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Estrous cycle modulation of nociceptive behaviors elicited by electrical stimulation and formalin

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Abstract

The impact of circulating ovarian hormones on nociceptive behaviors elicited by phasic and tonic stimuli was evaluated in rats using two behavioral tests: an operant escape task and the formalin test. The operant escape task was structured to separately evaluate hindlimb flexion reflexes, the latency of escape, and the amplitude of peak vocalization to a series of phasic electrocutaneous stimuli (0.05–0.8 mA), whereas the formalin test evaluated nociceptive behaviors elicited by tonic stimulation following a subcutaneous injection of dilute formalin (1%). Hindlimb reflex amplitude, escape latency, and peak vocalization varied across the estrous cycle, such that rats were most sensitive to electrical stimuli during proestrus (reflex and escape latency) and diestrus (vocalization). Furthermore, morphine-induced (3 mg/kg sc) attenuation of hindlimb reflex amplitude was sensitive to estrous cycling. During proestrus, morphine produced less attenuation of hindlimb reflex amplitude than during nonproestrus phases. However, estrous cycling did not alter nociceptive behaviors elicited by 1% formalin. These data support the notion that circulating ovarian hormones may differentially modulate behaviors associated with phasic and tonic pain.

Keywords: Ovarian hormones; Pain; Rat

1. Introduction

Circulating ovarian hormones have been shown to alter nociceptive behaviors and effects of opioid and nonopioid agents on these behaviors using a variety of models (Banerjee et al., 1983; Candido et al., 1992; Chatterjee et al., 1982; Frye et al., 1992; Kasson and George, 1984; Kepler et al., 1989; Romero et al., 1988a; Ryan and Maier, 1988; Simpkins, 1994). However, the findings of these investigations have been highly variable. Many investigators report no changes in nociceptive reflex thresholds across the estrous cycle or following ovariectomy (Beatty and Fessler, 1977; Marks and Hobbs, 1972; Marks et al., 1972), while others report an increased sensitivity to presumed nociceptive stimuli and decreased sensitivity to endogenous and exogenous opioids during periods of high-estrogen activity (i.e., during proestrus and estrus) (Drury and Gold, 1978; Frye et al., 1992; Frye et al., 1993; Martínez-Gómez et al., 1994). The inconsistency of these findings has produced much speculation as to whether reproductive hormones affect nociceptive and antinociceptive systems.

Much of the disparity between results arises from the number of variables known to alter nociceptive behaviors. In addition to gonadal hormones, age (Bodnar et al., 1988; Paré, 1969), weight (Beatty and Fessler, 1977; Marks and Hobbs, 1972; Marks et al., 1972), diet (Frye et al., 1992; Frye et al., 1993), species and strain of the animal (Paré, 1969; Tjölsen et al., 1992), behavioral test employed (e.g., hot plate, tailflick, flinch-jump), time of day the behavioral test is administered (Frederickson et al., 1977; Martínez-Gómez et al., 1994), stimulus modality and intensity and site of stimulus application (Martínez-Gómez et al., 1994; Ness at al., 1987), temperature of

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the room (Hole and Tjölsen, 1993), and novelty of the environment (Aloisi et al., 1994) have been shown to alter nociceptive behaviors. The need to evaluate interactions between these factors and hormonal states makes it difficult to discriminate the impact of ovarian hormones on nociception.

Estrous cycling has been shown to alter the sensitivity to endogenous (Romero et al., 1987; Ryan and Maier, 1988) and exogenous opioids (Banerjee et al., 1983; Berglund et al., 1988; Kasson and George, 1984; Kepler et al., 1989). During the preovulatory luteinizing hormone (LH) surge, opioid receptors desensitize, rendering even large doses of morphine unable to affect reflex responses or produce catalepsy or hypothermia (Banerjee et al., 1983; Berglund and Simpkins, 1988). The behavioral tests utilized previously to assess effects of morphine on nociception across the estrous cycle have been limited to circuits involved in spinally organized behaviors, mainly the tailflick reflex (Banerjee et al., 1983; Forman et al., 1989). Lesions or ablation of discrete regions of the central nervous system have shown that effects of morphine on nociceptive behaviors mediated by supraspinal and spinal circuits can be independently modulated (Ryan et al., 1985). Therefore, it is important to assess the effects of estrous cycling on morphine-induced alterations of nociceptive responses involving spinal and supraspinal circuits. There is some evidence that morphine attenuation of supraspinally organized behaviors can also vary as a function of estrous cycle (Forman et al., 1989).

The present study utilized an electrical stimulation paradigm that produces brief (phasic) pain sensations (Cooper and Vierck, 1986) and measures responses dependent upon spinal (reflex amplitude), rhinencephalic (vocalization), and cerebral cortical (operant escape task) sites (Borszcz, 1995). The formalin test produces tonic pain and was used to examine the effects of estrous cycling on nociceptive behaviors that are organized at spinal and rhomboencephalic levels (Matthies and Franklin, 1992). The use of two behavioral tests allows the impact of ovarian hormones to be assessed within and between stimulus paradigms while limiting the number of extraneous variables described above. Within each behavioral paradigm, spinally and supraspinally organized nociceptive behaviors were measured during estrous cycling. Between behavioral paradigms, differences in nociceptive behaviors elicited by two stimulus modalities (chemical and electrical) were assessed across the estrous cycle. Although different stimulus modalities have been used previously to investigate changes in nociceptive behaviors across the estrous cycle (Drury and Gold, 1978; Frye et al., 1992; Martínez-Gómez et al., 1994), differences in phasic (electrically evoked) and tonic (chemically evoked) nociceptive behaviors have not been compared directly. In addition to measuring changes in basal nociceptive responses, the effects of morphine were investigated across the estrous cycle using the electrical stimulation paradigm.

2. Methods

2.1. Subjects

Female Long–Evans rats (180–250 g, Charles River) were housed in groups of three and maintained on a 12 h light/12 h dark (lights on 07:00 h) cycle with food and water available ad libitum. Vaginal epithelia were obtained daily by lavage (10:00–11:00 h), and estrous phase was determined by cytology with low-power light microscopy (Diamond, 1970). Care and use of the animals in all described procedures were in accordance with protocols approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.2. Experiment 1: estrous cycle and electrical stimulation

2.2.1. Electrical stimulation paradigm

2.2.1.1. Apparatus. The electrical stimulation paradigm has been described previously (Vierck et al., 1995). Briefly, rats were suspended in a metal framework via a fitted jacket. The torso of the rat was supported by the jacket, while the rear half of the animal was supported by elastic straps attached to the jacket. Two arm holes in the jacket allowed free movement of the forelimbs. The left forepaw rested on the metal framework of the apparatus. The right forepaw rested on a counterweighted hinged lever, which was pressed by forepaw extension. Hindlimbs were tethered to the force transducers located beneath each hindlimb. Peak vocalizations were recorded using a microphone (Radio Shack) located near the rat's head.

Escapable stimuli were administered across the dorsal and ventral surfaces of each hindpaw. Contact with the skin was made through a 4 mm hole in adhesive squares filled with electrode paste. Stainless-steel electrodes were placed in contact with the electrode paste and fastened securely with surgical tape. Electrical stimulation was produced by a programmable stimulator that produced a constant-current sinusoid (60 Hz). The stimulus was interrupted to produce 50 ms trains at a rate of 4 Hz (50 ms on, 200 ms off) for a maximum duration of 5 s. The current delivery for each trial was measured to ensure stability throughout each testing session. Stimulus presentations were separated by an 18-s intertrial interval. A lockout period during the first 50 ms prevented the rat from avoiding the stimulus or reflexively terminating the stimulus prematurely.

Measurement of hindlimb flexion reflex amplitude has been described previously (submitted for publication). Briefly, the data collection window began after the first stimulus train (50 ms) and terminated immediately after the second train (250 ms from trial onset). Resting tension was recorded prior to each data collection window and was considered as a baseline for computation of reflex amplitude. Vocalizations were recorded during and immediately following the first 50 ms stimulus pulse in a

window from 25 to 200 ms. Stimulus intensities were recorded as nominal values (0.1 mA = 0.082 mA root mean squared), and peak reflex hindlimb and vocalization responses were recorded as voltage units. Customized software stored the latency to termination of each trial by a forelimb bar press, the peak amplitude of flexion reflexes of the stimulated and unstimulated hindlimb, and peak vocalization amplitudes.

2.2.1.2. Protocol. Six animals arrived at 3–4 weeks of age were trained for 3 months prior to testing and were 5–9 months old during these studies. Rats were trained to terminate hindpaw electrical stimuli by pressing a weighted lever with the right forepaw. Training was considered complete when rats consistently performed a stimulus intensity-dependent escape response. Generally, rats were 4–5 months of age at the completion of escape training.

A within-subjects design was used, and each rat was tested once per cycle phase over two estrous cycles. Testing occurred between 14:00 and 17:00 h. These times were chosen to examine the effects of nociceptive electrical stimuli during the LH surge, which occurs in the late afternoon during the proestrus phase (Butcher et al., 1974).

Rats were transported to the behavioral testing room several hours prior to the testing session. For all testing sessions, each animal received 40 electrical stimuli ranging in intensity from 0.05 to 0.8 mA. Stimuli were presented in a pseudorandomized order in blocks of 10 presentations per hindpaw. The left hindpaw received 0.05, 0.2, 0.4, 0.6, 0.8, 0.7, 0.5, 0.3, 0.1, and 0.3 mA; the right hindpaw received 0.05, 0.1, 0.3, 0.5, 0.7, 0.8, 0.6, 0.4, 0.2, and 0.4 mA. These two series were then reversed, with the left hindpaw receiving the latter stimulus block followed by the right hindpaw receiving the former.

2.3. Experiment 2: estrous cycle and the formalin test

2.3.1. Subjects

Female Long–Evans rats (180–200 g, Charles River) were housed in groups of two or three and maintained on a 12 h light/12 h dark (lights on 07:00 h) cycle with food and water available ad libitum. Estrous phase was determined daily (10:00–11:00 h) by vaginal lavage, with phases being determined by cytology. Care and use of the animals in all described procedures were in accordance with protocols approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.3.2. Protocol

Forty animals were handled and acclimated to the testing chamber on five separate occasions prior to use in the formalin test. The testing chamber was a $60 \times 60 \times 30$ cm Plexiglass box. Running water provided white noise throughout the testing session. Behaviors were recorded by a single observer using customized software, and the number of flinches and shakes and the duration of licking and biting of the injected hindpaw were recorded in 1 min bins for a total of 60 min. Two periods were considered, 0–5 min (acute phase) and 20–35 min (tonic phase) as described by Wheeler-Aceto and Cowan (1991).

All formalin tests were conducted between 16:00 and 19:00 h. Prior to each formalin test, rats were acclimated to the testing chamber for 15–30 min. Using an insulin syringe (0.5 ml, 27-{1\left/2} G), 50 μl of formalin (1%) was injected into the plantar surface of either hindpaw while rats were lightly restrained on a table top. Rats were returned to the testing chamber immediately. Data collection began 1 min after injection and continued for 60 min. At the conclusion of the testing session, rats were returned to their home cage.

2.4. Experiment 3: effects of morphine across the estrous cycle

2.4.1. Apparatus

The same electrical stimulation apparatus and paradigm described in Experiment 1 was used for this study. The latency to stimulus termination of each trial, peak reflex amplitude of the stimulated and unstimulated hindlimbs, and peak vocalizations were recorded for each stimulus presentation using customized software.

2.4.2. Protocol

Six rats were trained as described in Experiment 1. Rats were transported to the behavioral testing room several hours prior to the testing session. Morphine sulfate (obtained from NIDA) or physiological saline (0.9%) was administered subcutaneously 35–38 min prior to the first stimulus presentation of a session. The stimulus parameters described in Experiment 1 were used in this experiment. A within-subjects design was used where each rat was tested twice during proestrus and twice during the nonproestrus portion of the estrous cycle. Each rat was administered 3 mg/kg morphine once during each portion of the cycle, separated by at least 1 week to reduce the development of tolerance. Testing occurred between 14:00 and 17:00 h. These times were chosen to examine the antinociceptive effects of morphine during the LH surge, which occurs in the late afternoon during the proestrus phase (Freeman, 1988).

2.4.3. Data analyses

All data were sorted and graphed using Microsoft Excel and were analyzed using GBStat. A repeated-measures analysis of variance was used to assess estrous cycle differences in the electrical stimulation paradigm and the formalin test. A repeated-measures multivariate analysis of variance (MANOVA) assessed significant effects of morphine between estrous cycle phases (proestrus vs. nonproestrus) across a range of stimulus intensities on behavioral responses. Tukey's HSD procedure determined differences

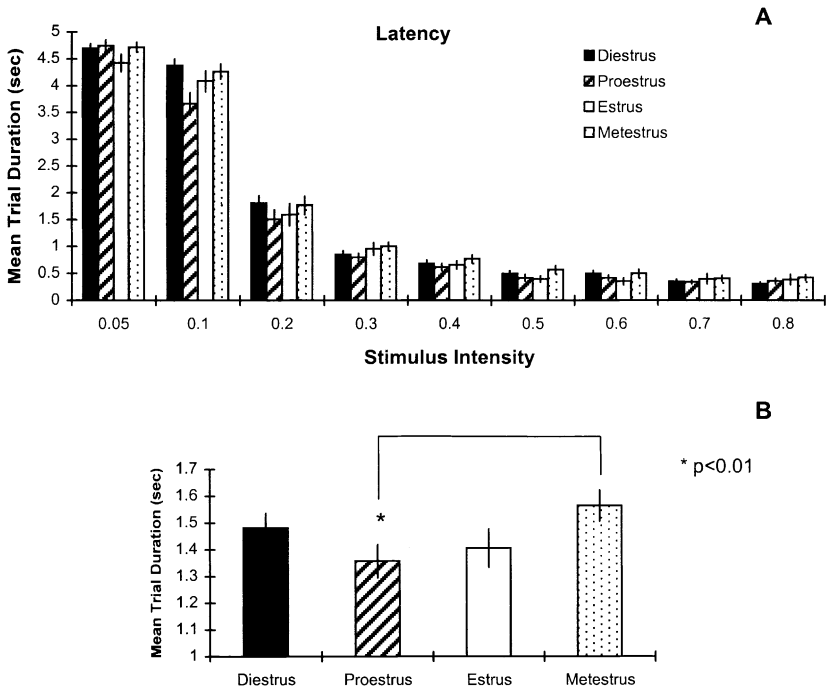

Fig. 1. Escape latencies across the estrous cycle. (A) The mean stimulus duration ± S.E.M. is illustrated for stimulus intensities ranging from 0.05 to 0.8 mA during each phase of the estrous cycle ($n = 6$). (B) Stimulus intensities were collapsed from (A) to illustrate the differences in mean stimulus durations ± S.E.M. of each estrous phase. The mean stimulus duration during proestrus (*) was significantly less than the mean stimulus duration during metestrus (brackets indicate comparison).

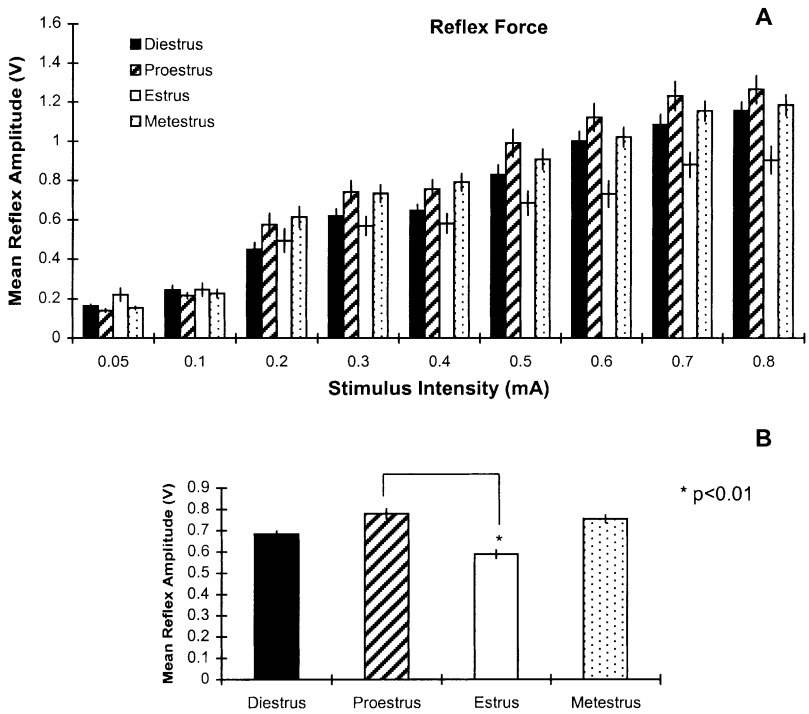

Fig. 2. Hindlimb reflex amplitude across the estrous cycle. (A) The mean reflex response ± S.E.M. across stimulus intensities (0.05 – 0.8 mA) is shown during estrous cycling ($n = 6$). (B) Stimulus intensities were collapsed from (A) to illustrate the differences in mean reflex response across the estrous cycle. The mean reflex amplitude was greatest during proestrus (*) when compared to estrus (brackets indicate comparison).

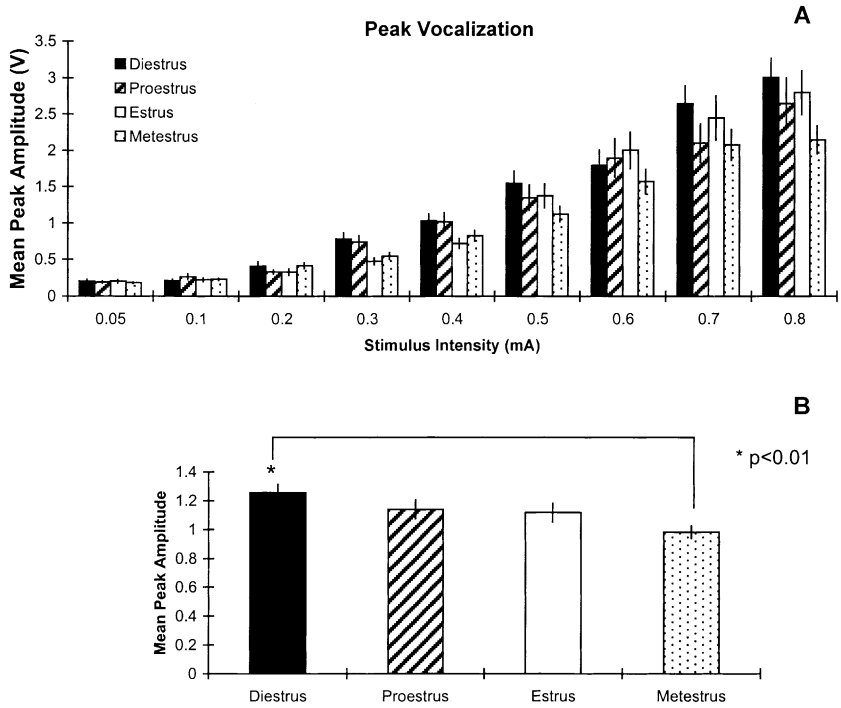

Fig. 3. Peak vocalization across the estrous cycle. (A) The mean peak amplitude ± S.E.M. for all stimulus intensities is shown across the estrous cycle ($n = 6$). (B) Stimulus intensities were collapsed from (A) to illustrate that no differences in vocalization occurred across the estrous cycle. The mean peak vocalization amplitude was greater during diestrus and proestrus (*) compared to metestrus (brackets indicate comparison).

between estrous phase, stimulus intensity, and morphine. Significance was set at $\alpha = .05$.

3. Results

3.1. Experiment 1: estrous cycle and the focal electrical stimulation paradigm

Estrous cycle modified the nociceptive responses elicited by focal electrical stimulation. Fig. 1 illustrates alterations of escape latency responses across the estrous cycle. When examined across stimulus intensities (Fig. 1B), escape latencies were significantly altered by estrous cycling [$F(3,8) = 4.82$, $P < .05$]. Post-hoc analysis revealed significantly faster escape responses during proestrus than during metestrus ($P < .01$). Fig. 2 illustrates hindlimb reflex amplitudes across the estrous cycle. Estrous cycle significantly altered reflex amplitude when examined across stimulus intensities [$F(3,8) = 4.36$, $P < .01$; Fig. 2B]. Reflex amplitude was greater during proestrus when compared to the reflex responses during diestrus ($P < .01$) and estrus ($P < .01$). Fig. 3 illustrates the significant effect of estrous cycle on peak vocalization amplitudes [$F(3,8) = 4.78$, $P < .05$]. Peak vocalization amplitudes were increased significantly during diestrus ($P < .01$) relative to metestrus (Fig. 3B).

3.2. Experiment 2: estrous cycle and the formalin test

Table 1 details nociceptive behaviors during the acute phase (0–5 min; left column) and the tonic phase (20–35

Table 1
The formalin test across the estrous cycle

	Acute phase (0–5 min)		Tonic phase (20–35 min)	
	Mean lick/bite (s)	Mean number flinch/shake	Mean lick/bite (s)	Mean number flinch/shake
Diestrus	77.5 ± 8.92	54.5 ± 7.67	77.7 ± 19.7	201.4 ± 29.9
Proestrus	98.2 ± 12.5	53.6 ± 9.75	95.0 ± 28.6	181.3 ± 23.4
Estrus	76.8 ± 6.28	68.5 ± 11.1	93.1 ± 26.6	195.1 ± 25.7
Metestrus	75.8 ± 12.0	57.9 ± 13.8	113.6 ± 25.9	210.5 ± 33.8

The formalin test was administered during each phase of the estrous cycle ($n = 10$ per estrous phase). Nociceptive behaviors were recorded for 60 min postinjection. The mean numbers of flinches and shakes ± S.E.M. during the acute (0–5 min) and tonic (20–30 min) phases of the formalin test are shown for each estrous phase. The mean values of duration of licking and biting ± S.E.M. during the acute (0–5 min) and tonic (20–30 min) phases of the formalin test are shown for each estrous phase.

min; right column) of the formalin test across the estrous cycle. Estrous cycle did not alter the duration of licking/biting during either the acute [$F(3,36) = 1.105$, $P > .05$] or tonic [$F(3,36) = 0.617$, $P > .05$] phase of the formalin test. Similarly, estrous cycling did not alter the mean number of flinches/shakes during the acute phase [$F(3,36) = 0.401$, $P > .05$] or tonic phase [$F(3,36) = 0.142$, $P > .05$].

3.3. Experiment 3: morphine, the estrous cycle, and the electrical stimulation paradigm

Fig. 4A illustrates the effect of morphine (3 mg/kg sc) on escape responses during the proestrus and nonproestrous phases. Morphine increased escape latencies [$F(2,8) = 5.35$, $P < .01$] and decreased hindlimb reflex amplitude [$F(2,8) =$

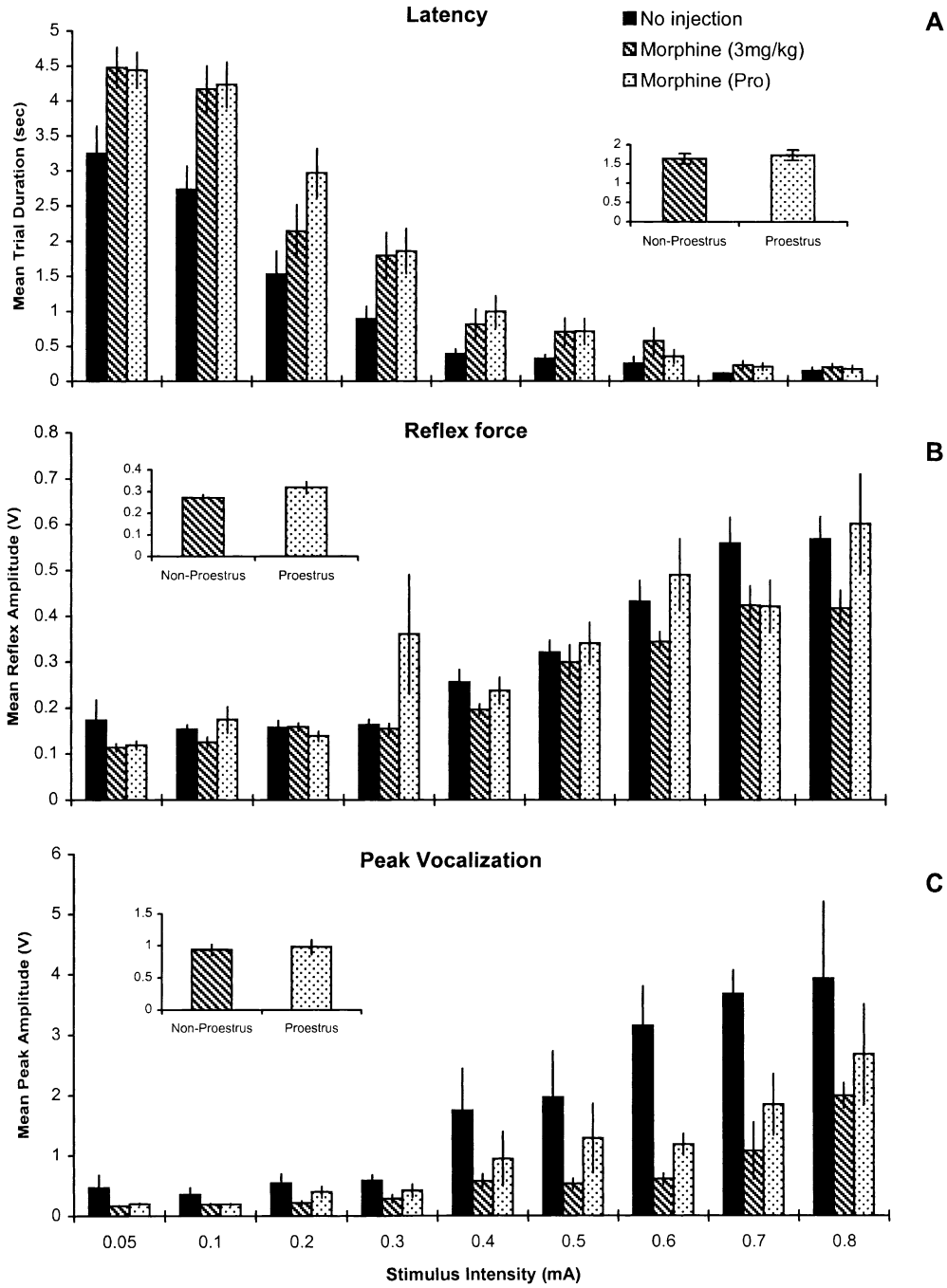

Fig. 4. Morphine-induced attenuation of electrically evoked behaviors was examined during proestrus and nonproestrus phases. (A) Mean stimulus duration ± S.E.M., (B) mean reflex amplitude ± S.E.M., and (C) mean peak vocalization ± S.E.M. following morphine administration (3 mg/kg sc) are shown ($n = 6$). Inserts represent stimulus intensities collapsed from each respective graph to illustrate phasic differences in morphine sensitivity.

3.52, $P<.05$] regardless of estrous phase. With respect to escape latency, no differences in morphine-induced hypoalgesia were observed between rats in the proestrus and nonproestrus phases [$F(1,8)=3.30$, $P>.05$]. However, hindlimb reflex responses demonstrated a differential morphine sensitivity between rats in proestrus and rats in nonproestrus [$F(1,8)=97.4$, $P<.001$]. Rats were less sensitive to the effects of morphine during proestrus than during nonproestrus phases (Fig. 4B). Morphine powerfully attenuated the amplitude of vocalizations irrespective of estrous phase [$F(2,8)=7.33$, $P<.01$], particularly for high stimulus levels. However, similar levels of vocalization were observed during proestrus and nonproestrus phases [$F(1,8)=0.19$, $P>.05$; Fig. 4C].

4. Discussion

Fluctuating ovarian hormones are known to alter numerous factors related to nociception, including nociceptive reflex thresholds (Drury and Gold, 1978; Forman et al., 1989; Frye et al., 1992; Martínez-Gómez et al., 1994), the number of hypothalamic μ-opioid receptors (Maggi et al., 1991, 1993), effects of endogenous opioid systems on nociceptive behaviors (Romero et al., 1987, 1988b; Romero and Bodnar, 1986; Ryan and Maier, 1988), and effects of exogenously applied opioids (Banerjee et al., 1983; Kasson and George, 1984; Kepler et al., 1989; Ratka and Simpkins, 1991). Our results concur and extend previous findings that cycling ovarian hormones can alter some nociceptive behaviors. In the electrical stimulation paradigm, the hindlimb reflex amplitude and operant escape latency were modified by estrous cycling, such that rats were most responsive to stimuli when estrogen concentrations were elevated (i.e., during proestrus). Also, the effect of morphine on one of these measures was attenuated during proestrus, when LH and estrogen concentrations are elevated. Flexion amplitude, but not escape latency or peak vocalization amplitude, was less sensitive to morphine during proestrus than during nonproestrus phases. In contrast to the hormonal effects on responses to phasic stimulation, estrous cycling altered neither the licking/biting nor the flinching/shaking behaviors in the formalin test.

4.1. Electrical stimulation: nociceptive behaviors and the estrous cycle

Our finding that reflex responses are greatest in amplitude during proestrus is in agreement with the findings of Drury and Gold (1978) who reported greater sensitivity of flinch thresholds to electric grid shock during periods of high circulating estrogen concentrations. Other investigators have reported similar alterations of reflex thresholds using the tailflick test (Frye et al., 1992; Martínez-Gómez et al., 1994). However, comparison of near threshold and suprathreshold responsivity for the flexion reflex in the present study showed that differences in reflex amplitude during proestrus and estrus were prominent only for suprathreshold stimulus intensities. This finding extends previous observations on ovarian cycling and reflex thresholds to demonstrate effects on reflexes elicited by stimuli that are nociceptive. Thresholds for flexion/withdrawal reflexes often are below nociceptive levels of stimulation (e.g., Cooper and Vierck, 1986, Yeomans et al., 1995), and therefore, measurement of only reflex threshold may not represent an effect on spinal nociceptive processing. In contrast, ovarian cycling affected reflex responses to levels of stimulation that were reliably escaped at minimal latencies. It is important to note that escape responses, which identified the aversive quality of the high stimulus intensities, involved motivated actions of a forelimb at latencies that distinguish these responses from the reflex actions of the stimulated hindlimb.

Consistent with the effect of ovarian cycling on reflex amplitude, operant escape exhibited cyclic fluctuations, such that escape latencies were faster during proestrus as compared to estrus. The influence of ovarian hormones on behaviors organized at supraspinal levels has not been studied extensively, and it is difficult to evaluate the threshold behaviors that have been utilized previously. Using electric grid shock, no differences in jump thresholds have been reported across the estrous cycle or following ovariectomy (Beatty and Fessler, 1977; Marks and Hobbs, 1972; Marks et al., 1972). Our finding that escape latencies did fluctuate across the estrous cycle does not contradict these reports. Escape latencies near threshold in our paradigm (0.05 and 0.1 mA) were not significantly different across the estrous cycle [$F(3,18)=0.518$, $P>.05$ at 0.05 mA; $F(3,18)=1.71$, $P>.05$ at 0.1 mA]. However, comparing escape latencies across the entire range of stimulus intensities from sub- to suprathreshold revealed significant differences between proestrus and metestrus (see Fig. 1) despite a ceiling effect for the highest intensities. A more substantial manipulation of ovarian hormone levels, produced by ovariectomy (submitted for publication), confirms the conclusion that ovarian hormones can modulate operant behaviors.

In the present study, the amplitude of early vocalizations (within 200 ms of trial onset) was monitored because later vocalizations would be confounded by repetitive electrical stimulation on trials that were not terminated by an early escape response. Early vocalizations have been shown to be organized at medullary levels (Borszcz et al., 1992), in contrast to later, more prolonged vocalizations that are dependent upon the integrity of rhinencephalic and diencephalic circuits (Hoffmeister, 1968). The short latency, brief vocalizations were graded in amplitude with stimulus intensity but occurred reliably only at the higher stimulus levels. Vocalization amplitudes were enhanced during diestrus when compared to metestrus. The reason for this difference in vocalization between these two phases is unknown.

4.2. The formalin test and estrous cycling

Although male and female rats have been reported to exhibit different nociceptive behaviors in response to formalin injection (Aloisi et al., 1994), the effects of circulating ovarian hormones on formalin-induced nociceptive behaviors have not been investigated previously. That neither of the nociceptive behaviors varied across the estrous cycle was an unexpected result, considering the number of studies reporting a decrease in nociceptive reflex thresholds during proestrus (Drury and Gold, 1978; Frye et al., 1992; Frye et al., 1993; Martínez-Gómez et al., 1994). However, there are numerous differences between these behavioral tests that may explain the disparity, including the nature of the nociceptive stimuli and the behaviors recorded.

Unlike the tailflick and flinch-jump tests that utilize phasic stimuli, the formalin test employs a tonic stimulus to elicit nociceptive behaviors. It has been suggested previously that different pain suppression mechanisms are utilized to modulate phasic and tonic stimuli (Abbott et al., 1982; Ryan et al., 1985). These modulatory systems may exhibit a differential sensitivity to ovarian hormones, such that pain suppression systems involved in the attenuation of phasic pain are sensitive to estrous status, whereas those systems responsible for the modulation of tonic pain are insensitive to ovarian state. This possibility is supported by our finding that hindlimb flexion and withdrawal responses elicited by phasic electrical stimuli but not by tonic formalin are sensitive to circulating ovarian hormones.

Alternatively, experimenter bias could account for the lack of ovarian hormone status on formalin-induced behaviors. This explanation is unlikely for several reasons. Our results are comparable to behaviors reported previously by numerous laboratories (Abbott et al., 1995; Wheeler-Aceto and Cowan, 1991). Furthermore, the observer was unaware of the estrous phase during the formalin test. The observer who recorded these behaviors has conducted nearly 100 formalin tests by the end of data collection, a strong argument against a lack of intrarater reliability. Taken together, these factors strongly suggest that experimenter bias cannot account for our observations.

The present results indicate that ovarian hormones modify nociceptive behaviors processed at different levels of the neuraxis. Electrically evoked flexion reflexes and operant pain sensitivity that depend respectively upon spinal and cortical processing were significantly attenuated during proestrus. However, licking-biting and flinching-shaking behaviors elicited by formalin were relatively unaffected. This combination of results suggests that stimulus modality is a more important factor than the nociceptive behavior recorded when examining the effects of ovarian hormones.

4.3. Morphine-induced effects and estrous cycling

A reduced effect of morphine was observed during proestrus for hindlimb reflex amplitude but not for escape latency or vocalization amplitude (Fig. 4). These results and differences in sensitivity of operant and reflex responses to ovarian cycling (Experiment 1) clearly show that neither spinal nor supraspinally organized "reflex" responses to nociceptive stimulation can substitute adequately for behavioral methods that evaluate consciously motivated escape of nociceptive stimulation and reveal effects on sensory magnitude. We have shown previously that reflex amplitude for rats is more sensitive to morphine than escape latency (Vincler et al., in press). The greater sensitivity of reflex amplitude to morphine may account for the attenuation of morphine's effects on this behavior during proestrus. This possibility could be evaluated in primates, where the relative sensitivity of flexion reflexes and operant escape responses to morphine is the reverse of that seen in rats, using nearly identical testing paradigms (Cooper and Vierck, 1986; Yeomans et al., 1995). Other studies have provided evidence that systems, which modulate supraspinally and spinally organized nociceptive behaviors, are independently regulated (e.g., Candido et al., 1992).

4.4. Estrous cycle, opioids, and other modulatory systems

Ovarian hormones have been shown previously to alter the effects of endogenous (Chatterjee et al., 1982; Romero et al., 1987; Ryan and Maier, 1988) or exogenous opioids (Banerjee et al., 1983; Berglund et al., 1988; Kasson and George, 1984; Kepler et al., 1989). That reduction of reflex amplitude by morphine was attenuated during proestrus, as compared to nonproestrus phases, corroborates the findings of Berglund et al. (1988) and Banerjee et al. (1983), but conflicts with observations by Kepler et al. (1989), who reported that estrous cycle failed to influence effects of morphine. This difference may be related to the larger proportion of behavioral tests that were administered during hours of maximal, rather than minimal, morphine sensitivity in the Kepler et al.'s (1989) study.

μ, and to a lesser extent δ, opioid receptors have been shown to desensitize during the preovulatory proestrus LH surge (Berglund et al., 1988). On proestrus afternoons, female rats become insensitive to antinociceptive, hypothermic, and cataleptic actions of morphine (Berglund et al., 1988). The mechanism of this reduced morphine responsivity is not clear. Estrogen receptors have been identified in brain areas involved in nociceptive processing such as the periaqueductal gray, arcuate nucleus, and amygdala (Mansour et al., 1988). Furthermore, subsets of β-endorphin- or dynorphin-containing neurons in the medial basal hypothalamus (MBH) contain estrogen receptors (Morrell et al., 1985). This implies that opioid-containing neurons in the MBH are sensitive to estrogen and render rats less sensitive to morphine during periods of high estrogen concentrations (i.e., during proestrus). This hypothesis is supported by the findings of Weiland and Wise (1990) who reported that μ-opiate binding sites are reduced in the MBH during the steroid-induced LH surge.

Furthermore, the MBH contains fibers that project to the lumbar cord and are thought to be involved in the processing of nociceptive information (Schwanzel-Fukuda et al., 1984). Therefore, the MBH provides a plausible site for opioid and gonadal steroid interactions.

In addition to the ovarian hormone effects on endogenous opioid systems, other systems that modulate nociceptive behaviors have also demonstrated sensitivity. The GABAergic system is sensitive to ovarian status according to some behavioral tests (McCarthy et al., 1990). It has been suggested that the activity of descending systems is under tonic inhibitory control of GABAergic neurons and that it is in these supraspinal sites that estrogen exerts its modulatory effects on spinally organized reflexes (McCarthy et al., 1990). The noradrenergic component of descending inhibitory systems is also sensitive to ovarian hormones (Kiefel and Bodnar, 1991). Although the effect of the α_2-adrenergic agonist, clonidine, does not vary significantly across the estrous cycle, ovariectomy increases effects of clonidine on the tailflick and jump tests (Kiefel and Bodnar, 1991). Estradiol-containing neurons have been identified in the MBH (Morrell et al., 1985) and in the lumbar area (Morrell et al., 1982) and may provide sites for adrenergic and gonadal steroid interactions.

This study has shown that cycling ovarian hormones can differentially alter operant pain sensitivity, vocalization amplitude, and segmental reflex responses to phasic electrical stimulation but not responses to tonic chemical stimulation. Focal application of electrical stimuli elicited behaviors that were more sensitive to the hormonal milieu than behaviors evoked by a focal injection of dilute formalin. The effect of morphine on electrically evoked reflex amplitude was attenuated during proestrus, while the effects on escape and vocalization responses to the same stimulus were unchanged. These results demonstrate that circulating ovarian hormones can affect pain sensations as well as nociceptive reflexes, but ovarian hormonal alterations of opioid modulation was preferentially expressed on spinal reflexes.

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