Disruption of conditioned sexual inhibition by alcohol in male rats

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This is to certify that the thesis prepared

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

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Abstract

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Previous studies have shown that male rats trained alternatively with a scented (almond) receptive female and an unscented non- receptive female display a conditioned ejaculatory preference (CEP) for females bearing the odor. This preference is observed in an open field where males are given the choice between two receptive females, one scented and the other unscented. Also, previous studies on alcohol demonstrated that acute treatment of low doses of alcohol can disinhibit male mounting behavior with sexually nonreceptive females. Here we examined whether CEP would be affected during the copulatory test after an acute treatment with alcohol. Sexually naïve male Long-Evans rats received conditioning sessions in bilevel chambers with an unscented receptive female or a scented non-receptive female. Two days after the last conditioning trial, males were injected with saline, alcohol $0.5g.kg^{-1}$ or alcohol $1g.kg^{-1}$, 45minutes before the beginning of the copulatory test. Following the preference test, males were exposed to the odor to assess brain activation by the conditioned inhibitory cue. Analyses showed that by the end of the conditioning phase, there was a greater sexual motivation before noninhibitory trials. During the copulatory test, saline-treated males developed CEP but not alcohol-treated males. Following the exposure to the conditioned odor, brains from conditioned animals revealed a differential pattern of Fos activation between saline and alcohol groups, especially in the NAccC, BLA and VMH. These data show that alcohol disrupts the display of an inhibitory CEP and it may involve specific brain regions.

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Introduction

Alcohol is one of the oldest and most commonly-consumed drugs. Its effects on perception, cognition, and behavior have been hypothesized about for centuries, but it is only recently that the systematic study of alcohol's effects on the brain has begun. Of all alcohol's effects, those on sexual behavior have generated enormous interest and commentary, but surprisingly an astonishingly sparse amount of direct experimental manipulation. The depressant effect of alcohol on the central nervous system is well-known and early literature has explored its inhibitory effects on human sexual behavior and reproduction (reviewed in Marshall 1941). There is a long history of alcohol's association with human sexual behavior highlighting its dual effects in terms of inhibited performance but also in terms of disinhibited arousal or desire (Pfaus *et al.* 2010).

Effects in men

In an early review, Abel (1980) discussed the impact of alcohol on the reproductive processes of men and women, and how those findings were still preliminary. Previous studies had established that large doses of alcohol decrease penile tumescence rate (Briddell and Wilson 1976), depress penile diameter increase (Farkas and Rosen 1976), decrease peak erection, mean erection (mean circumference of the erected penis) and increase the latency in the onset of an erection (Rubin and Henson 1976).

Despite an overwhelming evidence of inhibition, alcohol's dual effect of disinhibiting sexual desire while inhibiting the physiological sexual response remains a common belief. At higher doses, alcohol is assumed to depress central nervous system's activity but at low doses it may produce a release of inhibitions possibly leading to an

increase in sexual arousal in inhibited persons (Kaplan 1974). This was studied explicitly by Rubin and Henson (1976). Using a penile plethysmograph that measures variation of penile blood flow to assess sexual response, they showed that, after consumption of alcohol, men showed a significant decrease in the ability to perform sexually at higher doses but also an increase in sexual response during inhibitory conditions at low doses. In that study using repeated measure design, men were given up to three drinks and were asked to relax and enjoy an erotic film (non inhibitory test) or to inhibit their sexual excitation while watching the erotic film (inhibitory test). Penile tumescence was measured to assess their sexual response. During the non inhibitory test and following ingestion of the highest dose of alcohol (1.5 $g.kg^{-1}$), the subjects showed a significant decrease in sexual response. During the inhibitory test, when men were asked to inhibit their sexual response there was an increase in mean erections, following a moderate dose of alcohol (1 $g.kg^{-1}$). The investigators speculated that this phenomenon was sexual disinhibition.

Wilson (1977) questioned the disinhibitory effects of alcohol on sexual behavior by proposing a flaw in the experimental design. For disinhibition to occur inhibition must first be present, which could be either social or personal. Cultural background, social learning or personal beliefs could affect sexual response under the influence of alcohol. Hence, the results could be attributed to psychological factors rather than pharmacological effects of alcohol (Wilson 1977). During an experiment performed by Wilson and Niaura (1984) men were asked to suppress their sexual arousal while listening to an explicit erotic story and penile tumescence was measured. Participants experienced shorter latencies to the onset of an erection and to peak penile tumescence

following consumption of a moderate dose of alcohol (0.6 $g.kg^{-1}$). Later, in their review of the relationship between human sexual response and alcohol, Crowe and George (1989) proposed that low doses of alcohol might disinhibit sexual arousal at a psychological level and suppress physiological sexual response at higher doses.

Steele and Josephs (1990) proposed an overarching theory of "alcohol myopia" to explain potential disinhibitory effects of alcohol. Their theory states that alcohol provokes a decrease in cognitive and emotional function that limits attention to the most salient cues of the environment. They also argued that the nature of these cues would reflect the behavior induced by alcohol intoxication. By impairing the perception, thought, attention, cognition and emotions (Wallgren and Barry 1970), alcohol reduces the ability to process the meaning of surrounding cues and restricts the amount of cues processed at any one time. Therefore, when the internal (e.g. cultural background, learning) and external (e.g. environment, presence of other individuals) cues are in conflict (some are inhibitory while the others are appetitive), alcohol causes a shortsightedness leading the person to focus on the most provoking cues. In this case, disinhibition may occur if appetitive external (or internal) cues are more salient than inhibitory internal (or external) ones. Thus, due to "alcohol myopia", intoxicated individuals may behave in an inappropriate manner.

Although the effects of alcohol on human sexual behavior are best studied in humans, it is often difficult to do so in a meaningful way. In addition to ethical restrictions, the effects of alcohol on any behavior in humans are often confounded by expectations, beliefs, social learning and culture. Simply believing that alcohol is in a drink can influence expectancies and facilitate disinhibited behavior. Although external

cues can be modified experimentally (e.g. by changing in the instructions or the context), internal cues and expectations are almost impossible to impose. In contrast, animal models allow both internal and external cues to be imposed experimentally by conditioning and consistent inhibitory effects of alcohol have been observed on the sexual behavior of male rats.

Effects in male rats

Male rats display a characteristic pattern of sexual behavior that involves multiple mounts, intromissions, and ejaculations. Male rats also chase females in chambers where the females are allowed to solicit and pace the copulatory contact (Pfaus *et al.* 1990). Two distinct mechanisms (sexual arousal and ejaculation mechanisms) that compose male rat sexual behavior were defined (Beach 1956) which were later merged with the notion of precopulatory (appetitive) and copulatory (consummatory) sexual behaviors (Ball and Balthazart 2008, Pfaus 1996, 1999, Sachs 2007). During the precopulatory phase, male rats typically investigate the female's anogenital region. The copulatory phase consists in mounts (defined by anteroposterior pelvic thrusts without insertion of the penis into the vagina), intromissions (mounts with penile insertion) and after several intromissions, ejaculation (intromission with a longer thrust and expulsion of seminal fluid) (Beyer *et al.* 1981). Following an ejaculation, male rats are not able to immediately reengage copulatory behavior. The period between two bouts of sexual behavior is called the postejaculatory interval (PEI). It might last several minutes and its duration depends on the number of preceding ejaculatory series.

As observed in men, dose-dependent effects of alcohol also affect the sexual behavior of male rats. Hart (1969) examined the effects of alcohol on the sexual

responses and sexual reflexes of male rats that were either intact or that had a transected spinal cord. Using three different doses (1, 2 and 3 $g.kg^{-1}$), he observed that males with a transected spinal cord showed a decrease in the number of genital responses and intact males displayed significantly fewer ejaculations, when they were exposed to the highest dose of ethanol compared to saline treated animals. These results suggest that alcohol does not affect sexual motivation since intact males continued to mount, intromit and ejaculate but impacts on the capacity to perform sexually. Indeed, the first experiment of a subsequent study by Pfaus and Pinel (1989) showed that alcohol dose-dependently disrupted the sexual behavior of sexually experienced male rats. Low doses (0.25 and 0.5 $g.kg^{-1}$) increased mount, intromission, and ejaculation latencies, while higher doses (1 and 2 $g.kg^{-1}$) decreased the proportion of male rats that engaged in mounts, intromissions, or ejaculations. Those results were consistent with the human studies cited earlier showing the inhibitory effects of alcohol on sexual responses.

In the experimental studies on laboratory animals cited above, the emphasis was on the disruption of sexual behavior by alcohol. The first study to question Wilson's (1977) caveat about the disinhibitory effects of alcohol on the sexual behavior of male rats was the second experiment conducted by Pfaus and Pinel (1989), which examined sexual disinhibition specifically. To induce sexual inhibition, sexually experienced male rats were trained not to copulate with sexually nonreceptive females. The proportion of males that attempted to mount the nonreceptive females dropped from 80% on the 1^{st} trial, to 0% by the 5^{th} trial, whereas the proportions that mounted and ejaculated during the tests with receptive females remained at 100%. During the inhibitory test with nonreceptive females, males injected with the low dose mounted and ejaculated

significantly more compared to males in the saline group. Despite the fact that they learned to inhibit their copulatory behavior with a nonreceptive female, a single injection of a low dose of alcohol suppressed this learned inhibition. This study demonstrated that a low dose of alcohol does indeed possess disinhibitory properties on the sexual behavior of male rats provided sexual inhibition is present.

Another important parameter of sexual behavior in male rats is sexual motivation and desire distinct from copulatory activity. Mendelson and Pfaus (1989) reported that after several test sessions in bilevel chambers, males allowed to copulate with a receptive female show an increase in level changes during the habituation phase preceding the introduction of the female, and described this behavior as an index of sexual motivation. Using this measure of sexual behavior, Ferraro and Kiefer (2004) observed an increase of sexual motivation with alcohol. After an injection of ethanol or saline, male rats were placed in bilevel chambers, as in Mendelson and Pfaus (1989). They found an increase in the number of level changes following alcohol treatment (1 $g.kg^{-1}$) during the 5-min habituation prior to the test. They concluded that this dose of ethanol enhanced sexual motivation in male rats. Conversely, this dose of ethanol impaired sexual performance, resulting in fewer anogenital investigations and longer PEIs once males copulated with the females.

Alcohol myopia should not be excluded as an explanation in animals. Grant and Macdonald (2005) argued that findings in the literature on behavioral responses after ingestion of alcohol are not necessarily due to disinhibition per se, but could be the consequence of myopic processing. In alcohol myopia, the behavior depends on the salience of the cue, which is not the case in the theory of disinhibition. As in humans, the

cues can be external and internal. In the case of laboratory animals, external as well as internal cues can be experimentally imposed and monitored using conditioning. In studies of alcohol's effects on sexual behavior, both internal and external cues were appetitive (sexually experienced males with receptive females or manual stimulation of genitalia). In these cases, an inhibitory effect of alcohol was observed. It would be of interest to examine whether alcohol could disinhibit when the conflict is between cues that have an equal conditioning story.

In conclusion, the relation between alcohol and male sexual behavior and behavior in general is complex and depends on dose (Ferraro and Kiefer 2004, Pfaus and Pinel 1989) or simple expectancy, which is often culturally-based (Wilson 1977). Although alcohol's effects have been assumed to occur in cortical centers associated with higher-order inhibitory processing (e.g., medial prefrontal cortex (mPFC), there is currently no knowledge where alcohol might act in the brain.

Alcohol and brain function

In the early literature, behaviors considered as disorganized, impulsive, or inappropriate were assumed to be the consequence of frontal cortex dysfunction (e.g., (Bianchi 1922, Luria 1969). Anatomically, the frontal cortex, and in particular the prefrontal division, is well-placed to receive information from virtually all subcortical regions, and in turn, send information to other cortical and subcortical regions. In fact, the medial prefrontal cortex (mPFC) is considered the center of "executive function" (Banich *et al.* 2009, Miller and Cohen 2001). By acting as a global CNS depressant, with anesthetic and anxiolytic properties, alcohol was believed to act on the PFC to inhibit generally the cortical controls involved in executive function (Abrams and Wilson 1983,

Kaplan 1974, Wilson and Niaura 1984). However, despite these assumptions, the pharmacological effects of alcohol on inhibitory control in general and human sexual behavior in particular, have never been determined.

By affecting judgment, alcohol leads to uninhibited behavior (Chang *et al.* 1995). It was hypothesized that at higher doses, ethanol depresses cortical activity, impairing cognitive and executive functions (Kaplan 1974). Later studies demonstrated functional changes in abstinent and non-abstinent alcoholics (Grusser *et al.* 2004, Myrick *et al.* 2004), changes in the white and grey matter volume and executive dysfunction (Sullivan and Pfefferbaum 2005) in the prefrontal cortex. These were results of long term exposure to alcohol and without alcohol consumption. However, the short term mechanisms of alcohol as well as its specific site of action in the brain remain unclear. Studies on Fos induction in rodents have revealed brain areas activated after acute ethanol administration (Chang *et al.* 1995, Vilpoux *et al.* 2009), including the bed nucleus of the stria terminalis (BNST), paraventricular nucleus (PVN), the central amygdala (CeA), Edinger- Wesphal nucleus (Ew), locus coeruleus and the parabrachial nucleus (PB) (Chang *et al.* 1995). Fos activation was also observed in other regions such as the supraoptic nucleus (SON) but only at higher concentrations of alcohol. Interestingly, no difference of Fos activation in cortical regions was reported. More recently, Vilpoux *et al.* (2009) established a dose-dependent cartography of ethanol-sensitive brain regions in rodents using several strains of rats and mice. Long-Evans rats showed an increase in Fos activation two hours after an injection of 1 $g.kg^{-1}$ or 3 $g.kg^{-1}$ in the nucleus accumbens core (NAccC) and shell (NAccSh), PVN, LC, CeA, PB, nucleus of the solitary tract and the area prostema.

Apart from immunohistochemistry, other behavioral and electrophysiological studies provide evidence of alcohol's action on different systems of neurotransmission. In a study performed by Lovinger *et al.* (1989), alcohol was applied to hippocampal neurons slices and N-methyl-D-aspartate (NMDA) activation was reduced. They suggested that this inhibition of NMDA-activated current may be part of the causes leading to behavioral impairments observed during alcohol intoxication. Later, it was found that an injection of alcohol of 1 or 2 $g.kg^{-1}$ induced an increase in extracellular levels of dopamine and serotonin in the nucleus accumbens of rats (Yoshimoto *et al.* 1992). They concluded that alcohol could stimulate dopamine release in the NAcc and suggested that this alcohol effect could potentially be mediated by 5-hydroxytryptamine 3 (5-HT3) receptors (for a serotonin receptor subtype). Alcohol was also shown to influence neurotransmission of γ-aminobutyric acid type A ($GABA_A$) and glycine receptors by enhancing their inhibitory function (Mihic 1999, Mihic *et al.* 1997). Reviewing literature on the effects of alcohol on the brain,Vengeliene *et al.* (2008) stated that at low doses, alcohol has a primary effect on NMDA, $GABA_A$, neuronal nicotinic acetylcholine (ACh) receptors and on ion channels. By acting on these receptors, alcohol also acts on a larger scale to influence serotoninergic and dopaminergic neurotransmission. As a general depressant of the central nervous system, alcohol appears to increase inhibitory neurotransmission and decrease or inhibits excitatory neurotransmission.

The present experiments

The goal of this thesis was to examine the effects of two different doses of alcohol on male rats' conditioned sexual inhibition and on brain activation following exposure to a conditioned olfactory cue. Kippin *et al.* (1998) used an olfactory conditioning to induce

conditioned sexual inhibition in rats. To do so, they gave male rats conditioning sessions with an almond-scented nonreceptive female alternately with conditioning sessions with an unscented receptive female. On a final open field test with two receptive females, one almond-scented and one unscented, conditioned male rats ejaculated more frequently with the unscented female and a higher proportion of the males chose these females for their first ejaculation. Thus, male rats developed a conditioned ejaculatory preference for the unscented female but also a conditioned sexual inhibition towards the scented one. It was hypothesized that alcohol would disrupt this conditioned sexual inhibition. Therefore, conditioned male rats injected with saline would show an ejaculatory preference for a sexually receptive female, and to inhibit responding towards a female bearing an odor that was previously conditioned to predict non-receptivity but not the males treated with alcohol.

Alcohol was predicted to disrupt conditioned inhibition in male rats. Since males show an inhibition or a disinhibition of sexual behavior depending on the dose of alcohol injected, these alternate behaviors might be triggered by differential patterns of neural activation. Alcohol's inhibitory and disinhibitory effects on the brain remain unclear. Using immunocytochemistry, this thesis would be the first to assess the induction of Fos IR by the presentation of the conditioned olfactory cue associated with sexual inhibition following injections of saline or alcohol and to determine brain areas involved in alcohol's disruptive effects on conditioned inhibition.

Methods

Behavior

Animals

Males. Sexually naïve male Long-Evans rats (n=44) were obtained from Charles River, Canada (St Constant, QC). Rats weighed approximately 300g at the beginning of the experiment, and were housed in groups of 4 or 5 per cage with food (Purina Rat Chow) and water available *ad libitum*. They were maintained on a reverse 12hour dark-light cycle (lights on at 8:00pm) at ± 21°C. Behavioral tests were performed during the middle third of their dark cycle.

Females. Female Long-Evans rats (n=48) weighing approximately 200g were obtained from the same supplier as the males and were housed in pairs under the same lighting and temperature conditions as the males. To be able to experimentally monitor their sexual receptivity, females were bilaterally ovariectomized (OVX) after being anesthetized with an intraperitoneal (i.p.) injection (1mL/kg of body weight) of a mixture of ketamine (50mg/mL) and xylazine (4mg/mL) in a ratio of 4:3 respectively. OVX was performed via lumbar incision. The females were given a full week to recover from surgery. Following recovery, 38 females were placed in the testing chambers (see below) with sexually vigorous males for five 30-minute sessions of sexual training. Sexual receptivity was induced by subcutaneous injections of estradiol benzoate (10µg in 0.1mL of sesame oil) 48 hours and progesterone (500µg in 0.1mL of sesame oil) 4 hours prior to each behavioral test. The rest of the females were used as non-receptive stimuli (n=10) and did not receive any hormones or sexual training prior to the beginning of the experiment.

All animal procedures were approved by the Concordia University Animal Research Ethics Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Apparatus

Conditioning trials 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 (Mendelson and Gorzalka 1987). Copulatory preference tests took place in a large open field (123cm X 123cm X 146cm) with a layer of bedding covering the floor.
All conditioning trials and preference test were recorded on a video camera and later scored using a PC-based behavioral observation program (Cabilio 1996).

Ethanol treatment

Ethanol was prepared by diluting 95% ethyl alcohol in saline in order to obtain doses of 0.5 $g.kg^{-1}$ and 1 $g.kg^{-1}$ in a 25% v/v solution. The males in the control group were injected with saline solution in the same volume as the highest dose of alcohol. On the copulatory test day, 45 minutes prior to testing, each male was injected with their respective dose of alcohol or saline and were placed individually in a shoebox until testing began. The dose and time course were chosen based on previous evidence showing that blood alcohol levels in rodents are still high under these conditions (Goldstein 1983).

Procedure

Conditioning phase. Males were preexposed to the bilevel chambers for 15 minutes each day, for seven days before the beginning of the experiment. Following this habituation phase, males were given a total of 20 conditioning trials at 2-day intervals, ten with a receptive female and ten with a nonreceptive female. The access to an unscented receptive female or a scented nonreceptive female was alternated on each test day. For all the conditioning trials, each male was placed in a bilevel chamber for 5 minutes of habituation, after which a female in the appropriate condition was placed in the chamber for a 30 minute conditioning trial. Non-receptive females were scented with approximately 0.5 mL of almond extract applied to the back of their neck and 0.5 mL to the anogenital region. The two types of conditioning trials took place in two different rooms with bilevel chambers in each, in order to avoid any remaining almond odor in the chambers during trials with the unscented female.

Copulatory preference test. To habituate the animals to i.p. injections and because of the high volume of liquid injected, each male received an injection of saline in the same volume as the highest dose of ethanol 45 minutes before the last trial of each condition (unscented receptive and scented nonreceptive). This was made to ensure that the injections of alcohol or saline on the test day would not stress the animals and so would not bias the behaviors.

Two days after the last conditioning trial, male rats were randomly assigned to one of the three ethanol treatment groups: 0 $g.kg^{-1}$ alcohol (saline), 0.5 $g.kg^{-1}$ alcohol (A0.5) or 1 $g.kg^{-1}$ alcohol (A1).

Behavioral analysis

All conditioning sessions and copulatory test were videotaped, recorded and later scored. For behavior, each animal was scored individually. The frequency and latency of appetitive level changes during the 5 minutes habituation were recorded for all conditioning sessions (Mendelson and Pfaus 1989). For the last conditioning trial and the copulatory test, frequencies and latencies of mounts, intromissions and ejaculations were recorded and scored.

Statistical analyses

All statistical analyses were performed using SPSS Statistics 17.0 for PC.

To verify that the conditioning procedure did not bias the results, a one-way ANOVA was used to compare group differences in sexual behaviors on the final conditioning day. Following a significant overall main effect, group differences were analyzed using Tukey's *post-hoc* analysis.

A 3x2 mixed design analysis of variance (ANOVA) with ethanol treatment as a between subjects measure (three levels: 0, 0.5, 1 $g.kg^{-1}$) and female type as a within subjects measure (two levels: scented and unscented) was used to determine differences in the distribution and latencies of mounts, intromissions and ejaculations during the copulatory test followed by Tukey's *post-hoc* comparisons. Chi-square analyses were used to analyze the proportions of males that choose the unscented or the scented female for their first mount, first intromission and first ejaculation. For all analyses, the level of significance was set to $p<0.05$.

Immunocytochemistry

Fos activation by odor cue

Following the copulatory test, male rats were given two reconditioning trials in each condition (scented nonreceptive female or unscented receptive female). Two days after the last reconditioning trial, each male was injected with their previously assigned ethanol treatment and exposed 45 minutes later to 1mL of almond odor in the bedding of the exposure chamber (a Plexiglas shoebox cage) and on cotton gauze. At the end of the 60-minute exposure period, they were injected with an overdose of sodium pentobarbital (120 $mg.kg^{-1}$, 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 4hours then switched into a solution of 30% sucrose for 24 to 48hours (until they sunk). Brain were then stored at -80°C until slicing.

Fos immunocytochemistry.

Using a cryostat, each brain was sliced in 30 um 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). Brain sections were always rinsed in three 5-minute periods in 0.9% tris-buffered saline (TBS) between incubations. First, the sections were incubated in 30% hydrogen peroxide (H_2O_2) in TBS for 30 minutes at room temperature followed by a 2-hour incubation in a pre-blocking solution of 3% normal goat serum in 0.2% Triton TBS at room temperature. Next, sections were rinsed in 0.9% TBS and then incubated in a primary antibody solution made of 3% NGS and rabbit polyclonal anti-fos (Fos ab5, Calbiochem, Mississauga, ON; diluted 1:40,000) in 0.05%

Triton TBS for 72 hours at 4°C. After this time, brain sections were incubated for 1 hour in a secondary antibody mixture of 3% NGS and biotinylated goat anti-rabbit IgG (Vector Laboratories Canada, Burlington, ON; 1:200) in 0.05% triton TBS then in a solution of 3% NGS and avidin-biotinylated peroxidase complex (Vectastain ELITE ABC KIT, Vector Laboratories Canada; diluted 1:55) at 4°C for 2 hours. Immunoreactions were stained by incubation, at room temperature, in 50mM tris buffer for 10 minutes, then in a solution of 3,3'-diaminobenzidine (DAB) in 50mM tris buffer for 10 minutes and finally in a mixture of 8% nickel chloride, 3% H_2O_2 and DAB for 10 minutes. In the end, brain sections were then mounted on gel-coated slides and allowed to dry at least 48 hours. The slides were dehydrated in increasing concentrations of alcohol (70%, 90% and 100%, for 10 minutes each), cleared in xylene for 2 hours, cover-slipped and examined under a light microscope.

Histological analysis

Brain sections were examined at 40x magnification. Images of Fos-immunoreactive (IR) cells were capture using ScionImage, and were counted bilaterally from 5 sections per region per rat using Image J. Using the rat brain atlas of Paxinos and Watson (1998), Fos-IR cells were counted, in a standard area of 400μm^2, the prelimbic (PL; plates 7 to 10) and infralimbic (IL; plates 8 to 10) cortices, the piriform cortex (Pir; plates 11 to 18), the nucleus accumbens core (NAccC; plates 11 to 14) and shell (NAccSh; plates 11 to 14), the medial preoptic area (mPOA; plates 18 to 20), the medial amygdala (MeA; plates 26 to 30), the central amygdala (CeA; plates 26 to 30),the

basolateral amygdala (BLA; plates 26 to 30), the ventromedial hypothalamus (VMH; plates 29 to 34) and the ventral tegmental area (VTA; plates 40 to 44).

Statistical analysis

A mean from the 5 sections was calculated for each region for each rat. For each region, a one-way ANOVA was used to compare Fos activation between groups followed by comparisons using Tukey's method. For all analyses, the level of significance was set to $p<0.05$.

Results

Conditioning

Behavioral observations

All male rats reached ejaculation on the last conditioning trial with an unscented receptive female. Only three males mounted the scented nonreceptive female on the last trial. None of them intromitted or ejaculated with the nonreceptive female.

Final conditioning

The results of the last conditioning trial with a scented non receptive female and an unscented receptive female are shown in Figure 1. On the last trial, male rats display significantly more level changes before the trial with the receptive female than before the trial with the nonreceptive female, $t(34) = 4.639$, $p=0.000$ (Figure 1, top)

When males are assigned to their respective groups (saline, A0.5 or A1), there was no difference in sexual performance between males as the ANOVA revealed no significant differences between groups in the number of mounts (ANOVA, $F(2, 39) = 0.898$, $p=0.416$) and the number of ejaculations ($F(2, 39) = 0.735$, $p=0.486$). However, there was a significant difference between groups for the number of intromissions ($F(2, 39) = 3.603$, $p=0.037$). *Post-hoc* analyses using Tukey's method revealed that the males selected for the injection of 0.5 $g.kg^{-1}$ of ethanol intromitted more with the receptive female on the last conditioning trial compared to the rats selected to the injection of 1 $g.kg^{-1}$ of ethanol. (Figure 1 middle)

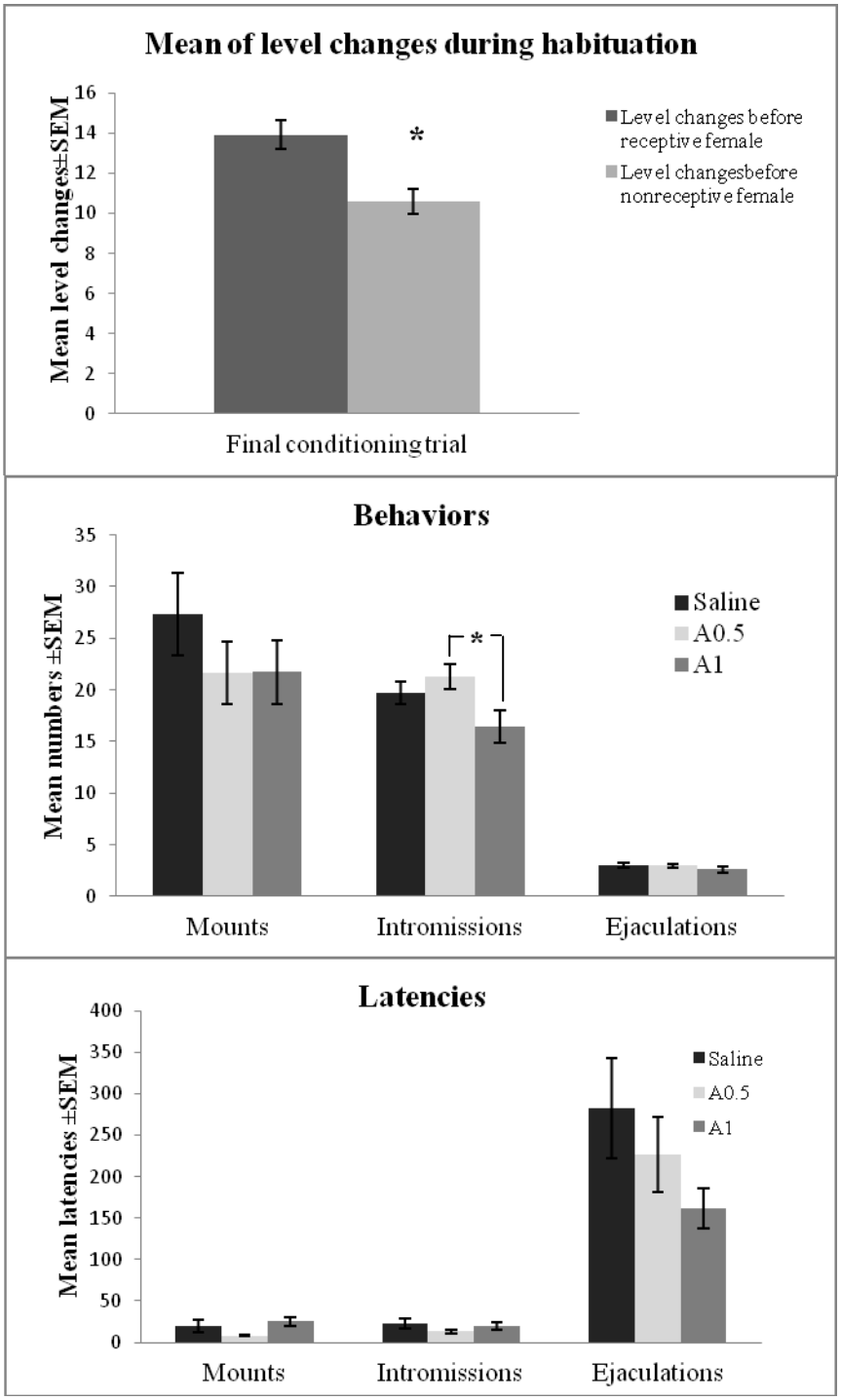

Figure 1. Mean number of level changes before the last conditioning trial with a receptive or a nonreceptive female (top). Mean number of mounts, intromissions and ejaculations during the last conditioning trial with a receptive female (middle). Mean latencies in seconds to first mount, intromission and ejaculation during the last conditioning trial with a receptive female (bottom). Error bars represent the standard errors. (*=$p<0.05$)

There was no difference between groups in the initiation of sexual behavior as confirmed by the ANOVA, for the latency to first mount ($F(2,39) = 2.787$, $p=0.075$), to first intromission ($F(2, 39)= 1.230$, $p=0.304$), or to ejaculation ($F(2,39) = 1.795$, $p=0.180$) (Figure 1, bottom)

Copulatory test

Behavioral observations

On the copulatory test day, one saline-treated male, and one A0.5 male did not ejaculate whereas four males from the A1 group did not ejaculate.

Open field

The data from the copulatory test are shown in Figure 2.

Mounts: Overall, males mounted more frequently the unscented female compared to the scented one. The mixed ANOVA confirmed a main effect of the female type ($F(1,41) = 10.307$, $p=0.03$). *Post-hoc* analyses using Tukey's method revealed that regardless of their groups, males mounted the no odor female significantly more often. The alcohol treatment didn't affect the frequency of mount as there was no significant difference between group ($F(2,41) = 0.006$, $p=0.994$). Also, the interaction between group and female type was not significant ($F (1,41) = 0.003$, $p=0.997$).

Intromissions: Overall, the unscented females received more intromissions than the scented one. Males in the A1 group intromitted less the females than males from the other groups. The significance of these observations were confirmed by the mixed ANOVA as there were main effects of female type ($F(1,41) = 5.528$, $p=0.024$) and of the group ($F(2,41) = 4.770$, $p=0.014$). *Post-hoc* analyses using Tukey's method revealed that

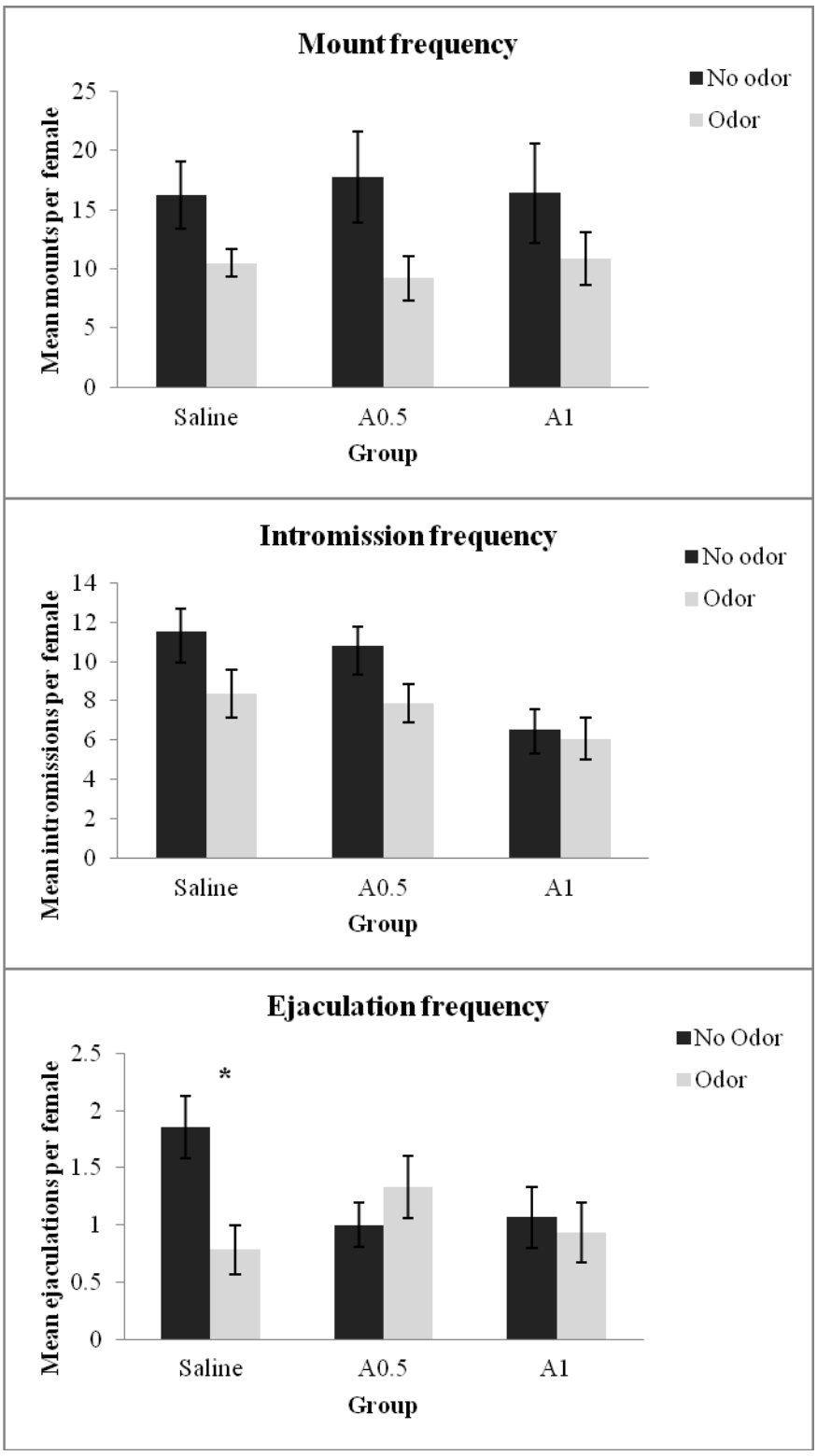

Figure 2. Distribution of mean mounts, intromissions and ejaculations per female during the copulatory test. Error bars represent the standard errors. (*=$p<0.05$)

all males intromitted significantly more the unscented female, and that male rats injected with $1g.kg^{-1}$ of alcohol intromitted less frequently than rats from the two other groups. The interaction between group and female type was not significant ($F(2,41) = 0.864$, $p=0.429$).

Ejaculations: Overall, neither the alcohol treatment nor the female type affected the frequency of ejaculation as there was no main effect of group ($F(2,41) = 1.402$, $p=0.258$) or of female type ($F(1,41) =0.899$, $p=0.349$). Interestingly, males in the saline group displayed significantly more ejaculations with the unscented female compared to the scented one. The significance was confirmed by the mixed ANOVA ($F(2,41) =3.831$, $p=0.030$). *Post-hoc* comparisons revealed that there was a significant difference in the number of ejaculation between the unscented and the scented female for males in the saline group but no significant differences were observed for the alcohol groups.

Figure 3 displays the selection of females for the first mount, first intromission and first ejaculation for each group.

First mount: There was no preference in the male's choice of female for the first mount, in any of the groups. This observation was confirmed by chi square analyses: saline group, scented versus unscented: χ^2 (1, N=14) = 0.286, p=0.593, group A0.5, scented versus unscented: $\chi^2(1,N=15) = 1.667$, p=0.197, and group A1, scented versus unscented: $\chi^2(1,N=15)=0.600$, p=0.439.

First intromission: Regardless of their group, males did not show a preference in their choice of female for their first intromission as confirmed by chi-square analysis:

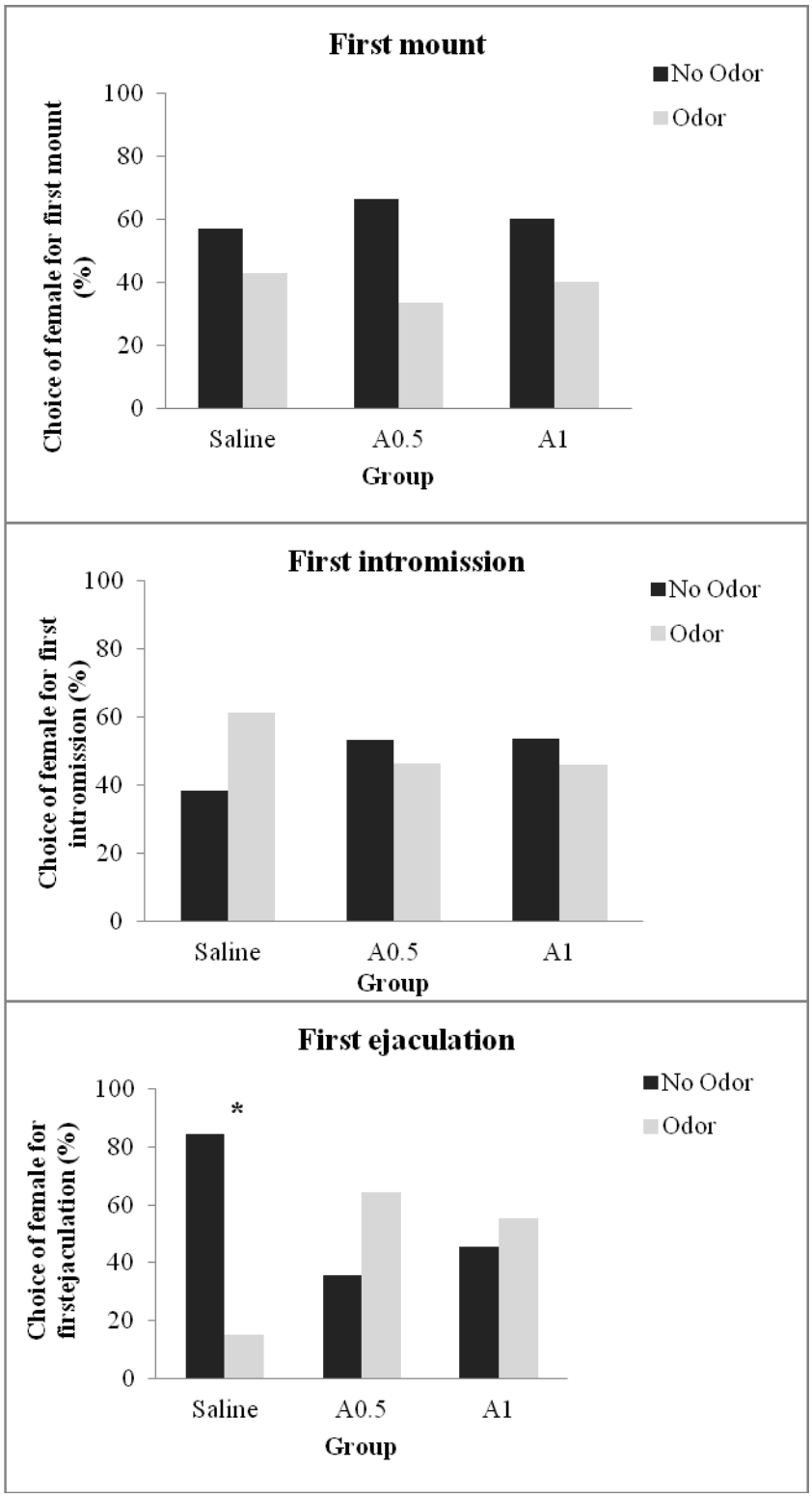

Figure 3. Males choice of female for their first mount, first intromission and first ejaculation during the copulatory test. (Proportions %) (*=p<0.05)

saline group $\chi^2(1, N=13) = 0.692$, p=0.405, group A0.5, $\chi^2(1, N=15) = 0.06$, p=0.796 and group A1, $\chi^2(1, N=13) = 0.077$, p=0.782.

First ejaculation: In the saline group, more males ejaculated first with the unscented female compared to the scented one as confirmed by chi square analysis $\chi^2(1, N=13) = 6.231$, p=0.013. On the other hand, the alcohol groups did not show a preference in the choice of female for their first ejaculation. This was confirmed by chi square analyses: group A0.5: $\chi^2(1, N=13) = 1.143$, p=0.285, group A1: $\chi^2(1, N=11) = 0.091$, p=0.763

Figure 4 shows the latencies to first mount, intromission and ejaculation.

Mount latency: The alcohol treatment and the females (scented or not) did not affect the latency to first mount as confirmed by the mixed ANOVA for female type ($F(1,41)=0.048$, p=0.827), group ($F(2,41) = 2.661$, p=0.082) and group X female type ($F(2,41) = 0.535$, p=0.590).

Intromission latency: Regardless their groups and the female, male rats didn't show a difference for the first intromission latency. This was confirmed by the mixed ANOVA for female type ($F(1,41)=0.118$, p=0.733), group ($F(2,41) = 0.123$, p=0.885) and female type X group ($F(2,41)=0.027$, p=0.973)

Ejaculation latency: Alcohol did not affect the latency to the first ejaculation nor did the females. The mixed ANOVA confirmed that there was no significant difference for female type ($F(1,41)=0.413$, p=0.524) , group ($F(2,41) = 0.976$, p=0.385) and female type X group ($F(2,41)= 0.270$, p=0.765).

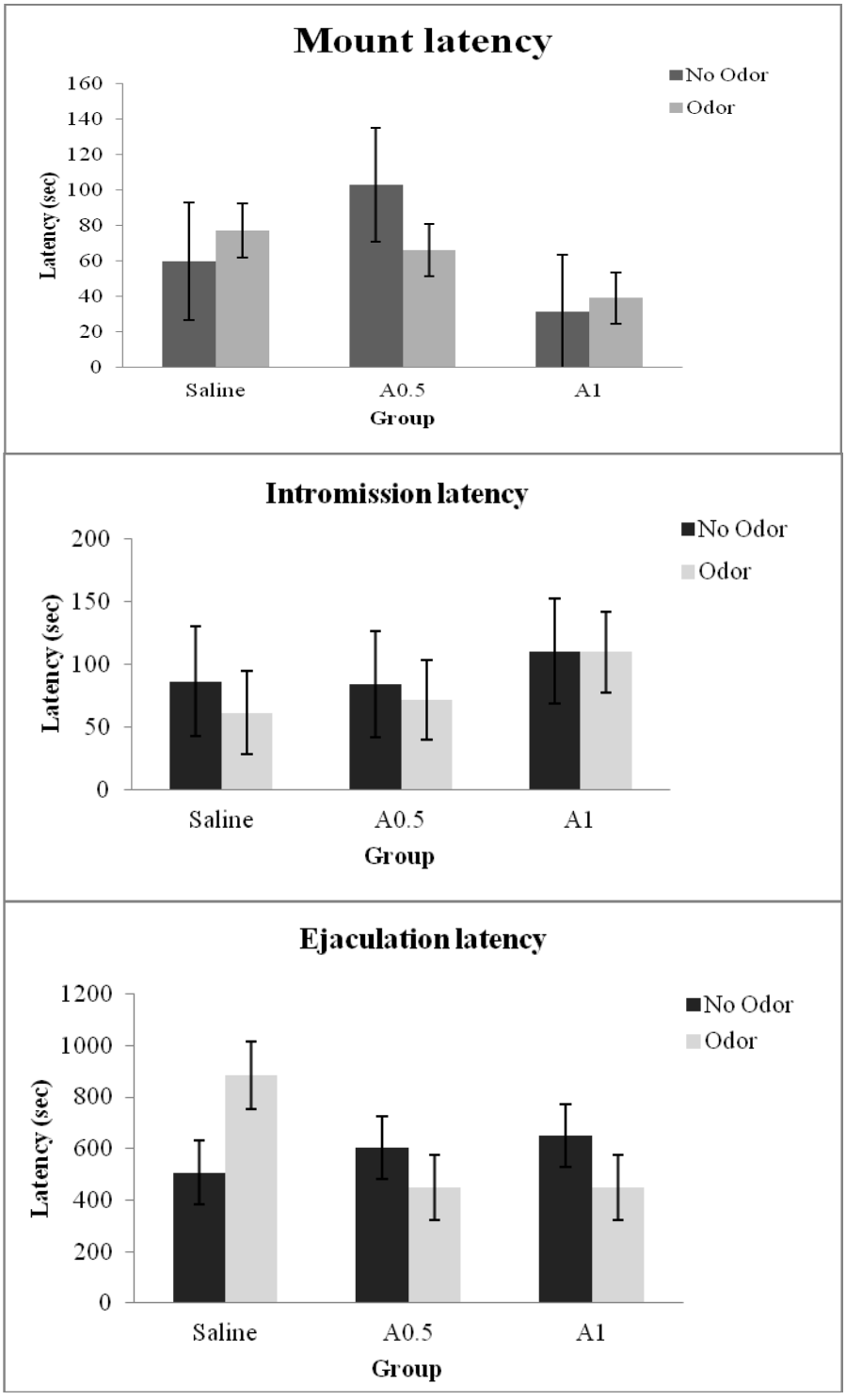

Figure 4: Mean latencies in seconds to first mount, intromission and ejaculation during the open field. Errors bars represent the standard errors.

Fos Immunoreactivity

Following an hour exposure to almond odor, there was a significant difference between groups in Fos IR in the NAccC ($F(2,14) = 26.422$, $p=0.000$), in the BLA ($F(2,14) = 4.235$, $p=0.041$ and in the VMH ($F(2,14) = 5.225$, $p=0.023$) but not in the other areas investigated. Fos IR can be observed on Figures 5 to 7.

The hour exposure to the cue did not induce significant differences between groups in the mean number of Fos IR cells in the PL ($F(2,14) = 2.646$, $p=0.112$) , IL ($F(2,14) = 0.744$, $p=0.496$), Pir ($F(2,14) = 2.191$, $p=0.154$) (Figure 5)

NAccC: The mean number of Fos IR cells in the NAccC was higher in the A0.5group compared to the other groups following the exposure to the almond odor alone as confirmed by the one way ANOVA ($F(2,14) = 26.422$, $p=0.000$). *Post-hoc* analyses using Tukey's method revealed a significant difference between the saline group versus A0.5 group and between A0.5 group versus A1 group. (Figure 5 and 7)

Exposure to the olfactory cue did not produce differences between groups in Fos activation in the NAccSh ($F(2,14) = 0.104$, $p=0.902$), MPOA ($F(2,14) = 0.596$, $p=0.566$), CeA ($F(2,14) = 0.395$, $p=0.682$), MeA ($F(2,14) = 1.657$, $p=0.231$), VTA ($F(2,14) = 0.279$, $p=0.761$). (Figure 5 and 6)

BLA: Males in the A0.5 group showed less Fos activation compared to the other groups. The ANOVA revealed a significant difference between groups in Fos IR cells in this area ($F(2,14)=4.235$, $p=0.041$). The significance in the BLA was verified by *post-hoc* analyses showing a significant difference between the A0.5 group and the A1 group but also a trend ($p=0.092$) between the saline group and the A0.5 group. (Figure 6 and 7)

VMH: Males in the A0.5 group exposed to the odor displayed less Fos IR cells in the VMH compared to males in the other groups. The significance of this observation was confirmed by one-way ANOVA ($F(2,14)=5.225$, $p=0.023$). *Post-hoc* analyses demonstrated a significant difference between the A0.5 group and the A1 group and a trend ($p=0.091$) between the saline group and the A0.5group. (Figure 6 and 7)

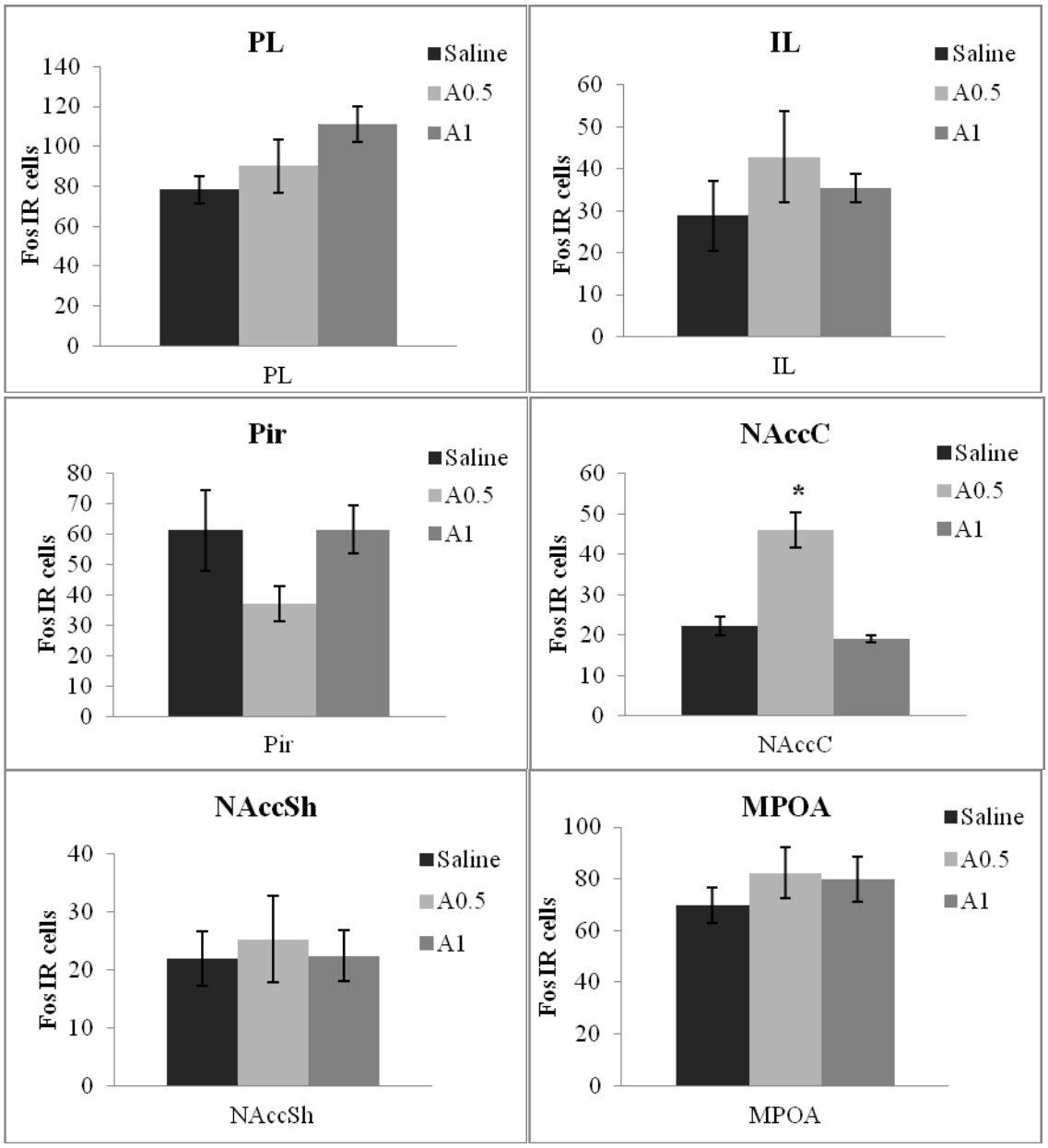

Figure 5: Mean Fos IR cells activated after an hour exposure to conditioned odor in the PL, IL, Pir, NAccC, NAccSh and MPOA. Error bars represent standard errors. (* = $p<0.05$)

Abbreviations: PL, prelimbic cortex; IL, infralimbic cortex; Pir, piriform cortex; NAccC, nucleus accumbens core; NAccSh , nucleus accumbens shell; MPOA , medial preoptic area.

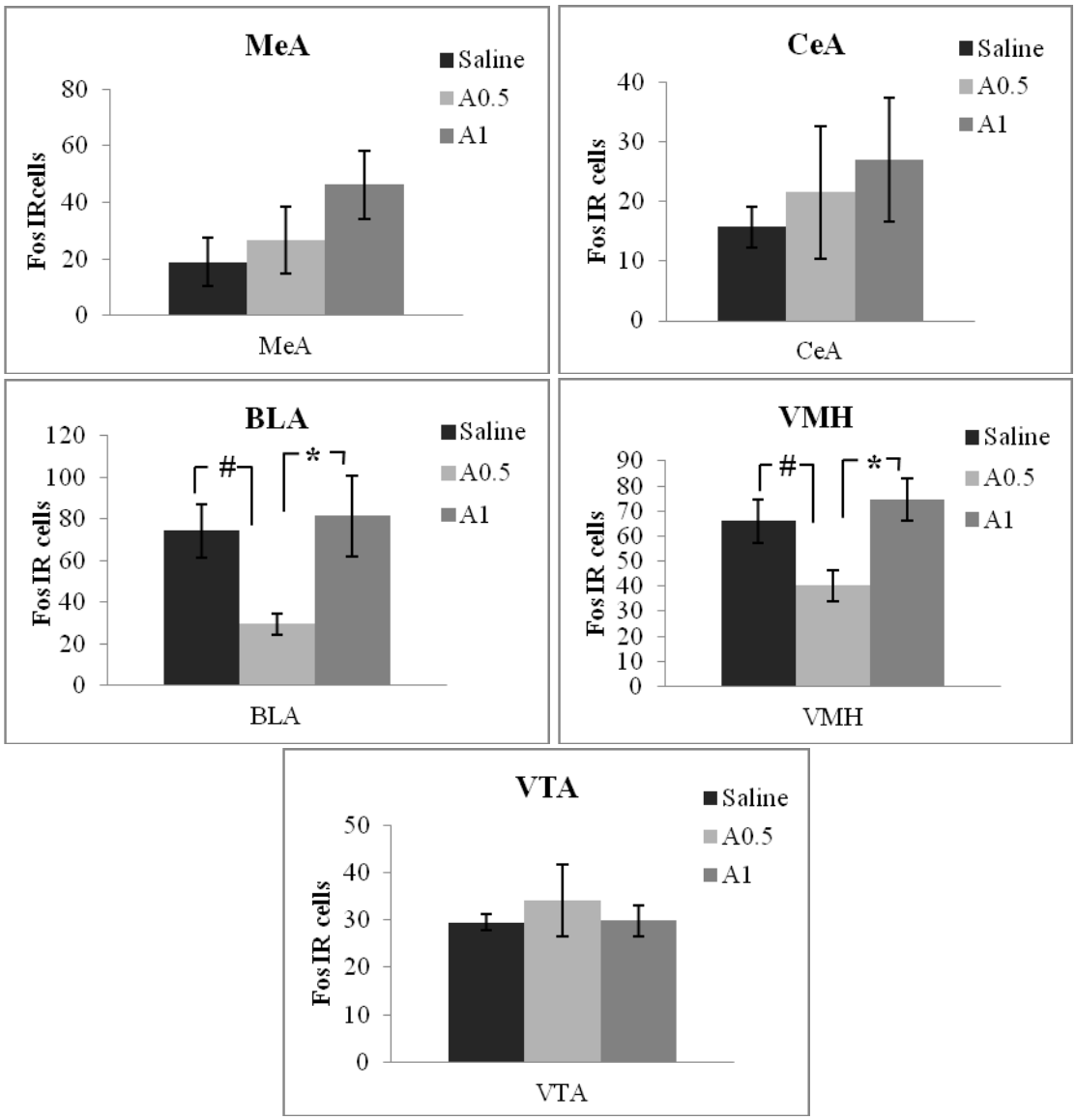

Figure 6: Mean Fos IR cells activated after an hour exposure to conditioned odor in the MeA, CeA, BLA, VMH and VTA. Error bars represent standard errors. (# = $p<0.1$; * = $p<0.05$)

Abbreviations: MeA, medial amygdala; CeA, central amygdala; BLA, basolateral amygdala; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

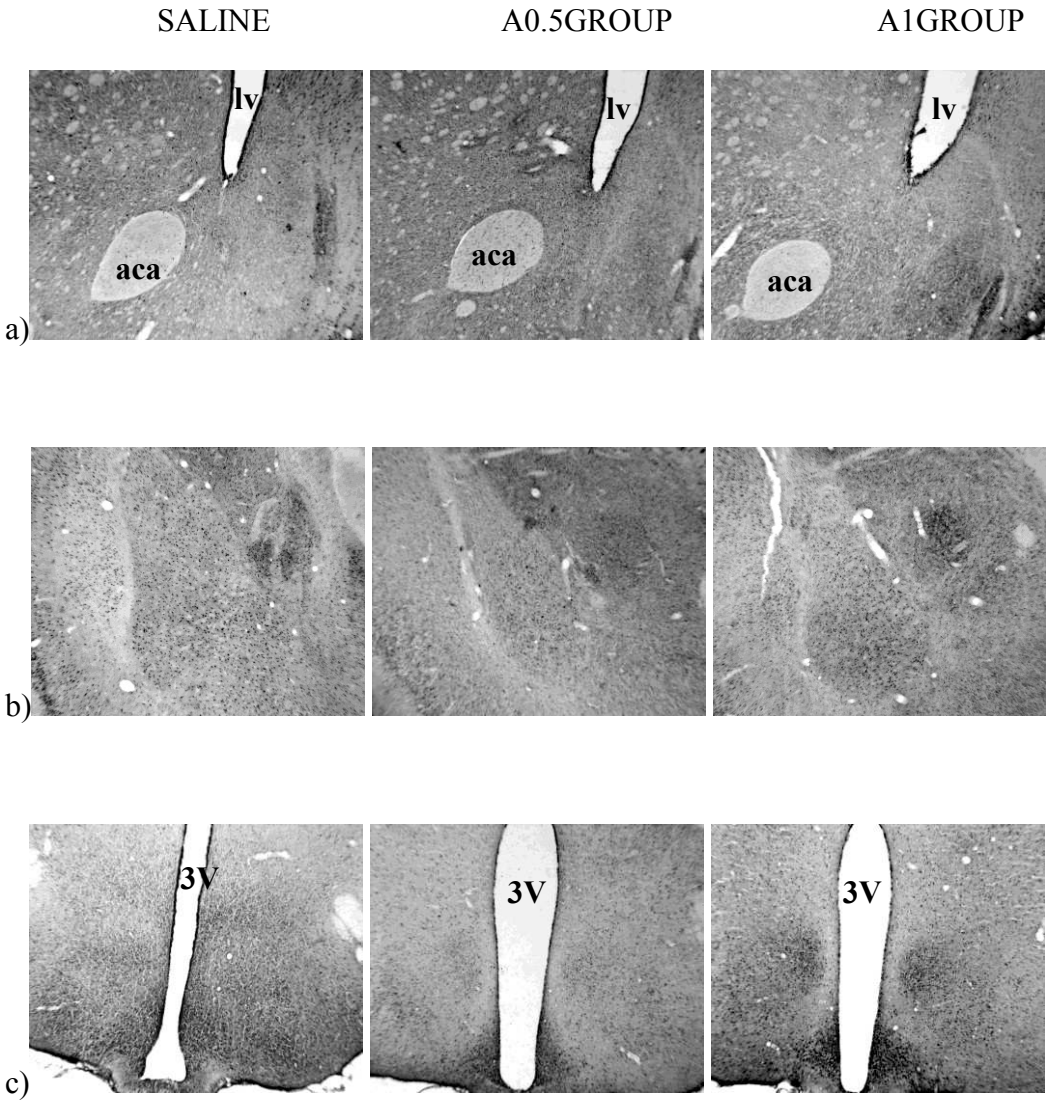

Figure 7: Photomicrographs of Fos activation in the NAccC (a), the BLA (b) and the VMH (c) after an hour exposure to the conditioned olfactory cue.
lv: lateral ventricle, aca: anterior commissure , 3V: third ventricle.

Discussion

For this study, it was predicted that following an injection with saline, male rats would show an ejaculatory preference towards the unscented female during the open-field. It was also hypothesized that males injected with alcohol would not show the preference and that the highest dose of alcohol would induce a disruption of copulatory behavior.

This thesis examined the effects of two different doses of alcohol on male rats' conditioned sexual inhibition and on brain activation following exposure to a conditioned olfactory cue. Males displayed more appetitive level changes on the last conditioning session with the unscented receptive female compared to the last conditioning with the scented nonreceptive female. Also, on the last conditioning with the nonreceptive female, only three males out of the 44 attempted to initiate copulatory behavior. On the copulatory test day, a greater proportion of male rats injected with saline chose the unscented female for their first ejaculation and their frequency of ejaculation was higher for these females. This ejaculatory preference was not observed with the males previously injected with alcohol regardless of dose. The present findings confirm the development of conditioned sexual inhibition toward the scented female in saline-treated males but also the disruptive effects of alcohol on this conditioned inhibition.

The present experiment replicates the effect first reported by Kippin *et al.* (1998) showing that male rats avoid ejaculating with sexually receptive females bearing an almond odor previously associated with sexually nonreceptive females. A significantly greater proportion of males injected with saline before the final open field test chose the

unscented female for their first ejaculation and ejaculated more frequently with the unscented female. During the open field, although males mounted and intromitted more with the unscented females, there was no significant difference within the saline group for these two behaviors. Thus the partner preference was the choice of female for ejaculation (CEP) but not for copulation per se.

Although the frequencies of mounts and intromissions were higher for the unscented females, males in the alcohol groups did not show an ejaculatory preference neither for their first ejaculation nor for the frequency of ejaculations. These data are consistent with previous studies using the dose of 0.5 $g.kg^{-1}$ of alcohol. In the experiments performed by Pfaus and Pinel (1989), male rats injected with this dose 45 minutes before the inhibitory test with a nonreceptive female test showed a significant increase in mounts and ejaculations compared to males injected with saline or 1 $g.kg^{-1}$ of alcohol. They concluded that a dose of 0.5 $g.kg^{-1}$ of alcohol disinhibits sexual behavior in male rats. The present results also demonstrate this phenomenon of disinhibition. In fact, males in the alcohol groups did not display CEP as they did not show preference or avoidance towards the female bearing the conditioned olfactory stimulus originally associated with no sexual activity.

One could argue that the lack of preference for the alcohol-treated males during the copulatory test of the present study could be due to differences between groups during the conditioning phase But analyses of the final conditioning trials demonstrated that only three males mounted the nonreceptive female during the last trial. Further, all males displayed significantly more level changes prior to the last conditioning session with an unscented receptive female than with the non receptive one bearing the almond odor.

This demonstrates a higher sexual motivation prior to the trial with the unscented female (Mendelson and Pfaus 1989). Finally, when males were randomly assigned to their groups, there was no difference in copulatory behaviors between groups. Thus, all rats learned to suppress their sexual behavior towards females bearing the conditioned olfactory stimulus associated with non receptivity of the female. The inhibition acquired during the conditioning phase was abolished following a single injection of alcohol. Thus, the absence of CEP during the copulatory preference test is due to the alcohol treatment and not to impairment in learning.

Although it is clear that male rats learned to inhibit their sexual behavior with a nonreceptive female bearing the almond odor, one alternative explanation for the alcohol-induced disruption of the olfactory conditioned inhibition is that alcohol might have disrupted olfactory processing in general by its anesthetic actions. However, previous studies have demonstrated that acute treatment with alcohol even at high dose (2 $g.kg^{-1}$ of alcohol) did not affect the expression of conditioned odor aversion. Rats conditioned to associate an olfactory stimulus with foot shock develop an aversion to this odor and do not show impairment in conditioned odor aversion after injection of a high dose of alcohol (Lopez *et al.* 1996, Molina *et al.* 1987). Therefore, acute alcohol intoxication does not appear to interfere with the olfactory conditioning, suggesting in the present thesis that an acute treatment with alcohol prior to the copulatory test can lead to a disinhibition of sexual behavior without impairing olfactory processing in general.

The present findings also fit with the theory of alcohol myopia and inhibition conflict proposed by Steele and Josephs (1990). This theory states that under the influence of alcohol, there is a reduction of inhibiting cues that require further processing

and the behavioral response is evoked by strong provoking cues. In the present study, during the open field, there was a strong conflict between internal inhibitory cues (conditioned inhibition towards the odor) and external appetitive cues (two receptive females). In this inhibition conflict, it appears that the presence of the two receptive females is more salient than the inhibition by the olfactory stimulus learned during the conditioning phase. Therefore alcohol intoxicated animals may not have dissociated the female bearing the conditioned olfactory cue from the other and copulate with both of them without preference.

Although the concentration of alcohol used was the same as in Pfaus and Pinel (1989), the highest dose of alcohol (1 $g.kg^{-1}$) had less of a disruptive effect on copulatory behavior in the present study. Like the A0.5 group, males from the A1 group did not show preference for a female. The latter group, however, intromitted with the females significantly less during the open-field but there was no significant difference in mount or ejaculation frequencies or latencies compared to the other groups. A potential explanation might be the open field context. Pfaus and Pinel (1989) observed that the proportion of male rats ejaculating decreased significantly after an injection of $1g.kg^{-1}$ of alcohol compared to the saline group and the two lower doses of alcohol. The major difference between this latter final test and the one in this thesis was the context. Pfaus and Pinel placed their males in a unilevel chamber with only one receptive female whereas in the copulatory test of the present experiment, male rats were placed in a context with two receptive females. The presence and availability of a second receptive female during the test may have modified the effects of the 1 $g.kg^{-1}$ dose of alcohol on sexual behavior. According to McClintock (1984), when more than one receptive female is available, male

rats' sexual behavior can be increased due to a "Coolidge effect". This phenomenon allows males to resume copulation after an ejaculation or after sexual satiety in presence of a novel female (Fisher 1962), suggesting that the males may be more sexually aroused with two females relative to one. The fact that male rats were in the presence of two receptive females for the first time may have produced this phenomenon and counterbalanced the disruptive effects of a high dose of alcohol on sexual behavior.

In addition to the differences observed in the behavior between the saline and the alcohol groups and based on previous immunohistological studies, it was hypothesized that the three groups would show a differential pattern of Fos induction in brain areas previously suggested to be important in male rats' sexual behavior. But there were no differences between groups in the medial preoptic area (MPOA), ventral tegmental area (VTA), nucleus accumbens (NAcc), in the prelimbic (PL) and infralimbic (IL) cortices, nucleus accumbens shell (NAccSh), medial amygdala (MeA), central amygdala (CeA) and piriform cortex (Pir) following an hour exposure to the conditioned olfactory cue. Males from the saline group showed a preference for the unscented female. Therefore the olfactory cue may induce an inhibitory state explaining the absence of Fos activation in areas involved in sexual behavior. Males in the alcohol groups did not display any ejaculatory preference to the females during the open field. This suggests that the olfactory cue was not associated with excitation or inhibition. However, males from the A0.5 group showed significantly more Fos IR cells in the nucleus accumbens core (NAccC) and significantly less in the basolateral amygdala (BLA) and the ventromedial hypothalamus (VMH) compared to males treated with saline or with $1 g.kg^{-1}$ of alcohol,

suggesting a possible involvement of these regions in the disinhibitory behavior observed during the copulatory test.

Following the exposure to the olfactory cue associated with inhibition during the conditioning phase, the A0.5 group showed significantly less Fos-IR cells in the BLA compared to the A1 group and fewer compared to the saline group. This area receives afferents from the cortex, hippocampus, and thalamus allowing integration and processing of conditioned and unconditioned stimuli (Cardinal *et al.* 2002). Many of these inputs are from sensory and somatosensory structures such as Pir, PL and IL. Although, there was no significant difference between groups for the Pir, the BLA and Pir showed the same pattern of Fos activation. The BLA has direct connections with the Pir (Johnson *et al.* 2000). This latter region, that receives information from the olfactory bulb, is involved in olfactory cue recognition. Kippin *et al.* (2003) found an increased Fos induction in both the Pir and BLA only when male rats were exposed to a conditioned olfactory cue previously associated with sexual reward. Taken together, these data suggest that the Pir not only receives olfactory inputs, but that it integrates that input to determine a "meaning" of the cue. The direct inputs from the Pir to the BLA allow the information to acquire its value (incentive or aversive) in the amygdala. The BLA is known to be involved in emotions and in emotional aspects of conditioning but also appears to be important for a conditioned stimulus to restore the value of its unconditioned stimulus (Cardinal *et al.* 2002). Males in the saline group showed a higher number of Fos–IR cells than the A0.5 group in the Pir and the BLA (a trend in this case) suggesting the acquisition of the aversive value of the conditioned odor.

The fact that males in the A0.5 group show less Fos induction in the Pir and BLA suggests that the processing of the olfactory cue may have failed in this group. The almond odor associated with sexual inhibition potentially lost its inhibitory value after an injection of 0.5 $g.kg^{-1}$ of alcohol. This could explain the disinhibition during the copulatory test.

Although both alcohol groups expressed the same pattern of sexual behavior during the copulatory test, there was a significant difference in Fos activation between these groups. This could be explained by the effects of alcohol on BLA neurons. Many studies have tried to characterize the site of action and the mechanisms of ethanol in the brain and on neurotransmission (Harris *et al.* 2009, Valenzuela 1997, Vengeliene *et al.* 2008). One of the most important research areas is the action of alcohol on GABAergic neurotransmission. Electrophysiological studies have demonstrated that the application of alcohol in BLA slices or isolated neurons increased the numbers of inhibitory post-synaptic currents and GABA release. Thus, alcohol activates GABAergic neurons and potentiates GABAergic transmission in the BLA (Zhu and Lovinger 2006). Although these results were on isolated neurons or BLA slices preparations, the potential action of alcohol on these neurons *in vivo* on behavior and in the present case on recognition of conditioned cue cannot be excluded. Therefore, Fos induction of BLA neurons following administration of 1 $g.kg^{-1}$ of alcohol and exposure to the olfactory cue might reflect alcohol's action on GABAergic neurons in the BLA.

At a first glance, the activation of the NAccC following exposure to the conditioned olfactory cue and an injection of 0.5 $g.kg^{-1}$ of alcohol may be inconsistent with previous studies on neural activation following exposure to sex-related olfactory

cues. Because of its implication in attributing salience to incentive cues (Berridge and Robinson 1998), its involvement in appetitive second-order conditioned approach behaviour (Setlow *et al.* 2002), and their findings showing NAccC activation following exposure to olfactory cues previously associated with sexual reward, Kippin and colleagues (2003) suggested that this brain region might be important in processing and acting in response to sexual stimuli. As part of the mesolimbic pathway that goes from the VTA to the prefrontal cortex, the NAccC is involved in all processes of reward and recognition of reward-related cue (Cardinal *et al.* 2002). But it is also involved in the olfactory system with direct projections from the BLA and the accessory olfactory bulb. The BLA largely sends inputs to the NAccC but also to the prefrontal cortex, thalamus, hippocampus (Sah *et al.* 2003). Interestingly, the BLA and NAccC displayed an opposite pattern of Fos induction. As mentioned previously, the olfactory information received and processed from the Pir and BLA to the NAccC acquired its conditioned value. Studies have also demonstrated the role of NAcc in reward seeking and consumption (Nicola *et al.* 2004) and in expectation of reward (Schultz *et al.* 2000). Therefore the NAcc is activated when a reward is expected or consumed. During the final open field test, the odor was presented on a sexually receptive female. It can be speculated that this single test where the inhibitory cue is associated with sexual arousal and reward may have acted as an extinction trial. Extinction appears to involve new learning (reviewed in Bouton 2004). Thus, in a new context (open field) and inner state (alcohol intoxication with the low dose), the olfactory cue may predict a new unconditioned stimulus (sexual reward). It is conceivable that exposure to the olfactory cue alone (new context) following alcohol administration (intoxication) induce expectation of the sexual reward,

so an activation of NAcc neurons. Therefore, the present data suggest that due to the disinhibitory effect of alcohol at a dose of $0.5 g.kg^{-1}$, the almond odor may have acquired an incentive value leading to the activation of NAccC neurons (reviewed in Carelli 2002, Day and Carelli 2007).

It should be kept in mind that alcohol alone has been shown to affect Fos IR in the NAccC (Vilpoux *et al.* 2009). Although the doses used in these studies (1.5 to 3 $g.kg^{-1}$) as well as the time course (2 hours following the injection) were different, it is possible that alcohol alone might have an effect of Fos IR in the present experiment. A further study will investigate the effect of alcohol alone using the same dosage in order to control the involvement of alcohol and of the cue in these Fos IR results.

The A1 group showed low Fos induction in NAccC neurons. The lack of Fos activation may not be due to the value of the conditioned stimulus but to the effect of alcohol itself. This low expression of Fos in the NAccC following administration of a high dose of alcohol is consistent with previous studies on the NAcc and ethanol. Criado *et al.* (1997) injected high doses of alcohol to male rats and recorded NAcc neurons activation after stimulation of the BLA. Alcohol induced a significant decrease in the number of NAcc neurons activated by the BLA but also a decrease in spontaneous activity in these neurons. This suggests that even though males from the A1 group have a high number of BLA neurons showing Fos activation, the dose injected may have inhibited BLA transmission to the NAccC neurons but also the spontaneous activity of these neurons explaining the low Fos activation in this region.

With its role in the control of lordosis (Pfaff and Sakuma 1979), its systems facilitating or inhibiting sexual receptivity (Pfaus *et al.* 2000) and its implication in

female-male mounting (Afonso *et al.* 2009), the VMH is known to be important in female rats' sexual behavior. However, this region also seems to play a role in male sexual behavior. In fact, bilateral lesions of VMH enhance sexual activity (Christensen *et al.* 1977) suggesting a tonic inhibition of male sexual behavior by the VMH. Hormonal manipulations in the VMH also affect sexual behavior in male rats. Harding and McGinnis (2003) showed that implants of testosterone in the VMH restore sexual behavior in castrated male rats and blockade of androgens receptors in the VMH, particularly in the dorsomedial part, impairs copulatory behavior. Immunohistological studies demonstrated Fos activation in the VMH following copulation (Coolen *et al.* 1996) or following exposure to estrous odor previously associated with copulation (Kippin *et al.* 2003).

The data from this thesis are consistent with the inhibition of male rats' sexual behavior by VMH suggested by Christensen *et al.* (1977). As activation of the VMH induces sexual inhibition in male rats, a strong Fos induction in the VMH of male rats treated with saline or with $1g.kg^{-1}$ of alcohol and exposed to the almond would suggest an a potential inhibition of copulatory behavior towards the scented female. Further, males from the A1 group didn't show conditioned inhibition towards the scented female during the copulatory test. Thus, activation of Fos IR cells in the VMH may not reflect conditioned sexual inhibition but copulatory behavior disruption. This would be consistent with Pfaus and Pinel (1989). They found an inhibitory effect of alcohol on sexual behavior at a dose of $1g.kg^{-1}$. In the context of exposure (without receptive females), the effect of the high dose of ethanol cannot be counterbalanced by any contextual effects. Therefore, the activation observed in the VMH may be the result of

the alcohol itself and could explain the disruptive effect of alcohol at that dose. Following the treatment with the 0.5 $g.kg^{-1}$ of alcohol, male rats did not display an inhibition towards the scented female and exhibited a low Fos induction in the VMH. As a possible center of tonic inhibition of male sexual behavior (Christensen *et al.* 1977), inactivation of this area would lead to a disinhibition of sexual behavior, as observed following an acute treatment with a low dose of alcohol

Interestingly, there was no difference between groups in Fos activation in the regions of the prefrontal cortex (PL and IL). It was assumed that environmental cues would activate internal representation within the PFC and that these representations were necessary to induce the appropriate behavior (Miller and Cohen 2001). By initiating inappropriate behaviors, alcohol was believed to act on the PFC to reduce its inhibitory influence (Abrams and Wilson 1983, Kaplan 1974, Wilson and Niaura 1984). This region is also important in the male sexual behavior (Agmo *et al.* 1995, Balfour *et al.* 2006, Hernandez-Gonzalez *et al.* 1997, Pfaus and Phillips 1991). However, recently, it was shown that PL and IL did not affect sexual performance in male rats but were necessary for behavioral inhibition in general (Davis *et al.* 2010). Males with lesions in the PL/IL did not show differences in the expression of copulatory behavior compared to sham animals. However, during the sex aversion conditioning, more males with PL/IL lesions display mounts, intromissions and ejaculations compared to the sham group. Davis *et al.* (2010) concluded that the PL and IL were necessary to suppress sexual reward seeking in aversive conditions. Also part of the reward circuit, the mPFC sends projections to the NAcc, VTA, BLA and can influence sexual motivation and reward (Balfour *et al.* 2006). The fact that PL and IL are involved in both sexual reward and

conditioned aversion could explain the lack of difference in Fos activation following exposure to the conditioned olfactory cue. It would be of interest to compare cell types activated during these conditions and their projections.

In conclusion, the findings of the present thesis suggest that alcohol can affect the conditioned inhibition of an ejaculatory preference by a mechanism of disinhibition. This effect occurs after an acute treatment and regardless the dose administered. The myopic state induced by alcohol altered the ejaculatory preference. This can also be observed at a neural level with a differential pattern of activation between groups. Male rats treated with the low dose of alcohol (0.5 $g.kg^{-1}$) showed a higher induction of Fos-IR in the NAccC, suggesting an expectation of sexual reward when these males are exposes to the olfactory cue and a decrease in the BLA and VMH compared to the males treated with saline. Interestingly, the areas showing a difference in Fos activation are involved in an olfactory pathway or in inhibition of sexual behavior. It appears that the effect of alcohol on sexual behavior is dependent on the nature of conditioned stimuli. It would be of interest to investigate the effects of alcohol on conditioned sexual inhibition using another sensory modality (e.g. somatosensory stimulus) but also in order to establish mechanism of sexual disinhibition by alcohol in the brain.

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