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FACTORS CONTROLLING THE TEMPORAL PATTERN OF FEMALE REPRODUCTIVE BEHAVIOR IN RATS

By

Liang-Yo Yang

A DISSERTATION

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ABSTRACT

FACTORS CONTROLLING THE TEMPORAL PATTERN OF FEMALE REPRODUCTIVE BEHAVIOR IN RATS

By

Liang-Yo Yang

When given the opportunity, the female rat regulates the copulatory speed. The female's return latencies and percentage exits following sexual contacts provide robust measures of her temporal copulatory behavior. One possible function of the female's pacing behavior is to facilitate successful reproduction. When the female controls the copulatory speed, the interintromission intervals are longer and fewer intromissions are sufficient to induce the progestational state of pregnancy. The female's postejaculatory refractory period (PER) is important to facilitate or allow sperm transport. If the female receives intromissions shortly following ejaculation, sperm transport will be stopped until the next ejaculation. The objectives of the experiments in this dissertation were to investigate (1) the relation of copulatory stimuli to the female's PER, (2) the relation among vaginocervical stimulation, uterine electromyographic (EMG) activity and female temporal copulatory behavior, and (3) whether the medial preoptic area (MPOA) of hypothalamus is involved in regulating female pacing behavior.

Female sexual behavior was tested in a two-compartment pacing chamber. Results indicated that the ejaculation duration and preejaculatory intromission frequency were positively correlated with the female's PER. Different hormone replacements also influenced the female's PER. No evidence was found to support the idea that the penile cup formation, the presence of a seminal plug, prostate secretions, or the number of pelvic thrusts during ejaculation contributed to the female's PER.

Consistent with previous reports, copulation had an immediate effect on uterine EMG activity. Moreover, the duration of uterine EMG activity associated with ejaculation was significantly longer than that associated with intromission or mount. This finding

coincides with the fact that the female's return latency following ejaculation is significantly longer than that following intromission or mount. Possibly the longer duration of uterine activity associated with ejaculation contributes to the female's PER.

The MPOA plays an important role in facilitating female sexual motivation in rats. MPOA lesions significantly increased the female's latency to return to the male's chamber following intromission and ejaculation. This finding suggests that MPOA lesions decrease female sexual motivation. Moreover, the significant increase of the female's return latencies following MPOA lesions parallels the change seen in male sexual behavior following MPOA lesions. It is suggested that the MPOA plays a similar role in regulating the temporal copulatory behavior of male and female rats.

To

my wife

Fu-Mei Chen

my daughter

Rena Yang

my parents

Chin-Nan Yang and Dwei Lin

and

my parents-in-law

Tsen-Ken Chen and Jui-Wun Hsiao

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LIST OF ABBREVIATION

AL	approach latency
BC	bulbocavernosus muscle
BCX	removal of bulbocavernosus muscle
IL	intromission latency
EB	estradiol benzoate
EL	ejaculation latency
EMG	electromyographic
ID	inner diameter
IF	intromission frequency
IL	intromission latency
III	interintromission interval
IM	intramuscular
IRL	intromission return latency
IP	intraperitoneal
ML	mount latency
MPOA	medial preoptic area of hypothalamus
MRL	mount return latency
OD	outer diameter
P	progesterone
PEI	postejaculatory interval
PER	postejaculatory refractory period
PX	removal of prostate glands
SCV	removal of seminal vesicles as well as coagulating glands and ligation of vas deferens
SNK	Student-Newman-Keuls
VMN	ventromedial nucleus of hypothalamus

INTRODUCTION

Different species of animals exhibit different patterns of copulatory behavior (Dewsbury, 1972). In rats, the male displays an intermittent copulatory pattern in which he delivers multiple intromissions (and/or multiple mounts) prior to ejaculation and achieves multiple ejaculations before sexual satiety (Dewsbury, 1972; Meisel and Sachs, 1994). Under a standard test situation, female sexual behavior is tested in a small test chamber from which she can not exit. The male rat has free access to the female and controls the timing of copulation. Lordosis behavior is the major measure of female sexual behavior. However, under test situations where the small testing chamber is divided into the male's chamber and an escape chamber available only to the female, the female can regulate the copulatory speed by exiting the male's chamber following sexual contacts (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1987, 1989, 1992; Erskine and Baum, 1982; Erskine et al., 1989; Fadem et al., 1979; Frye and Erskine, 1990; Gilman and Hitt, 1978; Krieger et al., 1976; Lange and Clemens, 1994; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a). Under the female-paced conditions, the female's return latencies and the percentage exits following sexual contacts, along with lordosis, provide robust measures of female sexual behavior.

One possible function of the temporal pattern of female sexual behavior is to facilitate successful pregnancy. When the female rat controls the timing of copulation, the interintromission intervals increase in length (Erskine, 1985; Erskine et al., 1989; Fadem and Barfield, 1982; Gilman et al., 1979) and fewer intromissions are sufficient to induce the progestational state of pregnancy in female rats (Erskine et al., 1989; Gilman et al., 1979). Since the regulation of the copulatory speed by the female rat facilitates successful

reproduction, it is important to understand what mechanisms underlie the regulation of the temporal pattern of female reproductive behavior. The present study aims to investigate (1) how stimulus factors affect the temporal pattern of female rat's sexual behavior, especially the female's postejaculatory refractory period (PER), (2) how uterine electromyographic (EMG) activity correlates with each type of sexual event and the temporal pattern of female sexual behavior, and (3) whether the medial preoptic area of the hypothalamus (MPOA) is involved in regulating female pacing behavior.

General Description of Female Rat's Sexual Behavior

The female rat displays a four or five day estrous cycle that is controlled by ovarian hormones (Carter, 1992; Nelson, 1995). The typical four-day estrous cycle consists of three stages: diestrus (48 hours) (Researchers further divide diestrus into diestrus I and diestrus II or metestrus and diestrus), proestrus (12 hours), and estrus (36 hours) (Carter, 1992; Nelson, 1995). Plasma estradiol levels gradually increase during diestrus, significantly increase during the morning and early afternoon of proestrus, and return rapidly to the baseline level during estrus (Smith et al., 1975). The significant increase of estradiol during the morning and early afternoon of proestrus and the beginning rise of progesterone during the afternoon of proestrus induce behavioral estrus in female rats (Smith et al., 1975; Young, 1961). In addition, the dramatic estradiol increase causes a surge of luteinizing hormone (LH) that in turn triggers ovulation on the evening of proestrus (Nelson, 1995). The coincidence of behavioral estrus and ovulation induced by ovarian hormones ensures successful reproduction.

In a standard test situation, female rat's sexual behavior is tested in a small test chamber where the male has free access to her and regulates the timing of mating. In behavioral estrus, the female rat hops and darts to attract the male's attention and initiate copulation (Beach, 1976; Madlafousek and Hlinak, 1977; Madlafousek et al., 1976;

Nelson, 1995). Consequently, the frequencies of hopping and darting have been used to measure the female's proceptivity which refers to "various reactions by the female toward the male which constitute her assumption of initiative in establishing or maintaining sexual interaction" (p. 105, Beach, 1976). In addition, the female rat shows lordosis behavior, the arching of her back and the elevation of her genitalia, in response to the male's mounting behavior. The display of lordosis behavior in female rats is necessary for successful penile insertion that ensures successful insemination (Diakow, 1974; Pfaff et al., 1978). Therefore, the lordosis quotient [(the number of lordosis responses in the first 10 mounts / 10) x 100] has been used to measure the female's receptivity which refers to the female's responses essential for successful intravaginal insemination (Beach, 1976) and has been the primary measure of female sexual behavior in rats (Pfaff et al., 1994).

In a two-compartment test situation, the latencies for the female to return to the male's chamber following sexual contacts and the percentage of times at which the female exits the male's chamber after each sexual contact provide robust measures of the temporal pattern of female sexual behavior. The female's latency to return to the male following a sexual contact is associated with the type of stimulation she receives from the male. Her return after an ejaculation (postejaculatory refractory period, PER) is significantly longer than that following a mount (mount return latency, MRL) or an intromission (intromission return latency, IRL) (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Lange and Clemens, 1994; Peirce and Nuttall, 1961a). In addition, her IRL is longer than her MRL (Bermant and Westbrook, 1966; Erskine, 1985; Krieger et al., 1976). Further, the female's MRL (Krieger et al., 1976), IRL (Bermant and Westbrook, 1966; Krieger et al., 1976), and PER (Bermant and Westbrook, 1966; Krieger et al., 1976) increase with the ejaculatory series. Finally, the female exits the male's chamber most frequently following an ejaculation and least frequently following a mount (Erskine, 1985, 1989; Krieger et al., 1976).

Significance of the Study of Temporal Pattern of Female Sexual Behavior

The study of the temporal aspects of female rat's sexual behavior is important in several respects. One important function of the temporal pattern of female sexual behavior is to facilitate successful pregnancy. In rats, the male delivers multiple intromissions before he ejaculates. These intromissions have at least two major functions. First, multiple intromissions facilitate sperm transport from the vagina to the uterus. It has been reported that if the female receives 0-1 intromission prior to ejaculation, no or few sperm are found in the uterus 3 hrs following ejaculation and no developing eggs are found in the uterus 2-4 days after copulation (Adler, 1969). In contrast, if the female receives two or more intromissions before ejaculation, many sperm are found in the uterus 3 hrs following ejaculation (Adler, 1969). Secondly, multiple intromissions induce the progestational state of pregnancy that is necessary for blastocyst implantation and successful pregnancy in female rats (Adler, 1969; Beach, 1965; Chester and Zucker, 1970; Wilson et al., 1965). In rats, the normal blood progesterone level of the female is not sufficient to support blastocyst implantation (Hashimoto et al., 1968). However, copulation can induce the secretion of progesterone to a level sufficient for blastocyst implantation (Adler et al., 1970). The number of intromissions required to induce the progestational state of pregnancy depends on the temporal pattern of copulation. In a standard test situation, the male rat controls the copulatory speed. Under this situation, approximately 10 intromissions are sufficient to induce the progestational state of pregnancy (Adler, 1969; Chester and Zucker, 1970; Edmonds et al., 1972; Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). In contrast, under a test situation where the female rat can escape from the male following sexual contacts, she regulates the timing of copulation. Under female-paced situation, the interintromission intervals are lengthened (Erskine, 1985; Erskine et al., 1989; Fadem and Barfield, 1982; Gilman et al., 1979) and the number of intromissions required for the induction of the progestational state of pregnancy is reduced

(approximately 5 intromissions in female-paced situation) (Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979).

In addition, one possible function of the female's postejaculatory refractory period is to facilitate or allow sperm transport from the vagina to the uterus. Under female-paced situations, the female rat leaves the male's chamber and shows a period of sexual inactivity following ejaculation: the postejaculatory refractory period. The female's postejaculatory refractory period is important for sperm transport. Sperm transport will be stopped until the next ejaculation if the female receives intromissions shortly after ejaculation. This is supported by the evidence that the number of sperm found in the uterus decreases significantly if the female receives an intromission within two minutes following ejaculation (Matthews & Adler, 1977).

Further, the study of female pacing behavior provides a possibility for comparing male and female sexual behavior under the same measurement scale. Traditionally, the latency and frequency of sexual contacts (mount, intromission, and ejaculation) are used to measure male rat's sexual behavior (Meisel & Sachs, 1994). The measures of male sexual behavior include mount latency (the latency from the introduction of the female to the first mount), intromission latency (the latency from the introduction of the female to the first intromission), ejaculation latency (the latency from the first intromission to ejaculation), mount frequency, intromission frequency, hit rate (intromission frequency / intromission frequency plus mount frequency), interintromission interval (III, the ejaculation latency divided by the intromission frequency), and the postejaculatory interval (PEI, the latency from ejaculation to the next intromission). In contrast, in a standard test situation, the frequency of lordosis but not the latency of sexual contacts is used to measure female sexual behavior because the male controls the timing of copulation (For more details, please see general description of female rat's sexual behavior in the previous section). However, under female paced situations, the female's latency to approach the male and her latencies to return to the male's chamber following sexual contacts appear comparable to the latency

measures of male sexual behavior. For example, the female's intromission return latency and postejaculatory refractory period are comparable to the male's interintromission interval and postejaculatory interval, respectively.

Finally, the investigation of female pacing behavior facilitates the study of sexual motivation in female rats. The distinction between female sexual ability and female sexual desire (sexual motivation) has been elaborated by Wallen (Wallen, 1990). Female sexual ability is defined as the female's ability to engage in copulation. Female sexual motivation refers to "the state underlying sexual initiation and sexual accommodation" (p. 234, Wallen, 1990). In rats, it has been demonstrated that it is impossible for the female to allow successful penile insertion without showing the lordosis posture (Diakow, 1974; Pfaff et al., 1978). Consequently, the display of lordosis behavior becomes the major measure of female sexual ability (Wallen, 1990). In female-paced situations, the female's accessibility and the temporal pattern of her sexual behavior are regulated by her sexual motivation (Wallen, 1990). In other words, the female's latencies to approach or return to the male following sexual contacts can provide measures of her sexual motivation.

Diversity of Testing Chambers in Studying Female Pacing Behavior

Different test situations have been used to measure the female's temporal copulatory behavior (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989, 1992; Fadem et al., 1979; Gilman and Hitt, 1978; Krieger et al., 1976; McClintock and Adler, 1978; McClintock and Anisko, 1982; McClintock et al., 1982; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a). These different test situations can be classified into two major categories. In one category, the temporal pattern of female sexual behavior is tested in a small test chamber and usually one male and one female are used. The test situations in this category can be further divided into three types. In some of the earlier studies, the females are trained to press a lever in order to get access to a sexually active male (Bermant,

1961; Bermant and Westbrook, 1966). In a second type of study, the female is provided with an escape chamber or platform to which she can retreat (Erskine, 1985, 1989, 1992; Gilman and Hitt, 1978; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a). The male is sharply hit on the nose when he tries to enter the escape chamber or platform. A third protocol involves tethering the male to one area of the test arena and allowing the female access to the male (Fadem et al., 1979; Krieger et al., 1976). In this category, the test situation is simpler and is most suitable for studying the mechanisms regulating the temporal pattern of female sexual behavior.

In the other category, the temporal pattern of female sexual behavior is tested in a seminatural environment (McClintock and Adler, 1978; McClintock and Anisko, 1982; McClintock et al., 1982). A group of intact animals (2 males and 5 females) are placed in a much larger seminatural testing arena and their mating behavior is videotaped. The female rat returns to the burrow or stays behind some barrier after she receives sexual contacts. The time the female stays away from the male following sexual contacts is used to measure her pacing behavior. Although the seminatural environment is more complicated, this seminatural testing setup mimics the natural environment and is most appropriate for studying the evolutionary significance of the mating strategy of females in nature.

Factors Influencing the Temporal Pattern of Female Sexual Behavior

Copulatory stimuli influence the temporal pattern of female rat's sexual behavior. In the previous section, it was noted that the female's PER is significantly longer than her IRL or MRL (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a). Although it has been reported that the presence of a seminal plug following ejaculation lengthens the female's PER (Bermant and Westbrook, 1966), the effect of a seminal plug on the female's PER is minimal in that study. The authors have suggested that other factors including the duration of penile

insertion, the number of deep thrusts and the tightness with which the male grasps the female during ejaculation might contribute to the female's PER. In addition, during ejaculation, the formation of the penile cup may generate some mechanical stimulation and contributes to the female's PER. Further, chemical substances released from the prostate may cause contraction of the vagina, cervix as well as uterus and induces the female's PER.

The temporal pattern of female rat's sexual behavior is affected by gonadal hormones (Fadem et al., 1979; Gilman and Hitt, 1978). In the presence of estradiol benzoate (EB), the increases in the progesterone (P) level reduce the return latencies following both intromissions and ejaculations (Fadem et al., 1979; Gilman and Hitt, 1978). In addition, increases in progesterone levels at different doses of EB decrease the frequency with which the female leaves the male's chamber after intromissions (Gilman and Hitt, 1978). Increases in EB do not affect the return latencies following intromissions or ejaculations nor do they affect the percentage of "exits" with which the female leaves the male's chamber following intromissions (Gilman and Hitt, 1978).

Different hormone replacement protocols have been used to prime the females into behavioral estrus in studying female pacing behavior (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976; Lange and Clemens, 1994). Basically these can be divided into two major categories. In one category, researchers give an injection of a high dose of estradiol benzoate (50 μg or 100 μg) followed by an injection of progesterone (0.5 or 1 mg) 48 or 72 hours later (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). In the other category, the females are brought into behavioral estrus by three consecutive injections of a low dose of EB (0.5 μg) followed by an injection of progesterone (0.5 mg) 24 hours later (Lange and Clemens, 1994). It has been noticed that the female's return latencies following sexual contacts are shorter in the study employing consecutive injections of low doses of estradiol benzoate followed by an injection of

progesterone 24 hours later (Lange and Clemens, 1994). For example, the female's PER is about 50 seconds in the study using this low dose of estradiol benzoate protocol (Lange and Clemens, 1994). In contrast, the females have a PER ranging from 90 seconds to several minutes in those studies using a high dose of estradiol benzoate followed by an injection of progesterone 48 or 72 hours later (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). In addition to the difference in hormonal treatments, different test chambers have been used in the above studies. Whether the difference in the female's return latencies in those studies using different hormone replacement protocols results from the difference in hormone treatments, the difference in testing chambers or both is not clear.

Sensory Innervation of Genitalia in Female Rats

In female rats, the pelvic nerve, pudendal nerve, and hypogastric nerve convey sensory stimulation from the external and internal genitalia. It has been reported that the pelvic nerve carries sensory stimulation from the vagina (Berkley et al., 1990; Berkley et al., 1993; Komisaruk et al., 1972; Peters et al., 1987), cervix (Berkley et al., 1990; Berkley et al., 1993; Komisaruk et al., 1972; Peters et al., 1987), uterus (Berkley et al., 1990; Berkley et al., 1993) and perineal skin (Peters et al., 1987). The hypogastric nerve responds to the mechanical stimulation of the cervix and three fifths of the uterus near the cervix (Berkley et al., 1988; Berkley et al., 1993; Peters et al., 1987). The pudendal nerve bears sensory stimulation from perineal skin, inner thigh, and clitoral sheath (Peters et al., 1987).

Fibers with mechanoreceptive fields are found along the vaginal canal to the uterine body (including the cervix), and more fibers with mechanoreceptive fields are found in the vaginocervical junction (Berkley et al., 1990). These fibers with mechanoreceptive fields respond best to the stimuli provided by a probe moving from vaginal opening toward vaginocervical junction (Berkley et al., 1990). The pelvic nerve fibers with

mechanoreceptive fields are significantly more sensitive to the mechanical stimulation of the cervix and uterus than the hypogastric nerve fibers (Berkley et al., 1993). A functional analysis of the pelvic nerve fibers and the hypogastric nerve fibers suggests that the former is closely bound to sensory and behavioral processes associated with copulation and pregnancy and the latter is closely bound to nociception and conception (Berkley et al., 1993).

Vaginocervical Stimulation, Uterine Activity and Female Sexual Behavior

In an earlier section, it has been mentioned that vaginocervical stimulation provided by the male during copulation is associated with the temporal pattern of female sexual behavior. In addition, vaginocervical stimulation provided by a male during copulation or provided by mechanical manual stimulation has been demonstrated to induce pseudopregnancy in female rats (Adler, 1969; Beach, 1965; Carlson and De Feo, 1965; Chester and Zucker, 1970; Edmonds et al., 1972; Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979; Wilson et al., 1965), and to shorten the period of behavioral estrus in female rats (Lodder and Zeilmaker, 1976).

The pelvic nerve is important for induction of pseudopregnancy by copulation or mechanical vaginocervical stimulation (Carlson and De Feo, 1965) as well as for abbreviation of behavioral estrus (Lodder and Zeilmaker, 1976). The pelvic nerve and the pudendal nerve have also been demonstrated to play an important role in the display of normal temporal patterns of sexual behavior in female rats (Emery and Whitney, 1985; Erskine, 1992). The pelvic neurectomy abolishes the induction of pseudopregnancy by mating or mechanical vaginocervical stimulation (Carlson and De Feo, 1965). In addition, bilateral transection of the pelvic nerve disrupts the female rat's pacing behavior (Emery and Whitney, 1985; Erskine, 1992). The females show no difference between the percentage exits following mounts and intromissions after transection of the pelvic nerve,

whereas control females show a higher percentage of exits following intromissions than following mounts (Erskine, 1992). Moreover, the number of intromissions and ejaculations that the male delivers during a constant time and the time that the female spends with the sexually active males increase significantly after transection of the pelvic nerve (Emery and Whitney, 1985). Furthermore, anesthetics applied locally to the vagina of female rats reduce their contact-response intervals following intromissions and ejaculations (Bermant and Westbrook, 1966). Finally, the female's return latencies following mounts, intromissions, and ejaculation are not different from one another after bilateral transection of the pelvic nerve and pudendal nerve (Erskine, 1992).

Vaginocervical stimulation plays an important role in the regulation of uterine EMG activity. Uterine contractions change with the course of copulation (Toner and Adler, 1986). Copulation has both an immediate effect and a delayed effect on uterine EMG activity (Toner and Adler, 1986). Copulation doubles the frequency of uterine contractions during active copulation but uterine activity returns to the premating level immediately after ejaculation, then increases significantly 5 minutes after ejaculation (Toner and Adler, 1986). These results are obtained under the situation where the male controls the copulatory rate. Although vaginocervical stimulation plays an important role in the control of uterine EMG activity and the temporal pattern of female sexual behavior, it is not known how uterine EMG activity is correlated with the temporal pattern of female sexual behavior. In addition, it is not clear, however, how uterine EMG activity correlates with each type of sexual contact (mount, intromission, and ejaculation).

Relation of MPOA to Female Sexual Behavior

The MPOA has been reported to play an important role in the control of female sexual behavior in rats. The display of lordosis behavior in female rats is inhibited by electrical stimulation of the medial preoptic area of hypothalamus (Moss et al., 1974;

Napoli et al., 1972). On the other hand, MPOA lesions have been reported to increase lordosis frequency in female rats treated with low doses of estradiol benzoate plus progesterone (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). Transections made anterior-dorsal to the MPOA also facilitate lordosis responding in female rats (Yamanouchi and Arai, 1977). Based on these results, it has been suggested that the MPOA inhibits female "sexual receptivity" (Powers and Valenstein, 1972). However, MPOA lesions significantly decrease the time that the female spends with the sexually active males (Whitney, 1986), suggesting that the MPOA may play a role in facilitating female sexual behavior.

Vaginocervical stimulation provided by a male rat during copulation, or by a glass rod, significantly increases c-fos expression in the MPOA of female rats (Pfaus et al., 1993; Wersinger et al., 1993). In addition, mechanical stimulation of the uterine cervix induces changes in the electrical activity of neurons in the MPOA (Haskins and Moss, 1983). The above findings strongly suggest that the MPOA may play a role in the regulation of female sexual behavior. The MPOA has been demonstrated to inhibit female lordosis responses. It is not known, however, whether the MPOA plays an important role in the regulation of temporal patterning of female sexual behavior. Since MPOA lesions have been demonstrated to decrease frequency of sexual contacts and the time that the female spends with the sexually active males during a constant time test when she can choose among sexually active males, castrated males and females (Whitney, 1986), the MPOA lesions are expected to significantly increase the female's return latencies following sexual contacts in a two-compartment pacing chamber.

The MPOA plays an important role in the regulation of male rat's sexual behavior. It has been reported that copulation significantly induces c-fos expression in the MPOA of male rats (Baum and Everitt, 1992; Wersinger et al., 1993). In addition, large MPOA lesions abolish male sexual behavior (Ginton and Merari, 1977; Heimer and Larsson, 1966/1967). It is also true that some males with MPOA lesions (some part of MPOA is

undamaged) can still copulate although their interintromission interval, ejaculation latency and postejaculatory interval are prolonged (Ginton and Merari, 1977). Moreover, electrical stimulation of the MPOA facilitates male rat's copulatory behavior (Malsbury, 1971). The above evidence suggests that the MPOA facilitates the temporal pattern of male sexual behavior in rats. Furthermore, the MPOA regulates the motor responses (lordosis behavior and mounting behavior) of sexual behavior similarly in both male and female rats. The MPOA lesions facilitate lordosis behavior in castrated male rats treated with estradiol benzoate and progesterone (Hennessey et al., 1986; Olster, 1993) just as they do in females (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). MPOA lesions also inhibit mounting in ovariectomized females treated with testosterone (Singer, 1968) just as they do in male rats (Ginton and Merari, 1977; Heimer and Larsson, 1966/1967). Therefore, MPOA may regulate the female's temporal copulatory pattern in the same way as it does in males.

Summary

When given the opportunity, the female rat can control the timing of copulation. One function of the temporal pattern of female sexual behavior is to facilitate successful pregnancy. Under female-paced situations, the interintromission intervals are longer and fewer intromissions are sufficient to elevate the blood progesterone to a level which is adequate for blastocyst implantation and maintenance of pregnancy. In addition, the female's postejaculatory refractory period is important for sperm transport from the vagina to the uterus. In the past thirty-five years, the temporal pattern of female sexual behavior has been studied to some extent. However, what mechanisms underlie the female's postejaculatory refractory period, how the uterine EMG activity correlates with different types of sexual contacts and the temporal pattern of female sexual behavior, and whether the MPOA regulates the temporal pattern of female sexual behavior are not well

understood. In the following experiments, several important questions about the temporal pattern of female rat's sexual behavior will be investigated including (1) how stimulus factors affect the female's postejaculatory refractory period, (2) how intromissions influence the female's individual return latencies following intromissions, (3) whether different hormonal regimes affect the temporal pattern of female sexual behavior, (4) how the uterine EMG activity correlates with the female pacing behavior, and (5) whether the MPOA regulates the female temporal copulatory pattern.

EXPERIMENT 1: RELATION OF INTROMISSIONS TO THE FEMALE'S POSTEJACULATORY REFRACTORY PERIOD IN RATS

ABSTRACT

YANG, L. Y. AND L. G. CLEMENS. Relation of intromissions to the female's postejaculatory refractory period in rats. PHYSIOL BEHAV 59(0) 000-000, 1996.- The objectives of this study were to investigate the temporal aspects of female sexual behavior during single and multiple ejaculatory tests. Females were tested in a two-compartment chamber where they could escape from the male following sexual contacts. In Experiment 1A, correlation analysis showed that the number of intromissions received by the female over three ejaculatory series was positively correlated with the female's postejaculatory refractory period (PER). In Experiment 1B, females receiving 2-4 intromissions before ejaculation had a PER that did not differ from those receiving 5-15 (average 10) intromissions preceding ejaculation. However, if the male ejaculated on the first or second intromission, the female's PER was significantly shorter than the other groups and did not differ from her return latency after an intromission without ejaculation. Females receiving 24-31 intromissions preceding ejaculation exhibited the longest PER. Analysis revealed that the number of intromissions received by females before ejaculation was positively correlated with the female's PER. We concluded that the male's ejaculatory reflex, seminal emission, and postejaculatory behavior alone without at least two preceding intromissions were not sufficient to induce a female's PER comparable to that seen after an ejaculation during normal copulation. In addition, the number of intromissions received by the female preceding ejaculation was positively correlated with the female's PER if the range of

intromission frequency was large enough.

INTRODUCTION

During copulation, the male rat displays an intermittent copulatory pattern that includes mounts, intromissions, and ejaculations (Dewsbury, 1972; Young, 1961). In a standard test situation, the male rat has free access to the female and thereby regulates the copulatory rate. In this situation, the receptive female rat displays soliciting behaviors such as hopping and darting and when mounted she responds with the lordosis posture to allow successful penile insertion (Beach, 1976; Young, 1961). This has resulted in the use of lordosis as the measure of the female sexual behavior. However, when the female is tested in a situation where she can escape from the male following a sexual contact, she "paces" the copulatory events (Bermant, 1961; Bermant and Westbrook, 1966; Clemens et al., 1995; Erskine, 1985, 1989; Erskine and Baum, 1982; Fadem et al., 1979; Gilman and Hitt, 1978; Krieger et al., 1976; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a; Yang and Clemens, 1994, 1995a, 1995b). Following an ejaculation, the female leaves the male's chamber for a period of time. This period of female sexual inactivity is referred to as her postejaculatory refractory period (PER). One important function of the female's PER is to allow sperm transport. This is supported by the evidence that sperm transport will be stopped until the next ejaculation if the female receives an intromission within two minutes following ejaculation (Matthews and Adler, 1977). On the other hand, multiple intromissions prior to ejaculation have been reported to facilitate sperm transport (Adler, 1969). However, the relation of multiple intromissions preceding ejaculation to the female's PER is poorly understood.

The female's latency to return to the male following a sexual contact is associated with the type of stimulation she receives from the male. Her return after an ejaculation (PER) is much longer than that following a mount (MRL) or intromission (IRL) (Bermant,

1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a; Yang and Clemens, 1994). In addition, her IRL is longer than her MRL (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a). The temporal pattern of female rat sexual behavior is also affected by gonadal hormones (Fadem et al., 1979; Gilman and Hitt, 1978). In the presence of estradiol benzoate (EB), the increases in the progesterone (P) levels reduce the return latencies following both intromissions and ejaculations (Fadem et al., 1979; Gilman and Hitt, 1978). In addition, increases in progesterone levels at different doses of EB decrease the frequency with which the female leaves the male's chamber after intromissions (Gilman and Hitt, 1978). Increases in EB alone do not affect the return latencies following intromissions or ejaculations nor do they affect the percentage of "exits" with which the female leaves the male's chamber following intromissions (Gilman and Hitt, 1978).

Different test situations have been used to measure the female's temporal copulatory behavior (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Erskine and Baum, 1982; Fadem et al., 1979; Gilman and Hitt, 1978; Krieger et al., 1976; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a). Basically these can be divided into three types. For some of the earlier studies, females were trained to press a lever in order to get access to a sexually active male (Bermant, 1961; Bermant and Westbrook, 1966). In a second category the female is provided with an escape chamber or platform to which she can retreat (Erskine, 1985, 1989; Erskine and Baum, 1982; Gilman and Hitt, 1978; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a). The males are sharply hit on the nose when they try to enter the escape chamber or platform. A third protocol involves tethering the male to one area of the test arena and allowing the female access to the male (Fadem et al., 1979; Krieger et al., 1976).

Although these different pacing paradigms have produced somewhat similar results, it is still the case that return latencies for the female are considerably longer in some reports (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976; Peirce and Nuttall,

1961a) than in others (Erskine, 1985, 1989). In Experiment 1A, we investigated whether the temporal aspects of female copulatory behavior tested in our modified pacing paradigm were similar to or different from those reported by others. In Experiment 1B, we examined whether the postejaculatory refractory period of the female rat was a response to the male's ejaculatory reflex or whether additional vaginocervical stimulation was necessary. Part of this work has been presented in abstract form (Yang and Clemens, 1994).

GENERAL METHODS

Animals

Sixty-day-old female and ninety-day-old male Long Evans rats were purchased from Charles River Laboratories (Wilmington, MA). All females were ovariectomized one week later upon arrival. Animals were housed in 16:8 hr light-dark cycle with lights off at 17:00-1:00. In general, females were housed three per cage and males were housed singly. Food and water were available ad lib. The temperature and humidity of the animal room were maintained at 72^0 F and 55%, respectively. The air in the animal room was exchanged 12 times per hour automatically. Ovariectomized females were brought into estrus by intramuscular (IM) injections of 0.5 μg estradiol benzoate (EB) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil (Sigma Chemical Co., St. Louis, MO) for three consecutive days and 24 hours later followed by an IM injection of 0.5 mg progesterone (P) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil. All female pacing behaviors were tested 4-7 hours after the injection of progesterone (2-5 hours after the lights off) under the dimly red illumination. All animals were tested once per week throughout the whole test period unless otherwise specified and had been tested at least twice in the testing chamber before data were collected. Only those females showing

pacing behavior in these preliminary tests were used in the experimental studies. In each test, a male was always placed in the testing chamber 15 minutes before a female was introduced. The approximate weights of the female and male rats were 200 - 300 g and 400 - 500 g, respectively, when the tests started.

Pacing Tests

To provide a test situation in which the female controlled the pacing of the copulation, females were tested in a two-compartment chamber that was divided by a Plexiglas barrier with 4 holes (3.5~4 cm x 3.5~4 cm) spaced along the bottom of the barrier. The dimensions of the male's chamber and escape chamber were 35.5 cm x 44.5 cm x 48.3 cm and 21.5 cm x 44.5 cm x 48.3 cm (Length x Width x Height), respectively. The escape holes were large enough to allow the female to pass through but prevented the larger male from following her.

Behavioral Measures

Several behavioral measures were recorded in the pacing tests unless otherwise specified: approach latency (AL), the latency for the female to enter the male's chamber after the start of the test (she was placed in the escape chamber at the beginning of the test); intromission latency (IL), the latency from the female's entry into the male's chamber to the first intromission; mount return latency (MRL), the latency for the female to re-enter the male's chamber after a mount; intromission return latency (IRL), the latency to return to the male's chamber following an intromission; postejaculatory refractory period (PER), the latency to re-enter the male's chamber after an ejaculation. All latencies were measured in seconds. The frequency with which the female left the male's chamber following a mount, intromission and ejaculation (percentage exit) was calculated by dividing the number of

exits by the number of each type of stimulus. All behavioral data were recorded on a computer disk.

EXPERIMENT 1A: THE TEMPORAL PATTERN OF FEMALE SEXUAL BEHAVIOR TESTED IN OUR MODIFIED PACING CHAMBER

The objective of this experiment was to investigate whether the female pacing behavior tested in our modified pacing paradigm was similar to or different from those reported previously (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a). Although these previous reports of female pacing behavior conveyed similar information, there were quantitative differences in the return latencies following a mount, intromission and ejaculation among these reports (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a). For example, the mean return latency following an ejaculation and an intromission in the first ejaculatory series was significantly shorter in some studies (Erskine, 1985, 1989) than in other studies (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976; Peirce and Nuttall, 1961a). In addition, the mean return latency following an ejaculation increased significantly over consecutive ejaculations in two studies (Bermant and Westbrook, 1966; Krieger et al., 1976) but it did not do so in another study (Bermant, 1961). The present experiment was designed to examine the features of female pacing behavior tested in our modified pacing paradigm.

METHOD

To obtain measures of normal female pacing behavior in our modified pacing paradigm, 67 male and 67 female Long Evans rats were used in this study. Each female was tested once with one male during this experiment. Measures of female pacing behavior

that included AL, IL, MRL, IRL, PER, and percentage exits following each specific sexual contact (mount, intromission, and ejaculation) for each female were recorded over three ejaculatory series. Mean of MRL as well as IRL and mean of percentage exits following mounts and intromissions for each ejaculatory series were calculated for each female and used for further analysis. MRL, IRL, PER, and percentage exits following sexual contacts were analyzed by ANOVA for repeated measures followed by Student-Newman-Keuls (SNK) post-hoc tests.

RESULTS

The mean latency for the female to enter the male's chamber after the start of the test (AL) and the mean latency from the female's entry into the male's chamber to the first intromission (IL) were 4.8 $\pm$ 0.4 seconds and 4.7 $\pm$ 0.5 seconds (mean $\pm$ SEM), respectively. The results of the female's MRL, IRL, and PER were summarized in Figure 1. ANOVA for repeated measures revealed a significant sexual contact effect [$F(2, 198) = 283.7$, $p<0.0001$], ejaculatory series effect [$F(2, 396) = 62.7$, $p<0.0001$], as well as sexual contact x ejaculatory series interaction [$F(4, 396) = 28.5$, $p<0.0001$]. The SNK post-hoc tests revealed that for each ejaculatory series, the PER was significantly longer than the MRL or IRL ($p<0.01$). The IRL was significantly longer than the MRL in the second and the third ejaculatory series ($p<0.01$) (Figure 1). The SNK post-hoc tests showed that the PER but not IRL or MRL increased significantly over consecutive ejaculatory series ($p<0.01$) (Figure 1).

Percentage exits, the number of times the female left the male's chamber following a mount, intromission, or ejaculation, were summarized in Figure 2. The percentage exits following sexual contacts were significantly different among different sexual contacts [$F(2, 198) = 193.3$, $p<0.0001$], and did not differ among three ejaculatory series [$F(2, 396) = 2.773$, $p = 0.06$]. There was no significant sexual contact x ejaculatory series interaction

Figure 1. Return latency following mounts, intromissions, and ejaculations for three ejaculatory series (N=67). The female's return latency following an ejaculation (PER) was significantly longer than her return latency following an intromission (IRL) and mount (MRL) for three ejaculatory series. The IRL was significantly longer than the MRL in the second and third ejaculatory series. The female's PER increased significantly as the ejaculatory series increased. The female's IRL increased slightly over three ejaculatory series but there was no significant increase between two continuous ejaculatory series. There was no significant increase in the female's MRL for three ejaculatory series. Data were analyzed by ANOVA for repeated measures. Bar represents mean $\pm$ SEM.
** $p<0.01$.

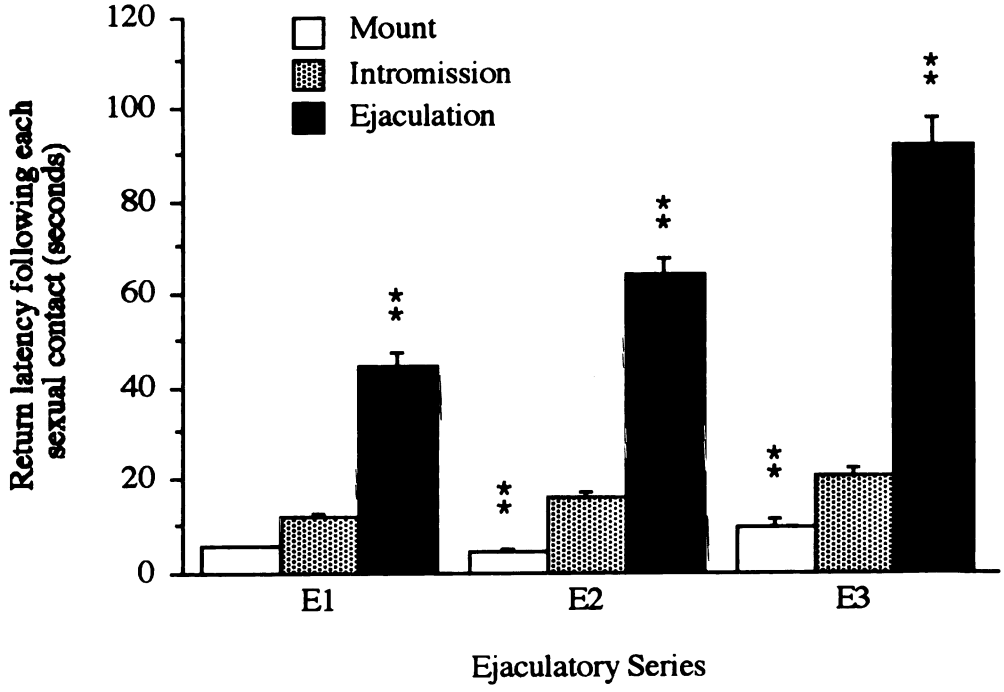

Mount
Intromission
Ejaculation
Return latency following each sexual contact (seconds)
120
100
80
60
40
20
0
E1
E2
E3
Ejaculatory Series

Figure 2. The percentage exits following mounts, intromissions, and ejaculations for three ejaculatory series. The percentage exit after ejaculation was significantly higher than that following intromissions which was higher than that following mounts. There was no significant difference among three ejaculatory series for the percentage exit after mounts, intromissions, and ejaculations. Data were analyzed by ANOVA for repeated measures. Bar represents mean $\pm$ SEM. ** $p<0.01$. N=67.

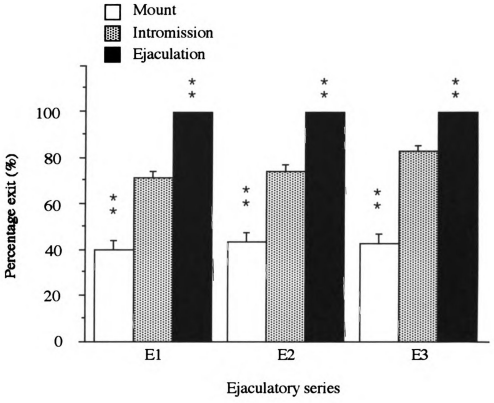
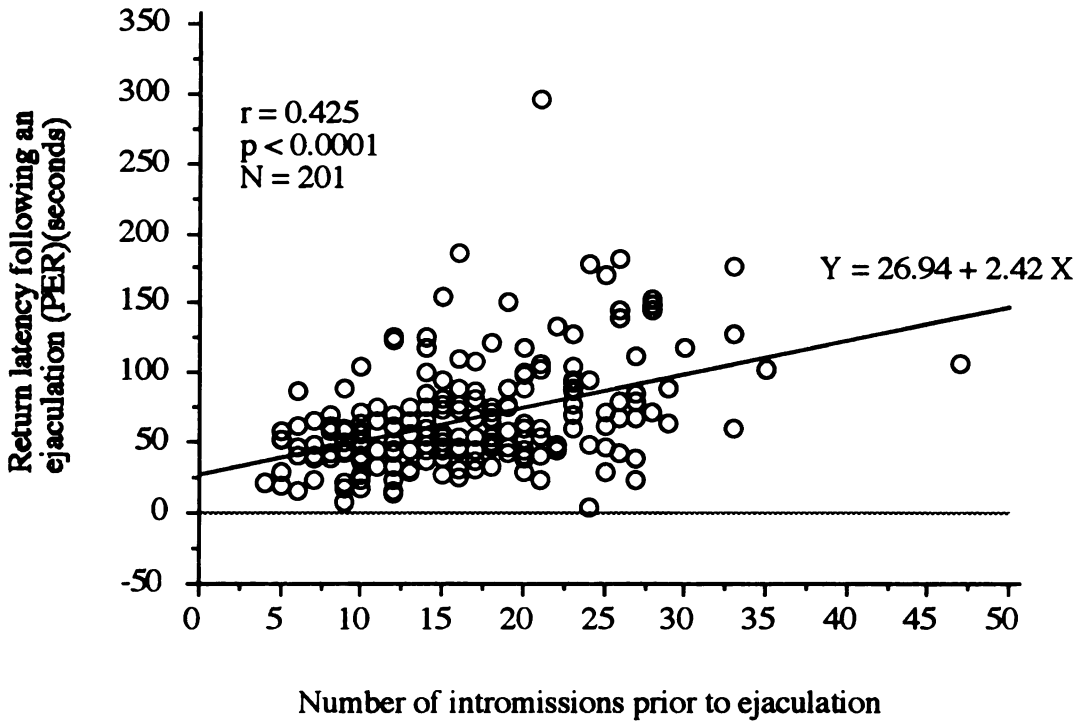

Mount
Intromission
Ejaculation
Percentage exit (%)
0
20
40
60
80
100
E1
E2
E3
Ejaculatory series

Figure 3. Relation of the number of intromissions prior to ejaculation and the female's return latency following an ejaculation (PER) for three ejaculatory series. There was a positive correlation between the number of intromissions received by the female before ejaculation over three ejaculatory series and the female's PER. Data were analyzed by Correlation analysis ($r=0.425$, $p<0.0001$). $N=201$.


Return latency following an ejaculation (PER)(seconds)
350
300
250
200
150
100
50
0
-50
r = 0.425
p < 0.0001
N = 201
Y = 26.94 + 2.42 X
0
5
10
15
20
25
30
35
40
45
50
Number of intromissions prior to ejaculation

effect [$F(4, 396) = 1.875$, $p = 0.114$] in the percentage exits following sexual contacts. The SNK post-hoc tests showed that for three ejaculatory series, the percentage exit following ejaculations was significantly higher than that following intromissions ($p<0.01$) and the percentage exit following intromissions was significantly higher than that following mounts ($p<0.01$) (Figure 2). There was no significant difference among 3 ejaculatory series for the percentage exits following mounts, intromissions and ejaculations (Figure 2). Correlation analysis did not show a significant relation between the number of intromissions received by the female prior to the first ejaculation and the female's PER ($r=0.151$, $p=0.225$). However, there was a significant positive correlation between the number of intromissions received by the female before ejaculation over three ejaculatory series and the female's PER ($r = 0.425$, $p<0.0001$) (Figure 3).

EXPERIMENT 1B: EFFECT OF DIFFERENT NUMBERS OF INTROMISSIONS ON THE FEMALE TEMPORAL COPULATORY BEHAVIOR

The objective of this experiment was to investigate whether different numbers of intromissions received by the female rat preceding ejaculation affected her PER or whether the ejaculatory reflex, seminal emission and the male's postejaculatory behavior were, in and of themselves, sufficient to induce a PER comparable to that seen after an ejaculation during normal copulation. While it has been reported that the female's PER is much longer than her MRL and IRL (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a; Yang and Clemens, 1994, 1995a), the factors that contribute to this difference have not been elucidated. In this experiment, we examined the effect of different numbers of intromissions received by the female prior to ejaculation on her PER.

METHOD

In separate biweekly tests, females were allowed to receive different numbers of intromissions before the male ejaculated: 0-1 intromission (0-1 I Group), 2-4 intromissions (2-4 I Group), 5-15 intromissions (5-15 I Group), and 24-31 intromissions (24-31 I Group). Each female was primed with EB and P once per week and randomly assigned to test with one male at the beginning of the experiment. Because each female received an ejaculation from the same male she mated at the beginning of the experiment throughout the entire test duration, we referred to "each female with the male providing ejaculation during copulation" as "female and male partners." Eighteen females and 9 males (27 additional stud males were used in the 24-31 intromission group to provide intromissions before the female was allowed to copulate with her "male partner") were used in this study. Each male had two "female partners" and was tested with one of two female partners on alternate weeks.

To obtain baseline measures of normal female pacing behavior (5-15 I Group), 9 females were tested with their "male partners" for one full ejaculatory series in the first week and another 9 females were tested in the second week. The ejaculation latency and intromission frequency of the males that were obtained in these tests were used to estimate when the male was close to ejaculation for the low intromission groups (0-1 I Group and 2-4 I Group). During the period of week 3 to week 6, the low number of intromissions in the 0-1 I Group and 2-4 I Group were achieved by pretesting the male with one "female partner" then replacing her with the experimental female, "the second female partner," when it was estimated that the male was close to ejaculation. During week 7 and 8, the higher number of intromissions in 24-31 I Group was achieved by pretesting the female with 3 stud males, each delivering 6 intromissions without ejaculation, then allowing her to copulate with her "male partner" until he ejaculated. Only the PER and IRL of those females receiving all four different numbers of intromissions prior to ejaculation (0-1 I, 2-4

I, 5-15 I and 24-31 I) were used for analysis. Mean of IRL was calculated for each female and used for further analysis. PER and IRL data were analyzed by ANOVA for repeated measures followed by Student-Newman-Keuls (SNK) post-hoc tests, respectively. Comparison of IRL and PER within each group except 0-1 I Group was achieved by paired t test. In addition, the relation of the number of intromissions prior to ejaculation in the four different situations to the female's PER was analyzed by Correlation analysis.

RESULTS

The mean return latencies following an intromission or ejaculation of females receiving different numbers of intromissions prior to ejaculation were summarized in Figure 4. Eleven of 18 females experienced all four intromission conditions preceding ejaculation during the eight-week experiment. Because only three animals had IRL in the 0-1 I Group due to no intromission or no leave after an intromission, the IRL data of 0-1 I Group were excluded for IRL analysis. In addition, three animals that did not have IRL in 2-4 I Group due to no exit after an intromission were excluded for IRL analysis. All the data were represented as mean $\pm$ SEM.

ANOVA for repeated measures showed that there was a significant difference among the PERs of the four different groups [$F(3, 30) = 20.454$, $p<0.0001$]. Student-Newman-Keuls (SNK) post-hoc tests revealed that the PER of 0-1 I Group was significantly shorter ($p<0.01$) than the other groups and that the PER of 24-31 I Group was significantly longer than that of 5-15 I Group ($p<0.05$), 2-4 I Group ($p<0.01$) and 0-1 I Group ($p<0.01$). However, the PER of females receiving 2-4 intromissions was not different from that of 5-15 I Group. The paired t test indicated that the female's IRL was significantly shorter than her PER in 2-4 I ($p<0.01$), 5-15 I ($p<0.0001$) and 24-31 I ($p<0.0001$) Groups (Figure 4). The t test showed that there was no difference between the PER of females receiving 0-1 intromission and the IRL of females receiving 5-15

Figure 4. Effect of intromissions on female's latency to return to the male following an ejaculation (PER, open bar, N=11) and following an intromission without ejaculation (IRL, solid bar, N=8). ** Return latency after an ejaculation was significantly shorter for 0-1 intromission group than the other groups ($p<0.01$). * Return latency after an ejaculation was significantly longer for the 24-31 intromission group than the 5-15 intromission group ($p<0.05$). # The PER of females receiving 24-31 intromissions was significantly longer than those receiving 0-1 or 2-4 intromissions ($p<0.01$). $ The female's IRL was significantly shorter than her PER in 2-4, 5-15 and 24-31 intromission groups ($p<0.01$, paired t test). There was no significant difference between the female's return latencies following an ejaculation of 2-4 and 5-15 intromission groups. There was no significant difference among the female's mean return latencies following an intromission of the different intromission groups. All data except comparison of IRL and PER within the same group were analyzed by ANOVA for repeated measures followed by SNK post-hoc tests. Bar represents mean $\pm$ SEM.

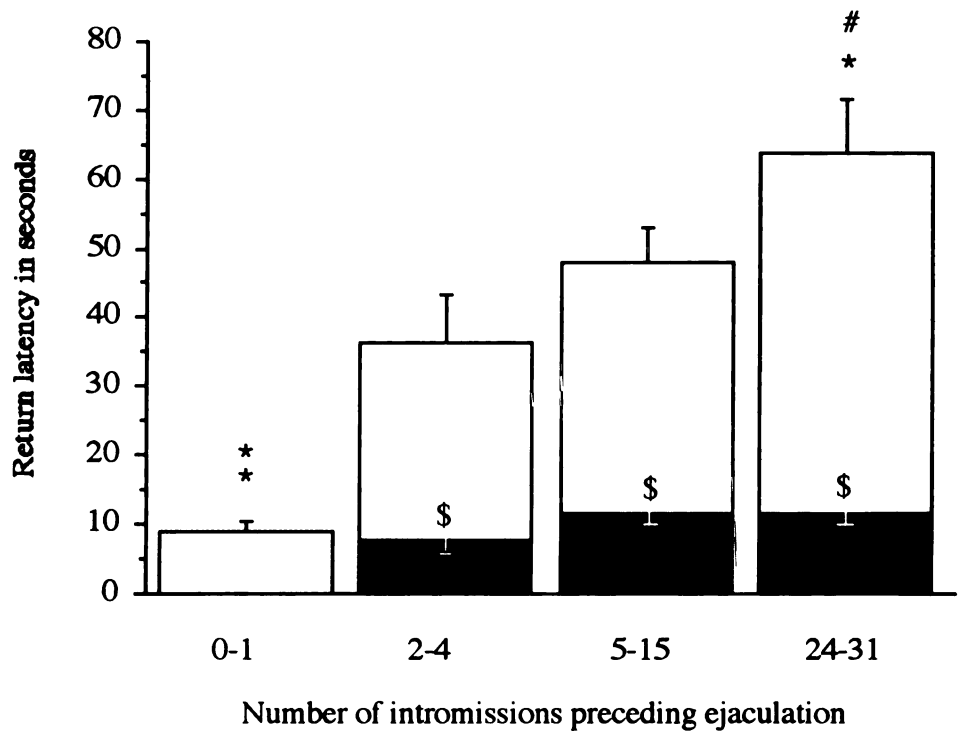
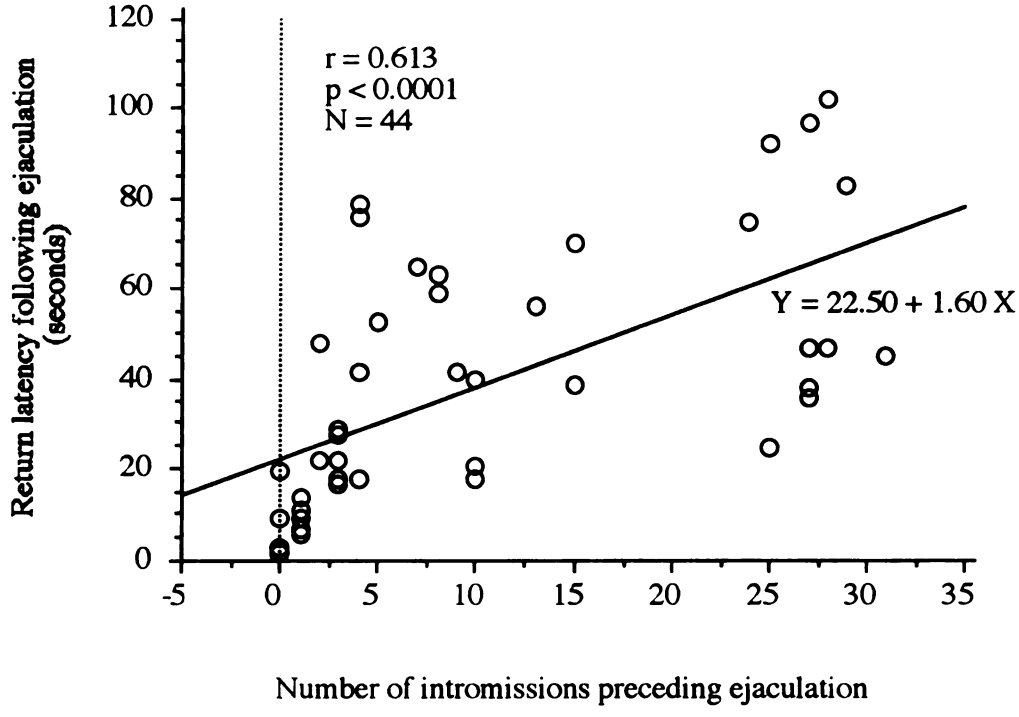

Return latency in seconds
80
70
60
50
40
30
20
10
0
#
*
*
*
$
$
$
0-1
2-4
5-15
24-31
Number of intromissions preceding ejaculation

Figure 5. The relation between the number of intromissions preceding ejaculation and the female's return latency following an ejaculation (PER). The number of intromissions received by the female prior to ejaculation in four groups was positively correlated with her PER. Data were analyzed by Correlation analysis ($r=0.613$, $p<0.0001$). N=44.


r = 0.613
p < 0.0001
N = 44
Y = 22.50 + 1.60 X
Return latency following ejaculation (seconds)
Number of intromissions preceding ejaculation
120
100
80
60
40
20
0
-5
0
5
10
15
20
25
30
35

intromissions [t=-0.926, p=0.366] (Figure 4). ANOVA for repeated measures showed that there was no significant difference among IRLs of three different groups (2-4, 5-15, and 24-31 I Groups) [F(2, 14) = 2.163, p=0.152] (Figure 4).

Figure 5 showed the relation of the number of intromissions (0-1, 2-4, 5-15 and 24-31) received by the females prior to ejaculation to the female's PER. Correlation analysis revealed that the number of intromissions received by the females prior to ejaculation was positively correlated with her PER ($r = 0.613$, $p<0.0001$).

DISCUSSION

In rats, the temporal pattern of female sexual behavior has physiological significance. The progestational state of pregnancy in female rats is more readily induced when the female controls the copulatory speed. When the female controls the timing of copulation, the interval between intromissions is longer (Erskine, 1985) and fewer intromissions are sufficient to induce the progestational state of pregnancy (Edmonds et al., 1972; Erskine et al., 1989; Gilman et al., 1979). In female paced tests (Erskine et al., 1989; Gilman et al., 1979) or enforced long interintromission interval (Edmonds et al., 1972), five intromissions are sufficient to trigger the progestational state of pregnancy in the female rats. However, five intromissions were insufficient to induce pseudopregnancy (Chester and Zucker, 1970; Edmonds et al., 1972; Erskine et al., 1989; Gilman et al., 1979) or pregnancy (Erskine et al., 1989) in non-paced female rats. Approximately, 10 intromissions are capable of inducing the progestational state of pregnancy in non-paced females (Edmonds et al., 1972; Erskine et al., 1989; Gilman et al., 1979).

In the present study, results show that the female's return latency following an ejaculation (PER) was much longer than her return latency following an intromission (IRL) or mount (MRL) in each of three ejaculatory series. The return latency following an intromission (IRL) was significantly longer than the MRL in the second and third

ejaculatory series. In general, these results are consistent with previous findings that have been reported using a variety of pacing test paradigms to measure female pacing behavior (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Peirce and Nuttall, 1961a). The PER, IRL, and MRL of our results are quite similar to those of some studies (Erskine, 1985, 1989) but are shorter than those reported by other research groups (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976; Peirce and Nuttall, 1961a). The MRL in our study did not increase over three ejaculatory series. This is consistent with the results of two studies (Bermant, 1961; Bermant and Westbrook, 1966) but different from those reported in another (Krieger et al., 1976). Most likely these differences in return latencies following specific sexual contacts reflect differences in the designs of test chambers as well as differences in hormone replacement protocols. For example, in some studies (Bermant, 1961; Bermant and Westbrook, 1966), the females were trained to press a lever to obtain access to a male. In this complex test situation that involves learning, it takes longer for the female to respond after each sexual contact than in the simpler paradigm used here. In addition, different hormone replacement protocols are used. In the present study, females receive low doses of EB (0.5 μg) for three consecutive days and 24 hours later followed by an injection of 0.5 mg progesterone. Other investigators often give a single injection of 50 or 100 ug EB 48 or 72 hrs prior to an injection of 0.5 or 1 mg progesterone. Four to eight hours later after the injection of progesterone, the females were tested for sexual behavior. These differences in the hormone replacement protocols may contribute to differences in the female's return latencies.

That the female's IRL is longer than her MRL most likely reflects the occurrence of penile insertion during an intromission but not during a mount (and/or the summation of intromission and mounting stimuli during an intromission). However, why the female's PER is much longer than her IRL is still not clear. Although it has been reported that the deposit of a seminal plug during ejaculation lengthened the female's PER, the effect of a

seminal plug on the female's PER was minimal in that study (Bermant and Westbrook, 1966). The authors suggested that the difference between PER and IRL might result from the differences between an ejaculation and an intromission including the differences between the contact durations, the numbers of deep thrusts, and the degree of tightness of the male's grasp during an ejaculation and an intromission (Bermant and Westbrook, 1966). It is also the case that the male rat emits an ultrasonic (22 kHz) vocalization after an ejaculation (Barfield and Greyer, 1972; Barfield and Greyer, 1975). Such vocalization might also contribute to the female's PER. However, the results in Experiment 1B demonstrate that the male's ejaculatory and postejaculatory behavior alone without at least two preceding intromissions are not sufficient to induce a PER comparable to that seen after an ejaculation during normal copulation.

In Experiment 1A, we found that the female's PER increased with consecutive ejaculations. This finding is consistent with some previous reports (Bermant and Westbrook, 1966; Krieger et al., 1976). In one study, the authors suggested that the increase in return latencies over consecutive ejaculatory series indicated a decline in the female's receptivity (Krieger et al., 1976). However, other measures of receptivity did not change. For example, lordosis quotients (100%) remained constant over the 3 ejaculatory series in their study (Krieger et al., 1976). We also did not observe any lordosis change over three ejaculatory series in our study. In addition, the MRL in the present study did not increase over the course of three ejaculations. If the receptivity of the female was decreasing, we would have expected to see the MRL increase. Given the correlation between intromission frequency (IF) and PER, it may be more likely that the increase in the PER during consecutive ejaculatory series results from an increase in the ejaculation duration which occurs in these cases (Peirce and Nuttall, 1961b). The increase in ejaculation duration would provide stronger vaginocervical stimulation which might result in a longer return latency following an ejaculation.

The female's PER was positively correlated with the number of intromissions she

received prior to ejaculation but this was only seen when there was a relatively large range of intromissions (0-31 in this study). In Experiment 1A, the female's PER obtained from the first ejaculatory series of normal copulation was not correlated with the number of intromissions she received. In Experiment 1B, however, when females received a large range of intromissions (0-31), the female's PER was significantly correlated with intromission frequency before the first ejaculation. In addition, in Experiment 1A, the female's PER over three ejaculatory series was positively correlated with the number of intromissions received by the female prior to ejaculation.

Following at least two preejaculatory intromissions, the ejaculatory reflex itself provides a more effective stimulus than an intromission in inducing a longer return latency. The stimulus of the ejaculatory reflex itself becomes more effective as the female receives more intromissions prior to ejaculation. This finding is supported by the evidence that the female's PER is significantly longer than her IRL in all groups except 0-1 intromission group. In addition, the female's PER in the 24-31 intromission group is significantly longer than that in the 2-4 and 5-15 intromission groups while there is no difference among the female's IRLs of these three (2-4, 5-15, and 24-31 I) groups (Figure 4). These results also imply that in addition to the preceding intromissions, some other factors play an important role in the increasing effectiveness of an ejaculatory reflex in lengthening the female's PER. Evidence shows that the male and female contact duration during ejaculation ("ejaculation duration") is positively correlated with the preejaculatory intromission frequency and the female's PER (Yang and Clemens, 1995b). That is, the female's PER is directly influenced by the "ejaculation duration" and indirectly regulated by the preejaculatory intromissions.

Multiple intromissions prior to ejaculation in male rats may have several functions. It has been proposed that one function of multiple intromissions is to induce the secretion of progesterone to facilitate blastocyst implantation (Beach, 1965). One group of researchers extended this hypothesis and postulated that vaginocervical stimulation

provided by the multiple intromissions induced the release of pituitary prolactin which in turn resulted in the secretion of progesterone (Wilson et al, 1965). This hypothesis was supported by numerous subsequent reports (Adler et al., 1970; Gorospe and Freeman, 1981; Hashimoto et al., 1968; Smith et al., 1975; Terkel and Sawyer, 1978). In addition, two groups of investigators proposed that another function of multiple intromissions prior to ejaculation in male rats was to facilitate the sperm transport through the cervix of the uterus (Adler, 1969; Chester and Zucker, 1970). This is supported by the finding that no or very few sperm were present in the uterus 1-3 hours after ejaculation and no developing eggs were found in the uterus 2-4 days after copulation if the male ejaculated on the first or second intromission (Adler, 1969). Females receiving 2 or more intromissions prior to ejaculation contained a large number of sperm in their uteri 1-3 hours after ejaculation and had developing eggs in their Fallopian tubes and uteri 2-4 days after copulation. Similar results were also reported by other researchers (Chester and Zucker, 1970). Further, multiple intromissions of male rats prior to ejaculation may serve to remove seminal plugs deposited by other competitors under naturalistic situations.

Another potential function of multiple intromissions in male rats may be to prepare the vaginal passage for the deep penile insertion during ejaculation. At least two consequences result from the deep penile insertion during ejaculation. The first consequence is a longer penis-vagina contact duration. It has been reported that an ejaculation duration is longer than an intromission duration (Erskine et al., 1989; Peirce and Nuttall, 1961b; Stone and Ferguson, 1940). Further, in Experiment 1B, we found that the ejaculation duration of males was shorter in the 0-1 intromission group than the other groups. This shorter ejaculation duration may reflect the possibility that initial intromissions are relatively shallow. In some cases, the male even ejaculated outside the female. A second consequence resulting from the deep penile insertion during ejaculation is a tight deposit of a seminal plug against cervix that, in turn, facilitates sperm transport from the cervix to the uterus. If the male ejaculates on the first or second intromission, the

lack of deep penile penetration may compromise the deposition of a seminal plug against the cervix with the result that very few or no sperm will be transported to the uterus. Several studies support this idea (Adler, 1969; Chester and Zucker, 1970; Matthews and Adler, 1977; Matthews and Adler, 1978). In addition to the studies that examined the relation of number of intromissions before ejaculation to the sperm transport from the cervix to the uterus (Adler, 1969; Chester and Zucker, 1970), two other studies investigated the relation between the position of a seminal plug in the vagina and the number of sperm transported to the uterus (Matthews and Adler, 1977; Matthews and Adler, 1978). The authors found that if the seminal plug sealed the cervix tightly, sperm were transported to the uterus in large numbers. Thus, while multiple intromissions are correlated with increased sperm transport, a second more proximal mechanism may be the deeper prolonged intromission that accompanies ejaculation and is itself a result of preceding intromissions.

In summary, different numbers of intromissions, preceding ejaculation, affect the female's PER. The number of intromissions received by the female preceding ejaculation is positively correlated with the female's PER if the range of intromission frequency is large enough (0-31 in this study). At least two preejaculatory intromissions are required to induce a PER comparable to the one following the first ejaculation during normal copulation. Additional intromissions received by the female prior to ejaculation may lengthen the "ejaculation duration" (the male and female contact duration during ejaculation) that in turn increases the length of the female's PER. One potential function of multiple intromissions in male rats during copulation may be to prepare the vaginal passage for the deep penile insertion that in turn results in a longer penis-vagina contact duration and a tight deposit of a seminal plug against the cervix of the uterus. This type of stimulation provided by deep penile insertion facilitates sperm transport through the cervix of the uterus and brings about the female's postejaculatory refractory period.

EXPERIMENT 2: FUNCTION OF INTROMISSIONS IN RELATION TO THE INTROMISSION RETURN LATENCY OF FEMALE RATS DURING PACED SEXUAL BEHAVIOR

ABSTRACT

YANG, L. Y. AND L. G. CLEMENS. Function of intromissions in relation to the intromission return latency of female rats during paced sexual behavior. PHYSIOL BEHAV 60(0):000-000, 1996.- The objectives of this study were to examine how multiple intromissions affect the temporal pattern of the female rat's copulatory behavior, in particular, her latency to return to the male following intromission (intromission return latency, IRL) and whether different hormone replacement regimens affect the temporal aspects of female copulatory behavior. Repeated intromissions alone, without ejaculation, often resulted in prolonged IRLs equal to the postejaculatory refractory period (PER). The first prolonged IRL occurred most frequently between the 24th and 44th intromission. The similar pattern of IRLs around the PER and the prolonged IRLs may indicate that the mechanisms mediating the occurrence of the prolonged IRL are similar to those for the PER. One possible function of the prolonged IRLs may be to facilitate the male's ejaculation after the female has received enough vaginocervical stimulation for the induction of the progestational state of pregnancy. Finally, females receiving a single dose of 50 μg estradiol benzoate (EB) followed by an injection of 0.5 mg progesterone (P) 48 hours later showed a significantly longer PER than those receiving three daily injections of 0.5 μg EB followed by an injection of 0.5 mg P 24 hours after the last EB injection.

INTRODUCTION

One function of the temporal pattern of female sexual behavior in the rat is to facilitate successful pregnancy. Following multiple intromissions, blood progesterone levels increase significantly in the female to maintain pregnancy (Adler et al., 1970). When the female is able to control the copulatory speed, the interintromission intervals are longer (Erskine, 1985; Erskine et al., 1989; Fadem and Barfield, 1982; Gilman et al., 1979) and fewer intromissions are sufficient to induce the progestational state of pregnancy (Erskine et al., 1989; Gilman et al., 1979). Following ejaculation, the female rat, if given the opportunity, stays away from the male for a longer period of time than she does after an intromission without ejaculation (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Fadem et al., 1979; Gilman and Hitt, 1978; Krieger et al., 1976; Peirce and Nuttall, 1961; Yang and Clemens, 1994, 1996a). This period of female sexual inactivity following ejaculation is referred to as the female's postejaculatory refractory period (PER), and its function is related to sperm transport: if the female receives an intromission within 2 minutes following ejaculation, sperm transport will be halted until the next ejaculation (Matthews and Adler, 1977).

In previous studies, we found that multiple intromissions preceding ejaculation affected the male's "ejaculation duration" (the duration of male-female contact during ejaculation) which in turn influenced the length of the female's postejaculatory refractory period (Yang and Clemens, 1995b, 1996a, 1996b). While two preejaculatory intromissions were sufficient for a normal PER to occur, additional intromissions lengthened the "ejaculation duration" that, in turn, was associated with an increase in the female's PER (Yang and Clemens, 1995b, 1996a, 1996b).

In the present study, we extended this analysis of intromission effects to determine how multiple intromissions affect the pattern of the female's individual IRLs over the course of copulation. In female paced situations, it has been observed that if a long IRL

occurs during the course of the third ejaculation, the male often ejaculates on the next intromission following this longer IRL (Personal observation). Moreover, experimentally enforced long interintromission intervals have been demonstrated to significantly reduce the number of intromissions prior to the first ejaculation in male rats (Bermant, 1964; Larsson, 1959). We suggest that, in addition to contributing to the induction of the progestational state of pregnancy, the female's timing of intromissions affects the probability of the male's ejaculation.

The temporal pattern of female sexual behavior is also affected by gonadal hormones (Fadem et al., 1979; Gilman and Hitt, 1978). Two hormone replacement regimens [0.5 μg estradiol benzoate (EB) x 3 + 0.5 mg progesterone (P) 24 hrs after the last EB injection and 50 or 100 μg EB + 0.5 or 1 mg P 48 or 72 hrs after EB injection] are often used to study the temporal pattern of female sexual behavior (Bermant, 1961; Bermant and Westbrook, 1966; Clemens et al., 1995; Krieger et al., 1976; Yang and Clemens, 1994, 1995a, 1995b, 1996a, 1996b). The general patterning of female sexual behavior was similar in these reports using these two hormone replacement protocols. However, the return latencies reported by us (Yang and Clemens, 1996a) were routinely shorter than those reported by others (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). For example, in our study the female's return latency following an ejaculation (Yang and Clemens, 1996a) was 47 seconds, which is significantly shorter than 90 seconds to several minutes reported by others (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). Whether this reflects the difference in hormone replacement regimens, or different test chambers is not clear.

In Experiment 2A, we examined how multiple intromissions affect the temporal pattern of the female rat's copulatory behavior, in particular, her latency to return to the male following intromission. Experiment 2B investigated whether different hormone replacement regimens affect the temporal aspects of female copulatory behavior. Part of this work has been presented in abstract form (Yang and Clemens, 1994).

GENERAL METHODS

Animals

Sixty-day-old female and ninety-day-old male Long Evans rats were purchased from Charles River Laboratories (Wilmington, MA). All females were ovariectomized one week after arrival in the laboratory. Animals were housed in 16:8 hr light-dark cycle with lights off at 17:00-1:00. In general, females were housed three per cage and males were housed singly. Food and water were available ad lib. Temperature and humidity of the animal room were maintained at 22^0 C and 55%, respectively. The air in the animal room was exchanged 12 times per hour automatically. Ovariectomized females were brought into estrus by intramuscular (IM) injections of 0.5 μg estradiol benzoate (EB) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil (Sigma Chemical Co., St. Louis, MO) for three consecutive days and 24 hours after the last EB injection followed by an IM injection of 0.5 mg progesterone (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil unless otherwise specified. All female pacing behavior was tested 4-7 hours after the injection of progesterone (2-5 hours after the lights off) under dimly red illumination. All behavioral tests were ended after the female returned to the male's chamber following her PER. Alternatively, the behavioral tests were terminated if the female did not leave the male's chamber within 60 seconds following ejaculation. All animals were tested once per week and had been tested twice in the testing chamber before data were collected. Males were always placed in the testing chamber 15 minutes before females were introduced (the test started). The approximate weights of the female and male rats were 200 - 300 g and 400 - 500 g, respectively, when the tests started.

Pacing Test Chamber

To provide a test situation in which the female could control the pacing of the copulation, females were tested in a two-compartment chamber that was divided by a Plexiglas barrier with 4 holes (3.5~4 cm x 3.5~4 cm) spaced along the bottom of the barrier. The dimensions of the male's chamber and escape chamber were 35.5 cm x 44.5 cm x 48.3 cm and 21.5 cm x 44.5 cm x 48.3 cm (Length x Width x Height), respectively. The escape holes were large enough to allow the female to pass through but prevented the larger male from following her.

Behavioral Measures

Several behavioral measures were recorded in the pacing tests unless otherwise specified: approach latency (AL), the latency for the female to enter the male's chamber after the start of the test (she was placed in the escape chamber at the beginning of the test); intromission latency (IL), the latency from the female's entry into the male's chamber to the first intromission; mount return latency (MRL), the latency for the female to re-enter the male's chamber after a mount; intromission return latency (IRL), the latency to return to the male's chamber following an intromission; postejaculatory refractory period (PER), the latency to re-enter the male's chamber after an ejaculation. All latencies were measured in seconds. The frequency with which the female left the male's chamber following a mount, intromission, or ejaculation (percentage exit) was calculated by dividing the number of exits by the number of each type of stimulus. All these behavioral data were recorded on a computer disk.

EXPERIMENT 2A: RELATION OF MULTIPLE INTROMISSIONS TO THE FEMALE'S INDIVIDUAL INTROMISSION RETURN LATENCY

In previous studies (Yang and Clemens, 1995b, 1996a, 1996b), we demonstrated that intromissions prior to ejaculation indirectly increased the female's PER by increasing the male's "ejaculation duration" (the duration of male-female contact during ejaculation). In this experiment, we extended our testing to determine (1) how multiple intromissions preceding ejaculation affect the pattern of the female's individual IRLs over three ejaculatory series, (2) how the female paces her sexual contacts with the male while receiving a large number of intromissions prior to ejaculation, and (3) whether repeated intromissions alone, without ejaculation, can induce a prolonged intromission return latency (prolonged IRL) that is comparable to the PER in length.

METHOD

Procedures

Females were given 2 or 3 tests at weekly intervals. Eleven males and eleven females plus 30 extra stud males (stud males used to deliver intromissions without ejaculation during the second and third tests) were used in this study. All females were tested once per week and had 2 complete pretests before data were collected.

A prolonged female's return latency following an intromission (prolonged intromission return latency, prolonged IRL) was defined as a return latency following an intromission that was equal to or larger than that female's PER following the first ejaculation during normal copulation. Alternatively, a return latency following an intromission equal to or larger than 47 seconds (mean PER of 67 females following the

first ejaculation during normal copulation under equivalent test conditions, Yang and Clemens, 1996a) was also regarded as a prolonged IRL only when the female's PER following the first ejaculation was larger than 60 seconds and no IRL was equal to or larger than the female's first PER.

To obtain baseline behavioral measures of normal female pacing behavior, each of eleven females was randomly assigned to copulate with one of 11 males for three ejaculatory series in the first week of this experiment. Because each female received an ejaculation from the same male she mated in the first week throughout the entire test duration, we referred to "each female with the male providing ejaculation during copulation" as "female and male partners". In a second test, one week later, after each female received 40 intromissions provided by 6 or 7 stud males, she was allowed to copulate with her "male partner" until she received an ejaculation and returned to the male's chamber following her PER. Females that did not show a prolonged IRL in the second test were tested in the third week. After each of these females received 70 intromissions provided by 12 to 15 stud males, she was allowed to mate with her "male partner" until she received an ejaculation and returned to the male's chamber following her PER. Each individual IRL instead of mean IRL of each female was recorded and used for analysis. In addition, the number of intromissions and the latency for the first prolonged IRL to occur during the second and third tests were recorded.

Statistics

Comparison of individual IRLs and PERs over three ejaculatory series during normal copulation was made by one way ANOVA analysis followed by Fisher's PLSD post-hoc tests. Comparison of individual IRLs and PER with the first prolonged IRL during the second and third tests was made by one-way ANOVA analysis followed by Fisher's PLSD post-hoc tests. An alpha level of 0.05 was used for all statistical tests.

Ideally, we would have used one way Repeated Measures ANOVA to analyze the individual IRLs and PERs. However, the fact that the female only left the male's chamber about 70% of times following intromission (Yang and Clemens, 1996a) resulted in a large number of missing values that made the use of one way Repeated Measures ANOVA impossible.

RESULTS

Figure 6 shows the general pattern of IRLs over three ejaculatory series obtained from 11 females in the first week. The number of intromissions required to achieve ejaculations varies with each animal. To effectively present these individual IRLs, they were divided into four sets of continuous IRLs and aligned by the first intromission and then by each of the three ejaculations. These four sets of continuous IRLs include: (1) the first four IRLs (1, 2, 3, 4), (2) the four IRLs right before and three IRLs right after the first ejaculation (L-3, L-2, L-1, L, E1, 1, 2, 3), (3) the three IRLs right before and two IRLs right after the second ejaculation (L-2, L-1, L, E2, 1, 2), and (4) the three IRLs right before the third ejaculation (L-2, L-1, L, E3) (Figure 1). L stands for the intromission right before ejaculation. E1, E2, and E3 represent the first, second, and third ejaculation, respectively. There was a significant difference among these IRLs and PERs [$F(21, 148) = 11.468$, $p<0.0001$]. The female's PER was significantly longer than any IRL in the same ejaculatory series (** $p<0.01$). All of these IRLs were significantly shorter than any of these three PERs ($p<0.05$). The female's PER increased significantly over three ejaculatory series (#$p<0.05$). Although there was a slight increase in the female's individual IRLs over three ejaculatory series, this increase was not statistically significant. Data are mean of individual IRL or PER $\pm$ SEM and analyzed by one-way ANOVA analysis followed by Fisher's PLSD post-hoc tests.

Ten of 11 females tested in the second and third experimental tests showed at least one prolonged IRL. Six of these 10 females had multiple prolonged IRLs. In the second test, eight of 11 females showed at least one prolonged IRL. The three females that did not show a prolonged IRL were tested in the third test. Eventually, two of them showed a prolonged IRL. Seven of 10 prolonged IRLs (the first prolonged IRL) were defined under the criterion: equal to or larger than that female's PER following the first ejaculation during normal copulation. The remaining three prolonged IRLs (the first prolonged IRL) were defined under the criterion: equal to or greater than 47 seconds.

The first prolonged IRL occurred most frequently between the 24th and 44th intromission (7 out of 10 females). Most of the first prolonged IRLs were achieved between 12-16 min. after the first intromission (7 out of 10 females). Figure 7 shows the female's IRLs at the beginning of the test, around the prolonged IRLs and at the end of the test in which the female received a large number of intromissions prior to ejaculation, and showed at least one prolonged IRL. The number of intromissions to achieve the first prolonged IRL varies with each animal. To effectively present these individual IRLs, the data points in Figure 7 were aligned by the first IRL, by the first prolonged IRL (N: the intromission inducing the first prolonged IRL), and by ejaculation (E). Figure 7 includes three sets of continuous IRLs: (1) the first three IRLs (1, 2, 3), (2) two IRLs right before and two IRLs right after the first prolonged IRL (N-2, N-1, N, N+1, N+2), and (3) three IRLs right before ejaculation (L-2, L-1, L, E). N, L, and E represent the intromission inducing the first prolonged IRL, the intromission right before ejaculation, and the ejaculation, respectively. Although the IRL increased slightly as the number of intromissions increased, this was not a statistically significant change. The first prolonged IRL was significantly longer than the first three IRLs as well as the IRLs just before and after it but significantly shorter than the PER (** $p<0.01$, * $p<0.05$ compared with the first prolonged IRL). Data are mean individual IRL or PER $\pm$ SEM and analyzed by one-way

Figure 6. Four representative portions of individual intromission return latencies (IRLs) and three PERs of females receiving three ejaculations are illustrated: (i) the first 4 IRLs (1, 2, 3, 4), (ii) 4 IRLs right before and 3 IRLs right after the 1st ejaculation (L-3, L-2, L-1, L, E1, 1, 2, 3), (iii) 3 IRLs right before and 2 IRLs right after the 2nd ejaculation (L-2, L-1, L, E2, 1, 2), and (iv) 3 IRLs right before the 3rd ejaculation (L-2, L-1, L, E3). Data are mean $\pm$ SEM and analyzed by one-way ANOVA followed by Fisher's PLSD post-hoc tests. L: the intromission right before ejaculation. E1, E2, and E3 stand for the 1st, 2nd and 3rd ejaculation, respectively. ** $p<0.01$, each individual IRL compared with PER in the same ejaculatory series; #$p<0.05$, comparison between adjacent PERs. The number of animals for each intromission or ejaculation is indicated in the same sequential order as in Figure 6: 5, 9, 7, 7, 8, 8, 7, 9, 11 (E1), 6, 5, 5, 7, 9, 10, 11 (E2), 2, 5, 8, 9, 11, 11 (E3). // indicates separation of 4 sets of continuous IRLs.

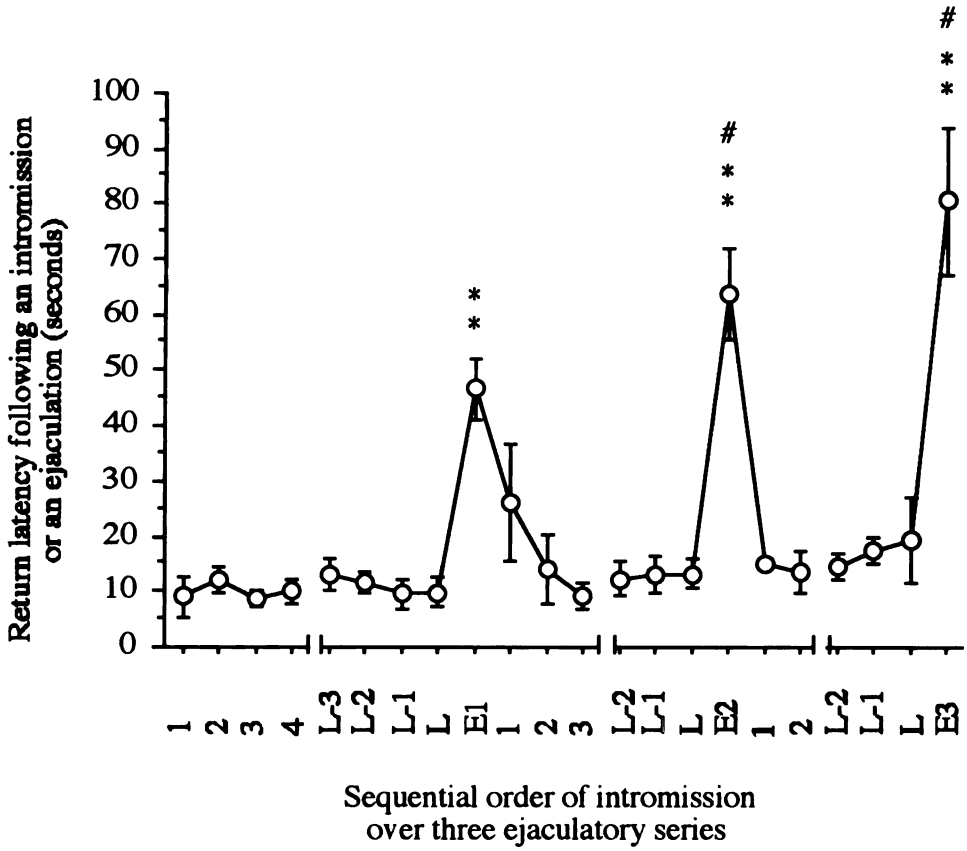

Return latency following an intromission or an ejaculation (seconds)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 L-3 L-2 L-1 L E1 1 2 3 L-2 L-1 L E2 1 2 L-2 L-1 L E3
Sequential order of intromission over three ejaculatory series

Figure 6. Four representative portions of individual intromission return latencies (IRLs) and three PERs of females receiving three ejaculations are illustrated: (i) the first 4 IRLs (1, 2, 3, 4), (ii) 4 IRLs right before and 3 IRLs right after the 1st ejaculation (L-3, L-2, L-1, L, E1, 1, 2, 3), (iii) 3 IRLs right before and 2 IRLs right after the 2nd ejaculation (L-2, L-1, L, E2, 1, 2), and (iv) 3 IRLs right before the 3rd ejaculation (L-2, L-1, L, E3). Data are mean $\pm$ SEM and analyzed by one-way ANOVA followed by Fisher's PLSD post-hoc tests. L: the intromission right before ejaculation. E1, E2, and E3 stand for the 1st, 2nd and 3rd ejaculation, respectively. ** $p<0.01$, each individual IRL compared with PER in the same ejaculatory series; #$p<0.05$, comparison between adjacent PERs. The number of animals for each intromission or ejaculation is indicated in the same sequential order as in Figure 6: 5, 9, 7, 7, 8, 8, 7, 9, 11 (E1), 6, 5, 5, 7, 9, 10, 11 (E2), 2, 5, 8, 9, 11, 11 (E3). // indicates separation of 4 sets of continuous IRLs.

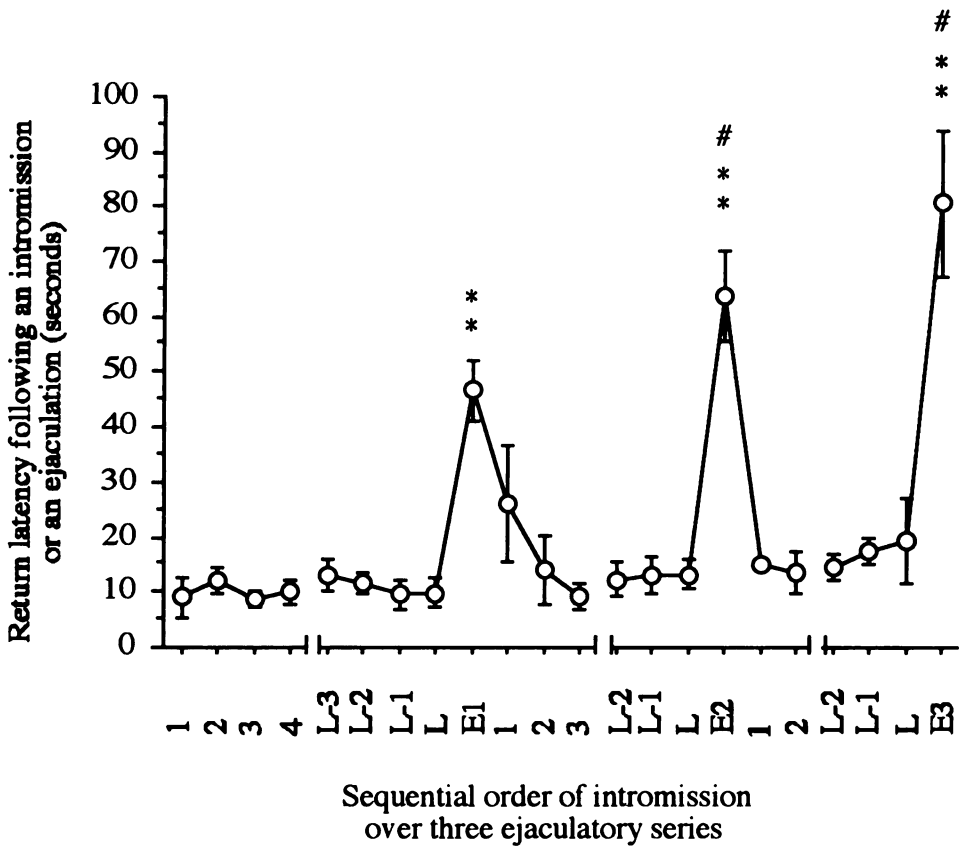
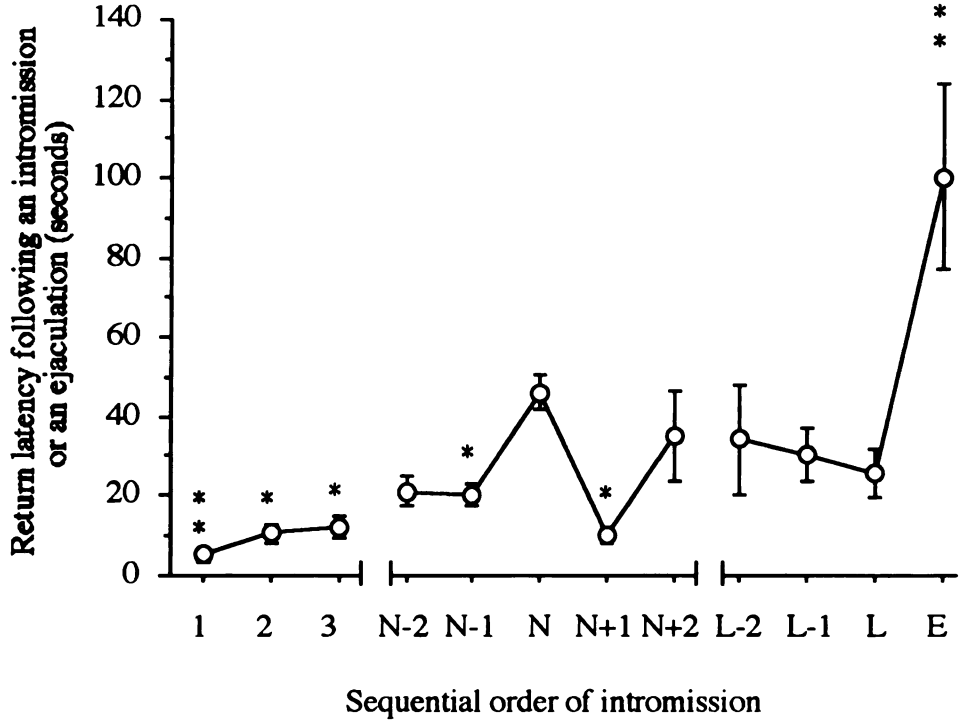

Return latency following an intromission or an ejaculation (seconds)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 L3 L2 L1 L E1 1 2 3 L2 L1 L E2 1 2 L2 L1 L E3
Sequential order of intromission over three ejaculatory series

Figure 7. Three representative portions of individual intromission return latencies (IRLs) of females receiving a large number of intromissions before ejaculation. These include 3 portions of consecutive IRLs: (i) the first 3 IRLs (1, 2, 3), (ii) 2 IRLs right before and 2 IRLs right after the first prolonged IRL (N-2, N-1, N, N+1, N+2), and (iii) 3 IRLs right before ejaculation (L-2, L-1, L, E). Data are mean $\pm$ SEM and analyzed by one-way ANOVA followed by Fisher's PLSD post-hoc tests. N: the intromission inducing the first prolonged IRL. L: the intromission right before ejaculation. E: ejaculation. ** $p<0.01$, * $p<0.05$ compared with the first prolonged IRL. The number of animals for each intromission or ejaculation is indicated in the same sequential order as in Figure 7: 6, 6, 8, 8, 8, 10 (N), 6, 6, 6, 7, 10, 9 (E). // indicates separation of 3 sets of continuous IRLs.


Return latency following an intromission or an ejaculation (seconds)
140
120
100
80
60
40
20
0
1
2
3
N-2
N-1
N
N+1
N+2
L-2
L-1
L
E
Sequential order of intromission

ANOVA analysis [$F(11, 78) = 7.338$, $p<0.0001$] followed by Fisher's PLSD post-hoc test.

EXPERIMENT 2B: INFLUENCE OF DIFFERENT HORMONE REPLACEMENTS ON THE FEMALE PACING BEHAVIOR

The objective of this experiment was to investigate whether different hormone replacement regimens affect the temporal aspects of female sexual behavior tested under the same test paradigm. The return latencies following a mount, intromission and ejaculation reported by us (Yang and Clemens, 1996a) were shorter than those reported by the other investigators (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). For example, the PER following the first ejaculation reported in our previous study (Yang and Clemens, 1996a) was 47 seconds, which is much shorter than 90 seconds to several minutes reported by others (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). There are some differences between our pacing behavior tests (Yang and Clemens, 1996a) and those of others (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976) which may account for these different results. One difference lies in the design of the pacing test chamber. In our previous study (Yang and Clemens, 1996a), the female can escape from the male easily from four escape holes along the bottom of the barrier separating the escape chamber from the male's chamber. In other studies (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976), females are trained to press a lever to gain access to the male (Bermant, 1961; Bermant and Westbrook, 1966) or the male is tethered to half of the test arena (Krieger et al., 1976). Another difference is the hormone replacement regimens. The hormone replacements used in the above studies (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976) were quite similar to one another. The females were brought into estrus by an injection of a high dose (50 or 100 μg) of EB followed by an injection of 0.5 or 1 mg P 48 or 72 hours after the EB injection.

However, in our study (Yang and Clemens, 1996a), females were primed with 0.5 μg EB for three consecutive days followed by an injection of 0.5 mg P 24 hours after the last EB injection. In the present experiment, we compared the effect of the hormone replacement regimen we have used (Clemens et al., 1995; Lange and Clemens, 1994; Yang and Clemens, 1994, 1995a, 1995b, 1996a, 1996b) with the one used by Krieger et al. (Krieger et al., 1976) on the temporal pattern of female sexual behavior.

METHOD

Procedures

Females were tested for female pacing behavior over a two-week test period in which two different hormone replacement regimens were used to prime the females for behavioral testing. Fourteen male and fourteen female Long Evans rats were used in this experiment. Each of 14 females was randomly assigned to copulate with one of 14 males in the first week of this experiment. Each female received an ejaculation from the same male she mated in the first week throughout the entire test duration. In the first week, the females were injected with 0.5 μg EB for 3 consecutive days followed by an injection of 0.5 mg P 24 hours after the last EB injection (0.5 μg EB x 3 + 0.5 mg P). The females were tested for female pacing behavior 4-7 hours after the injection of P. In the second week, the females were brought into estrus by a single injection of 50 μg EB followed by an injection of 0.5 mg P 48 hours after the EB injection (50 μg EB + 0.5 mg P). The females were tested for female pacing behavior 4-7 hours after the injection of P. Hormone treatments were not counterbalanced because the data obtained in our laboratory showed that repeated testing did not affect female pacing behavior when females were primed with 0.5 μg EB x 3 + 0.5 mg P.

Statistics

All behavioral data (AL, IL, MRL, IRL, PER and percentage exits following sexual contacts) obtained from two different hormone treatments were analyzed by paired t-test. An alpha level of 0.05 was used for all statistical tests.

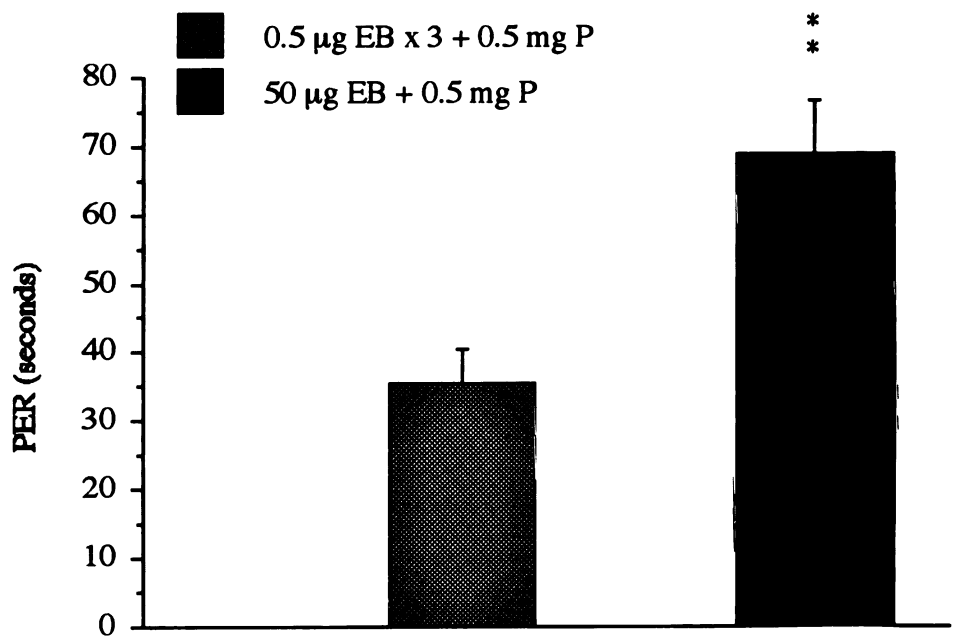
RESULTS

Figure 8 compares the PERs obtained from the females treated with two different hormone replacement regimens. The females receiving 50 μg EB + 0.5 mg P had a significantly longer PER than those receiving 0.5 μg EB x 3 + 0.5 mg P ($t=-4.448$, $p=0.0007$). No significant difference was found between these two groups in any of the other behavioral measures (AL, IL, MRL, IRL, and percentage exit following a mount, intromission, and ejaculation) recorded in this experiment.

DISCUSSION

In this study, we demonstrate that intromissions alone, without ejaculation, can induce the female to initiate prolonged latencies between intromissions. Females receiving a large number of intromissions prior to ejaculation showed one or more IRLs equal to or exceeding her first PER or the average PER of 47 seconds (Yang and Clemens, 1996a). Six of these 10 females had multiple prolonged IRLs. The first prolonged IRL occurred most frequently between the 24th and 44th intromission (7 out of 10 females) and 12 to 16 minutes after the first intromission (7 out of 10 females). The occurrence of prolonged IRL raises several interesting questions. First, what mechanisms bring about the prolonged IRL ? Secondly, what function do the prolonged IRLs play?

Figure 8. Comparison of the PER of females receiving two different hormone treatments. In the first week, the females received injections of 0.5 μg EB for three consecutive days 24 hours later followed by an injection of 0.5 mg progesterone. In the second week, the females were given an injection of 50 μg EB 48 hours later followed by an injection of 0.5 mg progesterone. Bar represents mean ± SEM. Data were analyzed by paired t test. ** P = 0.0007. N = 14.


0.5 µg EB x 3 + 0.5 mg P
50 µg EB + 0.5 mg P
**
PER (seconds)
80
70
60
50
40
30
20
10
0

The mechanisms that influence the occurrence of the prolonged IRL may be similar to those controlling the PER. The pattern of IRLs around the PER and the prolonged IRL in Figure 6 and Figure 7 strongly supports the idea that similar mechanisms may exist for the occurrence of the PER and prolonged IRL. In Figure 6, the IRLs were aligned by the first intromission and then by each of the three ejaculations. The female's return latency is normally short following intromission before ejaculation and then increases significantly immediately after ejaculation as a result of the long intromission duration that accompanies ejaculation (The ejaculatory intromission duration is significantly longer than the non-ejaculatory intromission duration) (Erskine et al., 1989). Following the postejaculatory refractory period, the female's return latency following intromission becomes short again. In Figure 7, the IRLs were aligned by the first three IRLs, by the first prolonged IRL (N: the intromission inducing the first prolonged IRL), and by ejaculation (E). The pattern of IRLs around the first prolonged IRL is similar to that around the PER seen in Figure 6. The first three IRLs in the test are short. Most of the first prolonged IRL occurs after 24 to 44 intromissions. Following the first prolonged IRL (the N IRL), the N+1 IRL is short as occurs after the PER. However, the lack of difference between the first prolonged IRL and the N+2 IRL as well as subsequent IRLs is puzzling. Analysis reveals that this is due largely to the occurrence of multiple prolonged IRLs by some females (6 of 10). The PER is associated with an increase in intromission duration during ejaculation. Possibly the prolonged IRL is also associated with a prolonged intromission duration.

One possible function of the female's prolonged IRLs may be to induce the male to ejaculate after the female has received enough vaginocervical stimulation for the induction of the progestational state of pregnancy. This is supported by several lines of evidence. First, prolonged IRLs only occur following a large number of intromissions that exceeds the number of intromissions necessary for the induction of the progestational state of pregnancy (approximately 5 in female-paced situation and 10 in non-paced situation) (Adler, 1969; Chester and Zucker, 1970; Edmonds et al., 1972; Erskine, 1989; Erskine et

al., 1989; Gilman et al., 1979). Second, experimentally enforced long interintromission intervals (1 to 5 minutes) have been reported to facilitate ejaculation by reducing the number of intromissions prior to the first ejaculation (Bermant, 1964; Larsson, 1959). The mean of the first prolonged IRL seen in the present study was about 50 seconds (Figure 7) which would be sufficient to facilitate the male's ejaculation under normal test conditions.

In Experiment 2B, different hormone replacement regimens were found to affect the female's PER but not her AL, IL, MRL, IRL and the frequency with which the female left the male's chamber after each sexual contact. Females receiving 50 μg EB + 0.5 mg P had a significantly longer PER than those receiving 0.5 μg EB x 3 + 0.5 mg P (Figure 8). The mean values of PER, IRL and MRL reported in our previous study (Yang and Clemens, 1996a) were shorter than those reported by other researchers (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976). For example, the mean PER and IRL in the first ejaculatory series reported in our previous study (Yang and Clemens, 1996a) were 47 seconds and 12 seconds, respectively. However, the mean PER and IRL in the first ejaculatory series reported in other studies (Bermant, 1961; Bermant and Westbrook, 1966; Krieger et al., 1976) were 90 seconds to several minutes and 40 seconds to over 1 minute, respectively. We suggest that the quantitative differences among the behavioral measures of female pacing behavior reported by different groups of researchers may reflect differences among the test paradigms as well as the hormone replacement regimens.

Increases in P levels but not in EB levels have been demonstrated to reduce the female's return latencies following intromissions and ejaculations (Fadem et al., 1979; Gilman and Hitt, 1978). The present finding that the 50 μg EB + 0.5 mg P treatment induced a significantly longer PER in the female than the 0.5 μg EB x 3 + 0.5 mg P treatment is not consistent with this earlier finding that increases in EB do not affect the female's return latency following an ejaculation (Gilman and Hitt, 1978). Differences in the time of hormone injection and in the dosages used may account for this discrepancy. First, the time for EB injection was different for the 50 μg EB + 0.5 mg P treatment and

the 0.5 μg EB x 3 + 0.5 mg P treatment in the present study. The 50 μg EB was injected 48 hours before P administration, whereas 0.5 μg EB was injected 72, 48, and 24 hours before P was injected. In contrast, the time for EB injection was the same for different dosages of EB in the earlier study: EB was always injected 44 hours prior to P injection (Gilman and Hitt, 1978). Secondly, in the present study, each female received an injection of 0.5 mg P, a dose that was not examined in the earlier study (Gilman and Hitt, 1978). Further, the dosage (50 μg EB per animal) used in the present study is double the highest dosage (90 μg/kg EB, approximately 22.5 μg EB per animal at a body weight of 250 g) used in the earlier study (Gilman and Hitt, 1978). Finally, the large difference in the EB dosages (0.5 μg EB x 3 and 50 μg EB) in the present study may also help to explain the difference in the PER of females treated with these two hormone regimens.

In the present study, the temporal aspects of female rat's copulatory behavior were found to vary in relation to hormone replacement regimens and intromission frequency. Females receiving a high dose of EB plus P show a significantly longer PER than those receiving three low doses of EB plus P. During copulatory bouts without ejaculation, it was found that after a large number of intromissions, females began to enforce prolonged intervals between intromissions. It is suggested that these prolonged intervals may function to facilitate ejaculation.

EXPERIMENT 3: INFLUENCE OF MALE RELATED STIMULI ON FEMALE POSTEJACULATORY REFRACTORY PERIOD IN RATS

ABSTRACT

YANG, L. Y. AND L. G. CLEMENS. Influence of male related stimuli on female postejaculatory refractory period in rats. Physiol. Behav. 60(0) 000-000, 1996.-When tested in a situation where the female rat can escape from the male during copulation, she "paces" her sexual contacts with the male. This pacing behavior is influenced by the type and quality of vaginocervical stimulation the female receives. One possible function of the female's return latency following an ejaculation (postejaculatory refractory period, PER) is to facilitate or allow sperm transport from the vagina to the uterus. The objective of this study was to investigate the effect of several male related stimuli on the female's PER. Females were tested in a two-compartment test chamber where they could escape from the male through one of the four openings along the bottom of the Plexiglas barrier. Experiment 3A examined the influence of the seminal plug, the penile cup and prostate secretions on the female's PER. Results showed that neither the seminal plug, the penile cup nor prostate secretions contributed to the female's PER. In addition, male sexual performance was not affected by any of the surgical treatments performed in this experiment. Experiment 3B investigated the relation among the male's ejaculation duration, preejaculatory intromission frequency, the number of pelvic thrusts during ejaculation, and the female's PER. Results indicated that both the female's PER and the male's ejaculation duration were found to be significantly shorter in tests where the male ejaculated on the first or second intromission. Moreover, the male's ejaculation duration, the preejaculatory

intromission frequency and the female's PER were significantly correlated with one another. Partial correlation analysis further suggests that preejaculatory intromissions affect the ejaculation duration which in turn influences the female's PER.

INTRODUCTION

In test situations where the female rat can escape from the male during copulation, she regulates the temporal pattern of her sexual contacts with the male (Bermant, 1961; Bermant and Westbrook, 1966; Clemens et al., 1995; Erskine, 1985, 1987, 1989, 1992; Erskine and Baum, 1982; Erskine et al., 1989; Fadem et al., 1979; Frye and Erskine, 1990; Gilman and Hitt, 1978; Krieger et al., 1976; McClintock and Adler, 1978; McClintock and Anisko, 1982; McClintock et al., 1982; Mermelstein and Becker, 1995; Peirce and Nuttall, 1961a; Yang and Clemens, 1994, 1995a, 1995b, 1996a, 1996c). The general pattern of this "female pacing behavior" shows that the female's return latency following an ejaculation, the postejaculatory refractory period (PER), is significantly longer than her return latency following an intromission (IRL) or a mount (MRL) (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985; Fadem et al., 1979; Krieger et al., 1976; Peirce and Nuttall, 1961a; Yang and Clemens, 1994, 1996a). In addition, the female's PER increases over consecutive ejaculatory series (Bermant and Westbrook, 1966; Krieger et al., 1976; Yang and Clemens, 1996a). One possible function of the female's PER is to facilitate or allow sperm transport from the vagina to the uterus. If the female receives intromissions shortly following ejaculation, sperm transport will be halted until the next ejaculation (Matthews and Adler, 1977). Since the female's PER is crucial for sperm transport from the vagina to the uterus, it is important to understand the mechanisms underlying the female's PER.

Paced coital stimulation is more effective in the induction of the progestational state of pregnancy in female rats than the same amount of non-paced coital stimulation. A high

number of intromissions (approximately 10 in non-paced situation) provided by the male during nonpaced copulation tests induces the progestational changes necessary for ovum implantation in the female rats (Adler, 1969; Chester and Zucker, 1970; Edmonds et al., 1972; Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). It has also been reported that the blood progesterone level of the female rat increases significantly after mating if the female receives a high number of intromissions (9 to 23) during copulation (Adler et al., 1970). In contrast, the females receiving two or fewer intromissions prior to ejaculation in nonpaced tests have a significantly lower percentage of pregnancy (Adler, 1969; Wilson et al., 1965). In addition, 5 intromissions are insufficient to induce pseudopregnancy in non-paced female rats (Chester and Zucker, 1970; Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). However, in female paced tests, five intromissions are adequate to trigger the progestational state of pregnancy in the female rats (Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). Female pacing of sexual behavior (Erskine, 1985; Erskine and Baum, 1982; Erskine et al., 1989) is more effective in shortening behavioral estrus than the same amount of non-paced coital stimulation (Blandau et al., 1941; Hardy and DeBold, 1972; Lodder and Zeilmaker, 1976). In female paced situations, ten intromissions are sufficient to shorten behavioral estrus (Erskine, 1985; Erskine and Baum, 1982; Erskine et al., 1989). In contrast, at least 25 intromissions are necessary for shortening of behavioral estrus in non-paced situations (Erskine and Baum, 1982).

Vaginocervical stimulation plays an important role in the regulation of pacing behavior in female rats. Following bilateral transection of the pelvic nerve and the pudendal nerve that innervate the vagina, cervix, uterus, perineal skin, inner thigh and clitoral sheath (Berkley et al., 1990; Berkley et al., 1993; Peters et al., 1987), females do not distinguish behaviorally among copulatory events; the return latencies following mount, intromission and ejaculation are not significantly different from one another (Erskine, 1992). Furthermore, anesthetics applied locally to the vagina reduce contact-response intervals following intromission and ejaculation (Bermant and Westbrook, 1966).

The female's PER is affected by the number of intromissions received prior to ejaculation (Yang and Clemens, 1994, 1996a). Females receiving 0-1 intromission before ejaculation have significantly shorter PERs that are not different from her IRLs. The PERs of females receiving 2-4 intromissions preceding ejaculation do not differ from those receiving 5-15 (average 10) intromissions. Females receiving 24-31 intromissions prior to ejaculation have significantly longer PERs than those receiving 5-15 (average 10) intromissions (Yang and Clemens, 1994, 1996a).

Although it has been demonstrated that at least two preejaculatory intromissions are necessary for the occurrence of a normal PER following the first ejaculation (Yang and Clemens, 1994, 1996a), the mechanisms underlying the female's PER are not well understood. However, since the female's pacing is heavily dependent upon vaginocervical stimulation, it seems likely that some form of sensory stimulation at the time of ejaculation induces the PER. Several male related stimuli might play a role in the induction of the PER. First, the deposit of a seminal plug in the vagina during ejaculation may contribute to the longer return latency following ejaculation. In rats, the ejaculate of the male coagulates and forms a seminal plug after ejaculation (Brooks, 1990; Luke and Coffey, 1994; Mann, 1981; Price and Williams-Ashman, 1961; Setchell et al., 1994; Wagner and Kistler, 1987; Walker, 1910a, 1910b; Williams-Ashman et al., 1977; Zorgniotti and Brendler, 1958). Possibly the seminal plug provides mechanical stimulation that contributes to the female's PER. Secondly, the formation of the penile cup during ejaculation may cause mechanical stimulation resulting in a longer return latency following ejaculation. In addition, chemical substances released from the prostate may stimulate the vagina, cervix as well as uterus and consequently cause vaginal and/or uterine responses that alter the female's return latency following ejaculation. Finally, the ejaculation duration may contribute to the female's PER. This is supported by the following evidence. It has been reported that the ejaculation duration (Peirce and Nuttall, 1961b) and the female's PER (Bermant and Westbrook, 1966; Krieger et al., 1976; Yang and Clemens, 1996a) increase over the course of copulation. In

addition, it was noticed that the ejaculation duration was shorter when the female received 0-1 intromission before ejaculation (Unpublished observation).

The objective of Experiment 3A was to examine the effect of the seminal plug, penile cup and prostate secretions on the female's PER. Experiment 3B investigated the relation among the male's ejaculation duration, the number of intromissions received prior to ejaculation, the number of pelvic thrusts during ejaculation, and the female's PER.

GENERAL METHODS

Animals

Sixty-day-old female and ninety-day-old male Long Evans rats were purchased from Charles River Laboratories (Wilmington, MA). All females were ovariectomized one week after arrival. Animals were housed in 16:8 hr light-dark cycle with lights off at 17:00. In general, females were housed three per cage and males were housed singly. Food and water were available ad lib. The temperature and humidity of the animal room were maintained at 22^0 C and 55%, respectively. The air in the animal room was exchanged 12 times per hour automatically. Ovariectomized females were brought into estrus by intramuscular (IM) injections of 0.5 μg estradiol benzoate (EB) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil (Sigma Chemical Co., St. Louis, MO) for three consecutive days and 24 hours later followed by an IM injection of 0.5 mg progesterone (P) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil. Female pacing tests were conducted 4-7 hours after the injection of progesterone (2-5 hours after the lights off) under dimly red illumination. Animals (males and females) were tested once per week throughout the whole test period and each female had been tested twice in the testing chamber before data were collected. Those females that did not exit the male's chamber at all following sexual contacts were excluded from the following studies. Only

those females that showed pacing behavior (over 95% of females) were used in the following studies. In each test, a male was placed in the testing chamber 15 minutes before a female was introduced. The approximate weights of the female and male rats were 200 - 300 g and 400 - 500 g, respectively, when testing began.

Pacing Tests

To provide a test situation in which the female could control the pacing of copulation, females were tested in a two-compartment chamber that was divided by a Plexiglas barrier with 4 holes (3.5~4 cm x 3.5~4 cm) spaced along the bottom of the barrier. The dimensions of the male's chamber and escape chamber were 35.5 cm x 44.5 cm x 48.3 cm and 21.5 cm x 44.5 cm x 48.3 cm (Length x Width x Height), respectively. The escape holes were large enough to allow the female to pass through but prevented the larger male from following her.

Behavioral Measures

Several behavioral measures were recorded in the pacing tests unless otherwise specified: approach latency (AL), the latency for the female to enter the male's chamber after the test started (she was placed in the escape chamber at the beginning of the test); mount latency (ML), the latency from the female's entry into the male's chamber to the first mount; intromission latency (IL), the latency from the female's entry into the male's chamber to the first intromission; mount return latency (MRL), the latency for the female to re-enter the male's chamber after a mount; intromission return latency (IRL), the latency to return to the male's chamber following an intromission; postejaculatory refractory period (PER), the latency to re-enter the male's chamber after an ejaculation. The frequency (percentage exit) with which the female left the male's chamber following mount,

intromission and ejaculation was calculated by dividing the number of exits by the number of each type of stimulus. All these behavioral data were recorded on a computer disk.

EXPERIMENT 3A: EFFECT OF STIMULUS FACTORS ON FEMALE POSTEJACULATORY REFRACTORY PERIOD

The objective of this experiment was to examine whether the deposit of a seminal plug, the formation of a penile cup, or the presence of prostate secretions during ejaculation influences the female's PER. In rats, the ejaculate of the male forms a seminal plug to seal the cervix after ejaculation. The formation of a seminal plug involves polymerization of proteins in the seminal vesicular secretion by an enzyme released from the coagulating glands (Brooks, 1990; Luke and Coffey, 1994; Mann, 1981; Price and Williams-Ashman, 1961; Setchell et al., 1994; Wagner and Kistler, 1987; Walker, 1910a, 1910b; Williams-Ashman et al., 1977; Zorgniotti and Brendler, 1958). Bilateral removal of seminal vesicles and coagulating glands prevents the formation of a seminal plug after ejaculation. Penile cup formation of male rats is observed during ejaculatory reflexes in *ex copula* tests (Hart and Odell, 1981). However, when the bulbocavernosus (BC) muscle was removed, formation of the penile cup was lost during *ex copula* tests (Hart and Melese-D'Hospital, 1983; Sachs, 1982, 1983). Indirect evidence suggests that removal of the BC muscle prevents the formation of the penile cup at the time of ejaculation during *in copula* tests because the weight of seminal plug attached to the penis is heavier in the males with BC removal than in control males (Sachs, 1982). In addition to the deposit of a seminal plug and the formation of a penile cup, chemical substance(s) released from prostate during ejaculation might cause contractions of the vagina and uterus and consequently affect the female's PER. Removal of the prostate was used to eliminate prostatic fluids.

MATERIALS AND METHODS

Procedures

Forty-three male and 43 female Long Evans rats were used in this study. Animals were randomly assigned into four groups: the control, SCV, SCV+BCX, and SCV+PX Groups. In the control Group, the males received anesthesia and laparotomy (N=11). In the SCV males, bilateral seminal vesicles and coagulating glands were removed and the vas deferens were bilaterally ligated and transected (N=11). In the SCV+BCX Group, in addition to the SCV treatment, the males received removal of bulbocavernosus muscle (N=10). In the SCV+PX males, in addition to the SCV treatment, the prostate glands were also removed (N=11).

In a three-week test period (the week before male surgery, one week after male surgery and two weeks after male surgery), female pacing behavior was tested once per week. Each female was randomly assigned to copulate with one male before male surgery and always tested with the same male she mated before male surgery throughout the entire test period. Therefore, we referred to each female with the male she mated as "female and male partners." Before male surgery, each female was allowed to copulate with her male partner until he ejaculated and then achieved one additional intromission. One week after male surgery, the male was allowed to have one post surgery experience test. During the experience test, the female was examined for the presence of a seminal plug in her vagina immediately following ejaculation. Two weeks after male surgery, each female was tested with her male partner until she received an ejaculation and one postejaculatory intromission. The temporal pattern of female sexual behavior was recorded and analyzed before male surgery and two weeks after male surgery. In addition, male sexual behavior was recorded before male surgery and two weeks after male surgery including mount frequency (MF), intromission frequency (IF), hit rate (number of intromissions / number of intromissions

plus number of mounts), mount latency (ML, the latency from the female's entry into the male's chamber to the first mount), intromission latency (IL, the latency from the female's entry into the male's chamber to the first intromission), interintromission interval (III, ejaculation latency divided by number of intromissions before ejaculation), ejaculation latency (EL, the latency from the first intromission to ejaculation), and postejaculatory interval (PEI, the latency from ejaculation to the next intromission).

Surgery

Male rats were anesthetized with intraperitoneal (IP) injection of Sodium Pentobarbital (Sigma Chemical Co., St. Louis, MO) at a dose of 50 mg per kg body weight and, if necessary, were given supplemental gas anesthetic, methoxyflurane (Metofane; Pitman-Moore Inc., Illinois). In the control Group, the males received a 3 to 4 cm midline incision on the abdomen and the seminal vesicles as well as coagulating glands were exposed and put back into the abdomen immediately after exposure. In the SCV Group, the males received a 3 to 4 cm midline incision on the abdomen and the seminal vesicles as well as coagulating glands were exposed and brought outside the body with fine forceps. A hemostat was used to hold the lower portion of the seminal vesicles and coagulating glands and 4-0 silk suture was applied to tie off the tissue below the hemostat. The seminal vesicles and coagulating glands were removed. In addition, the vas deferens were bilaterally ligated and transected. After surgery, the incision was sutured with 4-0 plain gut suture (Ethicon Inc., New Jersey), and antibiotics were applied to the wound before the skin was closed with wound clips. Each animal was checked and antibiotics were applied to the incision site daily for 5 days.

In the SCV+BCX Group, in addition to SCV treatment, the bulbocavernosus muscle was removed. A 2-3 cm midline incision was made in the skin of scrotum beginning 1 cm posterior to the penile sheath and running caudally. The testes of the

animal were pushed up into the body cavity and the incision was opened with the retractors. In order to expose the penile muscles, the fascia was cut; care was taken not to damage the bulbourethral glands. The bulbocavernosus muscle was then cut away and removed from the penile bulb. Antibiotics were applied to the surgical area and the skin around the incision. The incision was closed with wound clips (The procedures for BCX surgery is adapted from reference Sachs, 1982). Each animal was checked and antibiotics were applied to the incision site daily for 5 days.

In the SCV+PX Group, in addition to the SCV surgery, the prostate glands of males were removed. The procedures for this surgery were basically the same as those described earlier in the SCV treatment.

Statistics:

The data of female's percentage exits following mount, intromission as well as ejaculation and the male's hit rate were transformed by arc sine square root before analysis (Dixon and Massey, 1969). Comparison of the female pacing behavior (AL, MRL, IRL, PER, percentage exits following mount, intromission and ejaculation) among the four groups before male surgery and two weeks after male surgery was achieved by Mixed Factorial ANOVA (Two Way Repeated Measures ANOVA) followed by Tukey's post-hoc tests. Comparison of the male sexual behavior (MF, IF, hit rate, ML, IL, III, EL, and PEI) among the four groups before male surgery and two weeks after male surgery was analyzed by Mixed Factorial ANOVA (Two Way Repeated Measures ANOVA) followed by Tukey's post-hoc tests. An alpha level of 0.05 was used for all statistical tests.

RESULTS

No seminal plug was found in the vagina of females tested with experimental males one week after the SCV, SCV+BCX, or SCV+PX surgery, nor were significant differences found among the four treatment groups before male surgery and two weeks after male surgery in the female's AL, MRL, IRL, PER, and percentage exits following mount, intromission, and ejaculation. None of the measures of male sexual behavior recorded in this experiment changed following any surgical treatment except the interintromission interval (III) in the SCV+BCX Group. The III of males in the SCV+BCX Group was significantly longer after male surgery (39.4 $\pm$ 5.3 seconds) than before male surgery (30.0 $\pm$ 3.0 seconds) ($p<0.05$).

EXPERIMENT 3B: RELATION OF THE EJACULATION DURATION AND PREEJACULATORY INTROMISSIONS TO THE FEMALE'S POSTEJACULATORY REFRACTORY PERIOD

It has been reported that the duration of the male rat's ejaculatory reflex increases over successive ejaculatory series during copulation (Peirce and Nuttall, 1961b). Similarly, the female rat's PER increases over the course of copulation (Bermant and Westbrook, 1966; Krieger et al., 1976; Yang and Clemens, 1996a). In our previous study, we demonstrated that the PER of females receiving 0-1 intromission before ejaculation was significantly shorter than the other groups (2-4, 5-15, and 24-31 intromission groups) (Yang and Clemens, 1996a). We also found that the male's ejaculation duration was shorter when the female received only 0-1 intromission prior to ejaculation (Unpublished observation). These findings lend support to the idea that the male's ejaculation duration may contribute to the female's PER. Moreover, it is possible that the number of pelvic thrusts during ejaculation affects the male's ejaculation duration

and/or the female's PER. Although preejaculatory intromission frequency is not significantly correlated with the female's PER during the first ejaculatory series (Yang and Clemens, 1996a), preejaculatory intromission frequency was significantly correlated with the female's PER if the range of the preejaculatory intromission frequency was large enough (0-31 in that study) (Yang and Clemens, 1996a). It is possible that the preejaculatory intromissions may influence the male's ejaculation duration and/or the number of pelvic thrusts during ejaculation that in turn affect the female's PER. The objective of this experiment was to examine the relation among the ejaculation duration, the number of intromissions received prior to ejaculation, the number of pelvic thrusts during ejaculation, and the female's PER.

MATERIALS AND METHODS

Procedures:

In a two-week test period, the pacing behavior of sexually experienced female rats was recorded on a computer disk and videotaped with a camcorder. Eighteen male and 18 female Long Evans rats were used in this study. In the first week, each female was randomly assigned to copulate with one male until she received an ejaculation and returned to the male's chamber following her PER (the 4-18 I Group). Because each female was always tested with the same male she mated in the first week, we referred to the female and the male she mated as "the female and male partners". In the second week, each male was allowed to copulate with a stimulus female that was replaced with the "female partner" (experimental female) when it was estimated that the male was near ejaculation. This estimate was based on the preejaculatory intromission frequency obtained in the first week test. To examine whether the male's ejaculation duration and the female's PER are significantly shorter when the female receives 0-1 intromission prior to ejaculation, only the

data of those females that received 0 or 1 intromission prior to ejaculation were recorded and used for analysis (the 0-1 I Group). Mating treatments were not counterbalanced because the results obtained in our laboratory indicated that repeated testing did not affect the temporal pattern of female sexual behavior under the present hormone replacement regimen.

The male's ejaculation duration was defined as: the duration of male-female contact during the ejaculatory intromission and was obtained by playing back the videotape 30 times slower with a Hitachi VCR. The male's ejaculation duration refers specifically to the time period from the male's first pelvic thrust to the male's dismount from the female. Each ejaculation duration was measured three times during video analysis and the mean ejaculation duration was calculated and used for analysis. In addition, the number of pelvic thrusts during ejaculation was determined using the slow play-back of the videotape and only those animals with clearly identified pelvic thrusts were used for analysis of thrust frequency.

Statistics:

Comparison of the male's ejaculation durations between the 4-18 I Group and the 0-1 I Group and comparison of the female's PERs between the 4-18 I Group and the 0-1 I Group were achieved by paired t-test. The relations among the male's ejaculation duration, the preejaculatory intromission frequency, the number of pelvic thrusts during ejaculation and the female's PER were analyzed by Correlation analysis. An alpha level of 0.05 was used for all statistical tests.

RESULTS

In the first week, eighteen females were allowed to copulate for one full ejaculatory series and received a range of 4 to 18 intromissions prior to ejaculation (the 4-18 I Group). Two animals were excluded from analysis because one was rejected by the Outlier test and the other was excluded because of a loss of image focus caused by power failure of the camcorder. Therefore, data from 16 females were used for statistical analysis. The male's ejaculation duration was positively correlated with the female's PER ($r = 0.653$, $p = 0.0049$, $N = 16$). In addition, the number of intromissions received by the female prior to ejaculation was positively correlated with the male's ejaculation duration ($r = 0.643$, $p = 0.0059$, $N = 16$). However, the preejaculatory intromission frequency was not significantly correlated with the female's PER ($r = 0.270$, $p = 0.3187$, $N = 16$). This result replicated our previous finding that the number of preejaculatory intromissions was not significantly correlated with the female's PER in the first ejaculatory series (Yang and Clemens, 1996a). Twelve out of 16 males had pelvic thrusts clearly identified during ejaculation; thrust frequency ranged from 4 to 7 with a mean of 5.9. There was no significant correlation between the number of pelvic thrusts during ejaculation and the female's PER ($r = 0.396$, $p = 0.2083$, $N = 12$). Nor was thrust frequency during ejaculation significantly correlated with the male's ejaculation duration ($r = 0.445$, $p = 0.1512$, $N = 12$) or the preejaculatory intromission frequency ($r = -0.197$, $p = 0.5487$).

In the second week, 6 out of 16 females received 0-1 intromission before the male ejaculated (the 0-1 I Group) and the data of these 6 females were used for analysis. The data of females ($N = 10$) receiving more than 2 intromissions prior to ejaculation were not recorded and excluded from analysis. Figure 9 compares the male's ejaculation durations between the 4-18 I Group and the 0-1 I Group. The ejaculation duration of males in the 0-1 I Group was significantly shorter than that of the 4-18 I Group ($t = 4.065$, $p = 0.0097$, $N = 6$) (Figure 9). Figure 10 compares the female's PERs between the 4-18 I Group and the

0-1 I Group. The PER of females receiving 0-1 intromission prior to ejaculation was significantly shorter than that of the 4-18 I Group ($t = 4.155$, $p = 0.0089$, $N = 6$) (Figure 10). This result replicated our previous finding that the PER of females receiving 0-1 intromission prior to ejaculation was significantly shorter than the other groups (2-4, 5-15, and 24-31 intromission groups) (Yang and Clemens, 1996a).

When the data from week 1 (the 4-18 I Group) were combined with those of week 2 (the 0-1 I Group), the male's ejaculation duration was positively correlated with the female's PER ($r = 0.814$, $p < 0.0001$, $N = 22$) (Figure 11). In addition, the number of intromissions received by the female prior to ejaculation was positively correlated with the male's ejaculation duration ($r = 0.771$, $p < 0.0001$, $N = 22$) (Figure 12). Moreover, the preejaculatory intromission frequency became significantly correlated with the female's PER ($r = 0.611$, $p = 0.002$, $N = 22$) (Figure 13). This is consistent with our previous finding that preejaculatory intromission frequency was significantly correlated with the female's PER if the range of intromission frequency was large enough (0-31 in the previous study) (Yang and Clemens, 1996a). Furthermore, partial correlation analysis controlling the ejaculation duration indicated that the correlation between preejaculatory intromissions and the female's PER was close to zero ($r = -0.0453$, $N = 22$).

The number of pelvic thrusts during ejaculation can be identified in five of the 6 males in the 0-1 I Group; thrust frequency ranged from 6 to 7 with a mean of 6.4. After combining the data of the 4-18 I Group and the 0-1 I Group, the number of pelvic thrusts during ejaculation was not significantly correlated with either the ejaculation duration ($r = 0.137$, $p = 0.6064$, $N = 17$), the preejaculatory intromission frequency ($r = -0.304$, $p = 0.2407$, $N = 17$) or the female's PER ($r = 0.097$, $p = 0.7169$, $N = 17$).

Figure 9. compares the ejaculation duration of males between the 4-18 and the 0-1 intromission Groups (4-18 I and 0-1 I Groups). The male's ejaculation duration in the 4-18 I Group is significantly longer than that in the 0-1 I Group ($t = 4.065$, $p = 0.0097$, $N = 6$). Data are expressed as mean $\pm$ SEM and analyzed by paired t-test. ** $p<0.01$.

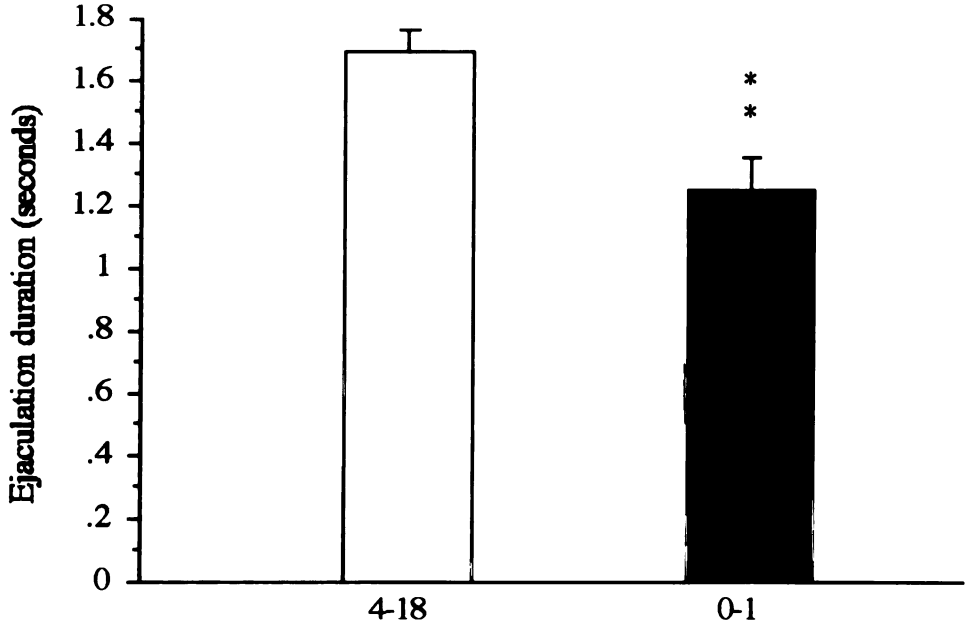

Ejaculation duration (seconds)
1.8
1.6
1.4
1.2
1
.8
.6
.4
.2
0
**
4-18
0-1
Number of intromissions prior to ejaculation

Figure 10. compares the postejaculatory refractory period (PER) of females between the 4-18 and the 0-1 intromission Groups (4-18 I and 0-1 I Groups). The PER of females in the 4-18 I Group is significantly longer than that in the 0-1 I Group ($t = 4.155$, $p = 0.0089$, $N = 6$). Data are expressed as mean $\pm$ SEM and analyzed by paired t-test. ** $p<0.01$.

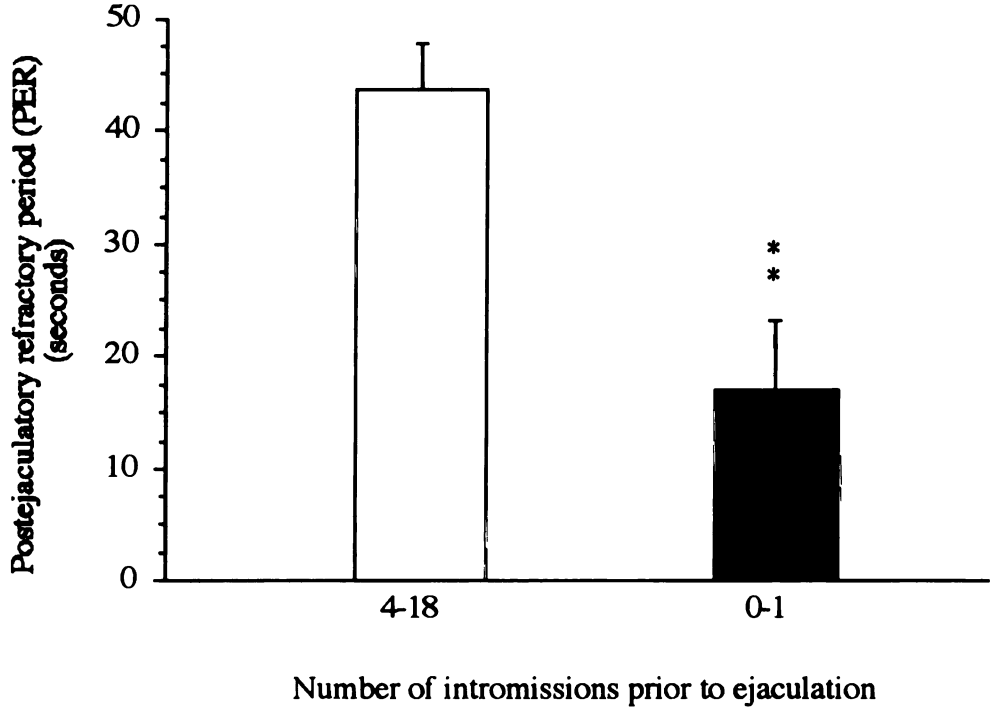

Postejaculatory refractory period (PER) (seconds)
50
40
30
20
10
0
**
4-18
0-1
Number of intromissions prior to ejaculation

Figure. 11. shows the relation of the male's ejaculation duration (ED) to the female's postejaculatory refractory period (PER). There is a significantly positive correlation between the male's ED and the female's PER ($r = 0.814$, $p<0.0001$, $N=22$).

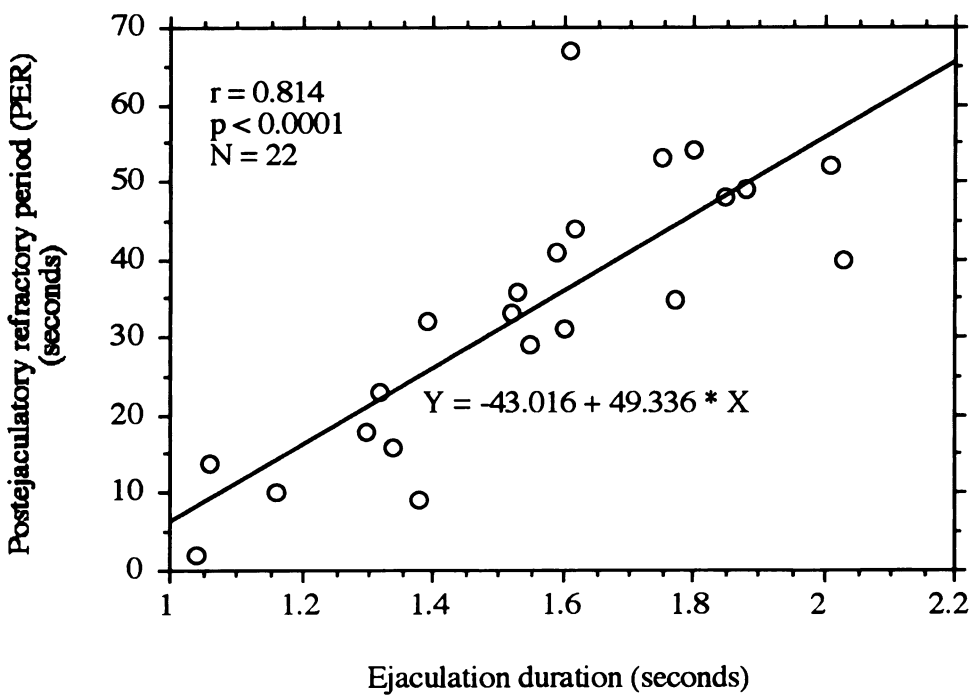

r = 0.814
p < 0.0001
N = 22
Y = -43.016 + 49.336 * X
Postejaculatory refractory period (PER) (seconds)
Ejaculation duration (seconds)
0
10
20
30
40
50
60
70
1
1.2
1.4
1.6
1.8
2
2.2

Figure. 12. shows the relation of the number of intromissions prior to ejaculation to the male's ejaculation duration. The preejaculatory intromission frequency is positively correlated with the male's ejaculation duration ($r = 0.771$, $p < 0.0001$, $N = 22$).

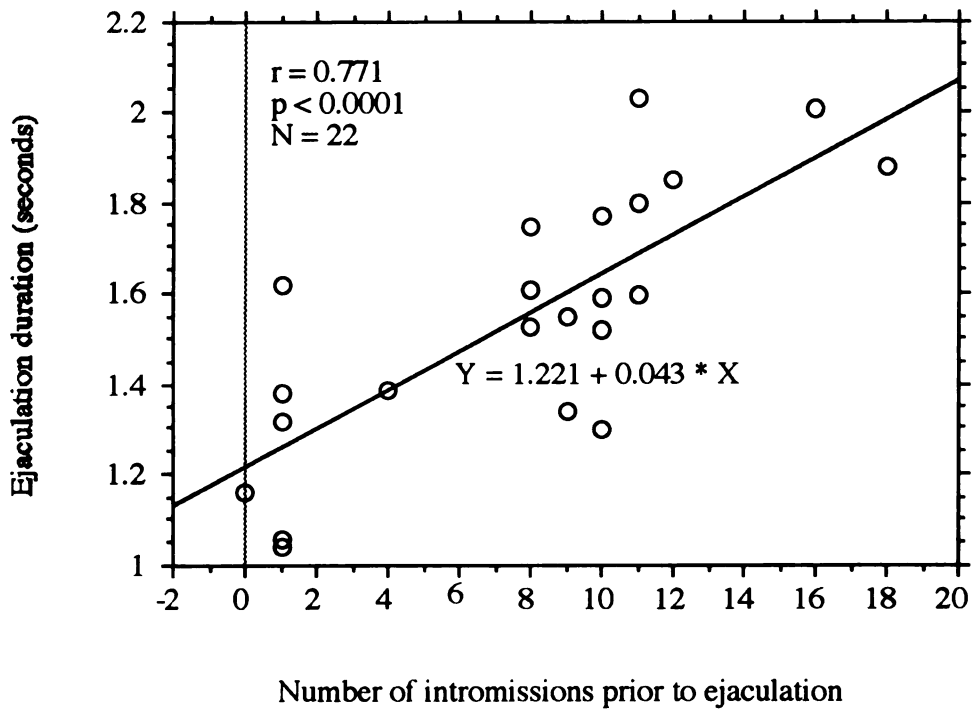
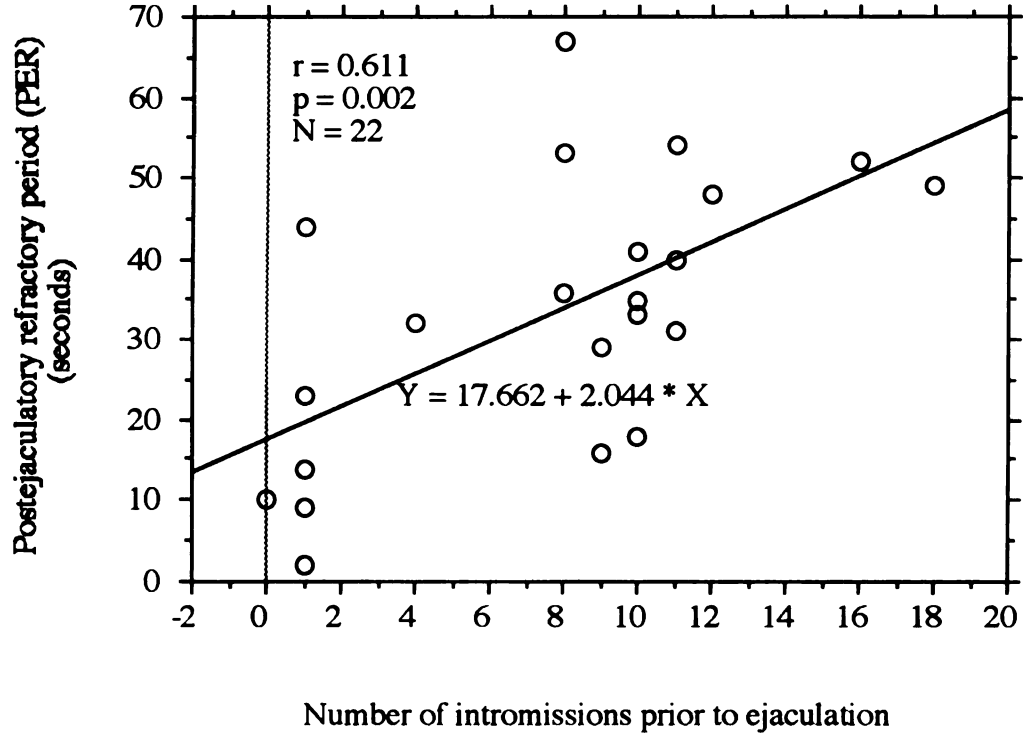

r = 0.771
p < 0.0001
N = 22
Y = 1.221 + 0.043 * X
Ejaculation duration (seconds)
Number of intromissions prior to ejaculation
2.2
2
1.8
1.6
1.4
1.2
1
-2
0
2
4
6
8
10
12
14
16
18
20

Figure 13. The relation of preejaculatory intromission frequency to the female's postejaculatory refractory period (PER). The preejaculatory intromission frequency is significantly correlated with the female's PER ($r = 0.611$, $p = 0.002$, $N = 22$).


r = 0.611
p = 0.002
N = 22
Y = 17.662 + 2.044 * X
Postejaculatory refractory period (PER) (seconds)
Number of intromissions prior to ejaculation
70
60
50
40
30
20
10
0
-2
0
2
4
6
8
10
12
14
16
18
20

DISCUSSION

The principal finding of this study is that the male's ejaculation duration is positively correlated with the female's PER. This finding is consistent with previous studies showing that the male's ejaculation duration increased over the course of consecutive ejaculatory series (Peirce and Nuttall, 1961b) and that the female's PER increased with consecutive ejaculatory series (Bermant and Westbrook, 1966; Krieger et al., 1976; Yang and Clemens, 1996a). Two methods have been used to measure the male's ejaculation duration: cinemagraphic analysis of copulation using frame analysis (Stone and Ferguson, 1940), and a moist contact method to activate electrical recording equipment (Erskine et al., 1989; Peirce and Nuttall, 1961b). The ejaculation duration of male rats as revealed by these methods was 1 to 4 seconds (Stone and Ferguson, 1940) and 1 to 2 seconds (Erskine et al., 1989; Peirce and Nuttall, 1961b), respectively, in the first ejaculatory series. In this study, the male's ejaculation duration: the time from the first pelvic thrust to the male's dismount from the female, is between 1 and 2.1 seconds in the first ejaculatory series.

The female's PER appears to be influenced directly by the male's ejaculation duration and indirectly by the number of intromissions received prior to ejaculation. Ejaculation duration was positively correlated with the number of intromissions received prior to ejaculation and the female's PER in the first ejaculatory series (the 4-18 I Group) and after combining the data of the 4-18 I Group and the 0-1 I Group. These findings support our previous notion that multiple intromissions of male rats prior to ejaculation may function to prepare the vaginal passage for the deep penile insertion that results in a longer penis-vagina contact during ejaculation (Yang and Clemens, 1996a). This longer penis-vagina contact in turn results in a longer return latency following ejaculation in the female. Although the male's ejaculation duration is significantly correlated with the female's PER in the first ejaculatory series, the number of preejaculatory intromissions is not, which

replicates our previous report (Yang and Clemens, 1996a). However, when the range of number of intromissions received by the female before ejaculation was increased beyond the normal range seen in the first ejaculatory series, intromission frequency was positively correlated with the female's PER (Yang and Clemens, 1996a). After combining the data of the 4-18 I Group and 0-1 I Group in the present study, the preejaculatory intromission frequency was positively correlated with the female's PER indicating that preejaculatory intromission frequency influences the female's PER. Further, partial correlation analysis controlling the ejaculation duration indicated that the correlation between the preejaculatory intromissions and the female's PER was close to zero. From these lines of evidence we suggest that the number of intromissions received prior to ejaculation affects the male's ejaculation duration which in turn influences the female's PER.

Further support for the idea that the male's ejaculation duration plays an important role in inducing the female's PER comes from experimental manipulation of intromission frequency preceding ejaculation. The male's ejaculation duration and the female's PER are significantly shorter when the female receives 0 or 1 intromission prior to ejaculation. These findings replicate our previous report (Yang and Clemens, 1996a) showing that when the female received 0 or 1 intromission before the male ejaculated, her PER was significantly shorter than the other groups (2-4, 5-15, and 24-31 intromission groups).

The influence of a longer ejaculation duration on the female's PER probably results from more intense stimulation due to the longer vagina-penis contact duration that may result from the deeper penile insertion during ejaculation. The longer vagina-penis contact associated with ejaculation or a longer PER does not appear to result from more pelvic thrusts because the number of pelvic thrusts during ejaculation is not significantly correlated with either the ejaculation duration or the female's PER. This finding also implies that the quality of pelvic thrusts may be more important than the frequency of pelvic thrusts in inducing the female's PER. Most likely the longer ejaculation duration and/or a deeper penile insertion generate a more intense stimulus which results in a longer PER.

Several lines of evidence support this notion. First, it has been reported that the vaginal canal close to the cervix is innervated by sensory afferent fibers (Nance et al., 1988). Secondly, fibers with mechanoreceptive fields that respond to thrusting stimuli are found along the vaginal canal in female rats (Berkley et al., 1990). In addition, evidence suggests that more fibers with mechanoreceptive fields are located near the vaginocervical junction than in other areas of vaginal canal (Berkley et al., 1990). Furthermore, a deeper penile insertion during ejaculation may simultaneously stimulate pelvic nerve fibers as well as fibers from the hypogastric nerve (Berkley et al., 1990; Berkley et al., 1993; Peters et al., 1987). However, a functional analysis of the respective roles played by these two nerves suggests that "pelvic nerve fibers seem closely tied to sensory and behavioral processes associated with mating and conception, whereas hypogastric fibers seem closely tied to pregnancy and nociception" (p. 533, Berkley et al., 1993).

Neither the seminal plug, prostate secretions, nor the penile cup formation appeared to contribute to the female's postejaculatory refractory period. In addition, the male's sexual performance was not affected by removal of the coagulating glands, seminal vesicles, prostate glands, or bulbocavernosus muscle. In rats, secretions from the seminal vesicles constituted a large portion of the male's ejaculate (Mann, 1964). The enzyme, transglutaminase, released from the coagulating glands, plays a major role in the formation of the copulatory plug (Luke and Coffey, 1994; Mann, 1981; Wagner and Kistler, 1987; Williams-Ashman, 1984; Williams-Ashman et al., 1977). Bilateral removal of the seminal vesicles and coagulating glands, therefore, prevents formation of a copulatory plug after ejaculation (This was confirmed by the evidence that no seminal plug was found in the vagina of the females that mated with the males after the SCV, SCV+BCX, and SCV+PX treatments). However, removal of both sets of tissue did not affect the measures of male sexual behavior or female pacing behavior including the female's PER. Extirpation of the BC muscle presumably eliminates the formation of penile cup during ejaculatory reflexes *in copula* tests. Removal of the bulbocavernosus muscle had no effect on the male sexual

performance or on the female temporal copulatory behavior including the female's PER. Although the prostatic secretions contribute considerable volume to the seminal plasma in rats, removal of the prostate did not affect sexual performance of either the male or female, nor did bilateral ligation and transection of vas deferens.

In summary, different types and qualities of vaginocervical stimulation received by the female affect her pacing behavior. The female's PER is significantly longer than her IRL and MRL. Different numbers of intromissions received by the female prior to ejaculation affect her PER. In the present study, our data show that neither the seminal plug, prostatic secretions, nor the penile cup contributes to the female's PER. In addition, removal of either the prostate glands, bulbocavernosus muscle, coagulating glands, or seminal vesicles does not affect the measures of male sexual behavior recorded in this study. The number of pelvic thrusts during ejaculation is not significantly correlated with either the male's ejaculation duration, the female's PER or the preejaculatory intromission frequency. However, the male's ejaculation duration is significantly correlated with the preejaculatory intromission frequency and the female's PER in the first ejaculatory series (the 4-18 I Group) and after combining the data of the 4-18 I Group and the 0-1 I Group. In contrast, the number of intromissions received prior to ejaculation is significantly correlated with the female's PER after combining the data of the 4-18 I Group and the 0-1 I Group. Partial correlation analysis controlling the ejaculation duration shows that no significant correlation exists between preejaculatory intromissions and the female's PER. These findings lead us to suggest that the female's PER is influenced directly by the male's ejaculation duration, indirectly by the number of intromissions received by the female prior to ejaculation and not at all by the amount of thrusting during ejaculation.

EXPERIMENT 4: CORRELATION OF UTERINE ACTIVITY WITH THE TEMPORAL PATTERN OF FEMALE SEXUAL BEHAVIOR IN RATS

ABSTRACT

In a standard test situation, it has been reported that copulation has both an immediate and a delayed effect on uterine contractions. The objective of the present study was to determine whether uterine activity correlates with different types of sexual contact and different temporal patterns of female sexual behavior. Electrodes implanted in the uterus were used to record electromyographic (EMG) activity while females were tested under a situation where they could escape from the male following sexual contacts. Results indicated that the duration of uterine activity associated with an ejaculation was significantly longer than that associated with an intromission or a mount. This finding coincides with the report that the female's return latency following an ejaculation is significantly longer than that following an intromission or a mount and that the intromission duration is longer during ejaculation than during non-ejaculatory intromissions. In addition, our results were consistent with the finding that mating has an immediate effect on uterine activity. Possibly the longer duration of uterine activity associated with ejaculation that reflects a more intense vaginocervical stimulation contributes to the fast sperm transport following ejaculation and the longer return latency following ejaculation.

INTRODUCTION

One function of the multiple intromission pattern of mating in rats is to facilitate sperm transport following ejaculation (Adler, 1969). When females received 0-1 intromission prior to ejaculation, no or few sperm were found in the uterus 3 hours following ejaculation. However, larger numbers of sperm were found in the uterus if the female received 2 or more intromissions prior to ejaculation (Adler, 1969). The vaginocervical stimulation received by the female rat during copulation increases uterine contractions. It has been reported that vaginocervical stimulation has both an immediate and a delayed effect on uterine electrical activity when the male controls the timing of copulation (Toner and Adler, 1986). Uterine contractions increased significantly during copulation, returned to the premating level following ejaculation, and increased again 5 minutes after ejaculation (Toner and Adler, 1986).

The objective of this experiment was to examine the relation of uterine electromyographic (EMG) activity to each sexual contact when the female is able to control the temporal pattern of female sexual behavior. While previous experiments have shown a relationship between various types of vaginocervical stimulation and female pacing behavior, the present study will provide insight into the relation of endogenous responses of the female to her mating behavior.

METHOD

Animals

Sixty-day-old female and ninety-day-old male Long Evans rats were purchased from Charles River Laboratories (Wilmington, MA). All females were ovariectomized one week after arrival in the laboratory. Animals were housed in 16:8 hr light-dark cycle with

lights off at 17:00. Animals (females and males) were housed singly. Food and water were available ad lib. The temperature and humidity of the animal room were maintained at 22^0 C and 55%, respectively. The air in the animal room was exchanged 12 times per hour automatically. Ovariectomized females were brought into estrus by intramuscular (IM) injections of 0.5 μg estradiol benzoate (EB) dissolved in 0.1 ml sesame oil (Sigma Chemical Co.) for three consecutive days and 24 hours later followed by an IM injection of 0.5 mg progesterone (P) dissolved in 0.1 ml sesame oil. All female pacing behavior was tested 4-7 hours after the injection of progesterone (2-5 hours after the lights off) under dimly red illumination. All animals were tested once per week throughout the whole test period and had been tested at least twice in the testing chamber before data were collected. Only those females showing pacing behavior in these preliminary tests were used in the experimental studies. The approximate weights of the female and male rats were 200 - 300 g and 500 - 600 g, respectively, when the tests started.

Pacing Tests and Behavioral Measures

Females were tested in our standard two-compartment test arena. Several behavioral measures were recorded in the pacing tests: mount return latency (MRL), the latency for the female to re-enter the male's chamber after a mount; intromission return latency (IRL), the latency to return to the male's chamber following an intromission; postejaculatory refractory period (PER), the latency to re-enter the male's chamber after an ejaculation. All latencies were measured in seconds.

Implant of electrodes:

Twenty female rats were implanted with electrodes in the uterus for this study. Females were anesthetized by injection of mixtures of Ketamine (44 mg/kg) and Xylazine (10 mg/kg).

A 3 to 4 cm midline incision on the abdomen was made with the scalpel and the uterus was exposed by muscle retractors. Two mm of Teflon was removed from each tip of Teflon-coated silver electrodes (0.003 in., bare diameter, Medwire, Mt. Vernon, NY) by a scalpel. Two electrodes were implanted into the uterus around the uterine bifurcation (near cervix) one at a time using a 25 gauge needle. The electrode was passed through a 25 gauge needle with the bare tip of the electrode bent backward and the 25 gauge needle along with the bent electrode wire was inserted into the uterus. The needle was then pulled back and the bare tip of the microelectrode was left in the uterus. The tips of the electrodes were 3 mm apart. Five mm of Teflon was removed from the free ends of the electrodes and these were passed through the lumen of a piece of polyethylene tubing (PE-50, ID: 0.58 mm, OD: 0.965 mm) (Clay Adams, Division of Becton Dickinson and Company Parsippany, New Jersey 07054). The PE tubing was secured to the abdomen muscles near the operated area via 4-0 gut suture to prevent the electrodes from coming free. The tubing with the electrode wires was passed subcutaneously around the abdomen, and along the back of the body to the head. Each of the free ends of electrodes was soldered to a pin of a miniature IC socket containing subminiature female connectors. A reference Teflon-coated silver wire with 5 mm bare tip was bent backward and inserted securely into the leg muscle by the same procedure previously described and passed through a second piece of PE tubing. The PE tubing was sutured to the adjacent muscle with 4-0 gut suture, then passed subcutaneously to the head where the free end of the electrode was soldered to a pin of the miniature IC socket. The miniature IC socket was anchored to the skull by tiny bone screws and dental acrylic. Antibiotics were applied to the skin around the incision and the

incision was closed with wound clips. The animal was injected with 5 mg Amoxicillin (Amoxi-inject, SmithKline Beecham, West Chester, PA) daily for 4 days starting from the day of surgery.

Recording of uterine electromyographic (EMG) activity:

One week after the implantation of the electrodes, females were tested with a male. Prior to copulation, the female's upper torso was fitted with a cloth harness (Stoelting 50556, Chicago, IL). The connectors of the miniature IC socket on the animal's head were attached to the leads on shielded-wires from a commutator (Stoelting 50506) mounted above the testing arena by means of a small spring. A shielded tether cable was used to protect the wires from animal's biting and was tightly attached to the cloth harness. The commutator, in turn, was connected to a Grass High Performance AC amplifier Model 7P511 (Grass Instrument Co., Quincy, MA 02169). The raw signal was filtered by half-amplitude low frequency filter to 10 Hz and half-amplitude high frequency filter to 3 kHz. The sensitivity was set in the range of 50 to 200 μv/mm.

The female was placed in the pacing chamber and uterine EMG activity was recorded for 30 minutes before the male was introduced. To prevent the male from entering the escape chamber, a cloth harness was put on the male's upper torso. Uterine EMG activity and the temporal pattern of female sexual behavior were recorded for one ejaculatory series. Recording was terminated 5 minutes after the ejaculation. The durations of uterine EMG activity associated with sexual contacts and the female's return latencies following sexual contacts were analyzed and reported.

Data analysis:

The duration of uterine activity associated with each type of sexual contact was recorded and analyzed by one way ANOVA followed by Fisher's PLSD post-hoc tests. Comparison of the MRL, IRL, and PER was achieved by one way ANOVA followed by Fisher's PLSD post-hoc tests. An alpha level of 0.05 was used for all statistical tests.

RESULTS

Uterine EMG activity of seven females was successfully monitored during copulation. Uterine activity of two females was recorded at a paper speed of 10 mm/min., while in five females, uterine EMG activity was recorded at a paper speed of 5 mm/sec or 10 mm/sec and these data were used for analysis of the duration of uterine activity associated with different types of sexual contacts.

Typical uterine EMG activity associated with each type of sexual contact recorded at a paper speed of 5 mm/sec is illustrated in Figure 14. The duration of uterine contractions associated with mounts, intromissions, and ejaculation is summarized in Figure 15. There was a significant difference among the durations of uterine contractions associated with sexual contacts [$F(2, 11) = 23.189$, $p<0.0001$]. The duration of uterine activity associated with an ejaculation was significantly longer than that associated with an intromission or a mount ($p<0.01$) (Figure 15). The duration of uterine contractile activity associated with an intromission was not significantly different from that associated with a mount (Figure 15). Data are expressed as mean $\pm$ SEM in seconds.

A significant difference existed among the return latencies following a mount, intromission, and ejaculation [$F(2,6) = 5.435$, $p = 0.045$]. The female's return latency following an ejaculation was significantly longer than that following an intromission or a mount ($p<0.05$) (Figure 16). The female's return latency following an intromission was

Figure 14. shows the typical uterine EMG activity associated with a mount, intromission, and ejaculation. The duration of uterine EMG activity associated with an ejaculation seemed longer than that associated with an intromission or a mount. E: ejaculation; I: intromission; M: mount. Amplitude: 1 cm = 1 mV. Time scale (paper speed): 1 cm = 2 seconds. Arrows indicate the approximate time for dismount.

Figure 15. Comparison of the durations of uterine EMG activity associated with a mount, intromission, and ejaculation. The duration of uterine EMG activity associated with an ejaculation was significantly longer than that associated with a mount or an intromission (** $p<0.01$). There was no significant difference found between durations of uterine EMG activity associated with a mount and an intromission. Data are expressed as mean $\pm$ SEM in seconds and analyzed by one way ANOVA followed by Fisher's PLSD post-hoc tests.

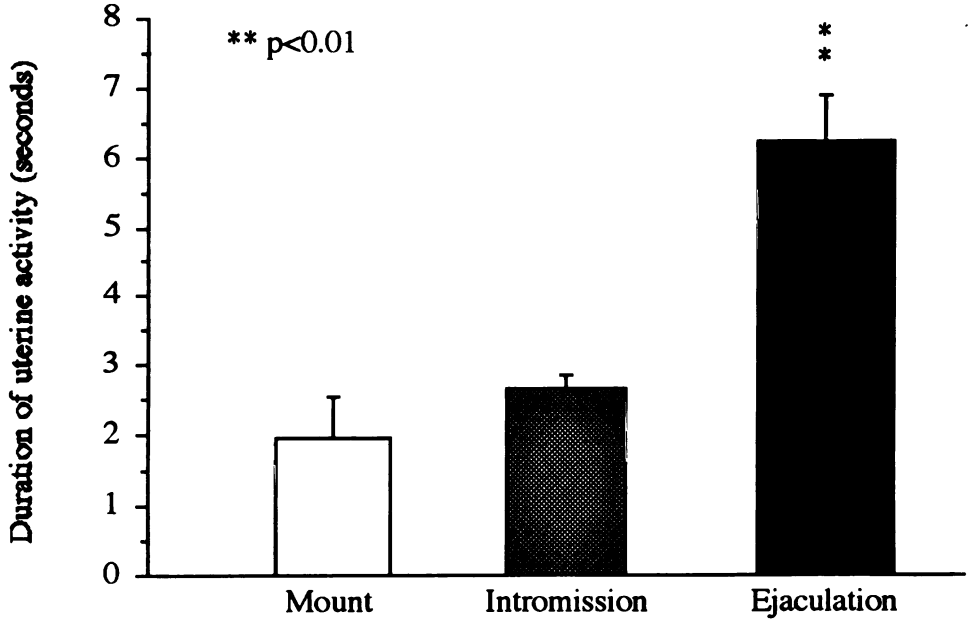
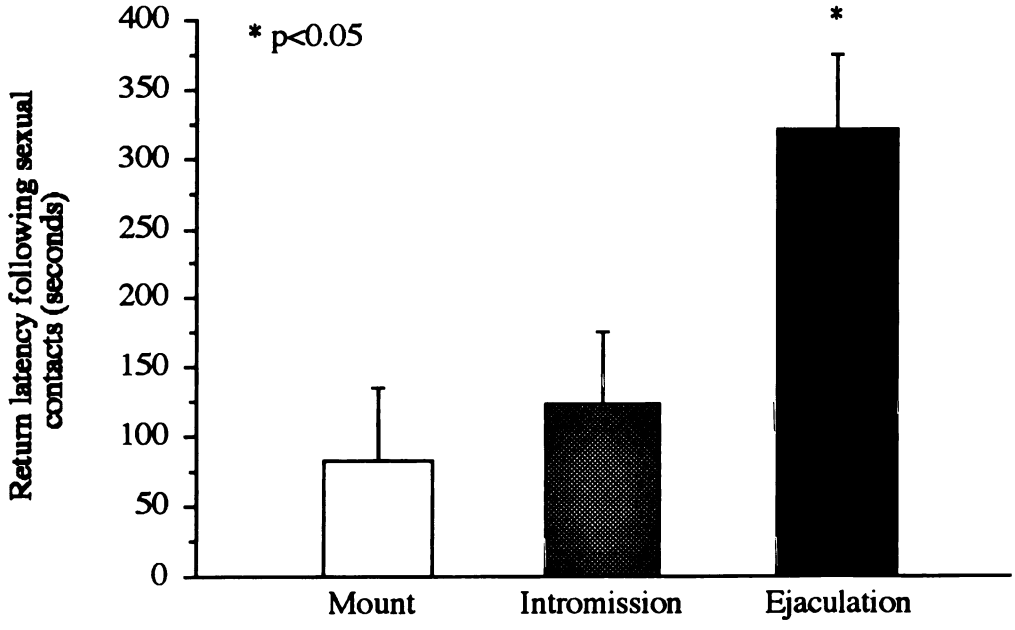

** p<0.01
**
Duration of uterine activity (seconds)
0
1
2
3
4
5
6
7
8
Mount
Intromission
Ejaculation

Figure 16. Comparison of the female's return latencies following a mount (MRL), intromission (IRL), and ejaculation (PER). The female's PER was significantly longer than her IRL or MRL (* $p<0.05$). Data are expressed as mean $\pm$ SEM in seconds and analyzed by one way ANOVA followed by Fisher's PLSD post-hoc tests.


Return latency following sexual contacts (seconds)
400
350
300
250
200
150
100
50
0
* p<0.05
*
Mount
Intromission
Ejaculation

not different from that following a mount (Figure 16). Data are expressed as mean ± SEM in seconds.

DISCUSSION

The major finding of the present study is to correlate uterine EMG activity with each type of sexual contact and the temporal pattern of female rat sexual behavior. The duration of uterine activity associated with an ejaculation was significantly longer than that associated with an intromission or a mount. This finding coincides with the result that the female's return latency following an ejaculation is significantly longer than that following an intromission or a mount (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985, 1989; Krieger et al., 1976; Lange and Clemens, 1994; Peirce and Nuttall, 1961a) and that the intromission duration is significantly longer during ejaculation than during non-ejaculatory intromissions (Erskine et al., 1989; Peirce and Nuttall, 1961b; Stone and Ferguson, 1940). Possibly the longer duration of uterine activity associated with ejaculation that reflects a more intense vaginocervical stimulation contributes to the female's PER.

The results of uterine EMG recordings are consistent with previous reports. In the present studies, few uterine contractions were observed before copulation. This is consistent with the previous finding that only some uterine contractile activity was observed during the evening of the proestrus stage (Talo and Karki, 1976) and before copulation (Toner and Adler, 1986). In the present study, uterine activity increased significantly during active copulation and returned to the premating level following ejaculation. This is also consistent with the previous report (Toner and Adler, 1986). In addition, the female's return latencies following sexual contacts follow the same pattern reported in other studies. The female's return latency following an ejaculation is significantly longer than that following an intromission or a mount (Bermant, 1961; Bermant and Westbrook, 1966;

Erskine, 1985, 1989; Krieger et al., 1976; Lange and Clemens, 1994; Peirce and Nuttall, 1961a). In contrast to our previous report, the female's return latencies following sexual contacts lengthened in the present study. This may reflect the fact that the females were recovering from electrode implants and adjusting to the harness as well as the tethering that was required during testing.

One possible function of the copulation-induced uterine activity may be to facilitate sperm transport in three ways. First, the immediate increase of uterine activity at the time of ejaculation probably facilitates sperm transport from the vagina to the uterus. This is supported by the evidence that sperm were found in the uterus as soon as 54 seconds following ejaculation (Hartman and Ball, 1930). Secondly, the immediate increase of uterine activity in response to active copulation may assist sperm deposited from previous ejaculation(s) to advance in the uterus at a higher speed. Finally, the delayed increase of uterine activity following multiple ejaculations (Toner and Adler, 1986) may serve to facilitate transport of preexisting sperm that were deposited from the previous ejaculation(s) in the uterus at a higher speed.

In summary, the duration of uterine EMG activity varies with each type of sexual contact. The duration of uterine contractions associated with an ejaculation was significantly longer than that associated with an intromission or a mount. Possibly this longer duration of uterine EMG activity accompanying an ejaculation results from a more intense stimulus and is responsible for the rapid sperm transport from the vagina to the uterus and the longer return latency following ejaculation

EXPERIMENT 5: SEXUAL MOTIVATION AND THE MEDIAL PREOPTIC AREA IN FEMALE RATS

ABSTRACT

Lesions of the medial preoptic area of hypothalamus (MPOA) have been reported to increase the lordosis response in female rats. The lordosis response of female rats is also facilitated by transections made anterior-dorsal to the MPOA. Based on these results, it has been suggested that the MPOA inhibits female rat lordosis behavior. However, MPOA lesions significantly decrease the time that the female rat spends with the male suggesting that the MPOA may play a role in enhancing female sexual behavior. The objective of this study was to investigate the relation of the MPOA to the temporal pattern of female sexual behavior in rats. In Experiment 5A, we investigated the effect of electrolytic (1.5 mA for 20 seconds) lesions of MPOA on female copulation when the female could regulate the timing of copulatory events (pacing). Results indicated that MPOA lesions significantly increased the female's latency to return to the male after an intromission (IRL) and after an ejaculation, the postejaculatory refractory period (PER). Experiment 5B examined the effect of ibotenic acid lesions (0.4 µl of ibotenic acid solution per side at a dose of 10 µg/µl 0.1 M phosphate buffer, pH 7.40) of MPOA on female pacing behavior. Results for chemical lesions were similar to those of electrolytic lesions suggesting that the alteration of timing in lesion females results from loss of MPOA cells and not fibers of passage. It has been reported that MPOA lesions that spare some part of MPOA significantly lengthened the male's interintromission interval (III) and postejaculatory interval (PEI). Our results with females parallel these effects of MPOA lesions in males. Based on these results, we

suggest that the MPOA plays a similar role in both males and females with regards to regulating the temporal pattern of copulation and possibly sexual motivation.

INTRODUCTION

Lordosis behavior, hopping, darting and the temporal pattern of copulatory events form the major components of female rat sexual behavior (Nelson, 1995; Pfaff, 1994). In a standard test situation where the female rat can not escape from the male during copulation, lordosis frequency, hopping, and darting serve as the primary measures of female sexual behavior (Beach, 1976; Nelson, 1995; Pfaff et al., 1994; Young, 1961). However, when given an opportunity, the female rat actively regulates the timing of copulatory events (Bermant, 1961; Bermant & Westbrook, 1966; Clemens et al., 1995; Erskine, 1985, 1987, 1989, 1992; Erskine & Baum, 1982; Erskine et al., 1989; Fadem et al., 1979; Frye & Erskine, 1990; Gilman & Hitt, 1978; Krieger et al., 1976; Mermelstein and Becker, 1995; Peirce & Nuttall, 1961a; Yang and Clemens, 1994, 1995a, & b, 1996a, b, & c). One possible function of the temporal copulatory pattern of female rats is to facilitate successful pregnancy. This is supported by the evidence that fewer intromissions are sufficient to induce the progestational state of pregnancy when the female rat controls the copulatory speed (Erskine et al., 1989; Gilman et al., 1979). Factors including gonadal hormones (Fadem et al., 1979; Gilman and Hitt, 1978), preejaculatory intromissions (Yang and Clemens, 1994, 1996a), and the ejaculation duration (Yang and Clemens, 1995b, 1996b) have been reported to influence the temporal copulatory pattern in female rats. However, what brain regions control the timing of female copulation is poorly understood.

The medial preoptic area of hypothalamus (MPOA) has been reported to play an important role in the regulation of female sexual behavior in rats. The MPOA lesions increase the lordosis response in female rats (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). The lordosis response of female rats is also facilitated after

transections made anterior-dorsal to the MPOA (Yamanouchi and Arai, 1977). Based on these results, it has been suggested that the MPOA plays an inhibitory role in the regulation of female lordosis behavior (Powers and Valenstein, 1972). However, MPOA lesions dramatically decrease the time that the female spends with the sexually active males when she can choose among sexually active males, castrated males and females (Whitney, 1986). This finding suggests that the MPOA may play a role in facilitating female sexual behavior. It is not known, however, whether the MPOA facilitates or inhibits the temporal pattern of female sexual behavior. The study of whether and how the MPOA regulates the temporal pattern of female sexual behavior is important at least in two aspects. First, it provides further understanding of how the MPOA controls some other aspects of female sexual behavior in addition to lordosis behavior. Secondly, it elucidates whether the same brain region regulates the temporal pattern of male and female sexual behavior in a parallel manner and allows comparison of the role of the same brain region in the male and female sexual behavior under similar measurement scales.

Vaginocervical stimulation plays an important role in the regulation of the temporal pattern of female rat sexual behavior. Following bilateral transection of the pelvic nerve and the pudendal nerve that carry sensory stimulation from the vagina, cervix, uterus, perineal skin, and clitoral sheath (Berkley et al., 1990; Berkley et al., 1993; Komisaruk et al., 1972; Peters et al., 1987), females do not distinguish among mounts, intromissions, and ejaculations; the return latencies following mounts, intromissions and ejaculations are not significantly different from one another (Erskine, 1992). In addition, anesthetics applied locally to the vagina of the female rats reduced their contact-response intervals following intromissions and ejaculations (Westbrook and Bermant, 1966). Based on these results, it is suggested that vaginocervical stimulation conveyed via the pelvic and pudendal nerves is important for the temporal pattern of female sexual behavior. Furthermore, evidence shows that the neurons in MPOA are activated during copulation. Vaginocervical stimulation provided by a male rat during copulation or a glass rod by the experimenter

significantly increases the c-fos expression in the MPOA of female rats (Pfaus et al., 1993; Wersinger et al., 1993). Mechanical stimulation of uterine cervix excites MPOA neurons (Haskins and Moss, 1983). That the neurons in the MPOA are activated during copulation and that vaginocervical stimulation is associated with the temporal copulatory events of female rats together suggest that the MPOA is a potential candidate in the regulation of the temporal pattern of female sexual behavior.

The MPOA also plays an important role in the regulation of male rat sexual behavior. It has been reported that copulation significantly induces c-fos expression in the MPOA of male rats (Baum and Everitt, 1992; Wersinger et al., 1993). In addition, large MPOA lesions abolished male sexual behavior (Giantonio et al., 1970; Ginton and Merari, 1977; Heimer and Larsson, 1966/1967; Hendricks and Scheetz, 1973; Lisk, 1968). It is also true that some males with large MPOA lesions sparing part of MPOA can still copulate although their interintromission interval (III), ejaculation latency (EL) and postejaculatory interval (PEI) lengthen (Ginton and Merari, 1977). Further, electrical stimulation of the MPOA facilitates male rat copulatory behavior (Malsbury, 1971). The above evidence suggests that the MPOA facilitates the temporal pattern of male sexual behavior in rats. Whether the MPOA exerts a parallel effect on the temporal pattern of female sexual behavior is not understood.

The objective of this study was to investigate the role of MPOA in the regulation of the temporal pattern of female sexual behavior in rats. In addition, we used the data collected in this study to determine whether the MPOA regulates the temporal aspects of male and female sexual behavior in a parallel manner. Experiment 5A examined the influence of electrolytic lesions of MPOA in the regulation of the temporal pattern of female rat sexual behavior. Experiment 5B investigated the effect of chemical (ibotenic acid) lesions of MPOA on the female pacing behavior in rats.

GENERAL METHODS

Animals

Sixty-day-old female and ninety-day-old male Long Evans rats were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). All females were ovariectomized one week later upon arrival. Animals were housed in 16:8 hr light-dark cycle with lights off at 17:00. Females were housed three per cage and males were housed singly. Food and water were available ad lib. The temperature and humidity of the animal room were maintained at 22^0 C and 55%, respectively. The air in the animal room was exchanged 12 times per hour automatically. Ovariectomized females were brought into estrus by intramuscular (IM) injections of 0.5 μg estradiol benzoate (EB) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil (Sigma Chemical Co., St. Louis, MO) for three consecutive days and 24 hours later followed by an IM injection of 0.5 mg progesterone (P) (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil. All female pacing behavior was tested 4-7 hours after the injection of progesterone (2-5 hours after the lights off) under the dimly red illumination. All animals were tested once per week throughout the whole test period and had been tested twice in the testing chamber before data were collected. Females that did not exit the male's chamber at all during the two experience tests were excluded from further studies. Only those females (over 95 %) showing pacing behavior in these preliminary tests were used in the experimental studies. In each test, a male was always placed in the testing chamber 15 minutes before a female was introduced. The approximate weights of the female and male rats were 200 - 300 g and 500 - 550 g, respectively, when the tests started.

Pacing Tests

To provide a test situation in which the female controlled the pacing of the copulation, females were tested in a two-compartment chamber which was divided by a Plexiglas barrier with 4 holes (3.5~4 cm x 3.5~4 cm) spaced along the bottom of the barrier. The dimensions of the male's chamber and escape chamber were 35.5 cm x 44.5 cm x 48.3 cm and 21.5 cm x 44.5 cm x 48.3 cm (Length x Width x Height), respectively. The escape holes were large enough to allow the female to pass through but prevented the larger male from following her.

Behavioral Measures

Several behavioral measures were recorded in the pacing tests: approach latency (AL), the latency for the female to enter the male's chamber after the start of the test (she was placed in the escape chamber at the beginning of the test); intromission latency (IL), the latency from the female's entry into the male's chamber to the first intromission; mount return latency (MRL), the latency for the female to re-enter the male's chamber after a mount; intromission return latency (IRL), the latency to return to the male's chamber following an intromission; postejaculatory refractory period (PER), the latency to re-enter the male's chamber after an ejaculation. All latencies were measured in seconds. The frequency with which the female left the male's chamber following a mount, intromission and ejaculation (percentage exit) was calculated by dividing the number of exits by the number of each type of stimulus. In addition, the female's lordosis behavior was recorded under paced situation and non-paced situation. Under paced situation, the female was allowed to copulate until she received an ejaculation and returned to the male's chamber following her PER. If the female did not leave the male's chamber following ejaculation, the test was ended 60 seconds after ejaculation. In addition, if the female did not receive an

ejaculation due to the long return latencies following intromissions within 15 minutes after the start of the test, the test was terminated. Immediately following paced test, the lordosis quotient of female rats was obtained by allowing the female to receive 10 mounts under non-paced situation (The barrier separating the male's chamber and the escape chamber was removed). The female lordosis quotient was calculated by the formula: (number of lordosis during the first 10 mounts / 10) x 100 or (number of lordosis / number of mounts) x 100 when the number of mounts was smaller than 10. All behavioral data were recorded on a computer disk.

EXPERIMENT 5A: INFLUENCE OF ELECTROLYTIC MPOA LESIONS ON THE FEMALE PACING BEHAVIOR

The objective of this experiment was to examine the effect of electrolytic lesions of MPOA on the temporal pattern of female sexual behavior in rats. It has been reported that the MPOA lesions potentiate the lordosis behavior (Hoshina et al., 1994; Whitney, 1986) and shorten the time that the female spends with the sexually active males during copulation (Whitney, 1986). In this experiment, we investigated the influence of electrolytic lesions (1.5 mA current for 20 seconds) of MPOA on the temporal copulatory pattern of female rats.

METHOD

Procedure:

Thirty-two male and 32 female Long Evans rats were used in this study. The MPOA coordinates (B: - 0.7 mm, L: $\pm$ 0.5 mm, V: - 8.2 mm) were determined empirically. Animals were randomly assigned into two groups: the MPOA lesioned group and the control group. In the MPOA lesioned group, females received bilateral electrolytic lesions (1.5 mA current for 20 seconds) of MPOA. The electrolytic lesions of MPOA were made

by Tungsten microelectrode with 8 degree tapered tip (diameter: 500 μm, catalog #5770, A-M Systems, Inc., Everett, WA) connected to 3500 Lesion Producing Device (Stoelting Co., Wood Dale, IL) (N=21). In the control group, the females received anesthesia and bilateral electrode insertion into the MPOA without passing any current (N=11).

In a three-week test period, the temporal pattern of copulation for each female was tested before surgery, and two weeks after surgery. Each female was randomly assigned to copulate with one male until she received an ejaculation and returned to the male's chamber following her PER in the first week of this experiment. Following the prelesion test, females received either MPOA lesions (N = 21) or sham lesions (N = 11). Throughout the rest of the test, each female was tested with the same male she mated in the first week of this experiment. One week after surgery, the female was allowed to have one post surgery experience test (without recording data) in female paced situations. Two weeks after surgery, each female was tested for the temporal pattern of female sexual behavior until she received an ejaculation and returned to the male's chamber following her PER. The temporal pattern of female sexual behavior was recorded and analyzed before surgery and two weeks after surgery. In the MPOA lesioned group, only those animals with more than 50 % of the MPOA destroyed on both sides were used for analysis.

Histology:

Following postsurgical behavioral tests (two weeks after surgery), all females were anesthetized with mixtures of Ketamine (44 mg/kg) and Xylazine (10 mg/kg) (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and perfused intracardially with 0.87% saline followed by 10% formalin. Brains were removed and post-fixed in 10% formalin for overnight. The brains were then transferred to 20% sucrose solution for cryoprotection

until they sank. Frozen coronal sections (50 μm) were taken and the lesioned site was verified following Nissl staining.

Statistics:

The data of the female's percentage exits following mount, intromission, and ejaculation were transformed by arc sine square root before analysis (Dixon and Massey, 1969). The measures of the temporal pattern of female sexual behavior obtained before surgery and two weeks after surgery were analyzed by Two Way Repeated Measures ANOVA followed by SNK post-hoc test. An alpha level of 0.05 was used for all statistical tests.

RESULTS

Electrolytic lesions of the MPOA destroyed an area of approximately 900 μm in the rostrocaudal direction and were limited to a region under the anterior commissure (Figure 17). Eight of 21 females that had more than 50% of the MPOA destroyed on both sides were used for analysis. In these 8 cases, large portions of bilateral MPOA and periventricular hypothalamic nucleus were damaged. In addition, variable amounts of septohypothalamic nucleus, parastrial nucleus, bed nucleus of the stria terminalis, lateral preoptic area, striohypothalamic nucleus, supraoptic nucleus and suprachiasmatic nucleus were destroyed. The optic chiasm was absent in the brain tissue of the electrolytic MPOA lesioned animals. This absence of the optic chiasm may not have resulted from the electrolytic lesions because the MPOA animals did not appear to have difficulty in seeing during behavioral test.

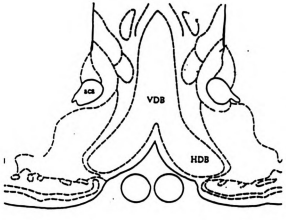
The mean return latencies following an intromission and an ejaculation in the

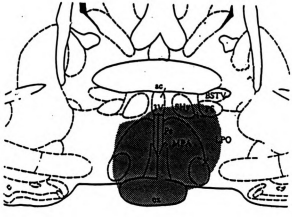
MPOA lesioned and sham-lesioned groups before surgery and after surgery are summarized in Figures 18 and 19, respectively. One of 8 MPOA lesioned females with destruction of both sides of MPOA larger than 50% was excluded from the PER analysis because the female's IRL was very long and the test was terminated before the male ejaculated. All the data are represented as mean $\pm$ SEM in seconds.

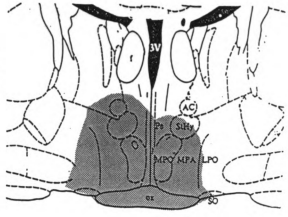
Two Way Repeated Measures ANOVA revealed a significant group effect [$F(1, 17) = 19.481$, $p = 0.0004$], surgery effect [$F(1, 17) = 16.652$, $p = 0.0008$], as well as group x surgery interaction [$F(1, 17) = 22.033$, $p = 0.0002$] for the female's IRL. The SNK post-hoc tests revealed that the postsurgical IRL of females with MPOA lesions was significantly longer than the other IRLs ($p<0.01$) (Figure 18).

Two Way Repeated Measures ANOVA also revealed a significant group effect [$F(1, 16) = 9.828$, $p = 0.0064$], surgery effect [$F(1, 16) = 7.868$, $p = 0.0127$], as well as group x surgery interaction [$F(1, 16) = 11.094$, $p = 0.0042$] for the female's PER. The SNK post-hoc tests revealed that the postsurgical PER of females with MPOA lesions was significantly longer than the other PERs ($p<0.01$) (Figure 19). The lordosis quotient of females tested under paced situation was not different from that tested under nonpaced situation, nor was there a significant difference in the female's lordosis quotients before surgery and after surgery. None of the other behavioral measures differed significantly before surgery and after surgery in the MPOA lesioned and control groups. Thirteen of 21 females receiving bilateral electrolytic MPOA lesions had one or both sides of MPOA damaged less than 50%. These females appeared to show normal temporal copulatory behavior.

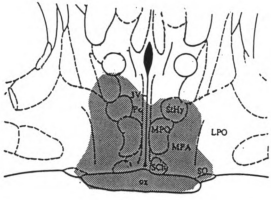
Figure 17. Composite diagrams of largest (shaded area) electrolytic MPOA lesions. Figures and nomenclature from Paxinos and Watson (1986). The Bregma coordinates for each figure on top row from left to right are + 0.20 mm, - 0.30 mm, and - 0.80 mm. The Bregma coordinates for each figure on bottom row from left to right are - 0.92 mm, and - 1.30 mm. 3V: 3rd ventricle; ac: anterior commissure; aca: anterior commissure, anterior; AHA: anterior hypothalamic area, anterior; BSTMPL: bed nucleus of the stria terminalis, medial division, posterolateral part; BSTV: bed nucleus of stria terminalis, ventral division; f: fornix; HDB: nucleus of the horizontal limb of the diagonal band of Broca; LA: lateroanterior hypothalamic nucleus; LH: lateral hypothalamic area; LPO: lateral preoptic area; MPA: medial preoptic area; MPO: medial preoptic nucleus; ox: optic chiasm; PaAP: paraventricular hypothalamic nucleus, anterior parvocellular part; Pe: periventricular hypothalamic nucleus; PS: parastrial nucleus; SCh: suprachiasmatic nucleus; SHy: septohypothalamic nucleus; StHy: striohypothalamic nucleus; SO: supraoptic nucleus; VDB: nucleus of the vertical limb of the diagonal band of Broca.


VDB
HDB


BSTV
Px
MPA
LPO


3V
AC
Px
SIHy
MPO
MPA
LPO
SO

1mm


3V
Px
SIHy
MPO
LPO
MPA
SCh
SO

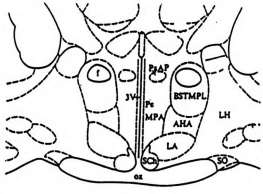

PrAF
3V
Px
MPA
BSTMPL
AHA
LH
LA
SCh
SO

Figure 18. summarizes the female's return latencies following an intromission (intromission return latency, IRL) before surgery and two weeks after surgery in the MPOA electrolytic lesioned group (N = 8) and the MPOA sham-lesioned group (N = 11). Two Way Repeated Measures ANOVA revealed a significant group effect [$F(1, 17) = 19.481$, $p = 0.0004$], surgery effect [$F(1, 17) = 16.652$, $p = 0.0008$], as well as group x surgery interaction [$F(1, 17) = 22.033$, $p = 0.0002$] for the female's IRL. The SNK post-hoc tests revealed that the postsurgical IRL of females with MPOA electrolytic lesions was significantly longer than the other IRLs ($p<0.01$). ** $p<0.01$.

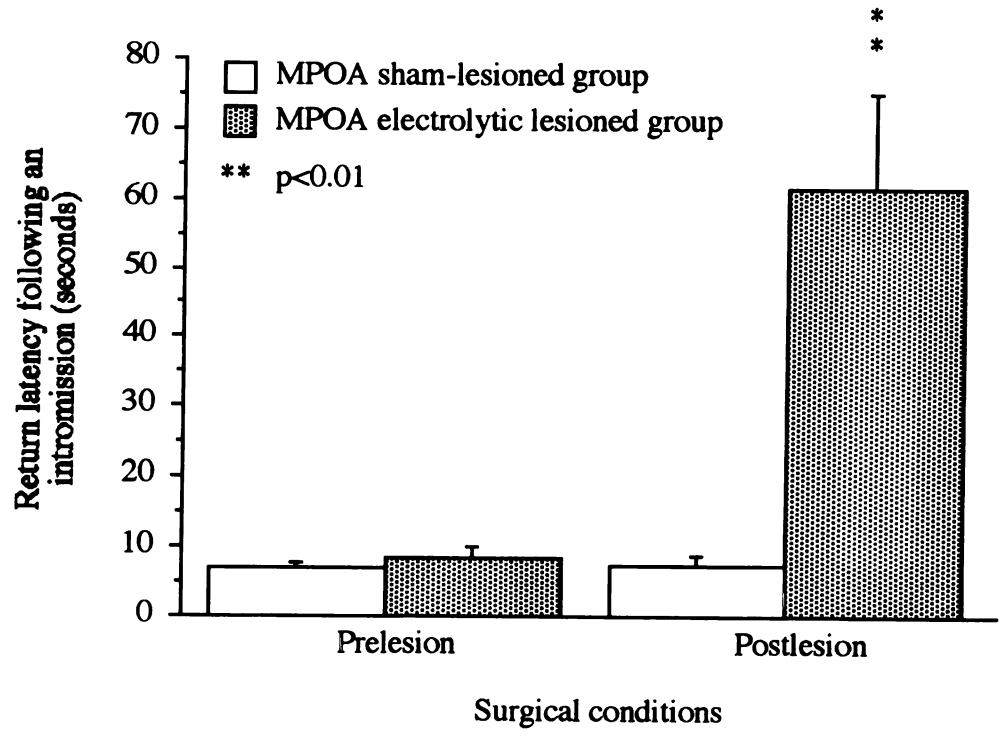
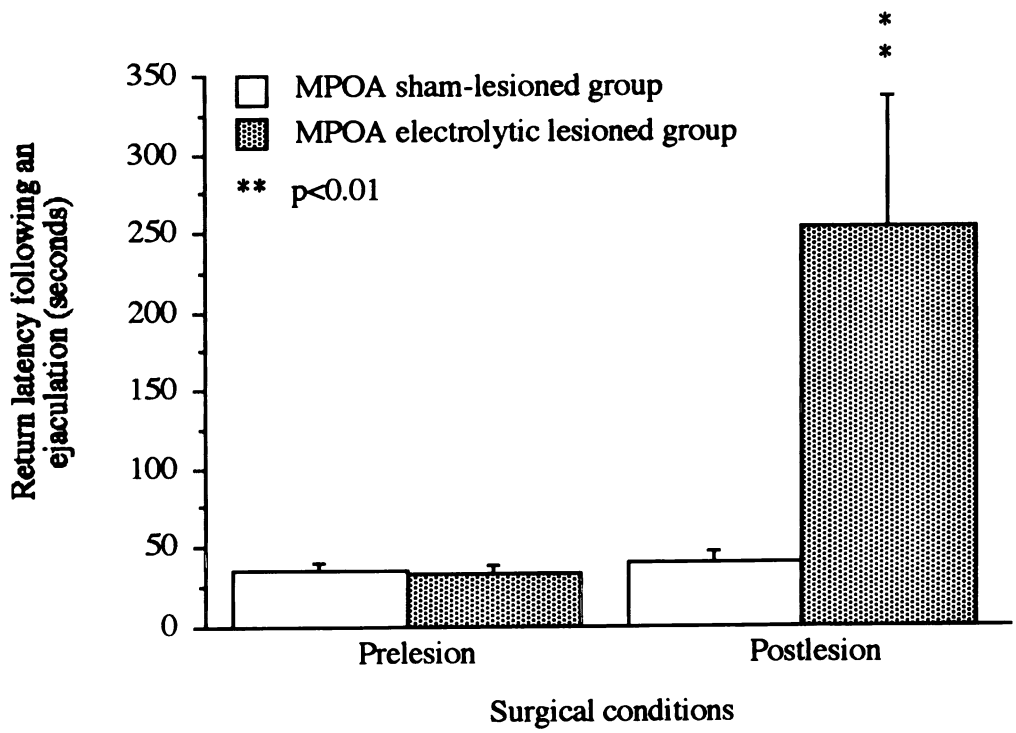

80
70
60
50
40
30
20
10
0
MPOA sham-lesioned group
MPOA electrolytic lesioned group
** p<0.01
* *
Return latency following an intromission (seconds)
Prelesion
Postlesion
Surgical conditions

Figure 19. shows the female's return latencies following an ejaculation (postejaculatory refractory period, PER) before surgery and two weeks after surgery in the MPOA electrolytic lesioned group (N = 7) and the MPOA sham-lesioned group (N = 11). Two Way Repeated Measures ANOVA revealed a significant group effect [$F(1, 16) = 9.828$, $p = 0.0064$], surgery effect [$F(1, 16) = 7.868$, $p = 0.0127$], as well as group x surgery interaction [$F(1, 16) = 11.094$, $p = 0.0042$] for the female's PER. The SNK post-hoc tests revealed that the postsurgical PER of females with MPOA lesions was significantly longer than the other PERs ($p<0.01$). ** $p<0.01$.


MPOA sham-lesioned group
MPOA electrolytic lesioned group
** p<0.01
Return latency following an ejaculation (seconds)
350
300
250
200
150
100
50
0
*
*
Prelesion
Postlesion
Surgical conditions

EXPERIMENT 5B: EFFECT OF CHEMICAL MPOA LESIONS ON THE FEMALE TEMPORAL COPULATORY BEHAVIOR

The objective of this experiment was to examine whether destruction of cell bodies in the MPOA but sparing the fibers passing through MPOA mimics the effects produced by electrolytic lesions of MPOA. In Experiment 5A, electrolytic lesions of MPOA significantly increased the female's PER and IRL. Both cell bodies in the MPOA and the fibers passing through that area can be destroyed by electrolytic lesions. The influence of electrolytic lesions of MPOA in the regulation of the temporal pattern of female sexual behavior may result from destruction of MPOA cell bodies and/or the fibers passing through that area. In contrast, chemical lesions can only destroy the cell bodies and spare the passing fibers (Schwarcz et al., 1979; Schwarcz et al., 1979). In this experiment, chemical (ibotenic acid) lesions of MPOA were performed in the females and the temporal pattern of female sexual behavior was recorded before surgery and two weeks after surgery.

METHOD

Procedure:

Nineteen female and nineteen male Long Evans rats were used in this experiment. Except for lesion procedures, the procedures in this experiment were the same as those of Experiment 5A. The MPOA microinjection coordinates (B: - 0.7 mm, L: $\pm$ 0.5 mm, V: - 7.6 mm) were aimed at the top of the MPOA and determined empirically. Animals were randomly assigned into two groups: the MPOA lesioned group (N = 10) and the control group (N = 9). In the MPOA lesioned group, the females received bilateral microinjection of ibotenic acid solution (10 μg ibotenic acid per μl 0.1 M phosphate buffer solution at pH

7.40) into MPOA via 1 μl microsyringe connected to a 26 gauge steel needle. A volume of 0.4 μl of ibotenic acid solution was microinjected into each site of MPOA via a microprocessor controlled syringe pump (Model 53100, Stoelting Co., Wood Dale, IL) over a 10-minute period. The syringe was left in place for another 10 minutes to avoid backflow of ibotenic acid into the needle track. In the control group, a volume of 0.4 μl 0.1 M phosphate buffer solution (pH 7.40) instead of ibotenic acid solution was microinjected into each site of MPOA via a microprocessor controlled syringe pump over a 10-minute period. The rest of the procedures were the same as those in the MPOA lesioned group.

The temporal pattern of female sexual behavior and lordosis responses were tested before surgery, and two weeks after surgery. In the MPOA lesioned group, only those females with chemical MPOA lesions destroying more than 50% of MPOA at both sides were included for analysis. The data obtained before surgery and two weeks after surgery were used for analysis.

Histology:

Following postsurgical behavioral tests (two weeks after surgery), all females were anesthetized with mixtures of Ketamine (44 mg/kg) and Xylazine (10 mg/kg) (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and perfused intracardially with 0.87% saline followed by 10% formalin. Brains were removed and post-fixed in 10% formalin for overnight. The brains were then transferred to 20% sucrose solution for cryoprotection until they sank. Frozen coronal sections (50 μm) were taken and the lesioned site was verified following Nissl staining.

Statistics:

The data of the female's percentage exits following mount, intromission, and ejaculation were transformed by arc sine square root before analysis (Dixon and Massey, 1969). The measures of the temporal pattern of female sexual behavior obtained before surgery and two weeks after surgery were analyzed by Two Way Repeated Measures ANOVA followed by SNK post-hoc test. An alpha level of 0.05 was used for all statistical tests.

RESULTS

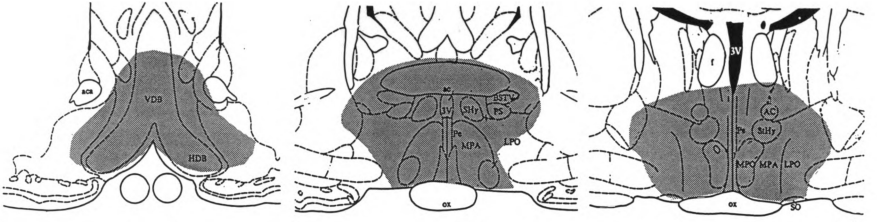
The ibotenic acid lesions of MPOA were characterized by disappearance of neurons and an enlarged third ventricle in thionin stained sections. Eight of 10 females had bilateral ibotenic lesions that were centered in the MPOA at the level of optic chiasm and destroyed more than 50% of MPOA on both sides. The ibotenic acid lesions damaged an area of approximately 1,700 μm in the rostrocaudal direction and were limited between the anterior commissure and the optic chiasm (Figure 20). The lesions included most of MPOA in these 8 animals. In addition, the lesions also covered variable amounts of the periventricular hypothalamic nucleus, septohypothalamic nucleus, parastrial nucleus, bed nucleus of stria terminalis, striohypothalamic nucleus, supraoptic nucleus, suprachiasmatic nucleus, lateral preoptic area, diagonal band, and the anterior hypothalamus. The lesions extended anteriorly into part of diagonal band and posteriorly into part of anterior hypothalamus. Two of these 8 females with MPOA lesions covering the entire MPOA as well as anterior commissure and extending laterally to a large portion of lateral preoptic area did not approach the male during the 15-minute postsurgery test. In contrast, two of 10 females had MPOA lesions less than 50% and were excluded from analysis.

Figure 21 summarizes the mean IRLs of the females with MPOA sham lesions and the females with MPOA ibotenic acid lesions before and after surgery. Two females with extensive ibotenic acid MPOA lesions including LPO were excluded from the IRL analysis because the female did not approach the male during 15-minute test period. These two females showed normal motor activity and moved around in the escape chamber during the 15-minute test period. In addition, while they often rejected the male, they showed normal lordosis behavior when they were mounted by the male after the barrier was removed. All the data are represented as mean $\pm$ SEM in seconds.

Two Way Repeated Measures ANOVA revealed a significant group effect [$F(1, 13) = 12.296$, $p = 0.0039$], surgery effect [$F(1, 13) = 10.510$, $p = 0.0064$], as well as group x surgery interaction [$F(1, 13) = 18.408$, $p = 0.0009$] for the female's IRL. The SNK post-hoc tests revealed that the postsurgical IRL of females with MPOA ibotenic acid lesions was significantly longer than the other IRLs ($p<0.01$) (Figure 21).

The female's PERs before and after surgery in the sham group and the MPOA ibotenic acid group are summarized in Figure 22. The two females with extensive MPOA ibotenic acid lesions that did not approach the male during the 15-minute test period were excluded from the PER analysis. There were a significant group effect [$F(1, 13) = 39.933$, $p < 0.0001$], surgery effect [$F(1, 13) = 30.108$, $p = 0.0001$], as well as group x surgery interaction [$F(1, 13) = 36.848$, $p < 0.0001$] for the female's PER. The SNK post-hoc tests revealed that the postsurgical PER of females with MPOA ibotenic acid lesions was significantly longer than the other PERs ($p<0.01$) (Figure 22). The lordosis quotient of females tested under the female paced situation was not different from that under the nonpaced situation. There was no significant difference in the female's lordosis quotient before and after surgery. For the other behavioral measures, there was also no significant difference before and after surgery in the MPOA lesioned and control groups.

Figure 20. Composite diagrams of largest (shaded area) ibotenic acid MPOA lesions. Figures and nomenclature from Paxinos and Watson. The Bregma coordinates for each figure on top row from left to right are + 0.20 mm, - 0.30 mm, and - 0.80 mm. The Bregma coordinates for each figure on bottom row from left to right are - 1.30 mm, and - 1.80 mm. 3V: 3rd ventricle; ac: anterior commissure; aca: anterior commissure, anterior; AHA: anterior hypothalamic area, anterior; AHC: anterior hypothalamic area, central part; BSTMPL: bed nucleus of the stria terminalis, medial division, posterolateral part; BSTV: bed nucleus of stria terminalis, ventral division; f: fornix; HDB: nucleus of the horizontal limb of the diagonal band of Broca; LA: lateroanterior hypothalamic nucleus; LH: lateral hypothalamic area; LPO: lateral preoptic area; MPA: medial preoptic area; MPO: medial preoptic nucleus; opt: optic tract; ox: optic chiasm; PaAP: paraventricular hypothalamic nucleus, anterior parvocellular part; Pe: periventricular hypothalamic nucleus; PS: parastrial nucleus; RCh: retrochiasmatic area; SCh: suprachiasmatic nucleus; SHy: septohypothalamic nucleus; StHy: striohypothalamic nucleus; SO: supraoptic nucleus; sox: supraoptic decussation; VDB: nucleus of the vertical limb of the diagonal band of Broca.


VDB
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ac
3V
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BSTV
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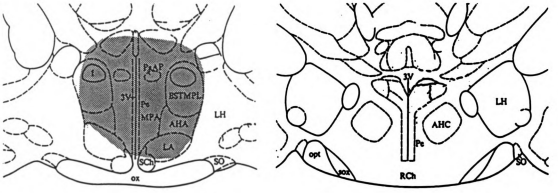
PMP
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LH
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AHC
opt
RCh

Figure 21. The female's return latencies following an intromission (intromission return latency, IRL) before surgery and two weeks after surgery in the MPOA chemical lesioned group (N = 6) and the MPOA sham-lesioned group (N = 9). Two Way Repeated Measures ANOVA revealed a significant group effect [$F(1, 13) = 12.296$, $p = 0.0039$], surgery effect [$F(1, 13) = 10.510$, $p = 0.0064$], as well as group x surgery interaction [$F(1, 13) = 18.408$, $p = 0.0009$] for the female's IRL. The SNK post-hoc tests revealed that the postsurgical IRL of females with MPOA ibotenic acid lesions was significantly longer than the other IRLs ($p<0.01$). ** $p<0.01$.

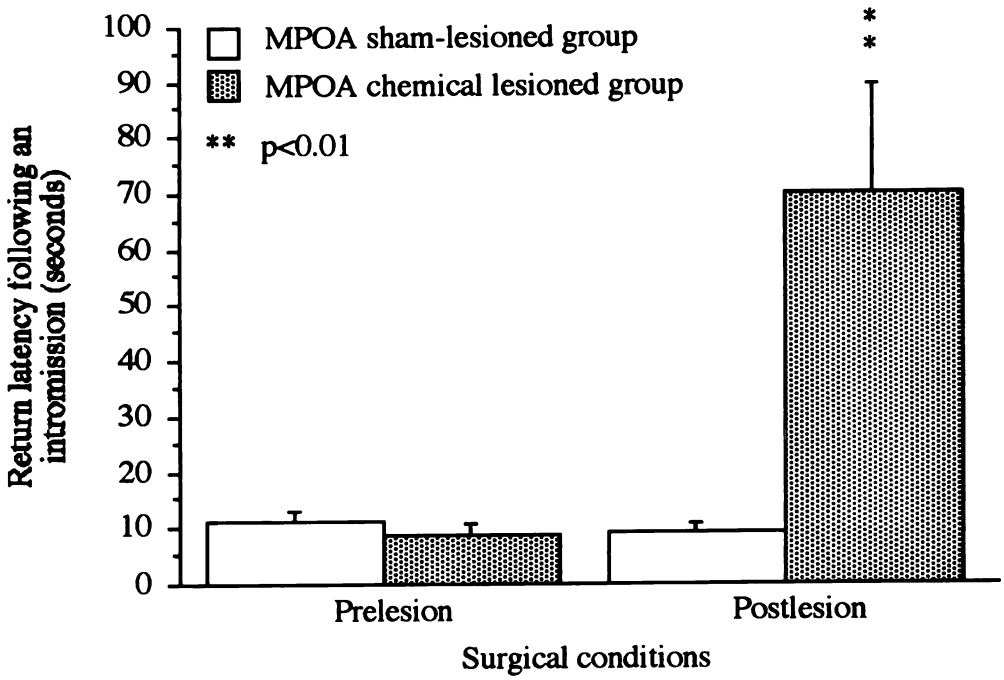
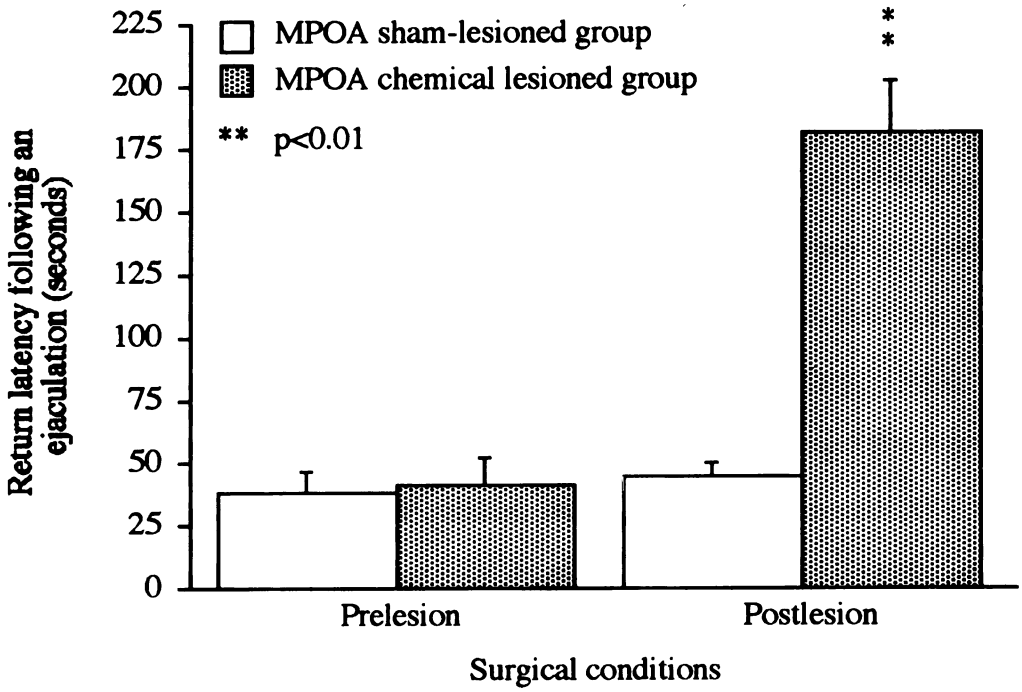

MPOA sham-lesioned group
MPOA chemical lesioned group
** p<0.01
Return latency following an intromission (seconds)
100
90
80
70
60
50
40
30
20
10
0
**
Prelesion
Postlesion
Surgical conditions

Figure 22. shows the female's return latencies following an ejaculation (postejaculatory refractory period, PER) before surgery and two weeks after surgery in the MPOA chemical lesioned group (N = 6) and the MPOA sham-lesioned group (N = 9). There were a significant group effect [$F(1, 13) = 39.933$, $p < 0.0001$], surgery effect [$F(1, 13) = 30.108$, $p = 0.0001$], as well as group x surgery interaction [$F(1, 13) = 36.848$, $p < 0.0001$] for the female's PER. The SNK post-hoc tests revealed that the postsurgical PER of females with MPOA chemical (ibotenic acid) lesions was significantly longer than the other PERs ($p<0.01$). ** $p<0.01$.


225
200
175
150
125
100
75
50
25
0
MPOA sham-lesioned group
MPOA chemical lesioned group
** p<0.01
**
Return latency following an ejaculation (seconds)
Prelesion
Postlesion
Surgical conditions

DISCUSSION

One of the major findings in this study is that the neurons in the MPOA influence the temporal pattern of female sexual behavior in rats. In Experiment 5A, electrolytic lesions of MPOA significantly increased the female's IRL and PER. This effect appears to result from destruction of cell bodies not fibers of passage since similar results were seen in Experiment 5B, using ibotenic acid lesions, which destroyed neurons in the MPOA but spared fibers of passage (Schwarcz et al., 1979; Schwarcz et al., 1979).

Sexual behavior of female rats has at least three major components: lordosis behavior, soliciting behavior and the temporal pattern of the female's approach to the male. The preoptic area appears to play a role in the occurrence of each of these components. For lordosis, it has been reported that MPOA lesions significantly increase the lordosis responding in female rats treated with low doses of estradiol benzoate plus progesterone (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). In the present study, the female's lordosis behavior did not change following MPOA lesions, due to the fact that the female's lordosis quotient was already high (around 90% to 100%) before surgery under the hormone replacement paradigm used in this laboratory (Clemens et al., 1981; Richmond and Clemens, 1986; Richmond and Clemens, 1988). Based on the finding that MPOA lesions increase lordosis frequency in female rats treated with low doses of EB plus P, it has been suggested that the MPOA exerts an inhibitory effect on female sexual receptivity (lordosis behavior) (Powers and Valenstein, 1972).

As for soliciting behavior, MPOA lesions did not affect the soliciting behavior (the frequency of hopping, darting and ear wiggling) in female rats under non-paced situations (Whitney, 1986). This is consistent with the results of a later study (Hoshina et al., 1994). However, under female-paced situations, MPOA lesions decreased soliciting behavior in female rats (Whitney, 1986).

With regard to the female's approach to the male, MPOA lesions have been demonstrated to shorten the time that the female spends with the sexually active males when she has the opportunity to choose among the sexually active males, castrated males, or females (Whitney, 1986). In the present study, we found that the MPOA lesions significantly increase the female's IRL and PER. This is consistent with the idea that the MPOA facilitates the female's approach to the male during copulation. Thus, it appears that the MPOA inhibits the lordosis responding when estrogen is low and facilitates the initiation of sexual interaction at higher levels of estrogen priming.

The MPOA may be important for facilitating sexual motivation in female rats. A distinction between sexual desire (sexual motivation) and sexual ability has been proposed (Wallen, 1990). Female sexual ability refers to the female's ability to engage in copulation. Female sexual motivation refers to "the state underlying sexual initiation and sexual accommodation" (p.234, Wallen, 1990). In female rats, the display of the lordosis response, the arching of the back and the raising of the hips and genitalia, is necessary for successful penile insertion (Diakow, 1974; Pfaff et al., 1978). Consequently, occurrence of the lordosis response has been used to measure the female's sexual ability. On the other hand, "female sexual motivation controls female availability and the temporal patterning of copulation" (p. 235, Wallen, 1990). Within this context, the increased IRL and PER can be interpreted as a decrease in sexual desire. In the present study, two females with extensive MPOA ibotenic acid lesions did not approach the male during 15-minute test but they showed motor activity with no apparent deficit and normal lordosis reflexes when mounted by a male after the barrier was removed. This suggests that the sexual motivation of these two females was absent but their sexual ability was unaffected. In male rats, the interintromission interval is often considered as an index of rearousal rate of his sexual motivation following a brief refractory period after an intromission (Beach, 1956; Meisel and Sachs, 1994). Correspondingly, in female rats, the female's IRL can be a measure for rearousal rate of her sexual motivation following a brief refractory period after an

intromission. Therefore, increases in the female's IRL as seen in the present study imply a decrease in her sexual arousal.

Another important finding in this study is that the MPOA regulates the temporal pattern of male and female sexual behavior in a similar manner. Traditionally, different measurement scales were used to study the male and female sexual behavior. In male rats, the frequency of sexual contacts (mount frequency, intromission frequency, and ejaculation frequency) and the latency of sexual contacts (mount latency, intromission latency, ejaculation latency, interintromission interval, and postejaculatory interval) were used to measure male sexual behavior (Meisel and Sachs, 1994). In female rats, the lordosis response to the male's mounting behavior was the major measure of female sexual behavior (Pfaff et al., 1994). Use of different measurement scales for male and female behavior has made comparison between sexes difficult. In the present study, we obtain measures for the female that are operationally similar to those used in studies of male sexual behavior. The female's intromission return latency is equivalent of the male's interintromission interval. The female's postejaculatory refractory period is equivalent of the male's postejaculatory interval. The increase of the IRL and PER in female rats following MPOA lesions parallels the increase of the interintromission interval and postejaculatory interval in male rats that still copulate following MPOA lesions sparing part of MPOA (Ginton and Merari, 1977). In addition, two females with MPOA ibotenic acid lesions did not approach the male during 15-minute test period. However, they displayed normal lordosis behavior when they were mounted by the male after the barrier was removed following 15-minute paced test. Likewise, males with large MPOA lesions did not copulate after lesion (Ginton and Merari, 1977; Heimer and Larsson, 1966/1967). These data together strongly support the idea that the MPOA regulates the temporal pattern of male and female sexual behavior in a parallel manner.

In addition to the temporal copulatory pattern, the MPOA regulates the lordosis behavior and mounting behavior of sexual behavior similarly in both male and female rats.

It is well documented that MPOA lesions facilitate lordosis behavior in female rats (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). Similarly, it is demonstrated that MPOA lesions facilitate lordosis in castrated male rats treated with estradiol benzoate and progesterone (Hennessey et al., 1986; Olster, 1993). In addition, large MPOA lesions abolished male sexual behavior in male rats (Heimer and Larsson, 1966/1967). Mounting behavior is the key feature of male rat sexual behavior. Therefore, it is doubtless to infer that MPOA lesions decrease mounting in male rats. Likewise, MPOA lesions have been reported to decrease mounting in female rats treated with testosterone (Singer, 1968). All the above evidence taken together suggests that the MPOA regulates the lordosis behavior and mounting behavior of male and female sexual behavior in a parallel manner.

In summary, we demonstrate that destruction of neurons in the MPOA rather than destruction of the fibers of passage significantly increases the female's IRL and PER. This finding suggests that neurons in the MPOA facilitate the temporal pattern of female rat sexual behavior. This is also consistent with the previous report that MPOA lesions significantly decreased the time that the female rat spent with the sexually active males. In addition, the MPOA regulates the temporal pattern of male and female sexual behavior in a similar manner. This is supported by the evidence that MPOA lesions significantly lengthen the III and PEI in male rats that still copulate and dramatically increase the IRL and PER in female rats. Moreover, in addition to the temporal copulatory pattern, the MPOA regulates the lordosis response and the mounting behavior of male and female rats in a like manner. Finally, following MPOA lesions, the dramatic increase in the female's IRL and the decrease in the time that the female spent with the sexually active males following MPOA lesions strongly support the idea that the MPOA plays an important role in facilitating female sexual motivation in rats.

GENERAL DISCUSSION

The present studies demonstrated that preejaculatory intromissions, the male's ejaculation duration, and variation in gonadal hormones affected the temporal pattern of female rat's sexual behavior. As intromission frequency prior to ejaculation increased, the male's ejaculation duration also increased and this was associated with an increase in the female's PER. In addition, uterine EMG activity also changed with the type of stimulus that the female received during copulation. The duration of the uterine contractions was longer during ejaculation than during intromission or mount. Finally, we demonstrated that the MPOA played a role in the regulation of the temporal pattern of female sexual behavior. Lesions (both electrolytic and chemical) of MPOA significantly increased the female's return latencies following intromission and ejaculation. This supports the idea that the MPOA facilitates female sexual motivation.

Significance of Female Temporal Sexual Behavior

The temporal pattern of female rat's sexual behavior has both behavioral and physiological significance. When the female rat regulates the timing of mating, the copulatory speed is slower (Erskine, 1985; Erskine et al., 1989; Fadem and Barfield, 1982; Gilman et al., 1979) and fewer intromissions are needed to induce the progestational state of pregnancy (Erskine et al., 1989; Gilman et al., 1979). In female paced situations, several studies have reported that five intromissions are sufficient to induce secretion of progesterone to a level sufficient for maintaining successful pregnancy (Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). In contrast, in male paced tests, approximately

10 intromissions were required for the induction of the progestational state of pregnancy (Adler, 1969; Chester and Zucker, 1970; Edmonds et al., 1972; Erskine, 1989; Erskine et al., 1989; Gilman et al., 1979). Intromissions were more effective in shortening the behavioral estrus of female rats in female paced situations than in nonpaced situations. In female paced tests, 10 intromissions were adequate to shorten the behavioral estrus (Erskine, 1985; Erskine and Baum, 1982; Erskine et al., 1989). In contrast, in male paced situations, shortening of behavioral estrus required at least 25 intromissions (Erskine and Baum, 1982).

Stimulus Factors that Affect Female Temporal Copulatory Behavior

In the present studies, we demonstrated that several factors affected the female's postejaculatory refractory period (PER). These factors include preejaculatory intromissions and the male's ejaculation duration.

Different numbers of intromissions received by the female rat prior to ejaculation affect the female's PER. In the present experiments, we demonstrated that the PER of females receiving 0-1 intromission prior to ejaculation was significantly shorter than that in the other groups (2-4, 5-15, and 24-31 intromission groups) and was not different from her intromission return latency. The females receiving two intromissions prior to ejaculation had a PER which was not different from that of females receiving 5-15 intromissions. The females receiving 24-31 intromissions before ejaculation had a significant longer PER than those in the other groups (0-1, 2-4, and 5-15 intromission groups). In addition, correlation analysis showed that preejaculatory intromission frequency was positively correlated with the female's PER when the range of intromissions received prior to ejaculation was large (0-31 and 0-18 in the present studies).

One possible function of multiple intromissions prior to ejaculation may be to prepare the vagina for a deep penile insertion during ejaculation that consequently induces a

longer ejaculation duration. A longer ejaculation duration in turns results in a longer PER. The male's ejaculation duration plays an important role in the induction of the female's PER. It has been reported that the male's ejaculation duration increases over multiple ejaculatory series (Peirce and Nuttall, 1961b). Similarly, the female's PER increases over the course of copulation (Bermant and Westbrook, 1966; Krieger et al., 1976). In the present experiments, it was demonstrated that the preejaculatory intromission frequency, the male's ejaculation duration, and the female's PER were positively correlated with one another. In addition, the ejaculation duration and the female's PER were affected by manipulating the number of intromissions prior to ejaculation. The male's ejaculation duration and the female's PER were significantly decreased if the female received 0-1 intromission prior to ejaculation. Furthermore, partial correlation controlling the ejaculation duration showed that the correlation between the preejaculatory intromission frequency and the female's PER was close to zero. These findings suggest that the preejaculatory intromissions influence the male's ejaculation duration that in turn affects the female's PER.

The effect of a longer ejaculation duration on the female's PER may result from more intense stimulation due to the longer vagina-penis contact duration and/or the deeper penile insertion rather than the number of pelvic thrusts during ejaculation. First, a longer ejaculation duration may result in more intense stimulation that in turn induces a longer PER. One anterograde tracing study shows that the L_6 and S_1 dorsal root ganglia send more afferent fibers to the vagina and the cervix (Nance et al., 1988). In addition, along the vaginal canal in female rats, fibers with mechanoreceptive fields that respond to moving stimuli are discovered (Berkley et al., 1990). The area near the vaginocervical junction contains more fibers with mechanoreceptive fields than other areas of vaginal canal (Berkley et al., 1990). These findings suggest that a longer stimulation of the area near vaginocervical junction resulting from a longer ejaculation duration can produce more intense stimulation and induces a longer PER. Secondly, that the number of pelvic thrusts

during ejaculation was not significantly correlated with the ejaculation duration and the female's PER suggests that a longer ejaculation duration does not result from more pelvic thrusts and that the quality of thrusting stimuli may be more important than the frequency of thrusting in inducing the female's PER. Finally, deeper penile insertion during ejaculation possibly contributes to a longer ejaculation duration. Deeper penile insertion during ejaculation can also simultaneously stimulate the pelvic nerve fibers as well as the hypogastric nerve sending sensory innervation to the vagina, cervix and uterus (Berkley et al., 1990; Berkley et al., 1993; Peters et al., 1987) and induces more intense stimulation. However, a functional analysis of the respective roles played by these two nerves suggests that "pelvic nerve fibers seem closely tied to sensory and behavioral processes associated with mating and conception, whereas hypogastric fibers seem closely tied to pregnancy and nociception" (p. 533, Berkley et al., 1993).

Intromissions have been demonstrated to affect the female's intromission return latency (IRL) in the present studies. Repeated intromissions alone, without ejaculation, could induce prolonged IRLs that in length are not different from the female's PER in the first ejaculatory series. The first prolonged IRL usually occurred between 24th to 44th intromission and 12 to 16 minutes after the first intromission. One possible function of these prolonged IRLs may be to induce the male to ejaculate after the female receives enough vaginocervical stimulation for induction of the progestational state of pregnancy. Several observations support this idea. First, the number of intromissions prior to the first ejaculation have been reduced experimentally by enforcing long interintromission intervals (1 to 5 minutes) (Bermant, 1964; Larsson, 1959), suggesting that long interintromission intervals can facilitate ejaculation. The mean of the first prolonged IRL seen in the present study (about 50 seconds) would be adequate to facilitate the male's ejaculation. Secondly, in the present experiments, the number of intromissions (usually 24 to 44 intromissions) required for the first prolonged IRL to occur far exceeded the number of intromissions necessary for inducing the progestational state of pregnancy. Further, in female paced

situations, it has been observed that if a prolonged IRL occurs during the third ejaculatory series, the male will try to get close to the female by protruding his head through the escape holes of the barrier and usually ejaculates on the next intromission when the female returns (Unpublished data).

None of the seminal plug, prostate secretions, and the penile cup formation affected the female's postejaculatory refractory period. In rats, an enormous portion of the male's ejaculate comes from secretions of the seminal vesicles (Mann, 1964). The formation of the copulatory plug involves polymerization of proteins in the seminal vesicular secretion by the transglutaminase released from the coagulating glands (Brooks, 1990; Luke and Coffey, 1994; Mann, 1981; Price and Williams-Ashman, 1961; Setchell et al., 1994; Wagner and Kistler, 1987; Walker, 1910a, 1910b; Williams-Ashman et al., 1977; Zorgniotti and Brendler, 1958). Therefore, the formation of a copulatory plug after ejaculation can be prevented by bilateral removal of the seminal vesicles and coagulating glands. However, the female's PER was not affected by removal of both sets of tissue.

The penile cup was observed during ejaculation in *ex copula* tests (Hart and Odell, 1981). Evidence suggests that the BC muscle is responsible for this flaring of the glans penis (Hart and Melese-D'Hospital, 1983; Sachs, 1982, 1983). Extirpation of the BC muscle presumably eliminates the formation of penile cup during ejaculatory reflexes *in copula* tests (Sachs, 1982). However, removal of the bulbocavernosus muscle did not affect the female's PER and we suggest that the formation of penile cup during ejaculation does not contribute to the female's PER.

To rule out the possibility that chemical substances released from the prostate may cause contraction of the vagina, cervix and uterus that in turn contributes to the female's PER, we removed the prostate. The female's PER was not affected by this operation.

Relation of Uterine Activity to Female Pacing Behavior

In a standard test situation where the male controls the timing of copulation, it has been demonstrated that copulation has both an immediate and a delayed effect on the uterine EMG activity. While little uterine EMG activity was observed before copulation, uterine EMG activity increased significantly during copulation, then returned to the premating level for several minutes following ejaculation. Five minutes following the ejaculation, however, uterine EMG activity again increased even without copulation (Toner and Adler, 1986).

In one of the experiments, we examined how the uterine EMG activity correlates with different types of sexual contacts and the temporal pattern of female sexual behavior. While we anticipated an increase in uterine EMG activity during the PER, we did not see it. However, we found that the duration of uterine EMG activity associated with ejaculation was significantly longer than that associated with intromission or mount. This finding coincides with the result that the female's PER is significantly longer than her IRL or MRL. It seems likely that the longer duration of uterine EMG activity associated with ejaculation reflecting a more intense vaginocervical stimulation contributes to the longer return latency following ejaculation.

We confirmed that copulation had an immediate effect on uterine EMG activity reported in an earlier study (Toner and Adler, 1986). Before copulation, little uterine EMG activity was observed. This is consistent with the previous finding that some uterine activity was observed during the evening of the proestrus stage (Talo and Karki, 1976) and before copulation (Toner and Adler, 1986). That little uterine activity was seen in the female's PER suggests that facilitation of sperm transport from the vagina to the uterus during the female's PER results from preventing the disturbance of sperm transport rather than from the increase of uterine EMG activity. During the active phase of copulation,

uterine contractions increased significantly, then returned to the premating level following ejaculation.

One possible function of the immediate increase and delayed increase of uterine EMG activity induced by copulation may be to facilitate sperm transport from the vagina to the uterus and to assist sperm to advance in the uterus at a faster speed. First, the longer duration of uterine activity associated with ejaculation may facilitate the newly deposited sperm from the vagina to the uterus. Secondly, the immediate increase of uterine EMG activity during active phase of copulation and the delayed increase of uterine contractions following copulation may serve to assist the sperm deposited from previous ejaculation(s) to advance in the uterus at a higher speed.

Central Neural Correlates of Female Temporal Copulatory Behavior

In rats, female sexual behavior is comprised of three major components: lordosis behavior, soliciting behavior (hopping and darting) and the temporal pattern of the female's approach to the male (Erskine, 1989; Nelson, 1995; Pfaff et al., 1994). The brain regions that regulate lordosis responding have been extensively studied (Pfaff et al., 1994). For example, the MPOA, ventromedial nucleus of the hypothalamus (VMN), frontal cortex, olfactory bulbs, and septum are important for the display of lordosis behavior. Several studies have reported that lesions of the MPOA increase the lordosis response in female rats treated with low doses of EB plus P (Hoshina et al., 1994; Powers and Valenstein, 1972; Whitney, 1986). Transections made anterior-dorsal to the MPOA also facilitate lordosis reflexes (Yamanouchi and Arai, 1977). Lesions of large frontal cortex, olfactory bulb, or septum facilitate lordosis behavior (Pfaff et al., 1994). In contrast, lesions of VMN inhibit the display of lordosis reflexes (Pfaff et al., 1994).

Compared to the studies measuring lordosis responses, brain regulation of the temporal pattern of female rat's sexual behavior is poorly understood. Two brain regions

have been reported to affect the temporal pattern of female sexual behavior: MPOA and VMN. MPOA lesions decreased the frequency of sexual contacts and the time that the female spent with the sexually active males (Whitney, 1986) when she could choose among sexually active males, castrated males and females. Similarly, VMN lesions decreased the female sexual contacts with the active males under similar test situations (Emery and Moss, 1984). These findings suggest that the MPOA and VMN may play a role in facilitating female sexual motivation.

Results of the present study extend the notion that the MPOA plays a role in the temporal copulatory behavior of female rats. Both electrolytic and chemical lesions of the MPOA significantly increased the female's return latencies following intromission and ejaculation. This finding suggests that the significant increase in the IRL and PER following MPOA lesions results from destruction of neurons in the MPOA rather than fibers of passage.

It seems likely that the MPOA plays an important role in facilitating sexual motivation in female rats. In a recent review, one author emphasized the distinction between female sexual desire (sexual motivation) and female sexual ability (Wallen, 1990). Female sexual ability refers to the female's ability to engage in copulation. Female sexual motivation refers to "the state underlying sexual initiation and sexual accommodation" (p. 234, Wallen, 1990). It is necessary for the female rat to display lordosis behavior to allow successful penile insertion (Diakow, 1974; Pfaff et al., 1978). As a result, the lordosis response has become the major measure of the female's sexual ability. On the other hand, the author proposed that the female temporal copulatory behavior was regulated by her sexual motivation (Wallen, 1990). Consequently, the temporal pattern of female rat sexual behavior can be used to measure her sexual motivation. Under such circumstances, the increase in the female's IRL and PER following MPOA lesions would indicate a decrease in her sexual motivation. This is consistent with the finding that two females with MPOA ibotenic acid lesions did not approach the male during the 15-minute test.

Comparison of MPOA Lesions in Male and Female Rats

The MPOA appears to regulate the temporal copulatory pattern of male and female rats in a parallel manner. Following large MPOA lesions, male sexual behavior was abolished (Giantonio et al., 1970; Ginton and Merari, 1977; Heimer and Larsson, 1966/1967; Hendricks and Scheetz, 1973; Lisk, 1968). With MPOA lesions sparing part of MPOA, some males continued to achieve intromissions and ejaculations. These males showed a significantly lengthened interintromission interval (III) (Heimer and Larsson, 1966/1967; Ginton and Merari, 1977; Stefanick and Davidson, 1987), ejaculation latency (EL) (Ginton and Merari, 1977; Stefanick and Davidson, 1987) and postejaculatory interval (PEI) (Ginton and Merari, 1977). In the present experiments, we demonstrated that the MPOA lesions significantly increased the female's IRL and PER. Larger MPOA lesions even abolished the female's sexual desire. The increase in the female's IRL and PER following MPOA lesions parallels the results of males following MPOA lesions. This comparison uses the same measurement scale to evaluate the role of MPOA in the male and female sexual behavior and is totally different from the traditional approach comparing different responses in the male (mounting) and the female (lordosis). These findings in the present study strongly support the idea that the MPOA regulates the temporal copulatory pattern of both the male and the female.

Future Directions

In the present experiments, the study of the role of MPOA in the regulation of female temporal copulatory behavior generated numerous questions about female sexual behavior. Future research should focus on what brain regions regulate the temporal pattern of female rat's sexual behavior, what neurotransmitters are critical for this regulation, and whether these brain regions function in a similar way in both the male and the female.

We have suggested that the MPOA functions to facilitate female sexual motivation. The next question that needs to be answered is what neurotransmitters in the MPOA are critical for this function. Evidence suggests that several neurotransmitters are logical candidates: dopamine and serotonin. In the preliminary studies, dopamine D1 antagonist (SCH 23390) seems to affect the temporal pattern of female sexual behavior when administered to the MPOA (unpublished data). Serotonin fibers have been shown in the MPOA (Yang and Clemens, 1993) and serotonin has been demonstrated to play a role in the female lordosis behavior (Pfaff et al., 1994) and male sexual behavior (Meisel and Sachs, 1994).

In addition to the MPOA, VMN lesions have also been shown to decrease the frequency of coital contacts with sexually active males (Emery and Moss, 1984). Therefore, the VMN is a good candidate for controlling the temporal pattern of female sexual behavior. In addition, copulation has been reported to increase c-fos expression in the VMN of male and female rats (Wersinger et al., 1993). This finding suggests that the VMN plays a role in both male and female sexual behavior. Since the data collected in the present experiments strongly support the view that the MPOA regulates the temporal pattern of male and female sexual behavior in a similar way, it is important to know whether other brain regions such as VMN control the male and female temporal copulatory pattern in a similar way. To compare the role of VMN in male and female sexual behavior can be achieved by investigating the role of VMN in the temporal copulatory behavior in both sexes using electrolytic and chemical lesion techniques.

Summary and Conclusions

In the present experiments, we examined the relation of copulatory stimulus factors to the temporal pattern of female rat sexual behavior. In addition, we investigated the relation of vaginocervical stimulation and uterine EMG activity to the female's temporal

copulatory pattern. Finally, we investigated whether the MPOA plays a role in the temporal pattern of female sexual behavior and whether the MPOA controls the temporal pattern of male and female sexual behavior in a parallel manner.

We demonstrated that the preejaculatory intromissions, the male's ejaculation duration and different hormone replacements affected the female rat's PER. The female's PER appeared not to be influenced by the deposit of a seminal plug, the penile cup formation, prostate secretions and the number of pelvic thrusts during ejaculation. The male's ejaculation duration, the preejaculatory intromission frequency and the female's PER were significantly correlated with one another. One possible function of multiple intromissions prior to ejaculation may be to prepare the vagina for a deep penile insertion during ejaculation. This deep penile insertion during ejaculation results in a longer penis-vagina contact duration (a longer ejaculation duration). A longer ejaculation duration causes a longer PER in the female.

The duration of uterine EMG activity associated with ejaculation was significantly longer than that associated with intromission or mount. This finding coincides with the result that the female's PER is significantly longer than her IRL or MRL. It is likely that the longer duration of uterine EMG activity associated with an ejaculation reflecting a more intense stimulus contributes to the female's PER. In addition, we confirmed the previous finding that copulation resulted in an immediate increase of uterine contractions. The possible function of the increased uterine activity induced by copulation may serve to facilitate newly deposited sperm to cross the cervix and to assist sperm deposited from previous ejaculations to advance in the uterus at a faster speed.

The MPOA is important for facilitating female temporal copulatory behavior and female sexual motivation in rats. Sexual motivation of female rats regulates their temporal copulatory pattern during copulation. The MPOA lesions significantly increased the female's IRL and PER. Two females with MPOA chemical lesions did not approach the male during the 15-minute test. These findings strongly support that the MPOA lesions

disrupt female pacing behavior and decrease female sexual motivation in rats. Both the electrolytic and chemical lesions of MPOA significantly increased the female's IRL and PER. This finding demonstrated that the effect of MPOA lesions on the female temporal copulatory pattern and female sexual motivation resulted from destruction of neurons in the MPOA, not fibers of passage. Finally, that the increase of the female's IRL and PER following MPOA lesions parallels the increase of the male's III and PEI following MPOA lesions strongly supports the view that the MPOA regulates the temporal pattern of male and female copulatory behavior in a similar manner.

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