EFFECTS OF ESTROGEN AND PROGESTERONE ON ACUTE NOCICEPTION RESPONSES AND THE DEVELOPMENT OF OPIOID TOLERANCE

By

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

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ABSTRACT

EFFECTS OF ESTROGEN AND PROGESTERONE ON ACUTE NOCICEPTION RESPONSES AND THE DEVELOPMENT OF OPIOID TOLERANCE

By

Lynne M. Kemen

Advisor: Professor Vanya Quinones Jenab

A growing body of anatomical, endocrinological and behavioral data suggests that there are significant differences between males and females in their responses to nociceptive stimuli and that steroidal hormones play an important role in modulating the response to such stimuli in females. We tested this possibility by experiments using ovariectomized (OVX) rats and administering estrogen, progesterone or estrogen plus progesterone with acute pain treatments. Estradiol increased and progesterone decreased the latency of responses to acute pain (hot water tail flick test) and the co-administration of estradiol and progesterone had dose-dependent effects. We then examined the latency when using morphine, U50, 488 and SNC80.

Overall, morphine increased the latency. Similarly, U50, 488 also increased the latency. SNC80, a δ opioid agonist affected latency to tail flick in a temperature and dose-dependant manner. To the degree that gonadal hormones influence nociceptive responses, this finding suggest that the κ and δ-opioid receptors might be more sensitive to gonadal hormone treatments that the μ-opioid

receptors. Estrogen and progesterone did not affect the acquisition of tolerance; levels of morphine-induced anti-nociception were similar between experimental groups. Furthermore, co-administration of both steroids did not alter the formation of tolerance in OVX rats. Administration of progesterone during the acquisition phase had no effect on the development of tolerance. However, when progesterone was administered during the expression phase, the latencies were significantly reduced when compared to rats receiving estrogen alone. Based on the complexity of the responses found in our study, this suggest that the roles of progesterone and estradiol as they vary throughout the estrus/menstrual cycle will not be uncovered until we are able to replicate the cycle rather than model the cycle with constant hormone levels.

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Chapter 1. Introduction

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage" [141]. Identifying the conditions mediating the response of individuals to painful stimulation has long been a central problem for pain research. The present dissertation examines the role of one such condition—the individual hormone fluctuations. Given the known role of gonadal (steroidal) hormones in the control of female reproductive behavior, we have chosen to address this problem using a rat model that facilitates manipulation of steroidal hormones and allows us to examine the interaction between these hormones and the subject's response to pain and sensitivity to opioid drugs. To provide some background on the anatomy and physiology of the pain system, we will begin by describing the properties of both peripheral receptors and central pain pathways.

Peripheral mechanisms of nociception

Melzack and Wall [140] describe acute pain as being comprised of a combination of tissue damage, pain, and anxiety. Whatever the proportion of these features, there is always involvement of one or more pain receptors (nociceptors) there are three basic types of nociceptors: thermal, mechanical, and polymodal [26]. Thermal nociceptors are thinly myelinated fast conducting (5-30 m/sec) Aδ fibers activated by extreme temperatures (<5°C or > 45°C [145];[174];[16]. Mechanical nociceptors are also-fast-conducting Aδ fibers, but are activated by pressure applied to the skin [140]. Polymodal nociceptors are slowly-conducting non-myelinated C fibers activated by mechanical, chemical or

thermal stimuli [64]. The Tail Flick Test (nociceptive thresholds) measures the latency to withdraw the half-immersed tail from a temperature controlled water bath. This is measured at several temperatures: “low intensity” is near threshold for C-fiber nociceptors; 50º and 52.5ºC are “medium intensity”. This activates some Aδ fiber nociceptors; at 55ºC, “high intensity”, Aδ fiber nociceptors are activated [37]. In normal skin, heat pain near threshold appears to be signaled by activity in C-fiber nociceptors [142]; [37]. Acute pain produces physiological effects within seconds [140].

Tail Flick Test (nociceptive thresholds)

- Measure the latency to withdraw the half immersed tail from a temperature controlled water bath

- Measured at three temperatures
  - 48°C “low intensity”
    - near threshold for C-fiber nociceptors
  - 52.5°C “medium intensity”
    - activates some Aδ fiber nociceptors
  - 55°C “high intensity”
    - activates Aδ fiber nociceptors

Table 1. Fibers activated at specific temperatures

Animal models of pain

Researchers interested in the modulation of pain have used two types of animal models, classified, respectively, as either somatic or visceral pain models. Somatic pain models are the most widely used and include acute nociceptive tests (hot plate, tail-flick) and pathological pain models [25];[204];[26]. In the latter category, pain can be induced in several ways: (1) persistent central pain can be induced by formalin or capsaicin [8];[89];[40] (2) chronic inflammatory pain by carrageenan, turpentine, UV-irradiation or Freund's complete adjuvant (FCA)[50]; [202] and (3) chronic neuropathic pain by damage or disturbance to a peripheral nerve [214];[24].A separate group of pain models represents particular diseases that feature pain as a prominent symptom e.g. diabetic neuropathy, [155];[54];[144];[177]. The most common stimulus used for pain research in any species is acute thermal stimulation [148] while acute thermal pain is unlikely to happen as a clinical entity, the heat-evoked flexion reflex is a good predictor of analgesic potential of drugs [57];[206]. Several authors [26];[42] have reported good correlations between animal and human responses to this type of pain and it has been postulated that the activation of neuronal mechanisms after acute pain is similar to some activated in human. Thus, the acute main model has been extensively used to determine neuronal mechanisms underlying pain responses.

One advantage of the warm water tail flick test is that it permits the animal to avoid the noxious stimulus by flicking the tail; a second is the relative stability of results with multiple repeated measurements. In a series of tests it was found that the results were reliably similar even when the time between tests was only ten minutes over a two-hour testing period [148].

Opioid Peptides Contribute to the Endogenous Pain Control System

Opiates as a form of central system analgesia have been used as a human analgesic for centuries [112] . Pert, Pasternak, and Snyder [163] were the first to demonstrate specific opiate receptors in the brain, confirming role for opiates in central nociceptive mechanisms. There are now thought to be three different types of opioid receptors: mu (μ), kappa (κ) and delta (δ). It has been postulated that they bind to the three distinct families of endogenous opioid peptides. Each class is cleaved from different precursor proteins and each is coded by separate genes and has a distinct anatomical distribution [140]. The 3 precursors are pre-proopiomelanocortin (POMC), pre-proenkephalin and pre-prodynorphin [47]. They act on 3 distinct receptor types in the brain and spinal cord. Enkephalins are considered the putative ligands for the δ- opioid receptors, β-endorphins for the μ-opioid receptors, and dynorphins for the κ - opioid receptors. The various types of opioid receptors are differently distributed within the central and peripheral nervous system. There is evidence for functional differences in these receptors in various structures [74];[214]. M-opioid receptors are closely linked with the naturally occurring or endogenous opioids and are probably most closely

associated with morphine [75]. Exactly how these receptors work with the central nervous system (CNS) is imperfectly understood [68];[129];[156]. Opioid descending pathways from the PAG matter to the midbrain and medulla activate serotonergic and enkephalin pathways to the dorsal horn of the spinal cord, which, in turn, exerts analgesic actions by inhibiting the release of substance P. Substance P is a peptide that acts as a sensory transmitter of pain impulses that are caused by tissue injury. The impulses travel from the injured tissue to the spinal cord and brain. Receptors for substance P are thought to be G-protein-coupled receptors (Figure 3). Endorphins and opioids act pre-synaptically on sensory neurons to inhibit the release of substance P on the primary afferent nociceptive neurons. [52];[114];[211]. The μ-opioid receptor has a high affinity for morphine and related opiate drugs [133];[221]. It binds β-endorphin, an endogenous opioid that activates the μ- opioid receptor. The μ-opioid receptors are found in areas of pain processing, including the periaqueductal gray and the dorsal horn of the spinal cord [133];[221]. The δ-opioid receptors are concentrated in the vas deferens as well as discrete areas of the CNS, in patterns of distribution that are similar to the μ-opioids. The κ-opioid receptors have a different distribution pattern, with sparse distribution in the periaqueductal gray, nucleus of the raphe, spinal trigeminal nucleus and the dorsal horn of the spinal cord. K-opioid receptors are thought to participate in spinal analgesia [133];[221].

How opioids work on a cellular level

At cellular level opioids decrease calcium ion entry, causing a decrease in presynaptic neurotransmitter release (e.g. substance P release from primary afferents in the spinal cord dorsal horn [175];[88](Figure 3). Opioids also encourage potassium ion efflux, causing hyperpolarization of postsynaptic neurons and a decrease in synaptic transmission [175];[88]. Faber and Sah [69] found that there is activation of slow calcium-activated potassium current [44];[109];[69]. The initial steps of opioid action are mediated through the activation of G protein-linked receptors. As is true for all G protein receptors, opioid receptors both activate and regulate multiple second messenger pathways associated with effector coupling, receptor trafficking, and nuclear signaling [198]. These initial effects are critical for understanding the early events leading to tolerance and dependence in cells that have opioid receptors. Equally important are network changes that occur as a result of the altered synaptic regulation that may affect downstream neurons that may not have opioid receptors.

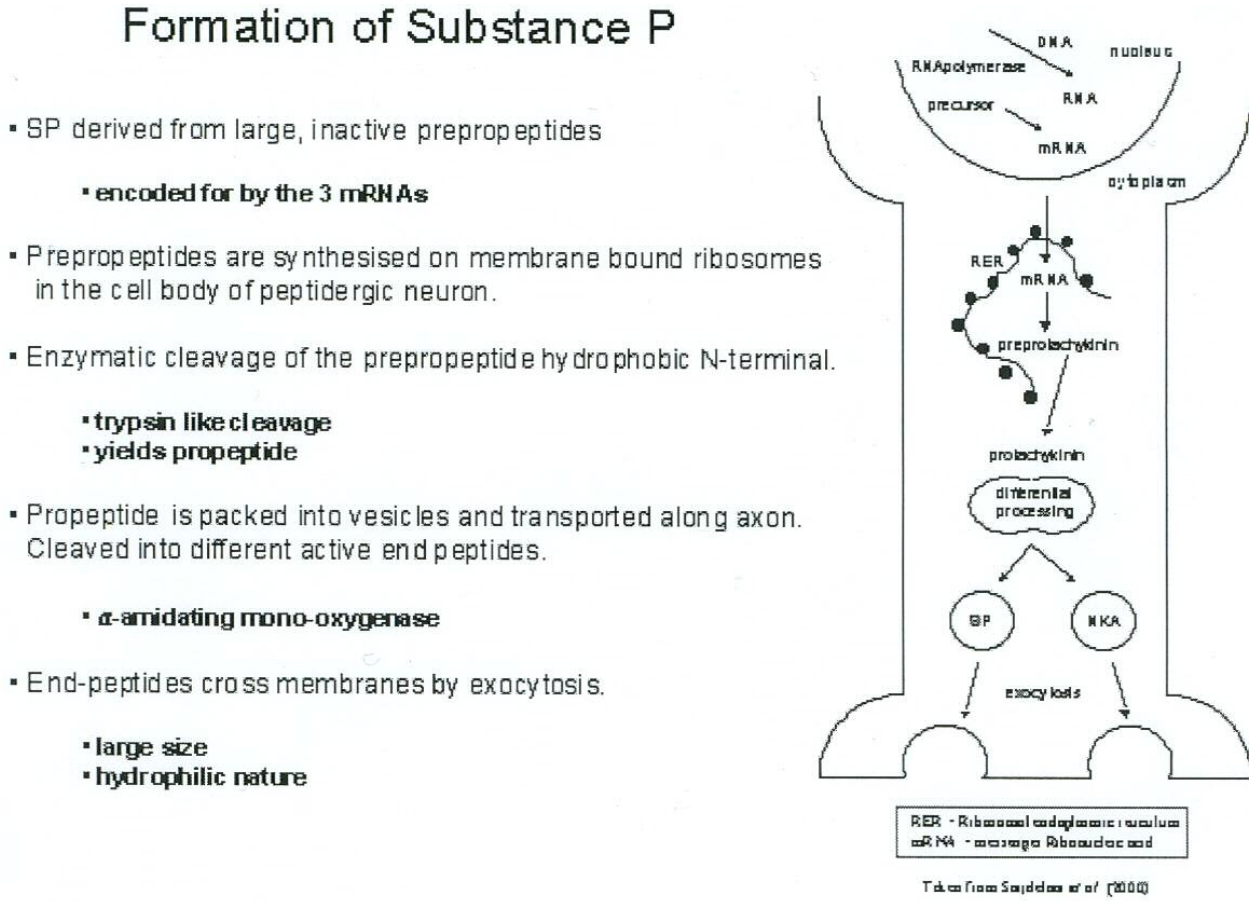


Figure 1. From: Snijdelaar et al,2000 [198].

Interactions between hormones and opioids

Hammer and Cheung, [102] note that gonadal steroid regulation of the hypothalamic opioids is a vitally important feedback system in reproduction. The pituitary secretion that releases gonadal steroids is controlled first by the hypothalamus. These are the same circuits that endogenous opioids use. Mateo et al [137] established that the cyclical expression of the MPN μ-receptors closely follow the estrus cycle fluctuations. Hammer et al [129] state that MPN μ-receptor density is linked to estrogen levels in a feedback relationship so that when one is elevated; the other is decreased (for example, during proestrus, when the estrogen levels are high, the μ-receptor density is low). Administration of naloxone into the medial preoptic area (MPOA) stimulates the release of luteinizing hormone. Maggi et al [131] also found that progesterone levels began to rise on the day of proestrus and returned to basal levels by the day of estrus and that there was a decline in the number of hypothalamic μ- receptors during this period. They draw the comparison to reports indicating the concentration of endogenous opioids follows the same pattern for female rats and monkeys.

Pfaff et al [165] have postulated that opioid peptides could over-ride pain responses and subsequently allow other behavior to occur in what would normally be considered a painful situation (mating). Pfaff et al have also speculated that the peptides might allow reflexes other than avoidance to manifest. Quinones-Jenab et al [172] examined whether estrogen treatment altered μ-opioid mRNA receptor levels in different areas of the forebrain of OVX

female rats by injecting a single 10μg dose of 17β-estradiol. They found an increase in μ-opioid levels in the ventromedial nucleus of the hypothalamus and in the arcuate nucleus but no increases in the posterior medial nucleus of the amygdala, hippocampus or caudate putamen. They concluded that estrogenic regulation of μ-opioid in the CNS might account for a cascading of events controlling the reproductive behavior of rats, including the priming of lordotic behavior. However, these effects were not observed in delta opioid receptors [173]. It seems clear that endogenous opioids play a critical role in central nervous states that combine pain and sex. In particular, the ventrolateral PAG area of the brain is important, because it has of analgesia pathways. Bodner et al [34] illustrate how neural networks in the PAG control different behavioral outputs.They also note that these afferents may also create responses inhibiting escape behavior and limiting pain perception. Animal studies and human studies both found that elevated pain response thresholds were present in late pregnancy and parturition [92],[48],[7]. In fact, this elevated pain response threshold could be reproduced in non-pregnant rodents by injecting blood serum from the pregnant rodents [94]. K-opioid receptors mediate sex-steroid-induced antinociception according to Aloisi [7] due to their location in the spinal cord, i.e., they are present in high concentrations for both humans and rodents in the lumbosacral area. In a review articles Bodner [33] described different types of stress-induced analgesia as being either opioid or non-opioid mediated.

Hormones, Pain and Sex Differences

There are distinct differences in females and males in their response to nociceptive sensation and painful pathological conditions [147];[8];[53]. This area of research has attracted considerable interest both because of what it may reveal about mechanisms, particularly hormonal, modulating pain perception generally, as well as in helping understand how female mammals cope with the painful or potentially aversive stimuli that may accompany copulation, parturition, and nursing.

Despite a large number and variety of studies, evidence for sex differences in responses to pain in humans is conflicting. Some researchers have found that sex differences are relatively small [29], while others have found moderate effects [179] and still others have found considerable differences [78]. Bartok and Craft [18] note that popular and historical accounts suggest that women are less tolerant of pain than men are [10];[78]. Clinical studies actually indicate that women report pain more often, greater intensity of pain, and /or at lower thresholds for painful stimuli than do men [205];[65];[82];[71]. They also suggest that in humans, females showed a better ability to discriminate between heat intensities. However, Lautenbacher and Strian, [124] found no sex differences in heat-pain thresholds. Women had significantly lower warmth thresholds then men but similar cold thresholds; Lautenbacher and Rollmann [123] found no sex differences in heat pain, warmth and cold thresholds. They did find significant sex differences in electrical detection, pain and tolerance thresholds, with women having the lower thresholds. This inconsistency also appears with test utilizing cold thermal [218];[100] or with electrical stimulation

[126];[181]. Some controlled studies demonstrate clear sex differences in sensitivity to noxious stimuli, particularly mechanical pressure stimuli [18]. One possible difference that has been consistently noted is the complexity of the fluctuating role of steroid hormones in differences in pain sensitivity.

Studies of sex differences in pain sensitivity for rats produces varying results, as well. In part, the sex differences found seem to vary depending on the type of pain test administered. Sensitivity to electric shock seems to be greater for females than for males [158];[119];[120].In contrast, with tail withdrawal tests males appear to have greater sensitivity to pain [186];[111];[18].

To review some of the findings regarding sex differences in non-drug induced analgesia for rodents is to find contradictions, and there is a vast range of findings that seem to contradict each other (see Table 1).

Stimulus	Species, Strain-vendor"	Nociceptive test	Result	Procedural notes	Ref.
Stress:					
Cold Water Swim	Rat, SD	Tailflick	M > F	continuous vs. intermit. Swim	[46,47]
		jump threshold	M > F		
	Mouse, SW-B&K	Hotplate	M = F		[48]
	Mouse, SW-S, B6, D2-Jackson	Hotplate	M = F		[49]
	Deer mouse	Hotplate	M > F	Depended on breeding status	[50]
Restraint	Deer mouse	Hotplate	M > F		[51,52]
	Rat, Wistar	tail withdrawal	M > F	longer duration	[53]
	Rat, Wistar	formalin paw	F > M	second phase	[54]
Shock	Rat, F344-Harlan	tail withdrawal	M > F		[55]
	Mouse, Swiss-II	Tailflick	M > F		[56]
		Tail withdrawal	M = F		
		abd. constrict.	M = F		
Predator	Mouse, ?-RML	Hotplate	F > M	30-s exposure	[57]
			M > F	15-m exposure	
	Meadow vole	Hotplate	F > M	30-s exposure	[58]
			M = F	15-m exposure	
Predator odor	Deer mouse	Hotplate	M = F	30-s exposure	[59]
Biting fly	Deer mouse	Hotplate	M = F	5-m exposure	[60]
			M > F	30-m exposure	
"Novelty"	Deer mouse	Hotplate	M > F		[61]
"Competition"	Human, athlete	cold pressor	M = F		[62,63]
Athletic		radiant heat	M = F		
video game	Human, athlete and non-athlete	cold pressor	M > F	F no effect	[63]
		radiant heat	M = F	fingertip hyperalgesia, forearm analgesia	
Noise	Human	radiant heat	F > M	M hyperalgesia	[64]
Electroacupuncture:	Mouse, 10 in-bred strains-Jackson	Tailflick	M = F		[65]
Mating:	Hamster, Syrian	footshock paw shake/escape	M = F		[66]
Exercise:					
Isometric	Human	finger pressure	F > M		[67]
Treadmill	Human, athlete and non-athlete	cold pressor	F > M	M no effect	[63]
		radiant heat	M = F		
Wheel-running (20 days)	Rat, Long-Evans-CR	Tailflick	F > M*	*F no effect, M Hyperalgesia	[68]

[a] SD = Sprague-Dawley; SW = Swiss-Webster; B & K = Bantin and Kingman; II = Interfauna Iberica; RML = Rocky Mountain Laboratories; CR = Charles River.

Table 2. depicting differences in types of testing and differences of results regarding sexual dimorphism depending on test and strain of animal being used. From Craft,2003 [53]. *Table has been modified.*

For example, the type of nociceptive test done seems to affect whether males or females show a greater response to pain. Electrical shock experiments consistently indicate that females are more sensitive to pain than are males [185].

In active avoidance female rats acquire the response more quickly and are more resistant to extinction than males [22];[23];[61].

In an experiment using foot shock, Drago et al [63] found that passive avoidance latencies were longer in males than females. They discovered a positive correlation between the intensity of the shock and the length of avoidance latencies for males that did not exist for females. They postulated that while learning of the response was not different between males and females, that the retention of the learned response was different, with females showing. They concluded that female rats show a superior passive avoidance when ongoing operant behavior is suppressed by the present of aversion stimulation and that they show inferior passive avoidance when the retention of the response is not measured immediately after the aversive experience. These findings are consistent with other labs [41];[201] that examined sex-dependent effects of inescapable shock administration on behavior and subsequent escape performance in rats. In their experiment, they looked at the behavior of rats exposed to shock of varying lengths and found that male rats were more affected more profoundly than females by exposure to inescapable shocks. They found that duration was a more important factor than frequency of shock. The proposed explanation for the differences was that fluctuations in hormonal levels in the

females might account for their reactivity to shock. They further speculated that the reduced responsiveness to shocks in some of the females was the reason for why female's performance was unaffected by shock duration (interestingly, this experiment did not include lavage testing).

Romero and Bodnar [185] found that with continuous cold-water swim test (CCWS), males were significantly higher in magnitude of analgesia than females. In later experiments Romero et al [186]) worked with male and female gonadectomized rats found that both castration and ovariectomy significantly reduced the CCWS analgesia and further noted that castrated males showed analgesic magnitudes similar to those of intact females for both tail-flick and jump tests. They noted that there was a no change in activity levels during the swim for gonadectomized vs. non-gonadectomized rats for the CCWS, or in terms of hypothermia or change of body weight and they concluded that the differences in analgesia between the gonadectomized rats and non-gonadectomized rats was from the loss of gonadal steroids. They felt that the steroids play a facilitatory role in controlling stress-related analgesic responses.

Hormones, Pain and the female estrus and menstrual cycles

Regarding the correlation of pain levels for normally cycling humans, clinical tests show that women appear to have higher thresholds for pain during the luteal phase, when progesterone levels are high relative to estrogen levels [92];[48];[219];[29];[94];[176];[212]. In contrast, increased thresholds to pain have also been noted during the premenstrual phase, when progesterone levels are decreasing and also at the time of ovulation when estrogen levels are high and

progesterone levels are low [130];[135].Progesterone has been found to have anesthetic activity in mediating pain, while estrogen generally exerts excitation actions [193];[191].

As shown in Figure 4 [73], blood levels of estrogen and progesterone vary during the human female menstrual cycle. During the follicular phase, estrogen is initially at low levels, progressively rises at the end of this phase, and peaks at ovulation. In the luteal phase, estrogen levels are moderate and progesterone levels are high. Both estrogen and progesterone levels decline at the end of the luteal phase. Estrogen and progesterone fluctuate during the rat estrous cycle, but the pattern of these fluctuations differs from that in women. The rodent estrous cycle is divided into four phases. In diestrus, estrogen levels begin to rise and progesterone levels are low. In proestrus, estrogen levels peak and descend while progesterone levels rapidly peak and decline by the end of the phase. Estrogen and progesterone decline during estrus and begin to rise by the end of metestrus.

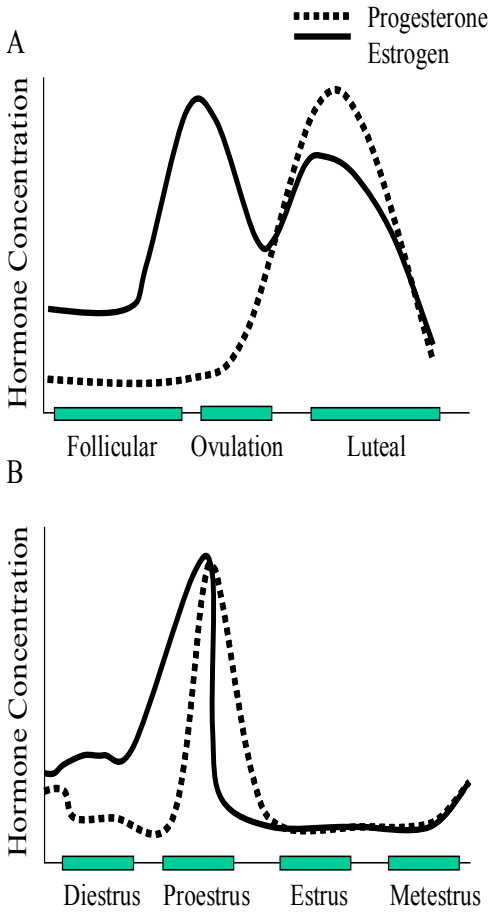


Figure 2. Adapted from E. Festa and V. Quinones-Jenab, 2004 [73].

Estrogen receptors are also present in the dorsal root ganglion of the spine. Patrone [159] suggests that estrogen modulates cutaneous sensation by acting directly on primary afferent cell body neurons. Estrogen receptor α is expressed in the small dorsal root ganglions [199] while estrogen receptor β is expressed in large, medium and small dorsal root ganglions [207];[128]. Liuzzi et al [128] suggests that loss of estrogen, such as occurs during menopause, may contribute to a decline in dorsal root ganglion neuronal function and may exacerbate ongoing neuropathic processes.

There is also evidence to suggest that the differing results of findings regarding pain and hormonal levels are related to the type of test (different actual types of pain administered or considered, as well as social and cultural differences that confound the findings). Research that examined the effects of these different paradigms of stress-induced analgesia also uncovered gender differences.

Neurons with steroid hormone receptors have been implicated in behavioral and endocrinological effects of reproduction [149];[150]. The responsiveness to the hormones estradiol and progesterone is not fixed i.e. there is evidence of plasticity in the number of receptors in each neuron of the brain [149;160];[150] studied the regulation of estrogen receptor mRNA in the brain of female rats during pregnancy and found many hormonal and behavioral changes that are unique to the condition of pregnancy. Onset of maternal behavior included among these characteristics: nesting, nursing, pup retrieval and protectiveness toward the pup) was one of the important behavioral changes

noted with the increase of estrogen. Based on prior studies by Giordano et al [95],Morrell et al [143] then examined identified the medial preoptic nucleus (MPN) and the caudal preoptic nucleus (CPN) as important areas to study over the course of pregnancy and they monitored the number of neurons expressing estrogen receptor mRNA during pregnancy.

In experiments with ovariectomized rats, Gordon and Soliman [98] used estradiol (E), progesterone (P) or a combination of E+P subcutaneously and were then tested for tail-flick and hotplate nociceptive thresholds. They found that the combined E+P produced significant increase in pain latency that was not seen for either steroid administered alone when using the tail-flick test and saw different results when using the hotplate method. In the hotplate method, rats treated with estradiol alone showed decreased latency to tail-flick, as did progesterone alone. Gordon and Soliman [98] postulated that peripheral sex steroid hormones were influencing central opioid activity and this is consistent with findings of others [220];[217]. Ratka and Simpkins [176] showed that ovariectomized rats treated with estradiol demonstrated increased sensitivity to thermal stimulation using the hotplate test. They theorize that hyperalgesia may occur as a result of the estradiol caused increase of endorphin levels in the hypothalamus previously shown by Forman et al, [81]. Ratka and Simpkins [176] also speculate that there is a reduction of opioid binding cites and cite findings by Wilkinson et al [220].

Further, pain thresholds in female rats may vary during different reproductive stages, [101]; [28];[168]. This suggests that the hormonal

environment may modulate nociceptive response of females [14];[39];[147];[157];[21]. Berkeley [28] notes that 45% of the articles in refereed neuroscience journals failed to mention the sex of their subjects. As Fillinghim [79] notes: these differences can be complex and variable and their exact nature is not always clear. Three important issues related to sex, gender, and pain are: 1) the magnitude of sex differences in pain 2) the mechanisms underlying these differences and 3) their clinical differences. Discrepancies between estrogen and progesterone effects on nociceptive response have been reported.

Some research reports increased pain sensitivity during the premenstrual (luteal) phases [171];[103];[77] while others have found that the increased pain sensitivity occurs during the post-menstrual (follicular) phases [130];[136];[98];[91]. In earlier research, scientists found conflicting results: Frye et al [84] found a decreased tail flick response for proestrus vs. metestrus, and increased tail flick response during pseudo pregnancy for OVX females with hormone replacement. Martinez-Gomez et al [136] found that there were decreased thresholds for estrus and metestrus vs. proestrus and diestrus. The different results may be that different tests were administered, that different strains of rat were used, that mice were being used *instead* of rats or that the method/amount of hormone replacement varied. Mogil et al [148] note that pain research as always been made more difficult because of the subjectivity of measuring pain. This is further compounded by the wide variation in handling techniques. If an animal is handled frequently and accustomed to being tested, there may be less stress for the animal and this may affect the animal's

response. Other researchers feel that frequent handling has the opposite effect and, in fact, increases the stress of the animal.

Effects of hormones and opioids on pain and other behaviors

Attempting to find a link between steroid hormones and various opioids led to several experiments. As with previous hormonal research, there are conflicting findings. Pfaus and Pfaff [167] performed an experiment in which they used various combinations of estradiol or progesterone in conjunction with μ-, δ and κ-opioid antagonists to examine sexual behaviors in the female rat. Using OVX rats, they treated the animals with injections of either estradiol or E+P and then treated them with one of the opioid agonists.

Baamonde et al [14], Islam et al [111] and Cicero et al [45] all found that male rats had greater antinociception than females to μ-opioids. Typically, male rats display a greater degree of antinociception at comparable doses of morphine. Cicero et al [46] show that the levels of morphine in the brain and in the blood of the animals was equal and postulate that the sex differences occur because sex hormones mediate the effect of the opioid. They feel that it is the organizational effects of steroids in the developing brain as opposed to activational effects caused in an adult rat. This was contradicted by Kepler et al [119] and Ali et al [5] who found no apparent sex differences. In their review paper, Bartok and Craft [18] found that different testing methods and different doses of injections were used and cite these variations as possible reasons for the discrepancies in findings. Terner et al [208] have also found inconsistencies of pain tolerance depending on the particular strains of rats. Female rats showed

less analgesic effects than matched males for both opioid and non-opioid mediated tests. Females also remained constant in their responses over the entire estrous cycle. In contrast, for different types of stress testing, this was not the case and responses varied significantly for foot shock tests in direct correlation to the phase of the estrous cycle [189].

Barrett et al [16] examined sex-related differences when testing mechanical nociception and antinociception produced by μ- and κ-opioid receptor agonists. In this experiment, gonadally intact males and females were tested using paw pressure. Males had higher nociceptive thresholds than females by 37%. Barrett et al [16] found that efficacy was more potent for male rats when μ-receptor agonists were used, but that sex differences were not apparent when κ-receptor agonists were used. They also noted that their results differed from others who used different types of nociceptive tests. For example, Cicero et al [45] found that there are typically male-female differences in the antinociceptive activity of μ-opioids with males displaying a greater degree of antinociception than do females at comparable doses of morphine when using the hot plate test. Bartok and Craft [18] used hot plate and tail flick tests to test μ-, κ- and δ-opioids. While they discovered sex differences, they attributed these differences to assay-, dose- and/or time factors. They found that females had higher hot plate and tail flick latencies in non-drug situations. Females had significantly longer latencies than males on the tail flick tests. They recalculated results for basic sex differences when testing the drug situations and found that κ-opioids produced greater

antinociceptive response in females when using the tail flick test that these effects appeared earlier when testing females. In contrast, males displayed greater antinociceptive response to δ-opioids with the hot plate test. Cook et al [51] built on these findings and found that μ-opioids were more potent in antinociception in their experiments that used thermal nociception rather than mechanical. They found that the magnitude of the sex differences found increased with decreases in the efficacy of the opioids and with increases in the nociceptive stimulus temperature (in this case, water temperature).

Gintzler and Bohan [93] did experiments in which they simulated pregnancy by using doses of estrogen and progesterone that would occur during pregnancy. In those experiments and in others performed by Dawson-Bascoa and Gintzler [59], they were able to induce levels of antinociception similar to that in pregnancy and significantly, to block those effects by administering naltrexone, a μ-opioid antagonist. They concluded that these results indicated that endogenous opioid antinociceptive systems could be activated by peripheral ovarian sex steroids, whose circulating concentration profile could be altered by pseudopregnancy [94].

Tolerance

Collett [49] defines tolerance as a phenomenon in which exposure to a drug results in the diminution of an effect or the need for a higher dose to maintain and affect. He further breaks down types of tolerance (see Table 2). As Inturrisi [110] stated on the development of tolerance to opioids, analgesia shows a shift to the right of the dose-response curve.

As efficacious as opioids are as analgesics, tolerance to the drug is rapid, radically decreases its effectiveness and increasing the required dosages [96]. With human subjects, the psychological aspect of pain is another factor. Most physicians and healthcare providers provide unlimited access to morphine and other opiates for the terminal patient, feeling that the problem of addiction and tolerance will outweigh requirement for the pain control [140]. While Melzack and Wall [31] are particularly vociferous in their statements that morphine tolerance is not a major issue for chronic pain, the problem of acute pain and tolerance poses a more serious risk for a patient and certainly the animal research indicates that tolerance builds a matter of days. Nevertheless, the issue of the body adapting to opioids should be addressed and has led many research scientists and pharmacologists to explore alternative medications.

The particular mechanisms of opioid tolerance are complex and we do not have a complete understanding of how they work. As explained by Brodsky et al, [36] there have been several in vivo attempts to see if the increased number of binding sites is responsible for the changes observed in chronic opioid treatment. As of this writing, there is no agreement regarding the meaning of the increase in receptors. There have been several studies that have considered how chronic treatment changes the cortex or striatum in adult animals. For example Devries et al [60] found that chronic treatment did not change the number of μ-opioid receptors in the striatum. Similarly, Elliott et al [66] did an experiment measuring latency to tail-flick with intact male mice, using two different paradigms. In the first study, they injected increasing amounts of morphine twice a day for 3 days

and in the second experiment, they used osmotic pumps that supplied continuous morphine over 7 days. In both paradigms, the percent analgesic showed a shift right, indicating that tolerance had occurred. Dextromethorphan, an NMDA receptor antagonist was able to attenuate or reverse tolerance in a dose-dependent manner. Elliott et al, [66] concluded that morphine tolerance requires a functional NMDA receptor and that blocking this receptor could prevent tolerance. Davis and Inturrisi [58] did a similar study in which they gave intact male rats and mice morphine in administration of morphine in several paradigms. The first paradigm consisted of s.c. injections of 7mg/kg of morphine for 5 days. Baseline tail flick latency was studied on days 1 and 5. The second paradigm examined tolerance to morphine using 3 times a day with an escalating dosage of morphine. Methadone was also injected in some of the rats to attenuate the response on an escalating dosage. They found that on day 1, 70% of the mice showed analgesic responses, but that by day 5, only 5% were responding to the lower dose for the first paradigm. This effect was blocked by administration of methadone; with the second paradigm, there was a 3-fold increase in the morphine ED_{50} response that was prevented by the co administration of the methadone. Davis and Inturrisi concluded that systematically co-administered methadone could counteract the effects of systematically administered morphine and that the NMDA receptor antagonist could block the effect of NMDA-induced hyperalgesia.

Types of opioid tolerance
Inate
Acquired
Pharmacokinetic
Pharmacodynamic
Learned tolerance
Behavioral
Conditioned

Table 3. Types of opioid tolerance, from Collett, 1998 [49]

Sex differences in the development of tolerance

Kasson and George [115] determined that tolerance to morphine was slightly more rapid in males than in females. Craft et al [56] using gonadally intact male and female rats demonstrated that females had significantly longer hotplate latencies than males. Moreover, after tail-flick no sex differences were observed. When the length of chronic morphine dosing was extended to 2 weeks, male rats developed greater tolerance to and dependence on morphine than did females. Craft et al also noted that chronic morphine treatment disrupted the estrous cycle of female rats However, other studies of the development of tolerance to opioid compounds have shown that pain thresholds in female rats may vary during different reproductive stages [101];[197]. This suggests that the hormonal environment may modulate nociceptive response of females [16];[39];[147];[78];[146;216]. Shekunova and Bespalov [169] did not find significant differences between male and female rats when they tested for sex and estrous cycles affecting tail flick testing, but did find that development of acute tolerance was greatly affected by sex and estrous cycle stages. They found that acute tolerance was most pronounced in proestrus females and completely reversed by ovariectomy. They postulate that earlier findings by others [9]; [35] that did not find significant differences made the mistake of ignoring the estrous cycles and that accounted for the difference in findings.

Specific Aims

As discussed, a growing body of anatomical, endocrinological and behavioral data suggests (1) that there are significant differences between males and females in their responses to nociceptive stimuli and (2) that steroidal hormones play an important role in modulating the response to such stimuli in females. Due to their endocrinological profile, female rats provide a useful model for studying the interactions between hormones and nociception-induced CNS alternations. However, the direct effect of estrogen and progesterone in normally cycling females is difficult to study due to the cyclic manner in which steroid hormones fluctuate during different stages of the estrous cycle. To determine the possible modulation of ovarian steroids to the response of pain stimuli, we propose to utilize ovariectomized (OVX) rats and administer estrogen, progesterone or estrogen plus progesterone with acute pain treatments.

Our working hypothesis is that estrogen and progesterone modulate cellular and molecular mechanisms underlying nociceptive response and opioid tolerance in females. We hypothesize that estradiol will increase and progesterone decrease latency of responses to acute pain and that co-administration of estradiol and progesterone will have dose-dependent effects.

Results from this study may help to develop better and more effective pharmacological treatments for females. Furthermore, this study will also provide insight into nociception and analgesia in females utilizing estrogen or progesterone based anti-conceptive or estrogen replacement treatment after menopause. To test these postulates, the following aims are proposed:

Specific Aim One: To test the hypothesis that estrogen and progesterone are involved in the modulation of analgesia and nociception.

Previously reported discrepancies between the interactions between estrogen and progesterone effects on nociceptive response may reflect a dose, time of administration and paradigm of steroid replacement that affect the female response to nociception. First we will determine the optimal doses of steroid replacement paradigm needed to identify the threshold effects of steroids on nociception (using a standard tail flick response). The aim of our first set of experiments is to identify the critical concentration of estradiol and progesterone and their combinations that induce analgesia in female rats. We will first determine the optimal estradiol and progesterone doses to produce analgesia. The range of doses chosen for these experiments corresponds to physiological doses previously shown to affect the level of opioid peptide and receptors in OVX animals [83]. The steroid replacement paradigm chosen for subsequent experiments of this study will correspond to a dose that produces an analgesic response to during both acute and chronic behavioral testing.

Specific Aim Two: To determine to what extent opioid regulation of nociceptive response is affected by gonadal hormones:

We will determine how nociception responses to tail flick is affected by central administration (s.c. and i.v.c) of different opioid agonists and antagonists in ovariectomized rats co-administered with either vehicle, estrogen, progesterone, or estrogen plus progesterone. This will allow us to determine

whether opioid receptors play a key role in the regulation of nociception in female rats and identify candidate receptors.

Specific Aim Three: **To determine if ovarian hormones affect the development of opioid tolerance.**

To determine how steroids affect the development of tolerance to opioids, we will study the interactions between chronic morphine administration and different steroid replacement paradigms. The experimental design will follow those established for male rats by other investigators. These studies will help to determine the effect of steroids on opioid tolerance in female rats.

Chapter 2. The Effects of Estrogen and Progesterone Replacement on Acute Nociception in Ovariectomized Female Rats

Introduction

There are distinct differences in females and males in their response to nociceptive sensation and painful pathological conditions. Further, pain thresholds in female rats may vary according to the estrous cycle [185];[147];[8];[55]. For example, females have increased latencies to tail flick and decreased thresholds in the hot plate and warm water bath, suggesting that the hormonal environment may modulate nociceptive responses [39];[147];[78];[85];[146];[215] As noted in the introduction, the results found by various labs are often in conflict. For example, Martinez- Gomez et al [135], reported that tail flick latencies were shorter during times when estrogen levels are lowest (metestrus and estrus), but Kayser et al [117] found that there were decreased thresholds to mechanical and thermal pain during proestrus and estrus. Kepler et al [119] found that there were no changes in nociceptive sensitivities throughout the cycle. Pregnancy studies note that both humans and rodents are able to withstand greater levels of pain in the last stages of pregnancy [132]. At this time, both estrogen and progesterone levels are elevated [195].

This set of experiments will be conducted to establish a dose-response relationship between estrogen's and progesterone's effects on acute nociceptive responses using a warm water bath tail flick test in female ovariectomized rats.

However, we will first conduct a time course study to determine the point at which hormone levels stabilize and remain constant in the blood. These studies will determine the doses of hormone to be used in subsequent pharmacological experiments.

Methods

Animals

Eight week old, ovariectomized Sprague-Dawley rats (Taconic, Germantown, New York) were double-housed in standard plastic cages (20x20x41cm ^{3}) layered with beta chips under a 12-h light/12-h dark cycle (lights on at 9 A.M.) with food and water available ad libitum. Animals were handled daily for 5 minutes to reduce the stress induced by subsequent handling during the behavioral testing. All animal procedures were in accordance with the "Principles of Laboratory Animal Care" (NIH publication 85-23, Bethesda, MD, 1996) and approved by the Institutional Animal Care and Use Committee at Hunter College.

Hormones

Cholesterol, 17β estradiol and progesterone were purchased from Sigma (St. Louis, MO).

Hormone Replacement

One week after arrival, animals were anaesthetized and SILASTIC brand capsules (1 cm or 1.5 cm, 0.058 in. ID X 0.077 in. OD, Dow Corning) were implanted subcutaneously (s.c.) in the interscapular region following methods previously described [11];[162]. To prepare capsules, SILASTIC medical grade

tubing was filled with hormone or vehicle (cholesterol). The ends of the tubes were sealed with medical adhesive silicone. This manner of hormone delivery was chosen to avoid saturation of hormone in the animals and to achieve stable plasma levels of hormone. Doses of estradiol (17 β) were 10%, 20%, 30% and 40 % (in a mixture of estradiol and cholesterol). Progesterone was administered in a similar manner, using SILASTIC capsules of 100% progesterone in lengths of 1, 1.5, 3cm and 9cm, and control capsules contained cholesterol (100%). For co-administration of estradiol and progesterone, rats received hormone doses of either 10% estradiol and 1.5 cm progesterone (low-low); 10% estradiol and 3 cm progesterone (low-high); 40% estradiol and 1.5 cm progesterone (high-low) or 40% estradiol and 3 cm progesterone (high-high). These doses have been shown to fall within the range of serum levels during the estrous cycle [83];[166]. All animals were allowed one week to recover from the surgery.

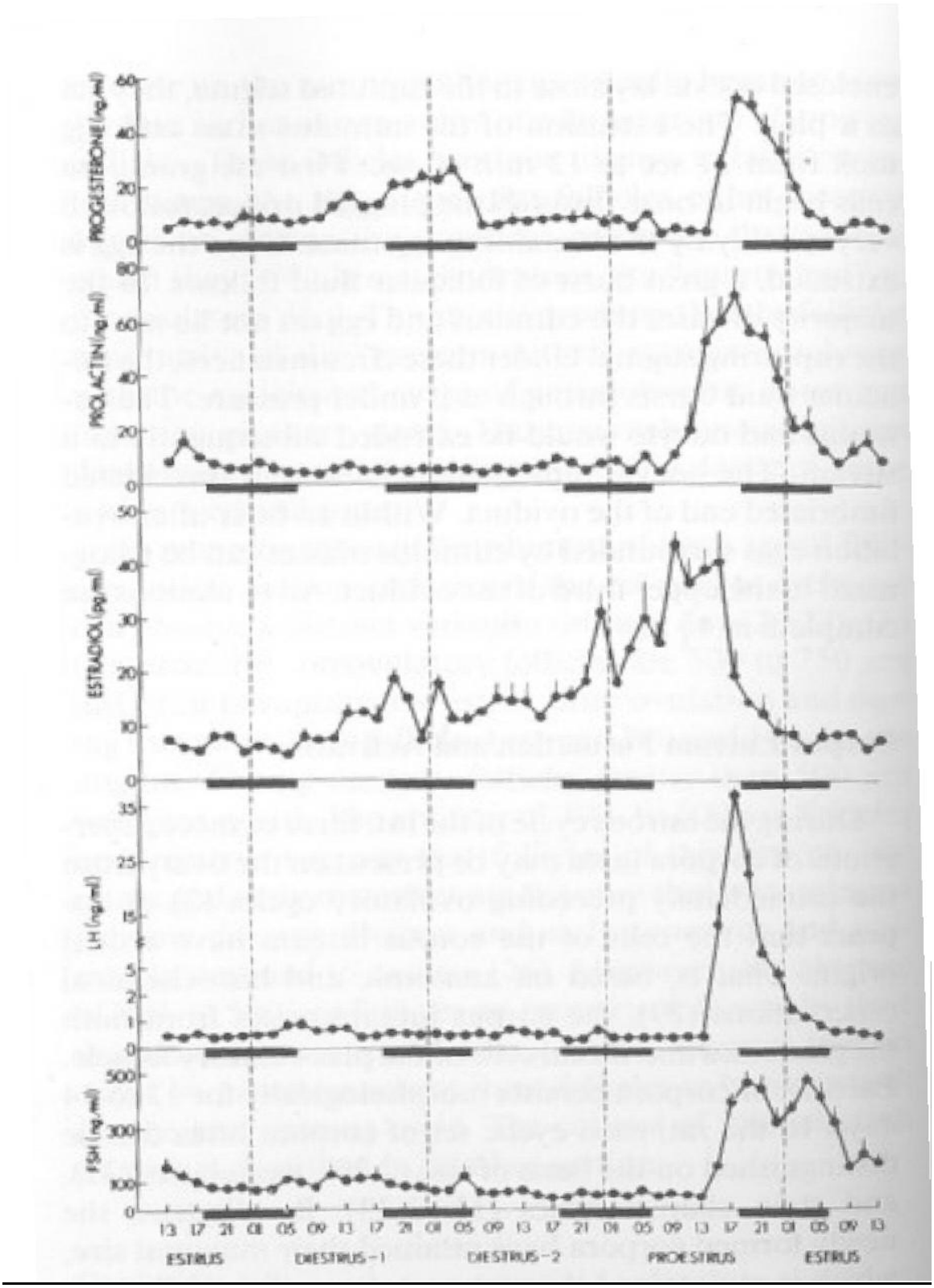


Figure 3. Pfaff,D.W. and Schwartz-Giblin,S., Cellular mechanism of female reproductive behavior. In: E.Knobil, J.Neill (Eds.), The physiology of reproduction, Raven, New York, 1995,

	Estrogen	Progesterone
Proestrus (approx)	45 pg/mL`	60 ng/mL
Estrus (approx)	10 pg/mL	10 ng/mL
Diestrus (approx)	17 pg/mL	22 ng/mL

Table 4. Physiological levels of estrogen and progesterone at various stages of the estrus cycle (adapted from Pfaff et al, 2002 [164]).

Estrogen	
10%	60 pg/mL
20%	200 pg/mL
30%	125 pg/m
40%	300 pg/mL
Progesterone	
1cm	7ng/mL
1.5cm	10ng/mL
3cm	17ng/mL
9cm	15ng/mL

Table 5. Strength of silastic implants (adapted from Pfaff et al, 2002 [164]).

Behavioral Testing

In order to measure the integrated response of gonadal hormone effects on nociception, we used the hot water tail-flick test to measure antinociceptive responses. Water temperatures used were 48°, 50°, 52.5° and 55°C. The rat was held above the water with the tail submerged half way in the water. The animal was held in this position until the tail flicks or until 15 seconds passed. At this point, the testing was discontinued to avoid tissue damage to the tail. Pre-testing for acclimation purposes was conducted. Actual testing was done on the animals by repeating the measurement three times and taking the mean as the result for the individual animal [35];[121].

Radioimmunoassay

Following a brief exposure to CO_2 (15 seconds), rats were decapitated and trunk blood was collected. Blood was centrifuged at 3,000 RPM for 30 minutes at 4ºC. Serum was collected and stored at –80 ºC until used. Serum levels of estradiol and progesterone were analyzed per the manufacturer's instructions using Estradiol Double Antibody and Progesterone Coat-Count kits obtained from Diagnostic Products (Los Angeles, CA). Results were determined using a log-logit analysis within GRAPHPAD PRISM (GraphPad Software, CA, USA). Levels were expressed as pg/mL for estrogen and as ng/mL for progesterone.

Statistical Analysis

One-way analysis of variance (ANOVA) were used to determine the effects of hormone dose on serum levels of estradiol (10%, 20%, 30% and 40 %

(in a mixture of estradiol and cholesterol).) or100% progesterone in lengths of 1, 1.5, 3cm and 9cm). For time course determination of estradiol and progesterone levels, one- way ANOVAS were used to determine the effects of estradiol (40%) or progesterone (3 cm) on time following implantation (2, 7, 14, 21 and 28 days).

Two-way analyses of variance (ANOVAs) were used to determine the effects of temperature (48°, 50°, 52.5°, 55° C) x hormone (for estradiol: 0, 10,20,40%; for progesterone: 0, 1, 1.5,3,9cm; for combined E+P 0, 10%E+1 cm P; 10%E+ 3cm P; 40%E+ 1 cm P; 40% E+3cm P) on tail flick. For post hoc analysis, Tukey tests were performed when appropriate, with all significances to the $p > .05$ levels.

Results

Both estradiol and progesterone serum levels increased according to the length and/or concentration of the implanted capsules (Fig.5). In rats treated with 10%, 20%, 30% and 40%, serum levels of estradiol were greater than rats with cholesterol implants [$F(4,33)= 12.13$, $p <0.0001$; Fig. 5A.] Furthermore, rats receiving 40% estradiol had higher serum levels than all other estradiol-treated groups ($p<0.05$). In rats treated with 1.5, 3 or 9 cm implants, serum levels of progesterone were greater than rats with cholesterol implants [$F(4,33)=9.563$, $p<0.0001$; Fig.5B]. Steady levels of both hormones were obtained 7 days past capsule implantation (Fig. 6A and 6B).

Tail-flick latencies decreased as a function of temperature in both estradiol and progesterone treated rats [estradiol; $F(4, 34) =51.51$, $p<0.0001$; progesterone; $F(4, 55) =12.96$, $p<0.0001$; Fig.7 and Fig.8]. Estradiol did not

affect acute nociceptive responses at the highest temperature tested. However, at 48°, 50° and 52.5° C, there was an increased tail flick latency as compared with cholesterol treated rats independent of dose ($p<0.05$). At 48° C, estradiol doses of 20, 30 and 40% produced higher tail flick latencies as compared to control and 10% estradiol ($p< 0.05$; Fig 3). At 52.5° C, the highest dose of estradiol, 40% implant, produced the longest latency to tail flick ($p<0.001$; Fig. 7C). Progesterone, on the other hand, did not affect tail flick latencies at any temperatures or doses tested ($p>0.05$; Fig. 8).

However, when estradiol and progesterone were co-administered, a significant effect of hormone, independent of dose, was observed at 50°C [$F=4.586$, $p<0.0001$] where tail flick latencies were increased: Fig. 9.

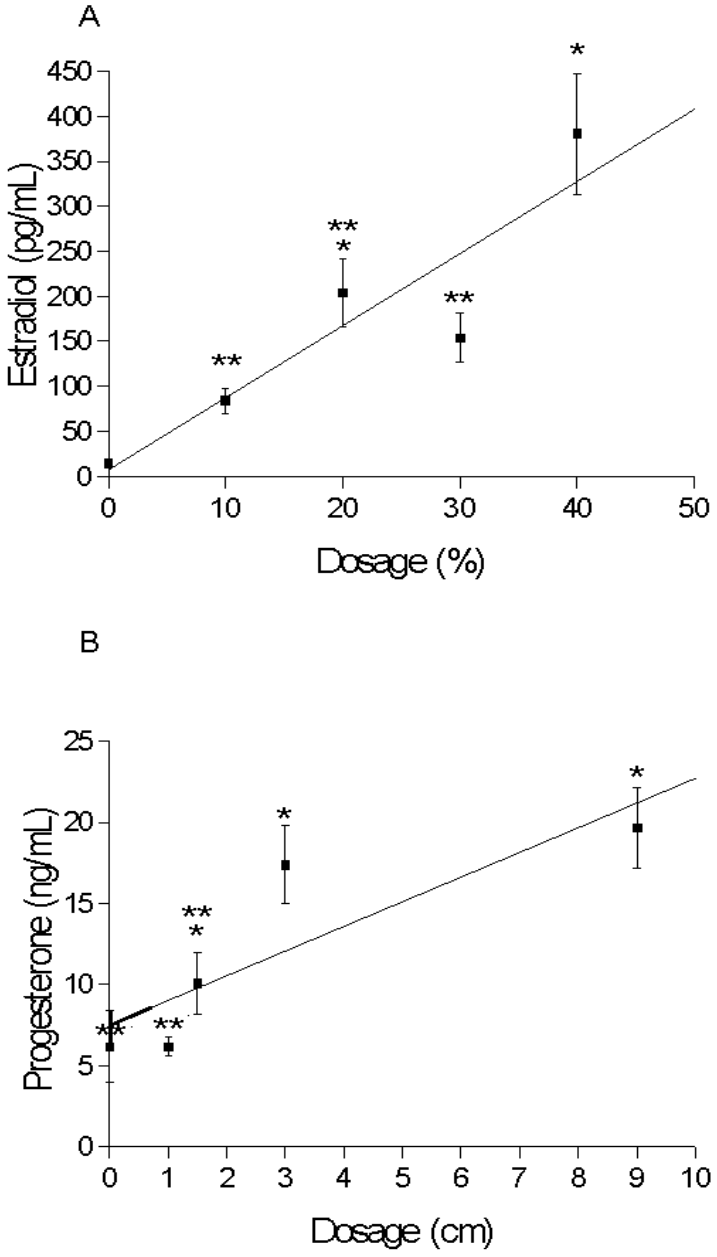


FIGURE 4A: Estradiol serum levels (pg/mL), in female OVX Sprague Dawley rats administered in doses of 10, 20, 30, and 40% for estradiol (* indicates 0 is different from 20 and 40%; ** indicates 40 is different from 10, 20 and 30%). FIGURE 4B: and progesterone (ng/mL) in 1, 1.5,3 and 9 cm lengths (* indicates 0 is different from 1.5,3 and 9cm; ** indicates that 0, 1, and 1.5 are different from 3 and 9cm). Animals were sacrificed 7 days after implantation and RIAs were run to quantitate estradiol and progesterone serum concentrations.

A

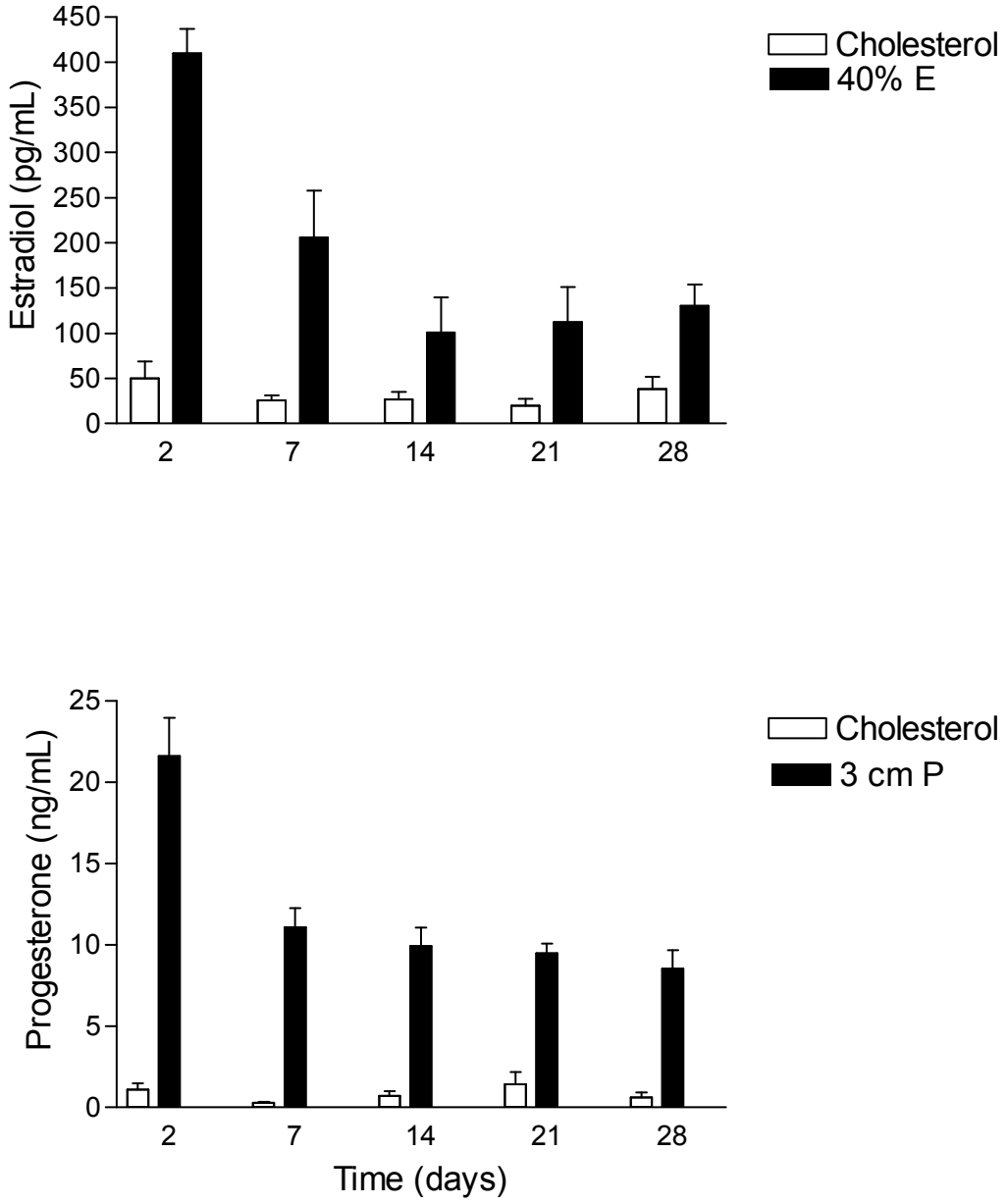


FIGURE 5A: Estradiol (pg/mL) and FIGURE 5B: progesterone serum levels (ng/mL) in female Sprague Dawley rats after 2, 7,14,21,28 days (n=3-7)

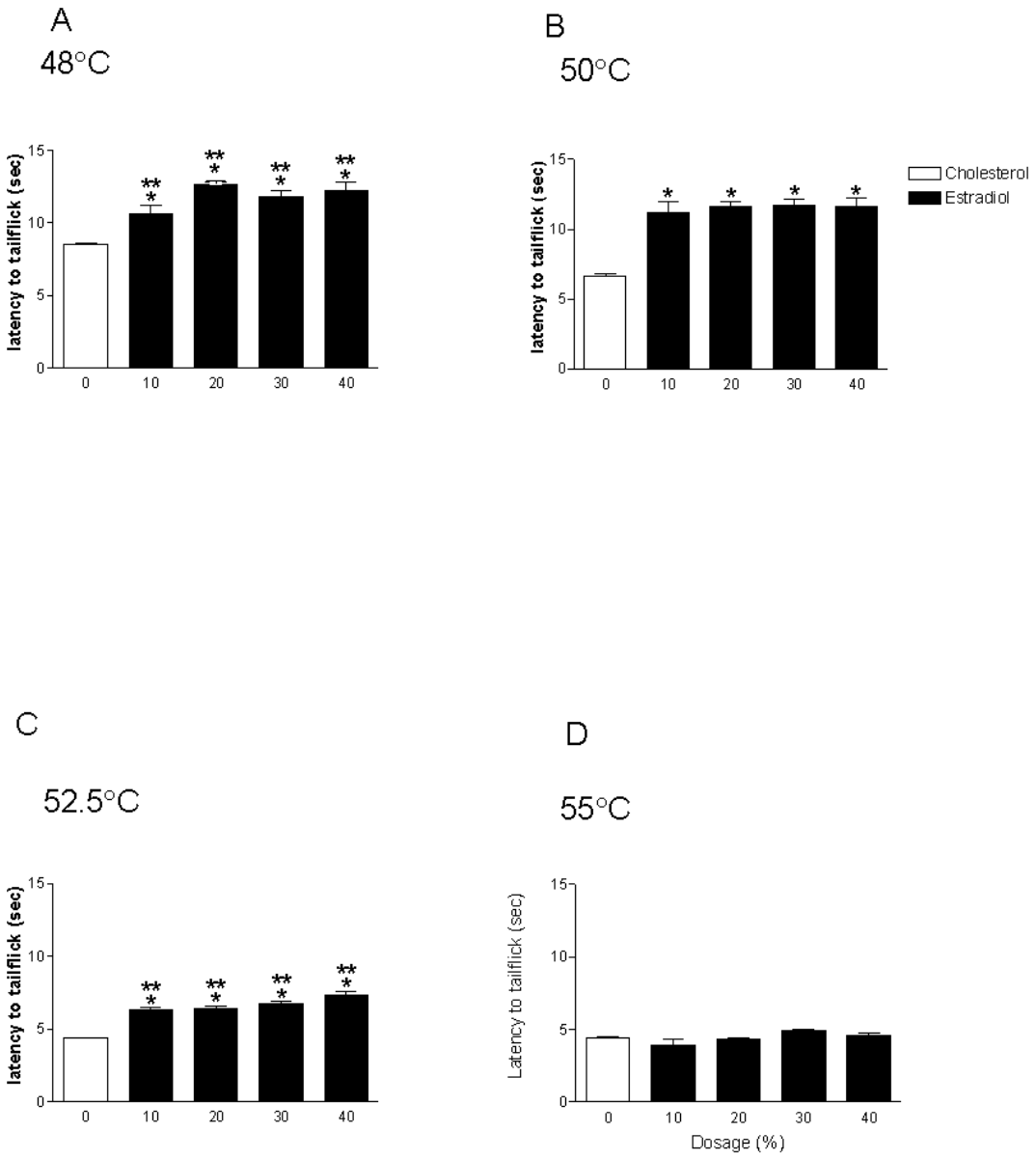


FIGURE 6: Effects of Estradiol on acute nociception at 48°, 50° and 52.5° C in female Sprague Dawley rats (n=6-20). At 48° C there is no significance between the control (cholesterol) and the estradiol implants. At 50° C there is significance between the control and all of the strengths of estradiol implants with increased latency to tail flick. It appears that all of the strengths of estradiol are similar to each other in their effect. At 52.5° C, there is significance between the control and all strengths, with increased latency to tail flick seen in all estradiol. Additionally, there is significance between the 10%, 20% and 30% vs. 40% estradiol with the highest strength showing the largest latency to tail flick.

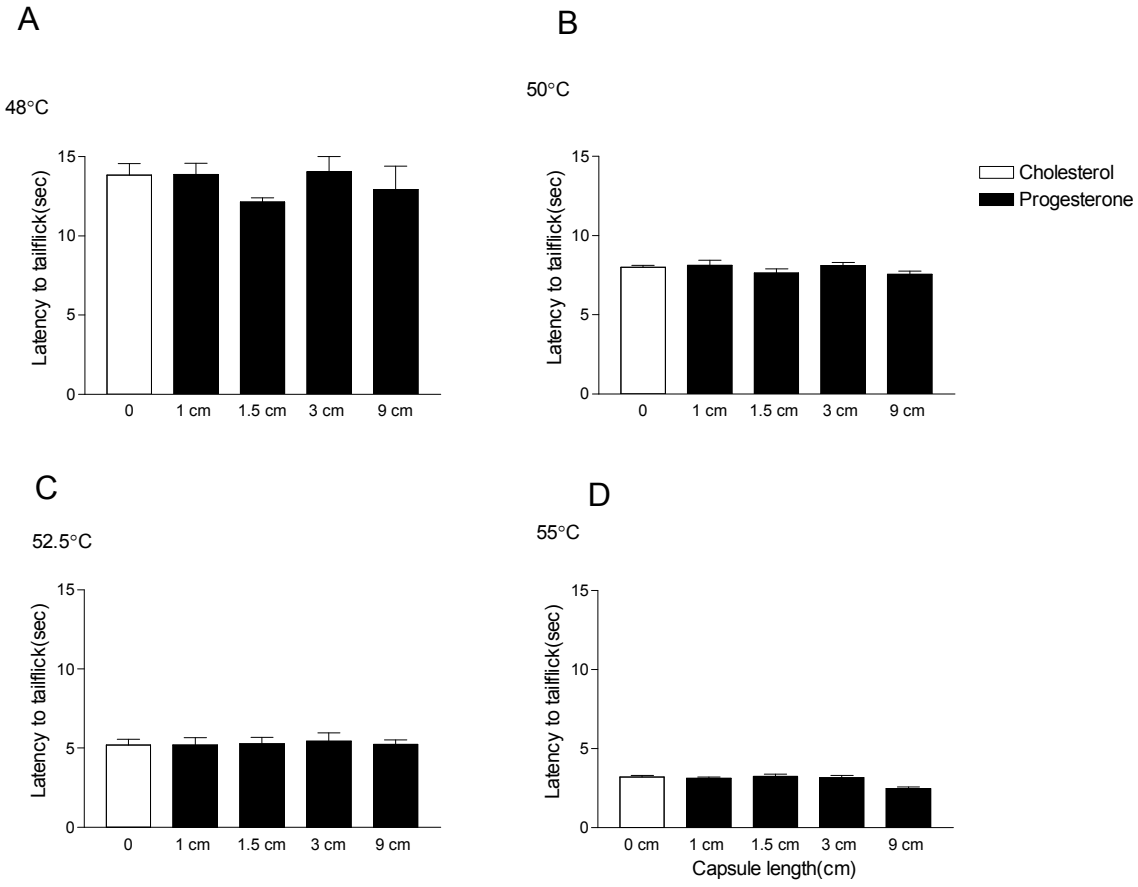


FIGURE 7: Effects of progesterone replacement on acute nociception at 48°C (A), 50°C (B), 52.5°(C) and 55°C (D) in female OVX Sprague Dawley rats (n=8). Doses of progesterone administered are 1, 1.5,3 and 9-cm length Silastic capsules of 100% progesterone. Zero represents 100% cholesterol.

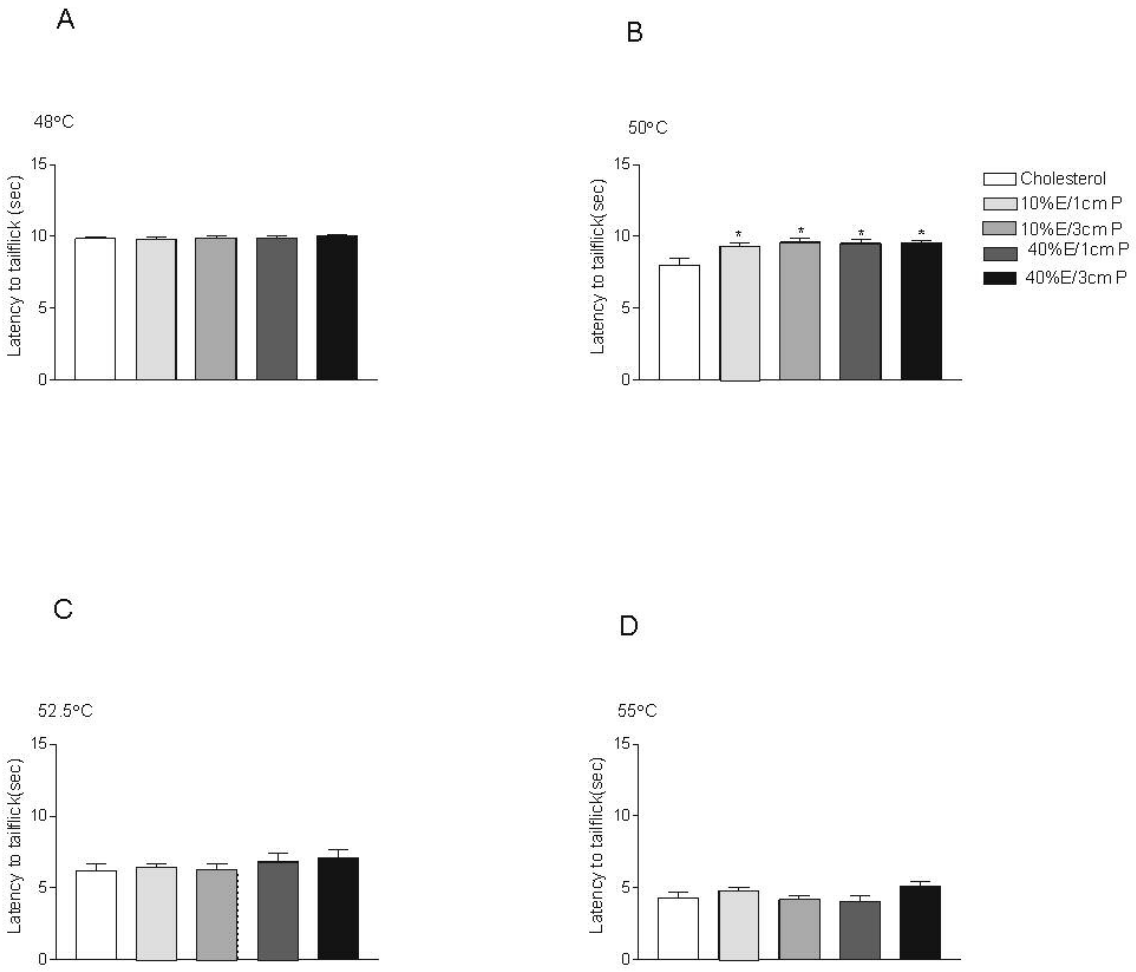


FIGURE 8: Effects of estradiol and progesterone on acute nociception at 48°C, 50°C, 52.5°C and 55°C in female OVX Sprague Dawley rats (n=6-10). * Represents significant effect of all doses at 50°C ($p<0.05$) when compared to control. Doses administered are 1cm 100% cholesterol; 10% estradiol with 1.5cm length progesterone; 10 % estradiol with 3-cm length progesterone; 40% estradiol with 1.5cm length progesterone and 40% estradiol with 3cm-length progesterone. All estradiol capsules are 1 cm in length.

Discussion

The first part of this set of experiments established that there is a direct relationship between hormone dose and blood levels. These results are in agreement with previous studies that have demonstrated estradiol and progesterone serum levels increase and stabilize in a dose- and time-dependent manner following capsule implantation [70];[192]. Moreover, serum levels of estradiol and progesterone are within the physiological range found in the normal cycling female rat [70]. For all hormone replacement groups, by day 7, the level of hormone found in the animal's serum was saturated and remained stable at least until day 28 when the experiment was discontinued. Thus, at any behavioral testing point in between 7-28 days hormone levels of both estradiol and progesterone remained constant.

The next set of experiments tested the effects of hormone and water temperature on tail-flick latencies in female rats. For all groups tested, when the test temperature was 55° C, tail-flick latencies were too abrupt to discern any hormone effects between groups. It was unlikely that either of these temperatures were optimal for assessing ovarian hormone effects on nociceptive responses. However, ovarian hormone effects emerged at 48°, 50° and 52.5°C. At temperatures of 48°, 50° and 52.5° C estradiol increased tail-flick latencies where the degree of the analgesic effect was greatest at 50° C independent of hormone dose. At 52.5°C, the highest dose of estradiol induced significantly greater analgesia than all other estradiol doses. Moreover, at 48° C, higher doses of estradiol increased a greater latency to tail flick as compared to low

estradiol and cholesterol treated rats. Previous studies have produced conflicting results where estrogen injections have produced no effect, increases and decreases in pain sensitivity in OVX female rats ([63]; [86]; [148]). These discrepancies appear to be related to a number of factors including, but not limited to, dose, administration paradigm and pain assay. For example, estradiol that is injected s.c. produces an increase in pain latency when using tail-flick methodology, but a decrease in pain sensitivity when using hot plate methods [98]. The results of the current study aimed to clarify inconsistencies in the literature by replacing hormone through implantation, which has the advantage of producing steady-state levels of hormone similar to those found in the normally cycling female animal. The intention of our paradigm was not to mimic the estrous cycle per se, but to have a consistent level of hormone rather than a cyclic pattern produced by injection paradigms which may result in differing hormone actions, i.e., genomic versus non-genomic effects of hormones on the opioid system. Thus, though the mechanisms through which a steady-state level of estradiol acts to produce analgesia are unclear, our study confirms that estrogens have anti-nociceptive effects, at least in this particular acute pain model.

Progesterone has been recognized as having an analgesic effect during nociceptive stimulation when administered alone [127]. In the current study, female OVX rats with progesterone replacement did not have an altered tail-flick response at any of the temperatures tested. Other studies have found that pain latency was significantly changed in a fluctuating manner over a period of days

when progesterone was injected [98],[116]. These differences likely arise due to administration paradigm discrepancies, though our results with estradiol mirror those using an injection paradigm and tail-flick test. Studies have demonstrated that progesterone's effects can be rapid, occurring in as little as 30 minutes following a physiological injection of progesterone that restored analgesia in female mice [203]. Few studies have examined chronic progesterone exposure and pain responses in animal models. Other studies suggest that progesterone effects on estradiol are facilitory, causing an augmentation of the analgesic response [139]. The combined estradiol and progesterone showed significance for all combinations of hormone dose strength at 50˚ C. Gordon and Soliman [98] also found that E+P increased pain latencies. They note that the levels of estradiol and progesterone during gestation may trigger the activation of a spinal opioid system and speculate that this may be responsible for the decreased pain sensitivity. Gordon and Soliman [97] previously speculated that the release of endogenous opiates may play a role in the analgesic effects of estradiol and progesterone. Others have shown that different temperatures appear to stimulate different pain pathways (see introduction).

Having tested the effects of hormones without opiates, we now wanted to examine the role that different types of opioids would have on the latency to tail flick. The effect of the μ-receptors found in morphine will be examined in the following chapter.

Chapter 3 Effects of estradiol and progesterone on morphine-induced antinociception

Introduction

Morphine is a potent opioid agonist that binds to mu-receptors. It is, and has been the preferred opioid of choice when trying to reduce pain [214]. As previously discussed in the introduction, studies have suggested that reproductive hormones are important modulators of the opioid system and, in turn, are important modulators of opioid-induced analgesia [108];[3]. For example, estrogen increases μ-opioid receptors mRNA and protein levels in the central nervous system. These effects have been attributed to both translational and transcriptional effects. To date, few studies have examined the rate of estrogen, progesterone and/or their co-administration on pain responses. The aim of this study is to determine to what extent estrogen and other gonadal hormonal hormones alter μ-opioid receptor behavioral responses. Using the warm water tail flick model, a dose-response study of estrogen, progesterone and E + P on morphine-induced analgesia will be done.

Methods

Animals

Eight week old, ovariectomized Sprague-Dawley rats (Taconic, Germantown, New York) were double-housed in standard plastic cages (20 x 20 x 41cm ^{3}) layered with beta chips Light cycle was a 12-h light/12-h dark cycle (lights on at 9 A.M.). Rats had food and water available ad libitum. Animals were

handled daily for 5 minutes for a week prior to manipulations to reduce the stress induced by subsequent handling during the behavioral testing.

Drugs and Hormones

Morphine 25 mg/mL was purchased from Henry Schein (Indianapolis, IN). Cholesterol, 17β estradiol, and progesterone were purchased from Sigma Chemical (St. Louis, MO).

Hormone Replacement

One week after arrival, animals were anaesthetized, and SILASTIC brand capsules (1 cm or 1.5 cm, 0.058 in. ID X 0.077 in. OD, Dow Corning) were implanted subcutaneously in the interscapular region following methods previously described [11];[161]. This manner of hormone delivery was chosen to avoid saturation of hormone in the animals and to achieve stable plasma levels of hormone. Doses of 17 β estradiol were 10%, 20%, 30% and 40 % (in a mixture of estradiol and cholesterol). Progesterone was administered in a similar manner, using SILASTIC capsules of 100% progesterone in lengths of 1, 1.5, 3 and 9 cm. Control capsules for progesterone or estradiol contained cholesterol (100%) with a 1 cm length. For co-administration of estradiol and progesterone, rats received hormone doses of either 10% estradiol and 1.5 cm progesterone (low-low); 10% estradiol and 3 cm progesterone (low-high); 40% estradiol and 1.5 cm progesterone (high-low) or 40% estradiol and 3 cm progesterone (high-high). These doses have been shown to fall within the range of serum levels during the estrous cycle [83]; [165] All animals were allowed one week to recover from the surgery.

Behavioral Testing Cumulative Dose Response (CDR)

To determine baseline activity, animals were tested for latency to tail flick with the water bath temperature at 55º C. The following day, animals were injected with incremental doses of morphine (1.4, 2.5, 4.5, 8.0, 14.0 and 25 mg/mL). Thirty minutes after morphine administration, the latency to tail flick was determined for each rat with a cut-off of twice the animal's baseline latency. Consecutive increments of morphine were administered 30 minutes after the prior behavioral testing. When the rats reached their cut-off level, they received no further incremental doses and all testing for that animal was finished.

Behavioral Testing Dose Response Morphine (DRC)

Baseline activity was determined at 48°, 50° and 52.5º C. For all behavioral determinations, 3 tests at each temperature were used with a 20 second cutoff. The following day, morphine was administered subcutaneously at 0, 1, 3 or 9 mg/kg. Thirty minutes after morphine pretreatment, the tail flick test was administered three times with 20 second intervals between testing. Each of the water temperatures were done for each animal with testing being done in ascending order on subsequent days.

Statistical Analysis

For the dose response curve, a concentration percent or quantal concentration effect curve was done. The concentration of a drug that produces a specified effect (in this case, the amount of morphine that was administered that sufficiently made the rat unable to tail flick in twice the time achieved in the drug-

free base-line). The data was analyzed using a program in Dr Charles Inturrisi's laboratory at Cornell Medical, BLISS-21. Two-way analyses of variance (ANOVAs) were used to determine the effects of hormone. Doses of estradiol were 1cm of 10% estradiol; progesterone was 1.5 cm of 100% progesterone and the combination of estradiol and progesterone was done with 1cm 10% estradiol plus 1.5 cm of progesterone by morphine dose (0, 1, 3, and 9 mg/kg) on tail flick at each temperature (48°, 50°, 52.5° C). For post hoc analysis, Tukey tests were performed when appropriate. Significance was at the $P < 0.05$ level.

Results

As shown in Fig.10D, although morphine dose response curves were shifted to the left after estradiol replacement and to the right after for progesterone administration(Fig.10E), no statistically significant changes were observed for ED_{50} of both hormones when compared to saline controls (p=0.16 for estradiol and 0.07 for progesterone). However, morphine ED_{50} was significantly increased after all E+P tested groups (Fig. 10F).

Overall, morphine increased the latency to tail flick (48˚C: [F (9, 32) =3.365, p=0.0010; Fig. 11A] 50˚C: [F (9, 32) =2.456, p=0.0128; Fig. 11B]; 52.5˚C: [F (9, 32) =2.410, p=0.0145; Fig. 11C]. However, at 48˚C, morphine effects were neither dose dependent nor hormone specific. At 50˚C, morphine effects on the latency to tail flick were dose dependent only on rats pre-treated with the estrogen plus progesterone treatment with 9 mg/kg of morphine increased the latency to tail flick when compared to all other treatment groups and 1 + 3 mg/kg

doses. At 52.5˚C, morphine effects were dose-dependent regardless of the hormone treatment (Fig. 11C).

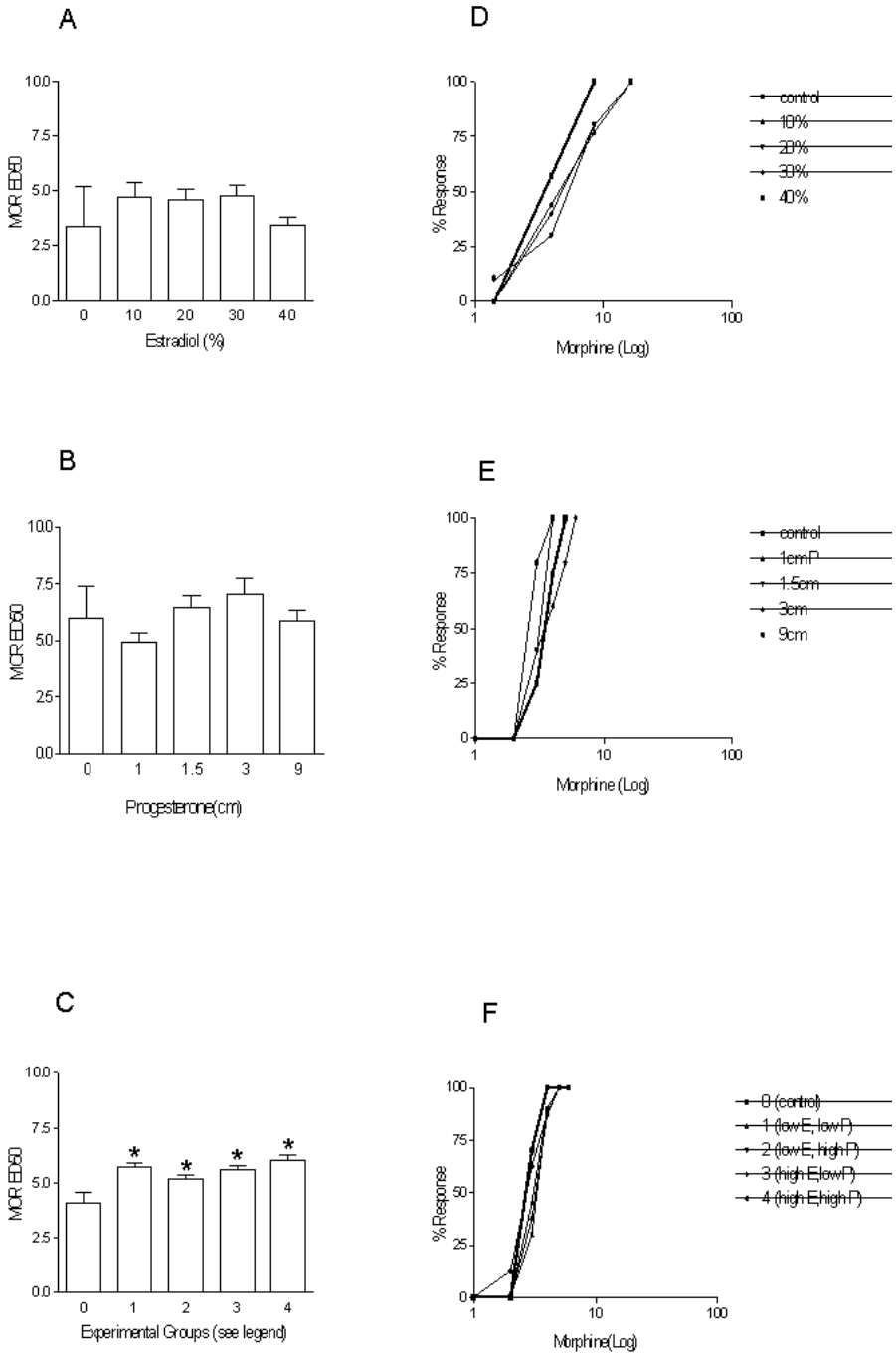


Figure 9. Mean ED_{50} and DRC (± SEM) after estrogen (A, D), progesterone (B, E) and E+P (C, F) replacement. Estradiol replacement concentrations are 10, 20, 30, 40%. For progesterone, replacement concentrations are 1, 1.5,3 and 9cm length capsules. Replacement concentrations of estradiol and progesterone for the group numbers are as follows: 1=10% E/1.5cm P; 2= 10%E/3cm P; 3=40% E/1.5cm P; 4= 40%E, 3cm P (n=8). * represents p>0.05.

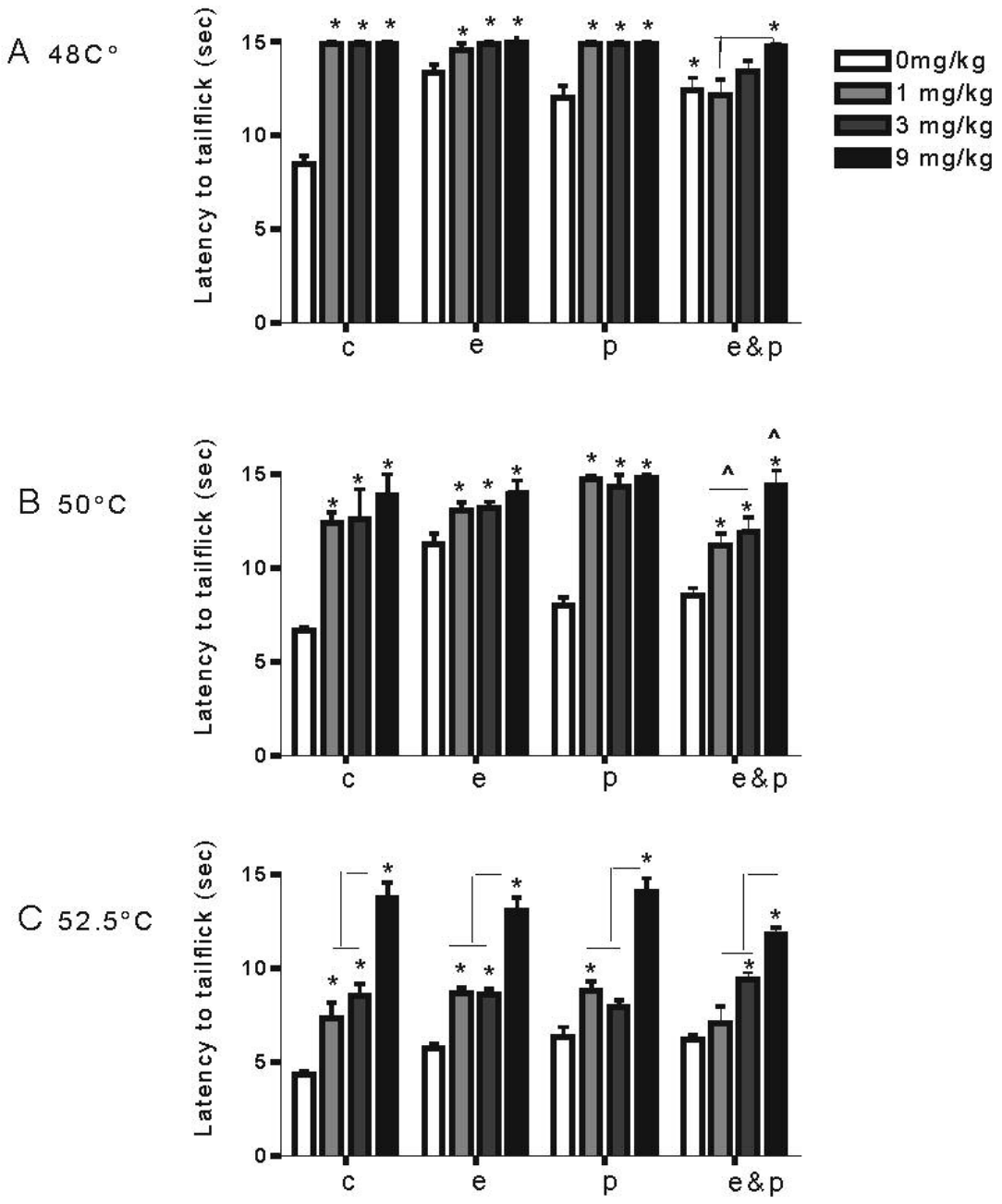


Figure 10. Dose response for morphine in the latency to tail flick at (A) 48º C, (B) 50º C, (C) 52.5º C in rats receiving E, P or E+P. Morphine doses were 0, 1, 3 and 9 mg/kg. Hormone replacements were as follows: cholesterol (100%); estradiol 1cm of 10% E; progesterone 1.5 cm 100 %. E+P doses were co-administration of both estradiol and progesterone.* represents statistically significant differences to saline. N=6-10 per group.

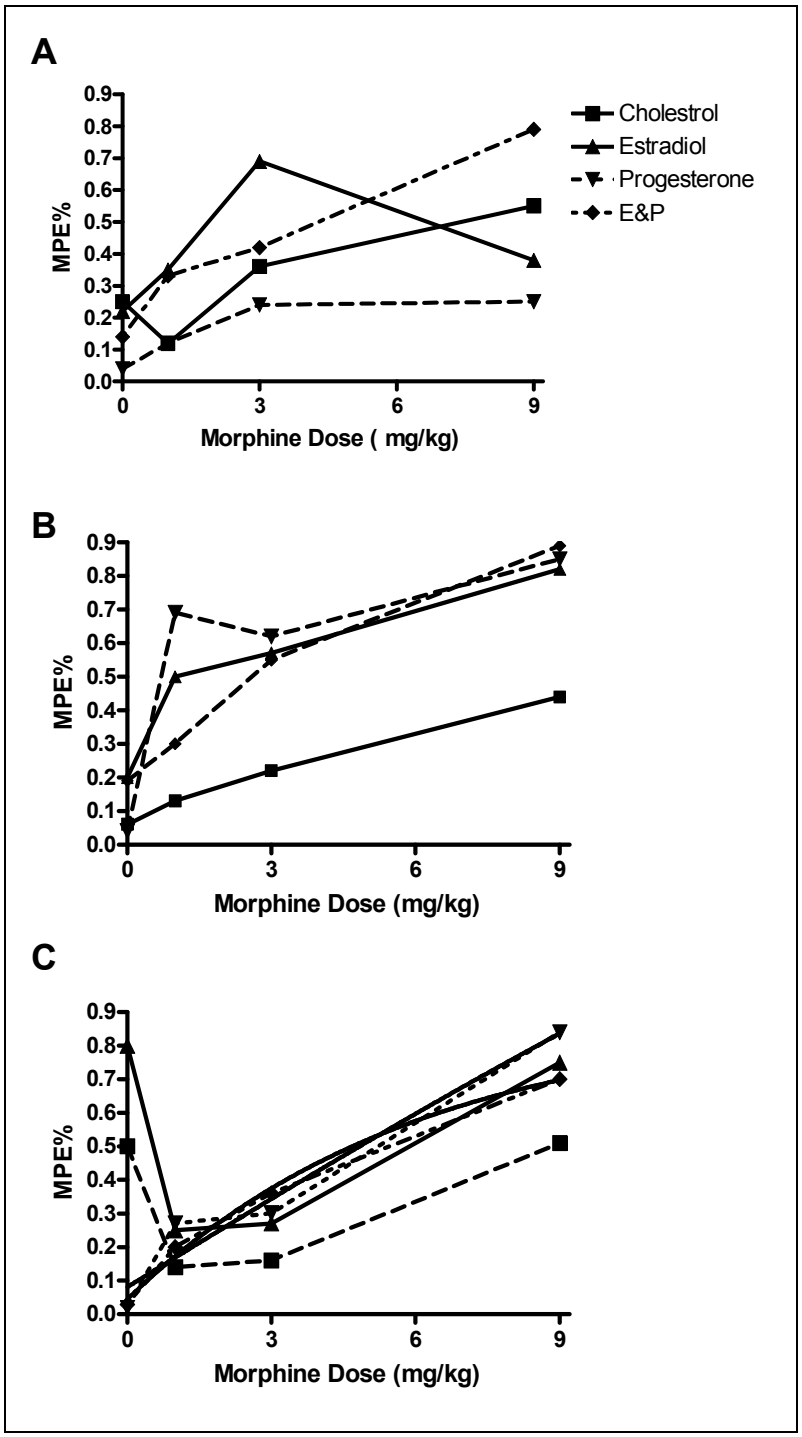


Figure 11. MPE% for **A** 48°C, **B** 50°C, **C** 52.5°C for morphine

morphine	cholesterol	estradiol	progesterone	E+P
48°C	1020	dnc	11.64	4.6087e-017
50°C	7.1090e-027	7.02250e-013	0.9157	97.72
52°C	291.1	270.0	9.9650e-017	6.6076e+019

Table 6. ED50 for morphine

Discussion

Consistent with our hypothesis that E+P would have a significant effect on nociceptive responses, we have shown that co-administration of E+P does cause increased sensitivity to morphine-induced analgesia. This finding is consistent with the finding of both clinical and rodent research [83]. Fillinghim and Maixner [78] found several clinical cases where women's estrus cycle affected pain thresholds; Aloisi [6] suggested that sex steroid hormones exert a profound influence on the morphine-induced analgesia. Moreover, the gross structure of the hippocampal neurons is modified by steroid hormones, as indicated by the modifications occurring in female rats during the estrous cycle phases. Further, our results regarding estradiol- treated ovariectomized rats agrees with Kasson and George [115]. Ovariectomized rats were significantly more responsive acutely to morphine and developed tolerance less rapidly than estradiol-treated females. However, alterations of gonadal hormones in males did not affect morphine responses. These results indicate that morphine responses vary considerably between strains of animals and are influenced by gonadal hormones of females, but not of males. Similarly, Ratka and Simpkins [176] did research using estradiol and progesterone pellets inserted either singly or in combination. They found that progesterone in high doses reduced morphine-induced antinociception and that it also enhanced the sensitivity to painful stimulation. They found that the effect of progesterone on morphine-induced antinociception was dose and time dependent; lower doses of progesterone potentiated morphine while a high dose antagonized the effects of the morphine.

Our results do not concur with them. This may in part be due to the strain of rat used and/or dose of progesterone.

Both estrogen and progesterone have been shown to regulate pain sensitivity in female rats. Ratka and Simpkins [177] demonstrated that OVX rats treated with estrogen showed increased sensitivity to the hot plate. However progesterone has been reported to produce both analgesia or to have no effect in the latency to tail flick. We did not observe any differentiated effect of estradiol or progesterone alone in morphine effects. Because estradiol and progesterone did not activate morphine responses, our results suggest that these observed behavioral responses are not based on a differential sensitivity of the μ-opioid receptor. Indeed, only when co-administered do they produce different effects than their respective controls and/or when administered alone, thus suggesting that the μ-opioid system is only differentially altered when both hormones are present.

The opioid system is modulated by ovarian hormones. For example, opioid receptors, including the μ-opioid receptor, have been shown to increase in protein and mRNA levels after estrogen treatment. However, short estradiol or progesterone administration has been shown to decrease μ-opioid binding in the pre-optic area. Thus, our group and others [164] have hypothesized that estrogen directly or indirectly influences the intensity of μ-opioid receptors in the central nervous system. However, we demonstrated that estrogen did not activate the morphine-induced analgesic responses, which included μ-opioid receptors, thus suggesting that although estrogen has the potential to alter levels

and/or to activate μ-opioid receptors, its effects do not extend to behavioral outcomes that control analgesia.

We, however, demonstrated that when co-administered, estrogen plus progesterone affect analgesic responses. As previously discussed, females have a complex endocrinological profile where E + P, under the regulation of hypothalamic and pituitary hormones fluctuate throughout the estrus cycle. Thus, interaction between gonadal hormones both in terms of concentrations and temperature relationships are, indeed, critical in the modulation if female behavioral activity as well as neuronal activity and central nervous system plasticity. Due to the overlap of motor mechanisms controlling the same aspects of pain and reproductive behaviors, pain and analgesic responses may be similarly regulated by interactions between estrogen and progesterone. Most studies have used a single dose and single hormone replacement to examine the role of estrogen and progesterone in pain and analgesia. Thus, our study demonstrated for the first time that it is possible that the differential effect of morphine during the estrus cycle indeed occurs through this estrogen and progesterone interactions. To what extent co-administration of estrogen and progesterone affected μ-opioid binding, protein levels, and/or mRNA transcription is yet to be determined. However, based on our observations, we can postulate that co-administration of the ovarian hormones altered μ-opioid receptor activation, thus producing a different behavioral outcome than when they are administered singly. Because, as shown in Figure 2, E+P affected the morphine effects at 50º C only, this suggests that the effect of these hormones is fiber

specific. This, in turn, suggests a distinct distribution of estrogen and progesterone receptors in different fiber types. Moreover, it also suggests that gonadal hormones may be involved in specific behavioral responses to distinct nociceptive stimuli, and thus, their effect is not ubiquitous.

Chapter 4 Effects of estradiol and progesterone on δ and κ-opioid-induced antinociception

Introduction

As recently reviewed by Stoffel et al [13] and Fillinghim et al [80], sex is an important determinant of nociceptive responses and sensitivity of antinociceptive effects of different opioid compounds. Generally, males are more sensitive to opioids than female rats. Barrett et al [17] demonstrated that μ-opioid receptors are less effective than κ-opioids to regulate sex and strain differences in antinociception. Gear et al [90] demonstrated that nalbuphine and butorphanol, both κ-opioid agonists produced significantly greater analgesia in females as compared to men; concluding that opioid analgesia is greater in females than men probably reflecting a difference in κ- opioid acting endogenous pain modulating circuits.

Gonadal hormones have been shown to play a pivotal role in the contribution of the dimorphic responses to pain [16]. Several studies have demonstrated that κ-opioid receptor densities in brain tissue were altered after estrogen administration [183] and during the estrous cycle [43]. Furthermore, after estrogen administration, δ-opioids receptors are altered at the binding, protein, and mRNA levels. Although many studies have been aimed to determine the pharmocokinetic factors affecting μ-opioid receptors in both male and female rats and the contribution of gonadal hormones, there is less evidence showing a direct role of gonadal hormones on δ and κ-opioid receptor activation.

Since in previous experiments in this dissertation, we demonstrated that estrogen and progesterone play a limited role on μ-opioid receptor modulation, this experiment was aimed to determine what if any δ and κ- opioid receptors play on modulating nociceptive responses in female rats.

Thus, our studies aimed to clarify ovarian hormone effects on other types of opioid-induced antinociception. The results from this experiment will help to determine which opioid receptors sub-types interact with ovarian hormones in the modulation of nociceptive responses in female rats. For κ- opioid agonists, we used U50, 488 (in doses of 1, 3 and 10 mg/kg); the δ- opioid agonist used was SNC80 (2.5, 5 and 10mg/kg); Saline was used as a vehicle dose. Water bath temperatures were 48°C, 50°C and 52.5°C.

Methods

Animals

Eight week old, ovariectomized Sprague-Dawley rats (Taconic, Germantown, New York) were double-housed in standard plastic cages (20 x 20 x 41 cm ^{3}) layered with beta chips Light cycle was a 12-h light/12-h dark cycle (lights on at 9 A.M.) Rats had food and water available ad libitum. Animals were handled daily for 5 minutes to reduce the stress induced by subsequent handling during the behavioral testing. All animal procedures were in accordance with the "Principles of Laboratory Animal Care" (NIH publication 85-23, Bethesda, MD, 1996) and approved by the Institutional Animal Care and Use Committee at Hunter College.

Drugs and Hormones

U50, 488 Hydrochloride and SNC80 are obtained from Tocris (Ellisville, MO). Cholesterol, 17β estradiol and progesterone were purchased from Sigma Chemical (St. Louis, MO).

Hormone Replacement

One week after arrival, animals were anaesthetized, and SILASTIC brand capsules, (1 cm or 1.5 cm, 0.058 in. ID X 0.077 in. OD, Dow Corning) were implanted subcutaneously (sc.) in the interscapular region following methods previously described ([11],[161]). To prepare capsules, SILASTIC medical grade tubing was filled with hormone or vehicle (cholesterol). The ends of the tubes were sealed with medical adhesive silicone. This manner of hormone delivery was chosen to avoid saturation of hormone in the animals and to achieve stable plasma levels of hormone. Doses of estradiol (17 β) were 10%, (in a mixture of estradiol and cholesterol). Progesterone was administered in a similar manner, using SILASTIC capsules of 100% progesterone in lengths of 1.5 cm, and control capsules for progesterone or estradiol contained cholesterol (100%) with a 1 cm length. For co-administration of estradiol and progesterone, rats received hormone doses of 10% estradiol and 1.5 cm progesterone. These doses have been shown to fall within the range of serum levels during the estrous cycle ([83],[165]). All animals were allowed one week to recover from the surgery.

Behavioral Testing

In order to measure the integrated response of gonadal hormone effects on nociception, we used the hot water tail-flick test to measure antinociceptive responses. Water temperatures used were 48°, 50° and 52.5° C. The rat was held above the water with the tail submerged half way in the water. The animal was held in this position until the tail flicks or until 15 seconds passed. At this point, the testing was discontinued to avoid tissue damage to the tail. Pre-testing for acclimation purposes was conducted. Actual testing was done on the animals by repeating the measurement three times and taking the mean as the result for the individual animal [35];[122].

Statistical Analysis

Two-way analysis of variance (ANOVAs) were used to determine the effects of temperature (48°, 50° and 52.5° C) x hormone (for estradiol: 0, 1 cm 10%; for progesterone: 0, 1.5cm) x dose of drug for SNC80 0,2.5,5,10 mg/kg; for U50, 488 0, 1,3,10 mg/kg).

Results

Effects of U50, 488, a κ- opioid agonist, on Latency to Tail flick

Figure 10 shows the tail withdrawal latencies for water temperatures ranging from 48° to 52.5° C in OVX female rats treated with U50, 488 and vehicle, estrogen, progesterone or co-administration of estrogen+progesterone. Analysis across hormone replacements and temperatures indicated a main effect of hormone. Additionally effects were observed for temperatures, where a

hormone by temperature interaction was obtained for 50° C. At 50° C, while lower doses of U50, 488 produced analgesia higher doses produced analgesia in all other hormone replacement groups. However, while at 52.5° C U50, 488 did not produce analgesic responses in estrogen treated rats, it did in all other treatment groups (where 9 mg/kg consistently produced double latencies than vehicle groups). At 48° C with the exception of progesterone treatment, in all groups U50, 488 did not show dose dependent responses.

Overall, U50, 488 increased the latency to tail flick (48˚C: [F (9, 32) =3.69, p=0.0004; Fig.12A] 50˚C: [F (9, 32) =14.48, p=0.0001; Fig. 12B]; 52.5˚C: [F (9, 32) =8.43, p=0.0001; Fig. 12C]. At all temperatures, the hormones were dose dependent and hormone dependent. At 48˚C, the effect of the hormone was extremely significant and the dose was also significant. All hormones, with the exception of progesterone, at 1 mg/kg increased latencies to tail flick. At 50˚C, the hormone effect was extremely significant with estradiol at 1 mg/kg of morphine showing a ceiling effect. Progesterone showed a less robust latency to tail flick at all doses with the exception of 9 mg/kg. There was a ceiling effect at this dose. E+P showed a dose dependent effect. At 52.5 ˚C, there was a robust hormone effect in increased latency to tail flick, particularly with the highest dose of U50, 488. Estradiol showed a dose dependent effect, while the other hormones were essentially unaffected by dose.

Effects of SNC80, a delta opioid agonist, on Latency to Tail flick

Similar to κ opioid agonists, SNC80, a δ opioid agonist affected latency to tail flick in a temperature and dose-dependant manner (at 48°C, only the cholesterol showed *significant* increases in latency to tail flick with increased dose of SNC80). The drug effect is strongest in the cholesterol case at low temperature. For the hormone groups other than cholesterol, the latency to tail flick is very close to saturation in all cases. At increased temperatures, there is a hormone and drug effect showing increased latency to tail flick.

Overall, SNC80 increased the latency to tail flick (48˚C: [F (9, 32) =2.13, p=0.0310; Fig. 13A] 50˚C: [F (9, 32) =2. 56, p=0.0094; Fig. 13B]; 52.5˚C: [F (9, 32) =5.73, p=0.0001; Fig. 13C]. At 48˚C, there was an interaction between drug and hormone. There was a significant effect of hormone on latency to tail flick. There was no dose response effect for estradiol or progesterone when administered alone, but this effect appeared with E+P. All of the doses for estradiol, progesterone and E+P were close to saturation. At 50˚C, there were dose response curves for progesterone and E+P. All hormones caused an increased latency to tail flick and the 10 mg/kg of progesterone caused a ceiling effect. Estradiol showed a more robust effect with the 5 mg/kg dose, while the other doses were basically consistent. At 52.5˚C, there is a significant effect between hormone and dose. For estradiol, there was a marked increase of latency to tail flick at 5mg/kg and there is a slight dose response effect for the other strengths. Progesterone shows a decreased latency to tail flick with the 5mg/ kg dose and there is a slight inverse dose response curve with the

exception of the 10 mg/kg strength. E+P shows a dose response curve with 2.5 and 5 mg/kg being essentially the same.

Increases in tail flick latencies were observed with SNC80 administration in a dose-dependent manner in OVX rats at 50°C nociceptive stimuli, but not at 52.5° C. All of the steroid hormone replacements at the two higher temperatures increased latency to tail flick, with more remarkable results at 50°C. Taken together, these results suggest that δ opioid receptor agonist effects are affected by the hormonal replacement and well as by the temperature of the nociceptive stimulus. This suggests that δ opioid activation may play an important role in some nociceptive responses.

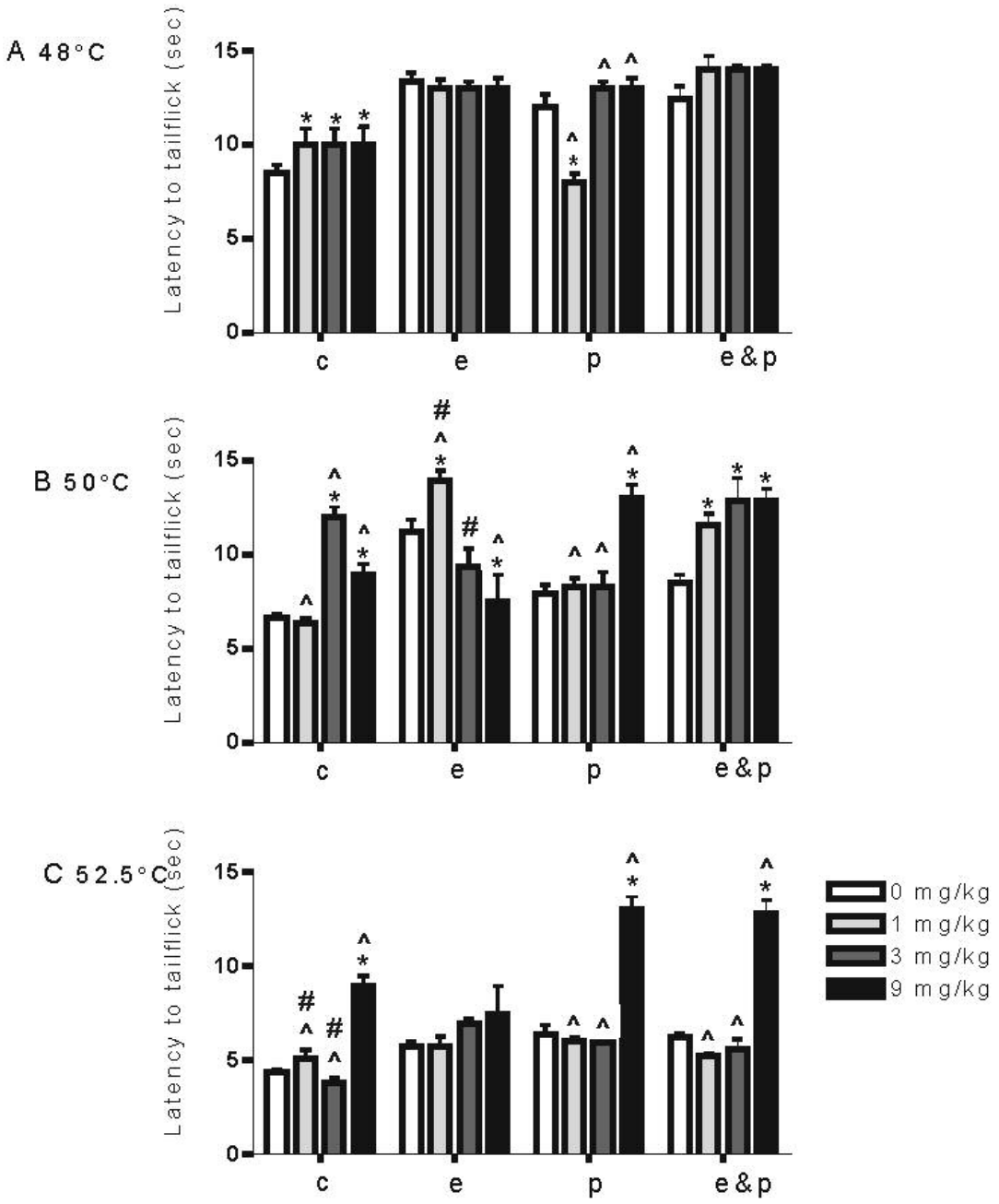


Figure 12. U50, 488 at doses 0, 1,3,10 mg/kg for temperatures of 48°, 50°, 52.5° C; at 48° in progesterone group, * indictes1mg/kg was significantly different from the control. ^ indicates doses 3&10 were significantly different from 1. At 50°, * indicates significant differences from saline. For cholesterol and estradiol ^ indicates significant differences between 1 and 3&10. For progesterone, ^ indicates significant differences between 10 and 1&3.At 52.5°C, * indicates significant differences from saline. ^ indicates differences between 3 and 0, 1, 10; ** indicates differences between 1&3. For progesterone, ^ indicates significant differences between 10 and 1&3; For E+P, ^ indicates significant differences between 10 and 1&3. N=6-8.

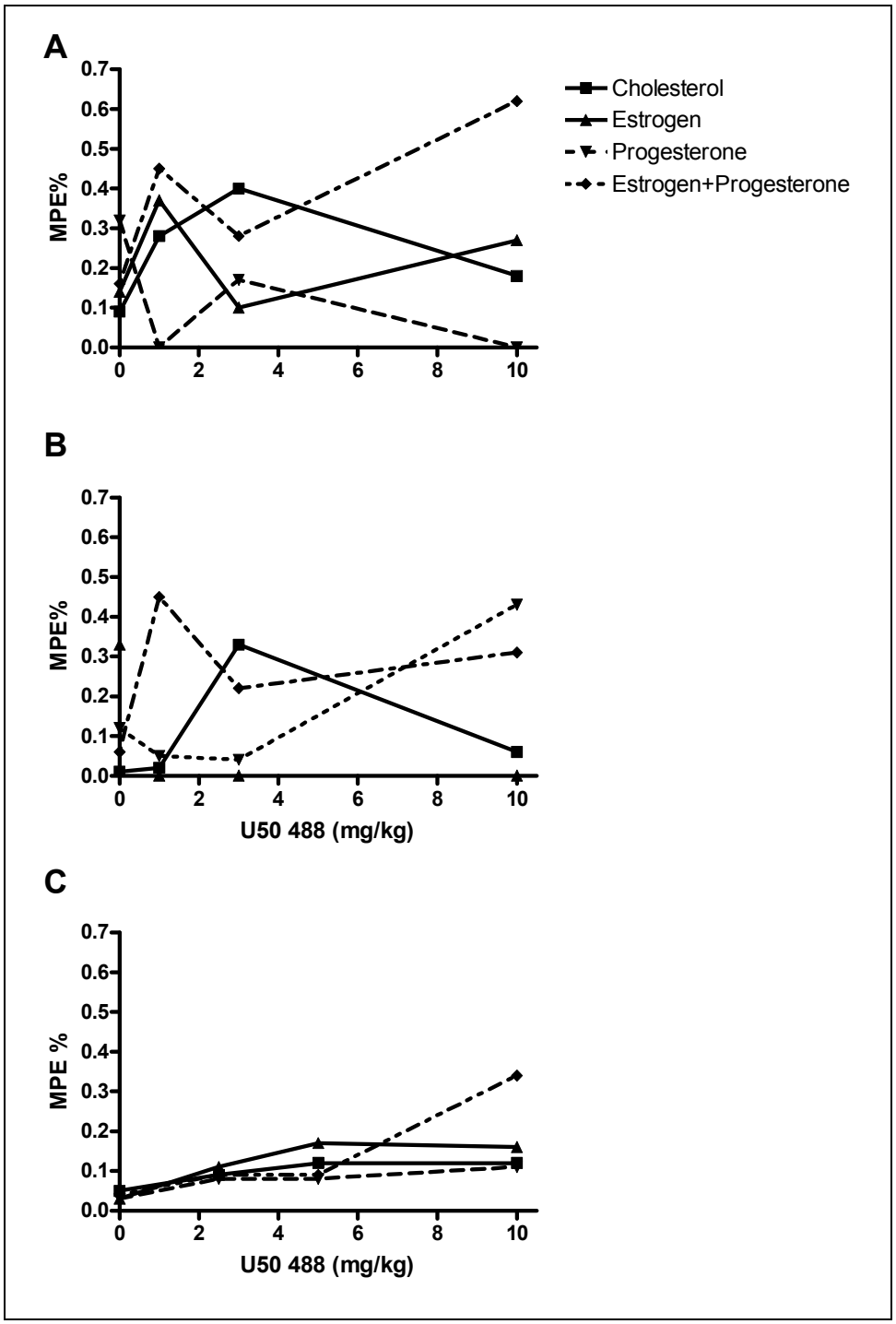


Figure 13. MPE% for U50 488 for **A** 48°C, **B** 50°C **C** 52.5°C

U50,488	cholesterol	estradiol	progesterone	E+P
48°C	dnc	dnc	dnc	2.2897-e+020
50°C	dnc	1.180	dnc	dnc
52°C	dnc	1518	1587	dnc

Table 7. ED50 for U50, 488

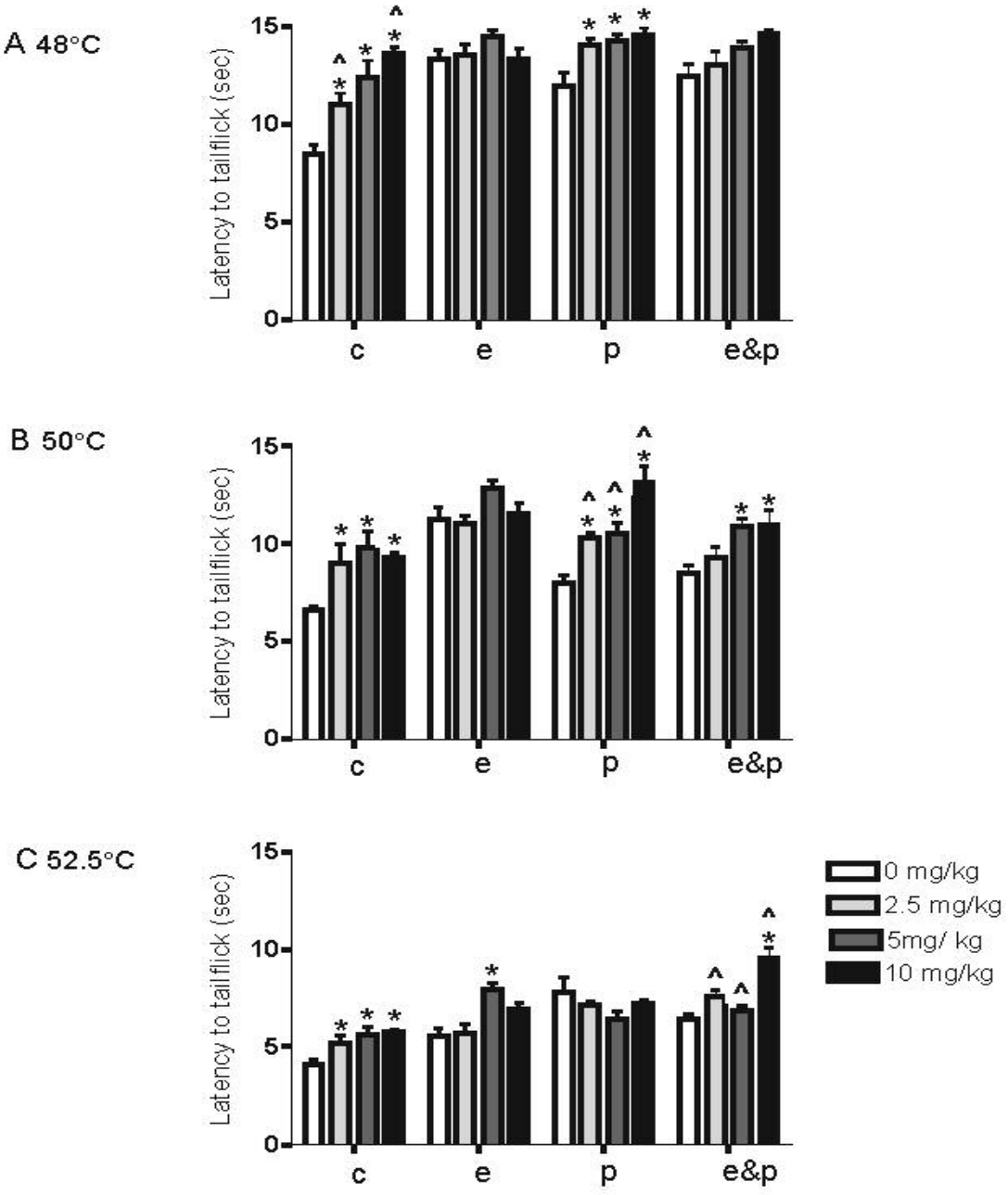


Figure 14: Effects of temperature (48, 50, and 52.5º C) and steroids on opioid modulation of tail flick represent animals pre-treated with SNC80 (0, 2.5,5,10 mg/kg) N is 6-10 per group. * represents statistically significant differences to saline. ^ represents differences to other doses within the same treatment group. E=estradiol (1 cm 10%), P=progesterone (1.5cm), E+P=co-administration of both estradiol and progesterone, C=cholesterol (vehicle, 1cm).

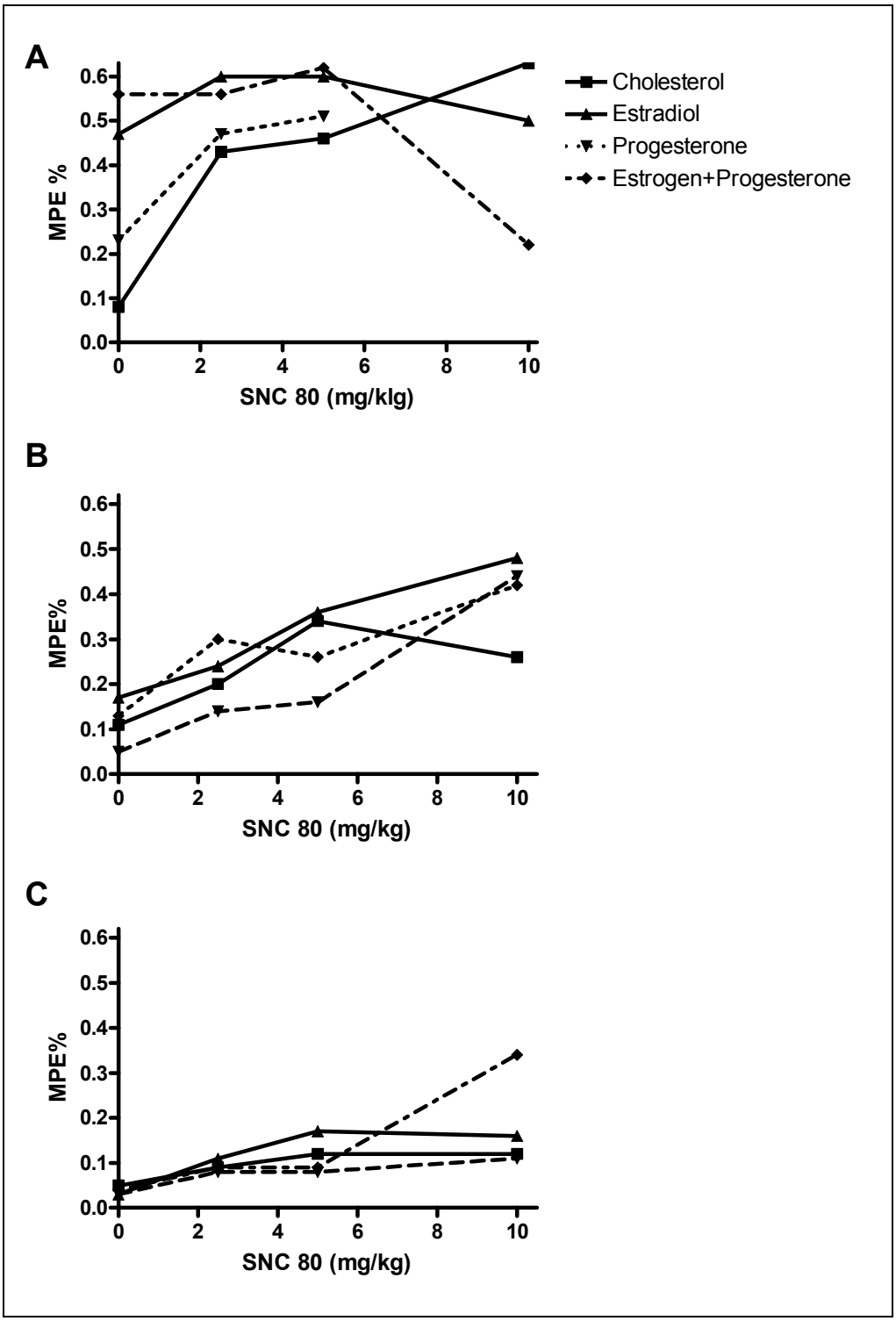


Figure 15. MPE% for SNC 80 for A 48°C, B 50°C and C 52.5°C

SNC80	cholesterol	estradiol	progesterone	E+P
48°C	9.2824e-015	dnc	dnc	5.3828e+009
50°C	326.3	12506	2.7249e+027	2.88856e-024
52°C	291.1	270.0	9.9650e-017	6.6076e+019

Table 8. ED50 for SNC80

Discussion

Unlike morphine, results indicated marked hormone effects in the potency and maximal effectiveness of κ and δ-opioid receptors in the behavioral effects related to nociceptive stimulus in females Thus, suggesting that the sex and gonadal hormone effects in nociceptive responses are in part mediated through activation of δ and κ-opioid receptors rather than μ-opioid receptors.

Differential effect of gonadal hormones was more apparent in tests conducted using low to moderate intensity nociception stimuli, where it has been previously shown by Barrett et al [17] that the opioids frequently produced sexual dimorphic responses. In addition, chronic estradiol administration enhanced the antinociceptive effects of U50 488 in thermal induced nociceptive responses [153]. Furthermore, Negus and Mello [153];[154] have previously shown that estrogen in combination with progesterone increases the potency and effectiveness of U50 488 in OVX rhesus monkey. They further postulated that estrogen enhanced κ- opioid nociception in females and that progesterone interact with estrogen to modulate behavioral responses to thermal stimuli. However, in our study we did not observed an increase in efficacy of the κ-agonist after co-administration with estradiol and progesterone. Most effects were observed with estrogen administration alone, where the ED_{50} was different after 50 °and 52.5° C test when compared to other treatments. Similar to Negus and Mello [153];[154], we postulate that estradiol levels appear to be the most important gonadal steroid hormone determinant of U50 488-induced antinociception in OVX females.

Few studies have addressed the role of delta opioid receptors in female nociceptive responses. Estrogen replacement altered SNC80 effects at all intensity of the nociceptive stimuli. However, at 52.5 °C, co administration of estrogen + progesterone produced the highest SNC 80 analgesic responses.

Thus, we postulate that although estrogen may mediate delta opioid nociception, the interaction between estrogen + progesterone during the reproductive cycle of females may indeed produced more profound effects on nociception.

Progesterone effects on U50, 488 induced antinociception were only observed in low intensity nociception stimuli. Our results suggest that progesterone also modulate κ- opioid receptor activity. Although because the effects were not observed at all stimuli, it suggested that progesterone may play a limited role on κ-opioid modulation. However, at a moderate intensity, progesterone altered the dose response profile of SNC80. And at higher intensity, it blocked SNC80 analgesic effects. This further suggests that progesterone effects on nociception involve the opioid receptor activation.

To the degree that gonadal hormones influence nociceptive responses, this finding suggest that the κ and δ-opioid receptors might be more sensitive to gonadal hormone treatments that the μ-opioid receptors. Future experiments must address the effect of hormone doses and duration of replacement treatments in the effects of opioid nociception in females. This in turn will further help to elucidate the levels by which gonadal hormones alter nociceptive responses during the female reproductive cycle.

Chapter 5. Estrogen has no effect but acute progesterone administration affects the acquisition but not expression of morphine tolerance in female rats

Introduction

It is now generally accepted that men and women exhibit differences in pain perception. Epidemiological studies have demonstrated that women exhibit greater perceptual responses, generally report higher pain levels and exhibit overall less tolerance than men to pain (see reviews Fillinghim and Maixner [78] and Unruh [1909]. In addition, pain symptomatologies of many diseases vary with the woman's reproductive status (i.e., puberty, across the menstrual cycle, and during and after menopause [151];[134];[210];[29;113]. Similar to humans, in rats, there are sex differences in basal nociceptive threshold and these differences can be hormonally modulated and shown to vary with the estrous cycle [85].

Morphine, a μ-opioid agonist, is the most widely used opioid analgesic ([140]; [141]; [62]). On of the most significant consequences of repeated morphine administration is analgesic tolerance; manifested as a shift to the right of the dose-responses curve or as a decrease in the intensity of the responses when a constant dose is repetitively administered [67]. Tolerance to opioids is characterized by a shorted duration and decreased intensity of the analgesia, euphoria, sedation and other effects caused by depression of the CNS, as well as by marked elevation in the average lethal dose [99]. Tolerance to

antinociceptive effects of opioid drugs has been demonstrated in humans and animals. In clinical settings the development of tolerance necessitates increases in the dose of morphine and other opioid agonists complicating the management of patients with persistent pain. Continuous administration of morphine will result in a decrease in the effects produced by a given dose of the drug in term of the magnitude of the response to thermal, mechanical, and chemical stimuli. Overall, tolerance to opioid have been demonstrated to be pharmacodynamic, time-and dose-dependent, receptor specific, and apparently reversible if the agonist is removed [42]. However, biological sex difference in the sensitivity of rats to antinociceptive effects of chronically administered morphine in female rats is not well understood. Further, although it has been hypothesized that gonadal hormones have a neuromodulatory role during the development of morphine tolerance, little is known of the role of gonadal hormones on the development and/or expression of antinociceptive tolerance to morphine. The aim of this study is to test this postulate.

Material and Methods

Animals

Eight week old, ovariectomized Sprague-Dawley rats (Taconic, Germantown, New York) were double-housed in standard plastic cages (20 x 20 x 41 cm^3) layered with beta chips Light cycle was a 12-h light/12-h dark cycle (lights on at 9 A.M.) Rats had food and water available ad libitum. Animals were handled daily for 5 minutes to reduce the stress induced by subsequent handling during the behavioral testing. All animal procedures were in accordance with the

"Principles of Laboratory Animal Care" (NIH publication 85-23, Bethesda, MD, 1996) and approved by the Institutional Animal Care and Use Committee at Hunter College.

Drugs and Hormones

Morphine 25 mg/mL was purchased from Henry Schein (Indianapolis, IN). Cholesterol, 17β estradiol, and progesterone were purchased from Sigma Chemical (St. Louis, MO).

Hormone Replacement for tolerance paradigm

One week after arrival, animals were anaesthetized, and SILASTIC brand capsules (1 cm or 1.5 cm, 0.058 in. ID X 0.077 in. OD, Dow Corning) were implanted subcutaneously in the interscapular region following methods previously described [11];[161]. This manner of hormone delivery was chosen to avoid saturation of hormone in the animals and to achieve stable plasma levels of hormone. Doses of 17 β estradiol were 10%, 20%, 30% and 40 % (in a mixture of estradiol and cholesterol). Progesterone was administered in a similar manner, using SILASTIC capsules of 100% progesterone in lengths of 1, 1.5, 3 and 9 cm. Control capsules for progesterone or estradiol contained cholesterol (100%) with a 1 cm length. For co-administration of estradiol and progesterone, rats received hormone doses of either 10% estradiol and 1.5 cm progesterone (low-low); 10% estradiol and 3 cm progesterone (low-high); 40% estradiol and 1.5 cm progesterone (high-low) or 40% estradiol and 3 cm progesterone (high-high). These doses have been shown to fall within the range of serum levels during the

estrous cycle [83];[165]. All animals were allowed one week to recover from the surgery.

Behavioral Testing

In order to measure the integrated response of gonadal hormone effects on nociception, we used the hot water tail-flick test to measure antinociceptive responses. Water temperatures used were 52.5° C. The rat was held above the water with the tail submerged half way in the water. The animal was held in this position until the tail flicks or until 15 seconds passed. At this point, the testing was discontinued to avoid tissue damage to the tail. Pre-testing for acclimation purposes was conducted. Actual testing was done on the animals by repeating the measurement three times and taking the mean as the result for the individual animal ([83],[166]).

Tolerance paradigms

One week after SILASTIC implants, OVX rats received 10 or 15 mg/kg of morphine s.c. twice daily (8:00 and 16:00 h). As shown in Figure 1, to determine the role of progesterone on the acquisition or development of morphine tolerance, one week after vehicle or estrogen replacement, rats received co administration of morphine and progesterone s.c. injections, through out the entire tolerance paradigm. A second cohort of rats received sesame oil (vehicle) and morphine and on day 8 received a single progesterone administration in conjunctions with morphine. The development of tolerance was determined by comparing the tail flick latencies between day 1 and 8.

Statistics

Two way ANOVAs were performed to examine the Two-way analyses of variance (ANOVAs) were used to determine the effects of hormone. For post hoc analysis, Tukey tests were performed when appropriate. Significance was at the $P < 0.05$ level. Tolerance testing was done with both 10 mg/kg morphine and 15 mg/kg morphine.

For the acquisition and expression part of the experiment, only cholesterol implants were used. Hormones were injected subcutaneously rather than implanted. Doses of estradiol were 1ml of 50 µg of estradiol in sesame oil solution and progesterone was 1 ml of 500 µg in solution with sesame oil. Morphine dose was 15 mg /kg on tail flick at 52.5° C. Hormone injections 60 min prior to either morphine or saline then test 30 min post that injection.

Results

As shown in Figure 2, OVX rats developed tolerance after repetitive administration of both 10 and 15 mg/kg of morphine. However, 15 mg/kg produced a stronger tolerance than the10 mg/kg of morphine. Estrogen and progesterone did not affect the acquisition of tolerance; levels of morphine-induced anti-nociception were similar between experimental groups. Furthermore, co-administration of both steroids did not alter the formation of tolerance in OVX rats. As shown in Figure 3, administration of progesterone during the acquisition phase had no effect on the development of tolerance. However, when progesterone was administered during the expression phase, the latencies to tail-flick were significantly reduced when compared to rats receiving estrogen alone.

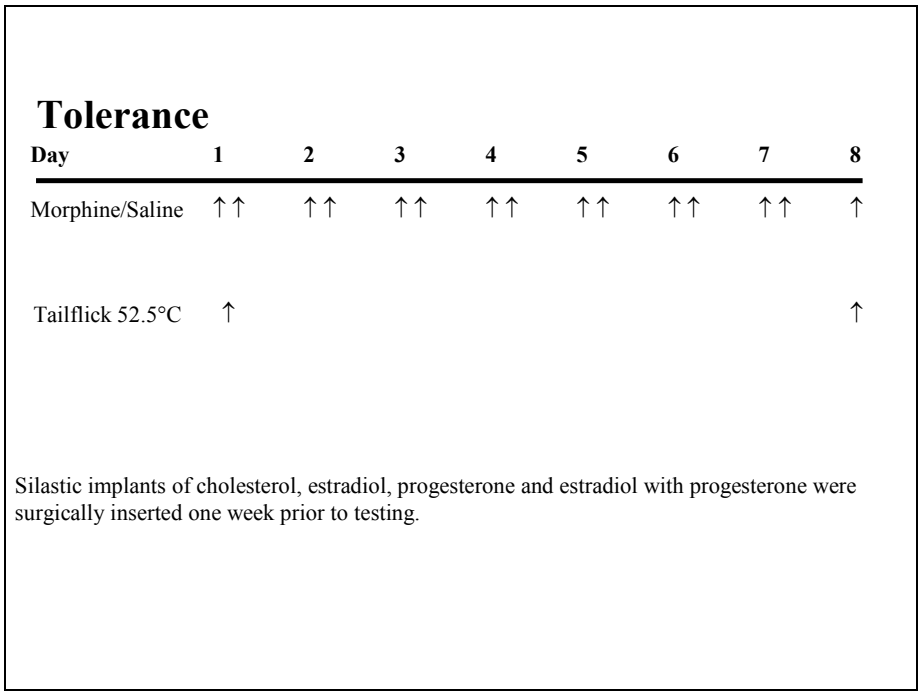


Table 9. Tolerance. Silastic implants of cholesterol, estradiol, progesterone and estradiol with progesterone were surgically inserted one week prior to testing. Tail flick water temperature was 52.5°C. N=6-8.

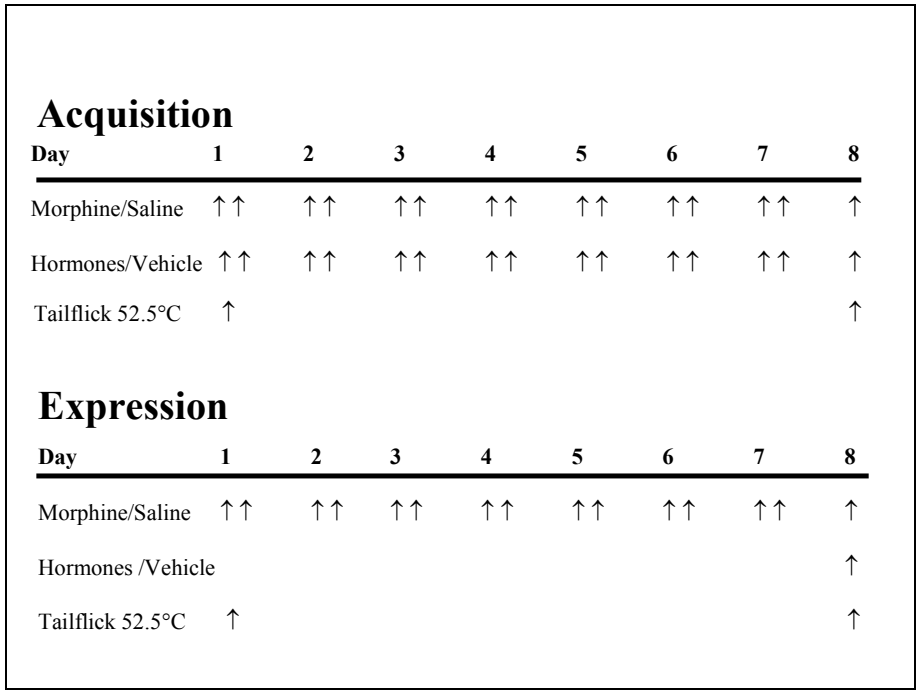
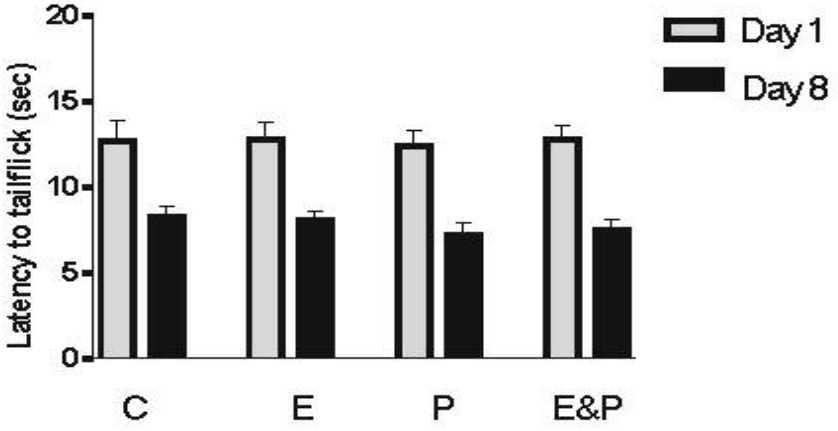


Table 10. Acquisition and Expression Flowcharts.

A



B

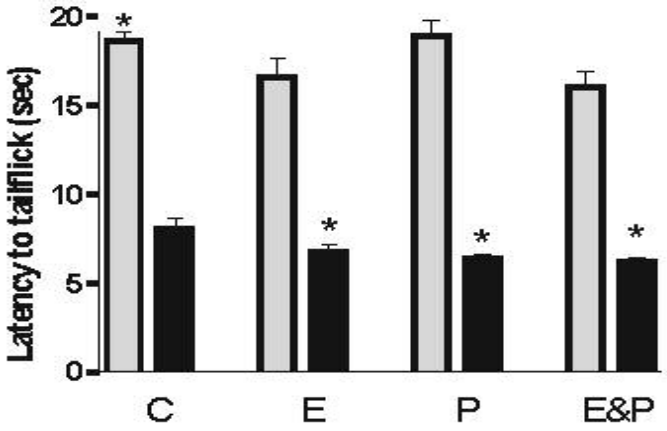


Figure 16. **A** 10 mg/kg morphine. **B** 15 mg/kg morphine. Tolerance is shown by decrease in latency to tail flick. While there is significance between day 1 and day 8, there is no significance between hormones N=12.

Acquisition

Pulsatory

Day	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Morphine/Saline		↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑
Hormones/Vehicle		↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑
Tailflick 52.5°C	↑								↑

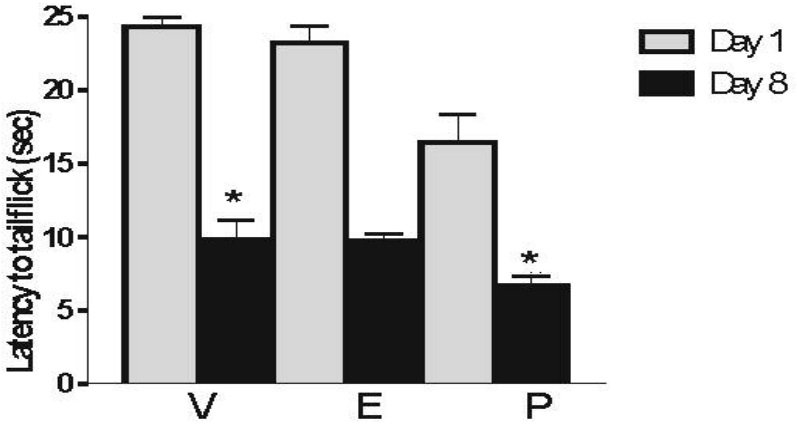
Chronic- used Silastic capsules inserted day 1

Day	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Morphine/Saline	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑
Hormones /Vehicle								
Tailflick 52.5°C	↑							↑

↑

Table 11. Acquisition pulsatory vs. chronic

A



B

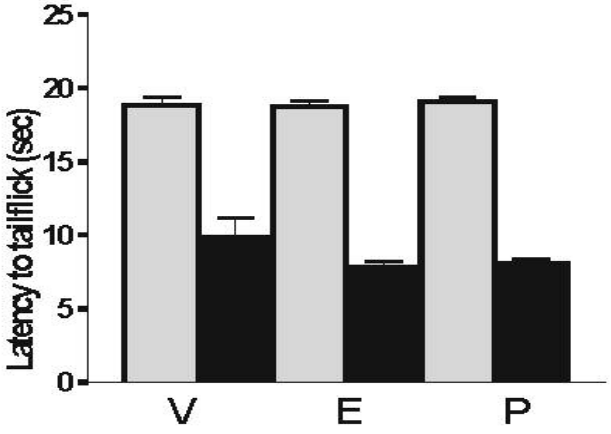


Figure 17. **A**. acquisition phase when progesterone was administered during the acquisition phase, the latencies to tail-flick were significantly reduced when compared to rats receiving estrogen alone administration of progesterone during the phase had no effect on the development of tolerance ($F= 5.574$, $p<0.05$) **B**. Expression phase. There is no significance between hormones. N=8-12

Discussion

To our knowledge this is the first report studying the effect of gonadal hormones in the development of morphine tolerance. Our results suggest that although female rats develop tolerance to morphine-induced anti-nociception, estrogen and progesterone, alone or in combination did not affect the development of morphine tolerance. However, when injected via s.c. administration progesterone altered the development/acquisition but not expression of morphine tolerance. Thus, the effects which ovarian hormones exert on the opioid system may vary according to the duration and/or manner of the steroid replacement. This is consistent with previous observations by Ratka and Simpkins [[176]; 2 days of progesterone administration reduced while 14 days of treatment, significantly increased morphine-antinociception.

Chronic morphine administration produced consistently higher levels anti-nociception in female rats than males [200];[56]. However, it has been recently reported that the rate of acquisition of tolerance was similar in male and female rats and that the there are sex-specific intrinsic reactivity of morphine which affect the desensitization to morphine during the development of tolerance [107]. Furthermore, as reviewed in Fillingim and Ness [79] some investigators report that the estrous cycle affects nociceptive responses and morphine potency however, these reports are contradictory. For example Stoffel et al [205] reported a similar potency of morphine during proestrus/diestrus and lower analgesia at estrous. However, while Kepler et al [118] reported no differences between estrus and diestrus, Berglund and Simpkins [27] reported a greater sensitivity to morphine at diestrus than in proestrus. The sexual dimorphic pattern in the development of morphine tolerance and estrous cycle-effects, in part, suggest that the hormonal regulation of morphine tolerance is complex

process. Based on our observations we concluded that although female rats develop tolerance to morphine-induced anti-nociception, fluctuations during the estrous cycle may play a limited role in the development of tolerance.

However, as reviewed in Fillingim and Ness [79], data on the effect of gonadal steroid hormone on acute and chronic morphine analgesia are also conflicting. As suggested by Mogil et al [146], although the use of varying methodologies clearly contributes to the inconsistencies in laboratory studies, this factor cannot account for all the contradictory reports. They further postulated that some experimental designs are simply not sufficiently powerful to detect existing differences, especially those of modest magnitude. For example the hormone doses and duration of replacement treatments may have profound effects on the development of tolerance. Herein is shown a differential effect of progesterone when administered via s.c. injections as compared to SILASTIC implants.

Previous data from this dissertation demonstrated that gonadal hormone played a limited role on acute morphine-antinociception. Herein, we demonstrated that they also played a limited role on the development of morphine tolerance. Thus, as suggested on Chapter 3, it is possible that gonadal hormones modulate other components of the opioid system such as the δ and κ-opioid receptors. However, all experimental manipulations were done at steady state levels of estrogen and progesterone. It is feasible that during a more dynamic state of hormone replacement where the levels of the hormones fluctuate, the gonadal hormone may regulate behaviors controlled by the μ-opioid receptors. This postulate in part is supported by the attenuation of antinociceptive responses by progesterone when administered via s.c. injections (a manner of administration that produces fluctuating serum levels of progesterone). Thus, further studies are needed to test this possibility.

Chapter 6. General Conclusions

We began this series of experiments because we were interested in the role of female hormones in the perception of pain. We narrowed the focus of the study to acute pain and selected the hot water tail flick test as our experimental paradigm. We divided the study into a series of experiments that would show how various components such as hormones and drugs act alone before combining them. Previous studies have shown that acute thermal pain is a good predictor of opioid analgesic potential and clinical efficacy [26]; [34]; [148]. Therefore, we were interested in doing these experiments to determine how activation of various opioid receptors would affect the perception of acute pain. The continued use of opioids by patients quickly leads to drug tolerance ([24]; [44]; [45]; [49]) so we also wanted to examine the effects of tolerance on the efficacy of morphine. Our first specific aim was to test the hypothesis that estrogen and progesterone are involved in the modulation of analgesia and nociception. To test this, we tested animals by using Silastic implants that delivered hormones in a steady state. We showed for the first time that serum levels of estradiol and progesterone initially increase with time but reach a constant serum level seven days post-surgery that remains constant up to 28 days, longer than the time of our experiments. The constant serum level attained increases proportionately with the dosage.

Our initial experiment was to determine the effect that different levels of hormone have on the pain as measured by the hot water tail flick test. We used hormone doses that provided hormone serum levels within the normal range of

cycling females. To this end, we worked with gonadectomized females so that we could control the amount of hormones available. We tested the effects of a series of water temperatures ranging from 48°-55°C and found that animals were most responsive to a temperature of 52.5°C. Similarly, when we tested a range of estradiol and progesterone implant dosages, we found that 10% estradiol and 1.5 cm progesterone provided the largest effects on pain perception. Having established the optimal water temperatures and steroid dosages, we further examined the effect of these factors in a drug-free state. We found that the latency to tail flick decreased as temperature increased. Additionally, we found that estradiol and progesterone, when supplied singly, increased the latency to tail flick when compared with the controls. We also found that E+P produced a hormone effect independent of the dose.

The next phase of experimentation was to determine to what extent opioid regulation of nociceptive response is affected by gonadal hormones. We began by using morphine, a μ opioid agonist. We first calculated a baseline dose response curve and a cumulative dose response curve. We found that E+P significantly increased sensitivity to morphine-induced analgesia, but these hormones did not have such an effect when administered singly. We concluded that morphine was more effective only when used with the combined E+P dose. This effect is consistent with the finding of both clinical and rodent research [83]. Because estradiol and progesterone did not activate morphine responses, our results suggest that these observed behavioral responses are not based on a differential sensitivity of the μ-opioid receptor. Indeed, only when co-administered

do they produce different effects than their respective controls and/or when administered alone, thus suggesting that the μ-opioid system is only differentially altered when both hormones are present.

Since μ opioid receptor agonists are not the only opioids available, and the δ and κ opioid receptors have been shown to have different efficacies and to affect different types of pain, we replicated the dose response curve and behavioral testing for both δ and κ opioid agonists. We found that the hormones have a significant effect on the action of the δ opioid receptor agonist used (U50, 488) increasing latency to tail flick and we concluded that the δ opioid receptor agonist was affected by hormonal replacement and by temperature of the nociceptive stimulus. It appears that the δ opioid activation may play an important role in some nociceptive responses ([104; [121]]. Similarly, the κ opioid agonist SNC80 effect showed a response to the hormones, producing an increased latency to tail flick ([144]; [153]; [43]; [16]; [17]).

Finally, we wanted to determine if ovarian hormones affect the development of opioid tolerance. Because morphine is among the most common pain medications given to patients, we did a further experiment to examine tolerance. Again, the animals were given Silastic implants of hormone for steady, continuous release. The injections of morphine were given twice a day and behavioral testing was done on day one and on day eight. In the first part of the tolerance experiment, we gave morphine in doses of 0, 10, 15 or 20 mg/kg. The dose of 20 mg/kg was found to be so potent that it totally blocked the tail flick response. We found that the dose of 15mg/kg increased the latency to tail flick without rendering the animal unable to respond. As expected, a week of twice daily morphine treatment created tolerance in the animals and diminished the latency to tail flick. In this experiment, we found that progesterone has an effect

on the baseline of reducing latency to tail flick, but neither E alone nor E+ P had any effect ([138]; [5]; [56]; [109]).

In a final experiment, we examined the differences between steady state and pulsatory (impulse) delivery of hormones. The difference between these two methods is that with the steady state method, there is constant delivery of hormone, whereas in the pulsatory method there is a sudden infusion of hormone and then no further hormone delivery once the injection is made. For this experiment, we again tested the effect on the acquisition of tolerance. For the expression part of the experiment, animals were given Silastic implants containing only cholesterol. Morphine was delivered twice daily (s.c.) and the hormones were injected only on the last day. This allowed us to look at the control situation (day 1) and to examine the effect of hormones delivered on the last day of testing (day 8). Estrogen or progesterone alone did not affect the acquisition of tolerance; levels of morphine-induced anti-nociception were similar between control and experimental groups. Co-administration of both steroids also did not alter the formation of tolerance. For the acquisition experiment, we compared two groups of animals that received hormones in one of two ways: the first was chronic, with Silastic implants that gave steady state, continuous release of hormone. The second experimental group received hormonal injections on the final day of testing. Both groups received twice daily s.c. injections of 15mg/kg morphine. For the group that had implants, we found that progesterone decreased latency to tail flick on both day one and on day eight. The groups that received injections of hormones showed no such effect and the estradiol, progesterone and control groups appeared virtually identical in terms of latency to tail flick.

Although hormones clearly modulate the responsiveness to pain, further experiments should be conducted to attempt to more fully understand the

complexity of the relationship between estradiol and progesterone ([136]; [18]; [45]; [46]). While there are advantages to delivering hormones at a steady rate, rather than in pulses, this does not mimic the dynamic hormone fluctuations of normal, cycling females. First experiments should be done using gonadectomized animals and control the cycling through injected steroids, but experiments using intact animals are also needed.

It would also be useful to try to induce and then to block antinociception by using μ, δ and κ-opioid receptor antagonists. This would indicate more clearly how the hormones are able to exert their effects. This would be particularly useful in terms of studying morphine, which while it is classified as a primarily μ opioid receptor agonist, also produces some κ opioid receptor agonist activity. The use of specific antagonists could help elucidate the effects of both types of receptors and might clarify their individual roles.

Previous experiments show different results, depending on the particular test administered and on the strain of rat/mouse used ([71]; [28]; [29]; [77]. This factor needs to be considered more seriously when designing an experimental paradigm to test antinociception. Other labs have attempted to solve this problem by running a number of different tests with a single strain of rodent or by administering the same test to a variety of strains of animal. The implication of this diversity of results indicates that there are more confounding variables at work. Particular strains of animals may have been bred for different behavioral and genetic purposes that affect their responses to drugs. Further, the complexity of the responses found in our study suggest that the roles of progesterone and estradiol as they vary throughout the estrus/menstrual cycle will not be uncovered until we are able to replicate the cycle rather than model the cycle with constant hormone levels.

Reference List

1. Adams,J.E., Naloxone reversal of analgesia produced by brain stimulation in the human., Pain, 2 (1976) 161-166.

2. Akil,H., Mayer,D.J., and Liebeskind,J.C., Antagonism of stimulation-produced analgesia by the narcotic antagonist naloxone, Science, 191 (1976) 961-962.

3. Akil,H., Watson,S.J., Young,E., Lewis,M.E., Khachaturian,H., and Walker,J.M., Endogenous opioids: biology and function., Ann. Rev. Neurosci., 7 (1984) 223-255.

4. al-Rodhan,N., Chipkin,R., and Yaksh,T.L., The antinociceptive effects of SCH-321615, a neutral endopeptidase (enkephalinase) inhibitor, microinjected into the periaqueductal, ventral medulla and amygdala., Brain Res, 520 (1990) 123-130.

5. Ali,B.H., Sharif,S.I., and Elkadi,A., Sex differences and the effects of gonadectomy on morphine-induced antinociception and dependence in rats and mice, Clin. Exp. Pharmacol. Physiol., 22 (1995) 342-344.

6. Aloisi,A.M., Sex differences in pain-induced effects on the septo-hippocampal system, Brain Res. Rev., 25 (1997) 397-406.

7. Aloisi,A.M., Sensory effects of gonadal hormones. In: Fillingim,R.B. (Ed.), Sex, Gender, and Pain, AISAP, Seattle, 2000, pp. 7-24.

8. Aloisi,A.M., Ceccarelli,I., and Lupo,C., Behavioural and hormonal effects of restraint stress and formalin test in male and female rats, Brain Res Bull, 47 (1998) 57-62.

9. Aloisi,A.M., Steenbergen,H.L., Van De Poll,N.E., and Farabollini,F., Sex-dependent effects of restraint on nociception and pituitary-adrenal hormones in the rat, Physiol. Behav., 55 (1994) 373-385.

10. American Medical Association Council on Scientific Affairs. Women's health: sex and gender-based differences in health and disease. 4, 1-30. 2000. Chicago, AMA.

11. Amico,J.A., Davis,A.M., and McCarthy,M.M., An ovarian steroid regimen that increases hypothalamic oxytocin expression alters [3H] muscimol binding in the hypothalamic supraoptic nucleus of the female rat, Brain Res, 857 (2000) 279-282.

12. Amir,S., Brown,Z.W., and Amit,Z., The role of endorphins in stress:evidence and speculations., Neurosci Biobehav Rev, 4 (1980) 77-86.

13. Amir,S., Solomon,R., and Amit,Z., The effect of acute and chronic naloxone administration on motor activation in the rat., Neuropharmacolagyrat, 18 (1979) 171-173.

14. Baamonde,A.I., Hidalgo,A., and Andres-Trelles,F., Sex-related differences in the effect of morphine and stress on visceral pain, Neuropharmacology, 28 (1989) 967-970.

15. Baizman,E.R., Cox,B.M., Osman,O.H., and Goldstein,A., Experimental alterations of endorphin levels in rat pituitary, Neuroendocrinol., 28 (1979) 402-414.

16. Barrett,A.C., Cook,C.D., Terner,J.M., Roach,E.L., Syvabthong,C., and Picker,M.J., Sex and rat strain determine sensitivity to K opioid-induced antinociception, Psychopharmacology, 160 (2002) 170-181.

17. Barrett,A.C., Smith,E.S., and Picker,M.J., Sex-related differences in mechanical nociception and antinociception produced by mu- and kappa-opioid receptor agonists in rats, Eur J Pharmacol, (2002) 163-173.

18. Bartok,R.E. and Craft,R.M., Sex differences in opioid antinociception, J. Pharm. Exp. Ther., 282 (1997) 769-778.

19. Basbaum,A.I. and Fields,H.L., Endogenous pain control mechanism: review and hypothesis, Ann. Neurol., 4 (1978) 451-462.

20. Basbaum,A.I. and Jessell,T.M., The Perception of Pain. In: Kandel,E.R., Schwartz,J.H., Jessell,T.M. (Eds.), Principles of Neural Science, McGraw-Hill, New York, 2000, pp. 472-491.

21. Baumann,M.H., Raley,T.J., Partilla,J.S., and Rothman,R.B., Biosynthesis of dopamine and serotonin in the rat brain after repeated cocaine injections: A microdissection mapping study. Synapse, 14 (1993) 40-50.

22. Beatty,W.W. and Beatty,P.A., Hormonal determinants of sex differences avoidance behavior and reactivity to electric shock in the rat., J Comp Physiol. Psychol., 73 (1970) 446-455.

23. Beatty,W.W. and Fessler,R.G., Gonadectomy and sensitivity to electric shock in the rat, Physiol. Behav., 19 (1977) 1-16.

24. Bennett,G.F., Neuropathic pain. In: Wall,P.D., Melzack,R. (Eds.), Textbook of Pain, Churchill Livingstone, New York, 1994.

25. Bennett,G.J., An animal model of neuropathic pain: a review, Muscle Nerve, 16 (1993) 1040-1048.

26. Bennett,G.J., Animal Models of Pain. In: Kruger. (Ed.), Methods of Pain Research, CRC Press, Boca Ratan, FLA, 2001, pp. 67-92.

27. Berglund,L.A. and Simpkins,J.W., Alterations in brain opiate receptor mechanisms on proestrous afternoon., Neuroendocrinol., 48 (1988) 394-400.

28. Berkley,K.J., Vive la difference, Trends Neurosci., (1992) 331-332.

29. Berkley,K.J., Sex differences in pain, Behav. Brain Sci, 20 (1997) 473-479.

30. Berne,R.M. and Levy,M.N., Principles of Physiology, Mosby, St Louis, 1988.

31. Bilsky,E.J., Montegut,M.J., Delon,C.L., and Reid,L.D., Opioidergic modulation of cocaine conditioned place preference, Life Sci., 50 (1992) PL85-PL92.

32. Bird,C.E. and Rieker,P.P., Gender matters: an integrated model for understanding men's and women's health, Soc Sci Med., 48 (1999) 745-755.

33. Bodnar,R.J., Neuropharmacological and neuroendocrine substrates of stress-induced analgesia., Ann NY Acad Sci, 467 (1986) 345-360.

34. Bodnar,R.J., Commons,K., and Pfaff,D.W., Central neural states relating sex and pain., John Hopkins University Press, Baltimore, MD, 2002.

35. Boyer,J.S., Morgan,M.M., and Craft,R.M., Sex differences in antinociception produced by morphine microinjections in the rostral ventromedial medulla., Brain Res., 796 (1998) 315-318.

36. Brodsky,M., Elliott,K.J., Hynansky,A., Jenab,S., and Inturrisi,C., Quantitation of mu-opioid receptor (MOR-1) mRNA in selected regions of the rat CNS, Neuroreport, 6 (1995) 725-729.

37. Campbell,J.N. and Meyer,A., Primary afferents and hyperplasia. In: Yakish,T.L. (Ed.), Spinal Afferent Processing, Plenum, New York, 1986, pp. 59-81.

38. Campbell,J.N., Raja,S.N., Cohen,R.H., Manning,D.C., Khan,A.A., and Mayer,R.A., Peripheral neural mechanisms of nociception. In: Wall,P.D., Melzack,R. (Eds.), Textbook of Pain, Churchill Livingstone, Edinburgh, 1989, pp. 22-45.

39. Candido,J., Lutfy,K., Billings,B., Sierra,V., Duttaroy,A., Inturrisi,C.E., and Yoburn,B.C., Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation, Pharmacol Biochem Behav, 42 (1992) 685-692.

40. Ceccarelli,I., Fiorenani,P., Massafra,C., and Aloisi,A.M., Long-term ovariectomy changes formalin-induced licking in female rats; the role of estrogens, Reprod Biol Endocrinol, 1 (2003) 1-24.

41. Chambers,K.C. and Sengstake,C.B., Sexually dimorphic extinction of a conditioned taste aversion in rats., Physiol Behav., 4 (1976) 181-185.

42. Chan,C.W. and Dalliere,M., Subjective pain sensation is linearly correlated with the flexion reflex in man, Brain Res, 479 (1989) 145.

43. Chang,P.C., Aicher,S.A., and Drake,C.T., Kappa opioid receptors in rat spinal cord vary across the estrous cycle., Brain Res, 861 (1999) 168-172.

44. Chen,X., Marrero,H.G., Murphy,R., Lin,Y.J., and Freedman,J.E., Altered gating of opiate receptor-modulated K+ channels on amygdala neurons of morphine-dependent rats., Proc Natl Acad Sci, 97 (2000) 14692-14696.

45. Cicero,T.J., Nock,B., and Meyer,E.R., Gender-related differences in the antiniciceptive properties of morphine., J. Pharm. Exp. Ther., 279 (1996) 267-273.

46. Cicero,T.J., Nock,B., O'Connor,L., and Meyer,E.R., Role of steroids in sex differences in morphine-induced analgesia: activational and organizational effects, J Pharmacol Exp Ther., 300 (2002) 695-701.

47. Civelli,O., Birnberg,N., Comb,M., Douglas,J., Lissitzky,M., Uhler,M., and Herbert,E., Regulation of opioid gene expression, Peptides, 4 (1983) 651-656.

48. Cogan,R. and Spinnato,J.A., Pain and discomfort thesholds in late pregnancy, Pain, 27 (1986) 63-68.

49. Collett,B.J., Opioid tolerance: the clinical perspective, Br J Anaesth., 81 (1998) 58-68.

50. Colpaert,F.C., Evidence that adjuvant arthritis in the rat is associated with chronic pain., Pain, 28 (1987) 201-222.

51. Cook,C.D., Barrett,A.C., Roach,E.L., Bowman,J.R., and Picker,M.J., Sex-related differences in the antinociceptive effects of opioids: importance of rat genotype, nociceptive intensity, and efficacy at the mu opioid receptor., Psychopharmacology, 150 (2000) 430-442.

52. CooperJ.R., Bloom,F.E., and Roth,R.H., Biochemical Basis of Neuropharmacology, Oxford University Press, New York, 1996.

53. Craft,R.M., Sex differences in drug-and non-drug-induced analgesia, Life Sci, (2003) 2675-2688.

54. Craft,R.M., Henley,S.R., Haaseth,R.C., Hruby,and V.J., and Porreca, F., Opioid antinociception in a rat model of visceral pain: systemic versus local drug administration., J. Pharmacol. Exp. Ther., 275 (1995) 1535-42.

55. Craft,R.M. and McNeil,D.M., Agonist/antagonist properties of nalbuphine, butorphanol and (-)pentazocine in male vs. female rats, Pharmacol Biochem Behav, (2003) 235-245.

56. Craft,R.M., Stratmann,J.A., Bartok,R.E., Walpole,T.I., and King,S.J., Sex differences in develolpment of morphine tolerance and dependence in the rat., Psychopharmacology, 143 (1999) 1-7.

57. D'Amour,F.E. and Smith,D.L., A method for determining the loss of pain sensation, J Pharmacol Exp Ther., 72 (1941) 74.

58. Davis,A.M. and Inturrisi,C.E., d-Methadone blocks morphine tolerance and N-methyl-D-Aspartate (NMDA)-induced hyperalgesia, J. Pharm. Exp. Ther., (1999).

59. Dawson-Basoa,M.B. and Gintzler,A.R., 17-Beta-Estradiol and progesterone modulate an intrinsic opioid analgesic system., Brain Res, 601 (1993) 241-245.

60. De Vries,T.J., Tjon Tien Ril,G.H., Van der Laan,J.W., Mulder,A.H., and Schoffelmeer,A.N., Chronic exposure to morphine and naltrexone induces changes in catecholaminergic neurotransmission in rat brain without altering mu-opioid receptor sensitivity., Life Sci., 52 (1993) 1685-1693.

61. Denti,A. and Epstein,A., Sex differences in the acquisition of two kinds of avoidance behavior in rats., Physiol Behav., 8 (1972) 611-615.

62. Diaz,J., How Drugs Influence Behavior. A Neuro-Behavioral Approach, Prentice Hall, Upper Saddle River, NJ, 1997.

63. Drago,F., Bohus,B., Scapagnini,U., and de Wied,D., Sexual dimorphism in passive avoidance behavior of rats: relation to body weight, age, shock intensity and retention interval., Physiol Behav., 24 (1980) 1161-1164.

64. Dubner,R. and Ruda,M.A., Activity-dependent neuronal plasticity following tissue injury and inflammation, Trends Neurosci., 15 (1992) 93-103.

65. Dubreuil,D. and Kohn,P., Reactivity and response to pain, Pers Individual Difference, 7 (1986) 907-909.

66. Elliot,K., Hynansky,A., and Inturrisi,C.E., Dextromethorphan attenuates and reverses analgesic tolerance to morphine, Pain, 61 (1994) 401-409.

67. Elliott,K.J., Brodsky,M., Hynansky,A., Foley,K.M., and Inturrisi,C.E., Dextromethorphan suppresses both formalin-induced nociceptive behavior and the formalin-induced increase in spinal cord c-fos mRNA, Pain, 59 (1994) 361-368.

68. Evans,C.C., Diversity Among the Opioid Receptors. In: Korenman., Barchas,J.D. (Eds.), Biological Basis of Substance Abuse, Oxford University Press, New York, 1993, pp. 31-48.

69. Faber,E.S. and Sah,P., Opioids inhibit lateral amygdala pyramidal neurons by enhancing a dendritic potassium current., Neurosci., 24 (2004) 3031-3039.

70. Febo,M., Jimenez-Rivera,C.A., and Segarra,A.C., Estrogen and opioids interact to modulate the locomotor response to cocaine in the female rat., Brain Res., 943 (2002) 151-161.

71. Feine,J.S., Bushnell,M.C., Miron,D., and Duncan,G.H., Sex differences in the perception of noxious heat stimuli., Pain, 44 (1991) 255-265.

72. Feldman,R.S., Meyer,J.S., and Quenzer,L.F., Principles of Neuropsychological Pharmacology, Sinauer, Sunderland, MA, 1997.

73. Festa,E.D. and Quinones-Jenab V, Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine, Horm. and Behav., 46 (2004) 509-519.

74. Fields,H.L., Pain, McGraw-Hill, New York, 1987.

75. Fields,H.L., State-dependent opioid control of pain, Nature Reviews, 5 (2004) 565-575.

76. Fields,H.L., Heinrichert,M.M., and Mason,P., Neurotransmitters in nociceptive modulatory circuits, Ann. Rev. Neurosci., 14 (1991) 219-245.

77. Fillingim,R.B., Maixner W., Girdler,S.S., Light,K.C., Harris,M.B., Sheps,D.S., and Mason,G.A., Ischemic but not thermal pain sensitivity varies across the menstrual cycle, Psychosom Med, 59 (1997) 512-520.

78. Fillingim,R.B. and Maixner,W., Gender differences in response to noxious stimuli, Pain Forum, 4 (1995) 209-221.

79. Fillingim,R.B. and Ness,T.J., Sex-related hormonal influences on pain and analgesic response., Neuroscience and Biobehavioral Reviews, 24 (2000) 485-501.

80. Fillingim,R.B., Ness,T.J., Glover,T.L., Campbell,C.M., Hastie,B.A., Price,D.D., and Staud,R., Morphine responses and experimental pain: sex differences in side effects and cardiovascular responses but not analgesia., Pain, 6 (2005) 116-124.

81. Forman,L.J., Marquis,D.E., and Stevens,R., The effect of chronic estrogen treatment on immunoreactive beta-endorphin levels in intact female rats, Proceedings for the Society for Experimental Biology and Medicine, 179 (1985) 365-372.

82. Frannson-Hall,C. and Kilbom,A., Sensitivity of the hand to surface pressure, Appl Ergo, 24 (1993) 181-189.

83. Freeman,M.E., The neuroendocrine control of the ovarian cycle of the rat. In: Knobil,E., Neill,J.D. (Eds.), The Physiology of Reproduction, Raven Press, Ltd., New York, 1994, pp. 613-658.

84. Frye,C.A., Bock,B.C., and Kanarek,R.B., Hormonal milieu affects tailflick latency in female rats and may be attenuated by access to sucrose, Physiol. Behav., 53 (1992) 27-32.

85. Frye,C.A., Cuevas,C.A., and Kanarek,R.B., Diet and estrous cycle influence pain sensitivity in rats., Pharmacol Biochem Behav, 45 (1993) 255-260.

86. Frye,C.A. and Duncan,J.E., Estradiol benzoate potentiates neuroactive steroids' effects on pain sensitivity, Pharmacol Biochem Behav, 53 (1994) 27-32.

87. Fujiwara,A., Lim,T.H., An,H.S., Tanaka,N., Jeon,C.H., Andersson,G.B., and Haughton,V.M., The effect of disc degeneration and facet joint osteoarthritis on the segmental flexibility of the lumbar spine, Spine., 25 (2000) 3036-3044.

88. Garcia,M.M. and Harlan,R.E., Stimulus-transcription coupling at the mu opiate receptor. In: Hammer,R. (Ed.), The Neurobiology of Opiates, CRC Press, Boca Raton, FL, 1992, pp. 257-299.

89. Gaumond,I., Arsenault,P., and Marchand,S., The role of sex hormones on formalin-induced nociceptive responses., Brain Res, 958 (2002) 139-145.

90. Gear,R.W., Miaskowski,C., Gordon,N.C., Paul,S.M., Heller,P.H., and Levine,J.D., Kappa-opioids produce significantly greater analgesia in women than in men., Nature Medicine, 2 (1996) 1248-1250.

91. Giamberardino,M.A., Affaitati,G., Valente,R., Iezzi,S., and Vecchiet,L., Changes in visceral pain reactivity as a function of estrous cycle in female rats with artificial ureteral calculosis, Brain Res, 744 (1997) 234-238.

92. Gintzler,A.R., Endorphin-mediated increases in pain threshold during pregnancy., Science, 210 (1980) 193-195.

93. Gintzler,A.R. and Bohan,M.C., Pain thresholds are elevated during pseudopregnancy., Brain Res, 507 (1990) 312-316.

94. Gintzler,A.R. and Liu,N.-J., Ovarian sex steroids activate antinociceptive systems and reveal gender-specific mechanisms. In: Fillingim,R.B. (Ed.), Sex, Gender, and Pain, IASP Press, Seattle, 2000, pp. 89-108.

95. Giordano,A.L., Siegel,H.I., and Rosenblatt,J.S., Nuclear estrogen receptor binding in microdissected brain regions of female rats during pregnancy: implications for maternal and sexual behavior, Physiol Behav., 50 (1991) 1263-1267.

96. Goodman,H.M., Basic Medical Endocrinology, Raven Press, New York, 1994.

97. Gordon,F.T. and Soliman,M.R.I., Diurnal variation in the acute effects of estradiol and progesterone on beta-endorphin and Met-enkephalin levels in specific brain regions of ovariectomized rats., Pharmacology., 49 (1994) 192-198.

98. Gordon,F.T. and Soliman,M.R.I., The effects of estradiol and progesterone on pain sensitivity and brain opioid receptors in ovariectomized rats, Horm Behav, 30 (1996) 244-250.

99. Hagbarth,K.E. and Kerr,D.I.B., Central influences on spinal afferent conduction, J Neurophysiol, 17 (1954) 295-307.

100. Hall,E.G. and Davies,S., Gender differences in perceived intensity and affect of pain between athletes and nonathletes, Percept Mot Skills, 73 (1991) 779-786.

101. Hammer,R.P., μ-opioid receptor binding in the medial preoptic area is cyclical and sexually dimorphic, Brain Res., 515 (1990) 187-192.

102. Hammer,R.P. and Cheung,S., Sex steroid regulation of hypothalamic opioid function. In: Micevych,P.E., Hammer,R.P. (Eds.), Cambridge University Press, New York, 1995, pp. 143-159.

103. Hapidou,E.G. and De Catanzaro,D., Sensitivity to cold pressor pain in dysmenorrheic and non-dysmenorrheic women as a function of menstrual cycle phase, Pain, 34 (1988) 277-283.

104. Harasawa,I., Fields,H.L., and Meng,I.D., Delta opioid receptor mediated actions in the rostral ventromedial medulla on tail flick latency and nociceptive modulatory neurons, Pain, 85 (2000) 255-262.

105. Haws,C.M., Williamson,A.M., and Fields,H.L., Putative nociceptive modulatory neurons in the dorsolateral pontomesencephalic reticular formation., Brain Res, 483 (1989) 272-282.

106. Heinricher,M.M., Cheng,Z.F., and Fields,H.L., Evidence for two classes of nociceptive modulating neurons in the pariaqueductal gray., J Neurosci, 7 (1987) 271-278.

107. Holtman Jr,J.R., Sloan,J.W., and Wala,E.P., Morphine tolerance in male and female rats, Pharmacol Biochem Behav, 2004 (2004) 517-523.

108. Hughes,J., Smith,T., Morgan,B., and Fothergill,L., Purification and properties of enkephalin- the possible endogenous ligand for the morphine receptor, Life Sci, 16 (1975) 1753-1758.

109. Inturrisi,C., Addiction and Pain: Assessment and Treatment Issues, Clin J of Pain, 18 supplement (2002) S3-S13.

110. Inturrisi,C.E., Brodsky,M., and Rasmussen,K., NMDA antagonists block c-fos expression during opioid withdrawal., Soc. Neurosci., 17 (1991) 1338.

111. Islam,A.K., Cooper,M.L., and Bodnar,R.J., Interactions among aging, gender and gonadectomy effects upon morphine antinociception in rats, Physiol. Behav., 54 (1993) 45-53.

112. Jaffe,J.H. and Martin,W.R., Opioid Analgesics and Antagonists. In: Goodman,A., Gilman,A. (Eds.), Goodman and Gilman's the Pharmacological Basics of Therapeutics, Pergamon Press, Inc., Elmsford, NY, 1990, pp. 508-510.

113. Jones,R.E., Human Reproductive Biology, Academic Press, San Diego, 1997.

114. Julien,R.M., A Primer of Drug Action, Worth Publishers, New York, 2001.

115. Kasson,B.G. and George,R., Endocrine influences on the actions of morphine:IV. effects of stress and strain, Life Sci., 34 (1984) 1613-1697.

116. Kaur,G. and Kulkarni,S.K., Evidence for serotonogic modulation of progesterone-induced hyperphagia, depression and algesia in female mice., Brain Res, 943 (2002) 206-215.

117. Kayser,V., Berkley,K.J., Keita,H., Gautron,M., and Guilbaud,G., Estrous and sex variations in vocalization thresholds to hindpaw and tail pressure stimulation in the rat, Brain Res, 742 (1996) 352-354.

118. Kepler,K.L., Kest,B., Kiefel,J.M., Cooper,M.L., and Bodnar,R.J., Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventicular morphine in rats., Pharmacol Biochem Behav, 34 (1989) 119-127.

119. Kepler,K.L., Standifer,K.M., Paul,D., Kest,B., Pasternak,G.W., and Bodnar,R.J., Gender effects and central opioid analgesia, Pain, 45 (1991) 87-94.

120. Kiefel,J.M. and Bodnar,R.J., Roles of gender and gonadectomy in pilocarpine and clonidine analgesia in rats., Pharmacol Biochem Behav., 41 (1992) 153-158.

121. Kovelowski,C.J., Bian,D., Hruby,V.J., Lai,J., Ossipov,M.H., and Porreca,F., Selective opioid delta agaonists elicit antinociceptive supraspinal/spinal synergy in the rat., Brain Res., 843 (1999) 12-17.

122. Kovelowski,C.J., Ossipov,M.H., Hruby,V.J., and Porreca,F., Lesions of the dorsolateral funiculus block supraspinal opioid delta receptor mediated antinociception in the rat., Pain, 943 (1999) 206-215.

123. Lautenbacher,S. and Rollman,G.B., Sex differences in responsiveness to painful and non-painful stimuli are dependent upon the stimulation method., Pain, 53 (1993) 255-264.

124. Lautenbacher,S. and Strian,F., Sex differences in pain and thermal sensitivity: the role of body size., Percept Psychophys, 50 (1991) 179-183.

125. Lenz,G.R., Evans,S.M., Walters,D.E., Hopfinger,A.J., and Hammond,D.L., Opiates, Academic Press, Orlando, FL, 1986.

126. Liberson,C.W. and Liberson,W.T., Sex differences in autonomic responses to electric shock., Psychophysiology., 12 (1975) 182-185.

127. Liu,N.-J. and Gintzler,A.R., Prolonged ovarian sex steroid treatment of male rats produces antinociception: identification of sex-based divergent analgesic mechanisms, Pain, 85 (2000) 273-281.

128. Liuzzi,F.J., Scoville,S.A., and Bufton,S.M., Effects of short-term estrogen replacement on trkA mRNA levels in axotomized dorsal root ganglion neurons, Exp Neurol, 159 (1999) 433-440.

129. Lord,J.A., Hughes,J., and Kosterlitz,H.W., Endogenous opioid peptides: Multiple agonists and receptors., Nature, 267 (1977) 495-499.

130. Machado,B.H. and Brody,M.J., Mechanisms of pressor response produced by stimulation of nucleus ambiguus., Am. J. Physiol., 259 (1990) R955-R962.

131. Maggi,R., Dondi,D., Rovati,G.E., Martini,L., Piva,F., and Limonta,P., Binding characteristics of hypothalamic mu opioid receptors throughout the estrous cycle in the rat., Neruoendocrinology, 58 (1993) 366-372.

132. Mann,G.E., Hormone control of prostaglandin F(2 alpha) production and oxytocin receptor concentrations in bovine endometrium in explant culture, Domest Anim Endocrinol, 20 (2001) 217-226.

133. Mansour,A., Khachaturian,H., Lewis,M.E., Akil,H., and Watson,S.J., Anatomy of CNS opioid receptors, Trends Neurosci., 11 (1988) 308-314.

134. Marcus,D.A., Interrelationships of neurochemicals, estrogen, and recurring headache, Pain, 62 (1995) 129-141.

135. Martinez-Gomez,M., Cruz,Y., Salas,M., Hudson,R., and Pacheco,P., Assessing pain threshold in the rat: changes with estrus and time of day, Physiol Behav, 55 (1994) 651-657.

136. Martinez-Gomez,M., Cruz,Y., Salas,M., Hudson,R., and Pacheco,P., Assessing pain thresholds in the rat: Changes with estrous and time of day, Physiol. Behav., 55 (1994) 651-657.

137. Mateo,A.R., Hijazi,M., and Hammer,R.P., Dynamic patterns of medial preoptic μ-opiate receptor regulation by gonadal steroid hormones, Neuroendocrinol., 55 (1992) 51-58.

138. Mayer,D.J. and Hayes,R.L., Stimulation-produced analgesia: development of tolerance and cross-tolerance to morphine., Science, 188 (1975) 941-943.

139. McCarthy,M.M., Caba,M., Komisurak,B.R., and Beyer,C., Modulation by estrogen and progesterone on the effect of muscimol on nociception in the spinal cord, Pharmacol. Biochem. Behav., 37 (1990) 123-128.

140. Melzack,R. and Wall,P.D., The Challenge of Pain, Penguin, London, 1982.

141. Merskey,H. and Bogduk,N., Classification of Chronic Pain:Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms, IASP Press, Seattle, 1994.

142. Meyer,R.A. and Campbell,J.N., Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand, Science, 213 (1981) 1527-1529.

143. Micevych,P. and Hammer,R., Estrogen receptor mRNA:neuroanatomical distribution and regulation in three behaviorally relevant physiological models. In: Micevych,P., Hammer,R. (Eds.), Neurobiological effects of sex steroid hormones, Cambridge University Press, Cambridge, 1995, pp. 57-84.

144. Millan,M.J., Kappa-opioid receptor-mediated antinociception in the rat. I. Comparative actions of mu- and kappa-opioids against noxious thermal, pressure and electrical stimuli, Pharmacol. Exp. Ther., 251 (1989) 334-341.

145. Millan,M.J., The induction of pain: an integrative review, Prog. Neurobiol., 57 (1999) 1-164.

146. Mogil,J.S., Chesler,E.J., Wilson,S.G., Juraska,J.M., and Sternberg,W.F., Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype., Neurosci Biobehav Rev, 24 (2000) 375-389.

147. Mogil,J.S., Sternberg,W.F., Kest,B., Marek,P., and Liebeskind,J.C., Sex differences in the antagonism of swim stress-induced analgesia: effects of gonadectomy and estrogen replacement., Pain, 53 (1993) 17-25.

148. Mogil,J.S., Wilson,S.G., and Wan,Y., Assessing Nociception in Murine Subjects. In: Kruger,L. (Ed.), Methods of Pain Research, CRC Press, Boca Raton,FLA, 2001, pp. 11-40.

149. Morrell,J.I., Kelly,D.B., and Pfaff,D.W., Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, Xenopus laevis.II. Estradiol., J Comp Neurol., 164 (2005) 63-77.

150. Morrell,J.I. and Pfaff,D.W., Immunocytochemistry of steroid hormone receiving cells in the central nervous system., Methods Enzymol., 103 (1983) 639-662.

151. Murray,A. and Holdcroft,A., Incidence and intensity of postpartum lower abdominal pain., BMJ, 298 (1989) 1619.

152. Musto,D., The American Disease: Origines of Narcotic Control, Oxford Press, New York, 1999, 432 pp.

153. Negus,S.S. and Mello,N.K., Effects of kappa opioid agonists on the discriminative stimulus effects of cocaine in rhesus monkeys, Experimental and Clinical Psychopharmacology, 7 (1999) 307-317.

154. Negus,S.S. and Mello,N.K., Effects of gonadal steroid hormone treatments on opioid antinociception in ovariectomized rhesus monkeys, Psychopharmacology, 159 (2002) 275-283.

155. Ness,T.J. and Gebhart,G.F., Visceral pain: a review of experimental studies, Pain, 41 (1990) 167.

156. North,A.R., Williams,J.T., Surprenant,A., and Christie,M.J., μ and δ receptors belong to a family of receptors that are coupled to potassium channels., Proc Natl Acad Sci U S A, 84 (1987) 5487-5491.

157. Ochi,T., Motoyama,Y., and Goto,T., The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models, Eur. J. Pharmacol., 391 (2000) 49-54.

158. Pare,W.P., Age, sex and strain differences in the aversive threshold to grid shock in the rat., Comp Physiol Psychol., 69 (1969) 214-218.

159. Patrone,C., Andersson,S., Korhonen,S., and Lindholm,D., Estrogen receptor-dependent regulation of sensory neuron survival in developing dorsal root ganglion., Proc Natl Acad Sci U S A, 96 (1999) 10905-10910.

160. Pearson,R.L., Ross,L.R., and Jacobson,C.D., Differential down-regulation of estrogen receptor-like immunoreactivity by estradiol in the female Brazilian opossum brain., Brain Res., 617 (1993) 171-175.

161. Perrotti,L.I., Russo,S.J., Fletcher,H., Chin,J., Webb,T., Jenab,S., and Quinones-Jenab,V., Ovarian hormones modulate cocaine induced locomotor and stereotypic activity, New York Academy of Science, 937 (2001) 202-216.

162. Perrotti,L.I., Russo,S.J., Fletcher,H., Chin,J., Webb,T., Jenab,S., and Quinones-Jenab,V., Ovarian hormones modulate cocaine-induced locomotor and stereotypic activity, Ann. N. Y. Acad. Sci., 937 (2001) 202-216.

163. Pert,C.B., Pasternak,G.W., and Snyder,S.H., Opiate agonists and antagonists discriminated by receptor binding in brain., Science, 182 (1973) 1359-1361.

164. Pfaff,D., Arnold,A.P., Etgan,A.M., Fahrbach,S.E., and Rubin,R.T., Hormones, Brain and Behavior, Vol. 5, Academic Press, San Diego, 2002.

165. Pfaff,D.W., Schwartz-Giblin,S., McCarthy,M.M., and Kow,L., Cellular and molecular mechanisms of female reproductive behavior. In: Knobil,E., Neill,J.D. (Eds.), Physiology of Reproduction, Raven Press, New York, 1994, pp. 107-220.

166. Pfaff,D.W. and Schwartz-Giblin,S., Cellular mechanism of female reproductive behavior. In: E.Knobil, J.Neill (Eds.), The physiology of reproduction, Raven, New York, 1995, pp. 1487-1568.

167. Pfaus,J. and Pfaff,D.W., μ–, δ-, and κ-opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone, Horm. and Behav., 26 (1992) 457-473.

168. Pfaus,J.G. and Pfaff,D.W., Mu-,delta-,and kappa-opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone., Horm Behav, 26 (1992) 457-73.

169. Philips,S.M. and Sherwin,B.B., Variations in memory function and sex steroid hormones across the menstrual cycle, Psychoneuroendocrinology, 17 (1992) 497-506.

170. Price,D.D., Psychological and neural mechanisms of pain, Raven, New York, 1988.

171. Procacci,P., Corte,M.D., Zoppi,M., and Maresca,M., Rhythmic changes of the cutaneous pain threshold in man. A general review., Chronobiologia., 1 (1974) 77-96.

172. Quinones-Jenab,V., Jenab,S., Ogawa,S., Inturrisi,C., and Pfaff,D.W., Estrogen regulation of μ-opioid receptor mRNA in the forebrain of female rats, Mol. Brain Res., 47 (1997) 134-138.

173. Quiñones-Jenab,V., Ogawa,S., Jenab,S., and Pfaff,D.W., Estrogen regulation of preproenkephalin messenger RNA in the forebrain of female mice, J. Chem. Neuroanatomy, 12 (1997) 29-36.

174. Raja,S.N., Meyer,R.A., Ringkamp,M., and Campbell,J.M., Peripheral neural mechanisms of nociception. In: Wall,P.D., Melzack,R. (Eds.), Textbook of Pain, Churchill Livingstone, Edinburgh, 1999, pp. 11-57.

175. Rang,H.P., Bevan,S., and Dray,A., Nociceptive periopheral neurosns: cellular properties. In: Wall,P.D., Melzack,R. (Eds.), Textbook of Pain, Churchill Livingstone, Edinburgh, 1994, pp. 57-78.

176. Ratka,A. and Simpkins,J.W., Effects of estradiol and progesterone on the sensitivity to pain and on morphine-induced antinociception in female rats., Horm Behav, 25 (1991) 217-228.

177. Ren,K. and Dubner,R., Inflammatory models of pain and hyperalgesia, ILAR Journal, 40 (1999).

178. Reynolds,D.V., Surgery in the rat during electrical analgesia produced by focal brain stimulation, Science, 164 (1969) 444-445.

179. Riley III,J.L., Robinson,M.E., Wise,E.A., Myers,C.D., and Fillingim,R.B., Sex differences in the perception of noxious expermental stimuli: a meta-analysis, Pain, 74 (1998) 181-187.

180. Roehrig,C.B., Let's take the high road., Internist., 25 (1984) 32-34.

181. Rollman,G.B. and Harris,G., The detectability, discriminability, and perceived magnitude of painful electrical shock, Percept Psychophys, 42 (1987) 257-268.

182. Romano,G.J., Harlan,R.E., Shivers,B.D., Howells,R.D., and Pfaff,D.W., Estrogen increases proenkephalin messenger ribonucleic acid levels in the ventromedial hypothalamus of the rat, Mol. Endo., 2 (1988) 1320-1328.

183. Romano,G.J., Mobbs,C.V., Lauber,A.H., Howells,R.D., and Pfaff,D.W., Differential regulation of proenkephalin gene expression by estrogen in the ventromedial hypothalamus of male and female rats: implications for the molecular basis of a sexually differentiated behavior, Brain Res., 536 (1990) 63-68.

184. Romano,G.J., Mobbs,C.V., and Pfaff,D.W., Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone, Mol. Brain Res., 5 (1989) 57-58.

185. Romero,M.T. and Bodnar,R.J., Gender differences in two forms of cold-water swim analgesia., Physiol Behav., 37 (1986) 893-897.

186. Romero,M.T., Kepler,K.L., Cooper,M.L., Komisaruk,B.R., and Bodnar,R.J., Modulation of gender-specific effects upon swim analgesia in gonadectomized rats, Physiol. Behav., 40 (1987) 39-45.

187. Rossi,G., Pan,Y.-X., Cheng,J., and Pasternak,G.W., Blockade of morphine analgesia by an antisense oligodeoxynucleotide against the mu receptor., Life Sci, 54 (1980) 375-379.

188. Rossi,G.C., Pasternak,G.W., and Bodnar,R.J., Synergistic brainstem interactions for morphine analgesia., Brain Res, 624 (1993) 171-180.

189. Ryan,S.M. and Maier,S.F., The estrous cycle and the estrogen modulate stress-induced analgesia., Behav. Neurosci., 102 (1988) 371-380.

190. Santagostino,A., Cocchi,D., Giagnoni,G., Gori,E., Muller,E., and Ferri,S., Some relationships between endorphins and pituitary hormones., Adv Biochem Psychopharmacol., 18 (1978) 175-181.

191. Schumacher,M. and Robert,F., Progesterone: synthesis, metabolism, mechanisms of action, and effects in the nervous system. In: Pfaff,D., Arnold,A.P., Etgen,A.M., Fahrbach,S.E., Rubin,R.T. (Eds.), Hormones, Brain and Behavior, Vol. 5, Academic Press, NY, 2002, pp. 683-746.

192. Sell,S.L., Scalzitti,J.M., Thomas,M.L., and Cunningham,K.A. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 293, 879-886. 2000.

193. Selye, H., Anaesthetic effect of steroid hormones, Proc Soc Exp Biol Me, 46 (1923) 116-121.

194. Selye,H., The physiology and pathology of exposure to stress., ACTA, Inc, Montreal, 1950.

195. Shekunova,E.V. and Bespalov,A.Y., Estrous cycle stage-dependent expression of acute tolerance to morphine analgesia in rats, Eur J Pharmacol, 486 (2004) 259-264.

196. Silberstein,S.D. and Lipton,R.B., Headache epidemiology. Emphasis on migraine, Neurol Clin., 14 (1996) 421-434.

197. Smiley,P.L., Johnson,M., Bush,L., Gibb,J.W., and Hanson,G.R., Effect of cocaine on extrapyramidal and limbic dynorphin system, J. Pharm. Exp. Ther., 253 (1990) 938-943.

198. Snijdelaar,D.G., Dirkson,R., Slappendel,R., and Crul,B.J., Substance P., Eur J Pain, 4 (2000) 121-135.

199. Sohrabji,F., Miranda,R.C., and Toran-Allerand,C.D., Estrogen differentially regulates estrogen and NGF receptor mRNAs in adult sensory neurons, J Neurosci, 14 (1994) 459-471.

200. South,S.M., Wright,A.W.E., Lau,M., Mather,L.E., and Smith,M.T., Sex-related differences in antinociception and tolerance development following chronic intravenous infusion of morphine in the rat: modulatory role of testosterone via morphine clearance, J. Pharmacol. Exp. Ther., 297 (2001) 446-457.

201. Steenbergen,H.L., Heinsbroek,R.P., Van Haaren,F., and Van De Poll,N.E., Sex-dependent effects of inescapable shock administration on behavior and subsequent escape performance in rats., Physiol Behav., 45 (1989) 781-787.

202. Stein,C., Millan,M.J., and Herz,A., Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds., Pharmacol Biochem Behav, 31 (1988) 451-455.

203. Sternberg,W.F., Ritchie,J., and Mogil,J.S., Qualitative sex differences in κ–opioid analgesia in mice are dependent on age, Neurosci Lett, 363 (2004) 178-181.

204. Sternberg,W.F. and Wachterman,M.W., Experimental studies of sex-related factors influencing nociceptive responses: nonhuman animal research. In: Fillingim,R.B. (Ed.), Sex, Gender, And Pain, IASP Press, Seattle, 2000, pp. 71-88.

205. Stoffel,E.C., Ulibarri,C., and Craft,R.M., Gonadal steroid hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats, Pain, 103 (2003) 285-302.

206. Taber,R.I., Predictive value of analgesic assays in mice and rats, Adv. Biochem. Psychopharmacol., 8 (1974) 191.

207. Taleghany,N., DonCarlos,L., Gollapudi,L., and Oblinger,M.M., Differential expression of estrogen receptor alpha and beta in rat dorsal root ganglion neurons., J Neurosci Res, 57 (1999) 603-615.

208. Terner,J.M., Barrett,A.C., Grossell,E., and Picker,M.J., Influence of gonadectomy on the antinociceptive effects of opioids in male and female rats, Psychopharmacology, 163 (2002) 183-193.

209. Tsou,K. and Jang,C.S., Studies on the site of analgesic action of morphine by intercerebral micro-injection., Sci. Sin., (1964) 1099-1109.

210. Unruh,A.M., Gender variations in clinical pain experience, Pain, 65 (1996) 123-167.

211. Urban,M.O. and Gebhart,G.F., Central mechanisms in pain., Med Clin North Am., 83 (1999) 585-596.

212. Vagell,M. and McGinnis,M., The role of gonadal steroid receptor activation in the restoration of sociosexual behavior in adult male rats, Horm Behav, 33 (1998) 163-179.

213. Wall,P.D., Pain: The Science of Suffering., Columbia University Press, New York, 2000.

214. Wall,P.D. and Melzack,R., Textbook of Pain, Churchill Livingstone, New York, 1994.

215. Wallas,S.L., Aswad,D.W., and Greegard,P., A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions, Nature, 301 (1983) 69-71.

216. Wallas,S.L., Aswad,D.W., and Greegard,P., A dopamine- and cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions, Nature, 301 (1983) 69-71.

217. Weiland,N.G. and Wise,P.M., Estrogen and progesterone regulate opiate receptor densities in multiple brain regions, Endocrinology, 126 (1990) 804-808.

218. Westcott,T.B., Huesz,L., Boswell,D., and Herold,P., Several variables of importance in the use of the cold pressor as a noxious stimulus in behavioral research., Percept Mot Skills, 44 (1977) 401-402.

219. Whipple,B., Josimovich,J.B., and Komisaruk,B.R., Sensory thresholds during the antepartum, intrapartum and postpartum periods., Int J Nurs Stud., 27 (1990) 213-221.

220. Wilkinson,M., Brawer,J.R., and Wilkinson,D.A., Gonadal steroid-induced modification of opiate binding sites in anterior hypothalamus of female rats, Biol Reprod, 32 (1985) 501-506.

221. Wollemann,M., Benhyde,S., and Simon,J., The kappa-opioid receptor: evidence for the different subtypes, Life Sci, 52 (1993) 599-611.

222. Yeung,J.C. and Rudy,T.A., Sites of antinociceptive action of systemically injected morphine: involvement of supraspinal loci as revealed by intracerebroventicular injection of naloxone., J Pharmacol Exp Ther., 215 (1980) 626-632.

223. Yeung,J.C., Yaksh,T.L., and Rudy,T.A., Concurrent mapping of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat., Pain, (1977) 23-40.