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THE ROLE OF DOPAMINE IN MOTIVATIONAL AND RECEPTIVE ASPECTS
OF FEMALE MOUSE SEXUAL BEHAVIOR

by

AMBER BRADSHAW HODGES

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment
of the requirements for the degree of Doctor of Philosophy, The City University of
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ABSTRACT

THE ROLE OF DOPAMINE IN MOTIVATIONAL AND RECEPTIVE ASPECTS
OF FEMALE MOUSE SEXUAL BEHAVIOR

by

Amber B. Hodges

Adviser: Victoria Luine, Ph.D.

A pacing paradigm investigated the modulatory role of monoamines in the mesolimbic dopamine system on copulatory behavior in female mice. Specifically whether there is a distinction between the neural regulatory mechanisms of pacing behavior and receptive behavior was determined. Pacing measures motivational and rewarding components of copulation by the female's contacts and withdrawals from the male, while receptivity is the willingness to engage in copulations and is measured by the number of sexual stimulations received. A pacing paradigm was established and female mice were observed to pace in a similar manner, but at a lesser rate, than female rats.

Dopamine (DA) and its pathways regulate copulatory behaviors in female rats [2, 3]. General and specific DA receptor agonists were used to investigate dopaminergic mediation of copulatory behavior in female mice. Three doses of apomorphine (APO; 20 μ g, 60 μ g and 120 μ g/kg), a general dopamine agonist, [4] were given and resulting effects on pacing behavior, receptive behavior, and dopamine and metabolite levels in brain areas measured. Treatment caused dose dependent increases in pacing and in DA levels in the Nacc and significantly lower

levels of HVA/DA in the striatum and Nacc. These results are consistent with previous studies in rats that show activation of DA terminals in these areas during reward. The lowest effective dose for altering pacing was 120 μ g/kg. APO treatment was associated with dose dependent decreases in receptivity and significant increases of HVA in the VMN with a lowest effective dose of 60 μ g/kg. The VMN is the CNS regulatory center for receptivity. Since pacing behavior involves locomotor behavior and APO affects locomotion, subjects were treated with APO and locomotion, anxiety and exploratory behavior were measured using an open field. APO dependent increases in locomotion and anxiety and decreases in exploratory behavior may contribute to its modulation of copulatory behaviors.

In conclusion, a pacing paradigm was established for mice, and results showed differential effects of DA (dose and brain region) on regulation of motivational and rewarding vs receptive components of female sexual behavior. This information can be utilized in clinical research on sexual dysfunction and drug abuse involving the DA mesolimbic "reward" pathway.

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I. Specific Aims

This research dissertation uses a pacing paradigm to investigate the modulatory role of monoamines in the mesolimbic dopamine system on copulatory behavior in female mice. There is particular interest in establishing a distinction between the neural regulatory mechanisms of pacing behavior and the neural regulatory mechanisms of receptive behavior. Additionally pacing behavior is being used to measure motivational and rewarding components of copulatory behavior. Dopamine (DA) and its pathways have been shown to regulate other motivational and rewarding behaviors [5]. Since pacing behavior in rodents is a motivational behavior [6], we are interested in determining if and/or how DA modulates this behavior.

Therefore the specific aims of this research dissertation are as follows:

- (1) Establishment and quantification of baseline pacing and receptive behavior in female mice. Pacing is a component of female rodent sexual behavior that is defined by the female approaching and withdrawing from the male, allowing her to control the frequency and amount of stimulation she receives from the male [7]. Pacing allows for the quantification of the motivational aspects involved in copulatory behavior. The pacing behavior can then be compared to receptive behaviors. Receptive behaviors allow the male to successfully copulate with the female. The lordosis posture and allowing mounts and intromissions are considered to be receptive behaviors (see [1, 7] for a review of rodent female sex behavior).
- (2) Investigation of the modulatory role of neurotransmitters on both pacing and receptive behaviors. Of particular interest is the regulatory role of DA and its metabolites in the nucleus accumbens (Nacc), striatum and ventromedial nucleus

of the hypothalamus (VMN). This aim was accomplished by quantifying pacing and receptive behavior and measuring levels of DA and metabolites in brain areas important for sexual behavior and motivation.

- (3) Investigation of the role of DA receptors mediating pacing and receptive components of copulatory behavior. The contribution of DA receptors to the regulation of pacing and receptive behaviors is of particular interest. This aim was accomplished by administering general and specific DA receptor agonists and measuring pacing and receptive behavior.

Although there are new animal models available in the form of transgenic and knockout mice, these genetic models have not been widely used in neuroendocrine studies because paradigms have not been established for mice[8]. Specifically, when examining sexual behavior, a pacing paradigm needs to be established. Once a pacing paradigm exists for wild type mice, it can be utilized to study the relationship between hormones, neurotransmitters and sexual behavior in female mice. With this information, we can continue to study the motivational and rewarding aspects of copulatory behavior in female rodents. Primarily these types of investigations have been confined to rats [1, 6, 7, 9]. However expanding our understanding of the various components of copulatory behavior to mice, will allow for further investigation of the underlying mechanisms of how neurotransmitters and hormones affect sexual behavior. Knockout and transgenic mice allow researchers to examine and gain an improved understanding of sexual behavior, fertility, infertility and sexual performance problems. Understanding these effects is also important because it gives information on how various medical treatments alter either neurotransmitter

levels or hormone levels, which affect sexual behavior in animals and humans. In a broader sense, investigating the regulatory mechanism underlying all components of copulatory behavior also gives information about the sexual side effects of drugs, used to treat mental diseases such as depression and anxiety disorders.

II. Overview of Sexual Receptivity in Female Rats

Studies examining rodent sexual behavior show that the females exhibit different degrees of receptivity in female rodents [10]. The female sexual response is dependent on her physiological state [11]. When the female is nonreponsive to the male mounting attempts, she displays active resistance or rejection behavior [9]. This can involve rearing on her hind legs and striking the male with her forepaws [9]. If the male is successful in mounting, the nonreponsive female may roll on her back, and dislodge the male [9]. She can also run away when under the male [12].

When the female is fully receptive, her behaviors are quite different. One of the most prominent receptive responses is the lordosis posture [13]. Lordosis is a reflexive posture that consists of the female planting her feet firmly on the ground and dorsiflexion of the vertebral column, causing her rear to be lifted in the air [11]. When her rear is lifted in the air, it allows the female genital to be more accessible to the male [12]. If the female is very receptive, her back is fully flexed causing her stomach area to touch the ground [12].

Female rodent sex behavior is divided into three categories which are proceptive [1], motivational [1, 6, 14] and receptive behavior [1]. Proceptive behavior is defined as behavioral cues displayed by the female to entice the male to come and mate with her [1]. There are two behaviors that have been observed in the receptive female rat, which are categorized as proceptive behaviors [1]. The first is a hopping/darting motion, which consists of a stiff legged hop and darting motion away from the male. The second is ear wiggling [1]. This consists of the female rat

shaking her head rapidly, which causes her ears to vibrate. These behaviors signal to the male that the female is willing and ready to mate [1].

Motivational behavior is the female displaying behavior that shows that she is interested in the male mating with her and is not just displaying a reflexive response [6]. Pacing is categorized as a motivated behavior [6, 14], because the female controls the frequency and amount of stimulation received from the male by using an escape mechanism to approach and withdraw from him.

Receptive behavior is facilitating successful mating by allowing accessibility of appropriate genitals. The lordosis posture is categorized as receptive behavior [12]. Receptivity can be measured using the lordosis quotient (number of lordosis postures divided by the number of mounts), lordosis quality score (quality of lordosis posture ranked from 0, no dorsoflexion of ventral column, to 3, dorsoflexion of ventral column and stomach touching the ground) [15] and number of stimulations (mounts, intromissions and ejaculations) received.

Lordosis

Initially, female rat sexual receptivity was quantified using a Copulatory quotient, a measure used to quantify the lordosis response when the female was mounted by the male [16]. Lordosis is a reflex behavior which is activated by the stimulation of the male's mount [11] (see Fig. 1). Estrogen and progesterone enhance the probability that lordosis will occur following a mount [10]. Many studies have shown that intact female rats will display lordosis during the proestrus phase of the estrus cycle [7, 12] (see Fig 2.). Ovariectomized (OVX) female rats, when

administered estrogen and progesterone in a pattern that mimics the natural estrus cycle will also display high levels of lordosis responding [7, 12, 16-18].

Figure 1: Female rat displaying lordosis posture. The Sprague Dawley (white) female rat is displaying the lordosis reflex when the Long Evans (black and white) male rat is mounting her. Her feet are planted firmly on the ground, dorsoflexion of vertebral column, and her head and rear are lifted in the air, allowing her genital to be accessible to the male [19].

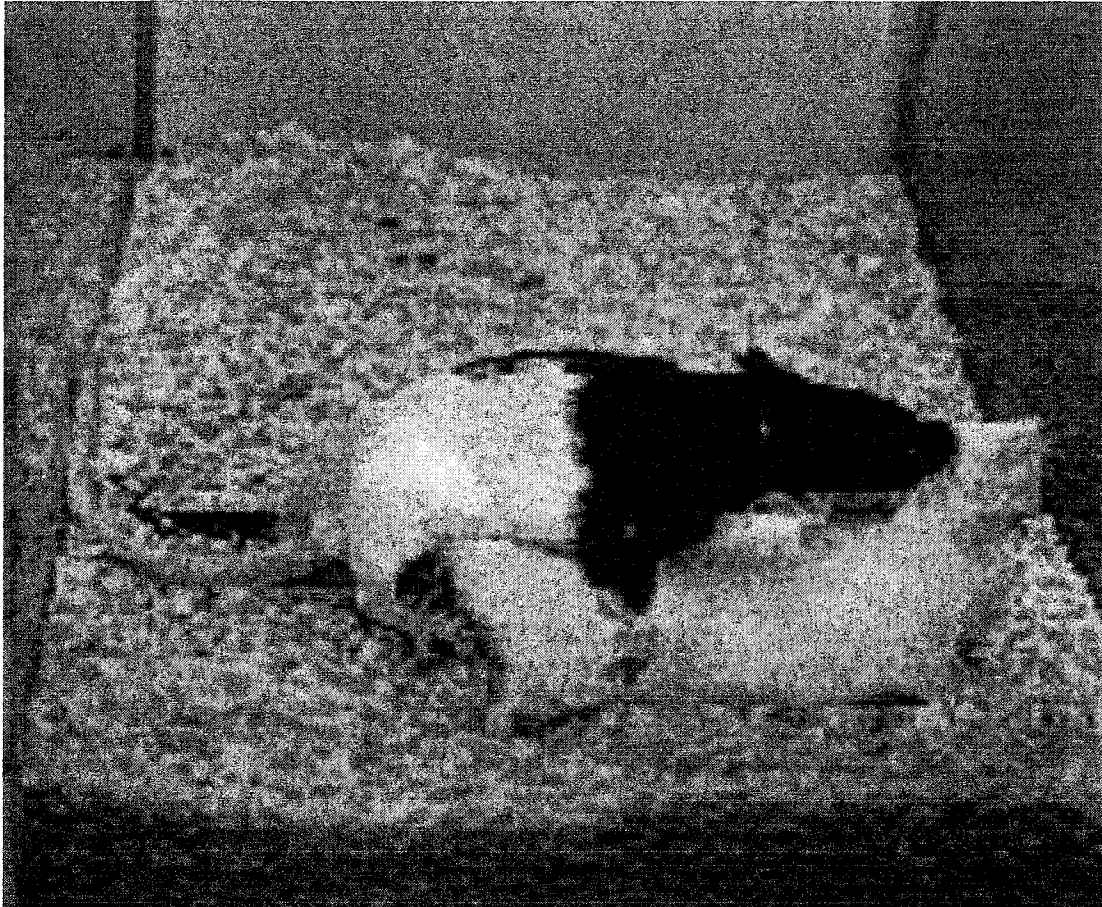
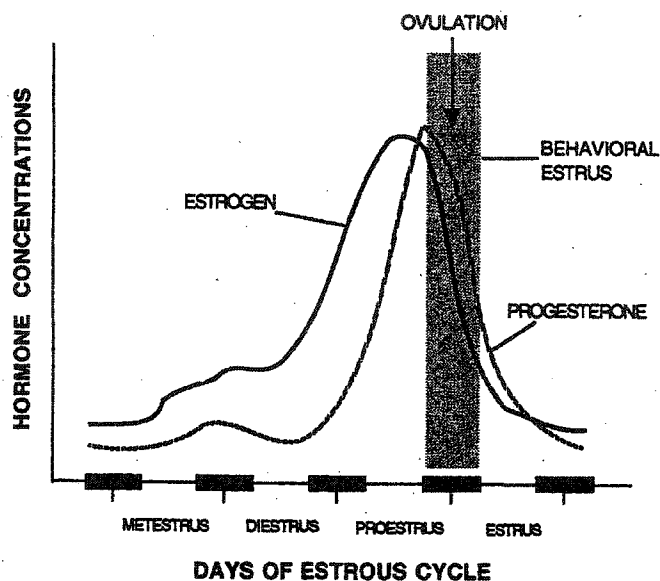


Figure 2: The days of the 4-day estrous cycle (metestrus, diestrus, proestrus, and estrus). The nights are represented by the dark bars on the X axis. Behavioral estrus usually begins on the night of proestrus. This study as well as others mimic the estrus cycle in OVX female rats by administering estrogen 48 and 24 hours before sexual testing and progesterone 5 hours before testing[1]



Additional experiments have examined the regulatory role of DA on lordosis behavior [20]. They found that when ovariectomized (OVX) female rats, primed with high doses of estrogen, are infused with DA receptor antagonists, haloperidol and α -flupenthixol, in the hypothalamus, lordosis behavior was significantly depressed [20]. Subsequently, when either apomorphine (APO), a general DA agonist, or DA were infused in the hypothalamus, lordosis was elevated [21]. In addition, when APO was administered in place of progesterone, lordosis behavior was also elevated [21].

When APO was administered to OVX females, primed with estrogen and progesterone, lordosis behavior increased [22]. There was also an increase when APO was given instead of progesterone [22]. Additional experiments substituting

DA agonists and antagonists for progesterone, found that APO and SKF 38393 (D₁ agonists) mimicked effects of progesterone and facilitated lordosis behavior in female rats. Quinpirole (D₂ agonist) had no effect on lordosis [23]. Similar to female rats, female hamsters show high levels of lordosis when administered D₂ receptor antagonists, sulpiride and raclopride [24]. However, place conditioning (a measurement of an appetitive process which signifies reward) was reduced or eliminated [24]. Overall, results of previous experiments show that administration of DA or its agonists increases receptivity in female rodents. Moreover, stimulation of D₁ receptors increases receptivity while stimulation of D₂ receptors decreases receptivity in female rodents.

Pacing

Data from studies on receptivity of female rats, led to research examining solicitation behaviors that interact with male copulatory behavior [7, 25]. Solicitation behavior can include the proceptive female behaviors of ear wiggling, darting and hopping. These behaviors are seen in receptive females who are not allowed to pace [7]. There is now evidence for different neural mechanisms that regulate proceptive, motivational and receptive sexual behaviors [7]. For the purposes of this research dissertation, the neural mechanisms that modulate pacing behavior will be discussed. Pacing mimics more closely the natural sexual behavior of rodents [7]. It is observed in natural settings and large environments, and therefore provides an assessment of more natural female sex behavior [2]. In laboratory experiments with rats, pacing occurs in chambers that have exit holes, which allow females to enter/exit the chamber of the male [7]. (see Figs. 3-4)

Figure 3: A schematic drawing of a pacing chamber used with female rats. The pacing chamber consists of a testing chamber separated by a Plexiglas barrier. The Plexiglas barrier has three enter/exits holes which are big enough for the female (white rat) to traverse both sides of the chamber but not the male (black and white rat). This allows the female an escape mechanism which allows her to control the amount and frequency of stimulation received [26].

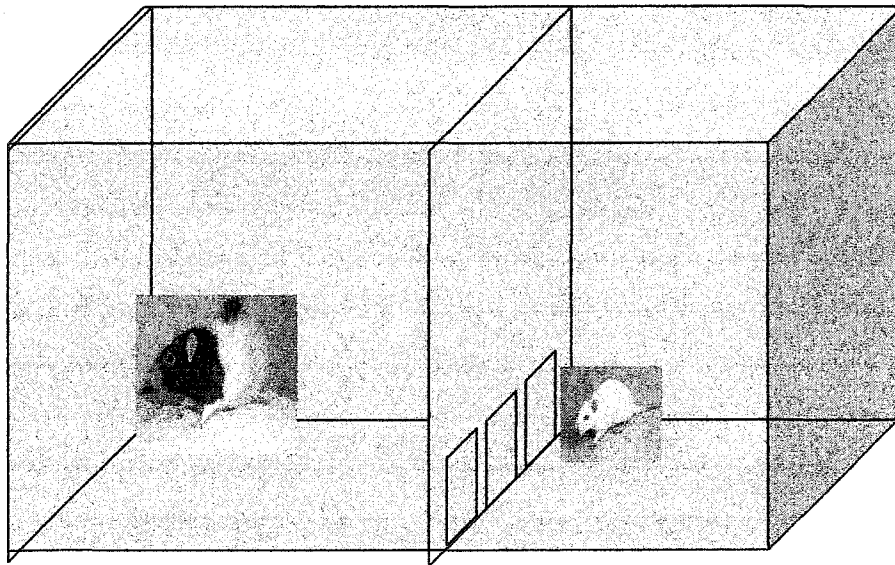


Figure 4: A schematic drawing of a bilevel pacing chamber. The testing chamber is narrow, which maintains a male and female rat in the optimal orientation for the observer to view sexual behavior, i.e., in the side view. Moreover, the chamber consists of an upper and lower level, which allows the females an avenue of escape from the male. Females can level change, by using the ladders on either side of the chamber to switch levels and escape the male. The chamber is used to evaluate lordosis and pacing behavior[27] .



The sexual stimulations given by the male to the female are mounts, intromissions and ejaculations [1]. The male begins interacting with the female by smelling her anal and genital area and attempting to mount her from behind [12]. A mount is when the male holds the female's hindquarters and thrusts without inserting his penis into the female's vagina [12]. Mounts occur first and provide the least stimulation [1]. If the female is receptive, intromissions are interspersed with the mounts [1]. Intromissions are the insertion of penis with or without ejaculations [12]. Copulation ends with ejaculation by the male, which is the most intense interaction [1]. The lordosis reflex is seen in receptive females in response to the male mount [11]. The receptivity of the female can be measured through the lordosis quotient (number of lordosis / number of mounts and intromissions) [16].

Pacing behavior is used to intersperse these stimulations [1]. If allowed, the female rodent will pace these stimulations by using an escape mechanism to approach and withdraw from the male [7]. This strategy allows her to control the frequency and amount of sexual stimulation she receives from the male [7]. As the intensity of the stimulation increases, generally the length of the time spent away from the male also increases [7]. The amount of time it takes the female to return to the male is referred to as a return latency [7]. Pacing and return latencies are mediated by vaginal stimulation [7]. When the vaginal area is anesthetized females do not pace, but they remain with the males throughout the bouts of stimulation [7]. Pacing behavior is seen in virgin rats as well as experienced rats, and return latencies are not altered by practice with or exposure to the male [7]. Also, estrogen and progesterone

administered to OVX female rats in a pattern mimicking the natural estrus cycle, can induce pacing behavior at the same level of naturally cycling female rats [28].

Reproductive Consequences of Pacing: Why do females pace? Why is this behavior interesting or relevant? Pacing behavior facilitates the likelihood of pregnancy or pseudopregnancy in female rats [18] [19]. Several studies have shown that fewer intromissions are needed to induce pregnancy or pseudopregnancy when sexual behavior is paced, compared to non paced sexual bouts [29]. In addition, if a female receives an ejaculation during a paced mating bout, she is more likely to get pregnant than if the sexual bout was not paced [28]. In pacing bouts, there are also longer inter-intromission intervals, which are more similar to natural settings, and are associated with a high proportion of females becoming pregnant [30]. Mating patterns, specifically intromissions, induce surges of serum prolactin, which persist in intact females for 12 days and define the period of pseudopregnancy [21]. In paced mating, less intromissions are needed to induce these serum prolactin surges compared to non-paced stimulation [29]. In addition, paced copulation increases the rate that estrous behavior declines toward the end of estrous[31]. Usually in female rats, large numbers of intromissions are needed to terminate estrus [29]. However, when allowed to pace, the vaginal-cervical stimulation seems to have a greater effect on estrus termination [31]. The need for less intromissions in order to induce pregnancy, pseudopregnancy, and termination of the estrus cycle, during paced copulation is due to the female rat being able to distinguish between the intensity of vaginal-cervical stimulation received during each copulatory mount [28]. Thus paced

stimulations overall seem to have more of an effect than non-paced stimulations on neuroendocrine changes that are induced by mating behavior [21].

Neural Basis of Pacing Behavior: Estrogen and progesterone are well known endocrine regulators of pacing behavior. However the neural regulation is much less understood. Some studies have investigated neural sites that are sensitive to estrogen and progesterone and therefore considered to be involved in pacing behavior.

Previous studies examining the neural regulation of lordosis found that the VMN mediates lordosis so it is a likely site to begin studying pacing behavior[32-36].

Implanting estradiol and progesterone in the VMN has been shown to stimulate lordosis in OVX female rats[37], while lesioning the VMN, reduces lordosis behavior [38, 39] and the number of approaches to the male [36]. The number of approaches did not increase when VMN lesioned females were treated with exogenous estrogen and progesterone [40]. This pattern suggests that damage to the VMN disrupts the pattern of approach to the male and ultimately disrupts the amount of coital stimulation that the female receives from the male.

Other studies have focused on the efferents projecting laterally from the VMN to the midbrain. Lesioning parts of the midbrain that receive efferents from the VMN have decreased or eliminated lordosis behavior, however, proceptive and motivational behaviors such as hopping, darting and pacing were not affected [39]. This was seen in both intact and OVX females [39]. Additionally, lesions in the ventral noradrenergic bundle (VNAB) eliminate lordosis but not precopulatory and pacing behaviors, and sometimes enhance these behaviors [40].

Because pacing underlies the female rat's ability to discriminate between different types of coital stimulation, pacing behavior may be controlled by areas in the brain that serve as terminals for vaginal-cervical sensory information. Additionally, since pacing behavior enhances the effect of mating stimulation that induces serum prolactin surges, the same brain areas might be involved [41]. The preoptic area of the hypothalamus (POA) tonically inhibits the nightly surge of serum prolactin following cervical stimulation and, when lesioned, these surges are repetitive [29]. Additionally, neurons responsive to vaginal-cervical stimulation are located in the POA [41]. But does it have a role on pacing behavior? When the POA was lesioned in females that are not allowed to escape from the male, lordosis was increased and enhanced [42]. However, when POA lesioned females were allowed to pace, there was no increase in lordosis. Additionally, there were decreases in time spent with sexually active male, number of copulatory mounts received and lordosis poses [43].

The data supports the idea of separate of neural controls of proceptive, motivational and receptive components of female rodent sexual behavior. When some areas of the brain VMN and VNAB are lesioned, lordosis behavior, but not pacing behavior is affected [39, 40]. In addition, brain areas, such as the POA, that are important for prolactin surges of pseudopregnancy and pregnancy [44] may also be important in pacing behaviors.

Description of Female Mice Sexual Behavior

Female mouse sexual behavior varies from female rat sexual behavior. Studies examining estrogen receptor knockout (ERKO) [45, 46] and progesterone

receptor knockout (PRKO) [47, 48] female mice and sexual behavior have described female mouse sexual behavior. Either wildtype [49, 50] or Swiss Webster [45, 51] males are used as stud males and there does not appear to be a difference in responsiveness from the female mice. The sexual tests can be conducted in the male home cage [45, 46, 51] or a separate testing chamber [49, 50]. If tested in a separate chamber, stud males have to be habituated for one hour in the test chamber before the female is introduced [49]. Testing time varies but sessions are usually thirty minutes long [45, 46] or terminated after a specific quantity of stimulations, such as 15 mounts [49].

Mounts in mice are defined as both male's forepaws on the female's rear [47]. Since the lordosis posture is not as rigid in female mice as in female rats, lordosis is defined in mice as all four paws being grounded, hind region elevated from the bottom of the test chamber and the dorsiflexion of the vertebral column. A lordosis quotient is calculated by dividing the number of lordosis postures by the number of mounts for each mouse [47]. Receptivity scores are calculated for each female mouse and are based on normal female behaviors such as squeaking, attempts to avoid mounts, rearing and kicking [47]. Receptivity scores given to wildtype females treated with estrogen and progesterone were approximately 65% and averaged 22 total mounts and 15 mounts with intromissions [50]. Subjects also showed 20% rejection behavior [46]. In addition to lordosis, sexual behavior in female mice also includes male and female approaches, female rejection behavior (fleeing, biting, kicking) and holding the still receptive posture with and without lordosis. During a thirty minute testing period female mice average approximately 35 sexual bouts

which are defined as sexual behaviors occurring in succession by the male, female or both mice until the mice are separated for more than half a body length [46].

When studying sexual behavior in mice, there are noteworthy strain differences that must be accounted for, in addition to experimental factors. Comparing the 129S6/Sv/Ev to the C57BL/6 strain is important because they are the parental strains of genetic mouse models [8]. When analyzing sexual behavior with these strains, several observations should be taken into account. When the female mouse is OVX, hormone replacement is necessary to achieve sexual behavior; however, estrogen alone will not induce high levels of lordosis and receptivity in either 129S6/Sv/Ev or C57BL/6 strains [48]. Administering progesterone along with estrogen to either strain results in high levels of sexual receptivity [48]. Lordosis quotients are higher for female mice primed with high doses of estrogen and administered progesterone than females primed with lower doses of estrogen [48].

To achieve maximal receptivity, both strains required weekly priming for six weeks with estrogen along with weekly testing [48]. However with equal hormone priming and testing, strain differences are still evident [48]. When high levels of estrogen and progesterone are administered, C57BL/6 females respond better than 129S6/Sv/Ev [48]. After six weeks of hormone treatment and testing, C57BL/6 mice have a LQ of approximately 80 while 129Sv/Ev mice have a LQ of approximately 60 [48]. Regardless of the strain, mice require sequential hormone treatment and testing experience to achieve maximal receptivity [48].

III. Dopamine and its Pathways

Sexual behavior in rodents has been used to examine the relationship between hormones, neurotransmitters levels and behavior. Our lab, as well as others, has examined sexual behavior in female rats to learn more about the role of neurotransmitters and hormones on sexual behavior [17, 18, 52-54]. These experiments have used pharmacological approaches such as administering agonists or antagonists or surgical approaches such as lesioning specific brain regions. DA and its pathways have been shown to regulate other motivational and reward behaviors, such as drug seeking behaviors [55-60]. Since pacing behavior in rodents is a motivational behavior, this dissertation wanted to determine whether DA contributes.

Dopamine Pathways

Mapping studies have estimated that DA constitutes eighty percent of the total brain catecholamine content [4]. Nerve cells containing DA are found in relatively rostral parts of the brain, including midbrain, hypothalamus and olfactory bulbs, and their projections are both ascending and descending [4]. There are many forebrain efferents projecting from the midbrain DA cell groups, and the substantial overlap of the midbrain and forebrain projections is sometimes labeled the mesotelencephalic DA system [4].

The DA system is divided into numerous pathways [4]. However for the purposes of this research dissertation, only three will be briefly described, the mesostriatal, mesolimbic and mesocortical, and the periventricular and diencephalospinal pathways.

Mesostriatal Pathway

Cells in the system originate in substantia nigra (ventral tegmental area (VTA)/A10 and A8 groups). The dorsal component (nigrostriatal pathway) ascends through the medial forebrain bundle followed by the internal capsule to innervate the caudate nucleus, and the putamen (collectively known as the striatum) [61]. There are approximately 10,000 DA cell bodies in A9 cell group on 1 side of the rat brain [62], however a single axon from one cell body can branch and result in many synaptic boutons in the striatum [63]. Numerous synaptic connections from the striatum make connections with medium sized spiny neurons that give rise to the output pathways of the striatum [64]. The ventral component cells originate in the VTA and projects to the nucleus accumbens (Nacc) [61]. The ventral component plays an important role in the reinforcing properties of psychostimulants and possibly other drugs of abuse [55]. DA cell bodies project to both the striatum and the Nacc and can have effects on motor functions.

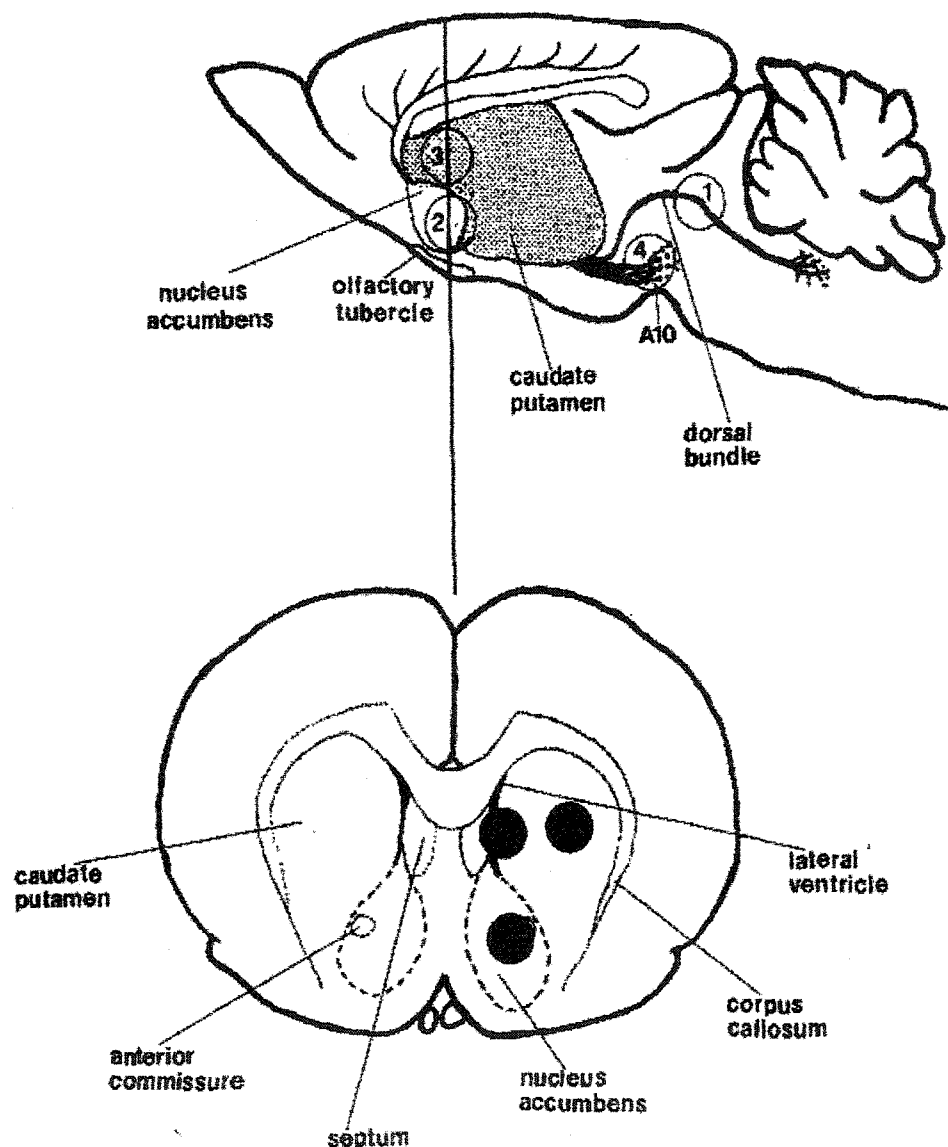
Mesolimbic System

This pathway arises from the DA neurons in the VTA [61]. The VTA is adjacent to the more prominent DA cell population of the substantia nigra [61]. The axons of these neurons project from the ventral tegmentum to limbic and cortical areas [61]. Specifically they ascend to hypothalamic tissue and distribute the axons to regions including the Nacc and prefrontal fields of the cerebral cortex [61]. They also project to the septum, amygdala, hippocampus, nucleus of the diagonal band, anterior olfactory nucleus, and limbic cortical areas [56]. (see Fig. 5)

The mesolimbic pathway constitutes a major part of the DA “reward” system [65]. This reward system is based on research that shows a DA surge in the Nacc,

when an animal is exposed to drugs of abuse [56]. It is also known that an animal will self stimulate to exhaustion if stimulatory microelectrodes are implanted along the trajectory of the medial forebrain bundle [56]. This evidence allowed the DA afferents to and from the Nacc, to be labeled as a “reward” system [56]. This system consists of DA cells in the VTA, being continually inhibited by GABA [56]. When disinhibited, DA is released and produces a reinforcement that is normally involved with natural rewarding stimuli such as food and sex [56]. When the term “reward” is used it describes behaviors that make an animal exhibit a self-reinforcing behavior. Reward does not describe a euphoric feeling [56].

Figure 5: Sagittal projection of the ascending dorsal noradrenergic bundle and the mesolimbic dopaminergic system depicting four lesion sites (upper panel). The lower panel displays a coronal section of the rat brain depicting three 6-hydroxydopamine injection sites into dopaminergic terminal fields that have been tested for their effects on cocaine self-administration. Coronal view corresponds to section A 8920. Reprinted from [66]



Pariventricular and diencephalospinal systems

Cells bodies for these systems originate in the A11 and are located in the periventricular gray of the posterior and dorsal hypothalamus and the caudal thalamus [4]. The local projections project to the medial thalamus and hypothalamic nuclei and make up the pariventricular DA system [4]. Some of the collateral branches of these fibers form the diencephalospinal system [4]. These branches descend through the dorsal longitudinal fasciculus and terminate in the spinal dorsal gray and the intermediolateral cell columns (where the sympathetic preganglionic neurons are located) [4]. Dual innervation of the pariventricular system (hypothalamic) and diencephalospinal system (sympathetic preganglionic cell groups) by the A11 dopaminergic neurons may allow for integrated control of central and autonomic components of complex motivated behavior such as sexual behavior [4].

Dopamine Receptors

All DA containing neural pathways contain DA receptors. DA receptors have at least six members, all belonging to the G protein coupled receptor superfamily [65]. Receptors are categorized as D₁-like and D₂-like [67]. D₁ and D₅ comprise the D₁-like receptors and D₂, D₃, and D₄ make up the D₂-like receptors [67]. The D₆ receptor is an isoform of D₂ [67]. (see Fig. 6)

D₁ receptors

D₁ receptors are distinguishable from *D₂* receptors because of their ability to stimulate adenylyl cyclase [68-70]. (see Fig. 6) These receptors have seven membrane spanning domains [65]. The highest density of the *D₁* receptor is found in the termination areas of the mesostriatal system (striatum, Nacc and olfactory tubules) and substantia nigra [4]. Intermediate levels have been found in some nuclei of the amygdala [4]. Low *D₁* levels are located in the neocortex, thalamus, cerebellum, hippocampus, septum and hypothalamus [4]. *D₁* receptors can have a low affinity or high affinity to agonists (including DA) because of receptor-G protein interactions [4]. High affinity occurs when the receptor is complexed with a G protein [4]. Antagonists do not differentiate between receptors complexed with a G protein and those that are not [4, 71].

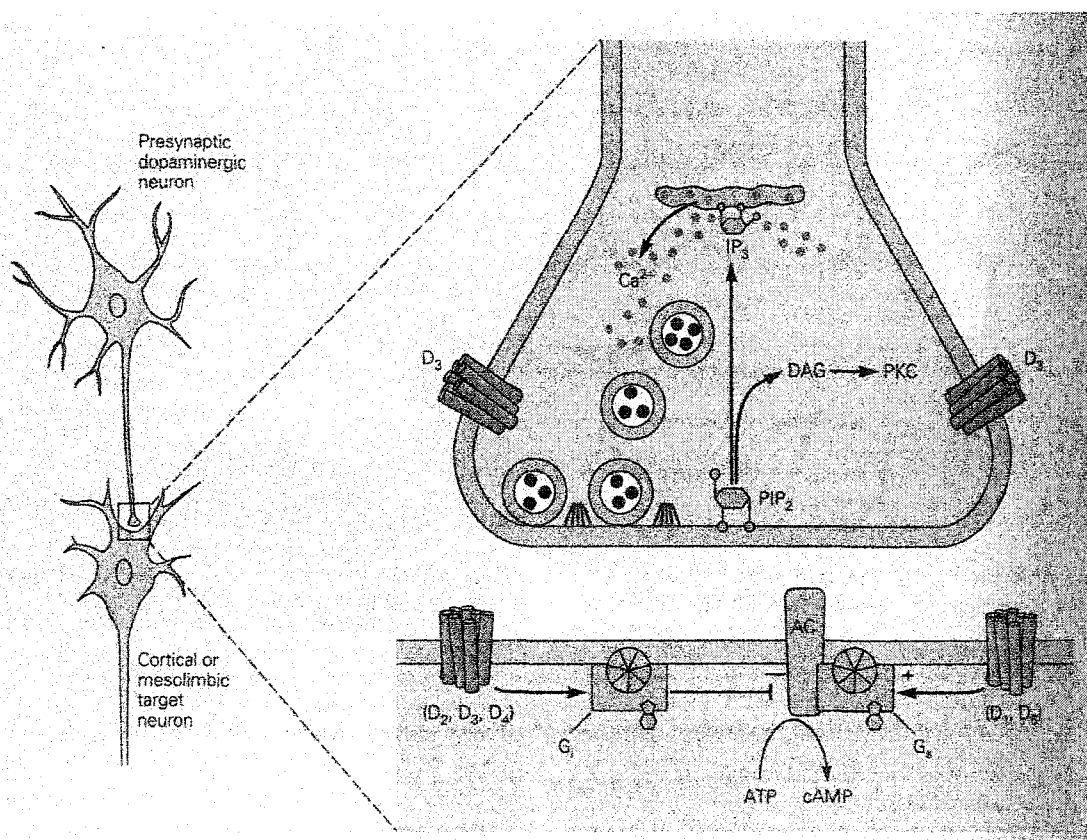
D₂ receptors

D₂ receptors have been identified because of their high affinity for antipsychotic drugs and their ability to either inhibit or not affect adenylyl cyclase. [67]. (see Fig. 6) *D₂* human receptors have a 96% homology with rat *D₂* receptors [72]. As mentioned before, there are two isoforms of *D₂* receptors (*D_{2L}* and *D_{2S}*). These two isoforms are produced from the same gene by alternative mRNA splicing with respect to the presence or absence of a stretch of 29 amino acids located on the third cytoplasmic loop of receptor [67]. Currently there is no evidence of any important pharmacological or functional differences between *D_{2L}* and *D_{2S}* [4].

In rats, the highest levels of *D₂* binding have been found in the striatum, Nacc, olfactory tubercle, substantia nigra, pars compacta, and glomerular layer of olfactory bulbs [4]. Intermediate levels have been found in the central nucleus of the

amygdala, lateral septum and the superior colliculus [4]. It is also interesting to note that in virtually all areas in which D_1 and D_2 receptor densities were directly compared, levels of D_1 receptors were distinctly greater [73].

Figure 6: Summary of the molecular events that lead to dopamine release. An increase of Ca^{2+} (as shown by the smaller dots) within the cell is a common trigger for the release of a neurotransmitter. The diagram shows that the D_1 and D_5 receptors activate (indicated by the +) adenylyl cyclase (AC) leading to the production of cAMP. The D_2 , D_3 , and D_4 receptors, on the other hand, inhibit it, as indicated by the ---] sign shown below. cAMP is a second messenger that activates other molecules within a cell, causing the cell to undergo certain changes in response to the increased presence of cAMP. Adapted from Kandel et al., 2000 [65].



Dopamine Receptor Mechanisms

Low ejection currents of DA or the selective D₁ agonist SKF 38393 had no effect on spontaneous activity of striatal neurons, but potentiated the increase in firing produced by the excitatory amino acid glutamate [74, 75]. This type of neuromodulatory action may be a mechanism whereby DA from the nigrostriatal pathway can enhance the excitatory glutamatergic input from the neocortex to the striatum (the corticostriatal pathway). At high ejection currents, both DA and selective D₁ and D₂ agonists mainly exert an inhibitory effect on spontaneous and glutamate-evoked nerve cell firing [75, 76]. However, some evidence shows that moderate stimulation of D₂ receptors can elicit an excitatory rather than an inhibitory response [76, 77]. More studies are needed to determine how these mechanisms relate to behavior regulated by DA systems.

Dopamine autoreceptors

DA autoreceptors are found in terminals, cell bodies and dendrites of DA neurons [78]. (see Fig. 7) Activation of presynaptic (terminal) DA autoreceptors inhibits both DA synthesis and release. Activation of somatodendritic DA autoreceptors causes a reduction in cell firing, which secondarily reduces transmitter release in terminal areas [79]. Dendrites of DA cells in substantia nigra release DA [80] and such release is stimulated by membrane depolarization or amphetamine administration [80, 81]. Dendritic DA release can thus be presumed to be a source of stimulation of somatodendritic autoreceptors, thereby modulating neuronal impulse flow and terminate DA release [79]. Early research suggested that DA autoreceptors effects are mediated by receptors with a D₂-like rather than a D₁ pharmacology [82,

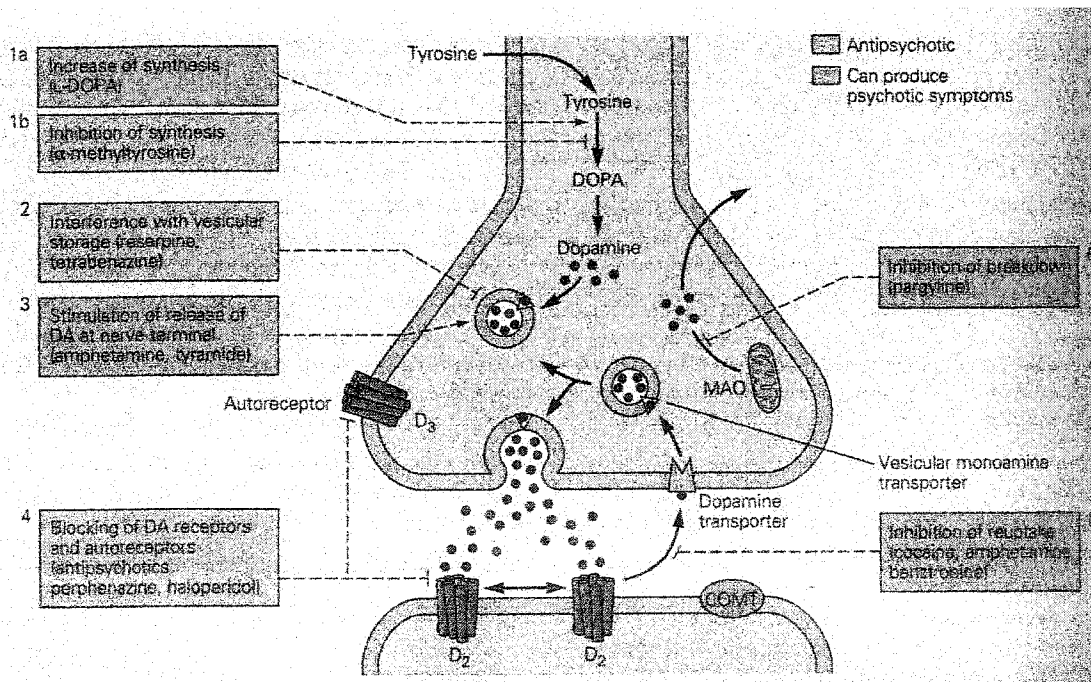
83]. Some research suggests that DA autoreceptors are distinct from classic D₂ receptors [84, 85]. Action of both somatodendritic autoreceptors and presynaptic inhibitory autoreceptors is mediated by increased opening of potassium channels [86, 87] which hyperpolarizes the cell membrane and decreases the firing rate of DA release. In contrast, autoreceptor mediated inhibition of DA synthesis seems to occur via a reduction of cAMP-dependent activation of tyrosine hydroxylase in nerve terminals [88, 89].

Autoreceptors are not found in all DA systems. In rats, autoreceptors appear to be on most ascending DA projections except for those terminating in prefrontal and cingulate cortices [90]. These differences may account for the higher firing rate of mesoprefrontal and mesocingulate fibers as compared with those of the mesostriatal system.

Figure 7: Presynaptic and postsynaptic area of a DA ending. The key steps in the synthesis and degradation of DA and the sites of action of various psychoactive substances at the DA synapse.

1. **Enzymatic synthesis.** The conversion of tyrosine to DOPA (dihydroxyphenylalanine) by tyrosine hydroxylase is stimulated by L-DOPA and blocked by the competitive inhibitor α -methyltyrosine.
2. **Storage.** Reserpine and tetrabenazine interfere with the uptake and storage of DA by the storage granules. Reserpine is an effective antipsychotic drug; the depletion of DA by reserpine is long lasting and the storage granules appear to be irreversibly damaged. Tetrabenazine also interferes with the uptake and storage mechanism of the granules but only transiently.
3. **Release.** Amphetamine and tyramide enhance DA release from dopaminergic neurons by blocking reuptake. Amphetamine induces a psychosis that is reversed by psychoactive drugs.
4. **Receptor interaction.** Typical antipsychotics such as perphenazine and haloperidol are particularly effective in blocking the D_2 and the presynaptic autoreceptors.
5. **Reuptake.** DA activity is terminated when DA is taken up into the presynaptic terminal. Amphetamines, cocaine and benzotropine are potent inhibitors of the reuptake mechanism.
6. **Degradation.** DA present in a free state within the presynaptic terminal can be degraded by the enzyme monoamine oxidase (MAO). Pargyline is an effective inhibitor of MAO. Some MAO is outside the dopaminergic neuron. DA can also be inactivated by the enzyme catechol-O-methyltransferase (COMT), which is believed to be localized outside the neuron in the postsynaptic cell.

Adapted from Kandel, Schwartz and Jessel 2000 [65]



Dopamine receptor agonists and antagonists

Apomorphine (APO) is a general DA receptor agonist [71]. APO treatment in humans can cause nausea and vomiting. Clinically, it has been used as an emetic after oral ingestion of certain poisons or drug overdoses [71]. Fortunately, rats and mice are incapable of vomiting. The mode of action of APO is to stimulate D₂ mediated receptors in the chemical trigger zone of the medulla oblongata of the brain [71]. Even though APO seems to affect D₂ mediated behavior effects, it is not overly selective for D₂ over D₁ receptors [71]. APO has a higher affinity for presynaptic autoreceptors than for postsynaptic DA receptors [71]. Low doses of APO diminish dopaminergic neurotransmission by selectively activating autoreceptors [4]. High doses stimulate dopaminergic functions by acting directly on postsynaptic receptors [4].

Many drugs that are agonists and antagonists of DA are available (see [4] for details). DA general antagonists of DA include haloperidol, pimozide, and flupenthixol. D₁ receptor agonists include SKF38393, SKF81297, SKF82958, and SKF82526. D₁ antagonists include SCH23390 (also has an affinity for 5-HT₂ receptors), SKF83566 and NNC-112, NNC-756 and SCH 39166. D₂ agonists include quinpirole and LY-163502, while D₂ antagonists include sulpride and raclopride [4].

Behavioral Effects of Apomorphine

Numerous studies have investigated APO's effects on various behaviors [91-97]. For the purposes of this dissertation, the behaviors of interest are sexual and locomotor behaviors. Locomotor behaviors are important to study because copulatory behavior, specifically pacing, involves locomotor activity and are an

essential part of pacing behavior. Generally, in both rats and mice, low doses of APO produce hypomotility and yawning, while higher doses produce stereotyped behaviors such as sniffing, licking and gnawing, [92]. Higher doses of APO enhance locomotor activity in a biphasic way in C57 mice and increase climbing behavior [93].

Particularly, 3mg/kg of APO produced an increase in locomotor activity and climbing behavior in comparison with saline[98].

Self administered stimulation thresholds were measured in rats treated with five doses of APO and trained to press a lever that delivered electrical stimulation to the lateral hypothalamus [99]. Low doses caused thresholds to increase while the two higher doses caused thresholds to drop [99]. The authors hypothesized that the change in the direction of the behavioral effect corresponds with the shift in APO's action from presynaptic activation to predominantly postsynaptic activation of DA receptors as the concentration of APO increases [99].

Rats administered low doses of APO showed increased yawning behavior which indicates activating of presynaptic DA receptors. Higher doses result in induced stereotyped sniffing and yawning which reflects an activation of pre and postsynaptic DA receptors [96]. The authors further investigated APO's effect on pre and postsynaptic DA receptors by administering the D₁ receptor agonist SKF 38393. SKF 39393 inhibited yawning induced by low doses of APO [96]. However this effect can be attenuated if the rat was pretreated with specific D₁ receptor antagonist SCH 23390 [96]. High doses of APO induced sniffing and this effect was potentiated when rats were pretreated with SKF 38393 [96]. These data indicate that D₁ receptor activation modulates both pre and postsynaptic effects of APO but the effect is in

opposite directions [96]. In a similar study, rats were treated for 21 days with the selective D₁ blocker SCH 23390. One, three, five and eleven weeks following the chronic treatment they received either a low hypomotility inducing dose or a high stereotyped behavior inducing dose of APO [97]. The rats always show an enhanced stereotyped response to the higher dose of APO, but never any change in their motility to the lower dose of APO [97]. The authors postulate that the results provide a behavioral correlate of the pre and postsynaptic modulation of APO by D₁ receptors [97]. A critical dose of APO (300 micrograms/kg s.c.) given immediately before placing rats into a novel environment produced initial sedation followed by enhanced locomotion [100]. When these same rats were treated with D₁ and D₂ antagonists, the APO induced mobility response was inhibited, indicating that APO stimulation can be antagonized by D₁ as well as D₂ receptor blockade [100]. However a high affinity for D₂ receptors seems vital for the blockage of APO inhibition [100].

APO effects behavior [91-96, 100], receptor activation [100] and DA metabolite levels [101, 102]. Chronically stressed C57BL/6 mice treated with two doses of APO showed decreased climbing behavior and reduced DA metabolites, DOPAC, HVA and 3-methoxytyramine (3-MT) concentrations in the caudate putamen and Nacc compared to non stressed mice. The authors suggest that chronic stress lead to a sensitization of mesolimbic DA presynaptic receptors possibly by an associated downregulation of postsynaptic DA receptors in the C57 strain [102].

Acute immobilization stress causes a significant reduction of spontaneous locomotion and a slight reduction of spontaneous climbing, while ten days of chronic stress decreased stress induced locomotion and increased stress induced spontaneous

climbing [94]. Twenty four hours after the last stressor, the results were reversed with increased locomotion behavior observed in the acutely stressed mice and decreased climbing behavior observed in the chronically stressed mice [94]. Following acute exposure to immobilization stress, DOPAC/DA and HVA/DA ratios were increased in the striatum and in the Nacc [101]. Finally, chronically stressed mice tested 24 hours after the last stressful experience, showed an increased sensitivity to the inhibitory effects of low doses of APO on climbing behavior and a decreased sensitivity to the inhibitory effects of the same doses of the DA agonist on locomotion [101]. These experiments show that APO can activate both pre and postsynaptic DA receptors depending on dose administered. In addition, stress can attenuate these APO induced behavioral changes which indicates that stress can modulate APO's activation of pre and postsynaptic receptors [94, 101].

Factors such as stress and dose are notable because they influence behavioral activation by pre and postsynaptic DA receptors. These factors can directly affect both pacing and receptive behaviors, causing unexpected increases or inhibition. Stress and dose are important to consider when examining the effects of DA or APO on female mice copulatory behavior. Currently, there are reports that APO both facilitates [22] or inhibits [103] copulatory behavior in female rodents . The discrepancy in reports is probably due to the aforementioned factors.

Behavioral Effects of Selective DA receptor agonists and antagonists

Systematic doses of D₁ agonists cause robust stimulation of grooming in rats [104]. Direct infusions to the striatum or Nacc can cause patterns of hyperactivity and stereotyped behavior. D₂ agonists, quinpirole or RU 24213 cause increases in

locomotion, sniffing, and snout contact [105-107]. These changes similar to APO behavioral effects but are not as robust. These effects can be blocked by both D₂ and D₁ antagonists as well as endogenous DA depletion [106, 108]. Combined administration of D₁ and D₂ selective agonists cause more robust effects than either singly [106, 109]. Both D₁ and D₂ selective antagonists can also cause catalepsy [110].

Dopamine, Estrogen and Progesterone

Administration of estrogen and progesterone to OVX female mice facilitates sexual behavior [48]. Research has shown that estrogen and progesterone have genomic and nongenomic effects that are mediated by interacting with DA receptors, uptake sites, and DA levels [23, 111-121]. Genomic effects have a long latency and require a specific hormone-receptor interaction, specifically translocation of the complex to the cell nucleus and subsequent induction of specific hormone-sensitive proteins [114]. Nongenomic effects are rapid in onset, recoverable and stereospecific [114]. Both types of effects are important to mention in the context of this dissertation because the behavioral paradigm used as well as past research requires that OVX female rodents are administered estrogen and progesterone either acutely or chronically [1, 11].

While the main focus of the current research was on DA concentrations in the striatum and Nacc because they are part of the mesolimbic “reward” system, the interaction between DA, estrogen and progesterone should also be considered. In the paradigm used in this dissertation, all animals were treated with estrogen and

progesterone to initiate sexual behavior. Therefore, the interaction effects of hormones with DA, its metabolites and its receptors warrant further discussion.

Estrogen and DA

Estrogen replacement is essential to achieve maximal receptive behavior in OVX female rodents. Estrogen has genomic effects in the brain including inducing progesterone receptors that facilitate copulatory behavior. Several studies have investigated the interaction between estrogen and DA levels [115, 122], receptors [120, 123] and transporters [117] in the mesolimbic DA system. When the female rat is OVX, extracellular DA concentrations and DA transporter density decreases [103, 122] and D₁ receptor density is decreased compared to gonadally intact rats [123]. In comparison, high affinity D₂ receptor sites increase and supersensitivity of striatal D₂ receptor binding sites develops over 2-3 months [120].

When estrogen is administered to OVX rats, there is a rapid release of DA in the striatum, an increase in striatal DA turnover and a downregulation of D₂ receptors in the striatum [118, 124]. Acute estrogen has also been shown to act directly on the Nacc to enhance potassium stimulated DA release [114]. In vivo voltammetry shows that local injections of 17 β -estradiol produce rapid and dramatic increases in stimulated DA overflow in the Nacc [114]. When a physiologically dose is given acutely to OVX female rats, DA turnover is increased in both the striatum and Nacc, while DA concentrations remain the same [118]. OVX females treated with chronic estrogen show a reduction of DA concentrations in both the striatum and Nacc, and no effect on DA turnover [121]. Although there is less research on estrogen effects in the Nacc, research suggests that the mechanisms are similar to the striatum [121].

When DA transporter levels were compared in short term versus long term OVX female rats before and after treatment with estrogen, several interesting results were found [117]. Compared to intact females, both short and long term OVX females showed a significant decrease in DAT density in the striatum, and the long term OVX females also showed a decrease in the Nacc [117]. DAT density was restored in striatum but not in the Nacc, of short term OVX females that were treated with estrogen [117]. Estrogen treatment did not change the DAT density levels in the striatum of the long term OVX females but it did restore levels in the Nacc [117].

This information is relevant to this dissertation for several reasons. All female mice used in these experiments were OVX and administered estrogen both acutely and chronically. The length of time that females were OVX before they receive hormones directly affects DA levels, its receptors and transporters. In view of the fact that this dissertation research measured DA, receptors and metabolites in the striatum and Nacc, the time between the ovariectomy and the estrogen administration is critical.

Progesterone and Dopamine Interactions

Several studies show that estrogen induced progesterone receptors mediate progesterone effects on sexual behavior in rodents [47, 48]. Progesterone receptors, like estrogen receptors are ligand inducible members of a superfamily of transcription factors that undergo significant conformational change upon ligand binding [47]. Studies using progesterone receptor antagonists and protein synthesis inhibitors consistently demonstrate the mediating role of intracellular progesterone receptors on sexual behavior[48] [47]. Progesterone's genomic effect on sexual behavior was

demonstrated in these studies [47]. However there is also evidence that progesterone has nongenomic effects on sexual behavior, possibly mediated through effects at the membrane [47]. The minimal receptivity response of progesterone receptor knockout mice (PRKO) given progesterone implies that membrane bound progesterone is not the primary mediator of the lordosis response in mice [47]. Instead, evidence suggests that intracellular genomic effects act together with membrane receptor effects to mediate sexual behavior in rodents [47].

Progesterone receptors are of particular interest because of their interaction with DA [125]. DA induced sexual behavior in mice requires an unoccupied progesterone receptor [47]. Positive correlations have been demonstrated between receptor concentrations in the hypothalamus and the maximal behavioral response, results which demonstrate the requirement for elevated levels of progesterone receptor for behavioral responsiveness to progesterone in mice [125].

Experiments with hormone primed PRKO have suggested crosstalk between progesterone receptors and DA [125]. Wildtype and PRKO were administered the D₁ agonist, SKF 39393, but only wildtype female mice exhibited high levels of lordosis.[125] The PRKO showed minimal responses to both progesterone and D₁ agonists [125]. These results suggest that progesterone receptors mediate DA's contribution to sexual behavior. [125].

DA is released in the striatum when estrogen primed female rats are treated with progesterone [9]. Furthermore after estrogen priming, a membrane associated protein with a high affinity for progesterone has been isolated from the striatum [9]. These effects of progesterone on striatal DA release are not seen without estrogen

priming [9]. Studies comparing the effect of repeated estrogen with either acute estrogen or progesterone treatment find prior exposure to estrogen enhances the effects of subsequent estrogen or progesterone treatments on AMPH-stimulated DA in dialysates from the dorsal lateral striatum[9] . In addition, significant increases of DA in the Nacc and striatum occurred concurrently with DA release in the VMN in the female during copulation [9].

IV. The Role of Dopamine in Sex Behavior

DA is a mediating factor in sexual behavior, specifically for incentive, motivation and reward. When sexually sluggish male rats are administered APO (a general DA agonist), copulatory behavior increases [126]. Sexually active male rats administered haloperidol (general DA antagonist), display decreased sexual behavior and motivation [127]. APO injected into the ventral tegmental area (VTA) delays onset of copulation and slows its rate. These males showed fewer complete copulatory behaviors (mounts, intromissions), and more misdirected copulatory attempts, implicating that the VTA affects motor but not motivational aspects of male rat sexual behavior [128, 129].

Experiments that administered selective DA agonists and antagonists in the medial preoptic area of male rats found the following results: the D₂ agonist, LY-163502 delayed the onset of copulation and slowed the rate of copulation [130]. A reduced number of vaginal intromissions were required to reach ejaculation [130]. The D₁ agonist, SKF-82526 had no effect alone or in combination with LY-163502 [130]. The D₁ antagonist SCH-23390 delayed the onset of copulation and decreased ejaculatory threshold (the number of vaginal intromissions required to trigger ejaculation) [130]. A low dose of D₂ agonist alone and with the D₁ antagonist delayed onset of copulation and reduced ejaculatory threshold [130]. This data suggests that stimulating D₂ receptors or blocking D₁ receptors delays onset of copulation and reduces ejaculatory threshold. Overall, this data indicates that DA, or its agonists, increase receptivity. Additionally stimulation of D₁ receptors increases receptivity, while stimulation of D₂ receptors decreases receptivity [130]

Additional studies have shown that the D₁ antagonist, SCH-23390 and the D₂ antagonist, raclopride injected into the medial preoptic area (MPOA) decreases the sexual motivation of male rats [131]. Additional evidence of DA modulating sexual behavior comes from work examining extracellular DA in the mPOA [132]. In male rats, there are changes in DA release during copulation in brain areas that play a role in reward and motivation aspects [132]. Specifically, DA is released in the mPOA during copulation and in other DA pathways during encounters with a receptive female [132]. These dopaminergic pathways underlie the motivational and reward aspects of sexual behavior. Specific increases are found in the nigrostriatal system, which enhances readiness to respond to stimuli as well as the mesolimbic system, which is critical for numerous types of motivation [133]. As mentioned previously, there are also increases in the mPOA which focuses motivation onto specific sexual targets, increases copulatory rate and efficiency, and coordinates genital reflexes [134]. The increase of DA in these three areas generally enhances sensorimotor integration by removing tonic inhibition. Therefore, steroid hormones increase the responsiveness of certain neurons but only after DA removes tonic inhibition.

Ascending DA projections to the Nacc are also involved in sexual behavior of the male rat. Several studies show an increase in DA in the Nacc of the male rat during sexual behaviors [135, 136] and it is important to determine if similar changes occur in females during sexual behavior. Extracellular DA increases in the Nacc of male rats during copulation as well as during exposure to stimuli that has been previously associated with a receptive female [135, 136]. These data suggest that, in

the male rat, DA release in the Nacc is associated with incentive or motivational behavior.

Findings that DA increased in both the mPOA and the Nacc during sexual behavior led researchers to examine if this effect occurs in female rats. DA transmission in female rats has not been studied as extensively as in male rats. In studies examining DA in the mPOA during female sexual behavior, DA levels were increased when a hormone primed female became receptive as well as when copulating with a male [137]. The increase of DA during copulation only occurred when the male and female were confined in the same arena [137]. However, when the female was allowed to pace her contacts with the male, no increase in DA was seen in the female mPOA during copulation [137]. DA levels in MPOA were also measured in vivo during sexual activity after priming with estrogen and progesterone. DA levels increased when females were primed with low levels of estrogen and given 500 µg of progesterone. Further increases in MPOA DA occurred when females copulated in non pacing chambers, and when they were allowed to pace, DA metabolites rose during copulation [137]. Single unit activities, recorded from Type 1 neurons in POA of female rats while copulating, were increased when the female rats initiated proceptive behavior [19]. Type 2 neurons showed brief activity when the male mounted [19]. Type 3 neurons fired in response to intromission, and type 4 were inhibited throughout lordosis [19]. Type 1-3 neurons were recorded from the transitional regions between the medial and lateral POAs [19]. Type 4 neurons were located more medially in the medial POA. Systemic injections of pimozide (a DA receptor blocker) diminished firing in type 1 neurons and abolished proceptivity [19].

The firing pattern in type 1 neurons appears to represent the motivational state of rat and has implications for consummatory value of penile intromission [19].

The role of DA transmission in both the striatum and Nacc has also been examined in estrogen and progesterone primed hamsters. Vagino-cervical stimulation was correlated with elevated DA levels in the Nacc [138]. Estrogen treated female rats also had enhanced increases of DA in Nacc compared to vehicle treated females [2]. DA levels, in striatum, increased more in OVX, hormone primed, female rats that paced copulation than in female rats that did not pace copulation [3]. Female rats that paced also showed a greater increase of DA in Nacc [3]. Lesions of the striatum reduces pacing and lesions to the shell of the Nacc causes failure of females to initiate sex prior to a sexual contact [139].

Results from numerous studies have shown an important modulatory role of DA on sexual behavior in female rats. These results have lead to this investigation of the regulatory role of DA on sexual behavior in female mice. Further investigations using mice will broaden the understanding of the role of DA in female sex behavior. Moreover, the possibility of investigating behavior in mice, which overexpress (transgenic) or do not express (knockout) specific components of DA neurons, provide a unique advantage of a mouse model. Establishment of a mouse model will allow further exploration of the regulatory roles of DA and its receptors on the reward system involved with sexual behavior. This new model will compliment current pharmacological studies already existing in with rats. The combination of information from rats and mice models may increase understanding of evolutionary and genetic differences underlying neural mechanisms of behavior.

V. Research Design and Methods - General Experimental Design

Animals

Animals used in this series of experiments were six week old C57BL/6N Tac or 129S6/Sv/Ev black female mice. Subjects were OVX at Taconic Farms prior to arrival at the Hunter College Animal Facility. Subjects were doubly housed with food and water ad lib and maintained on a 12-hour light/dark cycle. To ensure that males were larger than females, twelve week old Swiss Webster males served as stud males. Males were housed individually and received food and water ad lib.

Injections

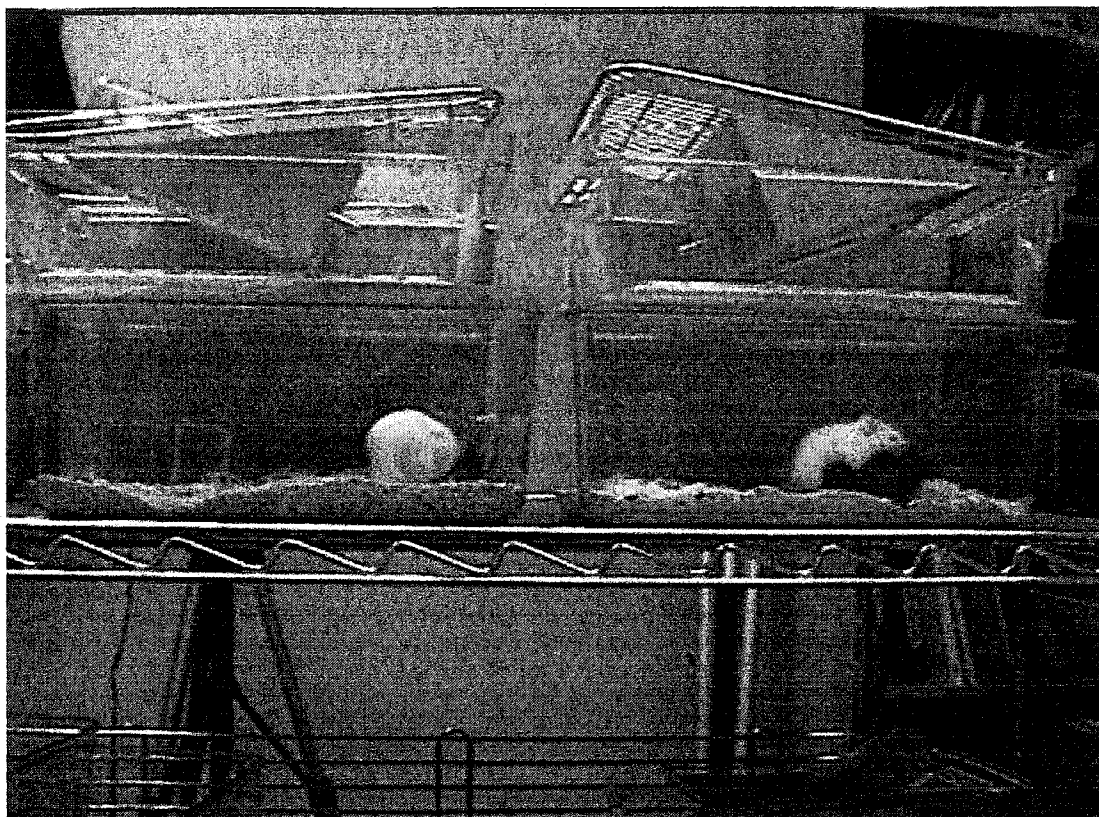
Each female received injections of 10 μg estradiol benzoate 48 and 24 hours before testing and 500 μg progesterone five hours before testing. These injections have been shown, in both rats [7, 139] and mice [46, 47, 50], to induce sexual receptivity. None of the males received injections.

Pacing Apparatus

The pacing apparatus consisted of the male's home cage with the addition of two Plexiglas cubes with a small opening. The openings are large enough for the female, but not the male, to enter and exit. The male's home cage is thirteen centimeters high, eighteen centimeters wide and twenty-nine centimeters long. Both the male and female had access to the entire cage. However, only the female had access to the inside of the cubes, but the males frequently tried to enter the cubes and sat on top of the cubes. The pacing cubes remained in the male's home cage during the testing session and were removed following completion of testing. During testing, the male's home cage was covered with Plexiglas instead of the wire top.

This change ensured that the subjects would not be distracted by food, water or climbing on the cage top. Because mice are exploratory, it was necessary to acclimate them to the chamber prior to testing. Pacing cubes remained in the female's homecage from a week after their arrival until they were sacrificed. This procedure allowed acclimation to the cube and ease of traversing through the small opening. The male was exposed to the pacing cubes five hours prior to testing, so that the male would be interested in the female, not the cubes, during sex behavior testing.

Figure 8: Pacing Chamber for Mice. Two chambers are shown. Each chamber consists of the male's homecage to which two Plexiglas cubes have been added, the cubes have small openings that only the female can fit through. The cubes allow the female an escape mechanism so that she can approach and withdraw from the male when desired. The top of the cage is replaced with a Plexiglass cover.



Behavioral Testing

Five hours after injection of progesterone, behavioral testing began. The pacing apparatus was placed in the male's home cage five hours prior to testing. The female was then placed in the male's home cage and the behavioral test began. All testing sessions were videotaped for behavioral scoring. Two observers recorded the exit latencies; return latencies after interactions such as mounts, intromissions and ejaculations as well as the number of mounts, intromissions and ejaculations and number of rejections. Exit latencies are the time it takes the female to leave and reenter the male chamber after stimulations. All sessions lasted thirty minutes or until ejaculation occurred. The percentage time in the male chamber, the percentage time in the pacing cubes, the percentage of leaves after each type of stimulation, the average time spent with the male, the average time spent in the pacing cubes, and the percentage of exits after stimulation were also calculated.

Each female was tested once a day, every four days with a maximum of two consecutive tests. All tests were performed under red light during the dark portion of their circadian cycle. After completion of testing, the female mice were sacrificed by rapid decapitation and their brains frozen on dry ice for further neurochemical analysis.

Monoamine Analysis

Brains were sectioned at 250-300 μ m at -6 to -8 $^{\circ}$ C in a microtome cryostat. Nacc, striatum, ventral medial nucleus, vertical diagonal band and amygdala brain areas were micropunched from sections according to the atlas of Franklin and Paxinos [140] with a 500 μ m diameter cannula while the slide rested on a microscope stage

which was maintained at -11.5°C as described by Luine et al., 1999 [141]. The monoamines and metabolites DA, DOPAC, HVA, serotonin, 5HIAA and norepinephrine were measured. Punched samples were placed in 1.5 ml Ependorf tubes with 60 μl of sodium acetate buffer containing α -methyl-DA (as internal standard). After freezing-thawing and centrifugation, the supernatant was removed and 2 μl of 1mg/ml ascorbate oxidase solution (Sigma) was added to each sample to minimize the HPLC front. Forty microliters was injected into a Waters Associates chromatographic system consisting of a Waters 2690 Separation Module, and Symmetry C_{18} -5 μm column. An ESA 5011 Colocomb 3100A electrochemical detector with the screening electrode set at +0.05 V and the detecting electrode at +0.35 V was used. Concentrations of neurotransmitters and metabolites were calculated by reference to standards using peak integration with a computer assisted Water Millennium system. Sample runs averaged 30-40 minutes. The pellet was dissolved in 100 μl of 0.2 N NaOH for protein determination by the Bradford method. Concentrations were expressed as $\text{pg}/\mu\text{g}$ protein [141].

Statistical Analysis

The behavioral data was scored and the number of mounts, intromissions, ejaculations, rejection behavior, returns latencies after each stimulation, total percent time spent with the male, total time spent in the neutral pacing cubes, exit latency, exit after stimulation and total exits after each type of stimulation for each female mouse were calculated. Descriptive statistics including the mean and standard error were obtained for each measure. One way ANOVA was used analyze data when a between subjects design was used and different groups of mice were treated with

different drugs or doses of the same drug. If ANOVA was significant at the $p < 0.05$ level, appropriate post hoc test were used to test differences between groups. Linear regression was used to correlate neurotransmitter levels to the number of stimulations received. Correlations at the $p < 0.05$ level were accepted as significant.

VI. Experiment 1 – Establishment of a Pacing Paradigm for Female Mice

Rationale

Extensive research has examined sexual behavior of female rats and shown that DA levels increase in specific brain areas when female rats are allowed to pace and copulate [2, 3, 9]. In comparison, little research has focused on sexual behavior of female mice. Data from female mouse studies will complement already existing pharmacological data from female rats. When a pacing paradigm for female mice is developed, it can be used with transgenic and knockout mice. 129S6/Sv/Ev females were selected for these experiments because knockouts are developed from 129 and C57 lines.

Specific Aims

1. Establish and quantify baseline pacing and receptive behavior in female mice.
2. Determine monoaminergic neurotransmitters that influence pacing.

Hypotheses

1. Female mice will pace in a manner similar to other small rodents.
2. DA and its metabolites, DOPAC and HVA, will increase in the striatum and Nacc when female mice are allowed to pace and copulate.

Animals

Female mice of a 129S6/Sv/Ev or C57BL/6N Tac background were used.

Injections

Female mice of the 129S6/Sv/Ev and C57BL/6N Tac were hormone primed with 10 µg of estrogen 24 and 48 hours before testing and 500 µg of progesterone five hours before testing.

Behavioral Testing

The subjects were tested as described in the general experimental methods.

Monoamine Analysis

Immediately following testing, brains removed, sectioned, micropunched and analyzed using HPLC analysis as described in the general experimental methods.

Statistical Analysis

Linear regression was used to correlate relationships between total stimulations and monoamine levels in the striatum, Nacc, VDB, and VMN as described in the general experimental methods.

Results

Female mice of the 129S6/Sv/Ev were not suitable for behavioral analysis because they were lethargic and exhibited low levels of copulatory behavior. These subjects showed little interest in the male and rarely used the pacing cubes. In addition, they showed low receptivity (few mounts or intromissions) (data not shown). Therefore C57 female mice were utilized for the rest of the experiments. This strain is also appropriate because they are used to create knockout mice.

Apparatus

Several apparatuses were evaluated for quantification of mouse pacing. The first attempt used a scaled down version of an established apparatus for testing of rat pacing [7] (see Fig 9a). This apparatus consisted of a chamber with Plexiglas barrier with three enter/exit holes that were too small for the males to fit through but were large enough for the females.

This apparatus was not suitable for analysis of mouse pacing. The females would traverse back and forth through the enter/exits holes when they were without the male. However, when males were placed in the chamber, many females would sniff at the holes, but never cross to the male side. Other females would cross to the male side but they never crossed back to the female side; thus they did not display pacing behavior. The greatest problem was that persistent males would attempt to cross through the enter/exit holes. Some males succeeded at squeezing through the holes, but others became stuck in the holes and had to be removed.

This apparatus was modified by removing the holes and substituting a solid wall of Plexiglas (see Fig. 9b). This Plexiglas barrier was raised above the bottom of the cage, and allowed for the females but not the males to fit under. Similar problems were encountered as in the first design. Some females would stick their head under the barrier, but not completely cross over to the male side. Other females crossed over to the male side but never crossed back over to the female side. Finally, some persistent males tried to cross over to the male side and became stuck under the barrier.

Though these first two apparatuses failed, a consistent trend was noted. Though the females did not use the enter/exit holes or the barrier to escape the male,

they did display other behaviors. Specifically, if the male approached a female and who was unwillingly to be mounted, she would crouch in a corner of the cage and reject him by kicking and rearing up on her hind legs. This strategy worked temporarily, but if the male was persistent, he could eventually push her out of the corner and overpower her.

Observing this interaction, a new apparatus that utilized the rejection behavior was devised (see Fig 9c). Plexiglas boxes (7 cm width, 6 cm height, and 7 cm depth) with small enter/exit holes that the female, but not the male, could fit through were constructed. These boxes were placed in two diagonal corners of the cage and allowed the female to escape the male, if desired. If the male attempted to follow her into the box or put his head into the box, she could kick or nip him to keep him away. Also, the Plexiglas box protected females from being pushed out of the corner. This configuration proved to be the best way to measure pacing behavior in female mice. The small boxes gave females an escape mechanism from the male and allowed the female to approach and withdraw from the male when she desired.

Figure 9a: Scaled down version of an established apparatus for testing of rat pacing. This apparatus consisted of a chamber with Plexiglas barrier with three enter/exit holes that were too small for the males to fit through but were large enough for the females.

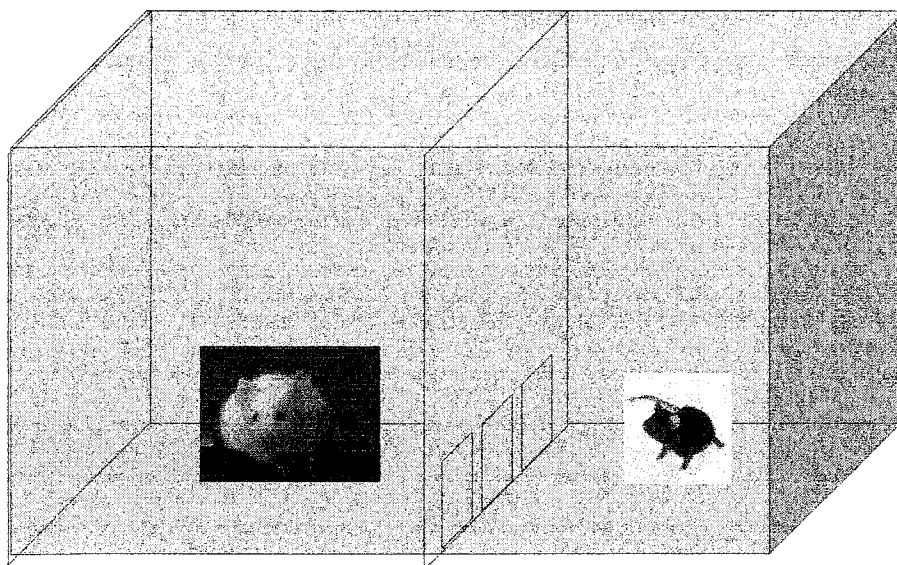


Figure 9b: Modified pacing apparatus for testing of mouse pacing. This apparatus was modified by removing the holes and substituting a solid wall of Plexiglas. The Plexiglas barrier was raised above the bottom of the cage, and allowed for the females but not the males to fit under.

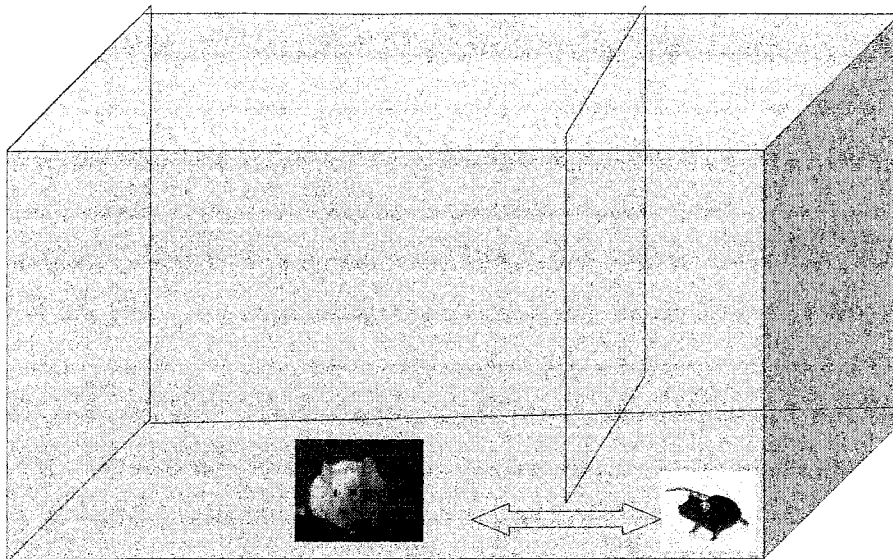
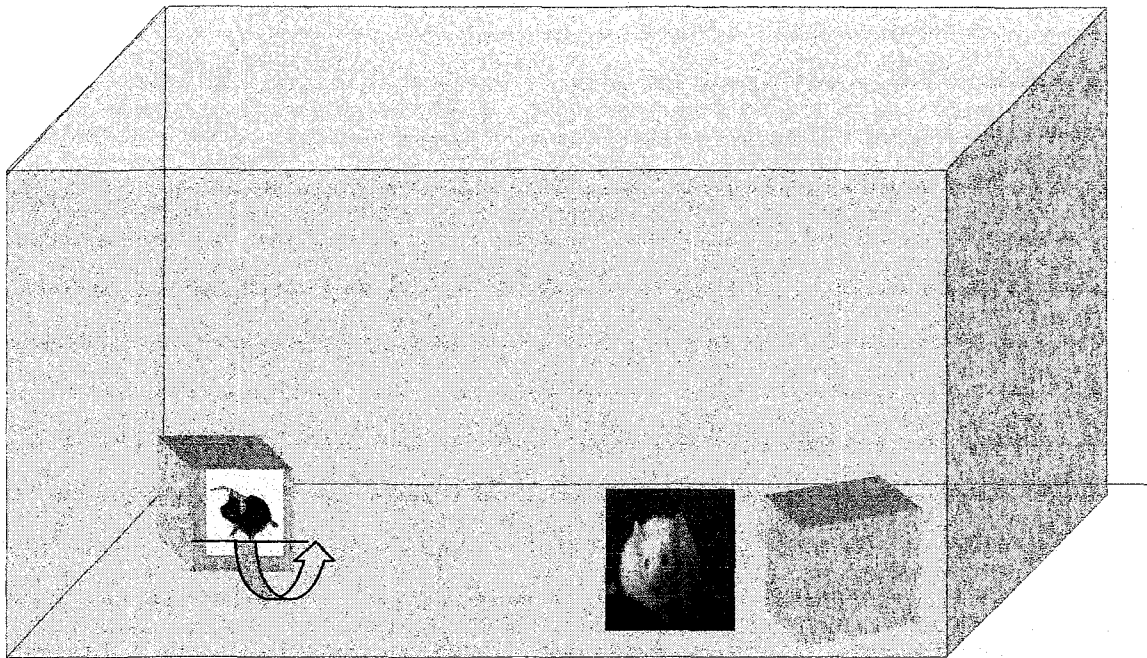


Figure 9c: Successful apparatus used in experiments. Plexiglas boxes (7 cm width, 6 cm height, and 7 cm depth) with small enter/exit holes that the female, but not the male, could fit through were constructed. These boxes were placed in two diagonal corners of the cage and allowed the female to escape the male, if desired.



Behavioral Measures in Pacing Females

Utilization of C57 females and Plexiglas escape cubes in the male home cage enabled measurement of female mouse pacing behavior. Pacing behavior in female rodents differs by species and most research has been done on pacing female rats (see Table 1). However, a description of female mice pacing behavior is also necessary and important to further understand female rodent copulatory behavior. Female mice pacing consists of entering and exiting two pacing cubes, which have a small enter/exit hole on one side to allow the female to escape and approach the male at will. In addition to entering and exiting the pacing cubes, the female mice also use rejection behavior, which consists of kicking, nipping and biting the male if he approaches her before she is ready to copulate. The pacing cubes, combined with the rejection behavior allow the female mouse to pace her copulations by providing an escape mechanism which allows her to control the amount and frequency of stimulation she receives from the male. Observations from this experiment have illustrated that female mice use both the pacing cubes and rejection behavior to escape the male. Therefore quantifying both the frequency of utilizing the cubes as well as the frequency of rejection behavior displayed provides an accurate assessment of pacing behavior in female mice. Pacing is quantified by calculating the latencies to return to the male after receiving a mount, intromission or ejaculation, total number of rejections and latency to return to the male after rejection behavior. The total amount of time spent with the male and in the pacing cubes is also calculated.

Table 1: Comparison of average Pacing Measures between Female rats and Mice. Mean and SEM. All latencies were measured in seconds.

1. Unpublished observations reported by Luine et al. , 1998 [142]
2. Observations reported by Erskine, 1989 [7]
3. Unpublished observations reported by Hodges et al., 2004

	Percent time Spent with Male	Percent time spent away from male	Return Latency after Mount	Return Latency after Intromission	Return Latency after Ejaculation	Percentage Exits after Mounts	Percentage Exits after Intromissions	Percentage Exits after Ejaculations
R a t s	55.00 ± 5.0 ₁	45.00 ± 5.0 ₁	14.33 ± 5.31 ₂	26.05 ± 3.83 ₂	49.00 ± 21.20 ₂	50.50 ± 11.47 ₂	73.57 ± 5.96 ₂	100.00 ± 0.0 ₂
M i c e	77.02 ± 7.37 ₃	12.12 ± 9.56	70.74 ± 1.58 ₃	55.29 ± 20.02 ₃	118.93 ± 12.26 ₃	49.09 ± 2.90 ₃	58.78 ± 33.28 ₃	100.00 ± 0.0 ₃

As shown in results, receptivity in female mice differs from receptivity in female rats. A female rat is receptive if she displays the lordosis posture in response to a male mount, which allows the male to successfully copulate [7]. Therefore, quantifying the frequency and quality of lordosis (calculating a lordosis score) serves as a measure of receptivity. Female mice do not consistently display the lordosis posture, which means that lordosis can not be easily used to measure receptivity. Instead, a female mouse is considered to be receptive if she remains still and allows the male to mount and intromit, without showing rejection behaviors. For the purposes of this series of experiments, female mice receptivity was categorized as low, medium and high. This categorization was developed from previous and current observations of female mouse sexual behavior in our laboratory (see Table 2).

Table 2: Evaluation of Female Mice Receptivity

This ranking system was developed to determine if a female mouse is displaying low, medium or high levels of receptivity. Data was derived from behavioral tests using estrogen and progesterone treated OVX mice using the mouse pacing paradigm of small Plexiglas cubes in the male homecage.

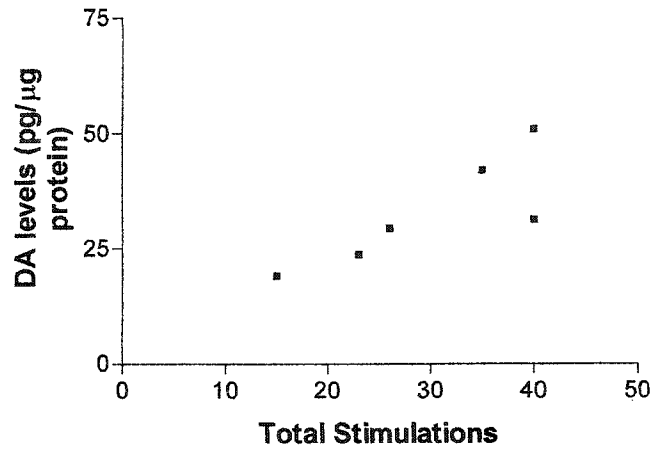
Behavior	Low	Medium	High
Mounts	0-1	2-5	6+
Intromissions	0-1	2-4	5+
Ejaculations	0	0	1
Rejection Behavior	5+	2-4	0-1

Monoamine and Metabolite Levels in Pacing Females

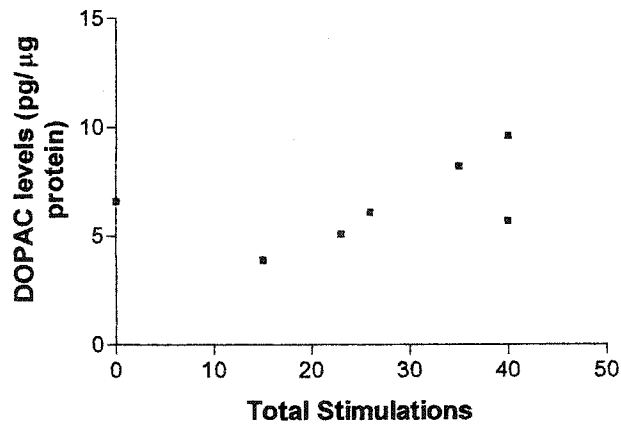
OVX females received estrogen and progesterone treatment and were tested for sexual behavior. Immediately following behavioral testing, mice were rapidly decapitated and their brains collected and frozen. Brains were sectioned at 250-300 μm and micropunched as described in the general experimental methods. Levels of monoamine levels and their metabolites (DA, DOPAC, HVA, HVA/DA 5-HT, 5-HIAA, NE) were measured in the striatum, Nacc, VMN, and amygdale using HPLC analysis described in the general experimental design. Though not significant, a trend was seen between total stimulations (mounts plus intromissions) and DA, HVA, and DOPAC levels in the Nacc (see Fig. 10). However, we did not find any other significant correlations or trends between any other monoamines and amount of stimulations or pacing parameters (see Figs. 11-13).

Figure 10: Levels of DA and metabolites in nucleus accumbens plotted versus total stimulations. Each data point represents one mouse. (n = 6). There was a significant correlation between DA and total stimulations ($r^2_{1,4} = 0.68$, $p < 0.05$). The correlation was not significant for metabolites and total stimulations including DOPAC and total stimulations ($r^2_{1,5} = 0.22$, $p = 0.30$), and HVA and total stimulations ($r^2_{1,5} = 0.10$, $p = 0.49$).

DA levels in Accumbens vs Total Stimulations



DOPAC levels in Accumbens vs Total Stimulations



HVA levels in Accumbens vs Total Stimulations

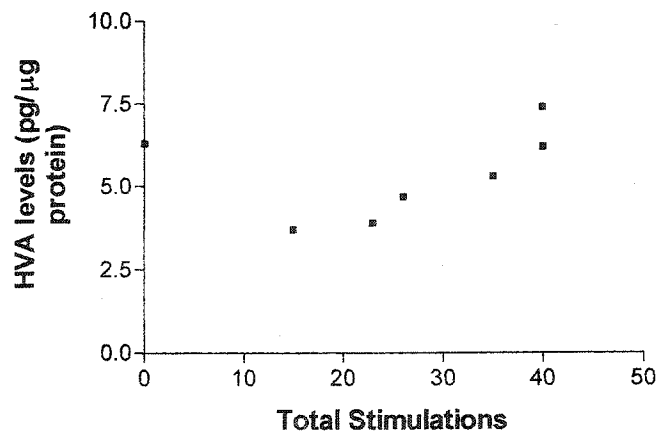


Figure 11: Levels of monoamine and metabolites in Amygdala plotted versus total stimulations. Each data point represents one mouse. (n = 5). There was no significant correlation found between the monoamine or metabolite levels and total stimulations in amygdala. This includes DA levels and total stimulations ($r^2_{1,4} = 0.02$, $p = 0.77$), NE levels and total stimulations ($r^2_{1,4} = 0.11$, $p = 0.51$), DOPAC levels and total stimulations ($r^2_{1,4} = 0.13$, $p = 0.47$), 5HIAA levels and total stimulations ($r^2_{1,4} = 0.06$, $p = 0.64$), HVA levels and total stimulations ($r^2_{1,4} = 0.12$, $p = 0.50$), 5HT levels and total stimulations ($r^2_{1,4} = 0.00$, $p = 0.98$), HVA/DA levels and total stimulations ($r^2_{1,4} = 0.05$, $p = 0.65$), and 5HIAA/5HT levels and total stimulations ($r^2_{1,4} = 0.37$, $p = 0.20$).

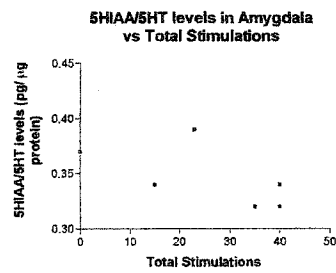
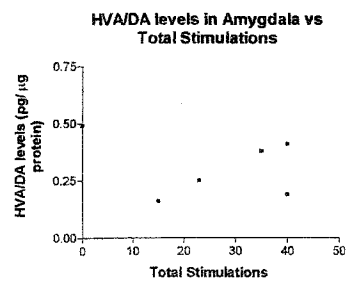
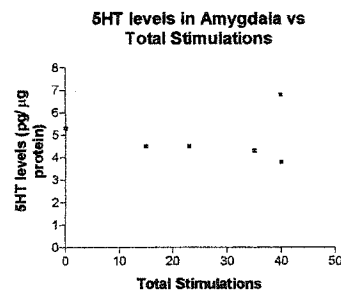
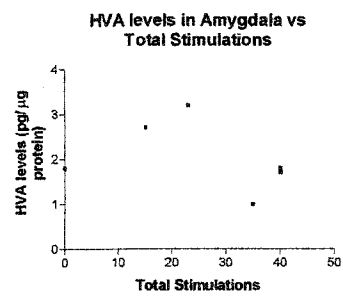
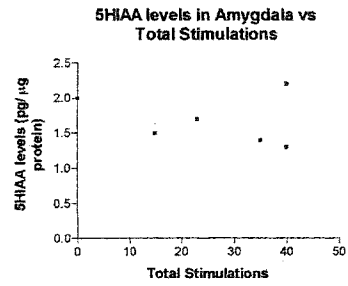
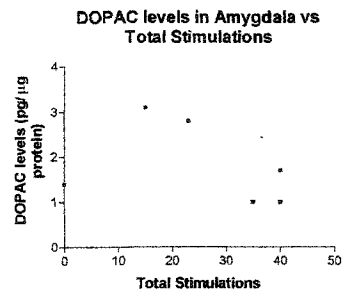
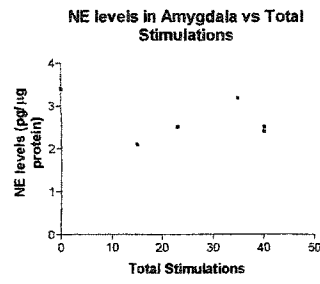
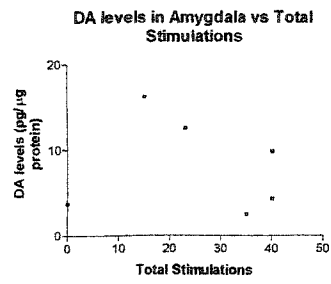


Figure 12: Levels of monoamine and metabolites in VDB plotted versus total stimulations. Each data point represents one female mouse. (n = 6). There was a positive correlation between 5HIAA/5HT levels and total stimulations in VDB ($r^2_{1,5} = 0.69$, $p < 0.02$) There was no significant correlation found between the monoamine or metabolite levels and total stimulations in VDB. This includes DA levels and total stimulations ($r^2_{1,5} = 0.01$, $p = 0.78$), NE levels and total stimulations ($r^2_{1,5} = 0.00$, $p = 0.91$), DOPAC levels and total stimulations ($r^2_{1,5} = 0.04$, $p = 0.65$), 5HIAA levels and total stimulations ($r^2_{1,5} = 0.13$, $p = 0.42$), HVA levels and total stimulations ($r^2_{1,5} = 0.05$, $p = 0.61$), 5HT levels and total stimulations ($r^2_{1,5} = 0.04$, $p = 0.66$), HVA/DA levels and total stimulations ($r^2_{1,5} = 0.19$, $p = 0.33$).

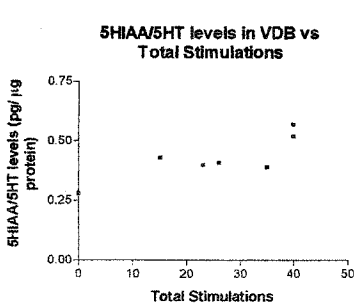
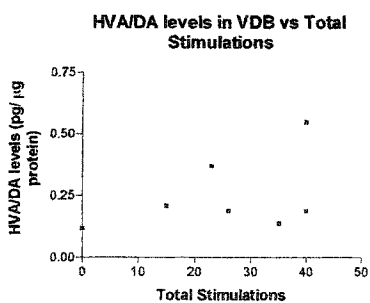
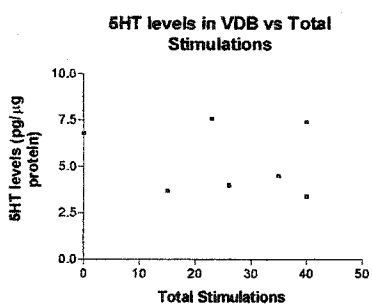
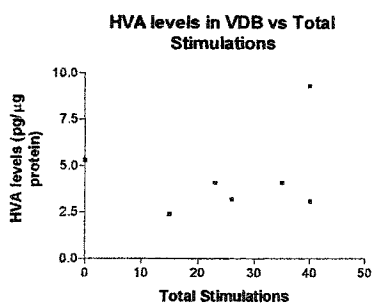
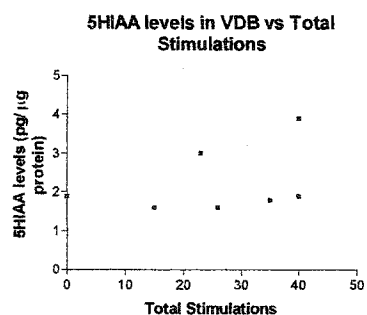
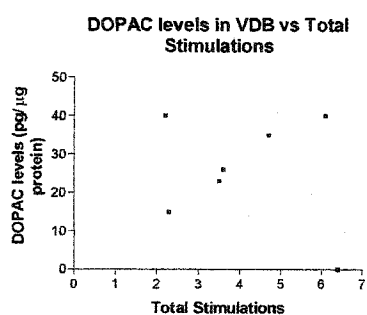
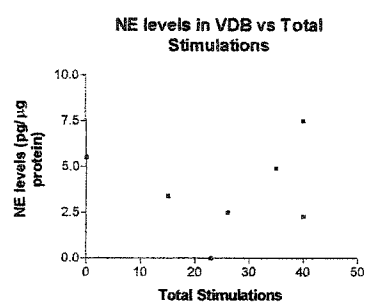
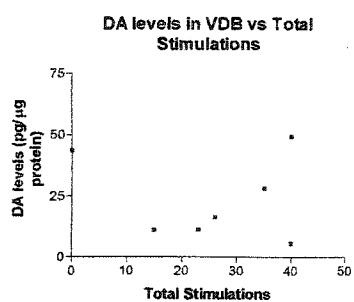
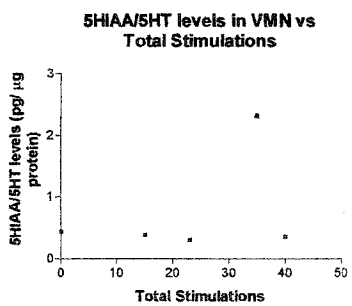
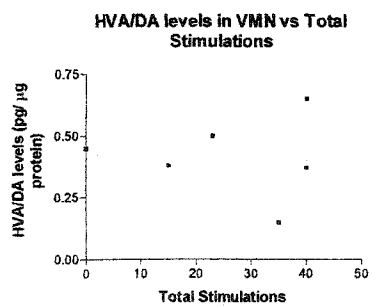
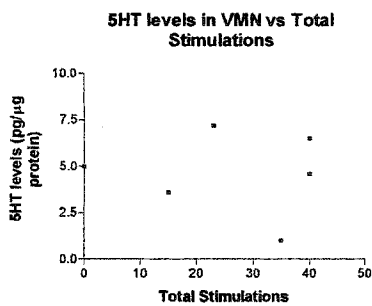
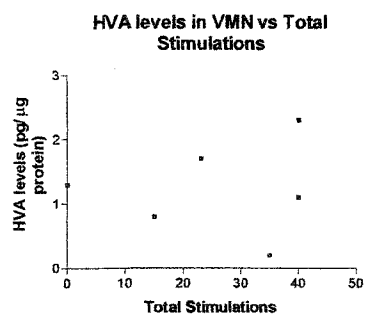
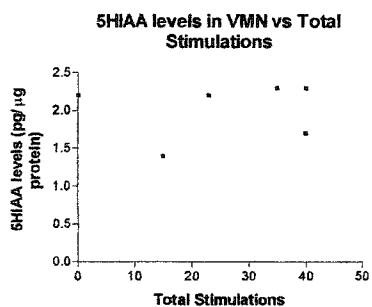
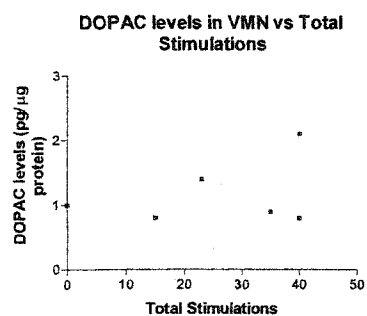
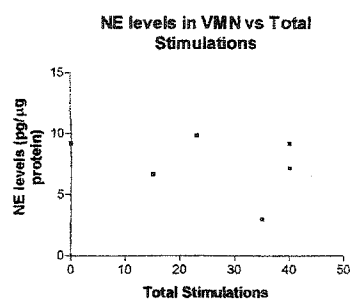
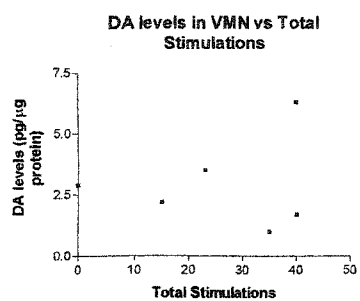


Figure 13: Levels of monoamine and metabolites in VMN plotted versus total stimulations. Each data point represents one mouse. (n = 5). There was no correlation found between monoamine levels and total stimulations in VMN. This includes DA levels and total stimulations ($r^2_{1,4} = 0.02$, $p = 0.80$), NE levels and total stimulations ($r^2_{1,4} = 0.10$, $p = 0.55$), DOPAC levels and total stimulations ($r^2_{1,4} = 0.10$, $p = 0.53$), 5HIAA levels and total stimulations ($r^2_{1,4} = 0.01$, $p = 0.82$), HVA levels and total stimulations ($r^2_{1,4} = 0.01$, $p = 0.86$), 5HT levels and total stimulations ($r^2_{1,4} = 0.00$, $p = 0.89$), HVA/DA levels and total stimulations ($r^2_{1,4} = 0.00$, $p = 0.94$), and 5HIAA/5HT levels and total stimulations ($r^2_{1,4} = 0.07$, $p = 0.62$)



Discussion

The results from this experiment demonstrated that female mice will pace when a suitable apparatus is used. Female mice pace at a lower rate compared to female rats (see Table 1). Observations of a lessened rate of pacing and receptivity as compared to female rats may be due to the amount of habituating sessions and hormone treatments. Hormonal treatments and habituating sessions were based on female rat behavior. In subsequent experiments, it was discovered that both the male and female mice need at least three training sessions to begin to show ample copulatory behavior. Studies examining sexual behavior in PRKO mice have shown that optimally, female mice need approximately six weeks of weekly hormone treatments and testing sessions to achieve maximal copulatory behavior [47], while rats only need a maximum of two hormone treatments and one training session before displaying maximal copulatory behavior [7]. The experiments discussed above were conducted before information regarding the optimal hormone and practice levels to achieve maximal copulatory behavior in mice was published. The mice in this experiment were not trained or administered hormone treatments for six weeks, which may explain why the female mice displayed low levels of receptivity (5 or more mounts and 6 more intromissions) compared to other experiments where female mice average 22 mounts and 15 mounts with intromissions [50].

Males, rats or mice undergo a “stud versus dud” test to determine if they will be suitable stimulus males for the experiment. If they do not show adequate copulatory behavior (mounts and intromissions) after three sessions with receptive females; they are not used with for experiments. Vigorous sexual behavior is important because the experimental females can show high levels of rejection behavior. If the stud males are

not good copulators, they will stop attempting copulation with the experimental females.

Male and female mice must also be acclimated to the pacing apparatus prior to testing. If they are not, they will spend the majority of the testing session exploring the pacing cubes instead of engaging in copulatory behaviors. The low levels of receptivity and pacing behavior observed in this experiment may be attributed to lack of prior habituating sessions and adequate hormone treatment. In subsequent experiments, more habituation sessions and higher hormone doses were given and greater behavior was found. (see subsequent experiments)

This experiment also examined monoamine levels in the striatum, Nacc, vertical diagonal band, amygdala and VMN in female C57 mice. A positive correlation was found between DA, DOPAC and HVA and total stimulations in the Nacc. These results are consistent with previous results showing that DA mediates copulatory behavior in female rats and hamsters [2, 24]. Previous research has shown elevated DA levels in the striatum and Nacc when rats or hamsters are allowed to copulate [3, 138]. These increases were even larger in the striatum and Nacc when females were allowed to pace their copulations. [2] [139] [3].

VII. Experiment 2 – Effects of Apomorphine (20 µg, 60 µg and 120 µg) Pacing and Receptive Components of Copulatory Behavior in C57 Female Mice

In the previous experiment, a method for measuring pacing behavior in female mice was established. Using this method allowed for several observations. The first is that female mice do pace in a similar manner, but at a lesser rate, than female rats (see Table 1). In addition, consistent with previous rat studies we found that DA and its metabolites increased in the Nacc as a function of sexual stimulations received (see Fig 10). This pattern indicated that DA in the Nacc, has a mediating role in copulatory behavior in female mice. Numerous female rat studies report similar results [2, 3, 139]. DA increases in both the striatum and Nacc when females have been exposed to male rats as well as when allowed to pace copulation [2, 3, 9, 122, 139].

APO is a general DA agonist that binds to both D₁ and D₂ receptors and can be used to examine the role of DA in regulating behaviors. Various doses of APO were administered (20, 60, 120 µg) and motivational and receptive components of copulatory behavior of female mice were measured.

Specific Aim

Assess the role of DA and its receptors in specific brain areas on receptive and motivational aspects of sexual behavior.

Hypothesis

Previous studies examining DA levels during copulation have found that DA increases in the striatum and Nacc during paced copulation [2, 3, 139]. Therefore it is hypothesized that administering APO, the DA receptor stimulating drug, to OVX female mice will cause an increase in pacing and receptive behavior.

Animals

Four groups of 7-14 OVX female C57 mice were used. The first group received saline and served as controls. The rest received 20 μ g, 60 μ g, or 120 μ g of APO. Animal care is outlined in the general experimental methods.

Injections

All female mice received 10 μ g of estrogen 48 and 24 hours before testing and 500 μ g of progesterone five hours before testing. Each group received a subcutaneous injection of APO or saline 15 minutes before behavioral testing began.

Experimental Methods

The behavioral testing methods are outlined in the general experimental methods.

Statistical Analysis

One way ANOVA was used to analyze data. If ANOVA was significant at $p < 0.05$, Fishers LSD, a post hoc test, was used to test specific differences between groups.

Results

Using the receptivity chart (see Table 2), all groups treated with APO displayed low to medium levels of receptivity while the control group displayed high levels of receptivity. The group treated with 60 μ g APO received significantly fewer mounts than the control group ($F_{3, 41} = 2.90, p < 0.05$) (see Fig 14). Intromissions were also decreased groups treated with APO, but this effect did not reach statistical significance.

Pacing was generally increased by APO. The group treated with 120 μ g of APO spent significantly more time in the neutral cubes after receiving an intromission than the control group, the 20 μ g APO group and the 60 μ g APO group ($F_{3, 13} = 6.83, p < 0.05$) (see Fig 15). Other measures of pacing did not show a statistically significant increase.

Figure 14: The effects of apomorphine on receptive behaviors. Data are mean \pm SE number or percentage of stimulations after treatment with saline, 20 μ g, 60 μ g or 120 μ g of APO (n = 7 to 14 per group). Data were analyzed by one way ANOVA. Fishers LSD, *post hoc test*, showed that the group treated with 60 μ g of APO received significantly less mounts than the control group ($F_{3,41} = 2.90$, $p < 0.05$).

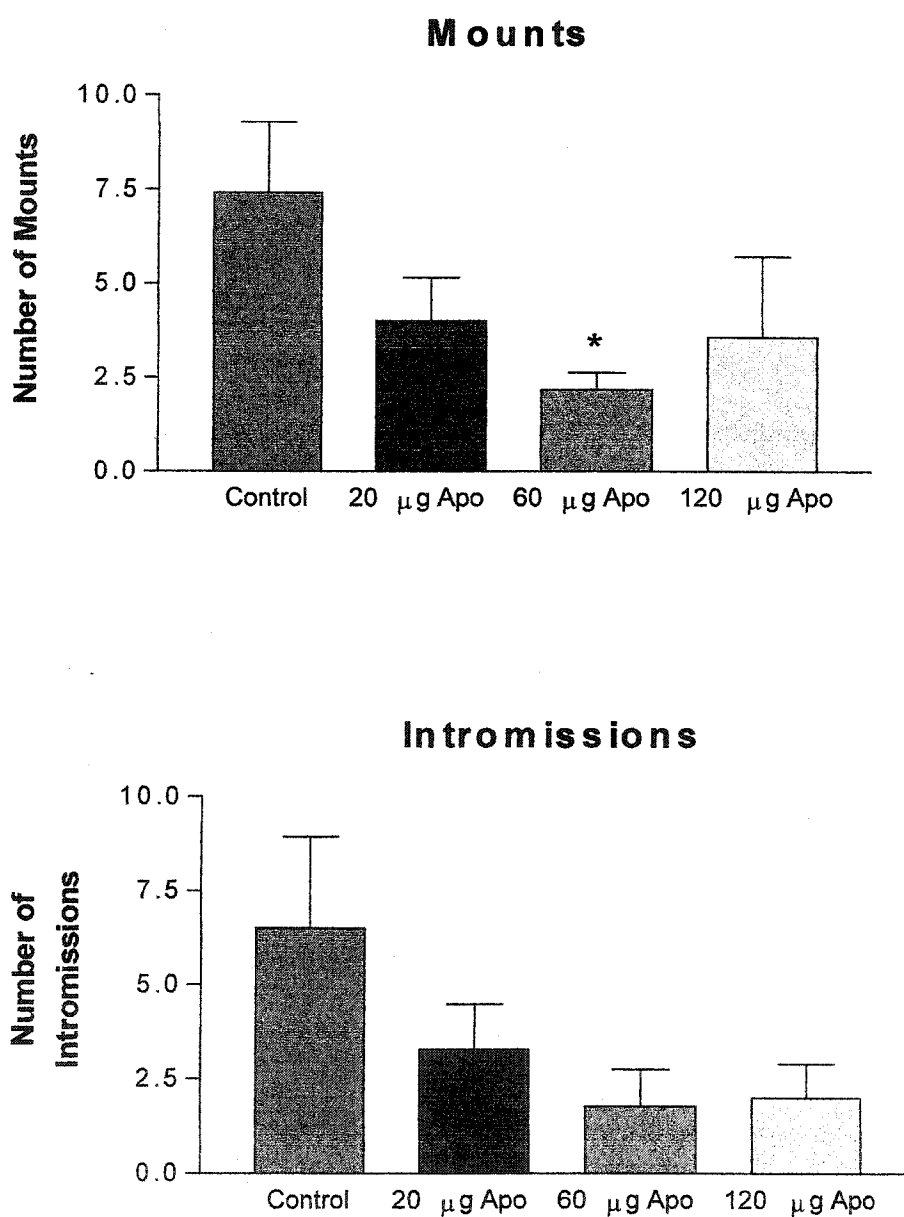
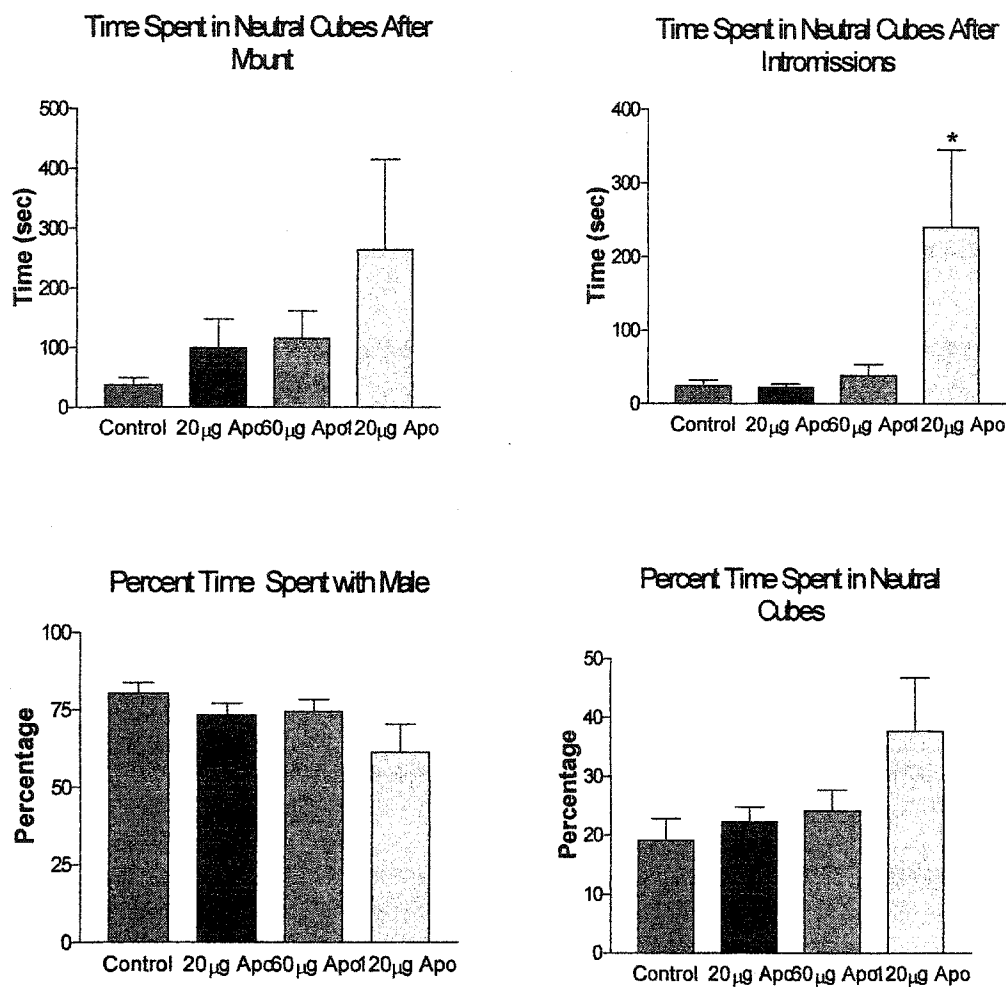


Figure 15: The effects of apomorphine on pacing behaviors. Data are mean \pm SE number or percentage of stimulations after treatment with saline, 20 μ g, 60 μ g or 120 μ g of APO (n = 7 to 14 per group). Data are analyzed by one way ANOVA. Fishers LSD, *post hoc* test, showed that the group treated with 120 μ g of APO spent significantly more time in the neutral cubes after receiving an intromission than the control or other treatment groups ($F_{3,13} = 6.83$, $p < 0.05$).



Discussion

Previous studies in female rats found increases in both pacing and receptive behavior when dopamine levels increased [2, 3, 9, 20, 22, 122]. Data from this experiment partially agreed with the results of those studies. The results illustrated that APO is associated with a dose dependent increase in pacing behavior (see Fig. 15), but a dose dependent decrease of receptive behavior (see Fig. 14). Specifically the group treated with 120 μg of APO spent significantly more time in the neutral cubes after receiving an intromission than the control and other treatment groups. Although not significant the group treated with 120 μg of APO spent more time in the neutral cubes after receiving a mount, less time overall with the male and a greater percentage of time overall in the neutral cubes (see Fig. 15).

Pacing parameters increased with APO treatment however receptivity decreased, particularly at the 60 μg dose. The group treated with 60 μg of APO received significantly less mounts than the control group. While not significant, the group treated with 60 μg of APO also received less intromissions compared to the control group. The dose dependent increase in pacing behavior seen at the 120 μg dose and dose dependent decrease in receptivity seen at the 60 μg dose indicates that receptive parameters are more sensitive than pacing parameters.

These relationships in the data may be explained by considering APO's affinity for pre and postsynaptic DA receptors and consequent effects on behavior. Generally, low doses of APO produce hypomotility while higher doses induce stereotyped behavior [92]. The dose dependent behavior may be related to the higher affinity of APO for presynaptic DA receptors than for postsynaptic DA receptors. At lower doses, APO acts

as an antagonist by activating presynaptic receptors. This causes an inhibition of both DA synthesis and release [4]. At higher doses, APO activates both presynaptic and postsynaptic receptors, increasing transmission and therefore acting as an agonist [4].

The activation of pre and postsynaptic behavior by APO has been shown with other behaviors. Self administration stimulation thresholds were measured in rats treated with five doses of APO and trained to press a lever that delivered electrical stimulation to the lateral hypothalamus [99]. Experiments examining the effects of increasing doses of APO on thresholds of self administered electrical stimulation to the hypothalamus found a significant behavioral difference at the lower doses versus the higher doses [99]. Low doses caused thresholds to increase while the two higher doses caused thresholds to decrease [99]. The authors indicate that increased thresholds at lower doses may reflect APO activating presynaptic receptors, which leads to an inhibitory effect and causes more stimulation to be necessary to have the same effect. Subsequently, the higher doses cause thresholds to decrease which indicates the shift of APO from activation of presynaptic receptors to postsynaptic receptors. This change in receptor stimulation causes less stimulation to result in the same effect [99]. Another study examining the effect of APO on locomotor activity found that there is a significant behavioral difference between low and high doses of APO [96]. Low doses induced yawning which reflected a selective activation of presynaptic DA receptors, while higher doses induced stereotyped sniffing and yawning indicating postsynaptic D₂ receptor activation [96].

Presynaptic behavioral inhibition as well as inhibition of DA release may underlie the decrease in receptivity induced by a lower dose of APO (60 µg). Hypomotility and decreased locomotor behavior may cause the female to display less

receptive behaviors. In addition, decreased receptivity can lead to increased pacing because the female is now utilizing the cubes to withdraw and escape the male. Higher doses of APO are recruiting postsynaptic receptors which will lead to increased DA synthesis and release. The increase in pacing behavior was seen in the highest dose (120 μg) of APO. In the current experiments with female mice and past experiments with female rats [2, 3], increased DA release is correlated with increased pacing behavior, inferring that pacing is a rewarding behavior. An increase in DA may explain past studies which reported that both male and female rats treated with APO showed an increase in receptive behavior [20, 22, 128, 129], however we did not see this change in behavior. When female mice were administered higher doses of APO (180 μg), we expected to see further increases in pacing behavior and increases in receptive behavior. However, no changes were found in either pacing or receptive behavior (unpublished observations).

Mechanisms which could account for these differences in the behavioral responses to APO between rats and mice are unknown. However, in the next section (Experiment 3), levels of DA and metabolites as well as other monamines were measured in brain areas of treated mice in order to better understand effects of APO.

VIII. Experiment 3 – Effects of APO (20 µg, 60 µg and 120 µg) of APO to female mice to examine neurochemical changes

Rationale

Experiment 2 showed significant behavioral differences between subjects treated with APO compared to control subjects (see Figs. 14-15). Subjects treated with higher doses of APO showed increased pacing behavior but decreased receptive behavior, as compared to controls. These differences in behavior may be attributed to the activation of pre and postsynaptic DA receptors. APO has a higher affinity for presynaptic DA autoreceptors than postsynaptic DA receptors and therefore at low doses, acts as an antagonist and decreases DA transmission [4]. At higher doses DA transmission is increased because APO acts as an agonist by acting on postsynaptic receptors [4].

These behavioral results could also be due to monoaminergic changes, particularly DA level changes in the striatum, Nacc and VMN which are induced by APO. Therefore, DA and metabolites were measured in brain areas after 20, 60, and 120 µg of APO. Monoaminergic changes may underlie the increases in pacing behavior and decreases in receptive behavior caused by APO and the differences in the behavioral responses between rats and mice.

Specific Aims

Assessment of effects of various doses of APO on DA levels and metabolites in the striatum and Nacc, areas responsible for pacing, and VMN, area responsible for receptivity.

Hypothesis

Experiment 3 showed treatment with APO caused a dose dependent increase in pacing behavior and a dose dependent decrease in receptive behavior. Receptivity was more sensitive to apomorphine than pacing because receptivity decreased at 60 μg while pacing increased at 120 μg . It is hypothesized that the treatment groups, specifically the group treated with 120 μg will have higher levels of DA in the Nacc and striatum than the control groups, while the group treated with 60 μg of APO will have higher levels of DA in the VMN.

Animals

Four groups of 6-10 OVX female C57 mice were used. The first group received saline and served as controls. The rest received 20 μg , 60 μg , or 120 μg of APO. Animal care is outlined in the general experimental methods.

Injections

All mice received 10 μg of estrogen 48 and 24 hours before sacrifice and 500 μg of progesterone five hours before sacrifice. Each group received a subcutaneous injection of APO or saline 30 minutes before sacrifice.

Experimental Methods

No behavioral tests were given. Thirty minutes after injection with APO or saline, the mice were sacrificed by rapid decapitation. Brains were quickly frozen for neurochemical analysis.

Statistical Analysis

One way ANOVA was used to analyze data. If ANOVA was significant at $p < 0.05$, Fishers LSD, a post hoc test, was used to test specific differences between groups.

Monoamine analysis

Brains were sectioned, micropunched, and analyzed for monoamine and metabolite levels using HPLC analysis as outlined in the general experimental methods.

Results

The results illustrate several significant findings in the striatum, Nacc and VMN. HVA/DA was significantly affected by APO treatment, and the group treated with 120 μg of APO had significantly lower HVA/DA levels in the striatum than the control and the group treated with 60 μg of APO ($F_{3,33} = 4.99$, $p < 0.005$) (see Fig 16). Serotonin was also affected by APO treatment and the groups treated with 20 μg and 60 μg of APO had significantly lower serotonin (5-HT) levels in the striatum than the control and group treated with 120 μg of APO ($F_{3,32} = 6.32$, $p < 0.001$) (see Fig 17). In the Nacc, 120 μg of APO treatment significantly increased DA levels as compared to both the control and 60 μg groups ($F_{3,23} = 5.57$, $p < 0.005$) (see Fig 18). Groups treated with 20 and 120 μg APO had significantly lower HVA/DA levels in the Nacc than the control group ($F_{3,23} = 3.66$, $p < 0.02$) (see Fig 18). Finally, the group treated with 60 μg of APO had significantly higher HVA levels in the VMN than the groups treated with 20 μg and 120 μg ($F_{3,20} = 2.95$, $p < 0.05$) (see Fig 19). DA and DOPAC in the VMN showed a similar pattern of change following APO, but the difference did not reach statistical significance.

Figure 16: The effects of apomorphine on DA and metabolites in striatum. Data are expressed as pg/ μ g protein (mean \pm SE; n = 6-10 per group). Data are analyzed by one way ANOVA. Fishers LSD, *post hoc* test, showed that HVA/DA levels were significantly lower in the group treated with 120 μ g of APO compared to both the control group and the group treated with 60 μ g of APO ($F_{3,33} = 4.99$, $p < 0.005$)

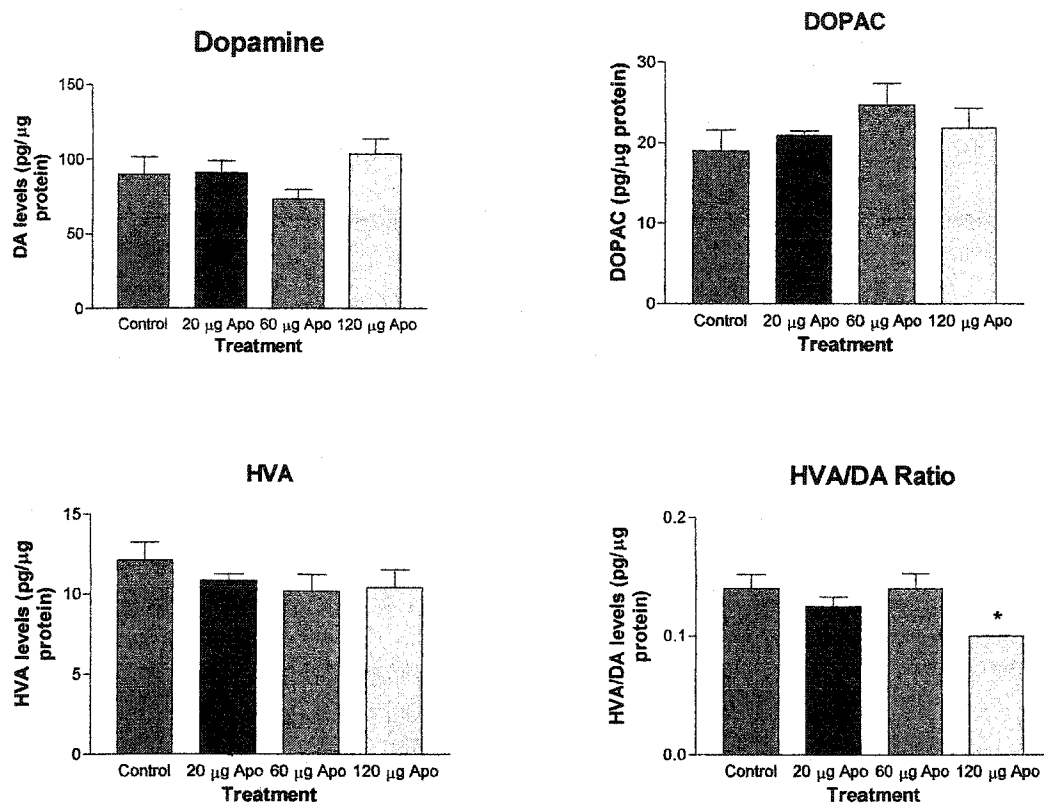


Figure 17: The effects of apomorphine on NE, 5-HT and metabolites in striatum. Data are expressed as pg/ μ g protein (mean \pm SE; n = 6-10 per group). Data are analyzed by one way ANOVA. Fishers LSD, *post hoc test*, showed the groups treated with 20 and 60 μ g of APO have significantly lower 5-HT levels than the control group and group treated with 120 μ g of APO ($F_{3,32} = 6.32, p < 0.001$).

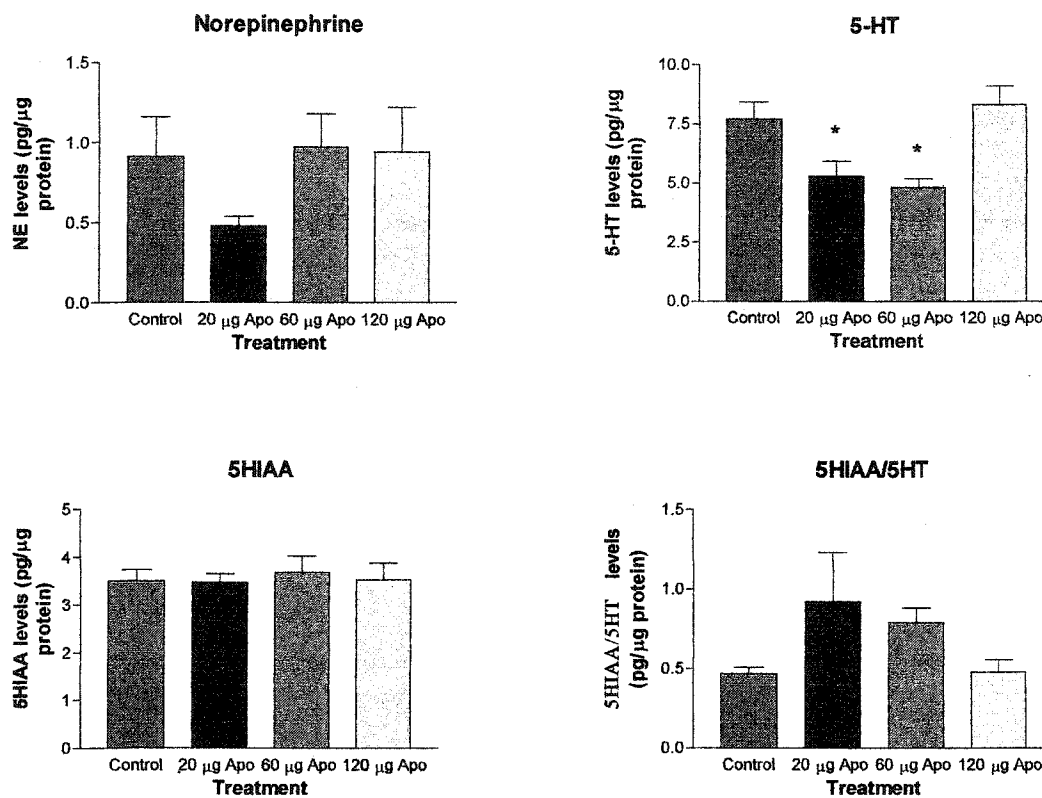


Figure 18: The effects of apomorphine on DA and metabolites in Nacc. Data are expressed as pg/ μ g protein (mean \pm SE; n = 6-10 per group). Data are analyzed by one way ANOVA. Fishers LSD, *post hoc* test, showed the group treated with 120 μ g APO had significantly higher levels of DA ($F_{3,23} = 5.57$, $p < 0.005$) and significantly lower levels of HVA/DA along with the group treated with 20 μ g of APO ($F_{3,23} = 3.66$, $p < 0.02$).

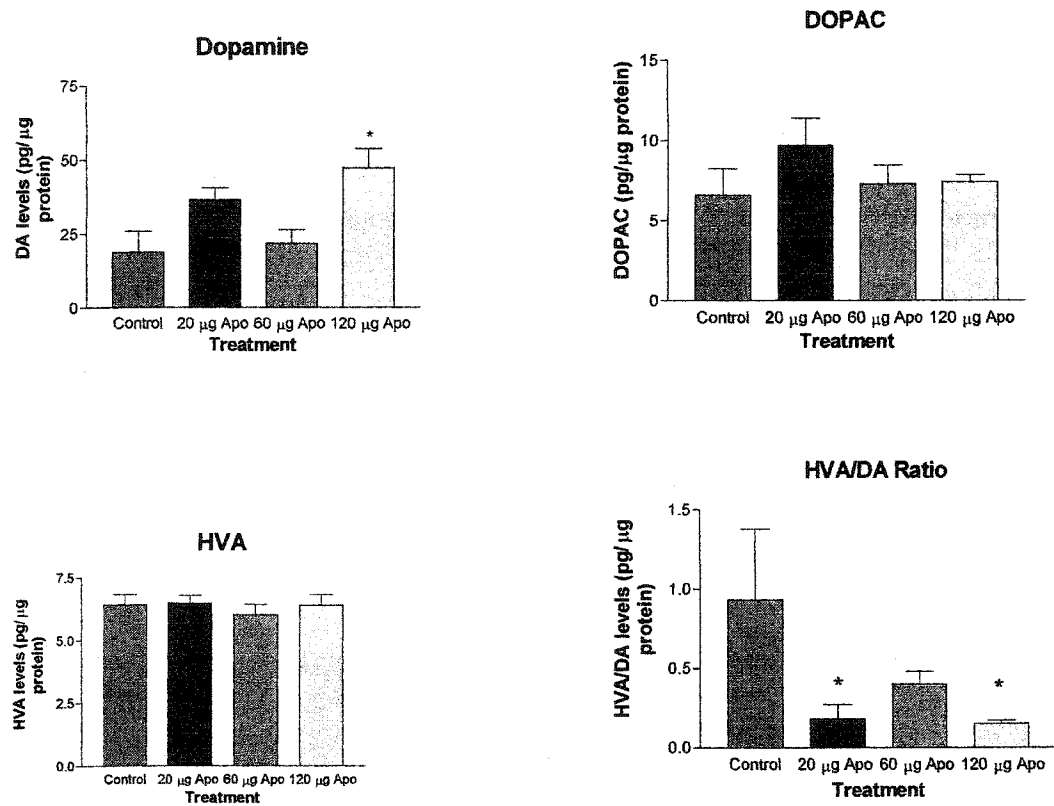
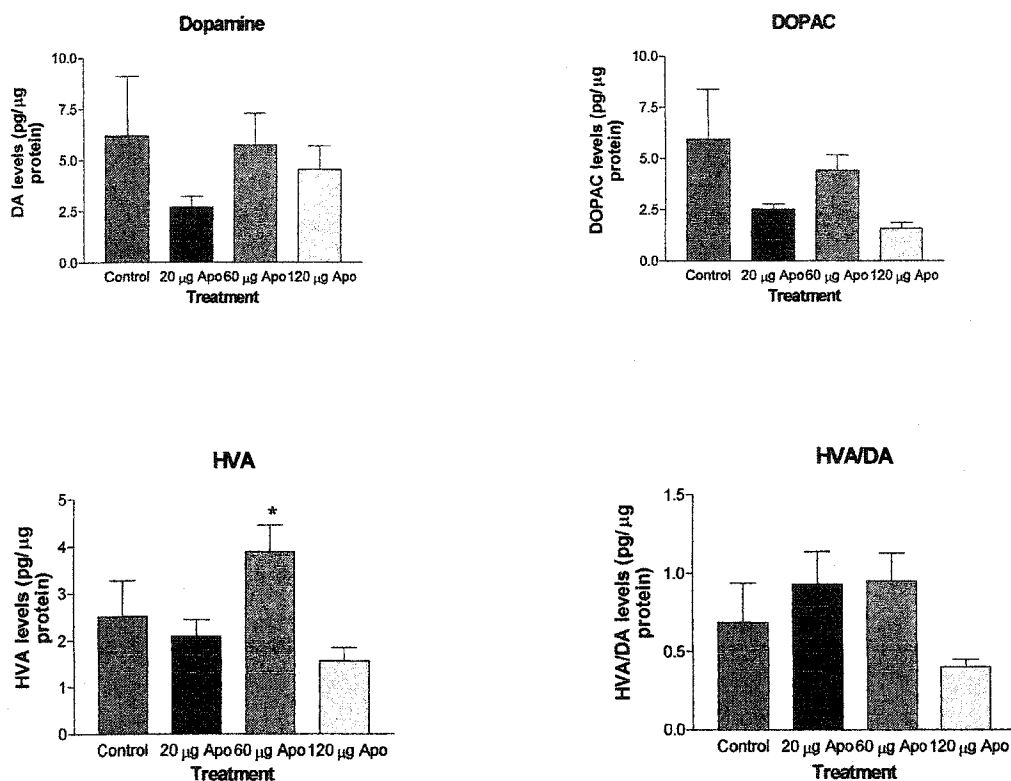


Figure 19: The effects of apomorphine on DA and metabolites in VMN. Data are expressed as pg/ μ g protein (mean \pm SE; n = 6-10 per group). Data are analyzed by one way ANOVA. Fishers LSD, *post hoc* test, showed the group treated with 60 μ g of APO had significantly higher levels of HVA in the VMN compared to groups treated with 20 μ g and 120 μ g of APO ($F_{3,20} = 2.95$, $p < 0.05$).



Discussion

Treatment with APO led to a number of dose dependent changes in monoamines in the striatum, Nacc and VMN. The group treated with 120 μ g of APO had significantly higher levels of DA in the Nacc and significantly lower HVA/DA levels in the striatum and Nacc compared to controls. The group treated with 60 μ g of APO had significantly higher HVA levels in the VMN than the groups treated with 20 μ g and 120 μ g. The groups treated with 20 μ g and 60 μ g of APO had significantly lower serotonin levels in the striatum than the control and group treated with 120 μ g of APO.

These monoaminergic results support the hypothesis and may provide an explanation for the behavioral results from Experiment 3. Again, the results of experiment 3 illustrated that APO caused a dose dependent increase in pacing behavior (see Fig 15) and a dose dependent decrease in receptive behavior (see Fig 14). In this experiment the group treated with 120 μ g APO had significantly higher levels of DA in the Nacc (see Fig 18), while that same dose induced significantly higher pacing behaviors (see Fig 15). In addition, they had significantly lower HVA/DA levels in both the striatum and Nacc (see Figs 16,18). The increase in Nacc DA and the decrease in the HVA/DA ratio indicate that APO induces increased DA release and that DA is not metabolized as rapidly as in controls (i.e. a decrease in turnover). Increased DA levels in the Nacc along with increased pacing agree with previous reports with female rats that pacing caused increased levels of DA in Nacc. The decrease in HVA/DA in the striatum at the 120 μ g dose of APO may have also contributed to the increased pacing of this group [2, 3, 139].

It is notable that treatment with 60 μ g of APO was associated with a significant increase of HVA levels in the VMN (see Fig. 19) because this group also displayed the lowest levels of receptivity. (see Fig 14) Inhibition of lordosis in female rats has previously been correlated with increases of DA in the ventromedial hypothalamus [143, 144] Therefore the decreased receptivity displayed by this group may be due to the increased activity of DA in this area.

APO treatment led to a u-shaped dose-response curve for 5-HT levels in the striatum: in the two lowest doses, 5-HT was decreased as compared to controls and the high dose of APO (see Fig. 17). 5-HT has an inhibitory effect on lordosis but this effect is centered in the VMN [145, 146]. No changes in the VMN 5-HT were noted. Striatal 5-HT and DA interact in some aspects of psychoactive drug effects [147, 148]. However, the pattern of these changes does not correlate with pacing changes. Thus, possible relationships of striatal 5-HT to the behaviors measured, if any, are unknown.

IX. Experiment 4 – APO effects on locomotion

Rationale

Experiment 2 demonstrated that APO had a dose dependent inhibitory effect on receptive behavior and a facilitatory effect on pacing behavior. This pattern could be explained by noting APO's dose dependent activation effect on pre and postsynaptic receptors [4]. At low doses, presynaptic DA receptors are activated while at high doses, postsynaptic DA receptors are activated [4]. Results of DA and metabolite measurements in Experiment 3 were consistent with this hypothesis. However activation of pre and postsynaptic receptors can also affect locomotor behavior [96]. Specifically APO can affect ambulations, grooming and stereotypy [92, 93].

While pacing behavior is a copulatory behavior [7] it also consists of locomotor behavior since the female must approach and withdraw from the male. APO effects on locomotor behavior, could have a profound effect on the female's ability to pace effectively. Therefore investigation of APO effects on locomotor behavior in female C57 mice is important for understanding the changes in copulatory behavior. In this experiment the same doses (20 μ g, 60 μ g, and 120 μ g) used in previous copulatory tests were used to examine the effect of APO on locomotor behavior in an open field apparatus. In addition, measures for exploration and anxiety were also made.

Specific Aim

Determine the effects of APO (20 μ g, 60 μ g, and 120 μ g) on locomotor behavior in female mice.

Hypothesis

Locomotor activity, exploration and anxiety will be correlated with APO dose, and changes may be related to pacing or receptivity.

Animals

Four groups of 8 OVX female C57 mice were used. The first group received saline and served as controls. The rest received 20 μ g, 60 μ g, or 120 μ g of APO. Animal care is outlined in the general experimental methods.

Injections

All mice received 10 μ g of estrogen 48 and 24 hours before testing and 500 μ g of progesterone 5 hours before testing. Each group received an injection of APO or saline 15 minutes before behavioral testing began.

Experimental Methods

Mice were first acclimated to the task by placing them on the open field apparatus for 15 minutes. The open field apparatus is a square (45cm by 45 cm), green, Plexiglas open field. The height of the four walls of the field is 20 cm. The field consists of a 4 by 4 grid and each grid is 10.5 cm by 10.5 cm.

Drug treatment and open field tests began 4 days after initial acclimation. Tabulations were made for grid visits, rears (mice standing on hind legs), wall climbs (mice standing on hind legs and placing front paws on wall), grooms (scratching and cleaning fur) and defecations.

Statistical Analysis

One way ANOVA was used to analyze data. If ANOVA was significant at $p < 0.05$, Fishers LSD, a post hoc test, were used to test specific differences between groups.

Results

All behavioral measures were altered in a dose-dependent manner by APO, and significant differences were found in several of the locomotion measures following APO treatment. Outer visits were increased by APO, and the group treated with 120 μg of APO had significantly more outer visits than the control or group treated with 20 μg of APO ($F_{3,28} = 4.34, p < 0.01$) (see Fig 20). In contrast, inner visits, a measure of anxiety, were decreased by APO. The groups treated with the highest doses of APO, 60 μg and 120 μg , had significantly less inner visits than the control group ($F_{3,28} = 5.01, p < 0.006$) (see Fig. 21). All groups treated with APO displayed significantly less rearing behavior than the control group ($F_{3,28} = 16.66, p < 0.0001$). Rearing is related to exploration. The groups treated with 60 μg and 120 μg of APO displayed significantly less grooming than the control group ($F_{3,28} = 8.28, p < 0.004$) (see Fig. 21). Finally, the group treated with 120 μg of APO displayed significantly less wall climbs than the control group ($F_{3,28} = 5.15, p < 0.005$) (see Fig. 21).

Figure 20: The effect of apomorphine on outer visit in open field apparatus. Data are mean \pm SE number of outer visits after treatment with saline, 20 μ g, 60 μ g or 120 μ g of APO (n = 8 per group). Data were analyzed by one way ANOVA. Fishers LSD *post hoc* test, showed the group treated with 120 μ g of APO had significantly more outer visits than the control or group treated with 20 μ g APO ($F_{3,28} = 4.34$, $p < 0.01$).

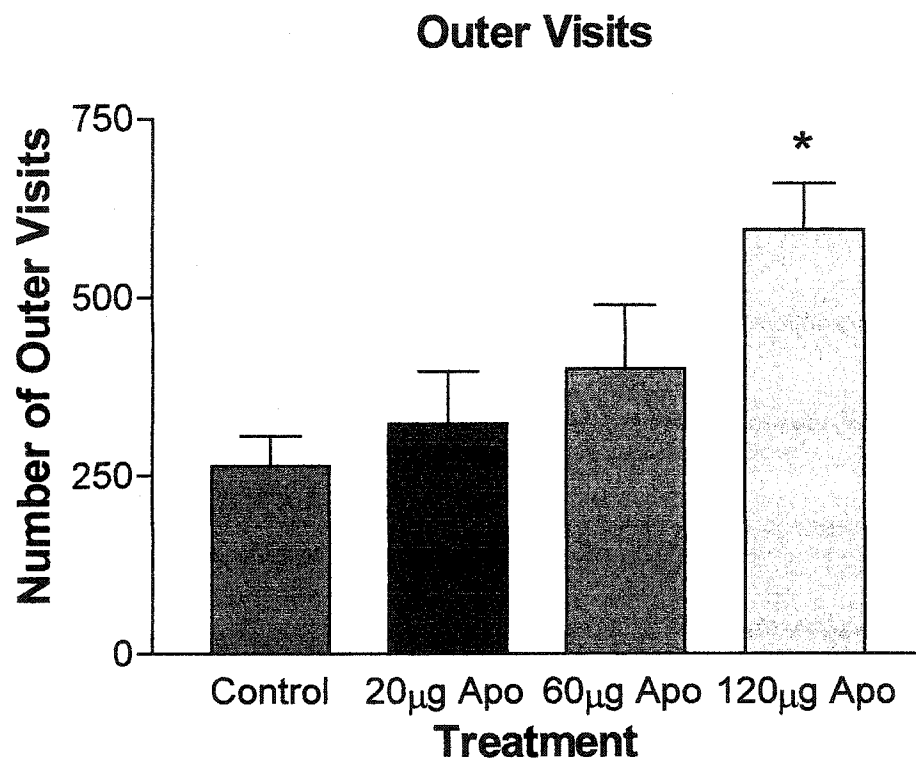


Figure 21: The effect of apomorphine on inner visits in open field apparatus. Data are mean \pm SE number of inner visits after treatment with saline, 20 μ g, 60 μ g or 120 μ g of APO (n = 8 per group). Data were analyzed by one way ANOVA. Fishers LSD *post hoc* test, showed the groups treated with 60 μ g and 120 μ g had significantly less inner visits than the control group ($F_{3,28} = 5.01$, $p < 0.006$).

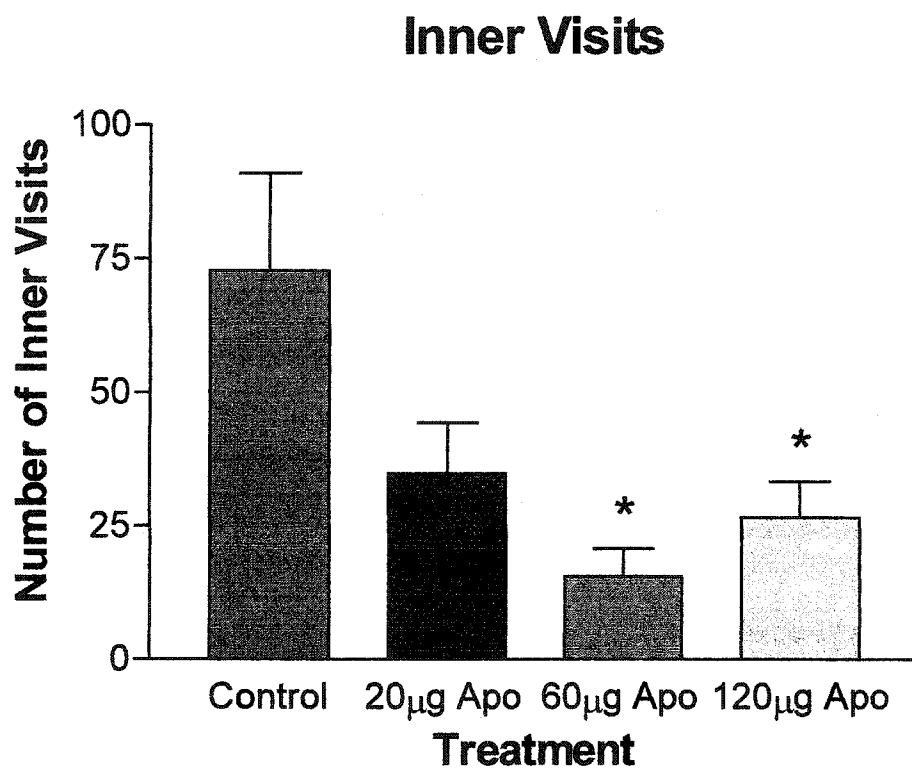
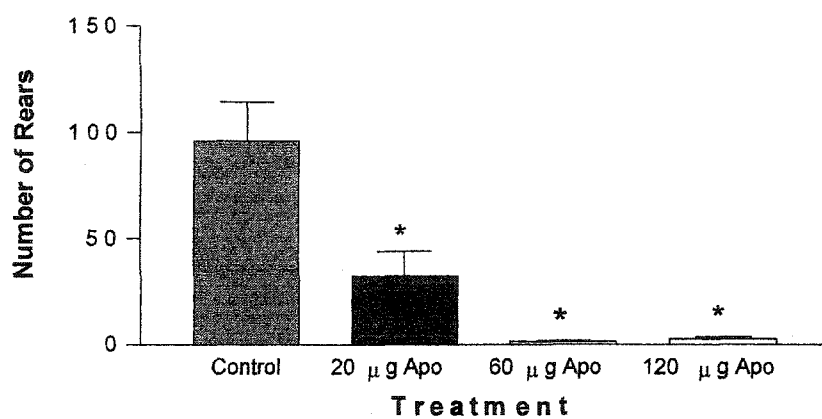
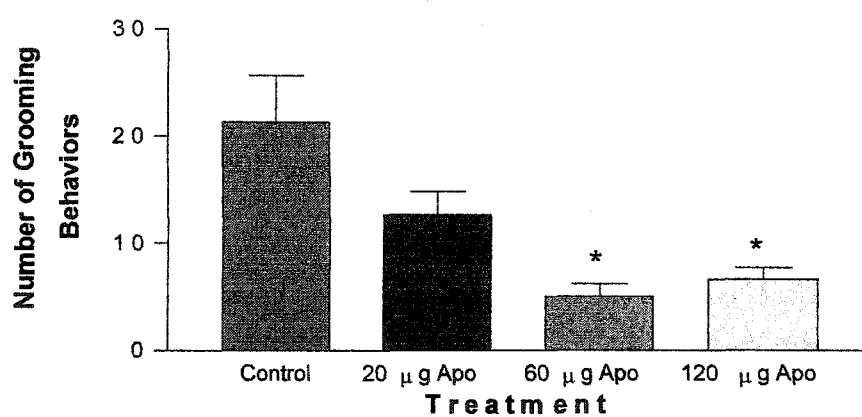


Figure 22: The effects of apomorphine on rearing behavior, wall climbing and grooming behavior. Data are mean \pm SE number of rears, wall climbs and grooming behavior after treatment with saline, 20 μ g, 60 μ g or 120 μ g of APO (n = 8 per group). Data were analyzed by one way ANOVA. Fishers LSD *post hoc test*, showed all groups treated with APO (20 μ g, 60 μ g and 120 μ g) displayed significantly less rearing ($F_{3,28} = 16.66$, $p < 0.0001$), while the groups treated with 60 μ g and 120 μ g APO showed significantly less grooming behaviors ($F_{3,28} = 8.28$, $p < 0.004$) than the control group. The group treated with 120 μ g displayed significantly less wall climbing behavior ($F_{3,28} = 5.15$, $p < 0.005$) than the control group.

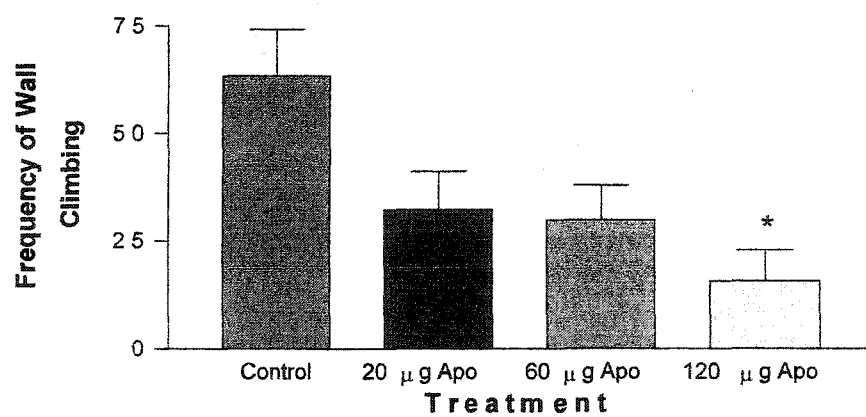
Rearing Behavior



Grooming



Wall Climbing Behavior



Discussion

The results support the hypothesis that behaviors on an open field, especially locomotion behavior would be correlated with APO dose. Outer visits increased with increasing APO doses, and the data showed a significant increase in outer visits, which is a measure of locomotor activity, in the group treated with the 120 μg of APO compared to controls (see Fig 20). In contrast, APO significantly decreased rearing and wall climbing, which are measures of exploratory behavior, compared to controls (see Fig 22). APO also significantly decreased the number of inner visits, an indirect measure of anxiety behavior [149], in the groups treated with 60 μg and 120 μg APO compared to controls (see Fig. 21). Finally APO decreased grooming behavior in the groups treated with 60 μg and 120 μg compared to controls (see Fig 22).

APO generally induces increased locomotion and stereotyped behavior such as sniffing and licking at high doses [94]. At lower doses, APO can induce hypomotility [92], however in C57 mice APO enhances locomotor activity [93]. The current data suggests that APO had a significant excitatory effect on locomotor activity (outer visits). However, APO had an inhibitory effect on exploratory behavior and grooming behavior. In addition APO increased anxiety levels (decreased inner visits). Comparing the number of inner visits in an open field behavior is an indirect measure of anxiety, but not an anxiety test [149]. This conclusion based on observations that rodents generally do not like wide open areas since they are more susceptible to predators. Instead they prefer a dark, enclosed area or in the case of the open field the area which is close to the wall[149].

In a previous experiment examining APO's effect on pacing and receptive behavior, a dose dependent increase in pacing behavior and a dose dependent decrease in

receptivity were seen. The same dose of APO (120 μg) was associated with an increase in locomotor activity (outer visits) and an increase in pacing behavior. Thus, changes in pacing behavior parallels increased outer visits in the open field paradigm. In addition a dose dependent decrease (60 μg) in receptivity was seen in Experiment 2 and there is a significant increase in anxiety (decreased inner visits) at the same dose in this experiment. The increase in anxiety may be related to the significant decrease in receptivity. If the female mouse is anxious, she may not show be able to remain still and allow the male to mount. Thus, less receptive behaviors may be shown. In addition, because of enhanced anxiety, she may show increased rejection behaviors such as nipping and kicking the male [47].

Anxiety was also increased following 120 μg of APO. This dose also significantly increased pacing behavior. The increased anxiety level may also contribute to increased pacing behavior, because subjects spend more time inside the cubes to escape the male. The mounting or intromitting of the female by the male may further increase anxiety levels and cause additional increased pacing behavior (retreating to the safe neutral cubes). Increased anxiety levels in conjunction with increased locomotor activity can lead to increased pacing behavior, but not decreased receptive behavior as seen at the 60 μg dose. Enhanced locomotion would not contribute to the immobilization required for lordosis. Decreased exploration (rearing and wall climbing) and grooming may also indicate increased anxiety levels which may also lead to increased pacing and decreased receptivity.

X. Experiment 5 – Effects of specific D₁ agonists and D₂ agonists on copulatory behavior in female mice.

Rationale

The previous three experiments have examined the effect of APO on copulatory and locomotion. APO is associated with a dose dependent increase in pacing behavior and a dose dependent decrease of receptive behavior. In addition, APO caused increased locomotion, increased anxiety and decreased exploration. The observed behaviors may be due to APO activating presynaptic and postsynaptic receptors which induce different behaviors. In addition the role of D₁ and D₂ receptors needs to be further investigated because they have been shown to modulate pre and postsynaptic effects [96].

D₁ and D₂ receptor activation also differentially effects sexual behavior in rodents [23, 24, 130, 131]. Treatment with D₁ agonists had no effect on male sexual behavior [130], but when substituted for progesterone in female rats, increased lordosis [23]. In addition, administration of D₂ agonists decreased receptivity in both male and female rats [23, 131]. The current experiment investigates the role of specific D₁ and D₂ agonists on pacing and receptive behavior in female mice.

Specific Aims

Determine the effects of D₁ and D₂ receptors on pacing and receptive sexual behaviors in mice.

Hypotheses:

Administering D₁ agonists will increase receptive but not pacing behavior. Administering D₂ agonists will decrease both receptive and pacing behaviors.

Animals

Seven groups of OVX female C57 mice were used. The first group received saline and served as controls. Three groups received increasing (0.3, 1.0, and 2.0 mg/kg) doses of SKF 38393 (D₁ agonist) [4] and three groups received increasing doses (.03, 0.1, 0.3 mg/kg) of quinpirole (D₂ agonists) [4]. Animal care is outlined in the general experimental methods

Injections

All mice received 10 µg of estrogen 48 and 24 hours before testing and 500 µg of progesterone 5 hours before testing. Each mouse is assigned to either the control group or one of the eight treatment groups and received either an injection of SKF 38393, quinpirole or saline 15 minutes before behavioral testing began.

Experimental Methods

The behavioral testing methods are outlined in the general experimental methods.

Statistical Analysis

One way ANOVA was used to analyze data. If ANOVA was significant at $p < 0.05$, Fishers LSD, a post hoc test, was used to test specific differences between groups.

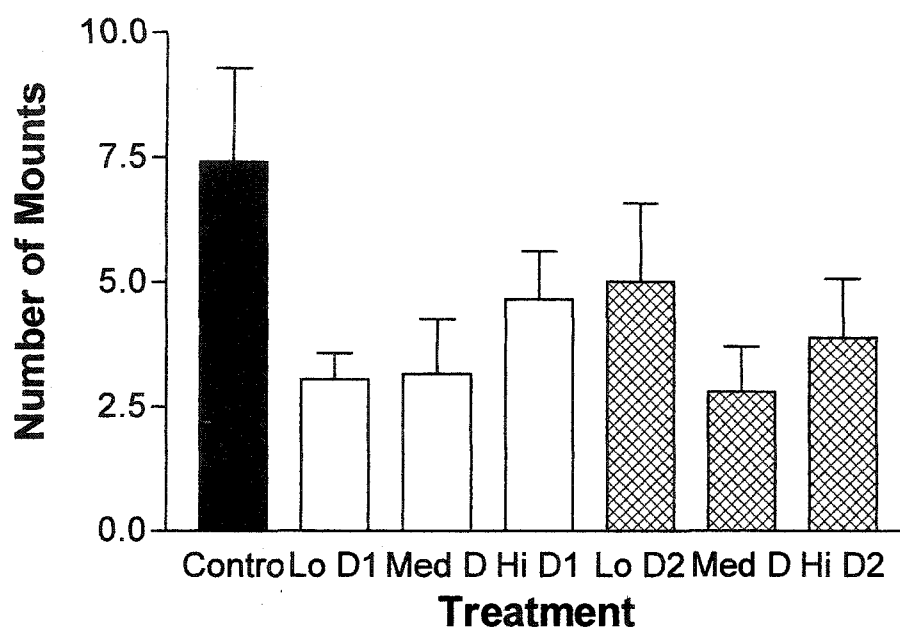
Results

There were no significant differences found between D₁ and D₂ treated subjects on any of the pacing or receptive measures. The group treated with the low dose of quinpirole spent a higher percentage of time after receiving an intromission with the male than the group treated with the medium dose of quinpirole ($F_{6, 119} = 3.28, p < 0.008$) (see Fig. 26). There were no significant differences found between any of the remaining groups on either of the receptive parameters including mounts ($F_{6, 125} = 0.71, p = 062$)

and intromissions ($F_{6, 125} = 0.54, p = 0.75$) (see Fig. 23). There were also no other significant differences found between any of the groups on any of the pacing parameters including time spent with the male ($F_{6, 125} = 1.27, p = 0.28$), time spent in the neutral cubes ($F_{6, 125} = 1.43, p = 0.22$), mount latency ($F_{6, 125} = 0.77, p = 0.57$), time spent with the male after receiving a mount ($F_{6, 125} = 1.74, p = 0.15$), time spent in the neutral cubes after receiving a mount ($F_{6, 125} = 1.60, p = 0.18$), percentage of times females exited after receiving a mount ($F_{6, 125} = 1.22, p = 0.31$), intromission latency ($F_{6, 119} = 1.57, p = 0.17$), time spent with the male after receiving an intromission ($F_{6, 119} = 1.43, p = 0.22$), time spent in the neutral cubes after receiving an intromission ($F_{6, 119} = 1.05, p = 0.39$) (see Figs. 24-26).

Figure 23: The effect of D₁ and D₂ agonists on receptive parameters. Data are mean ± SE number of stimulations after treatment with saline, SKF-38393 and quinpirole (n = 18-20 per group). The control group is represented by the solid bar. The groups treated with the D₁ agonist are represented by the open bars. The groups treated with the D₂ agonists are represented by the hatched bars. Data were analyzed using a one-way ANOVA. Treatment with SKF 38393 or quinpirole had no effect on mounts ($F_{6,125} = 0.71$, $p = 0.62$) or intromissions ($F_{6,125} = 0.54$, $p = 0.75$).

Mounts



Intrusions

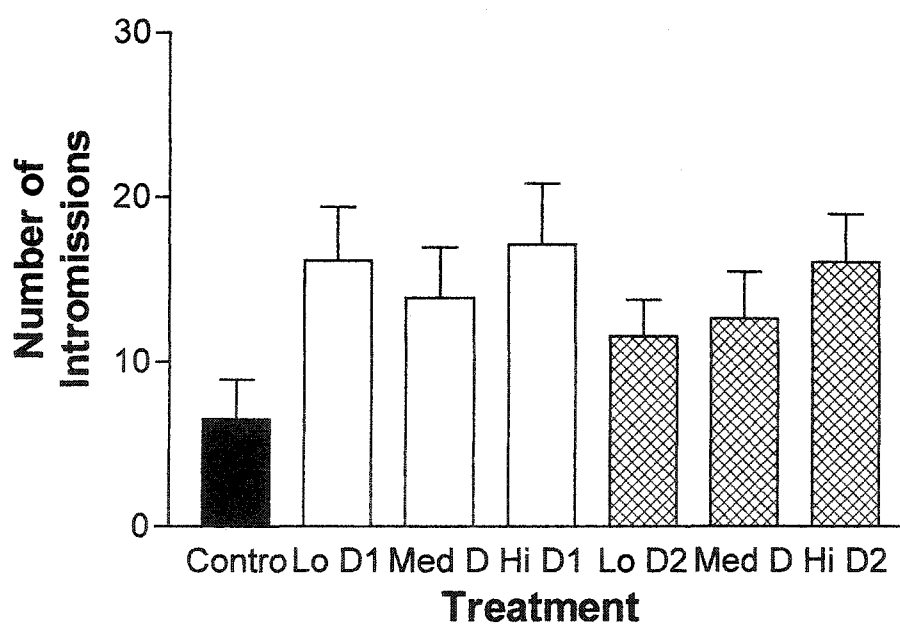
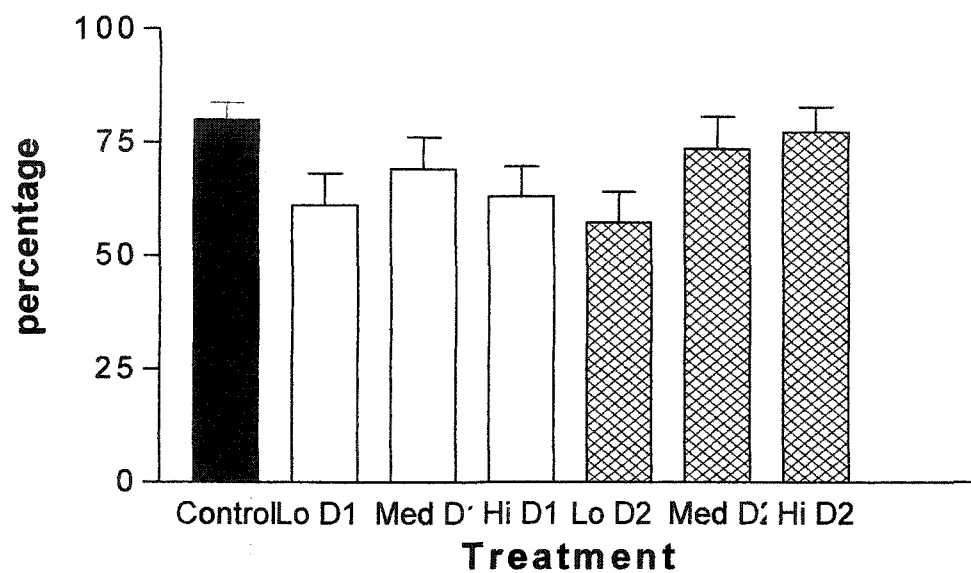


Figure 24: The effect of D₁ and D₂ agonists on percentage time spent with the male or in the neutral cubes. Data are mean ± SE percentage time after treatment with SKF 38393 or quinpirole (n = 20 per group). The control group is represented by the solid bar. The groups treated with the D₁ agonist are represented by the open bars. The groups treated with the D₂ agonists are represented by the hatched bars. Data were analyzed by one way ANOVA. Treatment with SKF 38393 or quinpirole had no effect on time spent with the male ($F_{6, 125} = 1.27, p = 0.28$), time spent in the neutral cubes ($F_{6, 125} = 1.43, p = 0.22$)

Percent time spent with male



Percent time spent in neutral cubes

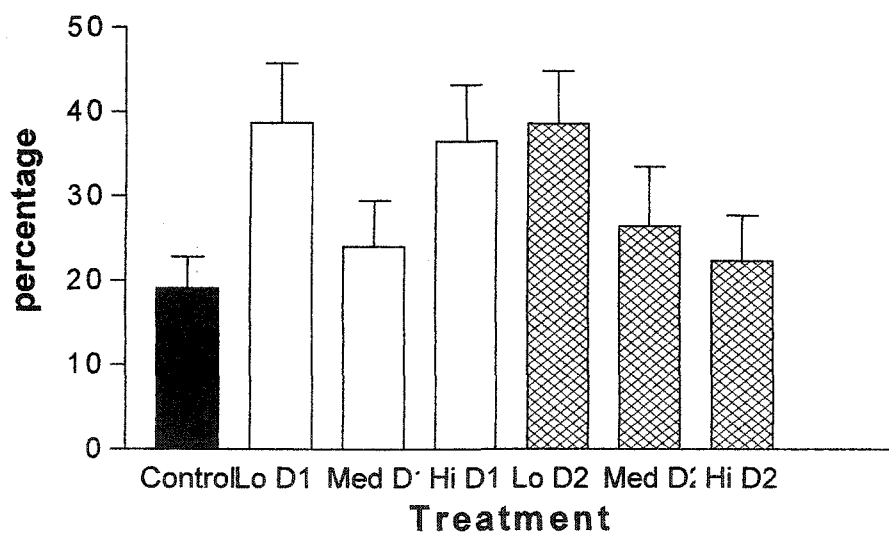
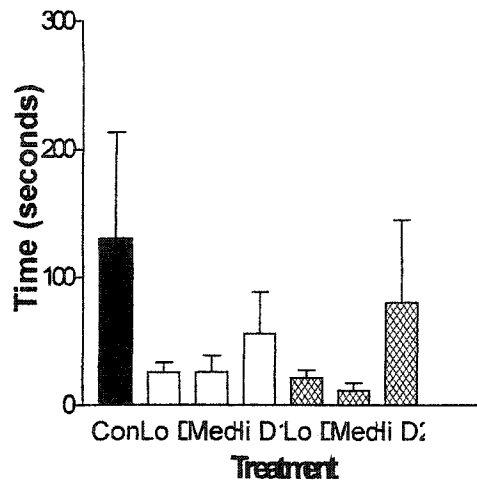


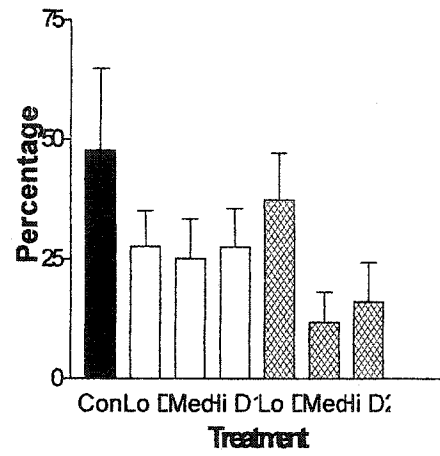
Figure 25: The effects of D₁ and D₂ agonists on pacing parameters associated with mounts.

Data are mean ± SE percentage time after treatment with SKF 38393 or quinpirole (n = 20 per group). The control group is represented by the solid bar. The groups treated with the D₁ agonist are represented by the open bars. The groups treated with the D₂ agonists are represented by the hatched bars. Data were analyzed using a one-way ANOVA. Treatment with SKF 38393 or quinpirole had no effect on pacing parameters associated with mounting including mount latency ($F_{6,125} = 0.77$, $p = 0.57$), time spent with the male after receiving a mount ($F_{6,125} = 1.74$, $p = 0.15$), time spent in the neutral cubes after receiving a mount ($F_{6,125} = 1.60$, $p = 0.18$), percentage of times females exited after receiving a mount ($F_{6,125} = 1.22$, $p = 0.31$)

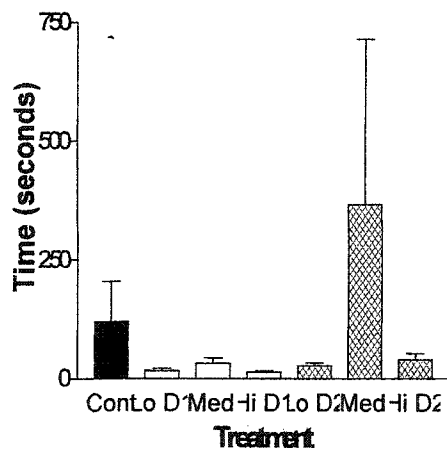
Munt Latency



Percent time spent with male after receiving a mount



Time spent with male after receiving amount



Time spent in neutral cubes after receiving a mount

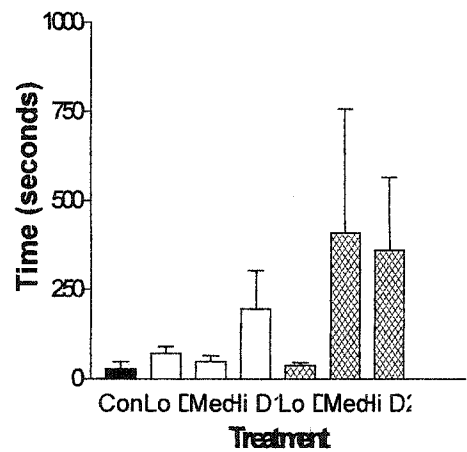
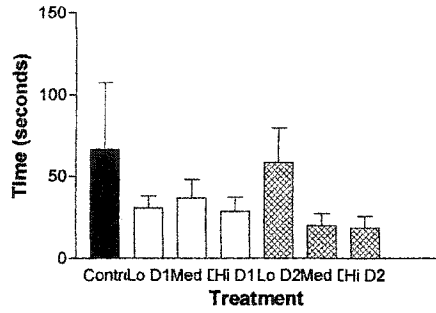
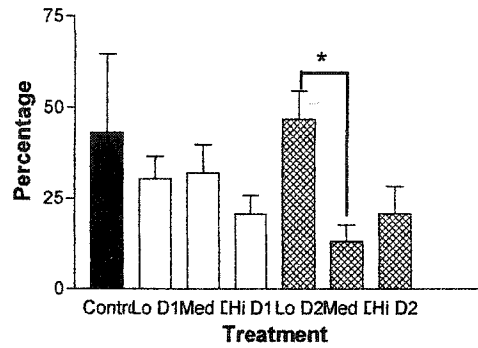


Figure 26: The effects of D₁ and D₂ agonists on pacing parameters associated with intromissions. Data are mean ± SE percentage time after treatment with SKF 38393 or quinpirole (n = 20 per group). The control group is represented by the solid bar. The groups treated with the D₁ agonist are represented by the open bars. The groups treated with the D₂ agonists are represented by the hatched bars. Data were analyzed using a one-way ANOVA. If ANOVA was significant at p < 0.05 level, Fishers LSD tested specific differences. Fishers LSD, *post hoc test*, showed the group treated with the low dose of the D₂ agonist spent a significantly higher percentage of time with the male after receiving an intromission than the group treated with the medium dose (F_{6,119} = 3.28, p < 0.008). There were no other significant finding on any intromission pacing parameters including intromission latency (F_{6,119} = 1.57, p = 0.17), time spent with the male after receiving an intromission (F_{6,119} = 1.43, p = 0.22), time spent in the neutral cubes after receiving an intromission (F_{6,119} = 1.05, p = 0.39)

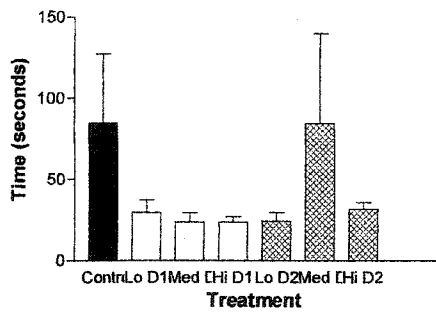
Intromission Latency



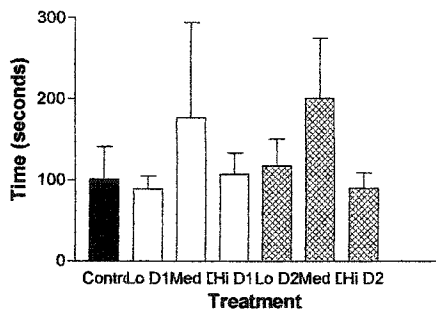
Percent time spent with male after receiving an intromission



Time spent with male after receiving an intromission



Time spent in neutral cubes after receiving an intromission



Discussion

In Experiment 2 which examined APO's mediating effects on copulatory behavior, subjects treated with 120 μ g of APO showed a dose dependent increase in pacing behavior, while subjects treated with 60 μ g of APO showed a dose dependent decrease in receptive behavior. D₁ and D₂ receptor involvement was hypothesized to have regulating effects, but further investigation was needed. Pacing and receptive behaviors were measured following D₁ and D₂ agonist treatment. Dose dependent increases in receptive behavior were expected after treatment with SKF 38393 [23] and dose dependent decreases in both receptivity and pacing were expected after treatment with quinpirole [23, 131]. However none of these expected changes were seen in either pacing or receptive behaviors. The group treated with the low dose of quinpirole spent significantly more time with the male after receiving a mount than the group treated with the medium dose (see Fig. 26). There were no other significant differences found in any of the pacing or receptivity parameters between any of the treatment groups (see Figs. 23-26). Several explanations may account for the results and they are discussed below.

Studies demonstrate that D₁ receptors mediate both pre and postsynaptic DA receptors [96]. Examining the effects of D₁ agonists on APO induced behavior has shown that D₁ agonists have different effects on low versus high doses of APO [96]. The authors indicate that low doses of APO induce yawning which reflects a selective activation of presynaptic DA receptors; while high doses of APO induce stereotyped sniffing which reflects postsynaptic D₂ receptor activation. SKF 38393, a D₁ receptor agonist, inhibited yawning induced by low doses of APO [96]. The inhibitory effect of SKF 38393 on APO induced yawning was attenuated by pretreatment with specific D₁ receptor antagonist SCH 23390 [96]. However, SKF 38393 potentiated sniffing induced

by the high doses of APO, without affecting gnawing [96]. These results indicate that D₁ receptor activation modulates both pre and postsynaptic effects of APO in opposite directions [96]. The authors imply that D₁ receptor activation decreases presynaptic effects but increases postsynaptic effects. When presynaptic DA receptors are stimulated DA synthesis is inhibited by blocking the activity of tyrosine hydroxylase, the rate limiting step in catecholamine synthesis [90, 150, 151]. In addition, presynaptic DA receptor activation blocks DA release from presynaptic membrane-enclosed storage vesicles and attenuates the firing rate of DA neurons [150, 151]. Activation of D₁ receptors inhibit presynaptic DA receptors activity, resulting in increased DA synthesis and release [4]. When postsynaptic DA receptors are stimulated, DA transmission increases. Therefore D₁ receptor activation of postsynaptic DA receptors may further increase DA transmission.

When female rodents are allowed to pace their copulations, DA levels are increased in the striatum and Nacc [2, 3, 139]. This observation infers that paced copulation is rewarding. However if there is already an increased level of DA available, paced copulations may not be necessary, therefore pacing may not increase as it is no longer needed for reward. This relationship may explain why increased pacing and receptive behavior was not present as the doses of SKF 38393 increased. If the increasing doses of SKF 38393 increased DA synthesis and release, by inhibiting presynaptic activity and stimulating postsynaptic activity, then receptive and pacing behaviors may no longer have any additional rewarding value.

D₂ receptors also modulate pre and postsynaptic receptor activity. An experiment examined the effects of D₁ and D₂ activation on pre and postsynaptic activity [100].

Administering APO immediately before placing rats into a novel environment produced a biphasic motility response (initial sedation followed by enhanced locomotion) [100].

Administering D₁ and D₂ receptor antagonists can block APO induced inhibition of locomotor behavior [100]. Antagonizing APO inhibition requires a higher affinity for D₂ receptors [100]. Generally activation of D₂ receptors causes locomotor inhibition [100]. Therefore increased D₂ binding and activation is needed to display increased locomotor behavior [100].

As previously indicated, there was no difference on either pacing or receptive behaviors within the groups that received quinpirole. This result may be due to the fact that the doses were not high enough to bind and activate sufficient D₂ receptors to inhibit behavior. The doses were intentionally kept low so undesirable behaviors such as immobility would not be induced [4]. In addition D₂ receptor activation of pre and postsynaptic receptor activity must be considered. Because of the low doses used, there may not have been enough available quinpirole to bind the needed amount of D₂ postsynaptic receptors and induce an increased behavioral effect. If the doses are inducing greater presynaptic receptor activity, there will either no change or a decrease in behavior.

Generally when D₂ agonists were administered to female rodents, copulatory behavior was decreased [23, 131]. The doses used in this experiment were based on experiments with rats investigating the effects of quinpirole on sex behavior and locomotion [131]. Similar results were expected in this experiment. D₂ binding and presynaptic D₂ activity is necessary to induce an inhibitory effect, while increased D₂ binding is required to induce an excitatory effect. The low doses of quinpirole may not

have been enough to stimulate D₂ receptors, pre or postsynaptically, which may explain why inhibition of pacing and receptivity was not seen. Though not measured in this experiment, the lack of D₂ binding may explain the lack of pacing and receptivity inhibition.

XI General Discussion and Conclusions

There were five experiments completed for this dissertation. Each gave information that was important and furthered understanding of how monoamines mediate copulatory behavior in the female mouse. Experiment 1 resulted in development of a pacing paradigm for female mice and was used to determine baseline pacing and receptive behaviors. Generally, female mice pace in a similar manner but at a lesser rate than female rats. However, unlike female rats that show the lordosis posture when tested after a small number of hormone treatments [12], female mice need weeks of hormone treatments to show receptive behaviors [47]. In addition, female mice demonstrated increased DA levels in the Nacc as receptivity increased. Increases in DA levels in the Nacc seen in this experiment as well as others [2, 3], led to further investigation of DA and its metabolites mediating pacing and receptivity.

APO was used to manipulate DA levels and investigate the resulting effect on pacing and receptive behavior. Female mice were treated with three doses of APO (20 μg , 60 μg and 120 μg) and behavioral tests were conducted. Treatment with 120 μg of APO caused increases in pacing while treatment with 60 μg caused decreases in receptivity. The increased pacing behavior agreed with data from previous studies with female rats [3, 139] and hamsters [24], but the decreased receptive behavior did not. The dose dependent relationships seen in the data may be explained by considering APO's affinity for pre or postsynaptic DA receptors. Low doses of APO are relatively selective for presynaptic DA receptors which inhibit DA synthesis and release, while higher doses of APO are relatively selective for postsynaptic DA receptors which have an excitatory effect on DA transmission and release [4]. The lower doses may have only activated

presynaptic DA receptors which may underlie the decreased receptivity seen at the 60 μg dose. The higher doses may have activated postsynaptic DA receptors which may underlie increased pacing behavior.

The results are also supported by the dopamine hypothesis of reward. The hypothesis states that both priming and reinforcing effects of rewarding brain stimuli seem to be at least partially DA dependent [152]. Priming is defined as the motivating effect of “free” reward given before an instrumental act [152]. Experiments have shown that DA and DA agonists can prime food, cocaine and heroin seeking behaviors [152]. The 120 μg dose of APO may have primed pacing behavior. DA also contributes to pre-reward motivational arousal [152]. Immediate dopaminergic action is an amplifying condition for pre-reward motivation [152]. Therefore, priming the animal with 120 μg of APO may have amplified pre-reward motivation which may have caused an increase in pacing behavior.

Experiment 2 examined the effects of increasing doses of APO on copulatory behavior in female mice, but the effects of the treatment on DA containing neurons was not measured. The following experiment (Exp 3) measured DA and metabolites in brain areas of mice treated with APO to better understand APO's effects. The group treated with 120 μg of APO had significantly higher levels of DA in the Nacc and significantly lower levels of HVA/DA in the striatum and Nacc compared to controls. Nacc DA increases and HVA/DA ratio decreases indicate that APO induces increased DA release and that DA is not metabolized as rapidly as in controls (i.e. a decrease in turnover). The same dose significantly increased pacing behavior. Thus the change in concentrations is correlated with the increased pacing seen in the previous experiments and previous

reports. This relationship suggests that in mice as in rats [2, 3, 139] and hamsters [24] the mesolimbic DA system contributes to paced reproductive behaviors.

Treatment with 60 μg of APO induced significant increases of HVA in the VMN. The same dose also induced the lowest levels of receptivity. HVA is a metabolite of DA, indicating that there was increased in DA activity. This results agrees with several studies that demonstrate an inhibitory effect of DA in the VMN on lordosis in female rats [143, 144]. Decreased receptivity may be due to increased DA activity in the VMN. This result is important because the VMN mediates reproductive behavior in female rodents [53, 54]. Therefore the decreased receptivity displayed by this group may be due to the increased activity of DA in this area. These results further support the hypothesis that there are different neural mechanisms underlying pacing and receptive components of sexual behavior. The increase in DA in the VMN may underlie the receptive component of female mouse behavior, while DA in the Nacc, may be the underlying mechanism of pacing behavior.

Pacing is a copulatory behavior but also consists of locomotor behavior. Numerous studies have demonstrated that APO has both excitatory and inhibitory effects on locomotor behavior [92, 93]. This information is relevant to this dissertation because pacing and receptive behavior heavily involves locomotor behavior. If locomotor behavior is compromised, copulatory behavior can be directly affected. Therefore it was important to examine the effects of the previously used doses (20 μg , 60 μg , and 120 μg) of APO on locomotion. An open field apparatus was used so that locomotion as well as exploratory behavior and anxiety was measured. APO increased locomotor behavior and anxiety, while decreasing exploratory behavior. At the 120 μg dose of APO, an increase

in locomotion, anxiety and pacing behavior were observed. Thus changes in pacing behavior parallels increased locomotion. Increased anxiety may also contribute to pacing, if the APO treated subjects are more anxious they may be more likely to pace as they may want to escape from the male. In addition, at the 60 μg dose, a decrease in receptivity was seen and there was also a significant increase in anxiety. The increase in anxiety may contribute to the significant decrease in receptivity.

The previous experiments focused on the effects of APO on copulatory and locomotor behaviors. APO increased pacing behavior, anxiety and locomotor behavior in a dose dependent manner while decreasing receptive and exploratory behavior in a dose dependent manner. These results led to further investigation of how DA mediates pacing and receptive behavior by specifically using D_1 and D_2 receptor agonists. D_1 and D_2 receptors differentially effect copulatory behavior in rats [23, 130, 131]. Dose dependent increases were expected after treatment with D_1 agonists and dose dependent decreases were expected after treatment with D_2 agonists [23, 130, 131]. However neither agonist had an effect on pacing or receptive behavior. D_1 and D_2 receptor activation effects on pre and postsynaptic DA receptors may explain the results.

Studies have shown that D_1 receptor activation decreases presynaptic effects [96]. When presynaptic DA receptors are stimulated, DA synthesis is inhibited because activity of tyrosine hydroxylase is blocked, DA release from presynaptic membrane-enclosed storage vesicles is blocked and the firing rate of DA neurons is attenuated [150, 151]. D_1 receptor activation inhibiting presynaptic DA receptors may result in increased DA synthesis and release [4]. Postsynaptic DA receptor activation increases DA transmission and D_1 stimulation of postsynaptic DA receptor may further increase DA transmission.

Generally pacing and receptive behaviors are correlated with increased DA in the Nacc and striatum [2, 3, 139]. D₁ agonists may have increased Nacc and striatum DA transmission in the female mice. If DA transmission increased because of treatment with D₁ agonists, less pacing behavior or receptive behavior may be needed for reward. This may explain why there were no changes in copulatory behavior in mice treated with the D₁ agonist.

Mice treated with the D₂ agonist displayed no changes in copulatory behavior. Generally D₂ binding and presynaptic D₂ activity are necessary to activate an inhibitory effect, while increased D₂ binding is required to activate an excitatory effect [100]. Expected decreases may not have been seen because low doses of quinpirole used may not have been enough to stimulate D₂ binding and activate presynaptic receptors which may explain the lack of change in behavior. Therefore the lack of decrease in both pacing and receptive behavior may be due to the lack of sufficient binding.

In conclusion, female mice display both receptive and pacing behaviors. DA regulates these behaviors through action in the Nacc, striatum and the hypothalamus. APO caused dose dependent increases in pacing and dose dependent decreases in receptivity. APO also caused dose dependent increases in DA levels in the Nacc and HVA in the VMN which correlated with the behavioral changes. Though D₁ and D₂ can influence pre and postsynaptic effects on copulatory behavior in opposite directions [23, 130, 131], there were no differences in either pacing or receptivity when female mice were treated with D₁ and D₂ agonists.

Future controlled studies are needed to examine the effects of copulatory behavior on DA transmission as well as the effect of DA transmission on copulatory behavior.

Further experiments measuring DA receptor binding after treatment with APO during sexual testing, particularly during pacing and receptive behaviors are needed to understand how DA receptors mediate these behaviors in female mice. Extracellular DA levels during pacing and receptivity behavior should also be measured in female mice as they have been done in female rats [2].

Though useful information was collected, there were shortfalls that may have affected results that warrant further discussion. Copulatory behavior in female mice was studied. However all experiments in this dissertation used female mice that were displaying low levels of receptivity. Mice need six weeks of hormone treatment and weekly testing before they show maximal receptive behavior [47]. Unlike female rats that will show maximal sexual behavior after being hormone primed and tested a small amount of times [12] mice need a longer period to become fully receptive [48]. The experiments for this dissertation were based on rat studies which is why female mice were tested after 2 weeks of hormone treatment and testing. Future experiments examining sexual behavior in female mice should allow ample time for hormone priming and weekly testing until maximum receptivity is achieved. If the experiments had allowed for increased hormone priming and weekly testing, it is expected that some of the results may have been altered.

Another potential confound was the potential activation of pre and postsynaptic DA receptors. Low doses of APO activate presynaptic DA receptors which cause a decrease of DA synthesis and release [4]. Therefore at low doses, APO acts as an antagonist. High doses of APO activate postsynaptic DA receptors and acts as a DA agonist. Being aware at what dose a drug acts as an agonist versus an antagonist is

crucial for maintaining the validity of the experiment. APO was administered at various doses and expected to act as an agonist. However, the lower doses may have been antagonistic. Drugs acting as antagonists when they are expected to act as agonists may compromise the data. In addition, any drug used during testing of pacing or receptive behavior should first be tested to establish if the drug affects locomotor activity. If the drug does affect locomotion, this change can interfere with the desired behavior of interest.

Finally, even though the pacing apparatus used in these experiments successfully allowed for observation and measurement of pacing behavior, it could be improved. The pacing cubes allowed for the female to escape the male and protected her on all sides. However, if the pacing cubes could be darkened, they may be more effective. Darkened cubes would take advantage of mice's preference to be in dark, hidden places [149]. Therefore the females would be less anxious and more comfortable entering the cubes and acclimate to them faster.

The information gathered from this dissertation can be broadened to clinical use. Currently clinical studies of human sexual behavior greatly depend on self reports of sexual desire, function and pleasure [153]. Studying the interaction between sexual behavior, neurotransmitter and endocrine regulation can be used to investigate sexual dysfunctions such as vaginismus (inability to relax vaginal walls for entry), dyspareunia (pain during intercourse), and infertility. Specifically, examining copulatory behavior using a female mouse pacing apparatus can provide further information. Information can be gathered to help investigate estrogen's effects on sexual desire in relation to menopause or women who are diagnosed with hypoactive sexual desire. It can also

increase our knowledge of the relationship between oral contraceptives that contain increased progesterone and subsequently have been reported to decrease sexual desire.

In addition many psychoactive medications that affect serotonin activity produce sexual side effects, particularly selective serotonin reuptake inhibitors (SSRIs) which act to increase serotonin levels and are associated with sexual side effects such as decreased libido [154]. Using a pacing paradigm with mice can help find remedies which reverse SSRIs induced sexual dysfunction. Additionally, antiparkinsonian and antipsychotic medication have been reported to impair sexual function [155]. However little research has been done to examine how DA mediates female sexual dysfunction. A pacing paradigm developed using female mice may be able to provide more insight.

Illicit drug use has been demonstrated to enhance [156] or inhibit sexual function [156-159]. Cocaine enhances DA activity by blocking presynaptic autoreceptors and is commonly believed to enhance sexual pleasure [160]. However chronic cocaine use can impair sexual function [157, 158]. In addition, withdrawal from cocaine can produce a temporary reduction in sexual desire [159].

Finally the various components of female mice copulatory behavior can be used as an additional model to study the DA mesolimbic “reward” system. Currently study of this system is mainly confined to drug addiction [55, 56] and food seeking models [161-163]. Copulatory behavior, particularly pacing behavior can be viewed as another motivated or rewarding behavior that can be manipulated to study the mediating effects of monoamines and metabolites within the mesolimbic DA system.

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