence that acute treatment with different drugs of abuse can disinhibit sexual behavior in rats that have learned to suppress it (reviewed in Pfaus et al., 2010). For example, male rats injected with methamphetamine prior to copulation that has been paired with gastrointestinal distress continue to copulate whereas males injected with saline stop copulating (Frohmader et al., 2010). Acute treatment with low-to-moderate doses of 95% alcohol has also been shown to disinhibit sexual behavior in male rats that had learned to suppress it with sexually non-receptive females (Pfaus and Pinel, 1989), and in males given access to the same conditioned inhibition paradigm used in the present study (Germé et al., in preparation). In the latter experiment, acute alcohol treatment (0.5 or 1.0 g/kg) was able to disrupt the CSI without affecting sexual behavior overall. This result is similar to that observed in the present study with d-amp, suggesting that both drugs can disinhibit suppressed sexual behavior at doses that do not significantly alter sexual responses. This is in agreement with previous research showing no effects of d-amp on copulatory behaviors of sexually intact male rats (Ågmo and Fernández, 1989; Ågmo and Villalpando, 1995). At the dosage used in the current study no difference in overall frequencies and latencies of mounts, intromissions and ejaculations was observed between the saline and the d-amp groups. It should be mentioned, however, that observations made during the copulatory test could be in favor of potential inhibitory effects of d-amp. Previous research associated d-amp with inhibitory effects on sexual behavior with an increase in time between intromissions and a marked increase in hyperactivity (Bignami, 1966). In the latter study, these effects were observed at doses above 2mg/kg. Despite the absence of noticeable hyperactivity (data not recorded) and lower doses used in the current study, more males from the 2 highest doses of d-amp (1 and 2 mg/kg) did not reach ejaculation during the copulatory test compared to the saline group. But again, when sexual behavior was initiated, no differences in any copulatory behaviors were revealed in these groups, regardless the dose revealing no effect of d-amp on sexual performance.

It could be argued that the absence of inhibition towards the scented females in the d-amp groups could be due to an alteration of the olfactory sense in the d-amp groups. In fact, it has been shown that administration of d-amp could alter olfactory acuity in humans (Goetzl and Stone, 1948) and olfactory detection in rats (Doty and Ferguson-Segall, 1987). However, the dosage of d-amp used in the studies mentioned were different from the ones used in the present experiment. More specifically, Doty and Ferguson-Segall (1987) revealed a dose-dependent

alteration of olfactory detection in their experiment. An increased performance was observed following an injection of 0.2mg/kg of d-amp whereas 1.6mg/kg of d-amp induced a decrease in olfactory performances. These dose-related changes in performances were not observed in the present experiment. Even males injected with 0.5mg/kg, considered as a low dose that could induce potential increased olfactory detection according to Doty and Ferguson-Segall (1987), showed a disruption of conditioned inhibition to the same extent as males injected with higher doses of d-amp (up to 2mg/kg). We argue, therefore, that the absence of inhibition towards the scented females was not due in the present study to an alteration of olfactory detection but to disinhibitory effects of d-amp on sexual behavior.

Given that drugs from two opposing classes (psychomotor stimulants and CNS depressants) produce a similar disinhibition, we wondered whether a common, or at least similar, mechanism might underlie the disinhibition observed. In both our previous alcohol study (Germé et al., in preparation) and the present study, Fos IR was examined in several cortical, limbic, and hypothalamic brain regions related to sexual motivation, arousal, and copulatory activity following exposure to the odor/drug pairing relative to the control treatment with saline, and additionally relative to saline or drug treatment in a no-odor condition. Analysis of Fos IR in these brain regions revealed a differential pattern of neural activation depending on the No Odor or Odor conditions and their d-amp group.

A significant difference was detected between groups in PirCx when males were exposed to the olfactory cue. In fact, in the Odor condition, males in the saline group showed higher Fos IR in this brain area compared to males in d-amp group. They show also significantly higher Fos IR compared to the saline group not exposed to the odor. PirCx serves as main olfactory cortex and receives direct inputs from the olfactory bulbs (reviewed in Bekkers and Suzuki, 2013). Previous research demonstrated that sexual behavior induced an increase in Fos IR in PirCx (Robertson et al., 1991). The greater Fos IR in this brain area was also observed when male rats were exposed to a neutral odor previously paired with sexual reward compared to unconditioned estrous odors (Kippin et al., 2003). In the latter study, it was suggested that this brain area could be activated by stimuli that have acquired incentive value. Although this is in agreement with the results from the saline group in the present study, it would appear that the PirCx controls both conditioned excitation and inhibition given that Fos IR is activated by the same olfactory cue acting as a

conditioned exciter (Kippin et al., 2003) or inhibitor (present study and Germé et al., in preparation). The absence of a difference in Fos expression between No Odor and Odor condition in the d-amp groups would suggest that the olfactory cue did not acquire an incentive value as strong as it did for saline group. This suggests that d-amp could act to disrupt the conditioned activation of this region. It would be interesting to examine whether acute d-amp could disrupt the kind of conditioned ejaculatory preference for an odor paired with sexual reward in male rats that Kippin et al. (1998) and Kippin and Pfaus (2001a,b) observed.

Fos IR was also different between the saline and d-amp groups and between the No Odor and Odor conditions in the mPOA. Exposure to the odor induced a significant decrease in the number of Fos IR cells in the mPOA of males in the saline group. This number was also significantly lower compared to males in the d-amp groups exposed to the cue. The mPOA is one of the most critical brain regions for the regulation of sexual behavior in males (reviewed in Hull et al, 2006). Several studies have shown Fos IR in the mPOA following sexual activity (Baum et Everitt, 1992; Robertson et al., 1991), exposure to estrous odors, and/or contact with estrous females (Baum and Everitt, 1992; Kelliher et al., 1999; Kippin et al., 2003), but did not show increased Fos induction when males were exposed to a sexually conditioned excitatory cue (Kippin et al., 2003). However, using voltammetry, dopamine release in this region has been observed during 5-10 min exposures of conditioned males to the conditioned excitatory olfactory cue (Blackburn et al., 1992). Conversely, lesions of the mPOA have been shown to suppress or impair male copulatory behaviors (reviewed in Hull et al., 2006; Hull and Rodríguez-Manzo, 2009). According to these previous findings, the marked decrease in neural activation in the mPOA observed in the saline group exposed to the olfactory cue therefore reflect the behavioural results during the copulatory test showing less sexual behaviors towards the scented females. The decreased Fos IR in the mPOA following exposure to a conditioned sexually inhibitory cue further demonstrate the role of mPOA in male sexual behavior and sexual reward. This absence of difference in Fos expression between exposure conditions in the d-amp group also reflect the behaviors observed during the open field and the indiscriminate copulatory behavior directed toward both females.

In the present study, NAcCore and NAcShell showed higher Fos expression when males rats were under the influence of d-amp compared to the saline group and but also when they were

exposed to the odor as opposed to the No Odor condition. The NAc is part of the mesolimbic dopaminergic pathway and is critical for the display of appetitive behaviors (Berridge and Robinson, 1998). As a drug known to act on this system, d-amp has been shown to induce Fos IR in the NAc (Graybiel et al., 1990; Rotllant et al., 2010). This is in accordance with findings of the present study showing higher Fos IR in the d-amp groups compared to the saline groups in both No Odor and Odor conditions. Regarding the findings in the Odor condition particularly, previous studies have also shown that odors associated with access to receptive females could enhance NAc neuronal responses (West et al., 1992) but also Fos IR was increased in NAc by copulation (Robertson et al., 1991) and by sex-related odors (Kippin et al., 2003). In the latter study, males were trained to associate a neutral odor to sexually receptive females. The goal was to compare brain activation elicited by estrous odors to activation following exposure to a sexually conditioned odor. Although exposure to estrous odors induced increased Fos expression in both sub regions of the NAc (among other brain regions), the sexually conditioned cue increased Fos IR in NAcCore sub region. Despite activating separate pathways, it was suggested that these odors activate a common set of neurons in NAcCore. Interestingly, in the present study, the odor presented to male rats was previously associated with non receptive females. However, males in d-amp groups exposed to this olfactory cue showed higher numbers of Fos IR cells than male rats treated with d-amp but not exposed to the olfactory cue. It has been suggested that the NAc is activated by both rewarding and aversive stimuli (Ikemoto and Panksepp, 1999). However, as males in d-amp group did not show aversion towards the scented female in the open field, these findings suggest that the olfactory cue previously associated with non receptive female may have acquired a new, drug-related positive incentive value.

Males under the influence of d-amp showed significantly higher Fos expression in the VTA compared to males injected with saline in both the No Odor and Odor condition. Due to the fact that no difference was observed between the odor conditions, the effects on the VTA are solely due to the influence of d-amp. The VTA is the origin of the mesolimbic dopaminergic pathway. Dopaminergic neurons arise from the VTA and connect it to the NAc and prefrontal cortex. Numerous studies have illustrated the important role of the VTA and the mesolimbic system in both appetitive and aversive drug-related behaviors (reviewed in Oliva and Wanat, 2016). And d-amp increases local dendritic DA release in the VTA (Byrnes and Wallace, 1997). It was also demonstrated that injections of d-amp dose-dependently induced Fos IR in VTA neurons

(Colussi-Mas et al., 2007; Rotllant et al., 2010). Although d-amp induced an increase in Fos IR in the present study, the dose-dependency was not observed. This could be explained by the doses used. In fact, in Rotllant et al (2010), the doses were 1.5 and 5mg/kg whereas in the present study the dosage range was much smaller (0.5, 1 and 2 mg/kg). To observe a dose dependent effect of d-amp on Fos expression in the VTA, higher doses or bigger gap between doses might be necessary. Nevertheless, the main effect of d-amp on Fos activation in the VTA was still present compared to the saline group suggesting an action of d-amp on VTA neurons.

There may also be a role of context in the present study. The final open field choice test was the first time males were under the influence of d-amp. Moreover, the inhibitory olfactory cue was present for the first time on a sexually receptive female (as opposed to a non receptive female during the conditioning phase in bilevel chambers). To obtain the Fos IR data, males were given reconditioning trials as before in bilevel chambers, but then exposed to the odor and previous d-amp dose in a familiar shoebox cage. The aim of this exposure was to understand the possible meaning acquired by the olfactory cue itself, without the presence of a female. The differential pattern of neural activation noticed is mainly between saline and d-amp groups and appears to reflect the differences between groups observed in behavior during the open field. It is possible that the Fos activation detected is due to a state-dependent association made during the open field where d-amp + odor meant access to a receptive female. Therefore, the neural activation pattern would reflect the expectancy of sexual reward under the influence of d- amp and in presence of this particular scent.

In our previous study of alcohol-induced disinhibition (Germé et al., in preparation), we observed similar effects to those of d-amp in the PirCx, NAcCore, and VTA. In contrast, the alcohol-odor pairing increased Fos IR in the mPOA, PL, but decreased it in the NAcShell and BLA. This suggests that a common pathway for disinhibition may well exist for both alcohol and amphetamine even if the neuropharmacological effects of the drugs differ in other brain regions. Although the precise nature of the disinhibition is not yet known, from the regions activated similarly, it may well involve a perturbation of the mesolimbic DA system that maintains sexual motivation and arousal for competent copulatory activity in the presence of powerful inhibitory cues.

Funding. This research was funded by a grant from the Canadian Institutes for Health Research (MOP-74563) to JGP and an infrastructure grant from Fonds de la Recherche en Santé du Québec to the Center for Studies in Behavioral Neurobiology at Concordia University.

Compliance with ethical standards. The authors declare that all animal procedures conformed to the guidelines of the Canadian Council for Animal Care. All procedures were approved by the Concordia University Animal Research Ethics Committee.

Conflict of Interest. The authors declare they have no conflicts of interest.

Chapter 3

Influence of nucleus accumbens DA on the disruption of conditioned sexual inhibition by alcohol in male rats.

Katuschia Germé [1], Julian Alvarez-Barkham [1], Lindsey R. Gossip [1], Cassandra Poirier [1], Tracey D'Cunha [1], Heshmat Rajabi [1], Shimon Amir [1], James G. Pfaus[1,2]

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

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

Running Head: Dopamine, Alcohol and sexual disinhibition

For publication in *Psychopharmacology*

Abstract

Rationale: In male rats, conditioned sexual inhibition (CSI) towards a scented (almond odor) female can be disrupted by an acute treatment of alcohol. When exposed to the inhibitory olfactory cue alone, males injected with a dose of 0.5g/kg of alcohol show greater Fos induction in the nucleus accumbens (NAc).

Objective: To assess the effects of low to moderate doses of alcohol on extracellular NAc dopamine (DA) concentrations and on neuronal activation in the presence of an inhibitory odor.

Methods: Using our established paradigm to induce CSI, sexually naïve male rats were trained to develop CSI to an odor (almond). They were then injected with saline or alcohol (0.5g/kg or 1g/kg) before the final copulatory test after which, males were implanted with microdialysis probes aimed at the NAc. They were then given access to the odor under the influence of saline or alcohol and extracellular levels of DA in the NAc as well as neuronal activation in the ventral tegmental area (VTA) were assessed.

Results: During the copulatory test, saline-treated males showed CSI towards the females bearing the almond odor. This conditioned inhibition was abolished in alcohol-treated males. Alcohol alone increased extracellular NAc DA 20 minutes after the injection. The olfactory cue alone had no effect on DA levels in saline-treated males; however, with 0.5g/kg of alcohol, the cue increased extracellular DA in the NAc but also increased VTA activation in all groups.

Conclusions: These findings suggest that enhanced transmission of NAc DA is involved in the disruptive effects of alcohol on CSI in male rats.

Key words: conditioned sexual inhibition, microdialysis, dopamine, fluorescence immunohistochemistry

Introduction

Much evidence links the nucleus accumbens (NAc) to various phases of male sexual behavior. However, its precise implication in the different processes of sexual behavior remains unclear. Previous studies using Fos immunoreactivity (Fos-IR) in male rats reported that the NAc is activated following one hour of copulation (Robertson et al., 1991). This increased neuronal activation in the NAc is again observed when males are exposed to estrous females as opposed to non receptive females or an empty arena (Lopez and Ettenberg, 2002) but also when exposed to sexually-relevant cues such as bedding soiled by a receptive female or neutral odors previously associated with copulation (Kippin et al., 2003; West et al., 1992). Accordingly, the neuronal activation in this brain region during or following exposure to sexually relevant cues suggests that the NAc may play a role in encoding cues, contexts, and rewards related to sexual behavior.

The NAc receives major dopaminergic inputs from the ventral tegmental area (VTA) (for review Salgado and Kaplitt, 2015). Dopamine (DA) in the NAc is known to play an important role in reward processes, aversion, motivated behaviors as well as in the acquisition of incentive properties of contextual cues (reviewed in Ikemoto and Panksepp, 1999). When it comes to its involvement in sexual behavior, DA does not seem to impact the execution of sexual behavior in male rats, at least not in the NAc (for review, Paredes and Ågmo, 2004). In fact, most studies looking at the effect of DA in the NAc revealed its involvement in anticipation and motivation to engage in copulation more than the expression and performances of sexual behavior *per se.* Mesolimbic DA may be necessary for the initiation of copulation, whereas mesostriatal DA would be essential for copulation (Pfaus and Phillips, 1989). Studies on DA antagonists mostly reveal an effect of these drugs on arousal and sexual motivation, as measured by level changes in bilevel chambers in some studies (Pfaus and Phillips, 1989; 1991), but not on sexual performance once initiated (Everitt, 1990; Mitchell and Gratton, 1994). On the other hand, DA agonists increase the proportion of sluggish males copulating and decrease the latency to mount, intromission and ejaculation (for review, Bitran and Hull, 1987), suggesting again that DA acts on the motivation to initiate sexual behavior rather than sexual performance once initiated. Alternatively, use of in vivo voltammetry (chronoamperometry) to detect oxidation of extracellular DA concentrations in the NAc revealed a phasic pattern of release during appetitive sexual behaviors, but a tonic release pattern during copulation that decreased sharply at

ejaculation (Blackburn et al, 1992). The ability of low doses of DA antagonists to disrupt appetitive but not consummatory sexual responses may therefore be due to their ability to compete with lower and phasic extracellular DA concentrations for DA receptors.

Lesions studies also confirm these previous findings. Sexually naive males with lesions in the NAc fail to intromit and ejaculate and do not develop conditioned excitement as measured by non-contact erections (Kippin et al., 2004). It has been suggested that the NAc might have an excitatory role in the regulation of sexual arousal. Similar findings have been reported following DA depleting lesions in the NAc, leading to increased latency to first erection with no changes in copulatory behaviors (Liu et al., 1998). DA transmission increases in the NAc of sexually experienced rats more particularly when they are placed in the mating chamber and their behaviors as well as DA output further intensify when they are exposed to receptive females behind a screen (Pfaus et al., 1990a). This DA increase is not due to novelty or locomotion but to the presentation of the female or to copulation (Damsma et al., 1992). Enhanced DA transmission is also associated to the Coolidge effect with reinitiating copulation in sexually satiated males as it has been demonstrated that there is an increase in DA release in NAc induced by presentation of a novel receptive female (Fiorino et al., 1997). Together, these findings strongly suggest the involvement of DA NAc in sexual arousal and sexual motivation.

Drugs of abuse, including alcohol (EtOH, or alcohol), act on the dopaminergic system by increasing DA levels in the NAc (Di Chiara and Imperato, 1988). Most psychoactive drugs are also recognized to influence sexual behavior and sexual arousal (Pfaus, 2009). However, few recent studies have looked at the influence of alcohol on sexual behavior and more particularly at the potential brain regions and neurotransmitters involved in these effects. Acute alcohol intoxication produces a wide range of effects on sexual behaviour in particular. While high doses inhibit sexual responses (Ferraro and Kiefer, 2004; Hart, 1969; Pfaus and Pinel, 1989), low doses appear to disinhibit sexual behavior (Germé et al., in preparation; Pfaus and Pinel., 1989). Yet, how exactly alcohol affects sexual behavior and in particular, how it induces its disinhibitory effect on sexual behavior remains unclear. Because of its known involvement in drug and natural reward-related processes, one neurotransmitter of interest is DA. Not only does alcohol increase neuronal activation in the NAc (Chang et al., 1995) but it also increases DA transmission in this brain region (Yoshimoto et al., 1991). As previously mentioned, increased DA levels in the NAc

is also associated with sexual arousal and sexual motivation. Hence, it would be interesting to observe whether alcohol-induced changes in DA transmission have an influence on sexual behavior in male rats.

Because studies reporting the effects of alcohol showed that high doses inhibit sexual responses mostly due to a decrease in locomotion and sleepiness (Ferraro and Kiefer, 2004; Pfaus and Pinel, 1989), studying the effects of low doses of alcohol would likely reveal mechanisms associated with sexual disinhibition. We have previously shown that low to medium doses of alcohol disrupt conditioned sexual inhibition (CSI) in male rats (Germé et al., in preparation). Furthermore, under the influence of EtOH and following exposure to a conditioned inhibitory cue, Fos-IR was significantly higher in the NAc compared to males injected with saline or to males not exposed to the olfactory cue. These findings suggest that activation of the NAc could be involved in the disinhibitory effect of alcohol on CSI. The goal of the present study was to examine the levels of extracellular DA in the NAc under the influence of alcohol alone and during exposure to a sexually relevant inhibitory olfactory cue. A second goal was to identify brain regions associated with changes in DA levels in the NAc.

Materials and Methods

Animals, surgery, and hormones. 40 sexually naive male Long-Evans rats (approximately 200-250g) were obtained from Charles River Canada, Inc (St. Constant, QC). They were housed in groups of 4 in Plexiglas cages with *ad libitum* access to food (Purina Rat Chow) and water.

Female Long-Evans rats were obtained from the same supplier. They were bilaterally ovariectomized via lumbar incisions using intraperitoneal (i.p.) injections (1ml/kg body weight) of ketamine hydrochloride (50mg/ml; Ketaset©, Wyeth Canada) and xylazine hydrochloride (4mg/ml; Rompun©, Bayer Healthcare) anesthetic mixed at a ratio of 4:3, respectively. After a week recovery, sexual receptivity was induced for half of the females by subcutaneous injections of estradiol benzoate (10µg) 48 hours and progesterone (500µg) 4 hours prior to each conditioning trials and copulatory test.

All rats were maintained on a reverse 12-hour dark-light cycle (lights on at 8:00pm) at 21 ± 1°C. All testing was carried out between 12:00pm and 5:00pm. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Research

Ethics Committee of Concordia University.

Apparatus. Conditioning trials took place in Plexiglas bilevel chambers (18cm x 25cm x 65cm) with a platform dividing the chamber into two levels (Mendelson and Gorzalka, 1987; Mendelson and Pfaus, 1989). The copulatory preference test took place in a large open field (123cm x 123cm x 96cm) with bedding covering the floor (described in Germé et al., in preparation; Kippin et al., 1998). All conditioning trials and preference test were recorded on a video camera and scored later using a PC-based behavioral observation program (Cabilio 1996).

Alcohol treatment. Ethyl alcohol (95%) was diluted in physiological saline (0.9%) to obtain doses of 0.5 g/kg and 1 g/kg in a 25% v/v solution. Males in the saline group received an i.p. injection of saline in the same volume as the highest dose of alcohol. On test day, each male was injected with their respective dose of alcohol 45 min prior to testing and left undisturbed until testing.

Procedures. *Conditioning phase.* Males were preexposed to the bilevel chambers for 15 minutes each day for seven days prior to the beginning of the experiment. Males were then given a total of 20 conditioning trials at 2-day intervals, alternating between an unscented receptive female and a scented (1 ml pure almond extract) nonreceptive female (ten trials each). Scent was applied on the back of the neck and the anogenital region. On a conditioning day, males were placed alone in a bilevel chamber for five minutes, after which a female in the appropriate sexual condition was introduced in the chamber for a 30-minute conditioning trial.

Open field test. Four days after the last conditioning trial with a receptive female, male rats were randomly assigned to one of the three alcohol treatment groups: 0 g/kg alcohol (saline), 0.5 g/kg alcohol (A0.5) or 1 g/kg alcohol (A1) and injected with their respective treatment 45 minutes before the test. Each male was then placed in a large open field and allowed to habituate for 5 minutes. Then two receptive females, one scented (almond extract) and one unscented, were placed simultaneously into the open field at equal distance from the male. During the 30-minute test, all copulatory behaviors and the females to which they were directed were recorded on a video camera and later scored using a PC-based behavioral observation program (Cabilio, 1996). Criteria for sexual behaviors were those described by Sachs and Barfield (1976) and Meisel and Sachs (1994) and Pfaus et al., (1990b).

Cannulation surgeries. Following the preference test, male rats were cannulated into the NAc. Rats were anaesthetized with Isofluorane (Inhalation Anaesthetic, Richmond Hill, ON, Canada). Two 21-gauge guide cannulae (Plastics One, Roanoke, VA) were lowered and positioned 1 mm above the left and right NAc (AP: +1.65mm, ML: ± 2.7mm, DV: -5.3mm from Bregma, angle 10°; Paxinos & Watson, 1998) and secured to the skull using dental cement, anchored by four stainless steel screws. A removable stainless-steel stylet (26-gauge; Plastics-One) was placed inside the guide cannula until microdialysis. Male rats were allowed a week recovery before microdialysis.

In vivo microdialysis and high-performance liquid chromatography (HPLC). Four Plexiglass chambers with stainless steel grid floors (hexagonal, 42 x 39 x 33.5cm^3, custom-made, Concordia University) were used for microdialysis. Each chamber was contained in wooden cubicles and lighting was provided on reverse cycle by overhead light bulbs.

Microdialysis probes. Microdialysis probes were made in the laboratory following the procedures of Sorge et al., 2005. They consisted of a 2mm long semi-permeable dialysis membrane (Fisher Scientific, 240 mm OD, 13 000 MW cut-off) attached to 22mm long 26-gauge stainless steel tubing. Small-diameter fused silica tubing extended internally through the probe, resting 0.5 mm from the tip of the probe. The steel tubing was attached to a 20-µm-diameter polyethylene (PE) tubing (35-40 cm long; Plastics-One, Roanoke, VA, USA). The other end of this PE tubing was connected to the stainless-steel shaft of a single-channel liquid swivel (HRS Scientific, Montréal, QC, Canada). The swivel was located above the wooden cubicle of each microdialysis chamber and was connected to a variable speed electric syringe infusion pump (Harvard Apparatus, South Natick, MA). Each probe was secured in place by stainless steel collars that were screwed onto the guide cannula. The external length of the PE tubing was protected by a steel spring casing.

Odor exposure and dialysate collection. The night before odor exposure, microdialysis probes were lowered into the NAc, using light isofluorane anaesthesia. Males were placed individually in the microdialysis chambers. To prevent occlusion before testing, probes were perfused with artificial cerebrospinal fluid (aCSF; 145mM Na^+, 2.7mM K^+, 1.22mM Ca^{2+}, 1.0mM Mg^{2+}, 150mM Cl^-, 2mM Na_2PO_4, pH 7.4 ± 0.1) at 1.0 µl/min for approximately 1 h. Samples were collected every 10 minutes during this time to ensure a good flow and no leakage of the probes.

The flow rate was then lowered to 0.2 µl/min overnight. The next day, the flow rate was returned to 1µl/min 2 hours prior to baseline sampling.

Dialysate samples were collected every 10 min from the beginning of baseline until the end of the exposure period (approximately 180 min total). Baseline samples were collected for 90 minutes. Following baseline, male rats received an acute injection of alcohol (0.5g/kg or 1g/kg) or saline i.p. depending on their respective group. Samples were collected for 50 minutes. Cotton gauze with 1ml of almond extract or lemon extract was introduced into the microdialysis chamber 50 minutes after the injection and left there for 30 minutes.

High-Performance Liquid Chromatography (HPLC). Dopamine (DA) levels were determined HPLC with electrochemical detection. Samples were loaded into a reverse-phase column (15 cm × 0.46 cm, Spherisorb-ODS, 5µm; Higgins Analytical, Mountain View, CA) through a manual injection port (Rheodyne 7125; Rheodyne LLC, Rhonert Park, CA; 20 µl loop). Oxidation and reduction currents for DA were measured with dual-channel ESA coulometric detectors (Coulochem III, with a model 5011 analytical cell) connected to a computer. The mobile phase (19% acetonitrile, 40mg 0.076M SDS, 0.1M EDTA, 0.058M $NaPO_4$, 0.03M citric acid, pH 3.35) was circulated by Waters 515 HPLC pumps (Lachine, Quebec, Canada) at a flow rate of 1.2ml/min. Analysis and integration of the data for DA were done through EZChrom Chromatography Data System (Scientific Software Inc., San Ramon, CA).

Fluoro-Gold and Immunofluorescence

Fluoro-Gold infusion. To determine the neural projections to the NAc, the retrograde tracer Fluoro-Gold (FG; Fluorochrome, LLC; Denver, CA, USA) was infused into the NAc (same coordinates as above). Rats were anaesthetized with isofluorane and a modified 27-gauge needle (2 mm longer than the guide cannula) was lowered to administer a 2% FG solution (dissolved in saline). The administration was done at a rate of 0.2µL/min for 1 minute using an infusion pump (Harvard Apparatus). The infusion cannula was left untouched for 10 minutes to allow absorption of FG. After removal of the infusion cannula, male rats were then left undisturbed in their home cage until sacrifice.

Odor exposure. Ten days after the FG infusion, males received an acute injection of alcohol (based on their respective alcohol treatment) and exposed 45 minutes later either to 1ml of

almond extract on cotton gauze or 1 ml of lemon extract in the exposure chamber. Males were allowed to remain undisturbed in the chamber for one hour. Following the exposure, males were injected with a lethal dose of sodium pentobarbital (120 mg/kg, i.p.) and intracardially perfused with 300mL of phosphate buffered saline (PBS) followed by 300mL of 4% paraformaldehyde (4% PFA). Brains were extracted and post-fixed in 4% PFA for four hours then switched into a solution of 30% sucrose for 24 to 48hours. Brains were then stored at -80°C until slicing.

Fluorescence immunohistochemistry (FIHC). FIHC was performed to examine a projection site (VTA), a neurotransmitter (DA) and a marker for neuronal activation (Fos). Thus, the FIHC would allow us to see if neurons activated in the VTA were dopaminergic neurons and if they were projecting to the NAc. As the retrograde tracer FG, used to determine efferent projections from the NAc to the VTA had auto fluorescent properties with an emission maximum of 461nm, visualized as a blue wavelength, no steps were included for its detection during FIHC procedures.

All steps were done at room temperature on a rotator. All rinses between FIHC steps were made in 0.1M Phosphate Buffer (PB) and consisted of one rinse for one minute then three rinses of 5 minutes each.

To visualize DA neurons, procedures started with 1-hour incubation in blocking solution (BS) consisting of 2% normal goat serum (NGS; Vector Laboratories, Cat# S-1000) and 0.4% Triton-X (Fisher Scientific, A00062) in 0.1M Phosphate buffer (PB). The sections were then incubated overnight in a solution of BS with a primary antibody recognizing tyrosine hydroxylase (TH) raised in mouse (1:8000; Sigma-Aldrich, T2928). The following day, sections were rinsed and then incubated for one hour with biotinylated goat anti-mouse IgG (1:200; Vector Laboratories, BA-9200) in BS. Slices were then rinsed and incubated with an avidin-biotin complex (1:1000; ABC-elite, Vector Laboratories, PK-6100) in 0.1M PB for one hour. Sections were rinsed and incubated with Streptavidin tagged Alexa488 (1:800; Life technologies, S32354) in BS for one hour.

To visualize Fos, the FIHC continued immediately after TH-FIHC. Following the incubation with streptavidin, sections were rinsed then incubated overnight with a primary antibody recognizing c-fos, raised in rabbit (1:40,000; Fos ab5, Calbiochem, Mississauga, ON). The following day, sections were rinsed and incubated for one hour with AlexaFluor 594-tagged

goat anti rabbit IgG (1:200, Life technologies, A11012) in BS. Sections were then rinsed, mounted onto gel coated slides and let to air dry overnight. The next day, the slides were coverslipped in dim light with Fluoromount (Sigma-Aldrich, F4680) then sealed with clear nail polish. The slides were stored in boxes at 4 ºC until microscope analysis.

Imaging. Microscopy images were taken using an Olympus Fluoview FV10i confocal laser microscope (Olympus, PA, USA). Three filters were used to observe FG, the neurons containing TH and the neurons activated (Fos): a 405 nm laser combined with a blue filter (Em 470nm), a 473nm laser with Alexa488 filter (Em 520nm) and a 559 nm laser with Alexa594 filter (Em 618nm) respectively. 0.75 m spacing z-stacks were converted to sum z-projections using Fiji software (Schindelin et al., 2012). Cell counting was performed using freeware Image J by taking 3 to 5 randomized counts per animal and creating an average count. These averaged counts were then combined for animals in each group. Images used to count were randomized throughout the VTA, but effort was made to find the largest amount of FG-positive neurons, as these were the least frequent.

Statistical analyses

All statistical analyses were performed using SPSS Statistics 25.0 for PC. For all analyses, the level of significance was set to $p<0.05$.

Behaviour. To compare the alcohol treatments and the differences in behaviour towards each female, a 3 (alcohol treatment) x 2 (female) mixed design ANOVA followed by Tukey's post-hoc comparisons. Partial eta squared (η^2_p) was reported as a measure of effect size. Chi-square analyses were used to assess the proportions of males choosing one of the two females for their first mount, intromission and ejaculation on the copulatory preference test.

Microdialysis. To determine the effect of alcohol and odor exposure on DA levels, baseline levels were determined by an average of the last three samples collected prior to the injection of alcohol and then converted to a percentage of the baseline. Changes from baseline were analyzed separately for each odor condition using a mixed design ANOVA with between subject factor of alcohol treatment (saline, A0.5, A1) and the within subject factor of time (-30 to + 80 minutes). A mixed design ANOVA with a between subject of odor (Almond, lemon) and a within subject factor of time was also performed to observe the potential differences in DA levels for each

group between the two odor conditions. Statistically significant main effects and interaction were followed by post hoc tests (Bonferroni correction). Partial eta squared (η^2_p) was reported as a measure of effect size.

FIHC. A one-way ANOVA was performed to compare the percentage of triple-labeled cells (Fos/TH/FG) in the VTA between the three alcohol groups when males were exposed to the lemon odor. Another one-way ANOVAs was performed to compare the proportions of triple-labelled (Fos/TH/FG) dopaminergic neurons in the VTA across the three alcohol treatments when rats were exposed to the almond extract. Both ANOVAs were followed by post hoc analyses (Bonferroni correction). Partial eta squared (η^2_p) was reported as a measure of effect size. Unpaired *t*-tests were used to assess differences between almond and lemon odors for each alcohol treatment. *Cohen's d* was reported as a measure of effect size in these analyses.

Results

Copulatory test. Of the 40 male rats in the experiment, two (n=2) from the A0.5 group and one (n=1) from the A1 group did not ejaculate during the final copulatory test. Therefore, they were excluded from the analyses.

The mean number of mounts, intromissions and ejaculations are represented in Figure 1.

During the final copulatory test, the mean number of mounts between the two females was significantly different. This was revealed by a main effect of female $F(1,34)=5.58$, $p=.024$, $\eta^2_p = .14$. Further analyses revealed that overall, the unscented females ($M=16.67$) received more mounts from the males compared to the scented female ($M=12.56$). There was no main effect of group and no significant interaction (group x female).

There was a significant difference between groups in the frequency of intromissions during the copulatory test as revealed by a significant main effect of group $F(2,34)=4.86$, $p=.014$, $\eta^2_p = .22$. Post hoc analyses revealed that the saline ($M=9.46$) and A0.5 ($M=9.58$) groups had significantly higher intromission frequencies than the A1 group ($M=6.92$). There was no main effect of female and no significant interaction group x female.

Overall, the mean number of ejaculations was not different between females and between groups as there was no significant main effect of female or group. However, there was a trend to a significant interaction (group x female) $F(2,34)=3.002$, $p=.063$, $\eta^2_P=.15$.

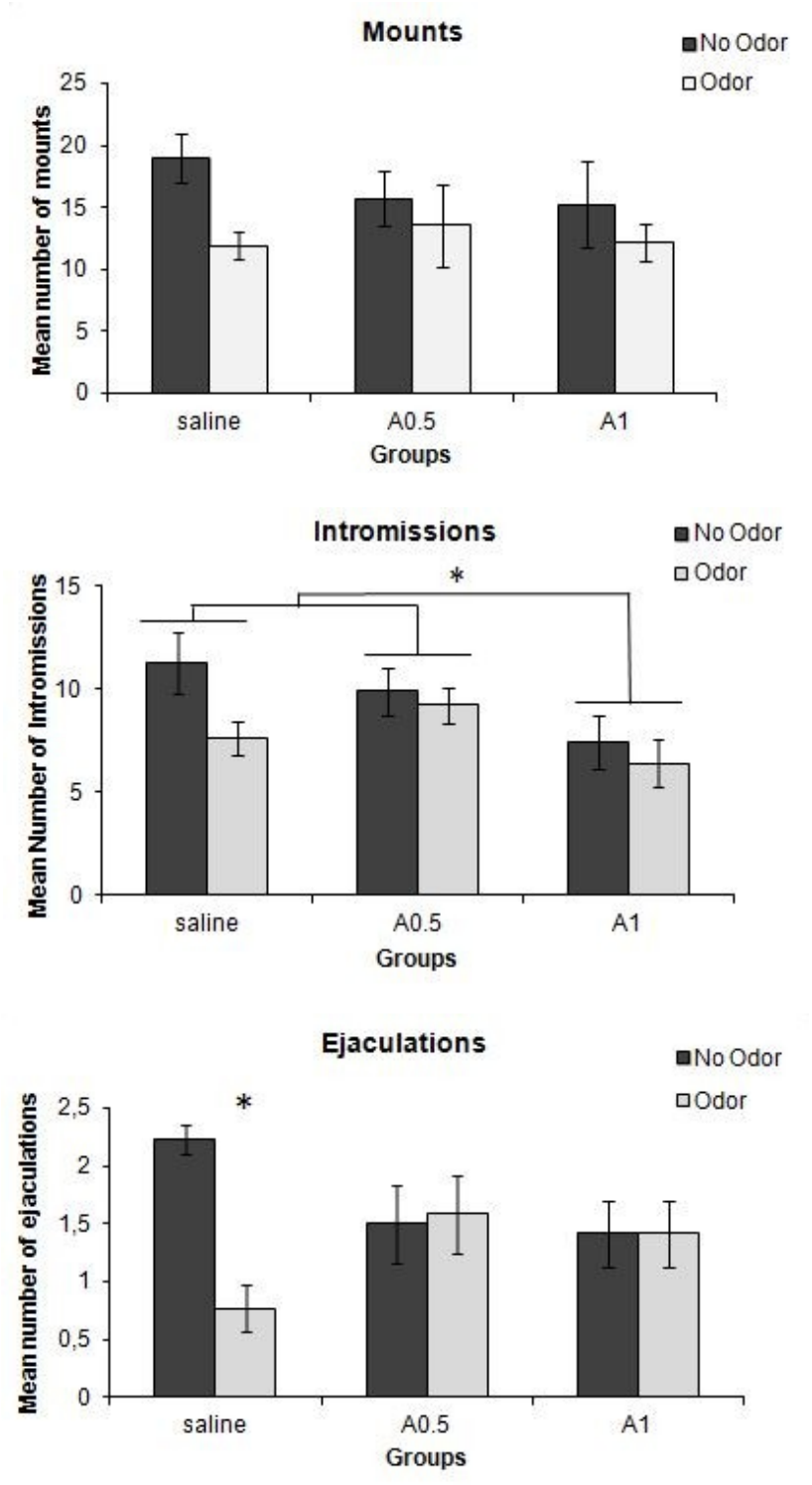


Figure 1. Mean of copulatory behaviors (± S.E.M) per group during the 30-minute copulatory test. Males in the A1 group intromit significantly less than males in the other two groups. Males in the saline ejaculated more frequently with the unscented (No Odor) female compared to the scented female. * $p<.05$

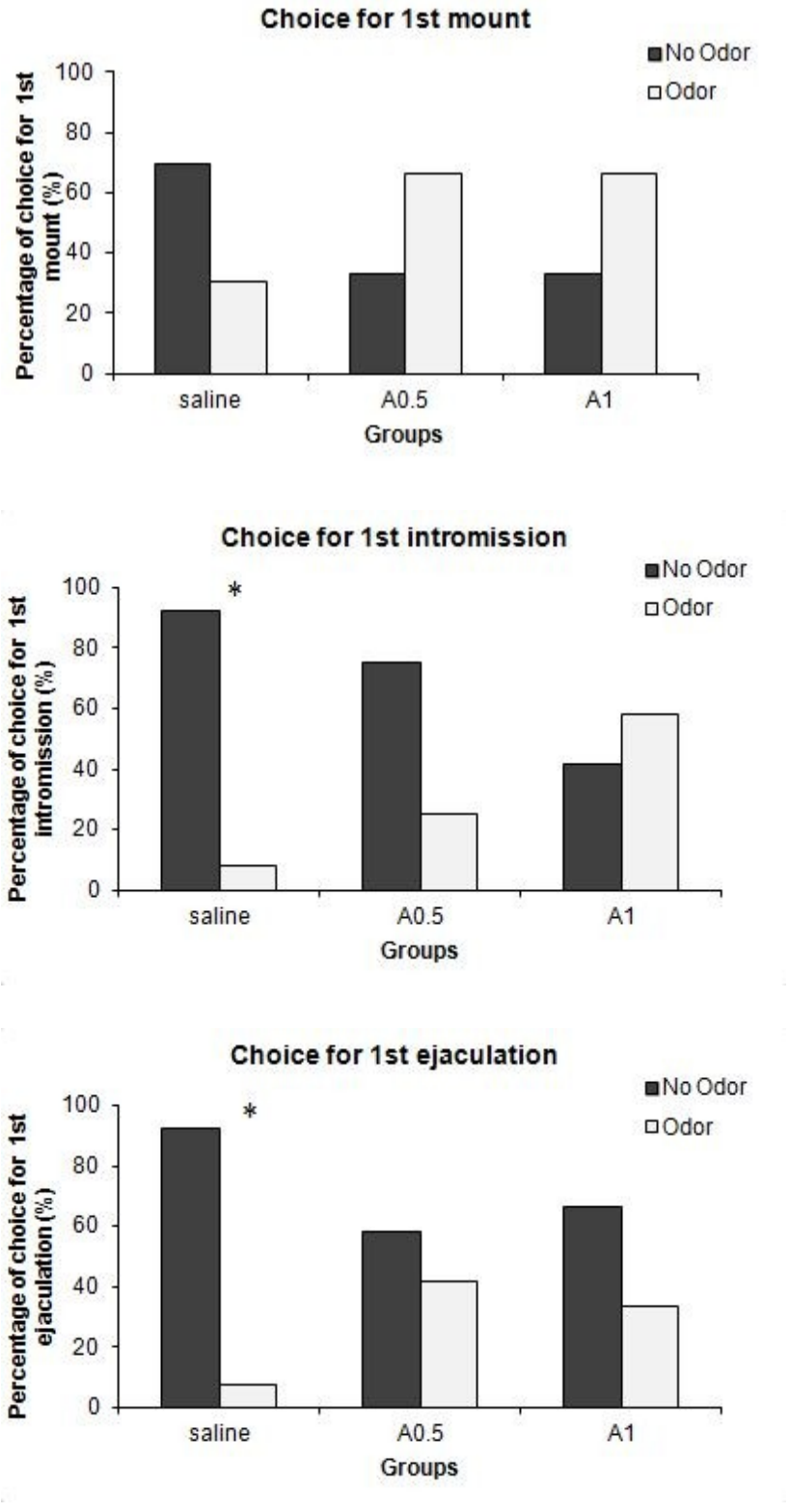


Figure 2. Percentage of choice for 1st mount, intromission and ejaculation. Saline-treated males chose the unscented females (No Odor) more frequently for their first intromission and ejaculation. * $p<.05$

Further analyses revealed that in the saline group only, the unscented female (No Odor; M=2.23) received significantly more ejaculations than the scented female (Odor; M=0.77).

There was no significant difference between groups or between females in the latency to first mount, first intromission or first ejaculation.

The percentages of males choosing either females for their first mount, intromission and ejaculation are shown in Figure 2. Regardless the dose, males treated with alcohol did not show a preference of female for their first mount, intromission or ejaculation. However, chi square analyses confirmed that males injected with saline prior to the copulatory test chose significantly more frequently the unscented female (No odor) for their first intromission χ^2 (1, N=13) = 9.31, p=.002 and their first ejaculation, χ^2 (1, N=13) = 9.31, p=.002, compared to the scented (Odor).

Microdialysis. All results from microdialysis are depicted in Figures 3 and 4.

Almond. Exposure to the almond odor induced a significant difference in extracellular levels of DA across time as demonstrated by a main effect of time F(12,180)= 5.47, p<.001, η^2_P =.27. There was also a significant difference in the concentration of DA in the NAc between groups F (2,15) =14.93, p<.001, η^2_P =.67. Finally, there was a significant interaction (group x time) F(24,180)=2.23, p=.002, η^2_P =.23 . Following up this significant interaction indicated that there was no significant difference between groups at the baseline time points and the extracellular DA levels of the saline group did not change over time. However, the mean levels of DA increased in the A0.5 and A1 groups following the alcohol injection compared to the saline group and to baseline levels and stayed significantly higher in the A0.5 group compared to baseline during exposure to the almond odor. These results are represented in Figure 3A.

Lemon. Figure 3B shows the results from exposure to lemon extract. During the lemon odor exposure condition, there was a significant difference in the mean levels of DA over time F(12,156)= 5.97, p<.001, η^2_P =.29. There was also a significant difference in DA concentration between groups as shown by a significant main effect of group F (2,13) =4.12, p=.037, η^2_P =.36. Finally, there was also a trend to a significant interaction (group x time) F(24,156)=1.57, p=.053, η^2_P =.17 . Post hoc analyses revealed that overall, both alcohol groups differed from baseline average at 20 and 30 min following injections. Also, the A1 group was significantly higher than

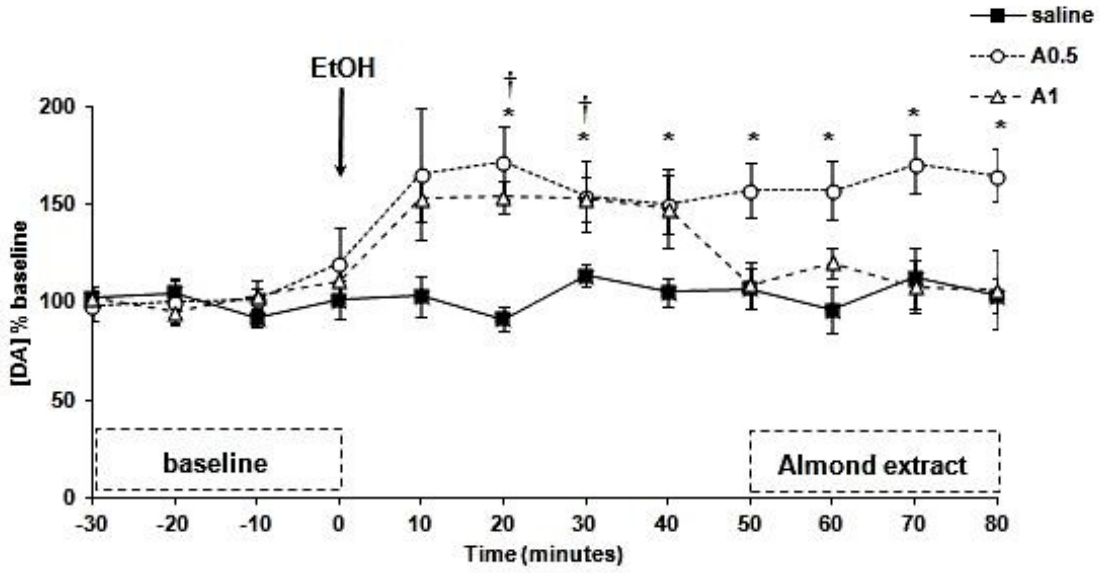
saline at 30 minutes following injection of alcohol. The extracellular DA levels of the saline group did not change over time compared to baseline.

Almond vs. lemon. The results for the comparison between odors are depicted in Figure 4. When comparing the exposure to almond and to lemon in the saline group, no difference over time and between odors was observed (Figure 4A). This was confirmed as there was no main effect of time $F(12,120)= .89$, $p=.563$, $\eta^2_P =.08$ and no main effect of odor $F(1,10)=.00$, $p=.999$, $\eta^2_P =.00$. Furthermore, the interaction (time x odor) was also not significant $F(12,120)=.38$, $p=.966$, $\eta^2_P =.04$.

In the A0.5 group, there was a significant main effect of time $F(12,120)=6.02$, $p<.001$, $\eta^2=.38$ and a significant main effect of odor $F(1,10)= 7.46$, $p=.021$, $\eta^2_P =.43$. There was no significant interaction (time x odor) $F(12,120)= 1.19$, $p=.291$, $\eta^2_P =.11$. Follow up analyses revealed that over time, extracellular levels of DA got significantly higher compared to the baseline average 20 minutes following the EtOH injection for both odors. However, the levels of DA in the NAc were significantly higher when male rats were exposed to the almond odor compared to the lemon odor (Figure 4B).

Extracellular levels of DA in the NAc changed over time in the A1 group as there was a main effect of time $F(12,120)=7.96$, $p<.001$, $\eta^2_P = .44$ but not between odor $F(1,10)= 1.13$, $p=.314$, $\eta^2_P =.10$. The interaction (time x odor) was also not significant $F(12,120)=.69$, $p=749$, $\eta^2_P =.06$. Post hoc analyses revealed that DA levels of males in the A1 group were significantly higher than baseline average in both conditions from 10 to 30 minutes following the EtOH injection and went back to levels similar to baseline average even during exposure to the odors (Figure 4C).

A . Exposure to almond odor



B. Exposure to lemon odor

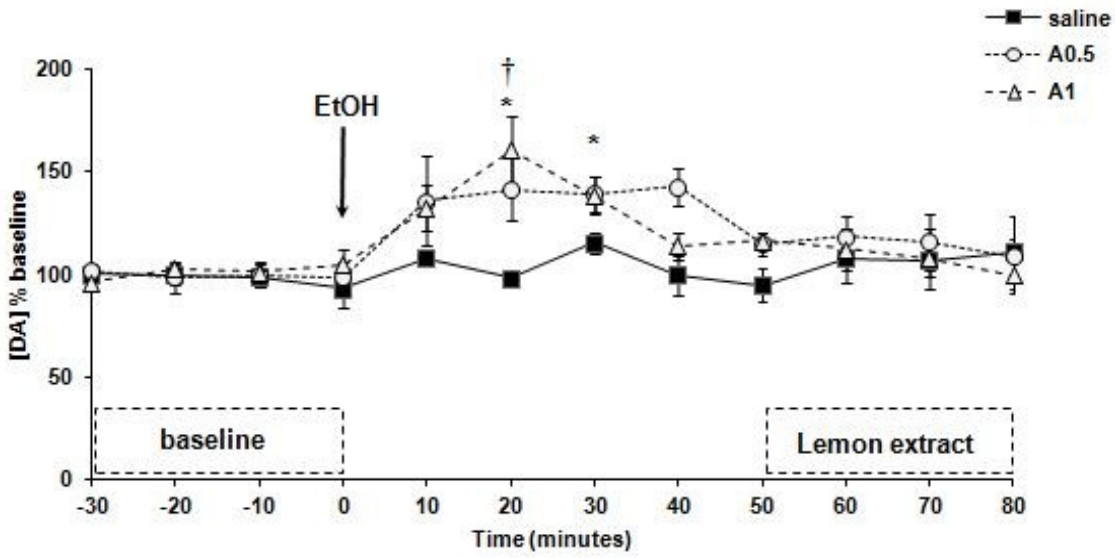
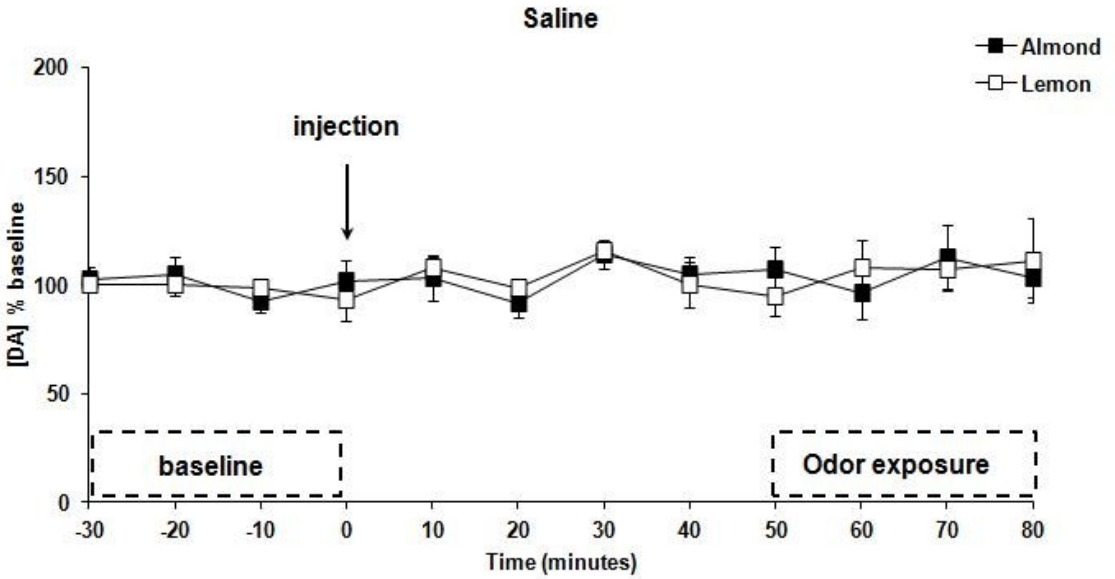
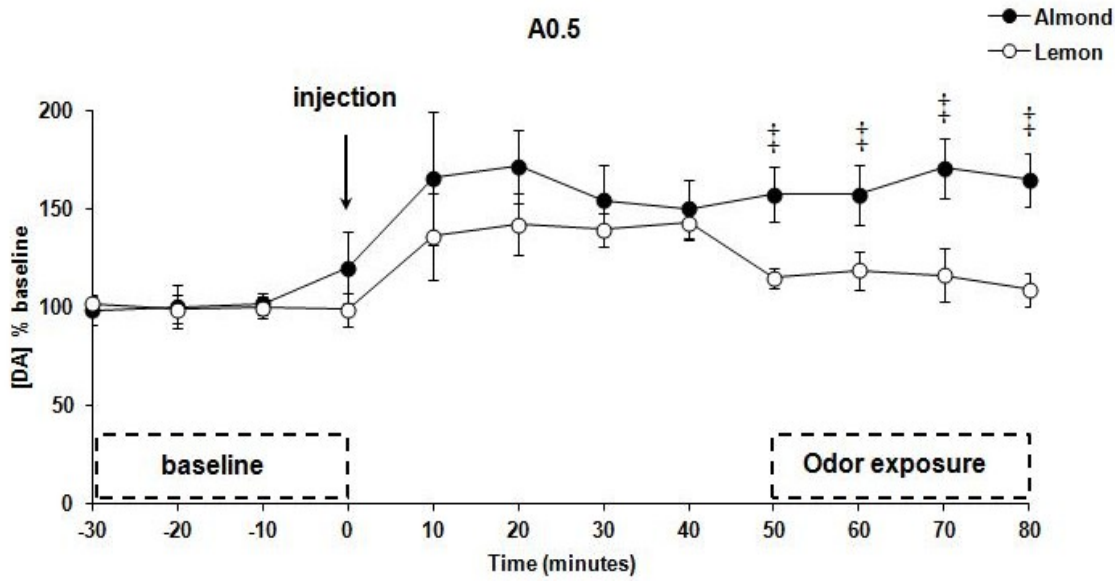


Figure 3. Extracellular dopamine levels in the nucleus accumbens following an injection of saline (n=6), alcohol 0.5g/kg (A0.5; n=6) or alcohol 1g/kg (A1; n=6) and exposure to (**A**) almond extract or (**B**) lemon extract, *p<.05 compared with baseline levels, † p<.05 compared to saline.

A.


Saline
Almond
Lemon
injection
[DA] % baseline
200
150
100
50
0
baseline
Odor exposure
-30
-20
-10
0
10
20
30
40
50
60
70
80
Time (minutes)

B.


A0.5
Almond
Lemon
injection
[DA] % baseline
200
150
100
50
0
baseline
Odor exposure
-30
-20
-10
0
10
20
30
40
50
60
70
80
Time (minutes)

C.

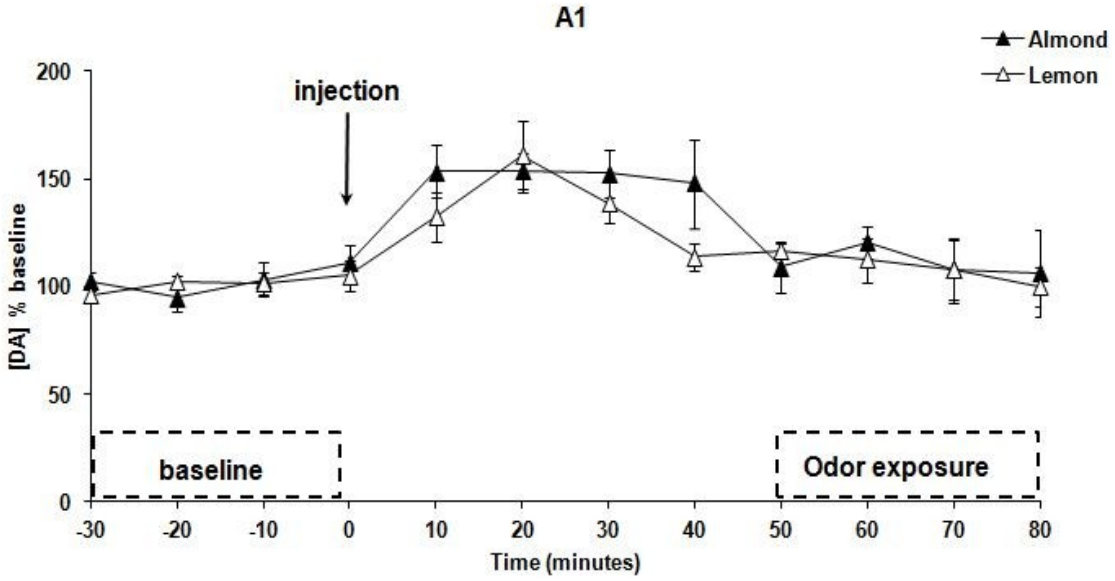


Figure 4. Comparison of extracellular dopamine levels in the nucleus accumbens between exposure to almond and lemon odors following an injection of (**A**) saline (n=6), (**B**) alcohol 0.5g/kg (A0.5; n=6) or (**C**) alcohol 1g/kg (A1; n=6), ‡ p<.05 compared to lemon.

Immunofluorescence.

Representative pictures of immunofluorescence are presented in Figure 5. The results of immunofluorescence are depicted in Figure 6.

Lemon odor. When male rats were exposed to the lemon extract, no significant difference was observed between groups in the proportion of VTA DA cells activated, $F(2,15)=1.197$, $p=.333$ $\eta^2_P=.16$.

Almond odor. There was a significant difference between groups when males were exposed for one hour to the almond odor, $F(2,16)= 32.23$, $p<.001$ $\eta^2_P=.82$. Post hoc analyses revealed that the proportion of VTA DA cells activated projecting to the NAc was significantly higher in the A0.5 group ($M= 69.39\%$) compared to the saline ($M=40.61\%$) and A1 ($M=31.30\%$) groups.

Lemon vs Almond odor. Males in the saline group exposed to the lemon odor showed significantly less activation of DA cells in the VTA compared to males in the saline group exposed to the almond odor, $t(10)= 2.58$, $p=.028$, *Cohen's d* =1.49.

Males injected with 0.5 g/kg of alcohol exposed to the lemon odor displayed less activated VTA DA neurons projecting to the NAc compared to males that received the same alcohol treatment but exposed to the almond odor, $t(10)=3.78$, $p=.004$, *Cohen's d*= 2.18.

Finally, when males in the A1 group were exposed to the lemon odor, there was a trend towards a significant lower proportion of triple labeled cells compared to males in the A1 group exposed to almond odor, $t(8)=2.27$, $p=.057$, *Cohen's d*= 1.45.

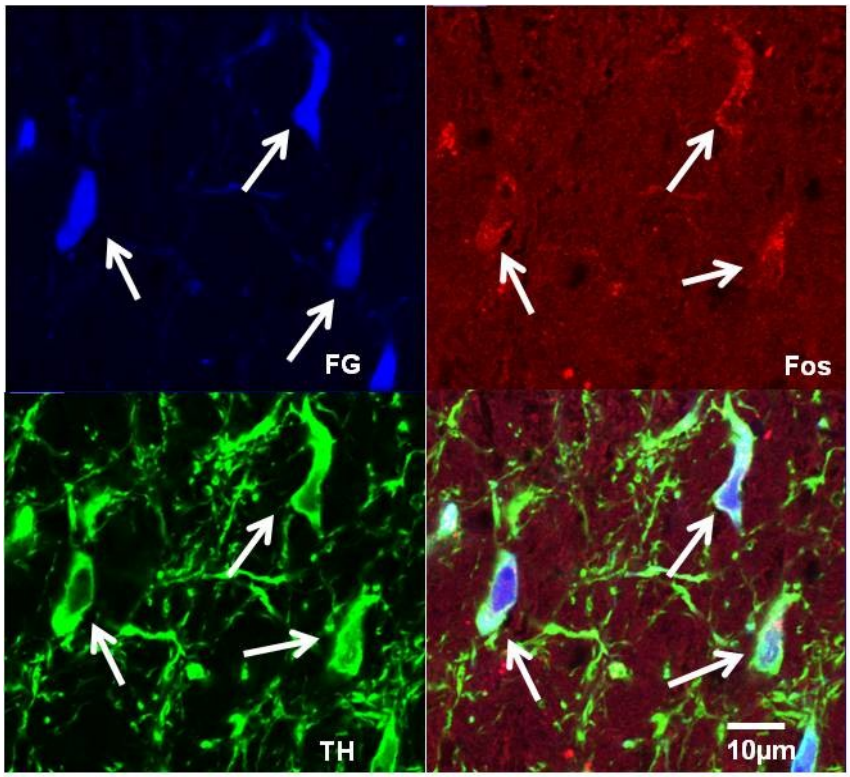


Figure 5. Representative fluorescent immunohistochemistry images, including examples of triple-labelled cells (arrows) in the VTA. Labeling includes FG: Fluoro-Gold; Fos: Fos protein; TH: tyrosine hydroxylase. (Animal treated with alcohol 0.5g/kg)

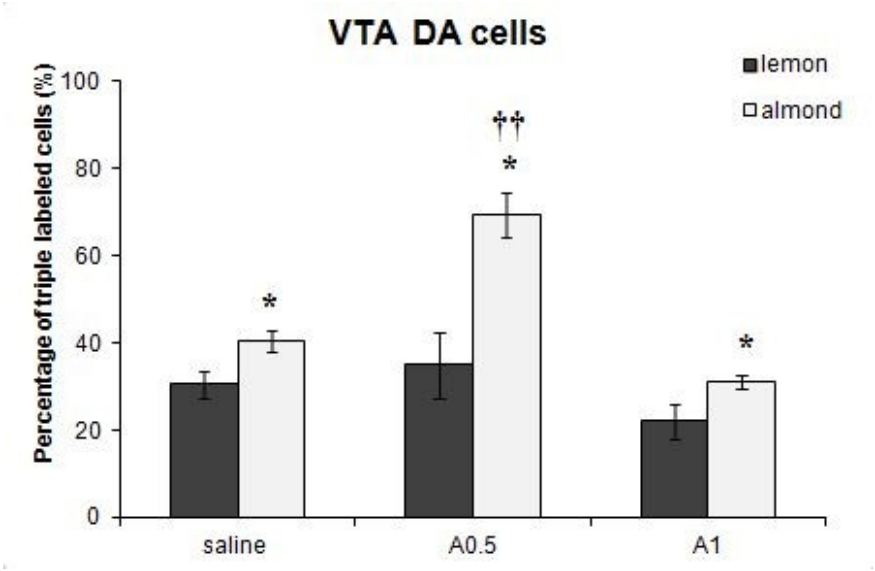


Figure 6. Mean percentage of VTA DA cells compared between groups (almond condition) or compared between odors (lemon vs. almond). Data represent means ± SEM. Almond odor condition †† $p<.001$; Lemon vs. almond * $p<.05$.

Discussion

The present study examined the effects of different doses of alcohol on extracellular levels of DA in the NAc when male rats were exposed to an odor previously associated with sexual inhibition. Male rats were trained to associate a neutral odor (almond) with nonreceptive females. Prior to a preference test with two receptive females, one scented and the other unscented, males were injected with saline or two different doses of alcohol. Male in the saline group preferentially copulated and ejaculated with the unscented female and also chose these females more frequently for their first intromissions and ejaculations revealing a preference for the unscented females that indicated a conditioned sexual inhibition (CSI) towards the scented females. Males injected with alcohol did not show this inhibition nor did they exhibit a preference for any of the females and copulated indiscriminately with both. These behavioral results replicate previous findings from our laboratory (Germé et al., in preparation) and from the literature (Pfaus and Pinel, 1989) showing that EtOH can disrupt conditioned inhibition, and thus further confirm the disinhibitory effects of low doses of alcohol on sexual behaviour.

The injection of alcohol, regardless the dose, induced an increase in extracellular DA levels in the NAc. These levels of DA were higher than baseline but also higher than those observed in

the saline group 20 minutes after the injection. These results add further evidence to the existing literature on the effects of alcohol on DA concentration in the NAc. As many other drugs of abuse, alcohol is known to increase DA levels in the NAc (Spanagel, 2009). This has been demonstrated in humans using positron emission tomography (PET) and the DA receptor ligand [^{11}C] raclopride (Boileau et al., 2004) or [^{18}F] Fallypride (Leurquin-Sterk et al., 2017). This was also shown in rodents with low to moderate doses of alcohol similar to those used in the present experiment inducing an increase in extracellular concentration of DA in the NAc and the striatum starting 20 minutes after the administration of alcohol (Blanchard et al., 1993; Di Chiara and Imperato, 1988; Imperato and Di Chiara, 1986; Yoshimoto et al., 1991). While some studies revealed that 1g/kg induced DA release similar to or below average (Blanchard et al. 1993), others showed increased levels following administration of the same dose (Di Chiara and Imperato, 1988). In the present experiment, males receiving 0.5 and 1 g/kg showed similar increase in extracellular DA following the injection, suggesting the higher doses (above 2.5 g/kg, as this dose also induced an increase according to Di Chiara and Imperato, 1988) are probably needed to observe a decrease in DA levels in the NAc.

The extracellular concentration of DA stayed at baseline levels in the saline group following the injection of saline but also following the exposure of both odors. DA levels went back to baseline levels 30 to 40 minutes following the EtOH injection in the A1 group and stayed similar to baseline levels during exposure of the almond or the lemon odors. However, males injected with 0.5g/kg of alcohol showed elevated DA levels during exposure to the almond odor. Because this was not the case for the other groups or with the lemon odor, it can be assumed that these increased levels of DA here are due to an interaction of the almond odor and this particular dose of alcohol (0.5g/kg). These results confirm previous findings from our laboratory (Germé et al., in preparation). In this latter experiment, only males injected with 0.5g/kg of EtOH showed an increased Fos activation in the NAc when exposed to the conditioned olfactory cue. No changes in Fos activation were observed in the NAc of the males receiving 1g/kg of alcohol. It was argued that, as opposed to 1g/kg, this low dose of alcohol was the one to use to fully witness the disruptive effects of alcohol on conditioned sexual inhibition without getting behavioral interference, as seen also in Pfaus and Pinel (1989). It should be mentioned that studies looking at the effects of alcohol on sexual behavior, revealed that the dose of 1g/kg was sufficient to decrease the number of male rats copulating (Ferraro and Kiefer, 2004; Pfaus and Pinel, 1989).

Therefore, in the present study, even though the behaviour of males in the A1 group was not affected by this dose of alcohol during the open field test, the physiology within the NAc might have been. This would also explain why, in the present experiment, DA concentrations in the A1 group did not change from baseline levels when males were exposed to the almond odor.

The NAc is known to be a critical brain area part of a network mediating the ability to form associations between reward and predictors. This region is required for a variety of reward-related behaviors, but also for processing rewards, interpreting, discriminating, and responding appropriately to cues (reviewed in Day and Carelli, 2007). When it comes to sexual reward in particular, several studies demonstrated increased DA levels in NAc when animals were exposed to cues predicting sexual reward. Interestingly, using voltammetry to measure DA levels, an early study demonstrated that the increase in DA release in the NAc could be natural and selective to sex-related olfactory stimuli (Louilot et al., 1991). In fact, in the latter experiment, NAc DA release in sexually naive male rats was higher when males were exposed for the first time to sexually receptive females' odors as opposed to odors of non receptive females or of other males. This reveals that DA release induced by natural reward could be unlearned. Similar results were obtained with sexually experienced males and revealed an increase in neural activation in the NAc following exposure to conditioned olfactory cues previously associated with sexual reward (Kippin et al., 2003) but also in DA levels in anticipation of copulation (Damsma et al., 1992; Pfaus et al., 1990a). DA levels appeared to increase before the introduction of the receptive females or during exposure to cues associated with possible upcoming sexual reward and to drop after copulation. Therefore, extracellular levels of DA were increasing in anticipation of sexual reward. In the present experiment, the almond odor was previously associated with non receptive females. However, during the copulatory test the odor was placed on a receptive female and male rats were allowed to copulate to ejaculation with these scented females. Ejaculation is the most reinforcing component of sexual behavior in male rats (Coolen et al., 2004; Pfaus et al., 2012) and appears to be required to develop partner preference and make the association between sexual reward and a neutral odor (Kippin and Pfaus, 2001). It is therefore possible that the almond odor, previously associated with no sexual reward during training, acquired during the copulatory test and most importantly, under the influence of alcohol, a positive valence. Then, subsequent exposures to alcohol and to the olfactory cue could potentially predict sexual reward. Despite the change in context (open field as opposed to microdialysis chamber), the microdialysis

test was the second time males were under the influence of alcohol and then exposed to the odor (the first time being the open field). Given the fact that during the open field the combination of alcohol and almond odor lead to sexual reward, the increased extracellular DA levels in the NAc observed during the exposure to the odor alone could reflect an anticipation of a potential receptive female and copulation. Consideration should be given to the fact that changes of DA levels in the NAc have also been associated with aversion (reviewed in Salamone, 1994). Yet, during the open field test in the present study, males under the influence of alcohol did not display any sort of aversive behavior or avoidance towards the scented females and copulated to ejaculation with them. Therefore, changes in extracellular DA levels in this case are not due to aversion. These findings suggest that: (1) because of its association to a receptive female during the open field, under the influence of alcohol, the olfactory cue is no longer perceived as inhibitory but rather excitatory, (2) one exposure (alcohol + olfactory cue + sexual reward during the open field) is sufficient to observe subsequent DA increase when males are intoxicated and exposed to the olfactory cue alone, increase that could be translated as possible anticipation of copulation.

Immunofluorescence was used to observe VTA cells containing TH staining as a marker of dopaminergic cells, Fos immunoreactive (Fos IR) cells as a marker of neuronal activation (Pfaus and Hebb, 1997) and the retrograde tracer Fluoro-gold (FG) (Schmued and Fallon, 1986) cells to identify cells projecting to the NAc. The percentage of activated VTA dopaminergic cells projecting to the NAc was higher in all three groups when males were exposed to the almond odor (inhibitory cue) compared to the lemon (neutral) odor. These results further confirm previous findings from our laboratory showing increased activation in the VTA when rats are exposed to the almond odor as opposed to no odor (Germé et al., in preparation). Previous studies showed increased activation of VTA neurons following exposure to sex-related cues (Balfour et al., 2004). In the latter study, however, because tracing methods were not used, it was speculated that these neurons projected to the NAc, due to the activation also in the NAc and previous evidence of the NAc being a major target of VTA dopaminergic cells. Here, the use of FG injected in the NAc, allowed an assessment of dopaminergic cells projecting from the VTA to the NAc and reveal that the activation of these neurons is increased when male rats are exposed to the almond odor, that can be considered as a sexually relevant cue. Aside from sex-related cues, dopaminergic neurons in the VTA are also activated by rewards or aversion (for review, Lammel

et al., 2014). This would explain this increase in neuronal activation in the VTA when exposed to the almond odor in all three groups despite the difference in behaviors towards the scented females between the saline (showing aversion) and the alcohol groups during the open field.

Furthermore, the percentage of triple-labeled cells was significantly higher in the A0.5 group when exposed to the almond odor compared to the other two groups. This is mimicking the changes detected in the microdialysis data. This suggests that the increased levels of DA in the NAc when rats have been injected with 0.5g/kg of alcohol and exposed to the olfactory cue observed during the microdialysis phase are due an increase in VTA DA cell activity. Therefore, the DA concentrations measured in the NAc likely originate from projection neurons in the VTA and not from the pool of DA present in NAc cell terminals. This strongly suggests that VTA and NAc Fos activation observed in our previous experiments (Germé et al., in preparation) as well as changes in DA levels in the NAc during exposure to the olfactory cue in the present study were due to the increased activation of VTA DA neurons projecting to the NAc.

It is recognized that alcohol affects VTA cells and previous studies have demonstrated that the alcohol-induced DA release in the NAc was most likely due to alcohol's effects on DA cells in the VTA (reviewed in Cui and Koob, 2017). However, despite an increased activation of VTA DA cells, no changes in DA levels were observed in the NAc of male rats that received 1g/kg of alcohol during exposure to the olfactory cue. Furthermore, no elevated activation was detected when male rats, regardless the alcohol dose, were exposed to the lemon odor. Hence, the higher percentage of VTA DA cells activated as well as the higher DA levels when male rats in the A0.5 group were exposed to the almond odor were not solely due to the effects of alcohol on VTA transmission but rather to the association between the intoxicated state at 0.5g/kg of EtOH and the exposure of the olfactory cue. These findings provide evidence that DA increase in NAc in a context of disinhibition by alcohol is mediated by VTA dopaminergic cell transmission and could reveal a potential change in the valence of a conditioned cue leading to an anticipation of sexual reward.

Studies have shown that VTA- NAc transmission is involved in reward-related behaviors (Volkow et al., 2017; Wise, 2008). However, NAc activation and DA release is also mediated by other brain regions raising further questions regarding the other neurotransmitters and brain regions potentially involved. The NAc receives inputs from several other brain regions,

including the basolateral amygdala (BLA), the medial prefrontal cortex (mPFC), the hippocampus. These regions mostly send glutamatergic and GABAergic projections to the NAc. As alcohol is also known to act on glutamatergic and GABAergic transmissions, these regions as well as these neurotransmitters could also influence the effects of alcohol on NAc DA and on sexual behavior. Therefore, further research is necessary to look at how exactly alcohol induces these changes and how these changes lead to behavioral disinhibition.

Funding. This research was funded by a grant from the Canadian Institutes for Health Research (MOP-74563) to JGP and an infrastructure grant from Fonds de la Recherche en Santé du Québec to the Center for Studies in Behavioral Neurobiology at Concordia University.

Compliance with ethical standards. The authors declare that all animal procedures conformed to the guidelines of the Canadian Council for Animal Care. All procedures were approved by the Concordia University Animal Research Ethics Committee.

Conflict of Interest. The authors declare they have no conflicts of interest.

Chapter 4

Nucleus accumbens glutamate and GABA levels do not mediate the disruptive effects of alcohol on conditioned sexual inhibition in male rats.

Katuschia Germé[1], Julian Alvarez-Barkam[1], Leah Cusack[1], Lindsey R. Gossip[1], Hannah S. Poupel[1], Heshmat Rajabi[1], Shimon Amir[1], James G. Pfaus[1,2]

[1]Centre for Studies in Behavioral Neurobiology, Department of Psychology,
Concordia University, Montréal, QC, H4B 1R6 Canada.
[2]Centro de Investigaciones Cerebrales, Universidad Veracruzana,
Xalapa CP 91193, VER, México.

Running Head: Glutamate, GABA, Alcohol and sexual disinhibition

For publication in *Psychopharmacology*

Abstract

Rationale: Conditioned sexual inhibition (CSI) can be induced in male rats when they are given access on alternate trials to a sexually nonreceptive female scented with a neutral odor (almond) and an unscented receptive female. On a final test, males are presented with two sexually receptive females, one scented one unscented. Untreated males show a preference to copulate with the unscented female and to avoid the scented female. In contrast, males treated with a low dose of alcohol before the final test show no significant preference for, or avoidance of, either female, thus confirming that alcohol can disinhibit the CSI. We have found previously that low doses of alcohol increase extracellular concentrations of dopamine in the nucleus accumbens (NAc) of male rats when exposed to the inhibitory odor alone.

Objective: To examine the effects of alcohol on extracellular concentrations of glutamate and GABA in the NAc during exposure to an inhibitory olfactory cue.

Methods: Sexually naïve male Long-Evans rats were conditioned for sexual inhibition to an odor (almond) using our established paradigm and then injected with saline or alcohol (0.5g/kg or 1g/kg) before a final choice test. Males were later implanted with microdialysis probes aimed at the NAc. Extracellular levels of glutamate and GABA were measured when rats were given access to the odor under the influence of saline or alcohol.

Results: Saline-treated males showed CSI toward the scented female. This conditioned inhibition was not observed in alcohol-treated males. The alcohol alone or in presence of the olfactory cue had no effect on glutamate and GABA concentrations. But a novel odor decreased GABA concentrations in the NAc in both saline- and alcohol- treated groups.

Conclusions: These findings suggest that the disinhibitory effects of alcohol on sexual behavior are not mediated by a modulation of glutamate or GABA neurotransmission in the NAc of male rats. However, a novel odor appears to decrease GABA transmission, suggesting a possible mechanism for orientation to novel stimuli that may or may not become conditioned.

Key words: conditioned sexual inhibition, microdialysis, glutamate, GABA

Introduction

Alcohol (EtOH; ethyl alcohol) has long been known to affect behavior and neurotransmission. While the dopaminergic (DA) system plays a crucial role in the rewarding effects of EtOH, other neurotransmitter systems are targets for alcohol's actions. For example, both glutamate (Glu) and γ-amino butyric acid (GABA) receptors are primary molecular targets of alcohol (for review Spanagel, 2009) and contribute to its intoxicating (Cui and Koob, 2017), its potential inhibitory effects of EtOH on memory (Dahchour and de Witte, 2000) as well as its rewarding and anxiolytic effects (Koob, 2004)**.** By acting on Glu and/or GABA, EtOH interacts with the main excitatory and inhibitory neurotransmission system in the CNS, respectively, particularly with these amino acids' action on their receptors, such as the GABAergic ligand-gated ion channels or ionotropic Glu receptors (for review Abrahao et al., 2017; Lovinger and Roberto, 2013).The general consensus regarding the effects of alcohol on Glu and GABA transmissions is that acute exposure to EtOH inhibits Glu and stimulates GABA systems while chronic exposure has the opposite effects on these neurotransmissions (reviewed in Rao et al., 2015).

The effects of EtOH on Glu transmission have been initially demonstrated via its effects on Glu *N*-methyl-D- aspartate (NMDA) receptors. Previous research has established that NMDA receptors antagonists had alcohol-like effects (reviewed in Krystal et al., 2003). While the exact binding site of alcohol molecules on NMDA receptors is still unknown, at doses leading to behavioral impairment, EtOH reduces ion currents induced by NMDA (Grant and Lovinger, 1995; Lovinger et al., 1989). Several studies have also reported effects of EtOH on extracellular levels of Glu in various brain regions and those effects of alcohol on Glu release appear dose-dependent. While acute low doses of alcohol increase Glu levels, higher doses decrease these levels in several brain regions (Carboni et al., 1993; Moghaddam and Bolinao, 1994; Yan et al., 1998). In contrast, GABA neurotransmission is enhanced by alcohol through its binding site on the $GABA_A$ receptor (Lobo and Harris, 2008). Early studies demonstrated that alcohol was able to increase the efficacy of GABA transmission (Davidoff, 1973). Since then, substantial evidence linked GABA transmission to the effects of alcohol (reviewed in Dahchour and de Witte, 2000; Koob, 2004).By enhancing GABAergic inhibitory neurotransmission, EtOH generates behavioral effects such as the impairment of motor coordination following low dose intoxication (Hanchar et

al., 2005) and those effects can be reversed by GABAA receptor antagonists (for review Koob, 2004).

Aside from their involvement in the behavioral effects of alcohol, both Glu and GABA have been shown to influence male rats' sexual behavior (review in Hull and Rodríguez-Manzo, 2009; Melis and Argiolas, 2011). Overall, Glu facilitates sexual behavior in male rats as increased Glu levels induce an increase in the number of ejaculations (Dominguez et al., 2006), and injections of Glu antagonists result in decreased sexual responses in male rats (Li et al., 2013). On the other hand, changes in GABA transmission by the stimulation of $GABA_A$ and $GABA_B$ receptors impairs sexual behavior (Agmo and Paredes, 1985) and antagonists of these receptors facilitate the expression of sexual behavior (Fernandez-Guasti et al., 1985; Rodríguez-Manzo and Canseco-Alba, 2017). Similar results were obtained when changes in Glu and GABA transmission were performed within specific regions important for sexual behavior and sexual reflexes such as the medial preoptic area (mPOA), the paraventricular nucleus of the hypothalamus (PVN) and the spinal cord (for review, Hull and Rodríguez-Manzo, 2009; 2017).

While the effects of alcohol on Glu and GABA transmission, and the influence of these neurotransmitters on male rats' sexual behavior, have been established, to our knowledge, no studies have looked at the involvement of these neurotransmitters on the effects of alcohol on sexual behavior. Beside anecdotal reports on the disinhibitory effects of alcohol on behavior, EtOH has been shown to disrupt sexual inhibition in humans (Rubin and Henson, 1976; Wilson and Niaura, 1984) as well as in male rats using first- and second-order conditioning (Pfaus and Pinel, 1989; Germé et al., in preparation). A single injection of low to medium doses of alcohol is able to disrupt conditioned sexual inhibition (CSI) and male rats would copulate indiscriminately with females even if they are bearing an olfactory cue previously associated with sexual inhibition (Germé et al., in preparation). We have previously demonstrated that increased dopamine (DA) in the nucleus accumbens (NAc) due to the activation of ventral tegmental area (VTA) dopaminergic neurons projecting to the NAc mediates the disinhibitory effects of alcohol on sexual behavior (Chapter 3). However, aside from VTA DA neurons activation, DA release can also be modulated by activation of Glu or GABA receptors (Zhang and Sulzer, 2012). Within the NAc, DA release is increased following administration of Glu receptors agonists (Chéramy et al., 1997; Imperato et al., 1990; Taber and Fibiger, 1995) and the probability of DA release is

decreased following administration of GABA receptors agonists (Pitman et al., 2014). The NAc receives Glu inputs from the prefrontal cortex (PFC), the amygdala, the hippocampus, the VTA as well as GABA from the VTA and the amygdala (Morales & Root, 2014; Pavuluri et al., 2017; Salgado and Kaplitt, 2015; Yamaguchi et al., 2011) and these projections can mediate DA release in this brain region. We have previously determined that exposure to alcohol and to an olfactory inhibitory cue induced an increase in Fos expression in the PFC, the NAc and the VTA and a decrease in Fos expression in the BLA (Germé et al., in preparation). The PFC sends Glu outputs to the NAc (Christie et al., 1985) and electrical stimulation of the PFC induces an increase in DA release in the NAc (Taber and Fibiger, 1995). This increase is mediated by Glu as the activation of metabotropic Glu receptors by local application of the agonist ACPD has an inhibitory effect on DA release elicited by PFC stimulation (Taber and Fibiger, 1995). Previous studies also reveal that terminals of glutamatergic afferents from the BLA are potentially close to DA terminals in the NAc (Johnson et al., 1994). Furthermore, Glu NMDA receptors have been shown to be located on DA axonal processes (Gracy & Pickel, 1996), suggesting that glutamatergic afferents from the BLA may directly modulate NAc DA transmission. On the other hand, aside from DA projections, the VTA sends GABA neurons to the NAc (Van Bockstaele and Pickel, 1995; Morales and Margiolis, 2017) targeting almost exclusively cholinergic interneurons and few MSNs (Brown et al., 2012) and modulation of cholinergic transmission in the NAc can regulate DA release in NAc (Cachope and Cheer, 2014).

Given the pattern of Fos activation previously observed in these brain areas during exposure to the olfactory cue and alcohol and their Glu and GABA projections to the NAc modulating DA release, the possible involvement of these two amino acids within this region in the disinhibitory effects of alcohol on sexual behavior cannot be excluded. In the present experiment, using the same paradigm of second-order conditioning to induce condition sexual inhibition (CSI) as Germé et al., (in preparation), we examined extracellular concentrations of Glu and GABA in the NAc, to determine whether these amino acids were affected by EtOH, potentially had a similar pattern of changes as seen previously with DA (Chapter 3) and could therefore contribute to the disinhibitory effects of alcohol on sexual behavior in male rats.

Materials and Methods

Animals, surgery, hormones

Males. 37 sexually naive male Long-Evans rats, weighing approximately 250g, were obtained from Charles River Canada, Inc (St. Constant, QC). They were housed in groups of 4 in Plexiglas cages with *ad libitum* access to food (Purina Rat Chow) and water.

Females. Female Long-Evans rats obtained from the same supplier, were bilaterally ovariectomized via lumbar incisions using intraperitoneal (i.p.) injections (1ml/kg body weight) of ketamine hydrochloride (50mg/ml; Ketaset©, Wyeth Canada) and xylazine hydrochloride (4mg/ml; Rompun©, Bayer Healthcare) anesthetic mixed at a ratio of 4:3, respectively. A week after ovariectomy, sexual receptivity was induced for half of the females by subcutaneous injections of estradiol benzoate (10μg) 48 hours and progesterone (500μg) 4 hours prior to each conditioning trials and copulatory test.

All rats were maintained on a reverse 12-hour dark-light cycle (lights on at 8:00pm) at 21 ± 1°C. All behavioral testing was carried out between 12:00pm and 5:00pm. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Research Ethics Committee at Concordia University.

Apparatus. Conditioning trials took place in Plexiglas bilevel chambers (18cm x 25cm x 65cm) with a platform dividing the chamber into two levels (Mendelson and Gorzalka, 1987; Mendelson and Pfaus, 1989). The preference test took place in a large open field (123cm x 123cm x 96cm) with bedding covering the floor (described in Germé et al., in preparation; Kippin et al., 1998). All conditioning trials and preference test were recorded on a video camera and scored later using a PC-based behavioral observation program (Cabilio, 1996).

Drug administration. 95% ethyl alcohol was diluted in saline to obtain doses of 0.5 g/kg and 1 g/kg in a 25% v/v solution. To avoid any bias due to the volume injected, males in the saline group received an i.p. injection of saline solution in the same volume as the highest dose of alcohol. On test days, each male was injected with their respective dose of alcohol 45 minutes before and left undisturbed until testing. The dose received by each rat was the same throughout the whole experiment.

Procedures. *Conditioning.* Procedures used for the conditioning are the same as those described in Germé et al., in preparation. To summarize, males were given 20 conditioning trials in bilevel chambers, at 2-day intervals, alternating between exposure to an unscented receptive female and a scented nonreceptive female, for a total of 20 conditioning trials. Nonreceptive females were scented with 1ml of pure almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) on the back of their neck and the anogenital region. On each conditioning day, males were placed alone in a bilevel chamber for five minutes, after which a female in the appropriate sexual condition was introduced in the chamber for a 30-minute conditioning trial.

*Copulatory preference test.*Copulatory preference tests took place in a large open field (123cm x 123cm x 96cm) with bedding covering the floor four days after the last conditioning trial with a receptive female. Male rats were assigned randomly to one of the two alcohol treatment groups, 0.5 g/kg alcohol (A0.5) or 1 g/kg alcohol (A1), or the saline control, and injected with their respective treatment 45 minutes before the copulatory preference test. Each male was then placed in the open field and allowed to habituate for 5 minutes after which two receptive females, one scented (almond extract) and one unscented, were placed simultaneously into the open field at equal distance from the male. During the 30-minute test, all copulatory behaviors and the females to which they were directed to were recorded on a video camera and later scored using a PC-based behavioral observation program (Cabilio, 1996). Criteria for sexual behaviors were those described by Sachs and Barnfeld (1976), Meisel and Sachs (1994), and Pfaus et al., (1990b).

Cannulation surgeries. Following the preference test, male rats received bilateral cannula placements aimed at the NAc. Rats were anaesthetized with Isofluorane (Inhalation Anaesthetic, Richmond Hill, ON, Canada). Two 21-gauge guide cannulae (Plastics One, Roanoke, VA) were lowered and positioned 1 mm above the left and right NAc (AP: +1.65mm, ML: ± 2.7mm, DV: -5.3mm from Bregma, angle 10°; Paxinos & Watson, 1998) and secured to the skull using dental cement, anchored by four stainless steel screws. A removable stainless steel stylet (26-gauge; Plastics-One) was placed inside the guide cannula until microdialysis. Male rats were allowed a week recovery before microdialysis.

In vivo microdialysis and high-performance liquid chromatography. Four Plexiglass chambers with stainless steel grid floors (hexagonal, 42 x 39 x 33.5cm^3, custom-made, Concordia

University) were used for microdialysis. Each chamber was contained in a wooden cubicle and lighting was provided on reverse cycle by overhead light bulbs.

Microdialysis probes. Microdialysis probes were made in the laboratory following the procedures by Sorge et al., 2005. They consisted of a 2mm long semi-permeable dialysis membrane (Fisher Scientific, 240 mm OD, 13 000 MW cut off) attached to 22mm long 26-gauge stainless steel tubing. Small-diameter fused silica tubing extended internally through the probe, resting 0.5 mm from the tip of the probe. The steel tubing was attached to a 20-µm-diameter polyethylene (PE) tubing (35-40 cm long; Plastics-One, Roanoke, VA, USA). The other end of this PE tubing was connected to the stainless steel shaft of a single-channel liquid swivel (HRS Scientific, Montreal, Qc, Canada). The swivel was located above the wooden cubicle of each microdialysis chamber and was connected to a variable speed electric syringe infusion pump (Harvard Apparatus, South Natick, MA). Each probe was secured in place by stainless steel collars that were screwed onto the guide cannula. The external length of the PE tubing was protected by a steel spring casing.

Odour exposure and dialysate collection. The night before the odour exposure, microdialysis probes were lowered into the NAc, using light isofluorane anaesthesia. Males were placed individually in a microdialysis chamber. To prevent occlusion before testing, probes were perfused with artificial cerebrospinal fluid (aCSF; 145mM Na^+, 2.7mM K^+, 1.22mM Ca^{2+}, 1.0mM Mg^{2+}, 150mM Cl^-, 2mM Na_2PO_4, pH 7.4 ± 0.1) at 1.0 µl/min for approximately 1 h. Samples were collected every 10 minutes during this time to ensure a good flow and no leakage of the probes. The flow rate was then lowered to 0.2 µL/min overnight. The next day, the flow rate was returned to 1µL/min 2 hours prior to baseline sampling.

Dialysate samples were collected every 10 minutes from beginning of baseline until the end of the exposure (approximately 180 minutes total). Baseline samples were collected for 90 minutes. Following baseline, male rats received an acute injection of saline or alcohol (0.5g/kg or 1g/kg, i.p.; depending on their respective group). Samples were collected for 50 minutes. Cotton gauze with 1mL of almond extract or lemon extract was introduced into the microdialysis chamber 50 minutes after the injection and left there for 40 minutes.

High-Performance Liquid Chromatography (HPLC). Glutamate and GABA levels were determined using HPLC with fluorescence detection. Dialysates were derivatized with *o*-

phthalaldehyde and manually injected into an injection port (Rheodyne 7125; Rheodyne LLC, Rhonert Park, CA; 20µl loop). Components were separated using a C-18 reverse-phase column (5 µm, 15 cm, 4 mm ID, Higgins Analytical). Glutamate and GABA were detected using Ultrafluor (Lab Alliance, Fisher Scientific, Montréal, QC, Canada) with an excitation wavelength of 340nm and an emission wavelength of 460nm. A phosphate buffer mobile phase was circulated through the HPLC system at a rate of 1.1mL/min by Waters 515 HPLC pumps. Between each sample, the column was flushed with a 40% acetonitrile buffer solution for approximately two minutes. Peaks of each amino acid of interest were integrated and quantified by EZ Chrom Chromatography Data System (Scientific Software Inc., San Ramon, CA, USA).

Statistical analyses

All statistical analyses were performed using SPSS Statistics 25.0 for PC.

Behavior. To compare the alcohol treatments and the differences in behavior towards each female, a 3 (alcohol treatment) x 2 (female) mixed design ANOVA followed by Tukey's post-hoc comparisons. Partial eta squared (η^2_p) was reported as a measure of effect size. Chi-square analyses were used to assess the proportions of males choosing one of the two females for their first mount, intromission and ejaculation on the copulatory preference test.

Microdialysis. To determine the effects of alcohol and odour exposure on Glu and GABA levels, baseline levels were determined by an average of the last three samples collected prior to the injection of EtOH and then converted to a percentage of the baseline. Changes from baseline were analyzed separately for each neurotransmitter and for each odour condition (almond or lemon) using a mixed design ANOVA for each odor with between subject factor of alcohol treatment (saline, A0.5, A1) and the within subject factor of time (baseline average, 0-80). Statistically significant main effects and interaction were followed by post hoc tests (Bonferroni correction).

For all analyses, the level of significance was set to $p<0.05$.

Results

Copulatory test.

Of the 37 male rats in the experiment, one from the saline group, two from the A0.5 group and two from the A1 group did not ejaculate during the final copulatory test. Therefore they were excluded from the analyses.

The mean number of mounts, intromissions and ejaculations are depicted in Figure 1.

During the final copulatory test, there was a difference in the mean number of mounts received by the two females. This was revealed by a main effect of female $F(1,29)=6.68$, $p=.015$, $\eta^2_p=.19$. Further analyses revealed that overall, the unscented females ($M=17.35$) received more mounts from the males compared to the scented female ($M=12.55$). There was no main effect of group and no significant interaction (group x female).

There was a significant difference between groups in the frequency of intromissions during the copulatory test as revealed by a significant main effect of group $F(2,29)=4.85$, $p=.015$, $\eta_p^2=.25$. Post hoc analyses revealed that the saline group ($M=9.14$) had overall significantly higher intromission frequencies than males in the A1 group ($M=6.23$). There was no main effect of female and no significant interaction group x female.

The mean number of ejaculations was not different between females and between groups as there was no significant main effect of female or group. However, there was a significant interaction (group x female) $F(2,29)=3.82$, $p=.034$, $\eta^2_p=.21$. Post hoc analyses revealed that in the saline group only, the unscented female (No Odor; $M=2.09$) received significantly more ejaculations than the scented female (Odor; $M=0.82$).

Regarding the latencies, no significant difference between groups or between females and no interaction (group x female) was observed in the latency to first mount or to first intromission. There were also no main effects of groups or of female in the latency to first ejaculation. However, there was a significant interaction (group x female) $F(2,29)=3.39$, $p=.047$, $\eta^2_p=.19$. Post hoc analyses revealed that, in the saline group, the unscented females ($M=491.72$) received the first ejaculation significantly faster than the scented female ($M=1077.30$).

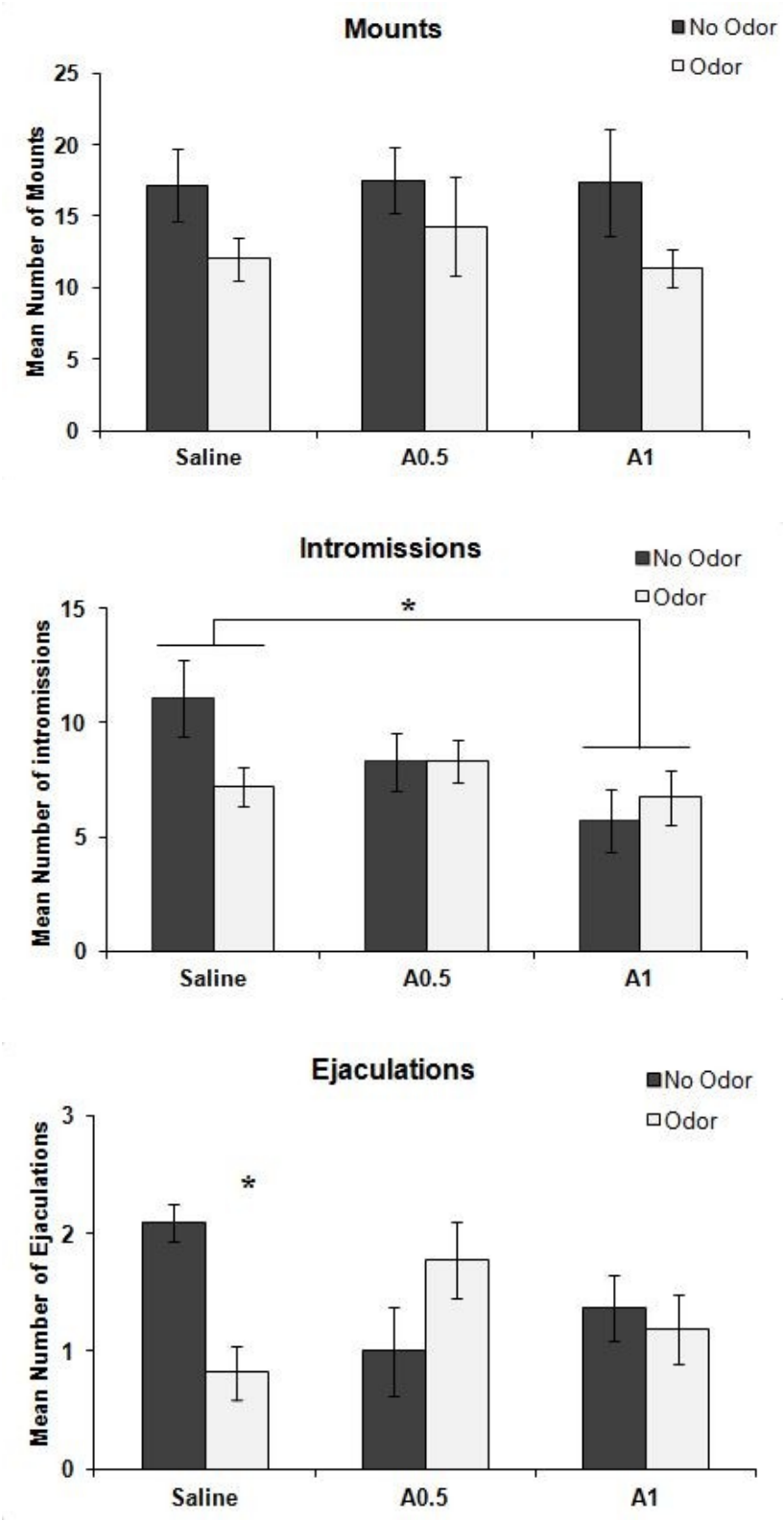


Figure 1. Mean copulatory behaviors (± S.E.M) during the 30 minute copulatory test. * $p<.05$

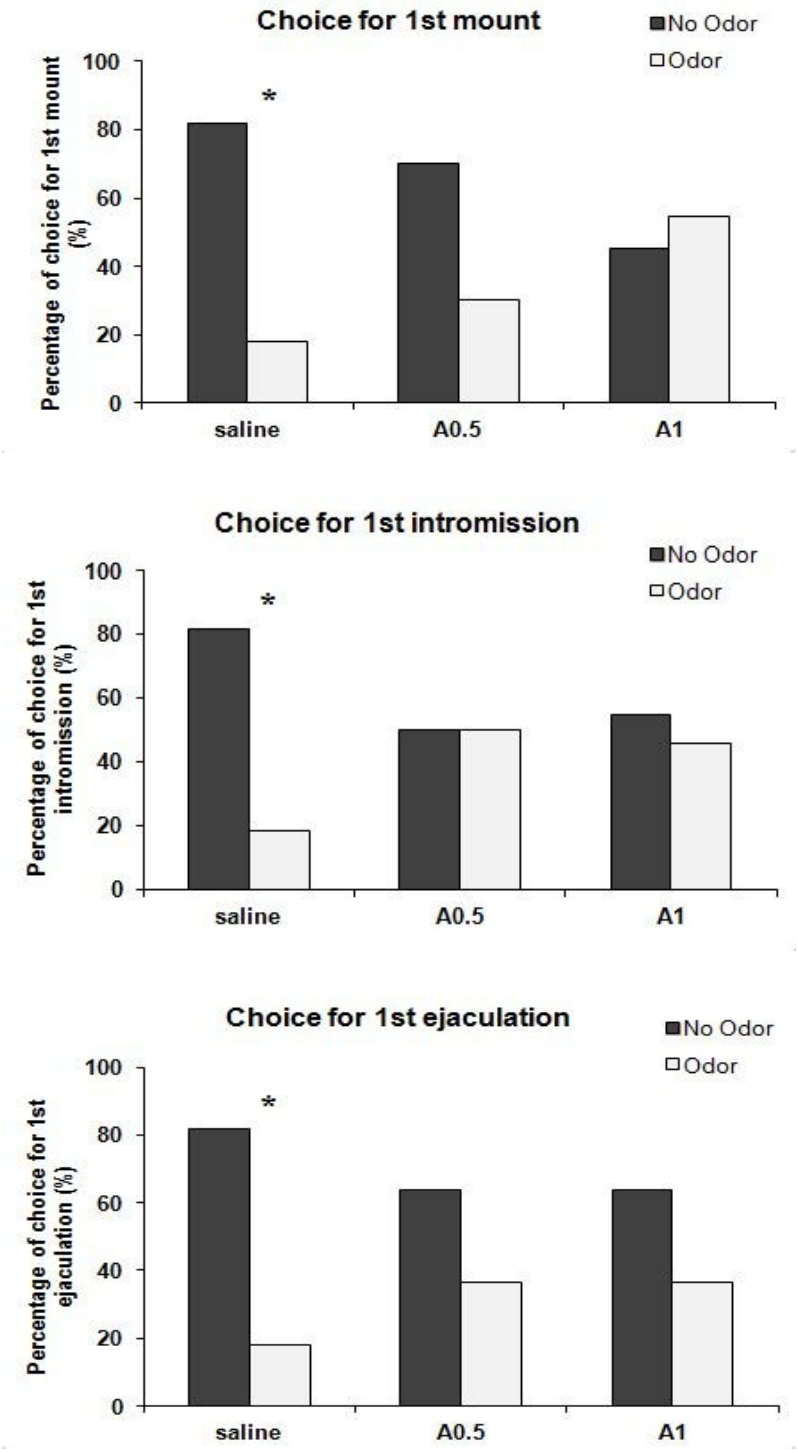


Figure 2. Percentage of choice for 1st mount, intromission and ejaculation. Males in the saline group chose the unscented females (No Odor) more frequently for their first mount, intromission and ejaculation * $p<.05$

The proportions of males choosing either females for their first mount, intromission and ejaculation are shown in Figure 2. There was a significantly higher percentage of males in the saline group choosing the unscented female as opposed to the scented female for their first mount χ^2 (1, N=11) = 4.45, p=.035, their first intromission χ^2 (1, N=11) = 4.45, p=.035 and their first ejaculation, χ^2 (1, N=11) = 4.45, p=.035 (Figure 2).

Microdialysis

Glutamate. None of the factors analyzed (alcohol treatment, time, odor) had an effect on the extracellular levels of Glu in the NAc. Results are depicted in Figure 3.

Glutamate concentrations during exposure to almond odor. Following the alcohol treatment and the exposure to the almond odor, there was a no significant difference across time in Glu levels as the main effect of time was not significant F(12,120)= 1.54, p=.119, η^2_p =.134. The Glu levels were also similar between groups as there was no significant main effect of group F (2,10) =2.21, p=.161, η^2_p =.31. Finally, the interaction (group x time) did not reach significance F(24,120)=0.72, p=.827, η^2_p =.13 .

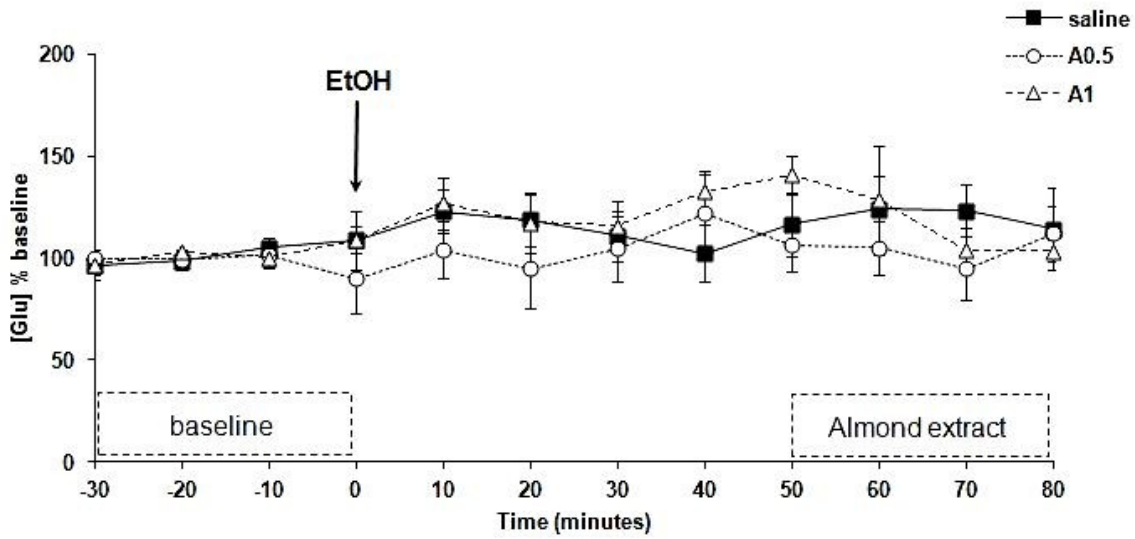
Glutamate concentrations during exposure to lemon odor. Neither the alcohol treatment nor the novel lemon odor had an effect on extracellular Glu concentrations as there were no significant main effects of group F(2,9) = .398, p=.683, η^2_p =.08 or time F(12,108)=1.52, p=.128, η^2_p =.14 . The interaction (group x time) was also not significant F(24,108)=.43, p=.990, η^2_p =.08.

GABA. Results from microdialysis for GABA levels are shown in Figure 4.
GABA concentrations during exposure to almond odor. Neither alcohol nor the exposure to the almond odor had a significant effect on the extracellular levels of GABA. There was no significant main effect of group F(2,10)= 1.68, p=.234, η^2_p =.25, or time F(12,120)=1.27, p=.243 η_p^2=.11. The interaction of group x time was not significant F(24,120)=.89, p=.613, η^2_p =.15.

GABA concentrations during exposure to lemon odor. There were changes in GABA levels in the NAc over time. There was a significant main effect of time F(12,108)=5.39, p<.001, η^2_p =.38. But the alcohol treatment alone did not affect GABA levels F(2,10)=.03, p=.975, η^2_p =.006. And the interaction was not significant F(24,108)=.25, p=.975, η^2_p =.05. Post hoc analyses revealed that the mean levels of extracellular GABA in NAc were significantly lower compared to baseline at

time 70 and 80 minutes (so 20 and 30 minutes following the introduction of the novel odor in the chamber) (Figure 4B).

A. Glutamate - Exposure to almond



B. Glutamate - Exposure to lemon

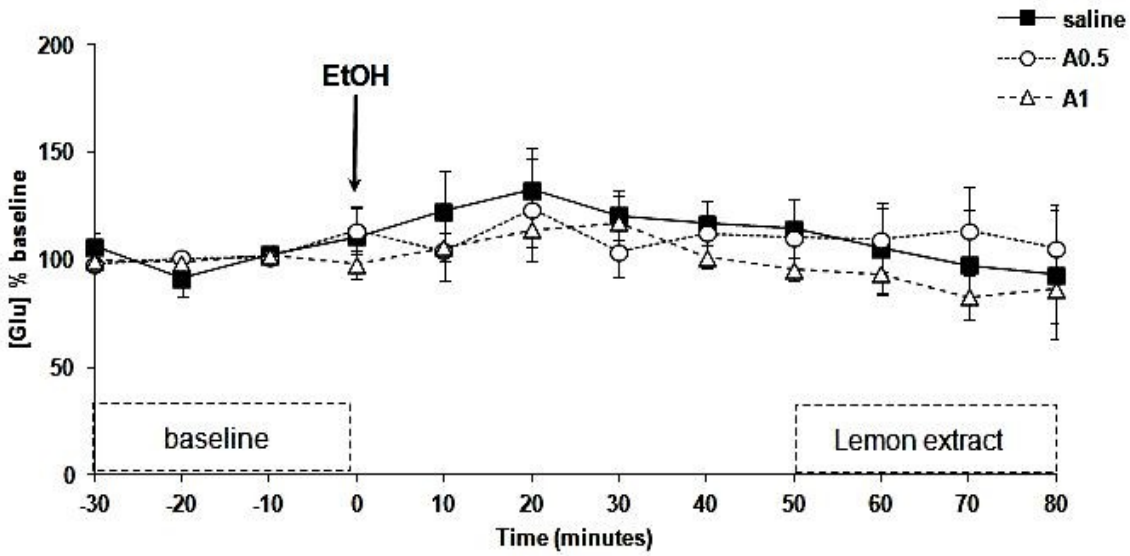
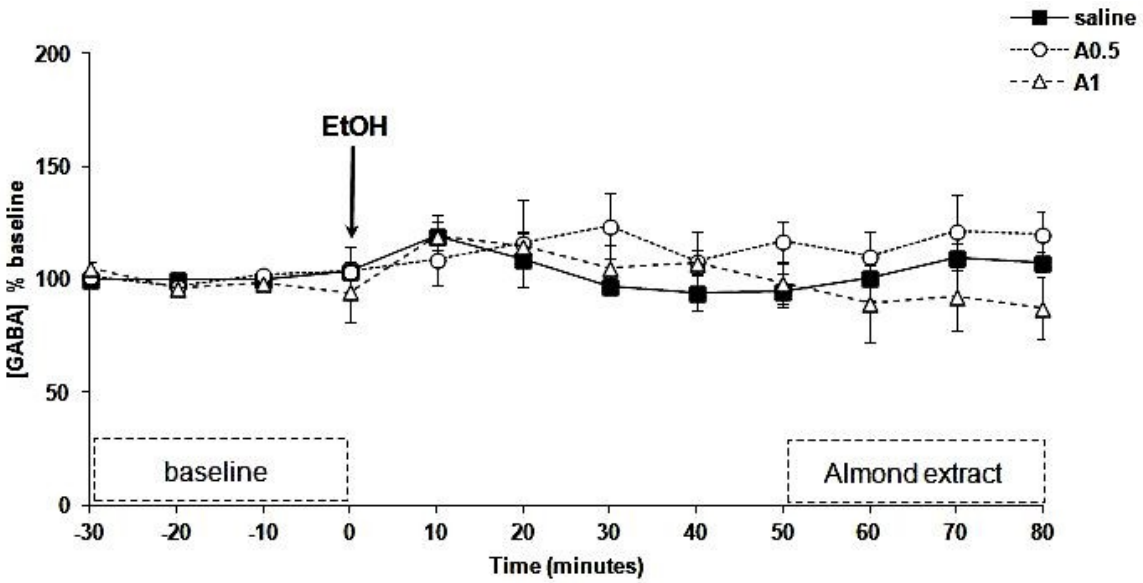


Figure 3. Extracellular glutamate levels in the nucleus accumbens following an injection of saline (n=6), alcohol 0.5g/kg (A0.5; n=5) or alcohol 1g/kg (A1; n=5) and exposure to (**A**) almond extract or (**B**) lemon extract.

A. GABA - Exposure to almond



B. GABA - Exposure to lemon

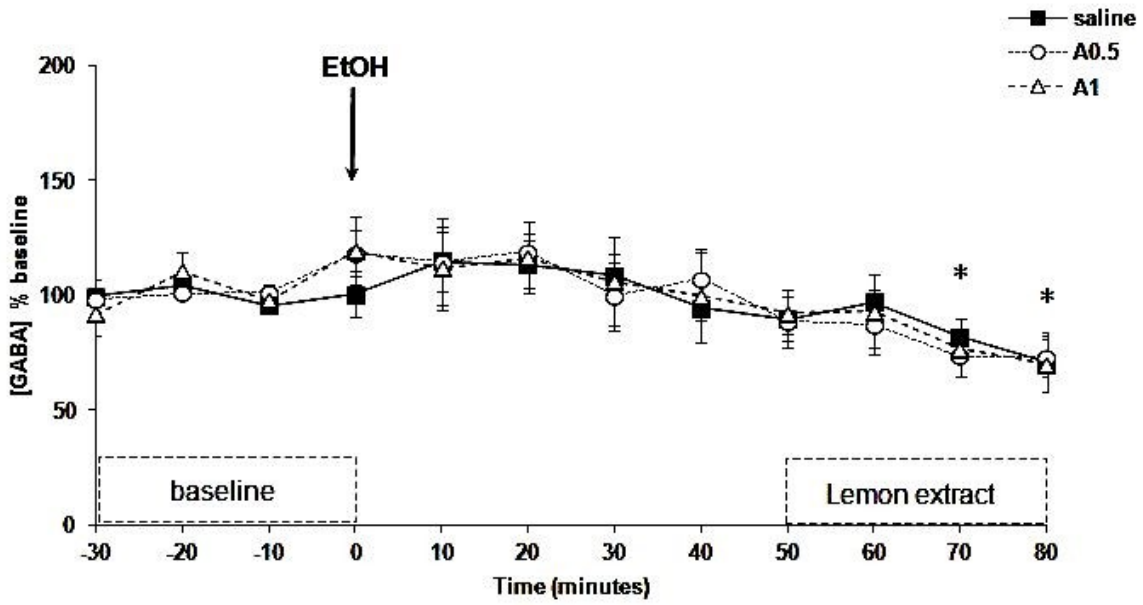


Figure 4. Extracellular GABA levels in the nucleus accumbens following an injection of saline (n=6), alcohol 0.5g/kg (A0.5; n=5) or alcohol 1g/kg (A1; n=5) and exposure to (**A**) almond extract or (**B**) lemon extract. *p<.05 compared to baseline

Discussion

The present study examined the effects of alcohol and an inhibitory olfactory cue on Glu and GABA transmission in the NAc and the potential implication of these neurotransmitters in the disinhibitory effects of EtOH on conditioned sexual inhibition (CSI) in male rats. We replicated our previous findings that a low dose of acute alcohol disinhibited an odor-based CSI. Male rats underwent alternating training with a scented (almond odor) non receptive female and an unscented receptive female. On the copulatory test where they had a choice between two receptive females, one scented with the inhibitory odor and the other unscented, saline-treated males intromitted and ejaculated more frequently with the unscented female compared to the scented female. Furthermore, a higher proportion of the males in the saline group preferentially chose the unscented female for their first mount, intromission and ejaculation compared to the scented female. These preferential behaviors were not observed in the alcohol-treated males as they copulated indiscriminately with both females during the copulatory test. The second part of this study investigated the potential role of NAc Glu and GABA in the disinhibitory effects of alcohol on sexual behavior. When male rats were injected with saline or EtOH, extracellular levels of Glu did not change from baseline over time even following exposure to the almond odor (inhibitory cue). Extracellular levels of GABA in the NAc were not altered significantly by any dose of alcohol or the conditioned inhibitory cue. However, to our surprise, GABA transmission decreased significantly when males were exposed to a neutral olfactory cue (lemon) regardless the alcohol treatment.

There are contradicting reports regarding the effects of acute low doses of alcohol on extracellular levels of Glu in the NAc using microdialysis. A biphasic response in Glu transmission following acute injections of EtOH was demonstrated in rats with elevated extracellular levels of Glu in the NAc at doses of 0.5 and 1g/kg of EtOH for up to two hours following the injection and decreased levels at doses of 2g/kg and above (Moghaddam and Bolinao, 1994). Similar decreases in extracellular Glu following 2g/kg of alcohol was also observed in other studies (Yan et al., 1998). Conversely, other investigators reported no changes in Glu transmission in the NAc after acute alcohol treatment of up to 3g/kg of alcohol (Dahchour et al., 1994; Kashkin and de Witte, 2004). The present study also found no effect of alcohol on Glu transmission in the NAc. What could explain this discrepancy? It has been shown previously

that strains of rats with high rates of alcohol self administration show increased Glu transmission (e.g., Selim and Bradberry, 1996). Male rats in the present experiment were randomly assigned to the alcohol groups. Their sensitivity to alcohol and predisposition for EtOH self-administration were not assessed before or after the alcohol treatment. It is possible that the rats in the present experiment did not have an unusual sensitivity to, or preference for, alcohol, and thus did not show changes in Glu transmission after alcohol injections.

As with Glu, previous studies show a varied effect of alcohol on GABA transmission depending on the brain region examined. For example, acute alcohol did not alter GABA transmission in regions such as the anterior cingulate cortex, the hippocampus, the ventral tegmental area, but decreased GABA transmission in the cerebellar nuclei and ventral pallidum (reviewed in Kelm et al., 2011). Low to moderate doses of alcohol used in this experiment did not alter extracellular levels of GABA in the NAc. This is in accordance with previous studies showing that low to moderate doses, similar to those used in the present experiment, did not affect GABA levels in the NAc (Dahchour et al., 1994; Heidbreder and de Witte, 1993; Kelm et al., 2011) while high doses of alcohol decreased extracellular levels of GABA in the NAc (Piepponen et al., 2002).

Exposure to either the conditioned (almond) or novel (lemon) odors did not affect Glu transmission in the NAc in any of the groups. And there was no difference in Glu levels between the two odors in all the groups. This suggests that Glu in the NAc do not play an intermediary role in the disinhibitory effects of alcohol on sexual behavior. However, it is also possible that fast Glu transmission could occur that is obscured by the 10-min microdialysis sampling rate in the present study. Indeed, other studies have shown rapid changes in NAc Glu transmission when rats are exposed to an unconditioned predator odor (Venton et al., 2006) or stimuli such as a tone, tail pinch, exposure to a conspecific, or cocaine (Wakabayashi and Kiyatkin, 2012) when using microdialysis with capillary electrophoresis or high-speed amperometry with enzyme-based biosensors, respectively.

As with Glu, the conditioned almond odor did not alter extracellular levels of GABA in the NAc. This suggests that GABA transmission in the NAc might not be a mediator in the disinhibitory effects of alcohol on CSI. However, unlike Glu, exposure to the novel lemon odor decreased GABA levels significantly in the NAc. Because this decrease was observed in both the

alcohol and saline control groups, it can be concluded that this phenomenon is not due to the administration of alcohol but to the novel lemon odor itself. Previous research has shown a decrease in NAc responses when rats were exposed to neutral odors (West et al., 1992). In that experiment, male rats were trained to associate odors with access to receptive females. They observed an increase in NAc neuronal responses when trained males were exposed to familiar odors associated with sexually receptive females. However, neutral odors that were not used during training and that rats had not been exposed to previously induced a decrease in NAc neuronal firing rates of trained males. West et al. discussed the possibility of NAc neuronal activity to decrease when rats are exposed to "non important" odors as a mechanism to focus attention on significant stimuli (e.g., reward-related stimuli) in the environment. This explanation would fit the findings of the present experiment. Rats were exposed to the novel lemon odor only during the microdialysis phase of the experiment. Although rats may have oriented to the lemon odor as a novel stimulus, its status as a neutral and unconditioned odor that was not followed by any reinforcer may have decreased GABA transmission as a mechanism of learned irrelevance. The NAc is mostly comprised of GABAergic medium spiny neurons and interneurons. It is therefore possible that the decrease in activity of these neurons observed in the experiment by West and colleagues could lead to the decrease in GABA levels detected in the present study when male rats are exposed to the neutral odor. On the other hand, as the almond odor did not induce decrease in GABA levels, this would also suggest that the inhibitory cue was not perceived as a neutral, non relevant odor by the male rats. Therefore, the absence of changes when male rats are exposed to the almond odor is not due to an absence in recognition but to its actual recognition as a relevant cue. This would also indicate that GABA in the NAc is not involved in the disinhibitory effects of alcohol on sexual inhibition.

The present experiment extends our previous findings on the neurochemical basis of alcohol's disinhibitory effect on male sexual behavior. While a previous experiment from our laboratory showed increase in NAc DA transmission in response to alcohol and the inhibitory odor (Chapter 3), the present experiment established that Glu and GABA in the NAc are not affected by alcohol with or without the conditioned olfactory cue. Therefore, these two amino acid systems in the NAc likely do not contribute to alcohol-induced sexual disinhibition in male rats. It should be noted that, Glu and GABA concentrations were observed only in the NAc as a potential link with our previous findings of increased DA transmission in this brain region

following exposure to alcohol with the conditioned olfactory cue. However, the levels of these amino acids are affected by alcohol in many other brain regions (Cui and Koob, 2017; Kelm et al., 2011) and these amino acids can interfere with the DA system and DA release in the NAc (Howland et al., 2002; Spanagel and Weiss, 1999; Spanagel, 2009) via other brain areas such as the VTA, the main source of NAc DA. In fact, previous studies demonstrated that facilitation of Glu transmission in the VTA by alcohol can increase DA release in NAc (Xiao et al., 2008) and that EtOH enhances GABA tone in the VTA modulating VTA-DA firing rate (Theile et al., 2011). Accordingly, the absence of changes in extracellular levels of Glu and GABA in the NAc does not mean the total non involvement of these neurotransmitters in the disruptive effects of alcohol on CSI. In addition to the VTA, other regions of interest such as the BLA and the PFC displaying differential pattern of Fos activation under the influence of alcohol and during exposure to the almond odor (Germé et al., in preparation), also play a mediatory role in DA release in the NAc (Johnson et al., 1994; Taber and Fibiger, 1995) and are affected by alcohol (for review, Abrahao et al., 2017). Thus, further investigation is needed to better understand how these brain regions, known to send glutamatergic and GABAergic projections and interact with the mesolimbic system, might mediate the effects of alcohol on conditioned sexual inhibition.

Funding. This research was funded by a grant from the Canadian Institutes for Health Research (MOP-74563) to JGP and an infrastructure grant from Fonds de la Recherche en Santé du Québec to the Center for Studies in Behavioral Neurobiology at Concordia University.

Compliance with ethical standards. The authors declare that all animal procedures conformed to the guidelines of the Canadian Council for Animal Care. All procedures were approved by the Concordia University Animal Research Ethics Committee.

Conflict of Interest. The authors declare they have no conflicts of interest.

General discussion

In this thesis, the neural mechanisms possibly underlying the disruptive effects of alcohol and d-amp on conditioned sexual inhibition in male rats were examined. Previous work from the laboratory showed that alcohol at low to medium doses induced a disruption of CSI and males exposed to alcohol and a conditioned olfactory cue displayed different neuronal activation in brain regions mediating sexual behavior and reward compared to saline-treated males (Germé et al., in preparation). Based on these results, and using the same paradigm to induce CSI, the purpose of this thesis was to further examine the disinhibition of sexual behavior in male rats using another drug, d-amp and to elucidate the brain regions and neurotransmitters potentially involved in drug-induced disruption of CSI.

Disruption of CSI by d-amp

The experiments of Chapter 2 were designed to investigate the effects of d-amp on CSI in male rats and the brain regions potentially mediating those effects. Regarding disinhibition of sexual behavior, anecdotal and scientific reports mention alcohol as a disinhibitory drug for human (Rubin and Henson, 1976; Wilson and Niaura, 1984) and for rodents (Pfaus and Pinel, 1989). However, psychomotor stimulants are also known to disinhibit sexual behavior. Because of its important potential for misuse and abuse (Heal et al., 2013), and its context of use associated with sexual activity (Skårner and Svensson, 2013), a study of the effect of d-amp on sexual behavior seemed necessary. Despite the differences in classification between alcohol and d-amp, and virtually opposing actions in the brain (Pfaus et al., 2010), d-amp had the same disinhibitory effect on CSI as alcohol. In fact, this first study showed that male rats treated with d-amp, regardless the dose administered before a copulatory choice test with two receptive females (one scented with an inhibitory olfactory cue and the other unscented), copulated with both females indiscriminately. In contrast, saline-treated males ejaculated preferentially with the unscented female, thus displaying a CSI toward the scented female.

The relationship between amphetamine use and sexual inhibition in humans seems still under debate (Chou et al., 2015; Pfaus et al., 2010; Skårner and Svensson, 2013). However, the animal literature has revealed a general agreement on the reversal effect of d-amp on disrupted or

inhibited sexual behavior. Doses of d-amp comparable to the ones used the present thesis were able to restore sexual behavior in male rats with lesion- and drug-induced disruption of sexual behavior (Butcher et al., 1969; Ågmo and Villalpando, 1995) or with sexual exhaustion (Soulairac and Soulairac, 1957). Furthermore, those studies suggested that d-amp interferes with sexual motivation but not with the expression of sexual behavior once initiated. Instead of disrupting sexual behavior in male rats using lesions or drugs, in this thesis, conditioning was used to induce sexual inhibition. This conditioning allowed a normal expression of sexual behavior but affected the motivation to engage in copulation with females bearing an inhibitory olfactory cue. As noted, d-amp was able to disrupt this conditioned inhibition without affecting copulatory performance. In contrast to males in the saline group that ejaculated preferentially with the unscented females and displayed CSI towards the scented female, d-amp treated males did not show conditioned inhibition towards the females bearing the olfactory cue and copulated with both females during the final choice test. Aside from this main difference between saline- and d-amp treated groups, no other behavioral distinction was observed in the expression of copulatory behaviors. These results supported the hypothesis that d-amp can disrupt CSI and be considered as a disinhibitory drug for sexual behavior. They also confirmed that the expression of sexual behavior is not affected by d-amp at the doses used (Ågmo and Villalpando, 1995). However, these results raise the question whether the disinhibitory effects of d-amp could be due to enhanced sexual motivation. Further investigation allowing a proper measure of sexual motivation (e.g., measure of level changes using bilevel chambers as in Mendelson and Pfaus (1989), but tested with only one female bearing the inhibitory cue to measure latencies to initiate copulation or using operant responses) would be needed to ascertain the role of sexual motivation in the disruption of CSI by d-amp and possibly other drugs.

To assess the brain regions involved in the disinhibitory effects of d-amp, Fos IR was used as a marker of neuronal activation to determine which brain regions were activated when male rats were under conditions leading to the disruption of CSI (drug + olfactory cue). When this technique was used in a previous experiment with alcohol and the inhibitory olfactory cue, increased Fos expression in the PFC, NAc and VTA and decreased neuronal activation in the BLA were reported (Germé et al., in preparation). The brain regions analyzed in the present study were chosen based on those findings but also on past literature revealing d-amp sensitive brain regions (Graybiel et al., 1990; Harlan and Garcia, 1998). Because both drugs induced a disruption

of CSI, it was expected to see some similar neuronal activation in addition to regions selective for alcohol and d-amp.

A significant difference in Fos expression was observed between d-amp and saline groups following exposure to the olfactory cue in the NAc, the mPOA, the PirCx and the VTA, suggesting the involvement of these brain regions in the disinhibitory effects of d-amp on sexual behavior. The exposure to the cue induced significantly lower Fos IR in the mPOA of the saline group compared to the d-amp group. Due to its crucial role in the regulation of male sexual behavior (Hull et al., 2006; Hull and Dominguez, 2007) and Fos induction in this region observed following exposure to estrous odor or estrous females (Kippin et al., 2003), the significantly lower Fos IR in the saline group exposed to the almond odor suggests that under conditions perceived as sexually inhibitory (presence of the conditioned olfactory cue) induce a decrease in neuronal activation in this region. The lack of difference in Fos expression between odor conditions in the d-amp groups reflects the behavioral results of the copulatory preference test and strongly suggests a possible absence of perception of the olfactory cue as sexually inhibitory which would result in the disruption of CSI by d-amp. This interpretation is further supported by the results of Fos expression detected in the PirCx. Neuronal activation in this brain area was significantly higher in the saline group exposed to the olfactory cue compared to the d-amp group, but also compared to the saline group not exposed to the odor. Again, no difference was observed between the two odor conditions in the d-amp groups. Because previous studies suggested activation of this brain area by olfactory stimuli that have acquired incentive value (Kippin et al., 2003), this would imply that in the d-amp groups the odor did not acquire as strong of an incentive value as it did for the saline group, again suggesting the lack of inhibitory meaning following treatment with d-amp. Together, the findings of Fos expression in the mPOA and in the PirCx suggest that the disinhibitory effects of d-amp are possibly due to a loss of inhibitory value of the olfactory cue.

Further in line with the hypothesis of a probable similar pathway for the disinhibitory effects of drugs on sexual behavior, the activation of two other regions under the influence of d-amp were coinciding with the neuronal activation previously observed with alcohol. Significantly more Fos was detected in the NAc and VTA when male rats were exposed to the olfactory cue and under the influence of d-amp in contrast to males treated with saline or males not exposed to

the odor. As this pattern of neuronal activation was also observed in our previous study with alcohol (Germé et al., in preparation), this suggests a possible disinhibitory network likely involving both regions, which are necessary for sexual reward, desire, and arousal.

Regardless the exposure (or non exposure) to the odor, Fos expression in the VTA was significantly higher in the d-amp groups compared to the saline group. These results were not surprising as the VTA has been shown to be activated following injections of d-amp (Colussi-Mas et al., 2007; Rotllant et al., 2010) and is also linked to drug-related behaviors such as d-amp behavioral sensitization or conditioned place preference for d-amp (reviewed in Oliva and Wanat, 2016). As there was no difference between the exposure conditions in the d-amp groups in the present study, it can be assumed that these results are due to the effects of d-amp on VTA neurons and not to the odors. Therefore, the involvement of VTA in the disruptive effects of d-amp on conditioned inhibition requires further study. For example, it is not clear whether Fos is activated within DA neurons or other neurochemical systems. Indeed, the VTA is composed of at least three neurochemical subpopulations: DA, GABA and Glu (Nairs-Roberts et al., 2008) and receives projections from several brain regions (Morales and Margiolis, 2017). The subpopulations of VTA neurons can be differentially activated based on the contextual cues, the neurotransmission within the VTA and the projections innervating the VTA (for review, Bariselli et al., 2016). Therefore, the lack of difference in Fos expression in that particular case does not necessarily mean no difference in subtype of neurons activated and further investigation is warranted to determine potential changes within those VTA subpopulations following exposure to the olfactory cue. This could be accomplished using double labelling for Fos as a nuclear marker of activation, and either tyrosine hydroxylase (for DA), GABA, or Glu as cytoplasmic stains to identify the neurochemical subtype.

The mesocorticolimbic DA pathway originates with cell bodies in the VTA and connects to several limbic structures, including the NAc. An injection of d-amp alone induced an increase in Fos IR in the latter, which replicated the effect observed in previous studies (Graybiel et al., 1990; Rotllant et al., 2010). However, the association of the olfactory cue and d-amp caused an even larger Fos expression in the NAc compared to the No Odor condition. The significant difference between the odor conditions observed in the NAc of the d-amp groups could indicate the relevance of the olfactory cue in this brain region, as odors previously associated with

receptive female or sexually relevant stimuli also induce an increase in Fos expression in this brain region (Robertson et al., 1991; Kippin et al., 2003; West et al., 1992). It could therefore be speculated that, under the influence of d-amp causing an increase in mesolimbic DA transmission, the odor lost its previous inhibitory association. This would also be in agreement with the aforementioned Fos IR pattern in the mPOA and the PirCx. The NAc is a critical region for the display of appetitive behaviors (Berridge and Robinson, 1998). Previous studies suggest that this brain region could play a role in assessing incentive value to conditioned stimuli (Kippin et al., 2003) and in determining the appropriate behavioral outcome to a context (Gruber et al., 2009). Significant increases in DA transmission occur in the NAc during free copulation in sexually experienced male rats (Damsma et al., 1992; Pfaus et al., 1990a). In contrast, male rats that are endogenous noncopulators, or those that show inhibited copulation due to castration, do not show increased DA transmission in this region or in the mPOA (Du et al., 1998; Wenkstern et al., 1993). Consistent with this, infusion of DA antagonists to this region inhibits free copulation in male rats (Pfaus and Phillips, 1991). Therefore, as a potential reflection of DA release by d-amp, the increased activation in the NAc occurring under the influence of d-amp and exposure to the olfactory cue may create a different context that inhibits the context dependency of the learned inhibitory association, leading to disinhibition.

To summarize, d-amp had a disinhibitory effect on sexual behavior in male rats without affecting sexual performance. Further, the neuronal activation in brain regions associated with anticipation of reward and sexual motivation induced by d-amp and exposure to an inhibitory cue reflects the behavioral results and suggests that under the influence of d-amp, the odor is no longer be perceived as inhibitory therefore leading to disinhibition of sexual behavior.

One trial conditioning and state-dependent learning

As suggested above, the disruption of CSI by d-amp may have occurred because the state of inhibition was itself disrupted by increased mesolimbic DA transmission. Might state dependency one-trial learning be at play for all drug-induced sexual disinhibition?

State-dependent learning refers to the phenomenon by which memory and retrieval of information happen in a particular state (i.e., drug-induced, mood, different context, etc.). Drug-induced state-dependent learning has been reported in humans as well as in rodents with

amphetamine, alcohol, and cocaine, among other drugs (reviewed in Radulovic et al., 2017). To relate this to the results of the present experiments with d-amp and our previous findings with alcohol (Germé et al., in preparation), the final choice test was the first time male rats were exposed to a drug (d-amp or alcohol) and to the olfactory cue on a *receptive* female. Thus, in contrast to the "sober" state during conditioning when the odor was on a *non receptive* female, in the drug-induced state, the olfactory cue was on a *receptive* female that males copulated with to ejaculation. As ejaculation has reinforcing properties and is needed to make an association between sexual reward and a cue (Pfaus et al., 2001; Pfaus et al., 2012), it is possible that in this particular drug-induced state, male rats associated the previously inhibitory olfactory cue with sexual reward. This could explain why subsequent exposures to the cue alone under the influence of drugs induce activation of brain regions involved in sexual behavior and reward such as the mesolimbic pathway.

According to Guthrie (1930), conditioning can occur following a single pairing of a stimulus and a response and might not require repeated exposure. This suggests that one exposure with the association of the drug and the olfactory cue on a *receptive* female leading to sexual reward (i.e., during the final choice test) could be sufficient to induce new learning in this new state. Thus, subsequent exposure to the new association (drug + olfactory cue) would activate an expectation of successful copulation and sexual reward. In fact, several studies have tested this with sexual conditioning. For example, Hilliard et al. (1997) provided evidence of one-trial sexual conditioning in Japanese quail. In their experiment, male Japanese quail received one exposure to a context associated with sexual reinforcement (access to a receptive female) for the paired group or to the context with no female (unpaired group). When tested in that context again with a test object instead of a female, males in the paired group spent more time in the test zone and mounted more frequently the object compared to the unpaired group (Hilliard et al., 1997). More recently, Quintana et al. (2018) showed that male rats given one trial of US preexposure (e.g., one trial of copulation with an unscented sexually receptive female to a single ejaculation) inhibits the ability of those males to associate an odor with sexual reward despite the fact that males copulated to ejaculation with scented receptive females for an additional nine trials. Both studies suggest that first experiences with sexual reward are sufficient for sexual conditioning. This raises the possibility that in a reward contrast shift, where a cue previously associated with inhibition (and presumably inhibited mesolimbic dopamine transmission) is now paired with

excitation (sexually receptive female) under the influence of disinhibited dopamine transmission, the cue may well acquire excitatory properties in a drug-associated state. However, as this new learning involves the use of drugs, state-dependent learning should be mentioned.

Despite the differences in testing environments (open field versus exposure chambers for Fos), the discrete and internal cues (almond odor and intoxication) were similar in all contexts. The first association of these cues in the copulatory test led to sexual reward. Then, later association of these cues induced neurochemical changes comparable to those observed in anticipation of sexual reward. Hence, the combination of one-trial conditioning and state-dependent learning would allow us to interpret these findings as anticipation of sexual reward in the drug groups upon subsequent exposure to the odor. Further behavioral tests are needed to confirm the possible combination of these phenomena in the disruptive effects of drugs on CSI and how this could influence the underlying neural activation.

Neurotransmitters in the NAc and drug-induced disruption of CSI

Role of DA in the NAc

Given its central role in conditioned, reward-related and drug-related behaviors (Carlezon and Thomas, 2009; Day and Carelli, 2007; Volkow et al., 2017) and increased neuronal activation following exposure to the olfactory cue and to both alcohol (Germé et al., in preparation) and d-amp (Chapter 2), the NAc was a brain region of particular interest in the present thesis. The use of Fos IR can only reveal neuronal activation without assessing the type of neurons activated and the neurotransmitter release in this brain region. Therefore, in Chapters 3 and 4, microdialysis was used to examine changes in extracellular concentrations of DA, Glu and GABA in the NAc when rats were under the influence of alcohol and exposed to the olfactory cue. Alcohol is known to increase extracellular DA levels in the NAc (Blanchard et al., 1993; Boileau et al., 2004; Pfaus et al., 2010) and this alcohol-induced increase in DA levels has also been observed in our findings. Interestingly, the DA levels stayed elevated when male rats were exposed to the olfactory cue under the influence of the low dose of 0.5g/kg of alcohol. These results agree with previous findings from our laboratory showing increased neural activation in this brain region under the same conditions (0.5g/kg alcohol + olfactory cue) compared to no odor but also compared to the other groups (Germé et al., in preparation).

DA transmission in the NAc is essential for the association between a salient cue and reward and serves to provide animals with reward prediction (Berridge and Robinson, 1998; Schultz, 2013). For this reason, DA is also one of the neurotransmitters essential for sexual behavior and its involvement in male sexual behavior has been studied extensively (for review, Hull and Rodríguez-Manzo, 2017; for a critique, see Paredes & Ågmo, 2004). The general consensus is that DA and DA receptor agonists within a low-to-moderate dose range facilitate sexual behavior. Several previous studies demonstrated increased extracellular DA levels changes in the NAc in anticipation of sexual reward, following exposure to sexually relevant stimuli and reward predictive cues (Damsma et al., 1992; Louilot et al., 1991; Pfaus et al., 1990a). Results from the present thesis established that extracellular DA levels are increased in the NAc under conditions leading to sexual disinhibition (drug + olfactory cue), suggesting a role of this neurotransmitter as disinhibitory for sexual motivation under inhibitory conditions. This argument is further supported by the literature showing that DA plays a role in reversal of natural sexual inhibition (sexual satiety) in male rats particularly in the NAc (Guadarrama-Bazante and Rodríguez-Manzo, 2019; Mas et al., 1995; Pfaus, 1999). This indicates that satiety and possibly other types of sexual inhibition rely on decreased DA transmission in this brain region. In fact, activation of NAc DA receptors facilitates sexual behavior in male rats with less sexual motivation but has no effect on males with normal levels of sexual motivation (Guadarrama-Bazante and Rodríguez-Manzo, 2019). Similar findings from earlier research showed the involvement of DA transmission in the NAc in the disruption of natural inhibition. The Coolidge effect establishes that the inhibition of male sexual behavior induced by several bouts of copulation can be delayed by presenting new females to the exhausted males (McClintock, 1984; Rodríguez-Manzo, 1999; Wilson et al., 1963). Thus, the Coolidge effect may work by activating DA systems, which in turn delays the induction of sexual inhibition. In agreement with this idea, Fiorino and colleagues demonstrated that DA levels were increased in the NAc upon presentation of new females to satiated males during a test of the Coolidge effect (Fiorino et al., 1997). Therefore, DA appears essential to alleviate natural sexual inhibition and to reinstate sexual motivation and behavior. Altogether, these previous data, in addition to the present findings, strongly suggest a crucial role DA transmission in the NAc (and perhaps elsewhere, e.g., mPOA) in drug-induced disruption of sexual inhibition possibly via changes in sexual motivation. To confirm this assumption, studies using the same conditioning paradigm and altering DA

transmission in the NAc during the open field or using operant conditioning and measuring sexual motivation would be necessary.

Role of Glu and GABA in the NAc

It was of interest to examine whether the transmission of either of these amino acids would be altered by drug, inhibitory conditioning, or their combination. It was hypothesized that the increase in DA levels in the NAc was potentially regulated by these amino acids within this region. This hypothesis was based on the known effects of alcohol on Glu and GABA transmission (see Chapter 4 Introduction) but also on previous evidence of the modulation of DA levels by these amino acids within this brain region, with increased DA release following administration of Glu receptors agonists (Imperato et al., 1990; Taber and Fibiger, 1995) and decreased probability of DA release following administration of GABA receptor agonists (Pitman et al., 2014). Furthermore, the NAc receives Glu and GABA inputs from several brain regions modulating DA levels (Pavuluri et al., 2017) and we have previously demonstrated that in some of these regions, in particular the PFC, the BLA and the VTA, exposure to the olfactory cue under the influence of alcohol induced changes in Fos IR (increase in the PFC and VTA, decrease in the BLA) compared to the saline-treated males or to no odor (Germé et al., in preparation). Thus, in the particular conditions of drug + olfactory cue, Glu and/or GABA projections from these brain regions could potentially modulate DA release in the NAc. However, neither alcohol nor the almond odor had any effect on extracellular Glu or GABA levels in the NAc in any of the groups, suggesting that altered transmission of these amino acids was not a driving force on disinhibited DA release under alcohol + almond odor conditions. Therefore, they do not contribute, at least not within the NAc, to the disinhibitory effects of alcohol on sexual behavior. However, their contribution in other brain regions cannot be excluded and will be discussed later.

To make sure that potential changes observed in neurotransmitter levels were specific to the conditioned meaning of the almond odor and not to the fact that it was simply an odor (as opposed to No Odor), male rats were exposed to a novel lemon extract. As with the effect of a novel odor on DA transmission in previous studies (Pfaus et al., 2012), neither Glu nor DA levels in the NAc were affected by exposure to the lemon odor. However, GABA levels decreased during lemon odor exposure in all three groups. The NAc is mainly composed on medium spiny neurons (MSNs) and interneurons which are largely GABAergic. Studies using microdialysis

demonstrated that changes in GABA levels in the NAc derived mainly from neurons releasing GABA by exocytic rather than metabolic mechanisms (Smith and Sharp, 1994), suggesting that GABA is released from NAc MSN collaterals and dendrites and/or interneurons or GABA projections from other brain regions. Given the fact that exposure to the novel lemon odor significantly decreased GABA levels in the NAc, this would indicate a decrease in GABA release rather than increased GABA uptake and metabolism. Based on the present results showing an absence of changes in their levels, it is safe to assume that DA and Glu are not involved in the novelty-induced decrease in GABA release. The origin of this decrease in GABA release is yet to be determined. However, in a study examining NAc neuronal activity in response to relevant and irrelevant cues, it was found that there was a decrease in NAc neuron responsiveness when animals were exposed to non relevant cues (West et al., 1992). Although both almond and lemon odors have been used as neutral stimuli to condition sexual excitation (Kippin et al., 1998; Pfaus et al., 2012), in the present study only the almond had acquired conditional properties. And, in contrast to the almond odor, the microdialysis experiment was the first exposure of male rats to the neutral lemon odor. Therefore, it is likely that the neutral odor was irrelevant to the males and thus induced no effect on DA or Glu, but a decrease in GABA transmission. With the ability of conditioned and unconditioned cues to elicit opposite patterns in NAc neuron activity among other evidence (reviewed in Day and Carelli, 2007), it can be speculated that the lemon odor reduced GABA transmission by decreasing the neuronal activation of GABA interneurons.

To summarize, exposure to the inhibitory cue and to alcohol at 0.5g/kg induced an increase in extracellular levels of DA in the NAc. In contrast, levels of Glu were not affected by the exposure conditions and GABA levels were decreased when male rats were exposed to the novel odor regardless the alcohol treatment confirming the dissociation between the conditioned cue and the novel odor. These findings suggest that: 1) DA release, but not Glu and GABA release, is involved in the disruptive effect of alcohol on sexual behavior; and 2) male rats perceive the difference between the conditioned cue and an unconditioned novel stimulus. This further confirms that the disinhibitory effects of alcohol are not due to an absence of distinction of the inhibitory cue.

From VTA DA neurons activation to NAc DA release

Extracellular levels of DA can be regulated within the NAc by many neurotransmitters and brain regions. However, as the NAc receives DA projections from DA cells in the VTA (Björklund & Lindvall, 1984) and Fos expression induced by both drugs (alcohol and d-amp) + olfactory cue was higher in the VTA, a closer look at the VTA seemed necessary. To supplement the microdialysis findings demonstrating an increase in the extracellular DA levels without changes in Glu and GABA levels in the NAc during exposure to alcohol and the conditioned inhibitory cue, we performed fluorescence immunohistochemistry (FIHC) to determine whether the activation of DA neurons in the VTA was mediating the increase in NAc DA levels. Triple labelling was used to assess DA neurons using TH expression, neuronal activation using Fos expression, and efferent projections to the NAc using the retrograde tracer FG. When male rats were exposed to the almond odor, the proportion of activated VTA DA cells projecting to the NAc was higher in all groups compared to males exposed to lemon odor. Previous research showed that VTA neurons are activated by reward and aversion (Lammel et al., 2014), but also by sexually relevant cues (Balfour et al., 2004). This adds further evidence that the almond odor is recognized as a relevant cue of the rats' environment as opposed to the novel lemon odor. Interestingly, the proportion of activated VTA DA cells was higher when males were injected with 0.5g/kg of alcohol compared to the other groups. Low to medium doses (0.5 -1 g/kg) of alcohol are known to increase the firing rate of VTA DA neurons (Gessa et al., 1985) and to increase DA release in the NAc (Blanchard et al., 1993; Boileau et al., 2004; Pfaus, 2009). In accordance with the aforementioned microdialysis results from the present thesis showing higher extracellular DA levels in the NAc at the 0.5 g/kg dose of alcohol, it is likely that the increased DA transmission was due to increased activation of DA neurons in the VTA.

It is worth mentioning that the results from the immunofluorescence also revealed two types of double labeled cells in the VTA. One type was FG + Fos-IR cells, indicating that the VTA sends projections other than DA to the NAc that are also activated. However, because the VTA is only composed of DA, Glu and GABA neurons (Nairs-Roberts et al., 2008) and that the levels of these amino acids were unchanged in the NAc in the present experiments, it is safe to assume that the activation of these non-DA projections had little to no effect on DA release in the NAc in the present studies. The second type is Fos + TH cells representing activated DA neurons projecting from the VTA to brain regions other than the NAc. These DA neurons are of interest because the VTA sends DA projections to several brain regions and receives projections back

from these regions (Morales and Margiolis, 2017) and these connections were likely involved in the alcohol-induced disinhibition.

When examining the results of Fos expression from Chapter 2 with d-amp and from our previous study with alcohol (Germé et al., in preparation), two brain regions showed consistently increased Fos-IR following exposure to the conditioned cue and the drug: the NAc and the VTA. These results suggest that the mesolimbic DA pathway plays a central role in the disinhibitory effects of both drugs on sexual behavior. An important next question is what activates this pathway to induce disinhibition? The present experiments ruled out Glu and GABA in the NAc, leaving then inputs to DA cell bodies in the VTA as the likely source.

Possible involvement of projections to VTA

Located in the midbrain, the VTA sends and receives projections from many brain regions (for review Morales and Margiolis, 2017) including the bed nucleus of the stria terminalis, the anterior cortex, the lateral hypothalamus but also the NAc, the mPFC and the amygdala among others. Their pattern of Fos activation under the influence of alcohol and following exposure to the conditioned cue (Germé et al., in preparation), their known efferences to the VTA for some of them (Geisler and Wise, 2008; Morales and Margiolis, 2017) and their influence on DA release in the NAc, make the PFC, the NAc, and the BLA appropriate starting points in our examination of the brain regions and neural mechanisms responsible for VTA DA neuronal activation under the same conditions (drug + almond odor).

PFC. Previous studies have shown that the PFC is involved in the expression of male sexual behavior (Ågmo et al., 1995; Balfour et al., 2006; Hernandez-Gonzalez et al., 1997), in appropriate conditioned sexual aversion (Davis et al., 2010). Furthermore, it has been demonstrated that sex-activated neurons within the VTA receive efferents from the PFC (Balfour et al., 2006). The PFC has a well-known role in learned behavioral inhibition as part of its control of executive function (for review, Aron, 2007). This region is also part of the mesocorticolimbic DA system and one of the most important Glu inputs to the VTA (Sesack and Pickel, 1992). Its direct projections to the NAc and to the VTA make the PFC a critical area in the modulation of DA release in the NAc (Carr and Sesack, 2000a). Stimulation of the PFC has been shown to increase the firing rate of DA neurons (Gariano and Groves, 1988) and more recent studies have

proposed that the Glu pathway from the PFC has a facilitatory effect on DA release in the NAc by acting on DA cells in the VTA (Carr and Sesack, 2000a). Previous work from our laboratory has revealed higher Fos IR in the prelimbic cortex (PL: a sub region of the PFC) when male rats are exposed to the olfactory cue compared to No Odor and an even higher Fos IR when rats are under the influence of EtOH compared to saline (Germé et al., in preparation). Further, PL has been shown to be involved in odor-reward associative learning (Tronel and Sara, 2002). Results from this thesis have revealed an increase in DA levels in the NAc following exposure to EtOH + odor. Taken together, this strongly suggests the mediating role of the PFC in the increase in NAc DA levels following exposure to the conditioned olfactory cue and EtOH and so in the disinhibitory effects of EtOH on sexual behavior.

NAc. As seen in several studies, including those of the present thesis, the VTA sends DA projections to the NAc. The VTA also sends GABAergic projections to the NAc (Carr and Sesack, 2000b) and receives reciprocal GABAergic projections from the MSNs in the NAc (Kalivas et al., 1993). These MSNs do not target directly DA neurons in the VTA (Xia et al., 2011), suggesting an indirect action of the NAc on DA neurons either by acting on VTA Glu or VTA GABA cells. The mechanisms involved in the action of acute alcohol on NAc MSNs have yet to be determined conclusively (for review Marty and Spigelmann, 2011). Thus, a speculation on the exact mechanisms of this feedback loop between the NAc and the VTA particularly in a context of alcohol-induced disinhibition of sexual behavior would not seem appropriate. However, a role for the feedback loop cannot be ignored given the fact that the NAc can regulate motivated behaviors through its MSNs (Wheeler and Carelli, 2009) and given the central role of the connections between the VTA and NAc in the phenomenon of disinhibition observed in this thesis. Further examination of the NAc projection neurons to the VTA (e.g., neuronal activation, GABA release pattern, or inactivation of these neurons) particularly during exposure to the olfactory cue would shed light on their possible involvement in the disinhibitory effects of alcohol and d-amp on sexual inhibition.

BLA. To date, evidence for direct projections from the BLA to the VTA is still lacking. However, modulation of DA release in the NAc through indirect connections between these two brain areas has been suggested (reviewed in Phillips et al., 2003). While findings showing unchanged DA release in the NAc following BLA activation (Jackson and Moghaddam, 2001) or

inactivation (Jones et al., 2010) exist, most studies agree on an interaction between the BLA and the NAc leading to the modulation of DA release in the NAc. The BLA sends direct Glu projections to the NAc (Brog et al., 1993; Kelley et al., 1982) and the increase in DA transmission following BLA stimulation appears dependent of Glu receptors in the NAc (Howland et al., 2002). However, because no changes in Glu levels were observed in the present thesis, potential modulation of DA levels by the BLA in our conditions of interest could come from its connections to PFC neurons (McDonald, 1991) that send outputs to the VTA. Despite sending Glu projections to the PFC, under basal conditions, stimulation of BLA inputs to the PFC inhibits PFC neurons firing (Floresco and Tse, 2007). Our previous results showed a decrease in neuronal activation in the BLA (Germé et al., in preparation) following exposure to alcohol and the almond odor. It is possible that in this context of alcohol + conditioned cue, this decrease in neuronal activation in the BLA could remove the inhibition onto PFC neurons, inducing a disinhibition of their activity. This was in fact observed in our previous experiment with an increase in Fos IR in the PL (sub region of the PFC) under the influence of alcohol and exposure to the odor (Germé et al., in preparation). As some PFC neurons project to the VTA and to DA neurons in particular (Carr and Sesack, 2000a), a disinhibition of these projections could lead to an activation of VTA DA neurons and ultimately to the increase of DA levels observed in the NAc in the present thesis. A study allowing specific inactivation of BLA → PFC neurons would confirm this hypothesis and would further elucidate the potential regulatory properties of these projections as well as the PFC →VTA → NAc projections mentioned above on the disruption of sexual inhibition in male rats.

Neurotransmission within the VTA leading to DA release in NAc

In addition to DA cell bodies, the VTA contains both Glu and GABA neurons (Nairs-Roberts et al., 2008) and it has been demonstrated that non-DA neurons within the VTA synapse locally onto DA neurons confirming local influence on DA neuron activity (Omelchenko and Sesack, 2009). Accordingly, it appears important to mention the potential influence of these amino acids on VTA DA neuronal activity particularly under the influence of alcohol and ultimately their potential to mediate the disinhibitory effects of drugs on sexual behavior.

Despite the absence of changes in Glu and GABA levels in the NAc when male rats are exposed to the conditioned olfactory cue under the influence of alcohol, VTA DA transmission as

well as DA levels can be regulated by Glu and GABA within the VTA (Spanagel and Weiss, 1999; Spanagel, 2009). As stimulation of $GABA_A$, $GABA_B$, and ionotropic Glu receptors within the VTA has been shown to increase DA levels in the NAc (Westerink et al., 1996), potential changes in Glu and GABA neurotransmission in the VTA mediating DA release in the NAc under disinhibitory conditions (drug + olfactory cue) should be considered.

The regulation of VTA cell activity including DA neurons projecting to the NAc is under the influence of Glu projections to the VTA (Geisler and Wise, 2008) and glutamatergic actions within the VTA are important for drug-related effects on DA neurons (Kauer, 2004). As observed in the NAc, alcohol has a biphasic effect on Glu levels in the VTA with acute exposure to low doses (e.g., 0.5g/kg) increasing extracellular levels of Glu in the VTA and higher doses (1g/kg and above) having the opposite effects (Ding et al, 2012). While the effects of alcohol on Glu levels appear associated to an increase of Glu release onto DA neurons in the VTA (Xiao et al., 2008), the origin of this Glu release has not been determined. It is known that alcohol inhibits glutamatergic NMDA receptor transmission and that NMDA receptors in the VTA are mainly on DA neurons (for review, see Morikawa and Morrisett, 2010). However, the precise effect of acute alcohol on NMDA receptors on DA neurons and how this effect could lead to DA release in the NAc remain unclear. Nonetheless, the influence of Glu in the VTA on DA release into the NAc observed in the present thesis under the influence of alcohol and during exposure to the conditioned cue cannot be excluded.

As previously mentioned, blockade of GABAergic transmission in the VTA by alcohol enhances DA transmission by increasing VTA DA neuronal activity (Xiao et al., 2007). $GABA_A$ receptors are located on DA neurons in the VTA and activation of these receptors can inhibit DA release in the NAc (Xi and Stein, 1998). These receptors are also detected on GABAergic interneurons in the VTA and systemic or intra VTA injections of $GABA_A$ agonist muscimol induce a significant increase in DA release in the NAc (Xi and Stein, 1998). Conversely, an inhibition of VTA DA neurons firing rate following injections of muscimol has also been reported (Theile et al., 2008; 2011). Alcohol stimulates VTA DA firing rate but at the same time enhances GABAergic transmission in this region. The existence of a balance between the direct inhibition via the activation of $GABA_A$ receptors on DA neurons and the indirect disinhibition through activation of these receptors on GABA interneurons to regulate DA release has been

suggested (Theile et al., 2011; Xi and Stein, 1998). $GABA_B$ receptors in the VTA can also influence DA release and activation of these receptors in the VTA inhibits the ability of opioids to evoke DA release in the NAc (Kalivas et al., 1990). Furthermore, infusions of $GABA_B$ receptor agonist into the VTA can inhibit DA neurons leading to a decrease in the display of EtOH-induced conditioned place preference (CPP) (Bechtholt and Cunningham, 2005), suggesting a role of GABA transmission in the VTA in the rewarding properties of alcohol. It has been proposed that DA neurons are involved in the memory of alcohol-associated environmental stimuli. Together these studies suggest the involvement of VTA GABA transmission in the modulation of DA release in the NAc by alcohol but also with alcohol-related conditional cues. The complex effects of alcohol on VTA DA neurons and on GABA transmission within the VTA to induce DA release, and how these phenomena could lead to the disruption of CSI, remain to be solved. However, GABA transmission within the VTA disrupts sexual inhibition and plays a role in the expression of male rats' sexual behavior as infusions of bicuculline, an antagonist of $GABA_A$ receptors induced sexual behavior in sexually exhausted males (Rodríguez-Manzo and Canseco-Alba, 2017). Therefore, the involvement of GABA in the VTA in the disinhibitory effects of alcohol and other drugs on sexual behavior should be considered. Further studies are necessary to examine GABA transmission in the VTA under the experimental conditions used in the present experiments, and the potential origin of GABA input to the VTA that might be involved.

It should be mentioned that previous research has established an interaction between GABA and opioids transmission in the VTA (Bechtholt and Cunningham, 2005). Because of the involvement of opioids transmission in sexual behaviour particularly in sexual motivation (Van Furth et al., 1995) and sexually conditioned olfactory stimulation (Ismail et al., 2009; Quintana et al., 2019), this neurotransmission system could be of interest. Several studies have linked opioids receptors manipulation within the VTA and drugs' effects on behavior or on DA transmission. VTA administration of μ opioids receptor (MOR) agonists stimulates VTA DA neuron firing and NAc DA release (Di Chiara and Imperato, 1988). A later study showed that the activation of MORs in the VTA could actually induce a disinhibition of DA neurons in the VTA possibly leading to DA release in the NAc (Balfour et al., 2004). On the other hand, naloxone, a nonselective opioids receptor antagonist, can attenuate alcohol-induced excitation of VTA DA neurons (Xiao et al., 2007), indicating the possible involvement of opioids and opioids receptors

activity in the effects of drugs on VTA DA transmission. It has been suggested that the activation of the mesolimbic pathway by sex and sex-related cues results mainly from MOR activation (as opposed to other opioids receptors). Using a paradigm to induce conditioned ejaculatory preference (CEP) for an olfactory cue, a recent study from our laboratory demonstrated that infusions of naloxone in the VTA before each trial of the conditioning phase can abolish CEP in male rats during a final preference test, suggesting that antagonism of MORs in the VTA removes incentive properties of the olfactory cue (Quintana et al., 2019). Earlier studies showed that blockade of opioids receptors within the VTA decreases sexual motivation measured by level changes (Van Furth et al., 1996). The aforementioned studies establish that not only MORs play a role in the regulation of DA release, in alcohol-induced DA neurons activation in the VTA, but also in incentive properties of sex-related cues, sexual reward, and sexual motivation. This suggests that the results observed in the present thesis linking the disinhibitory effects of alcohol to VTA DA transmission could involve changes in opioids transmission in the VTA and a closer examination of this system should be of interest.

How does this relate to human sexual disinhibition?

As mentioned in the Introduction (Chapter 1, p. 8), many factors can influence the relation between drugs and their effects on human sexual behavior and make it particularly complex and difficult to study. However, by using an animal model and removing some of the confounding factors, and by focusing solely on the behavioral effects of low to medium doses of drugs, results from this thesis have provided evidence that two drugs of virtually opposing effects on the brain (alcohol as a depressant vs. d-amp as a stimulant) have the same behavioral effects on sexual response and induce sexual disinhibition. It is also revealed that these effects are potentially due to increased sexual motivation. These findings side with studies in the human literature describing drug use in sexual contexts increase sexual desire (Volkow et al., 2007), and suggest that drug-induced increases in sexual motivation and sexual arousal can lead to sexual disinhibition. This is also concordant with reports of disinhibitory effects of sexual arousal (without the use of drugs) on decision making, leading to sexual disinhibition in men and women (Imhoff and Schmidt, 2014). Thus, as in rats, increased sexual desire and motivation in humans appear to lead to disinhibition of sexual behavior and this might be how drugs that augment the neurochemical substrates of sexual desire and arousal are inducing those effects.

As human sexual behavior requires the involvement of several brain regions and neurotransmitters systems, identifying the neural mechanisms involved in sexual disinhibition are quite challenging. Sexual disinhibition in humans can manifest itself as hypersexuality and/or engaging in inappropriate or risky sexual behaviors. Previous reports have mentioned the possible involvement of the PFC, the temporal lobes, and the amygdala, among others in sexual disinhibition. Indeed, lesions to, or removal of, these regions can lead to hypersexuality (reviewed in Baird et al., 2007). But to our knowledge, no studies have examined brain regions involved in sexual disinhibition under the influence of drugs. It is well known that virtually all drugs of abuse act on the human mesolimbic DA pathway (Koob, 2000; Volkow et al., 2004a; 2004b) and that this circuit is also one of the many systems involved in the regulation of human sexual behavior (Calabrò et al., 2019; Pfaus, 2009; Pfaus et al., 2012). However, how drug effects on this system can potentially lead to sexual disinhibition has not been established. Findings from this thesis provide strong evidence of a potentially crucial role of this pathway in drug-induced sexual disinhibition.

Disinhibition of human sexual behavior induced by drugs has been of particular interest in the past years due to the increase in sexually transmitted infections (STIs) and HIV transmission following drug consumption, as people are less likely to use a condom under the influence of drugs (Calsyn et al., 2010; Leigh and Stall, 1993) and to engage in drug-facilitated sexual assault (Lebeau, 2009; 2013). However, given the fact that sexual disinhibition is not only observed under the influence of drugs but also as inappropriate sexual behaviors and/or hypersexuality in undrugged individuals, these findings could be of use in clinical contexts. Identifying and understanding the neural mechanisms of sexual disinhibition induced by drugs appears particularly important as it might open new perspectives in the study of inappropriate sexual behaviors and the brain regions involved. In fact, inappropriate sexual behaviors and hypersexuality are comorbid with several neurological diseases, including dementia and temporal lobe epilepsy (Béreau, 2018; Chapman and Spitznagel, 2019; Cipriani et al., 2016). While several drugs have been tested in order treat these behaviors, defining pathway(s) for sexual disinhibition would help in identifying appropriate neurochemical targets to treat such behaviors in clinical populations.

Conclusions

The experiments in this thesis are a first attempt to elucidate the neural mechanisms underlying the disruption of sexual inhibition by two drugs, d-amp and alcohol. Identifying the neural processes and brain regions involved in drug-induced sexual disinhibition is of particular interest as it could give a new perspective in the studies on sexual behavior (excitation, inhibition and disinhibition) in a context of drug use but also potentially in a clinical context with patients exhibiting inappropriate sexual behaviors or hypersexuality.

Because of its known involvement in appetitive and drug-related processes, the mesolimbic DA system has provided an attractive pathway to study how drugs of abuse affect the brain to induce changes in sexual behavior. Although this pathway has been extensively studied, its role in the disinhibitory effects of drugs on sexual behavior and the exact mechanisms underlying these effects has not been confirmed. However, the findings of this thesis provide strong evidence of the significant involvement of mesolimbic DA pathway in the disinhibitory effects of drugs on sexual behavior. Future studies are needed with particular attention to DA levels and brain activation during the copulatory test as opposed to exposure to the cue. Therefore, the use of techniques such as DREADDS to activate or inactivate the mesolimbic DA projections from the VTA to the NAc as well as measuring sexual motivation during the copulatory test would allow a confirmation of the present findings.

Nonetheless, the known action of d-amp (possibly via its action on DA) on sexual motivation to reverse natural sexual inhibition, the fact that both EtOH and d-amp increase DA levels in the NAc, the known role of DA transmission on sexual motivation and the increase in DA levels via activation of VTA neurons by exposure to the drugs and the conditioned cue strongly suggest the crucial role of DA transmission in the disinhibitory effects of drugs on sexual behavior in male rats through a change in their sexual motivation.

References

Abel, E. L. (1980). A review of alcohol's effects on sex and reproduction. Drug Alcohol Depend, 5(5), 321-332. doi: 0376-8716(80)90158-1

Abrahao, K. P., Salinas, A. G., & Lovinger, D. M. (2017). Alcohol and the Brain: Neuronal Molecular Targets, Synapses, and Circuits. Neuron, 96(6), 1223-1238. doi: 10.1016/j.neuron.2017.10.032

Adinoff, B. (2004). Neurobiologic processes in drug reward and addiction. Harv Rev Psychiatry, 12(6), 305-320. doi: 10.1080/10673220490910844

Ågmo, A., & Fernandez, H. (1989). Dopamine and sexual behavior in the male rat: a reevaluation. J Neural Transm, 77(1), 21-37. doi: 10.1007/BF01255816

Ågmo, A., & Paredes, R. (1985). GABAergic drugs and sexual behaviour in the male rat. Eur J Pharmacol, 112(3), 371-378. doi: 10.1016/0014-2999(85)90783-6

Ågmo, A., & Villalpando, A. (1995). Central nervous stimulants facilitate sexual behavior in male rats with medial prefrontal cortex lesions. Brain Res, 696(1-2), 187-193. doi: 10.1016/0006-8993(95)00853-i

Ågmo, A., Villalpando, A., Picker, Z., & Fernandez, H. (1995). Lesions of the medial prefrontal cortex and sexual behavior in the male rat. Brain Res, 696(1-2), 177-186. doi: 10.1016/0006-8993(95)00852-h

Arias-Carrión, O., Stamelou, M., Murillo-Rodríguez, E. & Pöppel, E.Dopaminergic reward system: a short integrative review. Int Arch Med 3, 24 (2010). https://doi.org/10.1186/1755-7682-3-24

Aron, A. R. (2007). The neural basis of inhibition in cognitive control. Neuroscientist, 13(3), 214-228. doi: 10.1177/1073858407299288

Baird, A. D., Wilson, S. J., Bladin, P. F., Saling, M. M., & Reutens, D. C. (2007). Neurological control of human sexual behaviour: insights from lesion studies. Journal of neurology, neurosurgery, and psychiatry, 78(10), 1042–1049. https://doi.org/10.1136/jnnp.2006.107193

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(4), 718-730. doi: 10.1038/sj.npp.1300350

Balfour, M. E., Brown, J. L., Yu, L., & Coolen, L. M. (2006). Potential contributions of efferents from medial prefrontal cortex to neural activation following sexual behavior in the male rat. Neuroscience, 137(4), 1259-1276. doi: 10.1016/j.neuroscience.2005.11.013

Ball, G. F., & Balthazart, J. (2008). How useful is the appetitive and consummatory distinction for our understanding of the neuroendocrine control of sexual behavior? Horm Behav, 53(2), 307-31. doi: 10.1016/j.yhbeh.2007.09.023

Bancroft, J. (1999). Central inhibition of sexual response in the male: a theoretical perspective. Neurosci Biobehav Rev, 23(6), 763-784. doi: 10.1016/s0149-7634(99)00019-6

Bancroft, J., & Janssen, E. (2000). The dual control model of male sexual response: a theoretical approach to centrally mediated erectile dysfunction. Neuroscience and biobehavioral reviews, 24(5), 571–579. https://doi.org/10.1016/s0149-7634(00)00024-5

Bariselli, S., Glangetas, C., Tzanoulinou, S., & Bellone, C. (2016). Ventral tegmental area subcircuits process rewarding and aversive experiences. J Neurochem, 139(6), 1071-1080. doi: 10.1111/jnc.13779

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(3), 627-646. doi: 10.1016/0306-4522(92)90452-8

Beach, F. A. (1956). Characteristics of masculine "sex drive. Nebr Symp Motiv(4), 1-32.

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(2), 105-110. doi: 10.1037/h0046471

Bechtholt, A. J., & Cunningham, C. L. (2005). Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. Behav Neurosci, 119(1), 213-223. doi: 10.1037/0735-7044.119.1.213

Bekkers, J. M., & Suzuki, N. (2013). Neurons and circuits for odor processing in the piriform cortex. Trends Neurosci, 36(7), 429-438. doi: 10.1016/j.tins.2013.04.005

Bell, D. S., & Trethowan, W. H. (1961). Amphetamine addiction. The Journal of nervous and mental disease, 133, 489–496. https://doi.org/10.1097/00005053-196112000-00003

Béreau M. (2018). Hypersexuality in Neurological Disorders: From Disinhibition to Impulsivity. Frontiers of neurology and neuroscience, 41, 71–76. https://doi.org/10.1159/000475693

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(3), 309-369. doi: 10.1016/s0165-0173(98)00019-8

Beyer, C., Contreras, J. L., Morali, G., & Larsson, K. (1981). Effects of castration and sex steroid treatment on the motor copulatory pattern of the rat. Physiol Behav, 27(4), 727-730. doi: 10.1016/0031-9384(81)90247-x

Bignami, G. (1966). Pharmacologic influences on mating behavior in the male rat. Effects of d-amphetamine, LSD-25, strychnine, nicotine and various anticholinergic agents. Psychopharmacologia, 10(1), 44-58. doi: 10.1007/BF00401898

Bitran, D., & Hull, E. M. (1987). Pharmacological analysis of male rat sexual behavior. Neurosci Biobehav Rev, 11(4), 365-389. doi: 10.1016/s0149-7634(87)80008-8

Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. Trends Neurosci, 30(5), 194-202. doi: 10.1016/j.tins.2007.03.006

Björklund, A., & Lindvall, O. (1984). Dopamine-containing systems in the CNS. Classical transmitters in the CNS. Part I Handbook of chemical neuroanatomy: Elsevier, Amsterdam.

Björklund, D. F., & Harnishfeger, K. K. (1995). The evolution of inhibition mechanisms and their role in human cognition and behavior Interference and inhibition in cognition (pp. 141-173): Elsevier.

Blackburn, J. R., Pfaus, J. G., & Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. Prog Neurobiol, 39(3), 247-279. doi: 10.1016/0301-0082(92)90018-a

Blanchard, B. A., Steindorf, S., Wang, S., & Glick, S. D. (1993). Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. Alcohol Clin Exp Res, 17(5), 968-973. doi: 10.1111/j.1530-0277.1993.tb05650.x

Boileau, I., Assaad, J. M., Pihl, R. O., Benkelfat, C., Leyton, M., Diksic, M., . . . Dagher, A. (2003). Alcohol promotes dopamine release in the human nucleus accumbens. Synapse, 49(4), 226-231. doi: 10.1002/syn.10226

Bond, A.J. (1998) Drug-Induced Behavioural Disinhibition. Mol Diag Ther, 9,41-57. doi: 10.2165/00023210-19980910-00005

Bosma-Bleeker, M. H., & Blaauw, E. (2018). Substance use disorders and sexual behavior; the effects of alcohol and drugs on patients' sexual thoughts, feelings and behavior. Addictive behaviors, 87, 231–237. https://doi.org/10.1016/j.addbeh.2018.07.005

Braitenberg, V. (1986). Vehicles: Experiments in Synthetic Psychology. United Kingdom: MIT Press.

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol, 338(2), 255-278. doi: 10.1002/cne.903380209

Brown, R. E. (1974). Sexual arousal, the Coolidge effect and dominance in the rat (Rattus norvegicus). Animal Behaviour, 22(3), 634–637. https://doi.org/10.1016/S0003-3472(74)80009-6

Brown, M. T., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Luscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. Nature, 492(7429), 452-456. doi: 10.1038/nature11657

Buffum, J. (1982). Pharmacosexology: the effects of drugs on sexual function a review. J Psychoactive Drugs, 14(1-2), 5-44. doi: 10.1080/02791072.1982.10471907

Buffum, J. (1988). Substance abuse and high-risk sexual behavior: drugs and sex--the dark side. J Psychoactive Drugs, 20(2), 165-168. doi: 10.1080/02791072.1988.10524489

Buffum, J., Moser, C. and Smith, D. (1988) “Street drugs and sexual function.” In Handbook of Sexology, Volume 6: The Pharmacology and Endocrinology of Sexual Function. Edited by: Sisten, J. M.A. New York: Elsevier Science Publishers.

Butcher, L. L., Butcher, S. G., & Larsson, K. (1969). Effects of apomorphine, (+)-amphetamine, and nialamide on tetrabenazine-induced suppression of sexual behavior in the male rat. Eur J Pharmacol, 7(3), 283-288. doi: 10.1016/0014-2999(69)90093-4

Byrnes, J. J., & Wallace, L. J. (1997). Amphetamine-induced sensitization and release of dopamine in slices from the ventral tegmental area of rats is enhanced following administration of cholera toxin into the ventral tegmental area. Neurosci Lett, 223(1), 45-48. doi: 10.1016/s0304-3940(97)13399-7

Cabilio, S. (1996). Sexual behavior scoring program. Montreal: Concordia University.

Cachope, R., & Cheer, J. F. (2014). Local control of striatal dopamine release. Front Behav Neurosci, 8, 188. doi: 10.3389/fnbeh.2014.00188

Calabrò, R. S., Cacciola, A., Bruschetta, D., Milardi, D., Quattrini, F., Sciarrone, F., la Rosa, G., Bramanti, P., & Anastasi, G. (2019). Neuroanatomy and function of human sexual behavior: A neglected or unknown issue? Brain and behavior, 9(12), e01389. https://doi.org/10.1002/brb3.1389

Calsyn, D. A., Cousins, S. J., Hatch-Maillette, M. A., Forcehimes, A., Mandler, R., Doyle, S. R., & Woody, G. (2010). Sex under the influence of drugs or alcohol: common for men in substance abuse treatment and associated with high-risk sexual behavior. The American journal on addictions, 19(2), 119–127. https://doi.org/10.1111/j.1521-0391.2009.00022.x

Carboni, E., Imperato, A., Perezzani, L., & Di Chiara, G. (1989). Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience, 28(3), 653-661. doi: 10.1016/0306-4522(89)90012-2

Carboni, S., Isola, R., Gessa, G. L., & Rossetti, Z. L. (1993). Ethanol prevents the glutamate release induced by N-methyl-D-aspartate in the rat striatum. Neurosci Lett, 152(1-2), 133-136. doi: 10.1016/0304-3940(93)90501-b

Carlezon, W. A., Jr., & Thomas, M. J. (2009). Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. Neuropharmacology, 56 Suppl 1, 122-132. doi: 10.1016/j.neuropharm.2008.06.075

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

Carr, D. B., & Sesack, S. R. (2000 b). GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse, 38(2), 114-123. doi: 10.1002/1098-2396(200011)

Chan, W. L., Wood, D. M., & Dargan, P. I. (2015). Significant misuse of sildenafil in London nightclubs. Substance use & misuse, 50(11), 1390-1394.

Chance, M. R. A. (1962). Social behaviour and primate evolution. In M. F. A. Montagu (Ed.), Culture and the evolution of man. (M. F. A. Montagu ed., pp. 84-130). New York: Oxford University Press.

Chang, S. L., Patel, N. A., & Romero, A. A. (1995). Activation and desensitization of Fos immunoreactivity in the rat brain following ethanol administration. Brain Res, 679(1), 89-98. doi: 10.1016/0006-8993(95)00210-h

Chapman, K.R., & Spitznagel, M. (2019). Measurement of sexual disinhibition in dementia: A systematic review. International Journal of Geriatric Psychiatry, 34, 1747 - 1757.

Chéramy, A., L'Hirondel, M., Godeheu, G., Artaud, F., & Glowinski, J. (1998). Direct and indirect presynaptic control of dopamine release by excitatory amino acids. Amino Acids, 14(1-3), 63-68. doi: 10.1007/BF01345244

Chou, N. H., Huang, Y. J., & Jiann, B. P. (2015). The impact of illicit use of amphetamine on male sexual functions. The journal of sexual medicine, 12(8), 1694-1702.

Christie, M. J., James, L. B., & Beart, P. M. (1985). An excitant amino acid projection from the medial prefrontal cortex to the anterior part of nucleus accumbens in the rat. J Neurochem, 45(2), 477-482. doi: 10.1111/j.1471-4159.1985.tb04013.x

Cipriani, G., Ulivi, M., Danti, S., Lucetti, C., & Nuti, A. (2016). Sexual disinhibition and dementia. Psychogeriatrics: The Official Journal of the Japanese Psychogeriatric Society, 16(2), 145–153. https://doi.org/10.1111/psyg.12143

Clapton, E. (2007). *Clapton: The Autobiography*. New York: Broadway Books.

Colussi-Mas, J., Geisler, S., Zimmer, L., Zahm, D. S., & Berod, A. (2007). Activation of afferents to the ventral tegmental area in response to acute amphetamine: a double-labelling study. Eur J Neurosci, 26(4), 1011-1025. doi: 10.1111/j.1460-9568.2007.05738.x

Coolen, L. M., Fitzgerald, M. E., Yu, L., & Lehman, M. N. (2004). Activation of mu opioid receptors in the medial preoptic area following copulation in male rats. Neuroscience, 124(1), 11-21. doi: 10.1016/j.neuroscience.2003.10.045

Crone C. (1993). Reciprocal inhibition in man. Danish medical bulletin, 40(5), 571–581.

Crowe, L. C., & George, W. H. (1989). Alcohol and human sexuality: review and integration. Psychol Bull, 105(3), 374-386. doi: 10.1037/0033-2909.105.3.374

Cui, C., & Koob, G. F. (2017). Titrating Tipsy Targets: The Neurobiology of Low-Dose Alcohol. Trends Pharmacol Sci, 38(6), 556-568. doi: 10.1016/j.tips.2017.03.002

Dahchour, A., & De Witte, P. (2000). Ethanol and amino acids in the central nervous system: assessment of the pharmacological actions of acamprosate. Prog Neurobiol, 60(4), 343-362. doi: 10.1016/s0301-0082(99)00031-3

Dahchour, A., Quertemont, E., & De Witte, P. (1994). Acute ethanol increases taurine but neither glutamate nor GABA in the nucleus accumbens of male rats: a microdialysis study. Alcohol Alcohol, 29(5), 485-487.

Damsma, G., Pfaus, J. G., Wenkstern, D., Phillips, A. G., & Fibiger, H. C. (1992). Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats:

comparison with novelty and locomotion. Behav Neurosci, 106(1), 181-191. doi: 10.1037//0735-7044.106.1.181

Davidoff, R. A. (1973). Alcohol and presynaptic inhibition in an isolated spinal cord preparation. Arch Neurol, 28(1), 60-63. doi:10.1001/archneur.1973.00490190078011

Davis, J. F., Loos, M., Di Sebastiano, A. R., Brown, J. L., Lehman, M. N., & Coolen, L. M. (2010). Lesions of the medial prefrontal cortex cause maladaptive sexual behavior in male rats. Biol Psychiatry, 67(12), 1199-1204. doi: 10.1016/j.biopsych.2009.12.029

Day, J. J., & Carelli, R. M. (2007). The nucleus accumbens and Pavlovian reward learning. Neuroscientist, 13(2), 148-159. doi: 10.1177/1073858406295854

Di Chiara G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behavioural brain research, 137(1-2), 75–114. https://doi.org/10.1016/s0166-4328(02)00286-3

Di Chiara, 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(14), 5274-5278. doi: 10.1073/pnas.85.14.5274

Di Chiara, G., Tanda, G., Frau, R., & Carboni, E. (1993). On the preferential release of dopamine in the nucleus accumbens by amphetamine: further evidence obtained by vertically implanted concentric dialysis probes. Psychopharmacology, 112(2-3), 398-402.

Ding, Z. M., Engleman, E. A., Rodd, Z. A., & McBride, W. J. (2012). Ethanol increases glutamate neurotransmission in the posterior ventral tegmental area of female wistar rats. Alcohol Clin Exp Res, 36(4), 633-640. doi: 10.1111/j.1530-0277.2011.01665.x

Dominguez, J. M., Gil, M., & Hull, E. M. (2006). Preoptic glutamate facilitates male sexual behavior. J Neurosci, 26(6), 1699-1703. doi: 10.1523/JNEUROSCI.4176-05.2006

Doty, R. L., & Ferguson-Segall, M. (1987). Odor detection performance of rats following d-amphetamine treatment: a signal detection analysis. Psychopharmacology (Berl), 93(1), 87-93. doi: 10.1007/BF02439592

Du, J., Lorrain, D. S., & Hull, E. M. (1998). Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats. Brain research, 782(1-2), 11–17. https://doi.org/10.1016/s0006-8993(97)01144-x

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(2), 217-232. doi: 10.1016/s0149-7634(05)80222-2

Fernandez-Guasti, A., Larsson, K., & Beyer, C. (1985). Comparison of the effects of different isomers of bicuculline infused in the preoptic area on male rat sexual behavior. Experientia, 41(11), 1414-1416. doi: 10.1007/BF01950011

Fernandez-Guasti, A., & Rodriguez-Manzo, G. (2003). Pharmacological and physiological aspects of sexual exhaustion in male rats. Scand J Psychol, 44(3), 257-263. doi: 10.1111/1467-9450.00343

Ferraro, F. M., 3rd, & Kiefer, S. W. (2004). Behavioral analysis of male rat sexual motivation and performance following acute ethanol treatment. Pharmacol Biochem Behav, 78(3), 427-433. doi: 10.1016/j.pbb.2004.03.020

Fillmore, M. T., & Rush, C. R. (2002). Impaired inhibitory control of behavior in chronic cocaine users. Drug and alcohol dependence, 66(3), 265–273. https://doi.org/10.1016/s0376-8716(01)00206-x

Fillmore, M. T., Rush, C. R., & Marczinski, C. A. (2003). Effects of d-amphetamine on behavioral control in stimulant abusers: the role of prepotent response tendencies. Drug and alcohol dependence, 71(2), 143–152. https://doi.org/10.1016/s0376-8716(03)00089-9

Fiorino, D. F., Coury, A., & Phillips, A. G. (1997). Dynamic changes in nucleus accumbens dopamine efflux during the Coolidge effect in male rats. J Neurosci, 17(12), 4849-4855.

Fisher, D. G., Reynolds, G. L., Ware, M. R., & Napper, L. E. (2011). Methamphetamine and Viagra use: relationship to sexual risk behaviors. Arch Sex Behav, 40(2), 273-279. doi: 10.1007/s10508-009-9495-5

Floresco, S. B., & Tse, M. T. (2007). Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. J Neurosci, 27(8), 2045-2057. doi: 10.1523/JNEUROSCI.5474-06.2007

Frohmader, K. S., Pitchers, K. K., Balfour, M. E., & Coolen, L. M. (2010 a). Mixing pleasures: review of the effects of drugs on sex behavior in humans and animal models. Horm Behav, 58(1), 149-162. doi: 10.1016/j.yhbeh.2009.11.009

Frohmader, K. S., Bateman, K. L., Lehman, M. N., & Coolen, L. M. (2010 b). Effects of methamphetamine on sexual performance and compulsive sex behavior in male rats. Psychopharmacology (Berl), 212(1), 93-104. doi: 10.1007/s00213-010-1930-8

Gantt, W. H. (1940). Effect of alcohol on sexual reflexes in dogs. American Journal of Physiology, 129, 360.

Gantt, H. W. (1952). Effect of alcohol on the sexual reflexes of normal and neurotic male dogs. Psychosomatic Medicine, 14(3), 174-181.

Gantt, W. H. (1957). Acute effect of alcohol on automatic (sexual, secretory, cardiac) and somatic responses. In H. E. Himwich (Ed.), Alcoholism: Basic aspects and treatment (pp. 73–89). Washington, DC: American Association for the Advancement of Science.

Gariano, R. F., & Groves, P. M. (1988). Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. Brain Res, 462(1), 194-198. doi: 10.1016/0006-8993(88)90606-3

Geisler, S., & Wise, R. A. (2008). Functional implications of glutamatergic projections to the ventral tegmental area. Rev Neurosci, 19(4-5), 227-244. doi: 10.1515/revneuro.2008.19.4-5.227

George W. H. (2019). Alcohol and Sexual Health Behavior: "What We Know and How We Know It". Journal of sex research, 56(4-5), 409–424. https://doi.org/10.1080/00224499.2019.1588213

Germé, K., Hessing, N., & Pfaus, J. G. (in preparation). Acute alcohol disrupts conditioned sexual inhibition in the male rat.

Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., & Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res, 348(1), 201-203. doi: 10.1016/0006-8993(85)90381-6

Goetzl, F. R., & Stone, F. (1948). The influence of amphetamine sulfate upon olfactory acuity and appetite. Gastroenterology, 10(4), 708-713.

Gracy, K. N., & Pickel, V. M. (1996). Ultrastructural immunocytochemical localization of the N-methyl-D-aspartate receptor and tyrosine hydroxylase in the shell of the rat nucleus accumbens. Brain Res, 739(1-2), 169-181. doi: 10.1016/s0006-8993(96)00822-0

Grant, K. A., & Lovinger, D. M. (1995). Cellular and behavioral neurobiology of alcohol: receptor-mediated neuronal processes. Clin Neurosci, 3(3), 155-164.

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(17), 6912-6916. doi: 10.1073/pnas.87.17.6912

Gruber, A. J., Hussain, R. J., & O'Donnell, P. (2009). The nucleus accumbens: a switchboard for goal-directed behaviors. PLoS One, 4(4), e5062. doi: 10.1371/journal.pone.0005062

Guadarrama-Bazante, I. L., & Rodriguez-Manzo, G. (2019). Nucleus accumbens dopamine increases sexual motivation in sexually satiated male rats. Psychopharmacology (Berl), 236(4), 1303-1312. doi: 10.1007/s00213-018-5142-y

Guthrie, E. R. (1930). Conditioning as a principle of learning. Psychological review, 37(5), 412.

Hanchar, H. J., Dodson, P. D., Olsen, R. W., Otis, T. S., & Wallner, M. (2005). Alcohol-induced motor impairment caused by increased extrasynaptic GABA(A) receptor activity. Nat Neurosci, 8(3), 339-345. doi: 10.1038/nn1398

Harlan, R. E., & Garcia, M. M. (1998). Drugs of abuse and immediate-early genes in the forebrain. Mol Neurobiol, 16(3), 221-267. doi: 10.1007/BF02741385

Hart, B. L. (1968). Effects of Alcohol on Sexual Reflexes and Mating Behavior in the Male Dog. Quarterly Journal of Studies on Alcohol 29 (4):839-844.

Hart, B. L. (1969). Effects of alcohol on sexual reflexes and mating behavior in the male rat. Psychopharmacologia, 14(5), 377-382. doi: 10.1007/BF00403578

Heal, D. J., Smith, S. L., Gosden, J., & Nutt, D. J. (2013). Amphetamine, past and present--a pharmacological and clinical perspective. J Psychopharmacol, 27(6), 479-496. doi: 10.1177/0269881113482532

Heidbreder, C., & De Witte, P. (1993). Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens. Pharmacol Biochem Behav, 46(2), 477-481. doi: 10.1016/0091-3057(93)90383-5

Hernandez-Gonzalez, M., Guevara, M. A., Morali, G., & Cervantes, M. (1997). Subcortical multiple unit activity changes during rat male sexual behavior. Physiol Behav, 61(2), 285-291. doi: 10.1016/s0031-9384(96)00367-8

Hilliard, S., Nguyen, M., & Domjan, M. (1997). One-trial appetitive conditioning in the sexual behavior system. Psychon Bull Rev, 4(2), 237-241. doi: 10.3758/BF03209399

Hökfelt T, Johansson O, Fuxe K, Goldstein M, Park D. (1976) Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain. I. Tyrosine hydroxylase in the mes- and diencephalon. Medical Biology. 54(6):427-453.

Hooks, M. S., & Kalivas, P. W. (1995). The role of mesoaccumbens--pallidal circuitry in novelty-induced behavioral activation. Neuroscience, 64(3), 587-597. doi: 10.1016/0306-4522(94)00409-x

Howland, J. G., Taepavarapruk, P., & Phillips, A. G. (2002). Glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens by basolateral, but not central, nucleus of the amygdala in rats. J Neurosci, 22(3), 1137-1145.

Huey, E. D. (2020) A Critical Review of Behavioral and Emotional Disinhibition. The Journal of Nervous and Mental Disease. 208(4), 344-351. doi: 10.1097/NMD.0000000000001134

Hull, E. M., & Dominguez, J. M. (2007). Sexual behavior in male rodents. Horm Behav, 52(1), 45-55. doi: 10.1016/j.yhbeh.2007.03.030

Hull, E. M., Du, J., Lorrain, D. S., & Matuszewich, L. (1995). Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. J Neurosci, 15(11), 7465-7471.

Hull, E. M., & Rodríguez-Manzo, G. (2009). Male sexual behavior Hormones, brain and behavior, Vol. 1, 2nd ed. (pp. 5-65). San Diego, CA, US: Elsevier Academic Press.

Hull, E. M., & Rodríguez-Manzo, G. (2017). 1.01 - Male Sexual Behavior. In D. W. Pfaff & M. Joëls (Eds.), Hormones, Brain and Behavior (Third Edition) (pp. 1-57). Oxford: Academic Press.

Hull, E. M., Wood, R. I., & McKenna, K. E. (2006). Neurobiology of male sexual behavior. In J. Neill (Ed.), Physiology of Reproduction. New York: Elsevier.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev, 31(1), 6-41. doi: 10.1016/s0165-0173(99)00023-5

Imhoff, R., & Schmidt, A. F. (2014). Sexual disinhibition under sexual arousal: Evidence for domain specificity in men and women. Archives of Sexual Behavior, 43(6), 1123–1136. https://doi.org/10.1007/s10508-014-0329-8

Imperato, A., & Di Chiara, G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther, 239(1), 219-228.

Imperato, A., Honore, T., & Jensen, L. H. (1990). Dopamine release in the nucleus caudatus and in the nucleus accumbens is under glutamatergic control through non-NMDA receptors: a study in freely-moving rats. Brain Res, 530(2), 223-228. doi: 10.1016/0006-8993(90)91286-p

Ismail, N., Gelez, H., Lachapelle, I., & Pfaus, J. G. (2009). Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat. Physiology & behavior, 96(2), 201-208.

Janssen, E., & Bancroft, J. (2007). The dual control model: The role of sexual inhibition and excitation in sexual arousal and behavior. In E. Janssen (Ed.), The Kinsey Institute series. The psychophysiology of sex (p. 197–222). Indiana University Press.

Jackson, M. E., & Moghaddam, B. (2001). Amygdala regulation of nucleus accumbens dopamine output is governed by the prefrontal cortex. J Neurosci, 21(2), 676-681.

Jerison, H. J. (1973). Evolution of the brain and intelligence [by] Harry J. Jerison. New York: Academic Press.

Johnson, L. R., Aylward, R. L., Hussain, Z., & Totterdell, S. (1994). Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. Neuroscience, 61(4), 851-865. doi: 10.1016/0306-4522(94)90408-1

Jones, J. L., Day, J. J., Aragona, B. J., Wheeler, R. A., Wightman, R. M., & Carelli, R. M. (2010). Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding. Biol Psychiatry, 67(8), 737-744. doi: 10.1016/j.biopsych.2009.11.006

Kalivas, P. W., Churchill, L., & Klitenick, M. A. (1993). GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience, 57(4), 1047-1060. doi: 10.1016/0306-4522(93)90048-k

Kalivas, P. W., Duffy, P., & Eberhardt, H. (1990). Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J Pharmacol Exp Ther, 253(2), 858-866.

Källmén, H., & Gustafson, R. (1998). Alcohol and disinhibition. European addiction research, 4(4), 150–162. https://doi.org/10.1159/000018948

Kaplan, H. S. (1974). The new sex therapy: Active treatment of sexual dysfunctions. Oxford, England: Brunner/Mazel.

Kashkin, V. A., & De Witte, P. (2004). Ethanol but not acetaldehyde induced changes in brain taurine: a microdialysis study. Amino Acids, 26(2), 117-124. doi: 10.1007/s00726-003-0057-1

Kauer, J. A. (2004). Learning mechanisms in addiction: synaptic plasticity in the ventral tegmental area as a result of exposure to drugs of abuse. Annu Rev Physiol, 66, 447-475. doi: 10.1146/annurev.physiol.66.032102.112534

Kelley, A. E., Domesick, V. B., & Nauta, W. J. (1982). The amygdalostriatal projection in the rat--an anatomical study by anterograde and retrograde tracing methods. Neuroscience, 7(3), 615-630. doi: 10.1016/0306-4522(82)90067-7

Kelliher, K. R., Liu, Y. C., Baum, M. J., & Sachs, B. D. (1999). Neuronal Fos activation in olfactory bulb and forebrain of male rats having erections in the presence of inaccessible estrous females. Neuroscience, 92(3), 1025-1033. doi: 10.1016/s0306-4522(99)00050-0

Kelm, M. K., Criswell, H. E., & Breese, G. R. (2011). Ethanol-enhanced GABA release: a focus on G protein-coupled receptors. Brain Res Rev, 65(2), 113-123. doi: 10.1016/j.brainresrev.2010.09.003

Kippin, T. E., Cain, S. W., & Pfaus, J. G. (2003). Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. Neuroscience, 117(4), 971-979. doi: 10.1016/s0306-4522(02)00972-7

Kippin, T. E., & Pfaus, J. G. (2001 a). The development of olfactory conditioned ejaculatory preferences in the male rat. I. Nature of the unconditioned stimulus. Physiol Behav, 73(4), 457-469. doi: 10.1016/s0031-9384(01)00484-x

Kippin, T. E., & Pfaus, J. G. (2001 b). The nature of the conditioned response mediating olfactory conditioned ejaculatory preference in the male rat. Behav Brain Res, 122(1), 11-24. doi: 10.1016/s0166-4328(01)00162-0

Kippin, T. E., Samaha, A. N., Sotiropoulos, V., & Pfaus, J. G. (2001). The development of olfactory conditioned ejaculatory preferences in the male rat. II. Parametric manipulation of conditioning session number and duration. Physiol Behav, 73(4), 471-485. doi: 10.1016/s0031-9384(01)00485-1

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(3), 698-704. doi: 10.1111/j.0953-816x.2003.03160.x

Kippin, T. E., Talianakis, S., Schattmann, L., Bartholomew, S., & Pfaus, J. G. (1998). Olfactory conditioning of sexual behavior in the male rat (Rattus norvegicus). Journal of Comparative Psychology, 112(4), 389.

Kolb, B., & Whishaw, I. Q. (2021). Fundamentals of human neuropsychology.

Koob G. F. (2000). Neurobiology of addiction. Toward the development of new therapies. Annals of the New York Academy of Sciences, 909, 170–185. https://doi.org/10.1111/j.1749-6632.2000.tb06682.x

Koob, G. F. (2004). A role for GABA mechanisms in the motivational effects of alcohol. Biochem Pharmacol, 68(8), 1515-1525. doi: 10.1016/j.bcp.2004.07.031

Krystal, J. H., Petrakis, I. L., Mason, G., Trevisan, L., & D'Souza, D. C. (2003). N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment, and vulnerability. Pharmacol Ther, 99(1), 79-94. doi: 10.1016/s0163-7258(03)00054-8

Lammel, S., Lim, B. K., & Malenka, R. C. (2014). Reward and aversion in a heterogeneous midbrain dopamine system. Neuropharmacology, 76 Pt B, 351-359. doi: 10.1016/j.neuropharm.2013.03.019

Lebeau, M.A. (2009). Drug-Facilitated Sexual Assault. In Wiley Encyclopedia of Forensic Science (eds A. Jamieson and A. Moenssens). https://doi.org/10.1002/9780470061589.fsa408

LeBeau, M.A. (2013). Drug-Facilitated Crime. In Wiley Encyclopedia of Forensic Science (eds A. Jamieson and A. Moenssens). https://doi.org/10.1002/9780470061589.fsa408.pub2

Letzkus, J. J., Wolff, S. B. E., & Lüthi, A. (2015). Disinhibition, a Circuit Mechanism for Associative Learning and Memory. Neuron, 88(2), 264-276. https://doi.org/10.1016/j.neuron.2015.09.024

Leigh, B. C., & Stall, R. (1993). Substance use and risky sexual behavior for exposure to HIV. Issues in methodology, interpretation, and prevention. The American psychologist, 48(10), 1035–1045. https://doi.org/10.1037//0003-066x.48.10.1035

Leurquin-Sterk, G., Ceccarini, J., Crunelle, C. L., Weerasekera, A., de Laat, B., Himmelreich, U., . . . Van Laere, K. (2018). Cerebral dopaminergic and glutamatergic transmission relate to different subjective responses of acute alcohol intake: an in vivo multimodal imaging study. Addict Biol, 23(3), 931-944. doi: 10.1111/adb.12542

Li, X., Higley, A., Song, R., & Xi, Z. X. (2013). Effects of metabotropic glutamate receptor ligands on male sexual behavior in rats. Neuropharmacology, 66, 373-381. doi: 10.1016/j.neuropharm.2012.08.006

Liu, Y. C., Sachs, B. D., & Salamone, J. D. (1998). Sexual behavior in male rats after radiofrequency or dopamine-depleting lesions in nucleus accumbens. Pharmacol Biochem Behav, 60(2), 585-592. doi: 10.1016/s0091-3057(98)00022-7

Lobo, I. A., & Harris, R. A. (2008). GABA(A) receptors and alcohol. Pharmacol Biochem Behav, 90(1), 90-94. doi: 10.1016/j.pbb.2008.03.006

Lopez, H. H., & Ettenberg, A. (2002). Exposure to female rats produces differences in c-fos induction between sexually-naive and experienced male rats. Brain Res, 947(1), 57-66. doi: 10.1016/s0006-8993(02)02907-4

Louilot, A., Gonzalez-Mora, J. L., Guadalupe, T., & Mas, M. (1991). Sex-related olfactory stimuli induce a selective increase in dopamine release in the nucleus accumbens of male rats. A voltammetric study. Brain Res, 553(2), 313-317. doi: 10.1016/0006-8993(91)90841-i

Lovinger, D. M., & Roberto, M. (2013). Synaptic effects induced by alcohol. Curr Top Behav Neurosci, 13, 31-86. doi: 10.1007/7854_2011_143

Lovinger, D. M., White, G., & Weight, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science, 243(4899), 1721-1724. doi: 10.1126/science.2467382

Marty, V. N., & Spigelman, I. (2012). Effects of alcohol on the membrane excitability and synaptic transmission of medium spiny neurons in the nucleus accumbens. Alcohol, 46(4), 317-327. doi: 10.1016/j.alcohol.2011.12.002

Mas, M., Fumero, B., Fernandez-Vera, J. R., & Gonzalez-Mora, J. L. (1995 a). Neurochemical correlates of sexual exhaustion and recovery as assessed by in vivo microdialysis. Brain Res, 675(1-2), 13-19. doi: 10.1016/0006-8993(95)00029-p

Mas, M., Fumero, B., & Perez-Rodriguez, I. (1995 b). Induction of mating behavior by apomorphine in sexually sated rats. Eur J Pharmacol, 280(3), 331-334. doi: 10.1016/0014-2999(95)00270-u

McClintock, M. K. (1984). Group mating in the domestic rat as a context for sexual selection: Consequences for the analysis of sexual behavior and neuroendocrine responses Advances in the Study of Behavior (Vol. 14, pp. 1-50): Elsevier.

McDonald, A. (1991). Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience, 44(1), 1-14.

Meisel, R. L., & Sachs, B. D. (1994). The physiology of male sexual behavior. The Physiology and Reproduction, 2, 3-105.

Melis, M. R., & Argiolas, A. (1995). Dopamine and sexual behavior. Neurosci Biobehav Rev, 19(1), 19-38. doi: 10.1016/0149-7634(94)00020-2

Melis, M. R., & Argiolas, A. (2011). Central control of penile erection: a re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. Neurosci Biobehav Rev, 35(3), 939-955. doi: 10.1016/j.neubiorev.2010.10.014

Mendelson, S. D., & Gorzalka, B. B. (1987). An improved chamber for the observation and analysis of the sexual behavior of the female rat. Physiol Behav, 39(1), 67-71. doi: 10.1016/0031-9384(87)90345-3

Mendelson, S. D., & Pfaus, J. G. (1989). Level searching: a new assay of sexual motivation in the male rat. Physiol Behav, 45(2), 337-341. doi: 10.1016/0031-9384(89)90136-4

Miczek, K. A., de Boer, S. F., & Haller, J. (2013). Excessive aggression as model of violence: a critical evaluation of current preclinical methods. Psychopharmacology, 226(3), 445–458. https://doi.org/10.1007/s00213-013-3008-x

Mitchell, J. B., & Gratton, A. (1994). Involvement of mesolimbic dopamine neurons in sexual behaviors: implications for the neurobiology of motivation. Rev Neurosci, 5(4), 317-329. doi: 10.1515/revneuro.1994.5.4.317

Moghaddam, B., & Bolinao, M. L. (1994). Biphasic effect of ethanol on extracellular accumulation of glutamate in the hippocampus and the nucleus accumbens. Neurosci Lett, 178(1), 99-102. doi: 10.1016/0304-3940(94)90299-2

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev Neurosci, 18(2), 73-85. doi: 10.1038/nrn.2016.165

Morales, M., & Root, D. H. (2014). Glutamate neurons within the midbrain dopamine regions. Neuroscience, 282, 60-68. doi: https://doi.org/10.1016/j.neuroscience.2014.05.032

Morikawa, H., & Morrisett, R. A. (2010). Ethanol action on dopaminergic neurons in the ventral tegmental area: interaction with intrinsic ion channels and neurotransmitter inputs. Int Rev Neurobiol, 91, 235-288. doi: 10.1016/S0074-7742(10)91008-8

Nair-Roberts, R. G., Chatelain-Badie, S. D., Benson, E., White-Cooper, H., Bolam, J. P., & Ungless, M. A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience, 152(4), 1024-1031. doi: 10.1016/j.neuroscience.2008. 01.046

Oliva, I., & Wanat, M. J. (2016). Ventral Tegmental Area Afferents and Drug-Dependent Behaviors. Front Psychiatry, 7, 30. doi: 10.3389/fpsyt.2016.00030

Omelchenko, N., & Sesack, S. R. (2009). Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. Synapse, 63(10), 895-906. doi: 10.1002/syn.20668

Palha, A. P., & Esteves, M. (2008). Drugs of abuse and sexual functioning. Advances in psychosomatic medicine, 29, 131–149. https://doi.org/10.1159/000126628

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(3), 179-226. doi: 10.1016/j.pneurobio.2004.05.001

Paton, C. (2002). Benzodiazepines and disinhibition: a review. Psychiatric Bulletin, 26(12), 460-462.

Pavlov, I. P. (1927). Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Oxford, England: Oxford Univ. Press.

Pavuluri, M., Volpe, K., & Yuen, A. (2017). Nucleus accumbens and its role in reward and emotional circuitry: A potential hot mess in substance use and emotional disorders. AIMS Neuroscience, 4(1), 52-70.

Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic corrdinates. San Diego: Academic Press.

Peters, R. H., Koch, P. C., & Blythe, B. L. (1988). Differential effects of yohimbine and naloxone on copulatory behaviors of male rats. Behav Neurosci, 102(4), 559-564. doi: 10.1037//0735-7044.102.4.559

Peugh, J., & Belenko, S. (2001). Alcohol, drugs and sexual function: a review. J Psychoactive Drugs, 33(3), 223-232. doi: 10.1080/02791072.2001.10400569

Pfaus, J. G. (1996). Frank A. Beach award. Homologies of animal and human sexual behaviors. Horm Behav, 30(3), 187-200. doi: 10.1006/hbeh.1996.0024

Pfaus, J. G. (1999). Neurobiology of sexual behavior. Curr Opin Neurobiol, 9(6), 751-758. doi: 10.1016/s0959-4388(99)00034-3

Pfaus, J. G. (2009). Pathways of sexual desire. J Sex Med, 6(6), 1506-1533. doi: 10.1111/j.1743-6109.2009.01309.x

Pfaus, J. G., Damsma, G., Nomikos, G. G., Wenkstern, D. G., Blaha, C. D., Phillips, A. G., & Fibiger, H. C. (1990 a). Sexual behavior enhances central dopamine transmission in the male rat. Brain Res, 530(2), 345-348. doi: 10.1016/0006-8993(90)91309-5

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

Pfaus, J. G., Kippin, T. E., & Centeno, S. (2001). Conditioning and sexual behavior: a review. Horm Behav, 40(2), 291-321. doi: 10.1006/hbeh.2001.1686

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

Pfaus, J. G., Mendelson, S. D., & Phillips, A. G. (1990 b). A correlational and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat. Psychoneuroendocrinology, 15(5-6), 329-340. doi: 10.1016/0306-4530(90)90058-h

Pfaus, J. G., & Phillips, A. G. (1989). Differential effects of dopamine receptor antagonists on the sexual behavior of male rats. Psychopharmacology (Berl), 98(3), 363-368. doi: 10.1007/BF00451688

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

Pfaus, J. G., & Pinel, J. P. J. (1989). Alcohol inhibits and disinhibits sexual behavior in the male rat. Psychobiology, 17(2), 195-201. doi: 10.3758/bf03337836

Pfaus, J. G., Wilkins, M. F., Dipietro, N., Benibgui, M., Toledano, R., Rowe, A., & Couch, M. C. (2010). Inhibitory and disinhibitory effects of psychomotor stimulants and depressants on the sexual behavior of male and female rats. Horm Behav, 58(1), 163-176. doi: 10.1016/j.yhbeh.2009.10.004

Phillips, A. G., Ahn, S., & Howland, J. G. (2003). Amygdalar control of the mesocorticolimbic dopamine system: parallel pathways to motivated behavior. Neurosci Biobehav Rev, 27(6), 543-554. doi: 10.1016/j.neubiorev.2003.09.002

Piepponen, T. P., Kiianmaa, K., & Ahtee, L. (2002). Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats. Pharmacol Biochem Behav, 74(1), 21-30. doi: 10.1016/s0091-3057(02)00937-1

Pitman, K. A., Puil, E., & Borgland, S. L. (2014). GABA(B) modulation of dopamine release in the nucleus accumbens core. Eur J Neurosci, 40(10), 3472-3480. doi: 10.1111/ejn.12733

Quintana, G. R., Desbiens, S., Marceau, S., Kalantari, N., Bowden, J., & Pfaus, J. G. (2019). Conditioned partner preference in male and female rats for a somatosensory cue. Behav Neurosci, 133(2), 188-197. doi: 10.1037/bne00003002019-05623-001

Quintana, G. R., Jackson, M., Nasr, M., & Pfaus, J. G. (2018). Effect of CS preexposure on the conditioned ejaculatory preference of the male rat: behavioral analyses and neural correlates. Learn Mem, 25(10), 513-521. doi: 10.1101/lm.048108.11825/10/513

Radulovic, J., Jovasevic, V., & Meyer, M. A. (2017). Neurobiological mechanisms of state-dependent learning. Curr Opin Neurobiol, 45, 92-98. doi: 10.1016/j.conb.2017.05.013

Rao, P. S., Bell, R. L., Engleman, E. A., & Sari, Y. (2015). Targeting glutamate uptake to treat alcohol use disorders. Front Neurosci, 9, 144. doi: 10.3389/fnins.2015.00144

Rawson, R. A., Washton, A., Domier, C. P., & Reiber, C. (2002). Drugs and sexual effects: role of drug type and gender. J Subst Abuse Treat, 22(2), 103-108. doi: 10.1016/s0740-5472(01)00215-x

Reid, R., Davtian, M., Lenartowicz, A., Torrevillas, R., & Fong, T. (2013). Perspectives on the assessment and treatment of adult ADHD in hypersexual men. Neuropsychiatry, 3, 295-308. doi: 10.2217/npy.13.31

Rescorla, R. A. (1969). Conditioned inhibition of fear resulting from negative CS-US contingencies. J Comp Physiol Psychol, 67(4), 504-509. doi: 10.1037/h0027313

Rescorla, R. A. (1976). Stimulus generalization: some predictions from a model of Pavlovian conditioning. J Exp Psychol Anim Behav Process, 2(1), 88-96. doi: 10.1037//0097-7403.2.1.88

Rhodes, T., & Stimson, G. V. (1994). What is the relationship between drug taking and sexual risk? Social relations and social research. Sociology of Health & Illness, 16(2), 209-228. doi: https://doi.org/10.1111/1467-9566.ep11347330

Robertson, G. S., Pfaus, J. G., Atkinson, L. J., Matsumura, H., Phillips, A. G., & Fibiger, H. C. (1991). Sexual behavior increases c-fos expression in the forebrain of the male rat. Brain Res, 564(2), 352-357. doi: 10.1016/0006-8993(91)91477-i

Rodriguez-Manzo, G. (1999). Yohimbine interacts with the dopaminergic system to reverse sexual satiation: further evidence for a role of sexual motivation in sexual exhaustion. Eur J Pharmacol, 372(1), 1-8. doi: 10.1016/s0014-2999(99)00140-5

Rodriguez-Manzo, G., & Canseco-Alba, A. (2017). A new role for GABAergic transmission in the control of male rat sexual behavior expression. Behav Brain Res, 320, 21-29. doi: 10.1016/j.bbr.2016.11.041

Rodriguez-Manzo, G., & Fernandez-Guasti, A. (1995). Opioid antagonists and the sexual satiation phenomenon. Psychopharmacology (Berl), 122(2), 131-136. doi: 10.1007/BF02246087

Rodriguez-Nieto, G., Sack, A. T., Dewitte, M., Emmerling, F., & Schuhmann, T. (2019). Putting out the blaze: The neural mechanisms underlying sexual inhibition. PLoS One, 14(1), e0208809. doi: 10.1371/journal.pone.0208809

Room, R., Collins, G., editors. (1983) Alcohol and disinhibition: nature and meaning of the link. NIAAA Research Monograph No.12, DHSS Publications No. (ADM) 83-1246. Washington DC: Government Printing Office

Ross, M. W., & Williams, M. L. (2001). Sexual behavior and illicit drug use. Annu Rev Sex Res, 12, 290-310.

Rotllant, D., Marquez, C., Nadal, R., & Armario, A. (2010). The brain pattern of c-fos induction by two doses of amphetamine suggests different brain processing pathways and minor contribution of behavioural traits. Neuroscience, 168(3), 691-705. doi: 10.1016/j.neuroscience.2010.04.020

Rubin, H. B., & Henson, D. E. (1976). Effects of alcohol on male sexual responding. Psychopharmacologia, 47(2), 123-134. doi: 10.1007/BF00735810

Sachs, B. D. (2007). A contextual definition of male sexual arousal. Horm Behav, 51(5), 569-578. doi: 10.1016/j.yhbeh.2007.03.011

Salamone, J. D. (1994). The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behav Brain Res, 61(2), 117-133. doi: 10.1016/0166-4328(94)90153-8

Salgado, S., & Kaplitt, M. G. (2015). The Nucleus Accumbens: A Comprehensive Review. Stereotact Funct Neurosurg, 93(2), 75-93. doi: 10.1159/000368279

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nat Methods, 9(7), 676-682. doi: 10.1038/nmeth.2019

Schmued, L. C., & Fallon, J. H. (1986). Fluoro-Gold: a new fluorescent retrograde axonal tracer with numerous unique properties. Brain Res, 377(1), 147-154. doi: 10.1016/0006-8993(86)91199-6

Schultz, W. (2013). Updating dopamine reward signals. Curr Opin Neurobiol, 23(2), 229-238. doi: 10.1016/j.conb.2012.11.012

Selim, M., & Bradberry, C. W. (1996). Effect of ethanol on extracellular 5-HT and glutamate in the nucleus accumbens and prefrontal cortex: comparison between the Lewis and Fischer 344 rat strains. Brain Res, 716(1-2), 157-164. doi: 10.1016/0006-8993(95)01385-7

Sesack, S. R., & Pickel, V. M. (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol, 320(2), 145-160. doi: 10.1002/cne.903200202

Shafer, M. A. (1994). Sexual behavior and sexually transmitted diseases among male adolescents in detention. Sex Transm Dis, 21(3), 181-182. doi: 10.1097/00007435-199405000-00009

Skårner, A., & Svensson, B. (2013). Amphetamine use and Sexual Practices. Nordic Studies on Alcohol and Drugs, 30(5), 403-423. doi: 10.2478/nsad-2013-0035

Smith, S. E., & Sharp, T. (1994). An investigation of the origin of extracellular GABA in rat nucleus accumbens measured in vivo by microdialysis. J Neural Transm Gen Sect, 97(2), 161-171. doi: 10.1007/BF01277951

Sorge, R. E., Rajabi, H., & Stewart, J. (2005). Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement. Neuropsychopharmacology, 30(9), 1681-1692. doi: 10.1038/sj.npp.1300712

Soulairac, A., & Soulairac, M. L. (1957). [Action of amphetamine, adrenalin and atropine on the sexual behavior of male rats]. J Physiol (Paris), 49(1), 381-385.

Spanagel, R. (2009). Alcoholism: a systems approach from molecular physiology to addictive behavior. Physiol Rev, 89(2), 649-705. doi: 10.1152/physrev.00013.2008

Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: past and current status. Trends Neurosci, 22(11), 521-527. doi: 10.1016/s0166-2236(99)01447-2

Steele, C. M., & Josephs, R. A. (1990). Alcohol myopia. Its prized and dangerous effects. Am Psychol, 45(8), 921-933. doi: 10.1037//0003-066x.45.8.921

Taber, M. T., & Fibiger, H. C. (1995). Electrical stimulation of the prefrontal cortex increases dopamine release in the nucleus accumbens of the rat: modulation by metabotropic glutamate receptors. J Neurosci, 15(5 Pt 2), 3896-3904.

Teitelbaum, H. A., & Gantt, W. H. (1958). The effect of alcohol on sexual reflexes and sperm count in the dog. Q J Stud Alcohol, 19(3), 394-398.

Theile, J. W., Morikawa, H., Gonzales, R. A., & Morrisett, R. A. (2008). Ethanol enhances GABAergic transmission onto dopamine neurons in the ventral tegmental area of the rat. Alcohol Clin Exp Res, 32(6), 1040-1048. doi: 10.1111/j.1530-0277.2008.00665.x

Theile, J. W., Morikawa, H., Gonzales, R. A., & Morrisett, R. A. (2011). GABAergic transmission modulates ethanol excitation of ventral tegmental area dopamine neurons. Neuroscience, 172, 94-103. doi: 10.1016/j.neuroscience.2010.10.046

Toates, F. M. (1986). Motivational systems. Cambridge [Cambridgeshire] ; New York: Cambridge University Press.

Tronel, S., & Sara, S. J. (2002). Mapping of olfactory memory circuits: region-specific c-fos activation after odor-reward associative learning or after its retrieval. Learn Mem, 9(3), 105-111. doi: 10.1101/lm.47802

Van Bockstaele, E. J., & Pickel, V. M. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res, 682(1-2), 215-221. doi: 10.1016/0006-8993(95)00334-m

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(1), 123-134. doi: 10.1037//0735-7044.109.1.123

Venton, B. J., Robinson, T. E., & Kennedy, R. T. (2006). Transient changes in nucleus accumbens amino acid concentrations correlate with individual responsivity to the predator fox odor 2,5-dihydro-2,4,5-trimethylthiazoline. J Neurochem, 96(1), 236-246. doi: 10.1111/j.1471-4159.2005.03549.x

Volkow, N. D., Fowler, J. S., & Wang, G. J. (2004 a). The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. Neuropharmacology, 47 Suppl 1, 3-13. doi: 10.1016/j.neuropharm.2004.07.019

Volkow, N. D., Fowler, J. S., Wang, G. J., & Swanson, J. M. (2004 b). Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry, 9(6), 557-569. doi: 10.1038/sj.mp.4001507

Volkow, N. D., & Morales, M. (2015). The Brain on Drugs: From Reward to Addiction. Cell, 162(4), 712-725. doi: 10.1016/j.cell.2015.07.046

Volkow, N. D., Wang, G. J., Fowler, J. S., Telang, F., Jayne, M., & Wong, C. (2007). Stimulant-induced enhanced sexual desire as a potential contributing factor in HIV transmission. Am J Psychiatry, 164(1), 157-160. doi: 10.1176/ajp.2007.164.1.157

Volkow, N. D., Wiers, C. E., Shokri-Kojori, E., Tomasi, D., Wang, G. J., & Baler, R. (2017). Neurochemical and metabolic effects of acute and chronic alcohol in the human brain: Studies with positron emission tomography. Neuropharmacology, 122, 175-188. doi: 10.1016/j.neuropharm.2017.01.012

Wakabayashi, K. T., & Kiyatkin, E. A. (2012). Rapid changes in extracellular glutamate induced by natural arousing stimuli and intravenous cocaine in the nucleus accumbens shell and core. J Neurophysiol, 108(1), 285-299. doi: 10.1152/jn.01167.2011

Walley, R. E., & Weiden, T. D. (1973). Lateral inhibition and cognitive masking: a neuropsychological theory of attention. Psychol Rev, 80(4), 284-302. doi: 10.1037/h0035007

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(1), 41-46. doi: 10.1016/0006-8993(93)90426-n

West, C. H., Clancy, A. N., & Michael, R. P. (1992). Enhanced responses of nucleus accumbens neurons in male rats to novel odors associated with sexually receptive females. Brain Res, 585(1-2), 49-55. doi: 10.1016/0006-8993(92)91189-l

Westerink, B. H., Kwint, H. F., & deVries, J. B. (1996). The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J Neurosci, 16(8), 2605-2611.

Wheeler, R. A., & Carelli, R. M. (2009). Dissecting motivational circuitry to understand substance abuse. Neuropharmacology, 56 Suppl 1, 149-159. doi: 10.1016/j.neuropharm.2008.06.028

Wilson, G. T., & Niaura, R. (1984). Alcohol and the disinhibition of sexual responsiveness. J Stud Alcohol, 45(3), 219-224. doi: 10.15288/jsa.1984.45.219

Wilson, J. R., Kuehn, R. E., & Beach, F. A. (1963). Modification in the sexual behavior of male rats produced by changing the stimulus female. J Comp Physiol Psychol, 56, 636-644. doi: 10.1037/h0042469

Wise, R. A. (2008). Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox Res, 14(2-3), 169-183. doi: 10.1007/BF03033808

Xi, Z. X., & Stein, E. A. (1998). Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. Brain Res, 798(1-2), 156-165. doi: 10.1016/s0006-8993(98)00406-5

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. J Neurosci, 31(21), 7811-7816. doi: 10.1523/JNEUROSCI.1504-11.2011

Xiao, C., Shao, X. M., Olive, M. F., Griffin, W. C., 3rd, Li, K. Y., Krnjevic, K., . . . Ye, J. H. (2009). Ethanol facilitates glutamatergic transmission to dopamine neurons in the ventral tegmental area. Neuropsychopharmacology, 34(2), 307-318. doi: 10.1038/npp.2008.99

Xiao, C., Zhou, C., Li, K., & Ye, J. H. (2007). Presynaptic GABAA receptors facilitate GABAergic transmission to dopaminergic neurons in the ventral tegmental area of young rats. J Physiol, 580(Pt.3), 731-743. doi: 10.1113/jphysiol.2006.124099

Yamaguchi, T., Wang, H. L., Li, X., Ng, T. H., & Morales, M. (2011). Mesocorticolimbic glutamatergic pathway. J Neurosci, 31(23), 8476-8490. doi: 10.1523/JNEUROSCI.1598-11.2011

Yan, Q. S., Reith, M. E., Yan, S. G., & Jobe, P. C. (1998). Effect of systemic ethanol on basal and stimulated glutamate releases in the nucleus accumbens of freely moving Sprague-Dawley rats: a microdialysis study. Neurosci Lett, 258(1), 29-32. doi: 10.1016/s0304-3940(98)00840-4

Yoshimoto, K., McBride, W. J., Lumeng, L., & Li, T.-K. (1991). Alcohol Stimulates the Release of Dopamine and Serotonin in the Nucleus Accumbens. Alcohol, 9, 17-22.

Zhang, H., & Sulzer, D. (2012). Regulation of striatal dopamine release by presynaptic auto- and heteroreceptors. Basal Ganglia, 2(1), 5-13. doi: 10.1016/j.baga.2011.11.004