

# Difference in Fos Immunoreactivity in the Medial Preoptic Area of Male Rats and in the Ventromedial Hypothalamus of Female Rats, Post Copulation: The Effect of Sexual Experience on Fos Immunoreactivity

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*In the present study, the distribution of Fos immunoreactivity (IR) in the brain of female and male Sprague-Dawley rats, following one hour of copulation, was measured. The distribution of Fos-IR was induced by sexual behavior in the medial preoptic area (mPOA) of sexually experienced/inexperienced male rats. In addition Fos-IR was also augmented in the ventromedial hypothalamus (VMH) of sexually experienced/inexperienced female rats post-sexual behavior. An increased number of Fos-IR neurons were reported in sexually experienced rats as opposed to sexually inexperienced rats.*

## Introduction

-Fos is a cellular proto-oncogene, a gene that codes for cell growth regulation and differentiation with the potential of causing cancer, that belongs to a gene family of transcription factors. c-Fos transcribes mRNA that encodes for the expression of a particular DNA binding protein, Fos, within the brain (Robertson, Pfaus, Matsumura, Phillips & Fibiger, 1991). The neuronal expression

of c-fos is up-regulated or increased by a variety of physiological and pharmacological treatments (Morgan & Curran, 1989). For example, nociceptive stimulation (Hunt, Pini & Evan, 1987), light stimulation (Sager & Sharp, 1990), as well as caffeine stimulation (Nakajima, Daval, Morgan, Post & Maran-gos, 1989) have all been demonstrated to increase c-fos transcription within specific brain regions and are able to be detected by Fos immunocytochemistry, biochemical laboratory techniques where antibodies bind to specific protein sequences which then can be detected using various methods. c-Fos protein (Fos) has proven to be a valuable substrate in the role of investigative neuroscience, as it has been shown to be overly expressed in various regions of the brain when exposed to a range of stimuli. This research has led to the implication that Fos immunocytochemistry may be used in the mapping of metabolically functional pathways within the brain (Sager, Sharp & Curran, 1988).

The neural circuitry responsible for sexual behavior in both male and female rats appears to be similar in terms of the integration of sensory information. In male rats, the mPOA is suspected to be the brain region responsible for the integration of sensory and hormonal stimulation that proceed the onset of male sexual behavior, whereas the VMH appears to have a similar function within the female rat (Coolen, Peters, & Veening, 1996). Increases in Fos immunoreactivity, the measure of the reaction between an antigen and its antibody used for detection, have been reported in the mPOA after mating in male rats (Baum & Everitt, 1992). Bilateral lesions of the POA have been shown to severely disrupt the initiation of copulatory behavior in male rats (Everitt & Stacey, 1987). Similar studies in male rats have suggested that the lesion of the mPOA does not disrupt the motivational aspects of sexual behavior, whereas it actively inhibits the consummatory facets of sex in the form of copulatory apraxia, the inability to perform sex (Liu, Salamone & Sachs 1997). Investigators have also shown there to be an increase in Fos-IR following sexual behavior in

female rodents within the VMH (Pfaus, Kleopoulos, Mobbs, Gibbs & Pfaff 1993). Bilateral lesions of the VMH eliminate the induction of sexual receptiveness by estrogenic based treatments but do not eliminate the ability for a female rat to show lordosis, a posture of sexual receptiveness characterized by the voluntary inward curvature of the spine (Mathews & Edwards, 1977). The VMN is suspected not to be directly essential for the expression of hormonally induced lordosis behavior, but most likely serve as a neural- structure critical for a lordosis-modulating mechanism within the central dopaminergic system (Okada, Watanabe, Yamanouchi & Arai, 1979). Similar studies indicate the female rat VMH is critical for the modulation of proceptivity and receptivity, as opposed to motivation underlying sexual behavior. Thus according to the literature, the POA and VMH, in male and female rats respectively, may serve as appropriate brain regions in a probe for Fos protein using Fos immunocytochemistry following copulation.

Central dopaminergic systems have been shown to play an important role in the regulation of sexual behavior in rats. Dopamine receptor agonists, such as 3,4-dihydroxy-L-phenylalanine (L-DOPA, the precursor of Dopamine), increase central dopaminergic neurotransmission and increase sexual behavior as well (Bitran & Hull, 1987). The pro-sexual nature of dopamine receptor agonist in humans was first suggested by the observation of increased sexual activity in patients with Parkinson's disease who were being treated with L-dopa (Uitti, Tanner, Rajput, Goetz, Klawans & Thiesens, 1989). L-dopa also has been shown to facilitate erec-

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tions within human male test subjects (Goodwin, 1971). To the contrary, dopamine receptor antagonists have been shown to eliminate or disrupt proper male sexual function within male rats (Pfaus & Phillips, 1989). Psychomotor stimulants that augment

central dopamine neurotransmission increase the number of Fos-immunoreactive neurons in brain regions essential to the central dopaminergic systems, i.e. nucleus accumbens and striatum (Graybiel, Moratalla & Robertson, 1990). The literature suggests that an increase in dopamine neurotransmission in brain areas specific to the central dopaminergic system that underlie sexual behavior, particularly the POA (males) and VMH (females), experience an increase in the quantity of c-fos expressed after a period of copulation (Robertson et al., 1991).

The purpose of the present study was to determine the effects of sexual experience on the number of Fos-IR neurons within the mPOA and VMH of male and female rats post-copulatory behavior. The literature indicates that an increase in copulatory behavior will subsequently lead to an increase in the expression of c-fos in the brain regions specific for the integration of sensory information underlying sexual conduct within a female and male rat brain. Various studies have demonstrated that classical conditioning can produce sexual arousal. It has been reported that placing male rats into a chamber in which sexual intercourse had previously occurred significantly decreases the time interval for a male rat to display penile erections (Sachs and Garinello, 1978). Based on this knowledge, it seemed reasonable to expect that sexual experience would lead to an increase in the number of Fos-immunoreactive neurons within the POA in males and in the VMH in females. It was predicted that trained rats would participate more frequently in sexual behavior within a given period of time, and as a result, would express greater amounts of c-fos post-copulatory behavior. The inexperienced rats were anticipated to spend more time investigating their sexual partners as well as their environment as opposed to actually participating in sexual behavior. This ultimately would result in less c-fos being expressed within the critical brain structures of the female and male rat.

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Sexual receptivity was induced using the standard regime in the female rats(OVX)via a hormone treatment that consisted of a subcutaneous injection of 2  $\mu$ g estradiol benzoate 48 hours prior to each test, followed by a subcutaneous injection of progesterone (.5mg) 4-5 hours before each test.

### **TestsforSexualBehavior**

One week prior to the actual test day, sexually experienced intact male rats (n=4) had their sexual behavior compared to sexually inexperienced castrated male rats (n=4). In addition, the sexual behavior of sexually inexperienced non-hormonally treated OVX female rats (n=4) was compared to that of four sexually experienced hormonally treated OVX female rats (n=4). Both groups of males were given a 15-minute habituation period allowing them to adapt to the environment. Following this habituation period, a hormonally or non-hormonally treated OVX female rat was introduced to the environment. The hormonally treated female rats (OVX)were chosen to participate during the actual test, a week later, as the sexually experienced female group. The intact sexually experienced males were also used, as a sexually experienced group, in the following week's experiment from which data was obtained and analyzed. The male was allowed 10 mounts or 30 minutes with a female,while simultaneously both female and male had their sexual behavior graded based on their receptivity to copulatory behavior.

On the actual test day, males were assigned to one of two groups: intact males with previous sexual experience(n=4)and intact males with no previous exposure to females(n=4).Both groups were placed in an environment that was used in the previous week's comparison study for a habituation period of 15 minutes. The female rats were also divided into two groups: one consisting of OVX hormonally treated rats with previous sexual experience

(n=4) and the other consisting of OVX non-hormonally treated rats with no previous exposure to males(n=4). Both groups of female rats were introduced into the environment following the male rat's habituation period. The female rat's sexual behavior was assessed based on receptivity for 10 mounts. The male rat's sexual behavior was monitored for 30 minutes. The 2 rats were left in this environment for a total of one hour, where the intact males were allowed full access to the female rat.

## **Perfusion**

Immediately after the sexual behavior test the rats were anesthetized using pentobarbital(200mg/kgi.p.), treated intracardially with 1mL of heparin to prevent bloodclots, and perfused intracardially with 50mL of saline followed by 200 mL of 4% paraformaldehyde.

Brains were removed and stored in a refrigerated 20% sucrose/.1M sodium phosphate buffer for a period of 1 week.

## **Animals**

### **Method**

24 Sprague-Dawley rats (purchased from Charles River Laboratory) were used throughout the entire protocol of this experiment. The data that was analyzed from the present study involved a test using 16 of the 24 total rats. There were 12 male rats used in the current experiment, 8 of which were intact males, and 4 were castrated. The male rats, approximately 400-600 grams in weight, were all less than six months in age. All 12 females used throughout the procedure were ovariectomized (OVX), approximately 300-500 grams in weight and less than 6 months in age. Each rat was housed in an individual plastic tub, in a reversed light/dark cycle 12:12. The animal was housed in a vivarium at a temperature of 22° C, with food and water freely available.



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### Sectioning and Histology

One week following the original test day coronal sections were cut at  $40\mu\text{m}$  using a cryostat and collected for both male and female rats. Subsequently the sections were prepared for measuring Fosimmunoreactivity. (For procedure see: Robertson, G. S., Pfau, J. G., Atkinson, L. J., Matsumura, H., Phillips, A. G., and Fibiger, H. C. 1991 Sexual behavior increases c-fos expression in the forebrain of the male rat. *Brain Research*, 564, 352-357)

### Computer Analysis

Ensuing the staining procedure, the brain sections were analyzed using the computer software ImageJ and Mayachitra image. Using this software, the quantity of Fosimmunoreactive neurons was determined, the total area (in pixels) and density (count per pixel) were also reported.

### Results

Following sexual behavior, the induction of Fos-IR was measured in the mPOA of the 2 male rat groups. The descriptive statistics for the sexually experienced male rats that participated in 1-hour of copulatory behavior prior to perfusion depicted a mean Fos-IR count (the number of Fosimmunoreactive neurons) of  $263.5000 \pm 36.65492$  (SEM). The mean Fos-IR count for the inexperienced male rat exposed to the same conditions was  $28.7500 \pm 15.98632$ . Fos-IR was also measured in the VMH for the 2 female rat groups. The mean for the sexually experienced female rat showed a mean Fos-IR count of  $153.0000 \pm 34.21257$ . Inexperienced female rats under the same conditions had a mean Fos-IR count of  $38.2500 \pm 22.79757$ .

A one-way ANOVA test was used to evaluate the effect of sexual experience on c-fos expression in the POA (males) and VMH (females). The present study showed there to be a significant main effect of sexual experience on Fos immunoreactivity within the POA of male rats postcopulatory behavior,  $F_{1,6}=34.46062, p<0.05$ . This study also revealed a significant main effect of sexual experience on Fos-IR in the VMH of female rats following sexual behavior,  $F_{1,6}=7.79040, p<0.05$ .

## Discussion

The results of this study were consistent with the hypothesis presented. Sexual experience in male rats was found to have increased the number of Fos immunoreactive neurons within the mPOA. In addition, sexual experience in female rats (OVX) was observed to augment the number of Fos immunoreactive neurons in the VMH. The literature relevant to this topic supports the finding presented within this paper.

Rats depict a pattern of sexual behavior that has been appropriately described as “opportunistic,” as they will copulate under a variety of circumstances. Unlike the relative uninterrupted intervals of copulation that results in an orgasm in humans, rat copulation consists of bouts of intromissions coupled with a sequence of multiple ejaculations. The male rat’s ability to gain in-

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intromission is completely dependent on the willingness of a sexually responsive female (Pfaus, Kippin & Centeno, 2001). Using a female rat for a sexual behavior study that was not primed with an estradiol/progesterone treatment has been reported to result in increased intromission and mount latencies on the part of the male (Pfaus, 1996). This evidence supports the data that was collected in the present study. Sexually experienced female rats (OVX), primed for copulatory behavior, participated



more often in sexually related behavior as reflected by the Fos-IR data. The present study did not distinguish if the increased Fos-IR in the VMH of sexually experienced female rats was due to the estradiol/progesterone priming treatment due to previous exposure to male rats. A future study may involve determining which condition, sexual experience or hormonal priming, more directly increases sexual activity based on the Fos-IR observed within the female rat VMH following copulation.

Previous studies suggest copulatory experience of having a pronounced effect on sexual behavior and reproductive processes within male rats. When compared to sexually inexperienced males, sexually experienced males have been shown to possess larger testes (Drori & Folman, 1964), heavier penises (Herz, Folman, & Drori, 1969), as well as increased secretions from auxiliary sex glands (Drori & Folman, 1964). Similar to its role in physiological change, sexual experience has been observed to alter behavior in the male rat that is specific for copulation. One such behavior displayed by intact sexually experienced males was the ability to preferably select odors specific to receptive female over those of sexually non-receptive females. This differed from sexually inexperienced males, as they do not display a significant bias based on olfactory cues for receptive or non-receptive females (Carr, Loeb & Dissinger, 1965; Carr, Loeb & Wyllie, 1966). The literature suggests male rat copulation can be also affected by context. Sexually naïve males are more sensitive to the unfamiliarity of a testing situation and as a result will display longer mount and intromission latencies, whereas sexually experienced males are not susceptible to the same disruptive effects of novelty stress on copulation and will respond immediately to the presentation of a sexually receptive female with the initiation of copulation. Sexually inexperienced males, exposed to similar novelty conditions, will ignore a sexually receptive female for a long period of time (Pfaus & Wilkins, 1995). Lastly, male rats with sexual experience have been shown to respect

female sexual resistance and will either actively search for another available female or cease copulation completely (Pfaus, 1996). These studies suggested that sexually experienced male rats would spend more time participating in copulation, as opposed to non-sexually experienced male rats in the presence of a sexually receptive female. The results of the present study, regarding the quantity of Fos-IR neurons in the mPOA of male rats, confirm the observations reported in the literature. Previous studies indicate that sexual experience in the male rat increase subsequent sexual

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performance thus increasing the amount of sexual activity during a period of copulatory behavior has been shown to directly result in the escalation of the quantity of c-fos expressed within the mPOA of the male rat brain. A male rat's sexual experience not only induces change in physiological structures responsible for integrating sensory informational cues that proceeds sexual activity but in observable behavioral characteristics specific for sexual performance as well. Sexual experience in the male rat ultimately increases an individual's sexual performance during copulatory behavior, for it decreases mount and intromission latencies when compared to the copulatory activity of sexually naïve rats.

The current study indicates the male rat can learn when it is necessary to inhibit sexual advances when faced with a sexually nonresponsive female in an efficient manner based on prior experience. These results challenge the notion that rats cannot serve as a human model for sexual behavior because of a lack of advanced cognitive control of sexual behavior. In fact, rats that were sexually experienced displayed increased levels of sexual performance due to the fact they had acquired behavior specific for copulatory activity. In particular, behavior such as increased ability

to differentiate between sexually receptive and non-sexually receptive females and the inability to be affected by the context of an experimental environment amongst the presence of a receptive female are examples of two behavioral traits observed in sexually experienced male rats that would increase their sexual performance and ultimately manifest in an increase in c-fos expression in the mPOA. Using rats as a model for sexual behavior, a future study may investigate administering increasing doses of alcohol to male rats to assess how coital performance is affected by alcohol as a central nervous system depressant. By using c-fos activation as a means of metabolically marking the poly-synaptic pathway involved in copulatory behavior, it would be possible to examine the difference in synaptic activation histologically as well as quantitatively between groups of rats treated with alcohol and those who are not and compare it to their sexual performance. A measurement of Fos IR cells, as such, may provide insight to the afferent and efferent connections of the mPOA and indicate their importance in the integration of sexual and hormonal cues in the male rat. The behavioral aspect of this study could investigate alcohol's effects on sexual performance within experienced male rats and determine if there exists a similar relationship between alcohol consumption and its behavioral effects on male human sexual function.