Effects of Pheromones on Neuroendocrine Regulation of Reproductive Behavior in Male Rats

by

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ABSTRACT

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Neural regulation of male reproductive behavior has been studied extensively through the use of a variety of techniques. Studies have indicated that several brain areas are involved in the regulation of sexual behavior, including the amygdala, hypothalamus, nucleus accumbens, and the corpus striatum.

The amygdala and limbic forebrain structures, along with their connections to the hypothalamus and medial preoptic area, are important for the motivational processes that lead to the initiation of actions. The nucleus accumbens functions as the neural interface between areas of the brain processing the motivational state and those controlling the motor systems. Research has also suggested that dopamine (DA) and norepinephrine (NE) in these brain areas may be involved in the regulation of copulatory activity, but the precise role of these catecholamines has yet to be elucidated.

The present study analyzed the effects of sexual behavior and pheromone exposure on various behavioral parameters as well as on the catecholamine levels in the anterior amygdaloid area (AAA), anterior hypothalamic area (AHA), preoptic area (POA), nucleus accumbens (ACB), and caudate putamen (CPU). Sexually naive male rats were exposed to pheromones for three consecutive days, and then monitored for changes in various parameters of reproductive behavior when placed in an observation chamber with a receptive female. The effects of pheromone exposure were also studied through measurement of catecholamine concentrations (NE and DA) and some of their major metabolites (DOPAC and HVA) in homogenated isolates from discrete brain areas (AAA, AHA, POA, ACB, CPU) by HPLC-ED.

Results suggest that pheromonal exposure has minimal effect on sexual behavior in naive male rats. However, the increased levels of DOPAC found in the CPU and ACB suggest that exposure to pheromones may cause an increase in dopaminergic nerve activity in these brain areas. Determining the precise role of dopamine in the regulation of pheromonal cues will require further investigation.

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ABBREVIATION	DEFINITION
AAA -	anterior amygdaloid area
ACB -	nucleus accumbens
AHA -	anterior hypothalamic area
CPU -	caudate putamen
DA -	dopamine
DOPAC -	dihyroxyphenylacetic acid
E -	epinephrine
EL -	ejaculation latency
GABA -	gamma-aminobutyric acid
HPLC-ED -	high pressure liquid chromatography with electrochemical detection
HR -	hit rate
HVA -	homovanillic acid
I -	number of intromissions
III -	interintromission interval
IL -	intromission latency
M -	number of mounts
ML -	mount latency
MPOA -	medial preoptic area
NE -	norepinephrine
OVX -	ovariectomized
PEI -	post ejaculatory interval
POA -	preoptic area

CHAPTER I

INTRODUCTION

Animals initiate activity, including reproductive activity, for the purpose of biological adaptation and survival. The actions may be triggered by a variety of sensory stimuli from the external environment, or by signals from the internal environment. The actions resulting from these stimuli occur through complex processing and integrative activities of the central nervous system. These actions are comprised of two distinct components. The first component of behavior is known as the anticipatory or motivational stage. Following this stage, the animal displays increased motor activities, which are characteristic of the consummatory stage (Mogenson et al., 1980). Reproductive behavior in mammals, particularly rodents, has often been studied by researchers interested in neural regulation due to its easily distinguishable stereotypic patterns, and the ability to monitor changes in various aspects of this behavior under different experimental parameters.

MEASURES OF SEXUAL BEHAVIOR

The effects of various internal and external stimuli on

reproductive behavior in rodents can be measured in several different manners. One method is to measure the individual components of precopulatory and copulatory behavior in male rats visually.

PRECOPULATORY BEHAVIOR

Precopulatory behavior is commonly measured by the mount latency of the male. This is the most direct measure of male sexual motivation.

COPULATORY BEHAVIOR

There are a number of components of male copulatory behavior that can be observed to provide experimental data. A count of the number of intromissions preceding each ejaculation can be used to indicate the sensitivity of a hypothetical ejaculatory mechanism. Another useful measurement is a ratio referred to as the hit rate or copulatory efficiency. This measurement will indicate changes due to treatments affecting the male's penile sensitivity or erectile potential, as well as reflect the female's behavior. The mean interintromission interval is used as an index of the time required for the male's re-arousal of motivation following the refractory period after intromission.

In addition, the ejaculation latency is measured to assess the ejaculatory threshold of the male (Sachs and Meisel, 1988).

EXHAUSTION

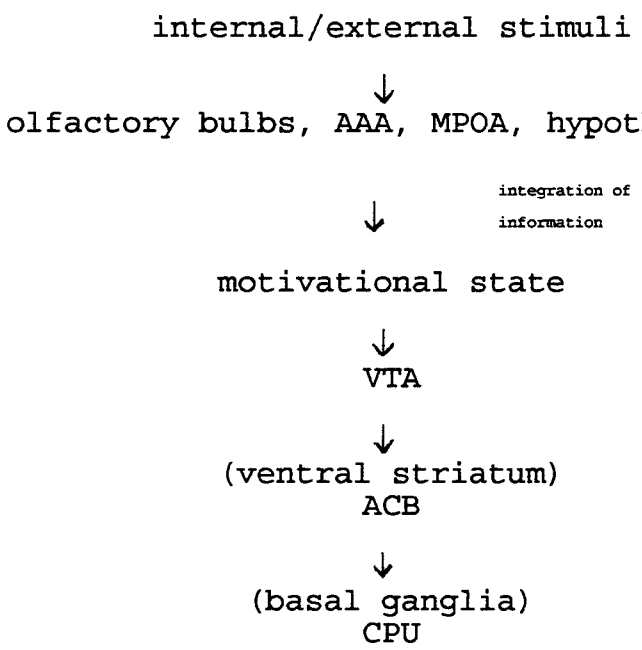
When males are allowed to behave freely with sexually receptive females, they will generally copulate through multiple ejaculatory series until they reach a state of exhaustion or sexual satiety. The method of defining exhaustion varies among different studies. Karen and Barfield (1975) determined a male to be exhausted when the animal failed to intromit within 30 minutes of the prior intromission or ejaculation, whereas Hoffman et al. (1987) allowed a 20 minute time period after ejaculation within which the male must not have mounted the female. This state has a prolonged effect in the males, for they remain sexually inactive for several days. Complete recovery occurs only after the animal has rested from sexual activity for 15 days (Rodriguez-Manzo and Fernandez-Guasti, 1994). The factors causing this refractoriness remain uncertain. There has been evidence of elevated levels of serotonin in the medial preoptic area of exhausted male rats (Hoffman et al., 1987), while another study showed increased levels of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) following sexual satiety. The increase in dopamine metabolite levels could be due to enhanced dopaminergic transmission, or possibly due to interference with dopamine transmission. This finding suggests that sexual refractoriness is not caused by factors such as motor inability or fatigue, but rather by a neural mechanism that affects motivation or sexual behavior (Mas et

al., 1995). A recent study has also shown the ability to reverse sexual exhaustion by affecting serotonergic and noradrenergic functions. Administration of a serotonin agonist facilitated restoration of copulation in exhausted animals, therefore seemingly affecting the consummatory component of behavior. Yohimbine, an α_2 antagonist which causes an increased noradrenaline release, caused an increase in the percentage of copulating animals, therefore affecting the motivational components of copulation (Rodriguez-Manzo and Fernandez-Guasti, 1994). Concurring with these results, McIntosh and Barfield (1984a, 1984b) found that pharmacologically or surgically decreasing central dopamine or norepinephrine levels results in longer refractory periods of normal intact male rats.

NEURAL REGULATION

Neural regulation of copulatory behavior in the male rat has been studied by the use of a variety of techniques including lesion studies (Chen and Bliss, 1974; Giantonio et al., 1970), electrical stimulation (Malsbury, 1971), effects of various pharmacologic agents (Dewsbury, 1975; Hull et al., 1986), and hormonal effects (Gandelman, 1983; Larsson, 1969). Thus far, research on neural regulation of copulation has focused on the forebrain pathways. Studies have shown that the limbic forebrain structures, which include the amygdala and hippocampus, along with their connections to the hypothalamus and medial preoptic area,

play a key role in "drives" and "motivational" processes leading to initiation of actions. However, the mechanism by which limbic processes translate to motor activities is uncertain. The forebrain structures relay the motivational state to the ventral tegmental area, from which it is transmitted to the nucleus accumbens. The nucleus accumbens is a key structure in the pathway, for it serves as the neural interface between brain areas processing the motivational state and those controlling the motor systems. From the accumbens, the information is passed on to the basal ganglia, which is involved in the programming phase of motor control. (Mogenson et al., 1980). The following flow chart is a general neural model for execution of motivated behaviors:



OLFACTORY BULBS

There are two anatomically distinct components to the olfactory bulbs in the rat. The main olfactory bulb is innervated by receptors located in the nasal epithelium, while the accessory bulb receives innervation from the vomeronasal organ. The effect of disruption of the main olfactory bulb can be observed by destruction of the nasal epithelium using zinc sulfate. The role of the accessory bulb can be studied through denervation; the vomeronasal nerve may be cut, or the vomeronasal organ may be removed. The olfactory bulbs play a key role in the neural control of copulation. Initially it was believed that removal of the olfactory bulbs had a purely sensory effect; anosmia. Research has shown, however, that the olfactory bulbs have integrative functions as well. A study done by Cain (1974) implicated the olfactory bulbs as having a role in limbic mechanisms. The study showed that olfactory bulbectomy eliminates interspecific aggression in rats, thus suggesting a role of the olfactory bulbs in the modulation of excitability in the forebrain. The main effect of removal of olfactory bulbs in male rats is on the initiation of sexual behavior; specifically, a decrease in the percentage of animals that will copulate to the point of ejaculation. The cause for this phenomenon has been attributed to an inability to initiate copulatory activity, along with an incapacity to sustain copulation after initiation of the behavior (Meisel et al., 1980). The effect of exogenous

administration of gonadotropin or testosterone was also studied, and showed no changes from the effects of olfactory bulbectomy on copulation (Larsson, 1969). This result indicated that copulatory dysfunction in olfactory bulbectomized rats was not due to an indirect effect of decreased gonadal output (Sachs and Meisel, 1988). In a study conducted by Pfeiffer and Johnston (1994) with male hamsters, removal of the vomeronasal organ resulted in the elimination of androgen surges caused by exposure to female odors in both naive and sexually experienced males. However, the males remained responsive to the females themselves. Lesions of both the vomeronasal system and the olfactory mucosa altered behavioral responses in the male by increasing the latency in initiation of investigation of the female's vaginal secretions.

AMYGDALA

The amygdala, which is a part of the limbic system, also plays an important role in neural regulation of copulation in males. Composed of a group of nuclei embedded in the temporal lobe located at the tail end of the caudate nucleus, the amygdala receives afferents from the genital region. In a study conducted by Turner and Herkenhaum (1991), each nucleus of the amygdala was characterized according to its method of information processing. From this, four amygdaloid systems were proposed. The first system, a unimodal corticomedial amygdaloid system, serves in relaying pheromonal information from the accessory

olfactory bulb to the hypothalamus and medial basal forebrain. The lateral-basomedial system functions in processing of input from various sensory modalities and distributing the information to the basal forebrain and hypothalamus, as occurs in the corticomedial system. The central system is responsible for concentrating viscerosensory information coming from multiple brainstem, thalamic, cortical, and amygdaloid sources, and combining this information with auditory and spinothalamic inputs from the thalamus and cortex. The unimodal basolateral system functions to relay viscerosensory information from the thalamus to the central nucleus of the amygdala and the lateral basal forebrain. The role of the amygdala in copulatory regulation has been assessed primarily by lesion studies in two regions of the amygdala: the corticomedial and the basolateral amygdala. Studies of lesion effects in the basolateral amygdala have found no disruption of copulatory activity; in one study, such lesions were found to in fact cause increased copulatory rate as compared to the control males (Harris and Sachs, 1975). However, corticomedial amygdaloid lesions were found to disrupt male copulatory activity. These lesions were found to increase the ejaculation latencies and decrease the number of ejaculations prior to sexual exhaustion in male rats (Giantonio et al., 1970). The increased ejaculation latencies caused by corticomedial amygdaloid lesions were due to an elevated number of intromissions prior to

ejaculation combined with longer time periods between intromissions (Harris and Sachs, 1975). Studies conducted in hamsters showed the location of the corticomedial lesions in the amygdala to yield varying effects on copulatory activity in males. Copulation was eliminated in animals possessing lesions of the rostral corticomedial amygdala, while caudal corticomedial amygdaloid lesions resulted in less severe suppression of copulation (Lehman and Winans, 1982). Corticomedial amygdaloid efferents of rats pass through the stria terminalis and innervate the bed nucleus of the stria terminalis and the medial preoptic area (Krettek and Price, 1978). The effects of lesions in the bed nucleus of the stria terminalis and in the corticomedial amygdala were found to be very similar. These results suggest that the neurons in the bed nucleus have a minimal, if any, role in the processing of copulatory information. The primary role of this nucleus is to convey information to other brain areas, such as the medial preoptic area, from the amygdala (Sachs and Meisel, 1988).

MEDIAL PREOPTIC AREA

The medial preoptic area's location in the brain is in the vicinity of the olfactory telencephalon, midbrain tegmentum, and the gonadotrophic mechanisms of the hypothalamic region. Thus, it is reasonable to assume that this brain area has some effect on neural regulation of copulation (Heimer and Larsson, 1966/67). This belief was confirmed in a study demonstrating that electrical

stimulation of the MPOA of male rats resulted in accelerated copulatory behavior (Malsbury, 1971). This behavior modification was seen as a reduction in the number of mounts and intromissions preceding ejaculation in rats receiving MPOA stimulations.

Lesion studies of the MPOA have shown this region to be of pivotal importance in neural control of reproductive behavior in the male rat. Studies conducted by Heimer and Larsson (1966/67) showed that large lesions of the medial preoptic area that extend to the rostral anterior hypothalamus eliminated copulatory behavior in male rats. Attempts to arouse males possessing this lesion by handling them, introduction of females, or testosterone therapy were all unsuccessful. Sachs and Meisel (1988) propose that the inability of MPOA lesioned males to copulate may be attributed to a failure to couple sensory information with the proper behavioral response, as opposed to a lack of sexual arousal. However, contrasting results were obtained by Chen and Bliss (1974), who performed unilateral and bilateral lesions of the MPOA either simultaneously or sequentially with bilateral mammillary lesions in sexually experienced male rats. They found mammillary lesions to have no effect on sexual behavior, as opposed to elimination of copulatory behavior with destruction of the MPOA. Partial destruction of the MPOA resulted in increased mount, intromission, and ejaculation latencies; however, the postejaculatory-refractory interval remained unchanged.

These findings led to the conclusion that the MPOA mediates sexual arousal mechanisms.

Van de Poll and van Dis (1977) conducted a study in which they varied the location of the lesion along the medial preoptic-anterior hypothalamic continuum and observed its effect on male copulatory behavior in rats. They found that bilateral lesions located at the transition between the MPOA and anterior hypothalamus resulted in severe copulatory deficits, while lesions that were in the rostral medial preoptic area had less severe disruption of copulation in male rats. Their results led them to conclude that the medial preoptic-anterior hypothalamic continuum is composed of a series of functionally and anatomically distinct units. In addition to this continuum, medial preoptic connections with the midbrain tegmentum have been found to be of importance for male sexual behavior (Brackett and Edwards, 1984). Efferents from the MPOA pass through the medial forebrain bundle and then pass through and/or terminate in the dorsolateral and ventral tegmentum of the midbrain. Brackett and Edwards (1984) showed that bilateral lesions of the dorsolateral tegmentum resulted in ablation of sexual behavior in male rats, as occurred with bilateral lesions of the MPOA. They duplicated this behavioral effect with a unilateral lesion of the MPOA combined with a contralateral lesion of the dorsolateral tegmentum. Therefore, they were able to conclude that the connections between the MPOA and the dorsolateral tegmentum were essential for copulatory

activity. In a related study, Edwards and Einhorn (1986) performed MPOA and dorsolateral tegmentum lesions that eliminated copulatory activity and decreased male rats' preference for a receptive female. These males were then castrated and given testosterone replacement therapy, which had no effect on restoration of preference for the females. Castrated control males without lesions, however, had restored copulatory activity and preference for receptive females when given testosterone. Therefore, they postulated that sex hormones may act on cells in the MPOA and cause increased sexual motivation and behavior. This may occur by a change in the activity of axons from the MPOA to the dorsolateral tegmentum, which primarily function in the mediation of sexual reward.

NUCLEUS ACCUMBENS

The nucleus accumbens is the area that is considered to be the neural interface between the limbic system, which regulates the motivational state, and the brain areas controlling the motor systems and execution of behaviors (Mogenson et al., 1980). There has been anatomical data and behavioral evidence indicating that the nucleus accumbens is innervated by dopaminergic neurons whose locus is in the ventral tegmental area. The ventral tegmental area receives neural connections from the limbic forebrain structures, including the amygdala and hippocampus. Various experiments have also demonstrated that the nucleus accumbens has GABAergic neural connections to the globus pallidus, which

is associated with the basal ganglia motor system (Mogenson et al., 1980). The flow of neural information through these brain areas begins with the messages from the limbic system concerning the motivational state of the animal, which are transmitted to the nucleus accumbens, and then conveyed to the globus pallidus for execution of appropriate actions.

Due to the association of the nucleus accumbens with motivated behaviors, much research has been done on the influence of the nucleus accumbens on sexual behavior, which is a motivated behavior. In castrated male rats, the dopamine levels in the nucleus accumbens were decreased according to a study completed by Mitchell and Stewart (1988). This decrease was able to be prevented by testosterone administration, implying an effect of testosterone on the mesolimbic dopamine system, which mediates sexual arousal. Louilot et al. (1991) looked at dopamine concentrations in the nucleus accumbens of naive male rats exposed to odors from a receptive female, non-receptive female, and an intact male, through the use of voltammetry. Their results showed a significant increase in dopamine levels only in the group exposed to olfactory stimuli from the receptive females, leading to the conclusion that the nucleus accumbens serves in the recognition and discrimination of relevant olfactory cues. These results were confirmed through a similar study using the same experimental parameters, but using in vivo brain microdialysis as their detection method (Wenkstern et al.,

1993). A related study indicated that dopamine levels in the nucleus accumbens increase when experienced male rats are placed in mating chambers with a screen partition between them and the receptive female (Pfaus et al., 1990). This seems to indicate that the odor alone was capable of eliciting the increased dopamine level seen in the nucleus accumbens. The increase was elevated even further in the males during copulation. Striatal dopamine transmission was only elevated significantly during copulatory activity. However, these results may have been due to the fact that mesostriatal dopamine function has been associated with motor variables. In this study, locomotion prior to introduction of females may have elevated the baseline dopamine levels in the striatum. On the other hand, meso-accumbens dopamine function has been correlated with incentive motivation. Exposure to the novel chamber and locomotion were eliminated as possible causes for this increase in dopamine concentration in the nucleus accumbens by monitoring dopamine levels during these conditions in the absence of female odor (Pfaus et al., 1990). Damsma et al. (1992) conducted a related study and achieved similar results that supported this data. However, they found that dopamine levels were not significantly increased in either the nucleus accumbens or the striatum by forced locomotion. Rather, the levels of dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were significantly increased in both regions during

locomotion. These findings further support the conclusion that the anticipatory and consummatory aspects of sexual activity are modulated by dopamine levels in the nucleus accumbens (Damsma et al., 1992), and supports the general role of the nucleus accumbens in processing cues from the animal's environment. However, Barr and Leipheimer (1993) found that bilateral lesions of the nucleus accumbens did not have inhibitory actions on copulatory behavior in male rats. These results serve to indicate that determination of the precise role of the nucleus accumbens in regulating sexual behavior requires further investigation.

NEUROTRANSMITTERS

Neurotransmitters are responsible for conducting impulses across synapses, neuromuscular junctions, and neuroglandular junctions. These chemicals are synthesized by the neuron, and are usually composed of amino acids. In the brain, there are numerous substances that are known or suspected to be neurotransmitters. These substances may be facilitatory, excitatory, or inhibitory to the postsynaptic neurons. Norepinephrine and dopamine are both neurotransmitters found in the brain that are subclassified as catecholamines, and can be further subclassified as monoamines (Tortora and Anagnostakos, 1987).

Many studies have implicated neurotransmitter influences on sexual behavior in males. Several results have shown that manipulations of various monoamine levels

have effects on copulatory behavior in male rats. Dewsbury (1975) demonstrated that depletion of monoamines by systemic injections of reserpine or tetrabenazine resulted in accelerated copulation in male rats. The reverse was also shown by inhibiting the degradative enzyme, monoamineoxidase, which caused increased monoamine levels resulting in decreased copulatory rate. However, this study did not pinpoint the effects of the individual transmitters, or allow insight into possible interactions among them. In addition, studies in which pharmacologic agents are used in the manipulation of neurotransmitter levels have the possibility of affecting the transmitter systems themselves. Therefore, results obtained through such studies must be analyzed carefully (Sachs and Meisel, 1988).

NOREPINEPHRINE

Norepinephrine is concentrated in a group of neurons called the locus coeruleus found in the brain stem, in close proximity to the fourth ventricle. These neurons project axons to the hypothalamus, cerebellum, cerebral cortex, and spinal cord (Tortora and Anagnostakos, 1987). The noradrenergic system's effect on copulatory regulation in males has been researched primarily through the use of an α_2-noradrenergic receptor antagonist, yohimbine. This compound has been shown to stimulate copulation in male rats. A study conducted by Clark et al. (1985a) indicated that yohimbine caused decreased interintromission intervals, resulting in a decreased ejaculatory latency. In a related

study, yohimbine facilitated intromissions in 50% of castrated males (Clark et al., 1985b). These results indicate that blockage of α_2-noradrenergic receptors stimulates copulation to occur, as well as accelerates various facets of copulation once initiated.

Prazosin, an α_1-noradrenergic receptor antagonist, has also been studied as to its effects on copulation (Clark et al., 1985b). The use of this compound, in sexually experienced rats slowed the copulation rate, as compared to a previous control test in the same males. Thus, it appears that blockage of α_1-noradrenergic receptors has a reciprocal effect on copulation as compared to yohimbine.

An α-noradrenergic receptor agonist, clonidine, was also studied as to its effects on sexual behavior. Administration of clonidine to intact sexually experienced male rats demonstrated a dose-related response on suppression of copulation; higher doses were more effective in elimination of intromission and ejaculation. Clonidine's effects were antagonized by yohimbine, but not by prazosin. Therefore, it was construed that clonidine was an α_2-noradrenergic receptor agonist. These results also suggest that α_1- and α_2-noradrenergic receptors have opposite effects on copulation, and that norepinephrine modulates copulation through both receptor system's interactions (Clark et al., 1985a).

Studies have also been done to pinpoint the effect of norepinephrine in various regions of the brain on male

sexual behavior. For example, Dluzen and Ramirez (1989) found that norepinephrine levels in the olfactory bulbs of freely behaving male rats tripled upon introduction of a receptive female, as measured by push-pull perfusion. No increase in norepinephrine was seen when the perfused males were exposed to other males, or in the absence of social stimulation. A subsequent study further isolated the role of olfactory bulb norepinephrine by demonstrating that depletion of olfactory bulb norepinephrine through the use of 6-OHDA disrupted the chemical cue, but not the social recognition response in male rats (Guan et al., 1993).

DOPAMINE

There have been numerous studies indicating that elevated dopamine levels facilitate copulation in males by increasing sexual arousal in the presence of the appropriate stimuli. Evidence supporting this conclusion was mainly derived from studies using administration of dopamine agonists, such as apomorphine and L-DOPA, which is a synthetic precursor of dopamine. These agonists serve to decrease the latency in initiation of copulation, decrease the number of intromissions prior to ejaculation, decrease ejaculatory latency and the post-ejaculatory interval, and stimulate copulatory behavior in sexually inactive male rats (Blackburn et al., 1992). For example, a study conducted by Tagliamonte et al. (1974) demonstrated that systemic administration of L-DOPA along with a peripheral decarboxylase inhibitor which maximizes L-DOPA availability

to the brain, resulted in reduced numbers of pre-ejaculatory intromissions, a reduced ejaculatory latency, and a reduced postejaculatory interval in male rats. This treatment also stimulated some inactive males to copulate to ejaculation. Apomorphine administrated systemically also stimulated copulation in sexually inactive males (Tagliamonte et al., 1974). Baum and Starr (1980) found that the dopamine-receptor agonists spiperidone and clozapine decreased the copulatory rate in male rats that had been castrated and given implants of testosterone, estradiol, or dihydroxytestosterone to maintain copulatory behavior. Localization of the dopaminergic activity within the brain has also been shown through the use of various dopamine agonists in specific brain areas. Hull et al. (1986) microinfused apomorphine into the medial preoptic area of male rats and found that this increased the copulatory rate. This result points to a facilitative role of MPOA dopamine in the regulation of copulatory activity in male rats.

The role of dopamine regulation of copulation in male rats has also been studied through methods measuring dopamine activity in the brain. Fumero et al., (1994) used microdialysis to measure the changes in the main dopamine metabolite, DOPAC, in three brain regions of copulating male rats. The DOPAC levels in the nucleus accumbens, medial preoptic area, and medial basal hypothalamus were fairly stable when the males were exposed to castrated females, but they increased during mating with receptive females,

suggesting a change in dopamine turnover in the forebrain areas associated with male sexual behavior. A study conducted by Hull et al. (1992) measured dopamine activity in the medial preoptic area of male rats by monitoring the levels of dopamine and its metabolites, DOPAC and HVA, before, during, and after copulation, using microdialysis. They found increased levels of the metabolites in microdialysates collected during copulation, which further supports previous findings indicating that MPOA dopamine enhances copulation in male rats. Mas et al. (1987) measured changes in the concentrations of dopamine and DOPAC in male rats following copulatory activity with receptive females as compared to the non-mating control males exposed to other males. High pressure liquid chromatography with electrochemical detection (HPLC-ED) was used to detect the levels of these compounds in the parietal cortex, preoptic region, mediobasal hypothalamus, and lumbosacral spinal cord. The results indicated that sexual arousal is associated with an increase in dopaminergic activity of the preoptic area, along with inhibition of monoaminergic signals in the lumbosacral cord. Ejaculation was associated with an increase in both serotonergic and dopaminergic activity in the preoptic region. In the study conducted by Hoffman et al. (1987) using HPLC-ED to detect levels of monoamines and metabolites in various brain regions also found increased dopamine concentrations in the medial preoptic area of both exhausted and active control male

rats, suggesting that this increase may have been due to locomotor activity.

A review by Melis and Argiolas (1995) elucidated the neuronal systems through which dopamine controls various aspects of sexual behavior. The nigrostriatal dopamine system, which originates in the substantia nigra and sends nerve endings to the striatum, was found to control sensory-motor coordination needed for copulation. The mesolimbic dopamine system, beginning in the ventral tegmental area and ending with innervation of the nucleus accumbens, along the mesocortical system are important primarily in sexual arousal and motivation. The incertohypothalamic dopamine system is key in regulation of consummatory behavior, primarily dealing with control of seminal emission and erectile performance, but it may also have some role in sexual motivation.

Overall, the results of these various studies suggest that the hypothalamic dopamine terminals are important in the facilitation of copulatory rate, ejaculation, and penile reflexes, whereas dopamine terminal regions in the forebrain, especially in the nucleus accumbens, play a key role in initiation of copulation and conditioned sexual arousal and motivation (Blackburn et al., 1992).

PHEROMONES

There have been many questions, as well as discoveries, regarding how pheromones influence reproduction in mammals.

It is known that many mammals depend upon chemical signals in order to identify members of the opposite sex and determine their reproductive status. Communication by these chemosignals depends upon reception and production of these compounds. The primary receptor for airborne chemosignals is known to be the main olfactory system. The receptors are ciliated neurons with axons that extend to the olfactory bulb, passing through the cribiform plate (Vandenbergh, 1988).

There are three chemoreceptor systems that may play a role in the reception of pheromones affecting reproductive behavior. The first of these systems is the septal organ, which is a patch of tissue on the nasal septum anterior to the main olfactory epithelium. The trigeminal system is thought to be involved in responses to strong, irritating odors, and it may also be responsible for modulating olfactory bulb activity. The function of the nervus terminalis system is less understood. The neurons of the nervus terminalis have been found to contain high levels of immunoreactive luteinizing hormone-releasing hormone (LHRH), indicating a possible significance of this structure for reproduction (Vandenbergh, 1988). However, a study by Wirsig-Wiechmann (1993) which used terminal nerve lesions to look at the involvement of the nervus terminalis in pheromonally induced testosterone surges in male hamsters found that these lesions did not alter the increase in serum testosterone levels following exposure to odor from an

estrous female. This result indicates that the nervus terminalis may not be involved in this pheromonally-induced neuroendocrine reflex.

The vomeronasal organ's chemosensory functions is also not well understood. There have been studies indicating that it may be important as a receptor for priming pheromones, which are chemosignals that drive reproductive functions (Meredith, 1983). Several reproductive events have been shown to be dependent on a functional vomeronasal organ. For example, Johns (1980) demonstrated that in vomeronasalectomized rats, the male priming effect is decreased. Pfieffer and Johnston (1994), found that removal of the vomeronasal organ in male hamsters eliminated androgen surges induced by exposure to female vaginal secretions in sexually experienced and naive animals.

Mammals are known to release a wide variety of odors to their environment from their skin glands as well as excretory products. However, not all body odors serve as chemosignals (Vandenbergh, 1988). Male rats have been shown to be able to differentiate between odors from a male versus a female, as well as show a preference for odor from an estrous female as opposed to a nonreceptive diestrous female (Vandenbergh, 1988). The male rat's odor detection performance may be enhanced by testosterone treatment (Gandelman, 1983), indicating that neural regions involved with preference for female odors may be mediated by androgen levels. Sexual experience or learning has also been found

to be important in male rats' discrimination and preference for estrous female urine as opposed to diestrous female urine (Carr et al., 1965).

There are definite advantages in emission of pheromones during or before the fertile time period for females. This allows the females to ensure a male's presence when required for conception to occur (Vandenbergh, 1988). Exposure of male rats to urine from estrous females, which contains pheromones, has been shown to result in increased precopulatory activity in males (Hlinak, 1984). In some species, the males also emit chemosignals that have an effect on reproductive behavior. However, these male pheromones seem to be more related to social than reproductive status, and may play a role in the female's mate selection process (Vandenbergh, 1988).

More recently, studies have investigated the localization of the pheromonal influences in the brain, and the effects of these chemosignals on various catecholamines. An earlier study conducted by Dluzen and Ramirez (1983) demonstrated that there were discrete changes in olfactory bulb norepinephrine and dopamine levels in male mice following exposure to females as opposed to males. These results implicated a possible role of the olfactory bulb in the processing and coding of chemical cues received during social interactions. In a related study (Dluzen and Ramirez, 1987), they implanted push-pull cannulas in the olfactory bulbs of freely moving male rats, and monitored

the in vivo release of catecholamines. Their results showed significant increases in the output of norepinephrine following the introduction of a receptive female, suggesting that norepinephrine may play a key role in the processing of information from chemical cues in the olfactory bulb. Mitchell and Gratton (1991) eliminated the social interaction aspect as a possible contributing factor to the changes in neurotransmitter levels by exposing male rats to the soiled bedding of other males, ovariectomized females, or estrous females. High speed chronoamperometry was used to measure oxidation and reduction currents of the monoamines as they were released within the nucleus accumbens and caudate putamen of freely moving male rats while they were exposed to the bedding. They found that the estrous bedding strongly increased the electrochemical signal obtained from the nucleus accumbens, and that dopamine was the primary contributor to this increase. These findings indicated that pheromones may facilitate sexual behavior by activating the mesolimbic dopamine system.

Although much progress has been made in elucidating the role of pheromones in sexual behavior and their mechanisms of action, there remain many questions yet to be answered. In the first aspect of the present study, the role of pheromonal stimulation on reproductive behavior in naive male rats was observed. Furthermore, the second aspect of the study compared the differences in catecholamine levels

and their metabolites in sexually experienced versus sexually exhausted male rats, and also analyzed the role of pheromonal exposure on the expression of these catecholamines and metabolites.

CHAPTER II

MATERIALS AND METHODS

EXPERIMENT 1

This experiment was designed to assess the effects of pheromonal stimulation on reproductive behavior in naive male rats.

ANIMALS

Twenty-nine sexually naive male Long-Evans rats age 6-12 months were housed separately in groups of two to four with a reversed 12h light: 12h dark cycle, with lights off between 1000 and 2200h. Food and water were available ad lib. Three female rats were bilaterally ovariectomized (OVX) and used as controls in the experiments. For each behavioral testing session, six female rats were brought into estrus by injection of 0.1 ml estradiol benzoate (50 μg/0.1 ml) 48 hours prior to testing and injection of 0.1 ml progesterone (500 μg/0.1 ml) 5-7 hours before testing. Urine and fresh soiled bedding were collected from control ovariectomized females and estrous females for exposure to males.

PROCEDURE

Twenty-nine naive male rats were randomly divided into control and experimental groups, with fifteen males exposed

to urine and soiled bedding of ovariectomized females (control group), and fourteen males exposed to urine and soiled bedding of estrous females (pheromone exposure group). Two different methods of exposure of males to female odors were used. In the first method, frozen collected urine from estrus or ovariectomized females was thawed and applied to the male rats' nose using a cotton swab at the same time each day for three consecutive days. In the second method, frozen urine collected from estrus or ovariectomized females was thawed and added to fresh bedding collected from estrus or ovariectomized females, respectively. The male rats were exposed to the bedding with urine for one hour each day for three consecutive days. On the fourth day, the male rats were placed into glass observation chambers and allowed to acclimate for five minutes. An estrous female was then added to the chamber, and the male was monitored for sexual behavior. The parameters observed were mount latency (ML), measured from the time of introduction of the female to the first mount; intromission latency (IL), recorded as the time from introduction of the female to the first intromission; number of mounts (M), counted as the total number of mounts preceding the first ejaculation; number of intromissions (I), counted as the total number of intromissions preceding the first ejaculation; hit rate (HR), calculated by dividing the number of intromissions by the sum of the total number of mounts plus number of intromissions; ejaculation

latency (EL), measured as the time from the first intromission to the first ejaculation; post ejaculatory interval (PEI), measured as the time from the first ejaculation to the following intromission; and interintromission interval (III), calculated as a ratio of the ejaculation latency to the number of intromissions. The average time span for sexually naive males to complete one ejaculatory series (from introduction of female to the first PEI) was approximately one hour.

The following table illustrates the various odor exposure groups, along with the number of animals exposed by each method.

GROUPS:

OVX ODOR EXPOSURE		PHEROMONE EXPOSURE	
METHOD	# OF MALES	METHOD	# OF MALES
- swab:	9	- swab:	8
- bedding:	6	- bedding:	6
TOTAL	15	TOTAL	14

STATISTICAL ANALYSIS

The differences in the percentages of males in each group that showed a positive response to receptive females were analyzed using a chi-squared test. The various parameters measured were analyzed for differences between the treatment groups by unpaired t-tests (Sigma Stat, Jandel Scientific).

EXPERIMENT 2

This experiment was designed to analyze the differences in catecholamine levels and their metabolites in sexually experienced versus sexually exhausted male rats, as well as assess the role of pheromonal exposure on the expression of these catecholamine and metabolite levels.

ANIMALS

Twenty-two sexually-experienced Long-Evans rats age 6-12 months were housed separately in groups of two to four with a reversed 12h light: 12h dark cycle, with lights off between 1000 and 2200h. Food and water were available ad lib.

Prior to the experiments, all males gained sexual experience by being paired with receptive females and ejaculating at least twice in separate test sessions. These males were then assigned to either receive ovariectomized odor exposure, or pheromone odor exposure. This odor exposure was administered by placing the males into clean glass observation chambers containing either fresh soiled bedding plus urine collected from an ovariectomized female, or fresh soiled bedding plus urine from an estrous female, respectively. Within each odor exposure group, half of the males were sexually experienced (control), and the other half were sexually refractory (exhausted). The exhaustion of the animals was also conducted prior to exposure of the animals to their experimentally assigned odor. The odor

exposure procedure occurred in the dark, with a minimum exposure time of one hour.

The following table indicates the number of animals assigned to the control and to the exhausted conditions within each odor exposure group.

GROUPS:

OVX ODOR EXPOSURE		PHEROMONE EXPOSURE	
CONDITION	# MALES	CONDITION	# MALES
- control:	6	- control:	5
- exhausted:	6	- exhausted:	5
TOTAL	12	TOTAL	10

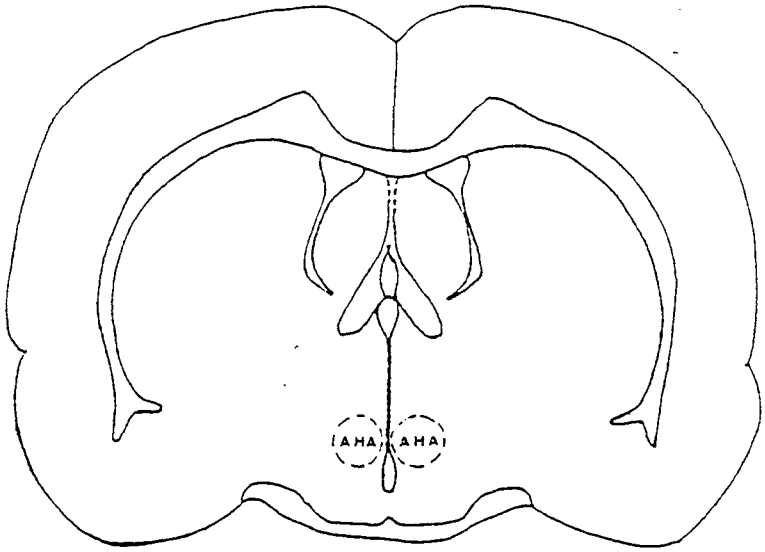
SEXUAL EXHAUSTION PROCEDURE

The males assigned to the sexually exhausted condition were placed in individual glass observation chambers. Following a five minute adaptation period, a receptive female was placed in each chamber. Each male was allowed multiple ejaculations with the same female until he failed to intromit during a one hour period after the last ejaculation. All males assigned to the sexually refractory parameter were exhausted on the day preceding tissue preparation. The exhaustion procedure for each rat required between 2 to 5 hours.

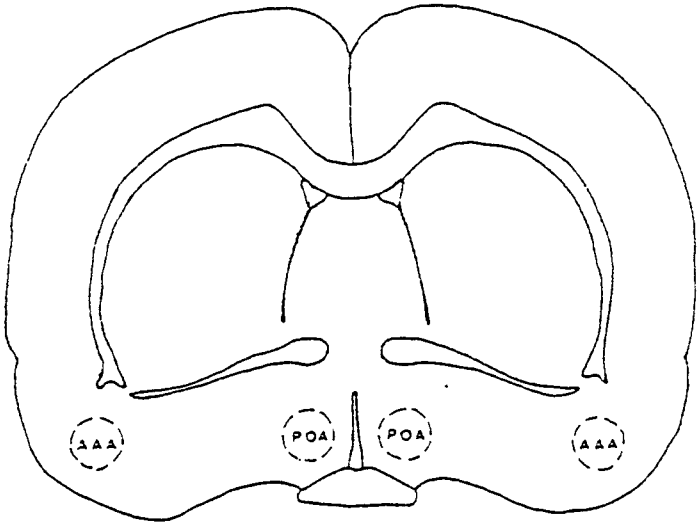
TISSUE PREPARATION

After the assigned bedding exposure, the males were anesthetized with carbon dioxide to the point of drowsiness. The males were then quickly decapitated and their brains were immediately extracted. Each brain was then placed in a beaker of ice cold 0.1 M phosphate buffered saline for

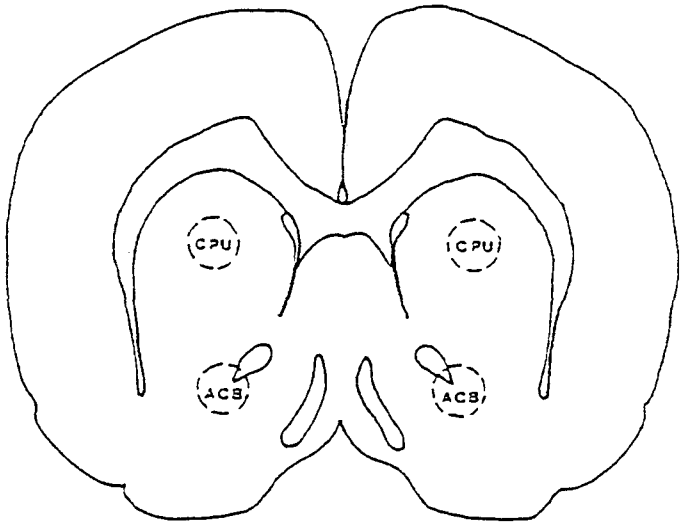
rinsing. The brain was then transferred to an ice cold petri dish in order to remove the meninges. Then, the brain was placed on a slicing apparatus and aligned. Cross-sections of the brain were made to isolate specific brain areas, using the Pelligrino atlas (1979) for coordinates and brain landmarks to ensure retrieval of each slice from the correct area. Beginning at approximately 3.0 mm anterior to bregma (at the point where the corpus callosum has just joined together) and proceeding posteriorly, three slices, each 1 mm in thickness, were made from each brain. Each slice was placed on a glass slide, and the slide was placed on dry ice to quick freeze the brain tissue. A method modified from Palkovits (1973) was used to make punches and isolate the desired brain areas from each slice. A blunt-ended 16 gauge needle with an inner diameter of approximately 1 mm was utilized to make two bilateral punches for each brain area on each frozen brain slice (Fig. 1). From the first slice, the caudate putamen (CPU) and the nucleus accumbens (ACB) were retrieved. From the second slice, the isolated brain areas were the preoptic area (POA) and the anterior amygdaloid area (AAA), while the last slice contained the anterior hypothalamic area (AHA). The two bilateral punches for each brain area were placed in one vial containing 500 μl 0.1 M perchloric acid, which preserves the catecholamines.



A.



B.



C.

1 mm

MEASUREMENT OF MONOAMINES AND METABOLITES

First, each vial was sonicated using the Fisher Sonic Dismembrator Model 300, until a homogenous solution was obtained. The vials were then microcentrifuged for five minutes to precipitate the tissue. Then, 150 μl of the supernatant, containing the catecholamines and metabolites, from each vial was transferred to a clean vial and analyzed for catecholamines and metabolites, using high pressure liquid chromatography with electrochemical detection (HPLC-ED). Endogenous concentrations of norepinephrine (NE), epinephrine (E), dihydroxyphenylacetic acid (DOPAC), dopamine (DA), and homovanillic acid (HVA), were measured by HPLC-ED. An ISCO Model 2300 HPLC pump delivered 0.9 ml/min filtered and degassed mobile phase (9.0% 0.2 M citric acid/0.24 M sodium acetate buffer, 10% methanol, and 1.8% $9x10^{-5}$ M EDTA/$6.25x10^{-4}$ M sodium octane sulfonate, adjusted to pH = 3.7). Chromatographic separation was performed on a 100mm Adsorbosphere Catecholamine 3U C_{18} column with an inside diameter of 4.6 mm. Catecholamines were oxidized at +0.9 V, and regression lines generated from standard curves were used to determine catecholamine concentrations (pg/μl) in sample aliquots. The minimum detectability limit for NE, E, and DOPAC was 0.78 pg/μl, and the limit for DA and HVA was 3.0 pg/μl. The values were reported as pg/μl/mg dry tissue weight.

STATISTICAL ANALYSIS

Catecholamine and metabolite levels were analyzed for

differences between treatment groups by 2-way analysis of variance. When significant main effects were detected, differences between groups were probed using the Student-Newman-Keuls test (Sigma Stat, Jandel Scientific).

CHAPTER III

RESULTS

EXPERIMENT 1: Effects of pheromone exposure on male sexual behavior.

No differences were found between the swab and bedding exposure methods, therefore these groups were combined for the analysis of the data. Data recorded from copulatory testing showed that there tended to be a higher number of males that responded positively to receptive females (i.e. copulated to ejaculation) in the pheromone-exposed group, although this difference was not found to be significantly different (Fig. 2). All of the other parameters were measured only in the males that were found to be responsive to estrous females in each group. Results showed no significant differences among treatment groups in any of the parameters measured during behavior testing (Fig. 3 - Fig. 8).

EXPERIMENT 2: Effects of pheromone exposure on catecholamine and metabolite levels in sexually experienced versus sexually exhausted male rats.

All the values measured for the catecholamines and metabolites in each brain area due to each treatment are reported in Table 1, and are given as the mean +/- S.E.M. The levels of E were not reported due to the fact that the

majority of the values obtained for E were below minimal detectable levels.

Caudate Putamen (CPU)

Although not significant, NE levels in the CPU of exhausted animals exposed to OVX bedding tended to be lower than levels found in the control animals exposed to OVX bedding. In addition, NE levels in the CPU of exhausted animals exposed to pheromones tended to be higher than NE levels in the exhausted animals exposed to OVX bedding (Fig. 9). With two-way ANOVA, a significant main effect due to pheromone exposure was seen in the DOPAC levels measured in the CPU ($f=5.026$, $p=0.0378$; Fig. 10). There also appeared to be a decrease in DA levels in the CPU of exhausted males following exposure to OVX bedding as compared to control males exposed to OVX bedding, although this difference was not found to be significant. Pheromonal treatment did not appear to have any significant effect on the expression of DA in the CPU (Fig. 11). No significant variations in the levels of HVA within the CPU were found between the groups (Table 1).

Nucleus Accumbens (ACB)

Significant differences in the levels of DOPAC in the ACB among groups were found due to pheromonal exposure ($f=9.909$, $p=0.0056$), with increased DOPAC levels observed following exposure to pheromones. However, no effect of exhaustion on DOPAC levels was seen (Fig. 12). There were also no differences in NE (Table 1), DA (Fig. 13), or HVA

(Table 1) levels due to either the pheromonal treatment or exhaustion in the ACB.

Preoptic Area (POA)

There were no significant differences found between groups in the levels of NE in the POA; however, it appeared as if the control males exposed to pheromones had the lowest levels of NE (Fig 14). There was also no significant difference in the levels of HVA among groups, although the control males exposed to pheromones appeared to have higher levels of HVA than control males exposed to OVX bedding (Fig. 15). The levels of DOPAC and DA in the POA of the animals were also not significantly affected by pheromonal exposure or exhaustion (Table 1).

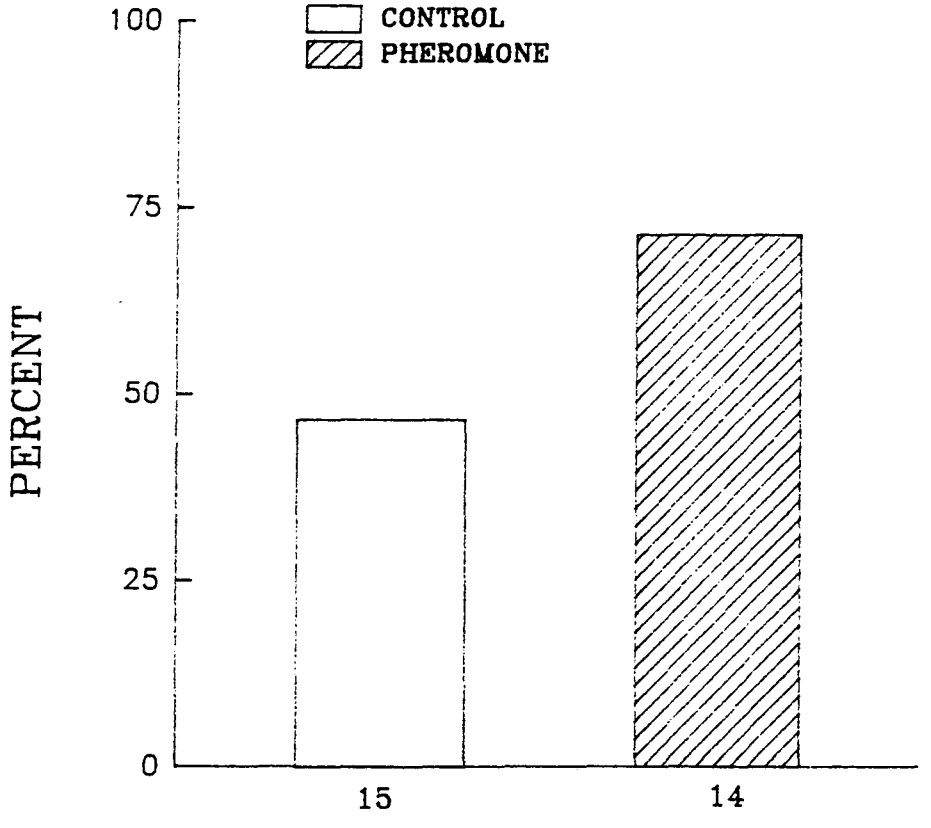
Anterior Amygdaloid Area (AAA)

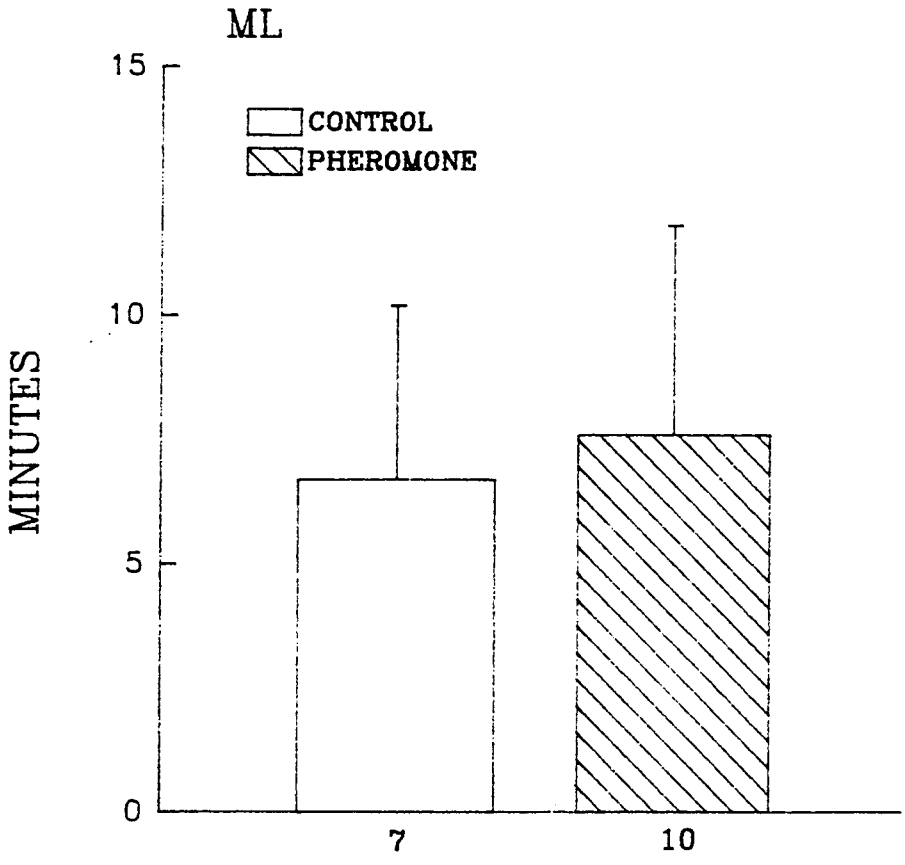
The levels of the catecholamines and metabolites measured were not found to be significantly affected by either pheromonal treatment or exhaustion in the AAA (Table 1).

Anterior Hypothalamic Area (AHA)

No significant differences in NE levels were obtained among the groups in the AHA; however, the control males exposed to pheromones had very low levels of NE as compared to the other treatment groups. Among the exhausted animals, NE levels appeared to be highest in the males exposed to pheromones (Fig. 16). The levels of DOPAC, DA, and HVA in the AHA had no significant variations among the different treatment groups (Table 1).

BRAIN AREA	TREATMENT	NE	DOPAC	DA	HVA
CPU	control+ovx	4.29 +/- 1.92	5.46 +/- 1.63	160.89+/-32.64	17.99+/- 6.10
	control+pher	6.71 +/- 5.40	13.79 +/- 6.38	88.60+/-29.43	23.56+/- 7.88
	exhaust+ovx	2.15 +/- 0.93	2.37 +/- 0.40	68.32+/- 9.06	14.03+/- 5.47
	exhaust+pher	8.88 +/- 5.05	7.97 +/- 1.57	84.53+/-50.22	10.51+/- 5.71
ACB	control+ovx	2.81 +/- 1.20	2.35 +/- 0.61	39.31+/- 7.75	10.59+/- 3.47
	control+pher	12.36 +/-11.56	7.19 +/- 2.19	42.73+/-26.33	13.44+/- 6.92
	exhaust+ovx	6.75 +/- 3.60	2.44 +/- 0.61	39.99+/-10.27	13.71+/- 4.94
	exhaust+pher	10.45 +/- 6.25	6.20 +/- 1.80	48.42+/-14.97	6.97+/- 2.20
POA	control+ovx	8.03 +/- 7.16	1.38 +/- 0.51	29.10+/-22.19	6.24+/- 1.89
	control+pher	1.76 +/- 0.49	4.23 +/- 2.26	15.40+/- 8.79	17.85+/- 7.99
	exhaust+ovx	2.65 +/- 1.44	3.32 +/- 2.23	4.48+/- 0.72	4.41+/- 0.73
	exhaust+pher	3.53 +/- 1.51	2.13 +/- 0.78	4.92+/- 2.03	6.30+/- 4.38
AAA	control+ovx	3.02 +/- 1.29	1.34 +/- 0.27	45.48+/- 13.57	10.03+/- 2.85
	control+pher	3.56 +/- 2.34	4.33 +/- 1.70	36.30+/- 19.73	14.35+/- 4.69
	exhaust+ovx	7.76 +/- 5.28	1.83 +/- 0.42	45.46+/- 16.86	12.78+/- 4.52
	exhaust+pher	3.42 +/- 2.08	1.66 +/- 0.84	13.12+/- 3.93	6.51+/- 4.63
AHA	control+ovx	10.06 +/- 5.85	1.23 +/- 0.27	4.73+/- 1.02	7.90+/- 1.47
	control+pher	0.94 +/- 0.18	0.94 +/- 0.18	3.60+/- 0.70	11.65+/- 3.85
	exhaust+ovx	12.82 +/- 6.29	1.47 +/- 0.35	4.75+/- 0.76	8.19+/- 2.08
	exhaust+pher	29.48 +/-17.11	1.65 +/- 0.36	6.36+/- 1.40	6.36+/- 1.40


CONTROL
PHEROMONE
100
75
50
25
0
PERCENT
15
14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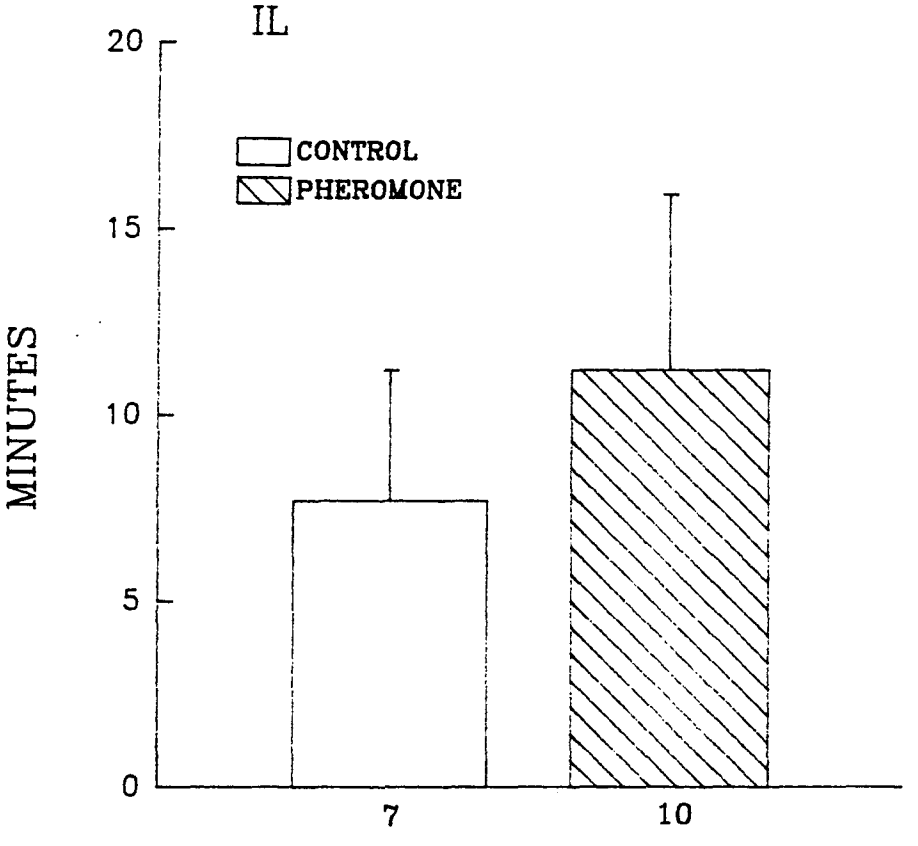
MINUTES
CONTROL
PHEROMONE
7
10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MINUTES
CONTROL
PHEROMONE
7
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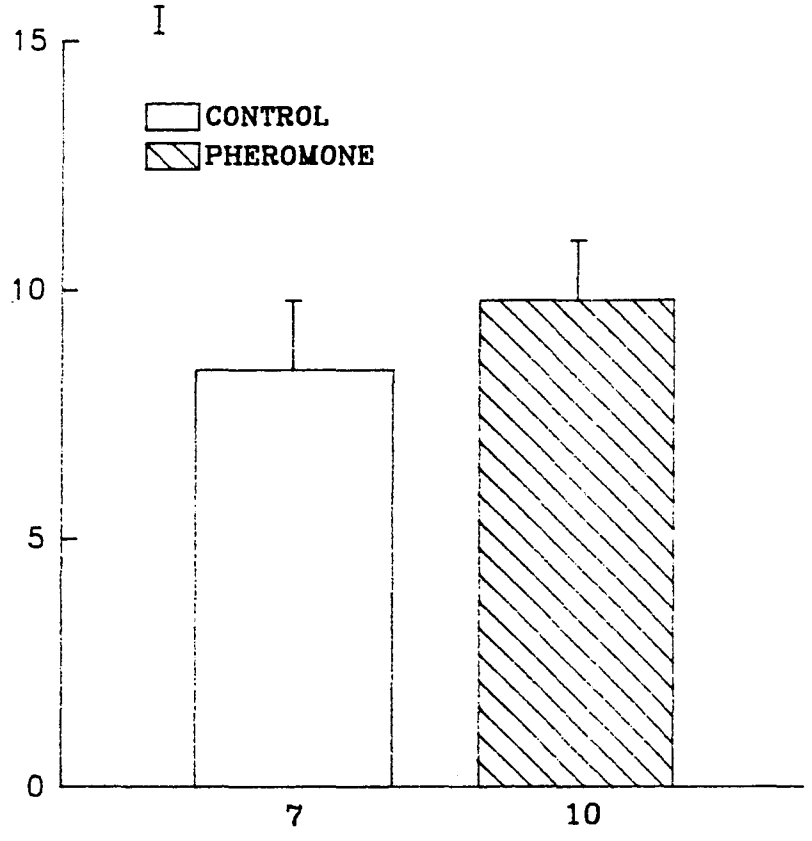
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CONTROL
PHEROMONE
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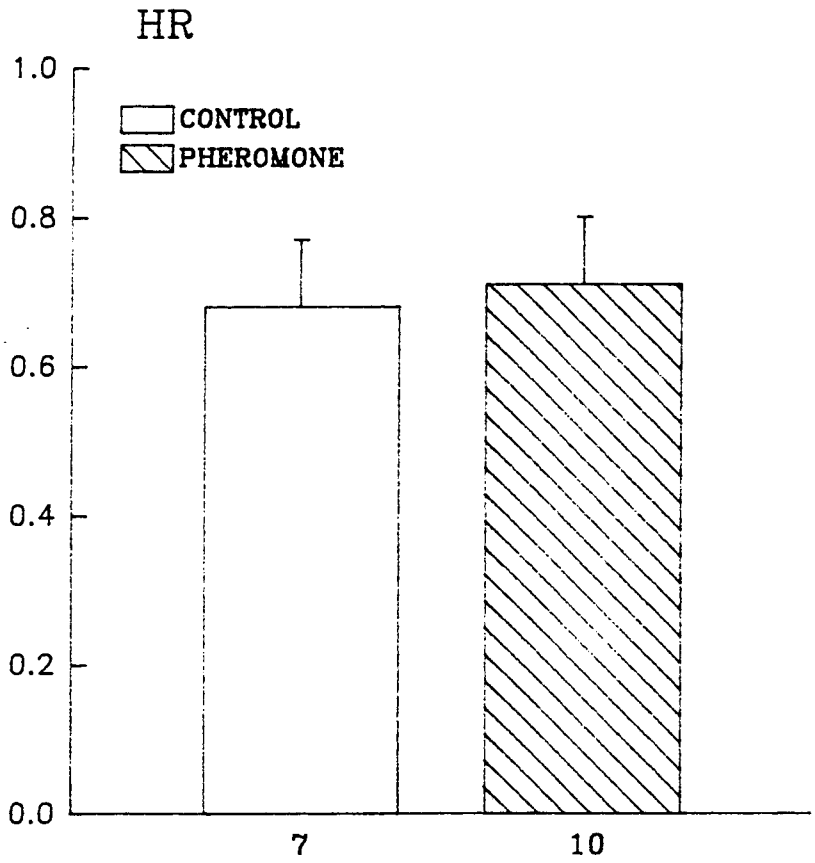
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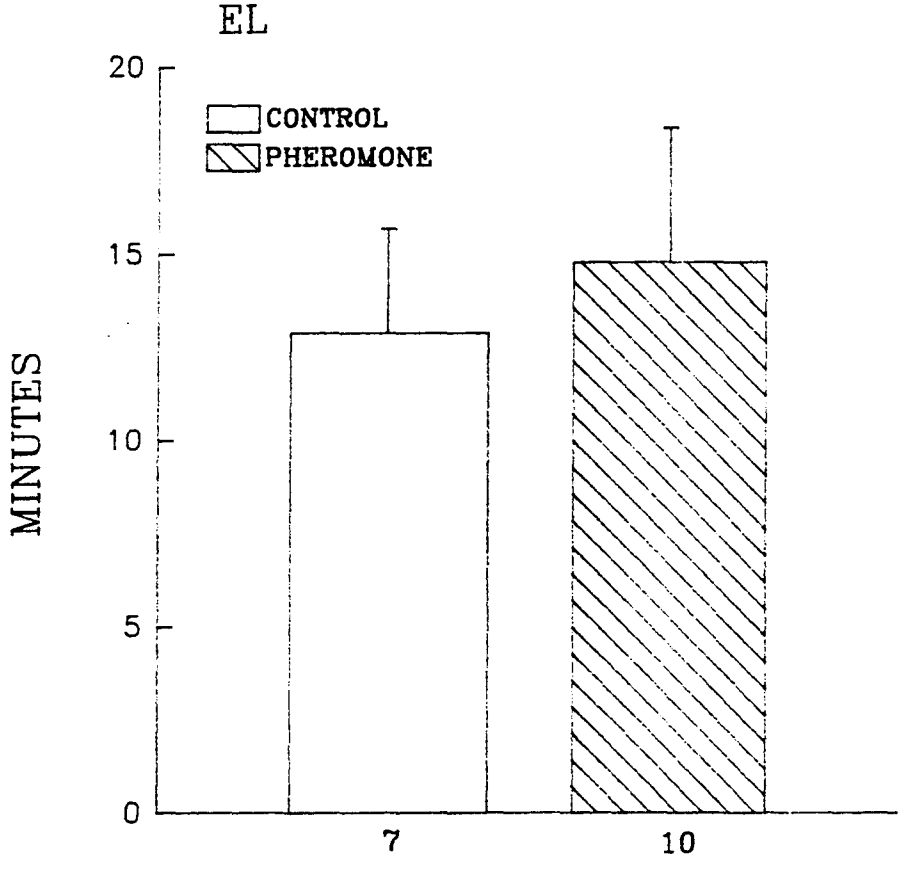
0.8
0.6
0.4
0.2
0.0
CONTROL
PHEROMONE
7
10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15
10
5
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MINUTES
CONTROL
PHEROMONE
7
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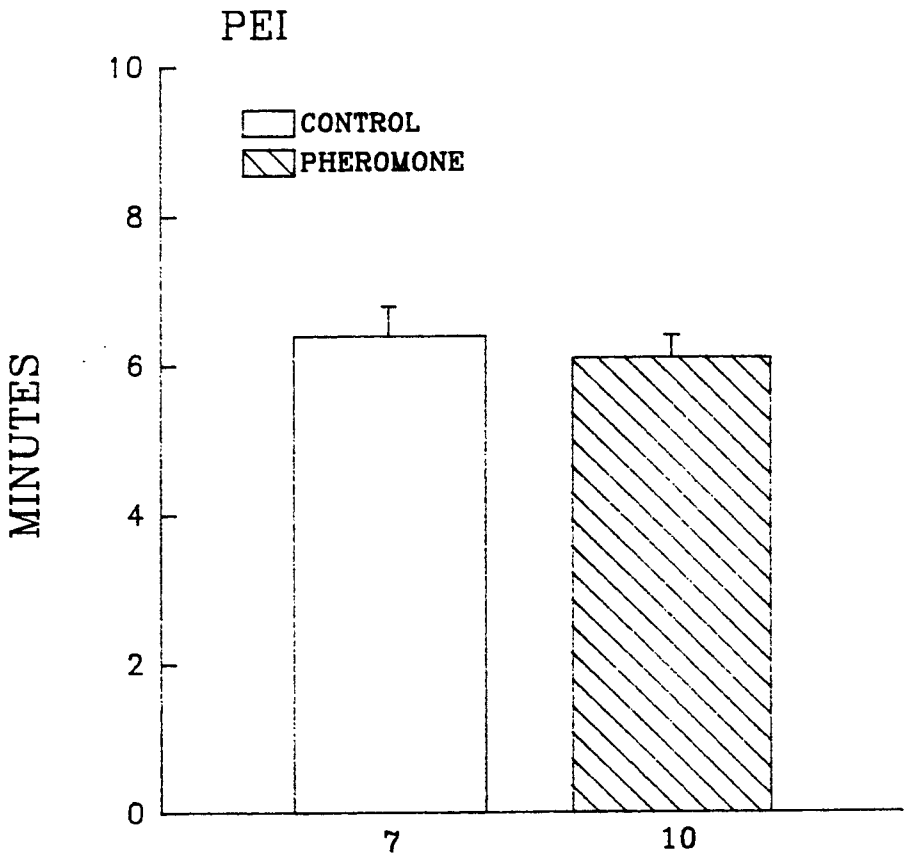
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PHEROMONE
MINUTES
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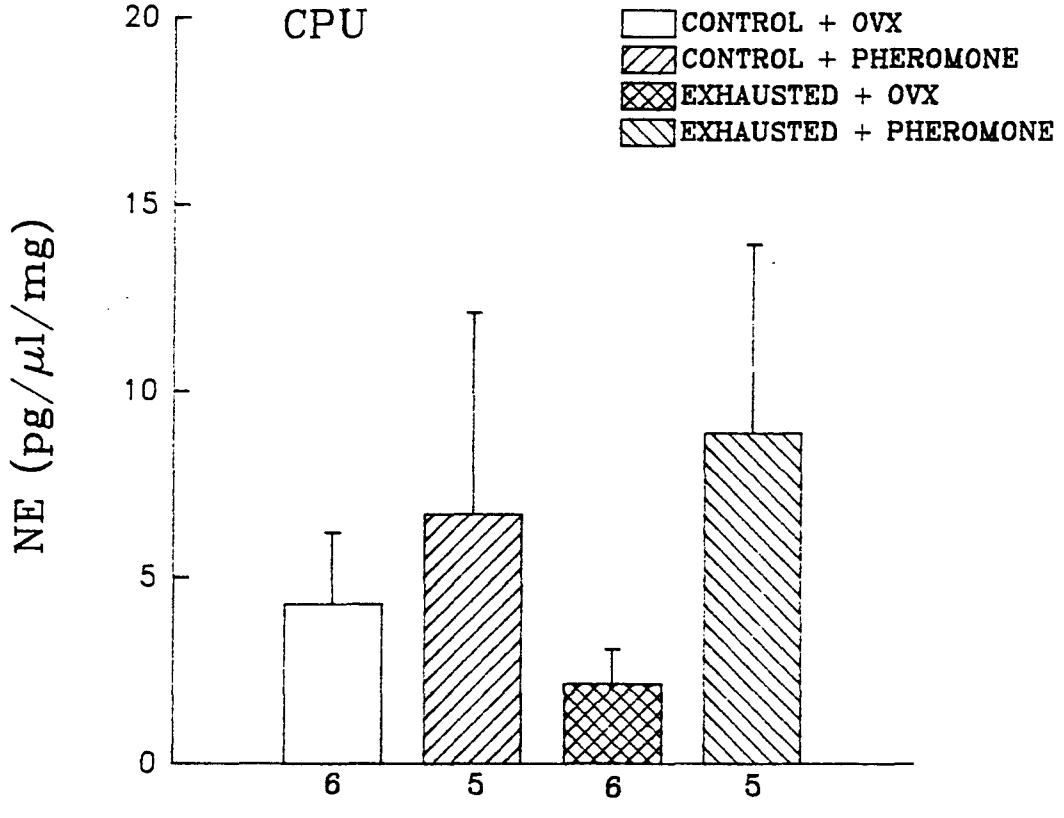
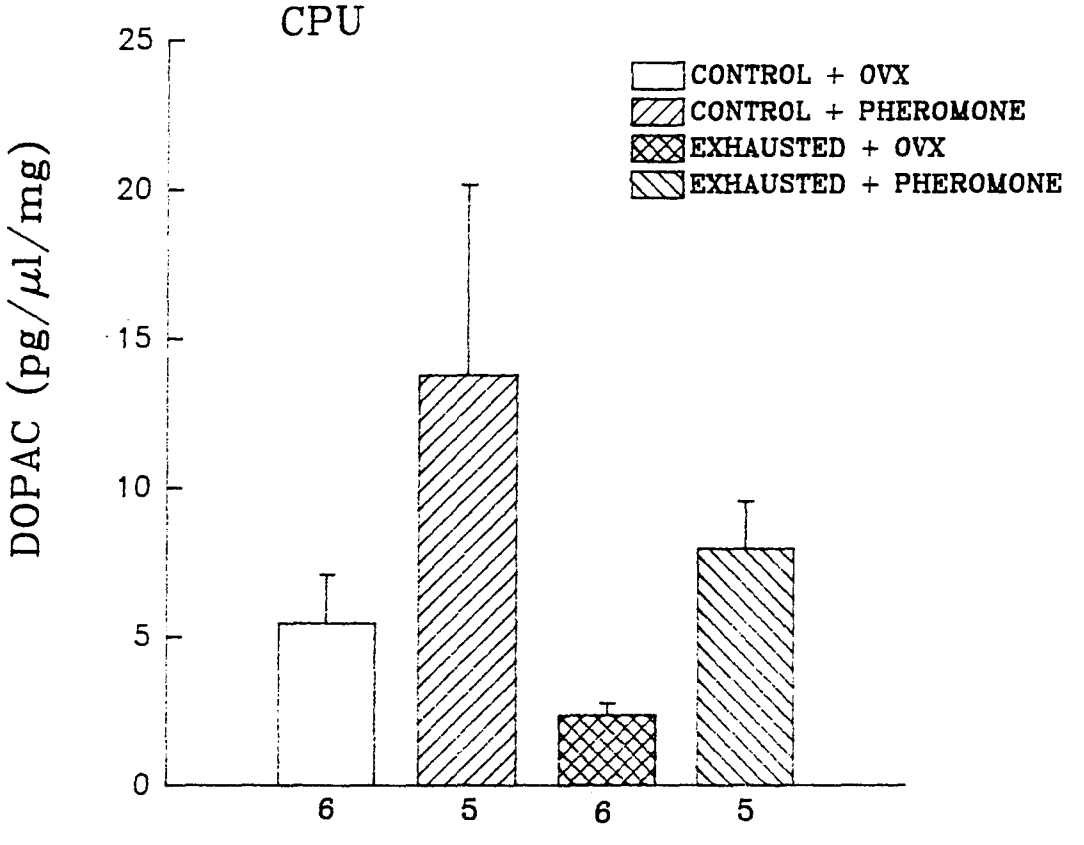
7
10


CPU
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
NE (pg/μl/mg)
20
15
10
5
0
6
5
6
5


CPU
DOPAC (pg/μl/mg)
25
20
15
10
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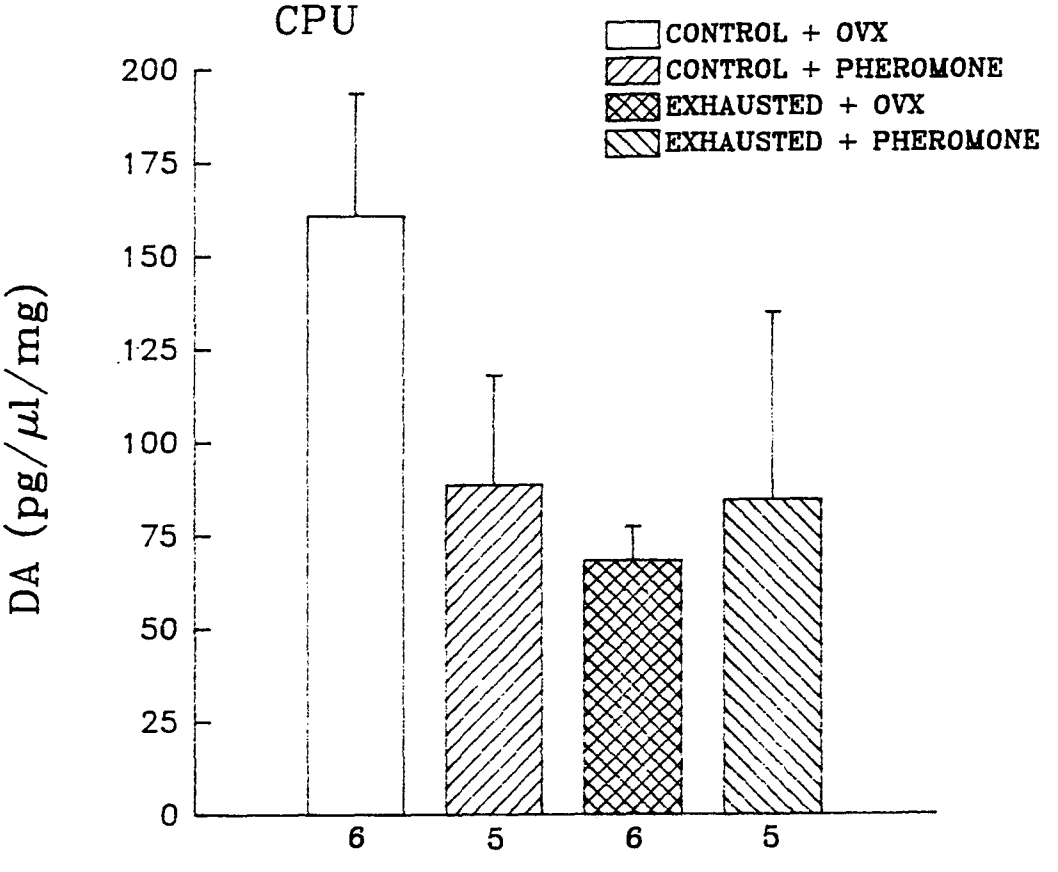
0
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
6
5
6
5


CPU
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
DA (pg/μl/mg)
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100
75
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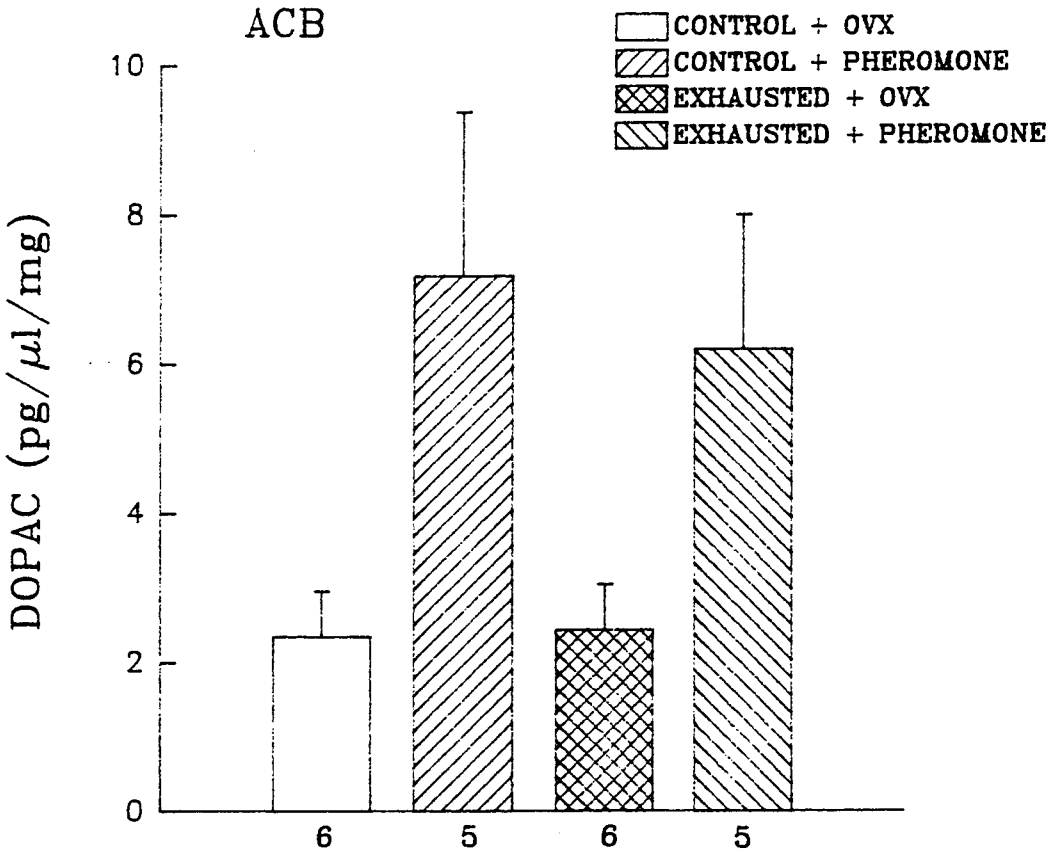
0
6
5
6
5


ACB
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
DOPAC (pg/μl/mg)
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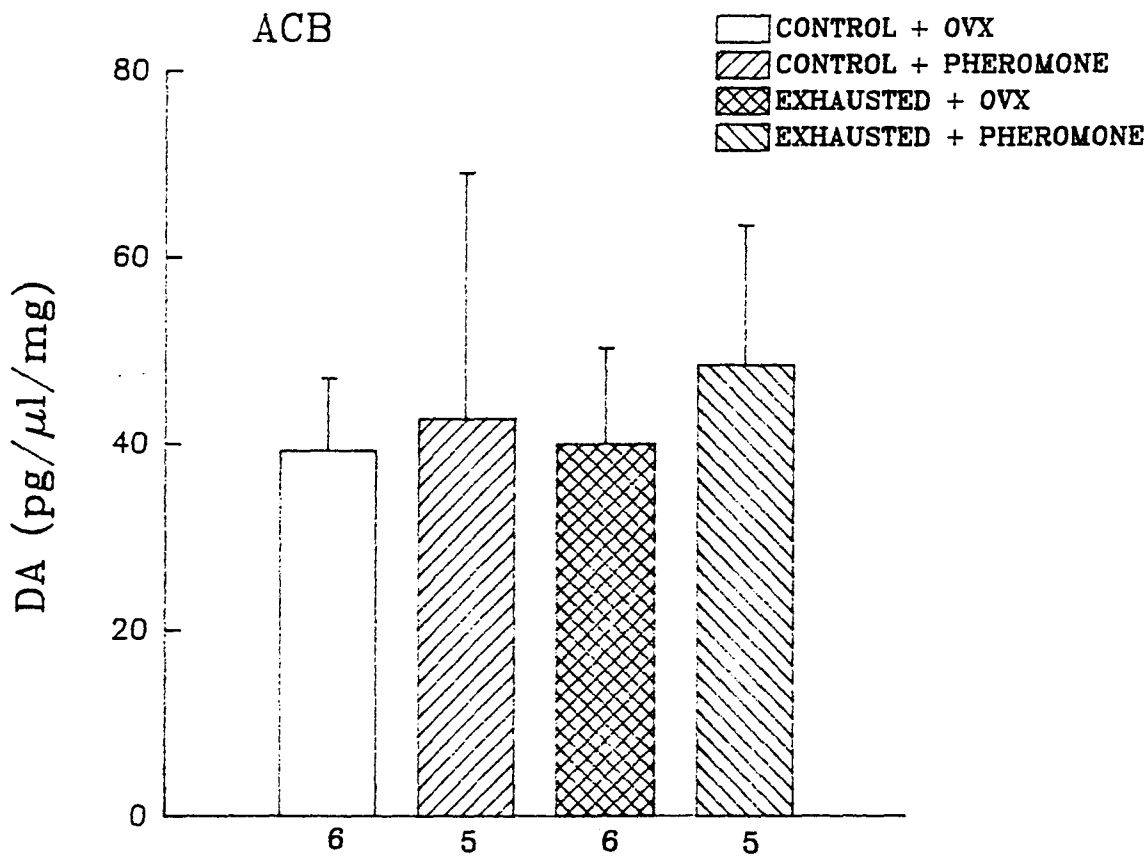
4
2
0
6
5
6
5


ACB
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
DA (pg/μl/mg)
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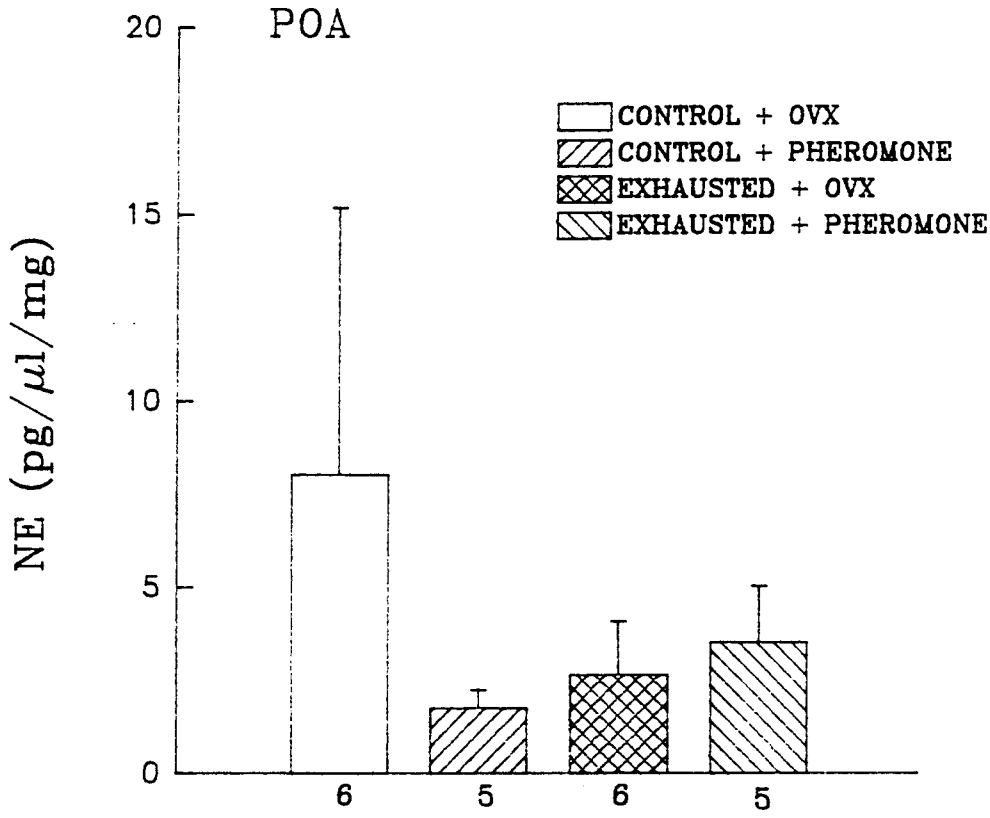
0
6
5
6
5


POA
NE (pg/μl/mg)
20
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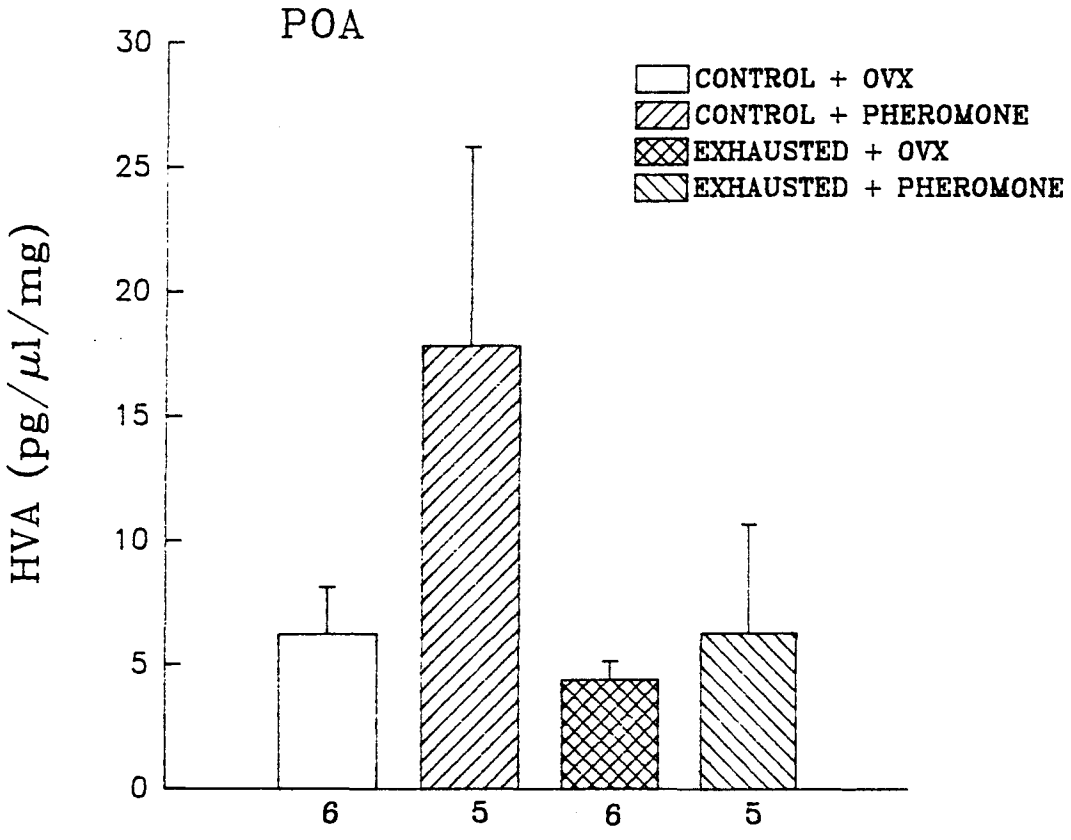
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
6
5
6
5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CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
HVA (pg/μl/mg)
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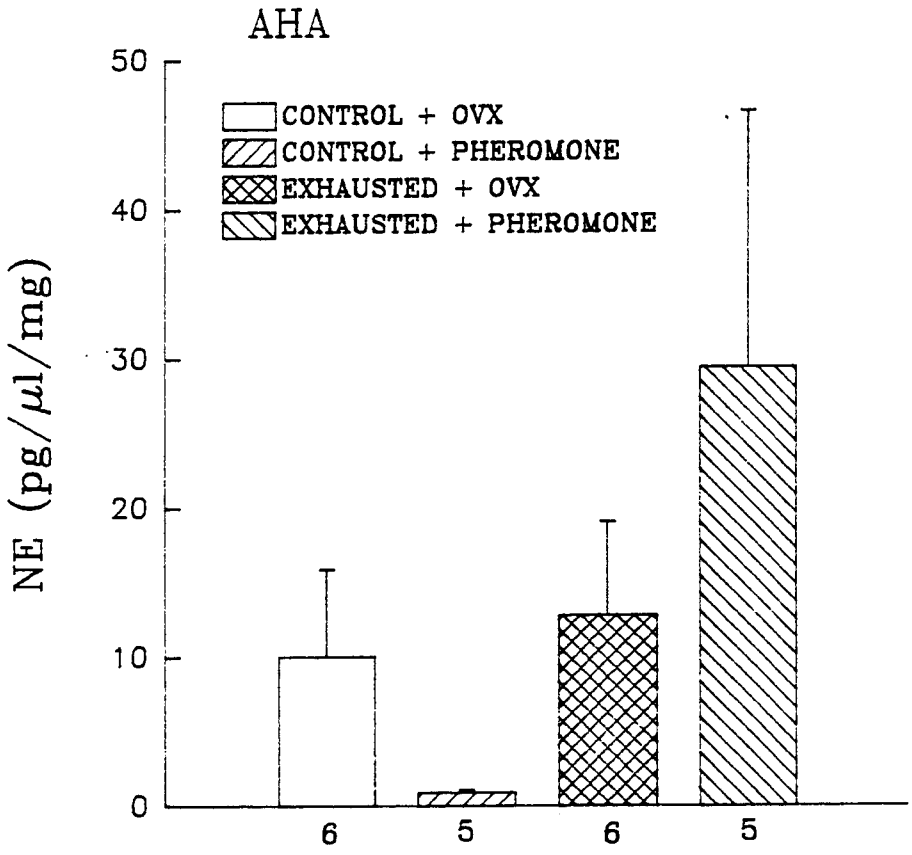
AHA
CONTROL + OVX
CONTROL + PHEROMONE
EXHAUSTED + OVX
EXHAUSTED + PHEROMONE
NE (pg/μl/mg)
50
40
30
20
10
0
6
5
6
5

CHAPTER IV

DISCUSSION

The present study was conducted to analyze the effects of pheromonal exposure on male sexual behavior, as well as on the neurochemical correlates of male sexual behavior. The effects of pheromones were examined by measuring changes in various parameters of sexual behavior following pheromone exposure in naive male rats, and also by analyzing levels of various catecholamines and their metabolites following pheromone exposure in both sexually experienced and sexually exhausted male rats.

Emission of pheromones by receptive females has been shown to have definite advantages. These chemical cues facilitate conception by attracting males to the females during the females' fertile time period (Vandenbergh, 1988). Hlinak (1984) found that exposure of sexually experienced male rats to estrous female odors stimulated increased precopulatory activity. Changes in precopulatory activity can be detected by differences in mount latency, which is the most direct measure of male sexual motivation (Sachs and Meisel, 1988). The current study found no significant differences between naive male rats exposed to estrous bedding containing pheromones and males exposed to the

control OVX bedding in data recorded for any of the parameters measured during copulatory testing. However, there appeared to be a greater percentage of males that responded to the point of ejaculation to the receptive females in the pheromone-exposed group, although this result was not statistically significant. The increase in the number of responders within this group may be attributed to increased motivation, which led to increased copulatory activity.

In the data obtained from the present study examining pheromonal effects on the levels of catecholamines and their metabolites in sexually experienced and sexually refractory males, various combinations of the neurotransmitter and metabolite levels have been found. Mas et al. (1987) interpreted several possibilities in the expression of these levels. They indicated that elevated levels of neurotransmitter content can be due to one of two factors: inhibited release or increased synthesis of the neurotransmitter. Enhanced synthesis is usually accompanied by increased levels of the metabolite as well. Increased levels of the catecholamine metabolites within the brain are usually an indication of enhanced catecholaminergic nerve activity. However, decreased metabolite levels are not an indication of inhibition of nerve firing.

The present findings in the caudate putamen, which is part of the striatum, indicate that NE and DA levels in exhausted males without pheromonal exposure tended to be

lower than the levels found in control animals exposed to OVX bedding, although this data was not found to be significant. This finding concurs with results obtained by McIntosh and Barfield (1984) indicating that decreasing levels of central NE and DA pharmacologically or surgically yields longer refractory periods in normal intact male rats. The present study also showed that exhausted males exposed to pheromones tended to have higher NE levels in the CPU than exhausted males exposed to OVX bedding, although these results were not significantly different. Similarly, Dluzen and Ramirez (1987) found significant increases in NE levels in the olfactory bulbs of male rats following introduction of receptive females. This suggests a role of NE in the processing of the pheromonal cue within the olfactory bulb, and possibly a related role in the CPU according to the current results. In addition, the current study found a significant difference due to pheromone exposure on DOPAC levels in the CPU. However, dopamine levels in the CPU were not found to be significantly affected by pheromonal treatment. In a similar study by Mas et al. (1987), elevated DOPAC levels along with consistent DA levels, as found in the present experiment, were interpreted as being due to enhanced dopaminergic nerve activity. This finding corresponds with the studies of Pfaus et al. (1990) that have associated DA function with motor activity. Their results indicated that striatal dopamine transmission was only significantly elevated during copulation. Also, the

current findings indicated that there were no significant variations in the HVA levels within the CPU between groups.

In the nucleus accumbens, a significant main effect due to pheromone exposure on DOPAC levels was seen, with increased levels in the pheromone-exposed groups. Similar results were also obtained in the CPU. As suggested by studies performed by Mas et al. (1987), increases in metabolite levels along with sustained DA levels could be caused by elevated dopaminergic nerve activity. Exhaustion, however, did not seem to affect DOPAC expression in the ACB. This result contradicts findings by Damsma et al. (1992), which associated increased locomotor activity with increased levels of DA metabolites in freely behaving male rats when measured by in vivo microdialysis. Thus, the decreased locomotion associated with exhaustion may have been expected to be associated with decreased DOPAC levels in sexually refractory males. The levels of NE, DA, and HVA in the ACB were not significantly affected by pheromonal exposure or exhaustion. Due to the association of the ACB with many facets of motivated behavior, the minimal effects of pheromonal exposure and exhaustion on these catecholamine and metabolite levels were unexpected. However, the present study reflected effects of pheromone exposure indirectly on DA levels in the CPU through the variations in the levels of the metabolite, DOPAC. Effects on DA levels were expected from results of in vivo voltammetry studies by Louilot et al. (1991) and Mitchell and Gratton (1991), as well as in

vivo microdialysis studies by Damsma et al. (1992) and Wenkstern et al. (1993), indicating that the anticipatory and consummatory aspects of sexual activity are regulated by DA levels in the ACB.

No significant variance in the NE levels was seen in the preoptic area among the different treatment groups. These results support findings in a similar study conducted by Hoffman et al. (1987), in which NE levels did not differ significantly in any of the brain areas tested, including the MPOA. Though significant differences in HVA levels in the POA were not found, it appeared as if pheromone exposure in the control animals resulted in higher levels of HVA than OVX exposure in the control males. As suggested by Mas et al. (1987), this apparent increased HVA level may be due to increased dopaminergic nerve activity. Such a result may be rationalized due to the suggested role of the MPOA in mediation of sexual arousal mechanisms (Chen and Bliss, 1974), which could theoretically be affected by pheromones. The levels of DOPAC and DA in the POA were not found to be significantly altered due to any treatments in the current study.

In the anterior amygdaloid area, no significant variations due to pheromonal treatment or sexual exhaustion were seen in the analysis of catecholamine and metabolite levels. These results were unexpected due to the multifaceted roles of the nuclei that compose the AAA.

The anterior hypothalamic area also showed no statistically significant variations in catecholamine and metabolite levels due to the different treatments. However, the pheromone-exposed control males displayed very low levels of NE as compared to the other treatment groups; whereas among the exhausted animals, NE levels appeared to be elevated in males exposed to pheromones. The cause for these apparent fluctuations in NE levels measured in the AHA due to pheromonal exposure among different treatment groups requires further research for explanation. These negative findings were unexpected due to the association of the AHA with sexual arousal (Mogenson et al., 1987).

In summary, results from this study demonstrate that pheromonal exposure has little effect on male sexual behavior in naive male rats. However, pheromone exposure significantly increased levels of DOPAC in both the CPU and ACB. This suggests that pheromone exposure may cause increases in dopaminergic nerve activity in these brain areas. Further studies are required to elucidate the role of dopamine in the regulation of male sexual behavior and the neurochemical processing of pheromone-induced chemical signalling.

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July 9, 1996

Dr. Robert Leipheimer
Department of Biological Sciences
UNIVERSITY

Dear Dr. Leipheimer:

Upon recommendation of the Animal Care and Use Committee, your proposals entitled "Hormonal and Behavioral Effects on the Dynamics of Dopamine Release in the Striatum and Nucleus Accumbens of the Rat Brain" (96-004); "The Role of Pheromones in Regulating Male Rat Reproductive Behavior" (96-005); "The Effects of Local Application of Tyramine on Dopamine Release in the Rat Striatum" (96-006); and "The Effects of MPP+ on the Dynamics of Dopamine Release and the Role of Estrogen as a Neuroprotectant in the Striatum of the Rat" (96-007) have been approved.

You must adhere to procedures described in your approved request; any modification must first be authorized by the Animal Care and Use Committee.

Sincerely,

Peter J. Kasvinsky
Dean of Graduate Studies

kb

c: Dr. Sobota, Chair, Biological Sciences

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YSU PROPOSAL# 96-005

APPROVALS

A. COMMITTEE MEMBERS

I have reviewed and evaluated this proposal according to the charge of the YSU IACUC and and responsibilities of Committee membership, with the following determination:

(CHECK ONE)
Proposal Approved _____ Proposal Disapproved _____

Proposal Approved with the Following Modifications _____

Required Modifications or reasons for disapproval:

Name_____
(Please Print)

Signature_____ Date _____
(IACUC Voting Member)

(After signing, please return ONLY this page to the Committee Chairperson. Retain proposal for your files.)

B. AUTHORIZATIONS

We have evaluated this proposal for conformity with University Policies and with PHS, USDA, and NIH Policies and determined that this proposal has been adequately reviewed, voted upon, and approved or disapproved as indicated above by the YSU IACUC.

Signature _____ Date _____
(Reviewing Subcommittee Chairperson)

Signature _____ Date JUNE 28/96
(Veterinary Consultant)

Signature _____ Date 7/8/96
(YSU IACUC Chairperson)
Acting Administrative Co-Chair