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GENDER DIFFERENCES IN ANALGESIC PROCESSES IN RATS: INFLUENCE OF GONADAL AND HORMONAL FACTORS

by

Maria-Teresa Romero

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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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

GENDER DIFFERENCES IN ANALGESIC PROCESSES IN RATS: INFLUENCE OF GONADAL AND HORMONAL FACTORS

by

Maria-Teresa Romero

Advisor: Professor Richard J. Bodnar

Gender specific differences are observed both in the perception of noxious stimuli and morphine analgesia in rats. Consequently, the purpose of the present dissertation was to determine whether gender and gonadal steroids play a modulatory role in analgesia processes. Pain thresholds were measured with the tail-flick and jump tests. The first experiment evaluated gender differences in continuous cold water swim (CCWS), intermittent cold water swim (ICWS), 2-Deoxy-D-Glucose (2DG) and systemic morphine analgesia. In order to control for body weight differences, adult age-matched male and female rats and a third groups of younger male rats, weight-matched to the female group were used as subjects. Female rats displayed significantly less analgesia than young and adult males following CCWS, ICWS and morphine. Gender differences failed to appear following central morphine administration, suggesting a peripheral mechanism for the gender differences observed in systemic morphine analgesia. Estrous cycle had no

effect on the magnitude of CCWS analgesia. The second study evaluated the role of gonadal status of the organism in the observed gender differences. Castration significantly reduced CCWS and ICWS analgesia to levels observed in the intact female. Ovariectomy reduced CCWS and ICWS analgesic magnitude further. This reduction could not be accounted for by changes in CCWS and ICWS hypothermia, activity during the swim or post-operative weight changes. Naloxone significantly reduced opioid-mediated ICWS analgesia in males, but not in females. Nonopioid-mediated CCWS analgesia was not affected by naloxone pretreatment. In the third experiment testosterone proprionate (TP) and estradiol benzoate (EB) were administered to intact and gonadectomized male and female rats. The magnitude of CCWS and ICWS analgesia was reinstated by TP treatment in castrated males and ovariectomized females. TP potentiated CCWS analgesia in intact males. EB attenuated CCWS analgesia in intact females and reinstated ICWS analgesia in ovariectomized females. These results suggest that TP, but not EB plays an important role in the gonadal modulation of both opioid-mediated and nonopioid-mediated forms of swim analgesia, but that supplements of neither steroids to intact rats consistently alters these analgesic responses.

¿A dónde vamos Pocho? Tu tampoco sabes, pero ¡aquí vamos!

A Pocho, Mami y a la "patota", Yolanda, Olga, Nilma y Puchi, todos igualmente responsables.

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Introduction

Various lines of evidence indicate the existence of multiple endogenous mechanisms that function to inhibit pain. These systems are usually characterized in terms of the involvement, or lack thereof, of opioid, hormonal and neural systems (Basbaum & Fields, 1984). Insights in the activation and characterization of intrinsic pain suppressing mechanisms have, in part, been provided by investigating the substrates of enviromentally-induced analgesia. Although the range of stimuli that modulate the response to pain is quite varied, much of this research has focused on stressful stimuli. These provide a means of reliably generating analgesia with different physiological and pharmacological profiles elicited depending upon the specific stressor employed or upon the parameters of the same stimuli (see reviews Bodnar, 1986; Watkins and Mayer, 1986). While much information has been gathered on the factors that influence the type, magnitude and duration of environmentally produced analgesia, related to either the parameters of the stimuli themselves or to the hormonal and neural substrates, little research has attempted to clarify the influence of subject variables on stress induced

analgesia (SIA). For example, most studies have traditionally employed young adult male rats as subjects, with the purpose of reducing variability and avoiding the cyclicity problems in female rats, therefore precluding the analysis of possible gender differences in SIA. Since an animal's gender influences its reactivity to noxious stimuli and its neuroendocrine and adaptive response to stress, the question arises as to whether gender and gonadal factors also influence analgesic stress responses.

Given the importance of endocrine factors in the response to stress in the young male rat and the modulatory role of gonadal hormones in pain sensitivity and morphine analgesia, the purpose of these series of experiments was to systematically compare and evaluate analgesic processes in male and female rats. Various procedures that induce opioid-mediated or nonopioid-mediated analgesia were examined, including analgesia induced by 1) morphine, 2) 2-deoxy-D-glucose (partially opioid), 3) intermittent cold-water swims (opioid) and 4) continuous cold-water swims (nonopoid). Multiple pain measures were employed, and other physiological responses related to stress were monitored to clarify the specificity of gender differences in these

responses. Second, the role of gonadal factors in two forms of SIA were evaluated by analyzing the effects of gonadectomy in male and female rats. Third, the role of steroid hormones in SIA was examined by administering testosterone or estradiol as replacement therapy in gonadectomized rats or as supplements in intact rats. The following sections will provide background information pertaining to the existence of multiple pain inhibitory systems and the different procedures that activate them, as well as the effects of gender, hormonal and gonadal factors upon pain sensitivity and morphine analgesia.

Multiple endogonous analgesia systems:

Compelling evidence for the existence of neural systems in the brain and spinal cord, which function to inhibit pain, came from reports that electrical stimulation of the medial brain stem produced potent analgesia in rats (Reynolds, 1969). Mayer and co-workers (1971) demonstrated that this form of analgesia represented a true pain inhibition and was not secondary to changes in other sensory or motor systems. Stimulation produced analgesia (SPA) was found to be similar to analgesia observed following intracerebral microinjections of morphine. The possibility that both

phenomena shared similar mechanisms of action was supported by mapping studies which suggested the involvement of the periaqueductal grey (PAG) (Jacquet & Lajtha, 1974; Sharpe et al, 1974), dorsal raphe nucleus, nucleus raphe magnus (Mayer & Price, 1976) and lateral hypothalamus (Balagura & Ralph, 1972) among other structures. Further, rats receiving repeated stimulation of the PAG displayed analgesic tolerance in a manner similar to that observed for repeated central injections of morphine (Mayer & Hayes, 1975). Rats made tolerant to morphine also showed decreased SPA, suggesting cross-tolerance between the two procedures. Subanalgesic levels of SPA combined with subanalgesic doses of morphine elicited analgesia (Samanin & Valzelli, 1971). Lesions placed in the dorsolateral funiculus of the spinal cord, a major descending bulbo-spinal projection, blocked both SPA and morphine analgesia (Basbaum et al., 1977). SPA and morphine analgesia elicited from the PAG produced similar excitatory action on neural activity in the nucleus raphe magnus (Oleson et al., 1978) and similar inhibitory action upon spinal substantia gelatinosa units (Urca & Liebeskind, 1979). Finally, neuropharmachological manipulations of monoaminergic systems produced similar effects upon SPA and morphine

analgesia (Mayer & Price, 1976).

Akil and co-workers (1976) initially reported that naloxone, a short acting opiate receptor antagonist, significantly reduced SPA (Oliveras et al. 1977, but see Yaksh et al., 1976; Pert & Walter, 1976). This observation led to the hypothesis that SPA resulted from the release of endogenous opiate-like substances. The characterization and localization of stereospecific binding sites for opiates in the central nervous system (Hiller et al., 1973; Pert & Snyder, 1973; Terenius, 1973), in areas overlaping anatomically with loci which supported SPA, and central morphine analgesia (Pert et al., 1976; Atweh & Kuhar, 1977a,b,c.) supported this hypothesis. Further, the enkepalins were isolated from neural tissue with properties of competitive binding to the opiate receptor and which were blocked by opiate receptor antagonists (Hughes et al, 1975). Later, other endogenous compounds, the endorphins and dynorphins, were found to share similar properties (Cox et al, 1975; Li & Chung, 1976; Akil et al, 1984). The precursors for endogenous opioid peptides were determined and localized: the proopiomelanocortin (POMC: beta-endorphin), proenkephalin (met-enkephalin) and prodynorphin (dynorphin) systems (Akil et al, 1984). Together with the

isolation and identification of opioid precursors, the existence of multiple subtypes of opioid receptors (mu, kappa, sigma, delta and epsilon) was suggested by pharmacological biochemical and physiological studies (e.g., Martin et al., 1976; Lord et al., 1977; Chang & Cuatrecasas, 1979). Again, the distribution in the brain of the endogenous opiate ligands and binding sites included those areas involved in the processing of pain (Hughes, 1975; Khachaturian et al., , 1985). Further, these endogenous opioids, when administered into relevant areas of the central nervous system increased pain thresholds. Enkephalins, administered either intraventricularly or into the central grey, induced mild analgesia that could be blocked by opiate antagonists (Belluzzi et al, 1976; Chang et al, 1976; Buscher et al., 1976). Central injection of beta-endorphin produced potent analgesia (Bradbury et al., 1976; Wei et al., 1977) and electrical stimulation of systems containing the peptide also increased pain thresholds (Watson et al., 1979; Lewis, 1986), effects which were reversed by naloxone. Dynorphin increased pain thresholds following intrathecal injections into the lumbo- sacral spinal cord, producing a strong and long lasting effect (Herman & Goldstein, 1985). Moreover, manipulations known to

produce analgesia, such as SPA, acupuncture and vaginal probing, were usually accompanied by an increase in endogenous opioids concentrations in blood or nervous tissue (Akil et al., 1984; Bragin, 1986; Rossier et al., 1977).

Environmentally Induced Analgesia:

The empirical evidence described above pointed to the existence of similar neural substrates for SPA and central morphine analgesia, and subsequently to the description of opioid-mediated pain inhibitory systems (Basbaum & Fields, 1984). Some research efforts concentrated on determining the adequate environmental stimuli that triggered these pain-inhibitory systems, since the presence of a physiological substrate mediating pain inhibition capable of being activated by invasive procedures did not necessarily imply that such a system served the same function under normal conditions. Although anecdotal descriptions of pain inhibition elicited by situations such as extreme arousal or anxiety, (see Chance, 1986), or by noxious stimuli (Melzack, 1975; Parsons & Goetzl, 1945) had been reported, the first animal models of environmentally produced analgesia were proposed by Rosecrans & Chance

(1976) and by Hayes and co-workers (1976). A number of stressors (e.g., rotation, electric shock and hypertonic saline injections) produced a transient analgesia in rats, while others (ether stress and oscillation) produced hyperalgesia. Therefore, intrinsic pain inhibition appeared to be activated by environmental stimulation.

However, more recent evidence suggested that not all endogenous pain mechanisms rely on an opioid mediated pathway; rather, multiple modulatory systems exist that involve different physiological substrates. The failure of naloxone to attenuate SPA reported by some laboratories (Yaksh et al., 1976; Pert & Walter, 1976) was explained by site-specificity (Cannon et al. 1982): only SPA elicited from ventral, but not dorsal, areas of the PAG was antagonized by naloxone. These results pointed to the existence of different anatomical pathways for SPA induced by stimulation of different anatomical areas (Lovick, 1985), and subsequently to the existence of opiate mediated and non-opiate mediated SPA. Finally, additional evidence for the existence of multiple intrinsic mechanisms of pain inhibition was derived from the study of the analgesic effect of various non-opiate peptides, including vasopressin (Berntson and Berson,

1980; Berson et al., 1983; Kordower & Bodnar, 1984) and neurotensin, (Clineschmidt & McGuffin, 1977; Clineschmidt et al., 1979) among others.

Research on stress-induced analgesia (SIA) led to similar conclusions. To determine the role of opioids in this type of analgesia, three main criteria have been used: reversibility by the opiate receptor antagonist naloxone; cross-tolerance with morphine analgesia; and development of tolerance with repeated administrations. Using these criteria, some laboratories reported involvement of opioid mechanisms in SIA (Akil et al, 1976; Bodnar et al., 1978a; Madden et al., 1977), while others did not (Bodnar et al., 1978c; Hayes et al., 1978). Subsequent studies indicated that important variables determining opioid involvement in SIA included either the stressor itself (e. g., electric shock, cold water swims, food deprivation, etc.), or different parameters of the same stressor (e. g., duration, intensity, frequency, localization, biological significance, predictability and controllability of the stimulus) (Hayes et al., 1978; Hayes & Katayama, 1986; Lewis et al., 1980b; Bodnar & Sikorszky, 1983; Watkins and Mayer, 1982; Grau et al., 1981; Jackson et al., 1979; Girardot & Hollaway, 1984). Lewis and co-workers (1980a)

initially reported that exposure to different parameters of inescapable footshock could selectively activate discrete opioid or non-opioid mediated pain-inhibitory systems. The analgesia following exposure to prolonged intermittent footshock was antagonized by pretreatment with naloxone (Lewis et al., 1980b). Further, tolerance and cross-tolerance with morphine analgesia developed after repeated exposure to prolonged intermittent footshock (Lewis et al., 1981). In contrast, analgesia induced by brief continuous footshock was not affected by high doses of naloxone, was not reduced in morphine-tolerant rats and showed no development of tolerance (Lewis et al., 1981; Terman et al., 1984). However, continuous footshock analgesia is not a unitary phenomenon, since variations in the intensity and duration of this shock can produce analgesia which is reversible by naloxone (Terman et al, 1984). It is clear, therefore, that various environmental stimuli (some of them stressful in nature) and different parameters of the same stimuli can activate either opiate mediated or nonopiate mediated endogenous pain inhibitory systems. The following section will describe physiological and pharmacological aspects of analgesia elicited by two stressors that were be used in these series of studies:

cold water swim and 2-deoxy-D-glucose administration.

Stress-Induced Analgesia: Physiological and Pharmacological characteristics

Various approaches have been utilized to determine the physiological mechanisms subserving pain inhibition induced by stressful stimuli. They include manipulation of the hypothalamic-hypophyseal-adrenal axis, lesions of relevant areas of the nervous system, as well as the use of a wide range of agonists and antagonists of the different neurotransmitter and neuromodulator systems. The outcome has been a characterization of the different types of SIA in terms of opioid involvement, neuroendocrine aspects and neurological correlates.

1. Continuous Cold Water Swim (CCWS) Analgesia:

Following acute cold-water swims (2-15 C for 3.5 min), rats display a profound and prolonged analgesia on the flinch-jump, tail-flick and operant liminal escape pain tests (Bodnar et al., 1978d). Warmer water temperatures (28 C), however, failed to alter pain thresholds, indicating that cold stress is the trigger for analgesia and not forced exercise. Although hypothermia is also observed following cold-water swims,

repeated daily exposures to CCWS over 14 days produced adaptation to the analgesic, but not the hypothermic effects (Bodnar et al., 1978f). This suggests that the stressful consequences of the swim, and not the swim per se, accounts for the increased pain thresholds.

Evidence indicates that CCWS analgesia is mediated by non-opiate systems. A lack of cross-tolerance is observed for CCWS and morphine analgesia: rats made tolerant to chronic morphine display a degree of CCWS analgesia similar to that of acutely exposed animals. Similarly, rats adapted to the analgesic effect of CCWS display a normal analgesic response to systemic morphine (Bodnar et al., 1978f; Girardot & Halloway, 1984a). In addition, naloxone doses ranging from 1 to 20 mg/kg, capable of fully blocking morphine analgesia, fails to reverse CCWS analgesia. (Bodnar et al, 1978c). It is interesting to note that partial attenuation of CCWS analgesia following naloxone occurs in some animals, but not others. This is explained, in part, by a significant positive correlation between the magnitude of CCWS analgesia and the ability of naloxone to attenuate the effect. This suggests that CCWS typically produces a nonopioid response, but in those animals in which an opioid mediated and nonopioid mediated response occurs,

the analgesia is larger and partially blocked by naloxone (Bodnar & Sikorsky, 1983). Since naloxone is a short acting, nonspecific opioid antagonist, its lack of effect on long-lasting CCWS analgesia may be difficult to evaluate. However, naltrexone, another opioid antagonist, which has a duration of action of at least 60 min, also fails to reverse the analgesic effect of CCWS (Girardot & Holloway, 1984b). Naloxazone, an irreversible mu-1 receptor antagonist, eliminates morphine analgesia but potentiates CCWS analgesia, indicating a clear dissociation of the mechanisms of action of the two procedures (Kirchgessner et al, 1982). Further, D-phenylalanine a putative anti-enkephalinase, increases morphine analgesia while decreasing CCWS analgesia (Bodnar et al., 1980e).

Since all stressful environmental stimuli activate the hypothalamic-hypophysial-adreno-cortical and sympathetic- medullary axes (Selye, 1956), neuro-hormonal manipulations would presumably affect various forms of SIA. In fact, destruction of the medial-basal hypothalamus by neonatal administration of the neurotoxin monosodium glutamate (MSG), reduces both CCWS analgesia and hypothermia in adult rats (Badillo-Martinez et al., 1984). This indicates an involvement of the medial basal

hypothalamus in the physiological response to stress. Hypophysectomy, in turn, attenuates the CCWS analgesic effect observed in intact animals across different pain tests, but fails to alter CCWS hypothermia (Bodnar et al, 1979a, b). This suggests a more specific effect of hypophysectomy on pain inhibitory mechanisms, as compared to the more global influence of medial-basal hypothalamic lesions on adaptation to stress. Further evidence implicated mediation of CCWS analgesia by the adrenocortical system. Removal of the intermediate and posterior lobes of the pituitary gland fails to alter CCWS analgesia while adrenalectomy potentiates it (Glusman et al., 1979; 1980). Further, adrenal demedullation and depletion of peripheral catecholamines with 6-hydroxydopamine fails to affect CCWS analgesia (Bodnar et al., 1982b). Manipulation of glucocorticoids alters this response. The synthetic glucocorticoid, dexamethasone, attenuates CCWS analgesia, while inhibition of corticosteroid synthesis with metyrapone potentiates it (Marek et al., 1980; Mousa et al., 1981; Mousa et al., 1983).

Neuropharmacological manipulations have provided information regarding the neural components of CCWS analgesia. Catecholamine involvement is suggested by the

reduction of CCWS analgesia by apomorphine, a domamine receptor agonist, and the potentiation of CCWS analgesia by dopamine receptor blockade (Bodnar et al., 1980b; Bodnar & Nicotera, 1982). CCWS analgesia is potentiated by clonidine, an alpha-noradrenergic receptor stimulant (Bodnar et al, 1983b), and desipramine, which blocks norepinephrine reuptake (Bodnar et al, 1985a). On the other hand, lesions placed in the noradrenergic locus coeruleus decrease CCWS analgesia (Bodnar et al. 1980a). Cholinergic involvement has also been implicated in that both scopolamine and methylscopolamine reduce CCWS analgesia (Sperber et al, 1986). Similarly, vasopressin seems to modulate CCWS analgesia since Brattleboro rats, genetically deficient in vasopressin, fail to display CCWS analgesia (Bodnar et al, 1980e). Also, central pretreatment with thyrotropin releasing hormone (TRH), its metabolite or analog potentiate CCWS analgesia probably through its interaction with the cholinergic system (Butler et al., 1986; Butler & Bodnar, 1987). In contrast, the GABAergic and serotoninergic systems appear not to be involved in CCWS analgesia (Brutus et al., 1979; Bodnar & Sperber, 1982; Bodnar et al, 1981a).

In summary, CCWS appears to activate mainly a nonopiate-mediated endogenous pain inhibitory system. The

characterization of the system goes beyond the lack of opioid involvement in that other neurohormonal substrates may act as modulatators of the analgesic effect of CCWS.

2. Intermittent Cold Water Swim (ICWS) analgesia:

As with many other stressors (see above), varying the parameters of the cold swim alters the nature of the analgesic effect produced. Girardot and Holloway (1984a) demonstrated that exposure to an intermittent swim (18 10-sec exposures in a 2 C bath) produces analgesia which was significantly reduced by naltrexone. Naltrexone fails to reduce CCWS analgesia, suggesting that analgesia following ICWS has an opioid mediated component not present in CCWS analgesia. Furthermore, unlike CCWS analgesia, rats made tolerant to morphine develop cross-tolerance to the analgesic effects of ICWS (Girardot & Holloway, 1984b). Finally, whereas daily exposure to ICWS produces adaptation to both the analgesic and hypothermic effects of this swim, chronic CCWS produces adaptation only to analgesic effects (Girardot & Holloway, 1985). It seems, therefore, that ICWS activates an opioid-mediated mechanism to produce analgesia, while CCWS analgesia is mainly mediated by nonopiate-neurohormonal mechanisms. The different

susbtrates mediating the two manipulations can to certain extent be explained by the differences in controllability and predictability of the stimuli: whereas CCWS has a clear begening and end, ICWS is characterized by a repeated pattern of presentation of the stimulus, which allows the organism to expect more stress and therefore prepare for it with a greater analgesia. Neuroendocrinological aspects still need to be investigated for ICWS analgesia and constitute part of the proposed experiments.

3. 2-Deoxy-D-Glucose (2DG) analgesia

Systemic and central administration of the non-metabolizable glucose analog 2DG initiate a number of stress related responses which include analgesia, glucoprivation, hyperphagia, hyperglycemia and peripheral sympatho-adrenal discharge (Bodnar et al, 1978a) Brown, 1962; Engeset & Ritter, 1980; Himsworth, 1970; Wick et al, 1957). The analgesic effect of 2DG appears to be secondary to the stressful, glucoprivic properties of the drug, since the magnitude of 2DG analgesia is reduced after pretreatment with alloxan, which appears to attenuate the central response to glucoprivation (Lubin et al., 1986).

2DG analgesia seems to possess both opioid and nonopioid properties. Evidence for opioid involvement comes from the observation that chronic injections of 2DG produce tolerance to the analgesic effect (Bodnar et al., 1978b). In addition, reciprocal crosstolerance develops between morphine and 2DG analgesia (Spiaggia et al., 1979), as does synergy between subanalgesic doses of 2DG and morphine (Bodnar et al, 1979c). Similar to morphine analgesia, 2DG analgesia is potentiated in hypophysectomized and neonatally MSG-treated rats (Badillo-Martinez et al., 1984), while attenuated in animals with lesions placed in the PAG (Bodnar et al, 1979c). However, 2DG and morphine analgesia also display dissimilar effects, while morphine analgesia is eliminated by naloxone pretreatment, 2DG analgesia remains unaltered (Bodnar et al., 1979c).

Similarities and differences also exist between nonopioid CCWS analgesia and 2DG analgesia. Neuropharmacological manipulations of the dopamine system produce comparable effects on 2DG analgesia as it does on CCWS analgesia: while dopamine receptor stimulation attenuates both responses (Bodnar et al, 1980), dopamine receptor blockade potentiates both form of analgesia (Bodnar & Nicotera, 1982). Increasing norepinephrine

avalability with desipramine produces opposite effects: 2DG analgesia is reduced while CCWS analgesia is potentiated (Bodnar et al., 1985, 1986). This opposite pattern of effects is also observed following pretreatment with muscarinic receptor anatagonists: 2DG analgesia is potentiated, while CCWS analgesia is reduced (Sperber et al., 1986). Likewise, neonatal MSG treatment potentiates 2DG analgesia while reducing CCWS analgesia (Badillo-Martinez et al., 1984). The suggestion of opioid involvement in 2DG analgesia is based on similarities found between morphine and 2DG analgesia. However, it appears that nonopioid substrates are also activated by 2DG injections, since certain phamacological manipulations affect CCWS and 2DG analgesia in a similar fashion. Thus, 2DG analgesia seems to be mediated by multiple mechanisms, some shared with morphine analgesia, and some with CCWS analgesia.

Although parametric, physiological and pharmacological factors that affect SIA have been studied extensively in the young adult male rat, little information has been collected regarding subject variables that may also alter the analgesic response to stress. The age of the organism has been found to influence analgesia following CCWS, 2DG, morphine

administration and hind-paw shock in that older (18 mo.) cohorts display smaller magnitudes of analgesia following these procedures than younger animals (6 mo.) (Kramer et al., 1985; Kramer & Bodnar, 1986a,b; Hamm & Knisely, 1986a; but see Hamm & Knisely, 1986b; Knisely & Hamm 1986). Since a number of other adaptive responses to stress are also affected in the aged organism, this suggests that analgesic mechanisms are part of a constellation of effects altered by aging.

This research identifies another subject-relevant variable, gender, which on the basis of studies of basal pain perception and opiate analgesia would appear to influence an organim's analgesic response to stress; the following section examines this evidence.

Gender Differences in Pain and Analgesic Processes

That gender differences exist in rats with respect to nociceptive stimulation has been well established: female rats display a lower shock threshold than male rats (Pare, 1969; Beatty & Beatty, 1970; Marks et al., 1972). This difference can be eliminated by neonatal androgenization of the female rat followed by steroid replacement therapy as adults or by neonatal castration

of the male rat. However, ovariectomy and/or testosterone administration in adulthood fails to produce an effect, indicating that gonadal hormones may play, mainly, an organizational role. The demasculinizing effect of neonatal castration on pain threshold can be reversed by testosterone administration in adulthood (Beatty & Fessler, 1976). Although it has been argued that gender differences in sensitivity to shock are secondary to gender differences in body weight (Pare, 1969; Marks & Hobbs, 1972), other investigators have demonstrated that gonadectomy performed in the adult rat, which produces marked weight changes in animals of the same sex, does not modify shock thresholds (Fessler & Beatty, 1976). It is interesting to note that gender differences in pain thresholds fail to occur in the tail-flick or hot plate tests (Nagase et al., 1985), indicating nociceptive test specificity. Circulating ovarian hormones also appear to influence sensitivity to shock, since females display threshold fluctuations across the estrous cycle. The period of highest responsivity corresponds to proestrous/estrous, when estrogen levels peak. Further, estrogen administration to ovariectomized females produces a decrease in shock sensitivity (Drury & Gold, 1978). The ventromedial hypothalamus, a sexually

dimorphic structure in the rat (Matsumoto & Arai, 1986), has been implicated in the neural control of gender differences in shock reactivity. Lesions placed in the ventro-medial nucleus lowers jump thresholds in male rats as compared to female rats without altering the latter's reactivity (Dennis, 1972).

Gender effects have also been observed in analgesic processes. Equimolar doses of morphine produce smaller magnitudes of analgesia in female relative to male rats (Badillo-Martinez et al., 1984). This difference may be modulated by gonadal hormones, since castration decreases morphine analgesia in the males, whereas testosterone sensitizes female rats to the analgesic effects of various doses of morphine (Pinsky et al., 1975; Chatterjee et al., 1982). Other laboratories, however, have been unable to replicate these results; procedural differences, as well as differenses in the age of the animals used, drug doses and type of nociceptive test employed may be responsible (Slivko & Stets, 1978; Kasson & George, 1983; Hahn, 1985). Castaration increases the availability of opiate receptors as well as the rate of metabolism of morphine in the rat brain. These alterations are reversed by testosterone replacement and are correlated with changes in morphine analgesic potency

(Hahn & Fishman, 1979; Hahn & Fishman, 1985; Hahn, 1985). Ovarian hormones also modulate morphine-induced analgesia since sensitivity of female rats to morphine vary during the different phases of the estrous cycle with sensitivity being highest during the late diestrous stage (Banerjee et al., 1983). Similarly, some forms of environmentally-induced analgesia are altered across estrous stage in the female rat, and are influenced by ovarian hormones. For example, analgesia induced by vaginal stimulation is markedly increased in ovariectomized females pretreated with estradiol (Crowley et al., 1976; Rothfeld et al, 1985). Further, Ryan and co-workers (1985) found that opioid shock-induced analgesia is greatest during the estrous phase and that estradiol administration enhances analgesia in ovariectomized animals. Since environmentally induced analgesia is thought to be linked to endogenous opiate systems (Akil et al., 1984), it is important to mention that gonadal hormones appear to have a modulatory action upon opioid systems. Levels of pituitary met-enkephalin in the male rat, for example, are twice that of the female rat. Gonadectomy reduces levels of the peptide in male rats, while increasing them in female rats. Estrogen replacement of ovariectomized females reversed the

effect. (Hong et al., 1982; Dupont et al., 1980). Beta-endorphin concentration in the midbrain of male rats is higher than in female rats, and pituitary levels of the peptide are reduced by castration (Lee et al., 1980).

Gender Differences in Response to Stress

Female rats exposed to ether stress show increased levels of plasma corticosterone relative to male rats. In contrast, adrenal corticosterone concentration following stress is greater in the male than in the female rat (Dunn et al., 1972). Further, when rats are placed in a conflict situation, male rats exhibit greater numbers of gastric ulcers than female rats (Sawrey & Long, 1962), suggesting greater sensitivity to the effects of stress. More recently, Menendez-Petterson and co-workers (1984) demonstrated that female rats exposed to stress (immobilization with pain) display a less intense response than males under the same conditions: oxygen uptake in various brain areas, adrenal gland weight and number of ulcers, all indicators of the stress response, were higher in male rats as compared to female rats.

The greater responsivity of males to stress has also been observed in humans. Adrenaline and nordrenaline secretion are considered sensitive indicators of

emotional arousal (Frankenhaeuser and Rissler, 1970). When males and females, between 8 and 22 years of age, were exposed to various stressful situations (e.g., a cognitive task or venipucture), adrenalin output of males increased whereas it remained constant in the females. Subjective experience (measured by the subjecs verbal report) and other physiological measures of stress were, however, similar in males and females (Frankenhaeuse et al., 1976; Johansson, 1972; Levi, 1972). Gender differences in pain perception and pain tolerance have also been reported in human subjects. Procacci and co-workers (1974) found women to be more sensitive than men to radiant heat-induced pain, and Woodrow and co-workers (1972) found that pain tolerance to pressure to the Achilles tendon and is higher among men as compared to women. Similar results were reported by Petrie (1967) and Otto & Dougher (1985) (see also, review by Notermans & Tophoff, 1967). Ovarian hormones may play a role in pain sensitivity in women (Herren, 1933), since pain sensitivity to pressure on the forearm has been observed to increase during the premenstrual period; sensitivity to electrical shock applied to the index finger is also increased during the same period (Tedford et al., 1977). In contrast, neither females receiving

oral contraceptives nor males exhibit cyclical changes. Pain thresholds to radiant heat were also observed to vary over a period of 30 days in young and menopausal woman, but not in women taking oral contraceptives (Procacci et al., 1972).

There is some indication that gender differences exist in relation to effectivness of analgesic medication, howeve, only a few compounds have been tested. The ability to perceive the analgesic effects of metamizole, the peripherally acting analgesic, vs placebo was greater in women than in men, probably reflecting the differential efficacy of the drug (Classen & Netter, 1985).

In summary, gender differences are observed in the response to noxious stimulation and in the adaptive and coping responses to stress. Gender differences have been described at behavioral, neuroendocrine, neuroanatomical and autonomic levels. Further, gonadal hormones appear to play an important role as modulators of these gender effects, and their influence can, in some instances, be traced to either early developmental stages, or to late adult stages. There is some indication that analgesic

processes are also differentially activated in males and females, but systematic study of these effects is still lacking.

Rationale

Gender and gonadal hormones appear to play a role in pain perception and analgesia. Further, the physiological and adaptive responses to stress differ in male and female rats. However, little is known about the influence of gender and of gonadal hormones on opioid and nonopioid pain inhibitory systems which are activated by environmental stressors. The purpose of these series of studies was to determine whether adult male and female rats display differential analgesic and stress-related responses, and if so, to examine whether such responses are modulated by gonadal hormones. Four analgesic manipulations with different physiological and pharmacological profiles were studied. Each yielded information on how different substrates of analgesia can be influenced by gender. The manipulations used were: CCWS, which produces a nonopioid/hormonally mediated analgesia; ICWS, which induces an opioid-mediated analgesia; 2DG administration, which produces analgesia that shares properties with opiate and nonopiate analgesia, and finally, morphine analgesia, the prototypic opiate-mediated analgesia which served as a basis for comparison with the other manipulations and which provided a replication for gender differences

previously described (Badillo-Martinez et al., 1984).

In the first series of experiments (1A), the magnitude of analgesia was compared in adult male and female rats exposed to the aforementioned manipulations. Since the responsivity to activation of opioid or nonopioid pain inhibitory systems might be differentially affected by the type of nociceptive measure, two tests were employed: the tail-flick test, which measures responsivity to heat (D'Amour and Smith, 1941), and the jump test, which measures responsivity to electric shock (Evans, 1961). These two tests presumably also differ in the level of the nervous system at which each acts: jump, supraspinally; tail-flick, spinally. Since body weight can affect pain thresholds (Marks & Hobbs, 1972; Pare, 1969), and since aged-matched adult male and female rats differ markedly in weight, this variable was controlled by including a group of younger (though sexually-mature) male rats of similar weight to adult females.

In examining gender differences in systemic morphine analgesia, it should be noted that although adult females and young males of similar body weight received similar amounts of the drug in Experiment 1A, absorption and metabolism of morphine may be different due to differences on adipose tissue content and liver enzymatic

activity (Hahn, 1985). To control for this difference, a second experiment (1B) was conducted in which groups of adult female and male rats received three equal doses of intracerebroventricular morphine, with subsequent determinations of the magnitude of morphine-induced analgesia.

Previous studies determined that ovarian hormones altered basal pain sensitivity (Drury & Gold, 1978), morphine analgesia (Banerjee et al., 1983) and tail-shock analgesia (Ryan et al, 1985). Therefore, a third experiment (1C) in this series determined whether CCWS analgesia varies thoughout the various phases of the estrus cycle. Although anecdotal observations (Bodnar & Komisaruk, 1984) suggest that the magnitude of CCWS analgesia does not change across the different stages of the cycle, it was important to attempt to replicate these observations. Female rats were tested for CCWS analgesia at the different phases of the estrous cycle, determined by vaginal smears, and the magnitude of analgesia compared.

The results of this set of experiments indicated that there are gender differences in SIA, and therefore, that opioid and/or nonopioid systems may be differentially activated in male and female rats.

Further, the results helped clarify the nature of gender diferences previously described for morphine analgesia. Finally, this set of experiments provided information concerning the role of ovarian hormones in CCWS analgesia.

Given that gonadal hormones modulate basal pain thresholds and morphine analgesia in rats (Beatty & Fessler, 1977; Chatterjee et al, 1982), it was likely that they also played a role in SIA. Therefore, the question of gonadal modulation of SIA was addressed in Experiment 2. For that purpose, adult male and female rats underwent gonadectomy and their analgesic responses to CCWS and ICWS were compared. These two manipulations were chosen because the different parameters of the same stressor (cold water baths) yield pharmacologically-different types of analgesia. The analgesic effects of the swims in gonadectomized rats were compared to the effects observed in sham-oparated controls in two pain tests. In addition, two other factors that might account for any gender-specific or gonadectomy-specific differences in the magnitude of analgesia were monitored: hypothermia following the swim (Bodnar et al., 1978) and activity during the swim (Girardot & Holloway, 1985). To further determine the

involvement and differential activation of opioid and nonopioid systems in male and female rats following stress, the opiate receptor antagonist, naloxone, was administered prior to each swim condition, and analgesic effects were subsequently measured and compared.

Previous reports have indicated that morphine analgesia is decreased with castration but reinstated after testosterone replacement (Banerjee et al., 1983). Testosterone-treated female rats exhibit greater morphine analgesia than vehicle treated animals (Chatterjee et al, 1982). Since testosterone has this effect on opiate-analgesia, it was expected that an opiate-mediated analgesic response like ICWS analgesia would also be decreased in castrated male and intact female rats as compared to control animals. It was unclear what this manipulation would do to nonopiate analgesia such as CCWS analgesia. Thus, the third experiment assessed the effect of steroid replacement therapy on CCWS and ICWS analgesia in both gonadectomized and intact animals. To administer the hormones, daily handling and injecting of the animal was required (see General Method). Since handling is known to cause changes in systems related to the respose to stress (Ader, 1975; Levine et al, 1967; Grota 1975) is was thought important to determine whether daily

injecting of the animal would produce changes in the analgesia induced by stress. Experiment 3A compared the magnitude of CCWS and ICWS analgesia displayed by intact nonhandled animals and those subjected to daily injections.

In Experiment 3B testosterone was administered daily to gonadectomized and intact male and female rats according to a regimen that reinstates sexual behavior in gonadectomized animals (Beyer et al., 1971; Beyer et al., 1973). Since various brain areas, particularly the medial preoptic area and ventromedial hypothalamus (McEwen, 1980) contain high concentrations of receptor sites for estradiol, and since opioid-mediated analgesia has been shown to be modulated by ovarian hormones (Ryan et al., 1986), the effects of daily estradiol administration on CCWS and ICWS analgesia in gonadectomized and intact animals was also examined. If gender differences in SIA are modulated by gonadal hormones, it would be expected that castration might eliminate any differences and testosterone administration would reinstate them.

These series of experiments may help clarify the role of gonadal hormones in the mediation of opioid and nonipioid forms of pain inhibition. Despite limitations in generalizing from acute animal studies to chronic

human pain states, these data may help to identify important gender differences in adaptive responses to stress and may have eventual clinical implications for a more individualized treatment of pain states.

General Method

A. Subjects

Male and female albino Sprague-Dawley rats served as subjects. All animals were the offspring of pregnant females purchased from Charles River Breeding Laboratories (Wilmington, MA) and were raised in the Queens College Vivarium. Different groups of animals were used for experiment 1A, 1B, 1C, 2, and 3. They were housed in same-sex pairs in wire mesh cages and were maintained on a 12 hr light/dark cycle. Ambient temperature will range from 21 to 25 C. Purina rat chow and water were available ad libitum.

B. Stressful Manipulations

1. Continuous Cold Water Swim: The animal was exposed to a forced swim for 3.5 min at a 2 C bath temperature (Bodnar et al, 1979a) in a plastic container (30 cm x 20 cm x 60 cm) to prevent escape. The water level was 25 cm above the floor so that the animal is forced to swim and the body, but not the head, of the animal is under water. After the swim, the animal was blotted dry and put in an individual wire mesh cage lined with dry paper towels.

2. Intermittent Cold Water Swim: The rat received eighteen pairs of 10-sec swims and 10-sec recovery periods over 6 min in a 2 C bath temperature (Girardot & Halloway, 1984a) in the previously-described container. The total time in the water was 3 min. Animals were treated identically after the swim, as indicated for CCWS.

3. 2-deoxy-D-glucose glucoprivation: Rats received 2DG (Sigma Labs: 600 mg/kg body weight in a 300 mg/ml normal saline solution, IP) (Bodnar et al, 1979b); only those animals which displayd abdominal writhing in response to the injection were used.

C. Surgical Procedures

1. Anesthesia: Each animal, in all surgical procedures, was pretreated with chlorpromazine HCL (3 mg/ml normal saline/kg body weight, IP) 10 min prior to anesthesia with Ketamine HCL (Parke-Davis: 100 mg/ml sterile water/kg body weight, IM).

2. Intracerebroventricular (ICV) cannulation (Experiment 1B): A stainless steel 22 gauge guide cannula (Plastic Products) was stereotaxically implanted so that its tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, the

coordinates were 0.5 anterior to the bregma suture, 1.3 mm lateral to the mid-sagital suture and 3.6 mm from the top of the skull. Three stainless steel screws and dental acrylic secured the cannula to the skull. Animals were allowed a minimum of seven days to recover from surgery.

3. Castration: After anesthetization, a midscrotal incision of 1.5 cm was made in male rats, and the testes and epididymal fat were removed. The testicular artery was tied to prevent bleeding and the incision closed with wound clips (Marks & Hobbs, 1972). A miminum of four weeks were allowed for recovery.

4. Ovariectomy: Ovaries, together with ovarian fat, and a small portion of the uterus were removed (to ensure completeness of the procedure) from female rats via a single dorsal incision. The ovarian artery was tied, with muscle and skin sutured in layers. A minimum of four weeks were allowed for recovery.

5. Sham surgery: Animals were anesthetized, approprite incisions made and subsequently closed. No organs were removed.

D. Histological Procedures

1. Determination of cannula placement: Following experimental testing, animals were killed with an

overdose of sodium pentobarbital (200 mg/kg). The brain was removed and left in 10% formalin for a minimum of 8 days. Then, it was blocked and sliced in 40 um coronal sections through the left lateral ventricle and stained with cresyl violet for cell body visualization. Sections were examined under light microscopy (10x magnifcation): only animals with properly placed cannulae will be included in the statistical analysis.

2. Post-mortem examination of accessory sex organs: After an overdose of sodium pentobarbital (200 mg/kg), ventral prostates and seminal vesicles of castrated and sham operated male animals were removed. Excess fat was dissected and the organs emptied of any secretion. The tissue was blotted dry and weighed to the nearest 0.01 g (Beyer et al, 1973). The same procedure was followed with the uteri of ovariectomized and sham operated females (Beyer & Komisaruk, 1971).

E. Nociceptive tests

1. Tail-flick test: This measure (D'Amour and Smith, 1941) utilizes a radiant heat source (IITC Company) mounted 8 cm dorsal and 4-10 cm proximal to the tip of the tail of a lightly restrained animal. The onset of the radiat heat stimulus activates a digital timer which is

stopped by the withdrawal of the animal's tail which exposes a photocell. The mean of the last three of four determinations, at 10 sec intervals, constitutes the value for each baseline session. Tail-flick latency is described as the time elapsed between the onset of the radiant heat stimulus ans thewithdrawal response of the animal. The intensity of the thermal stimulus will be set to produce baseline latencies between 2.5 and 3.5 sec. In order to avoid tissue damage, a trial was automatically terminated if the animal did not respond within 6 (Experiment 1) or 10 (Experiment 2 and 3) sec.

2. Jump Test: This measure of reactivity to electric shock (Evans, 1961) was assessed by placing each unrestrained animal in a 30 cm by 24 cm plexiglass chamber with a floor consisting of 16 grids, 1.5 cm apart. Electric shock was delivered to the feet of the animal by a 60 Hz constant shock generator (BRS/LVE) through a shock scrambler (Campden Instruments). Each trial begun with the animal receiving a 300 msec shock at a current intensity of 0.1 mA. Subsequent shocks were increased in 0.05 mA steps at 10 sec intervals. The jump threshold, for an individual trial, was defined as the lowest of two consecutive intensities at which the animal removed both hind paws simultaneously from the grids. Six

trials were determined during each session with the threshold calculated as the mean intensity of these six trials. Only ascending series of shocks were employed for ethical reasons and because suprathreshold intensities of electric shock produce analgesia (Watkins and Mayer, 1982). Further, it has repeatedly been demonstrated in our laboratory that this method does not result in errors of anticipation or habituation.

F. Core Temperature Determination

Rectal temperatures were obtained by inserting a 6 cm probe of a digital thermometer (Bailey Instruments) which was removed only after stable reading (0.1 C variability) was obtained. Since dissociations of CCWS analgesia and hypothermia have been reported (e.g., Bodnar et al., 1978c; Kramer and Bodnar, 1986), it was important to determine whether the two phenomena were independently controlled in male and female rats.

G. Activity levels

An independent observer, unaware of the gonadal status of the animal measured the time spent in active swimming (Type II behavior; Porsolt et al, 1978) as compared to passive behavior (Type III behavior) in which

the body of the animal is vertically oriented and only its head is kept out of the water. It was of interest to determine whether gender and gonadal status of the animal would affect the time spent in active swimming as opposed to passive behavior, and how activity would affect gender differences in CCWS and ICWS analgesia.

H. Drugs

1. Naloxone HCL (Endo Labs) was dissolved in normal saline (10 mg/ml normal saline) and administered at a subcutaneous 14 mg/kg dose; this dose was selected since opioid tail-shock and ICWS analgesia are best antagonized at this specific dose (Grau et al., 1981; Girardot & Holloway, 1984).

2. Morphine sulfate (MOR) (Pennick) dissolved in normal saline, (5 mg/ml normal saline) was injected at a 5 mg/kg dose (IP). This was the dose used in a previous study reporting gender differences in morphine analgesia (Badillo-Martinez et al., 1984). An ascending series of four doses was used ICV (experiment 1B): 1, 5, and 10 ug, dissolved in normal saline. A volume of 5 ul was injected. These doses were selected since they are known to produce mild to strong analgesia when injected centrally (Watanabe, 1970).

3. Testosterone Propionate (TP) (Sigma Labs) was dissolved in sesame oil (5 mg/ml) and injected SC at a dose of 2 mg/kg body weight. This dose is known to reinstate sexual behavior in castrated males (Beyer et al., 1973).

4. B-Estradiol 3-Benzoate (EB) (Sigma Labs) was dissolved in sesame oil (5 ug/ml). The dose used, 4 ug/kg elicits estrous and vaginal cornification in the ovariectomized female rat (Beyer et al., 1971).

I. Statistical analyses

A split plot analysis of variance (BMDP) was performed to assess main group effects among baseline and experimental conditions and between males and females. The same procedure was used to assess all interaction effects between groups and post-manipulation time course. When statistically significant differences emerged, Dunnett comparisons were made to determine which gorups differed through the time course. Since it was expected that some groups would differ in baseline measurements (Beatty & Beatty, 1970), the data were transformed to difference scores (experimental condition score minus baseline score) to partial out baseline variance and allow analysis of only the magnitude of change. Split

plot analyses of variance were subsequently used on difference scores with Dunn comparisons performed when appropriate.

Experiment I: Gender Differences in CCWS, ICWS, 2DG and Morphine Analgesia.

Experiment IA

Method

Subjects: Three groups of ten rats each served as subjects: adult males (100 days of age, 430-500 g), adult females (100 days of age, 225-265 g), and young, sexually mature males (60 days of age, 230-270 g). The first two groups were matched for age, and the last two groups were matched for weight.

Protocol: Baseline tail-flick latencies and jump thresholds were determined for each animal over four days with tail-flick latencies assessed before jump thresholds to minimize carry-over effects (Kelly, 1982). All rats were then exposed to the following four conditions at 3-7 hr into the light cycle: CCWS, ICWS, MOR (5 mg/kg, SC), and 2DG (600 mg/kg, IP). A minimum of eight days elapsed between conditions to prevent cross-adaptation effects from previous conditions (Bodnar et al, 1978; Bodnar & Sikorszky, 1983; Spiaggia et al., 1979). A within-subject design was employed to conserve on the number of animals and to reduce variability. Tail-flick latencies and jump

thresholds were measured 30, 60, 90 and 120 min after each condition with two additional data points (150 and 180 min) after MOR. All testing was carried out in the same room with ambient temperature between 22 and 25 C.

Results

CCWS Analgesia (Tail-flick Latencies): Significant differences were observed among groups ($F(2,27)= 4.80$, $p<.016$) and among baseline and post-swim values ($F(4,108)= 173.66$, $p<.001$). Relative to baseline latencies, which failed to differ among groups, CCWS significantly increased latencies across the post-swim time course in each group. To assess changes in the magnitude of analgesic effects among groups, difference scores for each post-swim value were derived by subtracting each baseline score from each corresponding post-swim score. This was done because baseline values differed among groups in some conditions (see below). Significant differences were observed across test times ($F(3,81)= 120.62$, $p<.001$). The left panel of Figure 1 illustrates the significantly smaller magnitude of analgesia 60 min following CCWS in both adult female and young male rats relative to adult males.

CCWS Analgesia (Jump Test): Significant differences

Figure 1. Magnitude of analgesia following CCWS in the tail-flick and jump tests in adult males, adult females and young males. Difference scores were calculated by subtracting the baseline score from the experimental condition score. The abscissa represents the time (min) after the swim when the animal was tested. The ordinate represents difference scores in tail-flick latencies (left panel) or jump thresholds (right panel). +Significantly lower than adult males; *Significantly lower than young males; ++Signicantly higher than adult males; Dunn comparisons, $p<.05$.

TAIL-FLICK DIFFERENCE SCORES (sec)
1
2
3
4
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES (mA)
.2
.4
.6
.8
POST-SWIM (min)
Groups (n=10)
• Adult males
▣ Adult females
○ Young males

in jump thresholds were observed among groups $F(2,27)=23.83$, $p<.001$), among baseline and post-swim values ($F(4,108)= 122.69$, $p<.001$) and for the interaction between groups and time course ($F(8,108)= 10.35$, $p<.001$). Female rats displayed significantly lower baseline jump thresholds relative to age-matched and weight-matched males which in turn did not differ from each other. CCWS significantly increased thresholds relative to baseline values 30 and 60 min following CCWS in all groups. The difference score analysis revealed significant differences in the magnitude of CCWS analgesia among groups ($F(2,27)= 5.71$, $p<.009$), across test times ($F(3,81)= 120.54$, $p<.001$) and for the interaction between groups and test times ($F(6,81)= 10.82$, $p<.001$). The right panel of Figure 1 illustrates the significantly smaller magnitude of CCWS analgesia in adult females relative to either young (30 and 60 min) or adult (30 min) male rats. The magnitude of the analgeisc effect on young male rats was significantly greater 30 min following CCWS than in adult males.

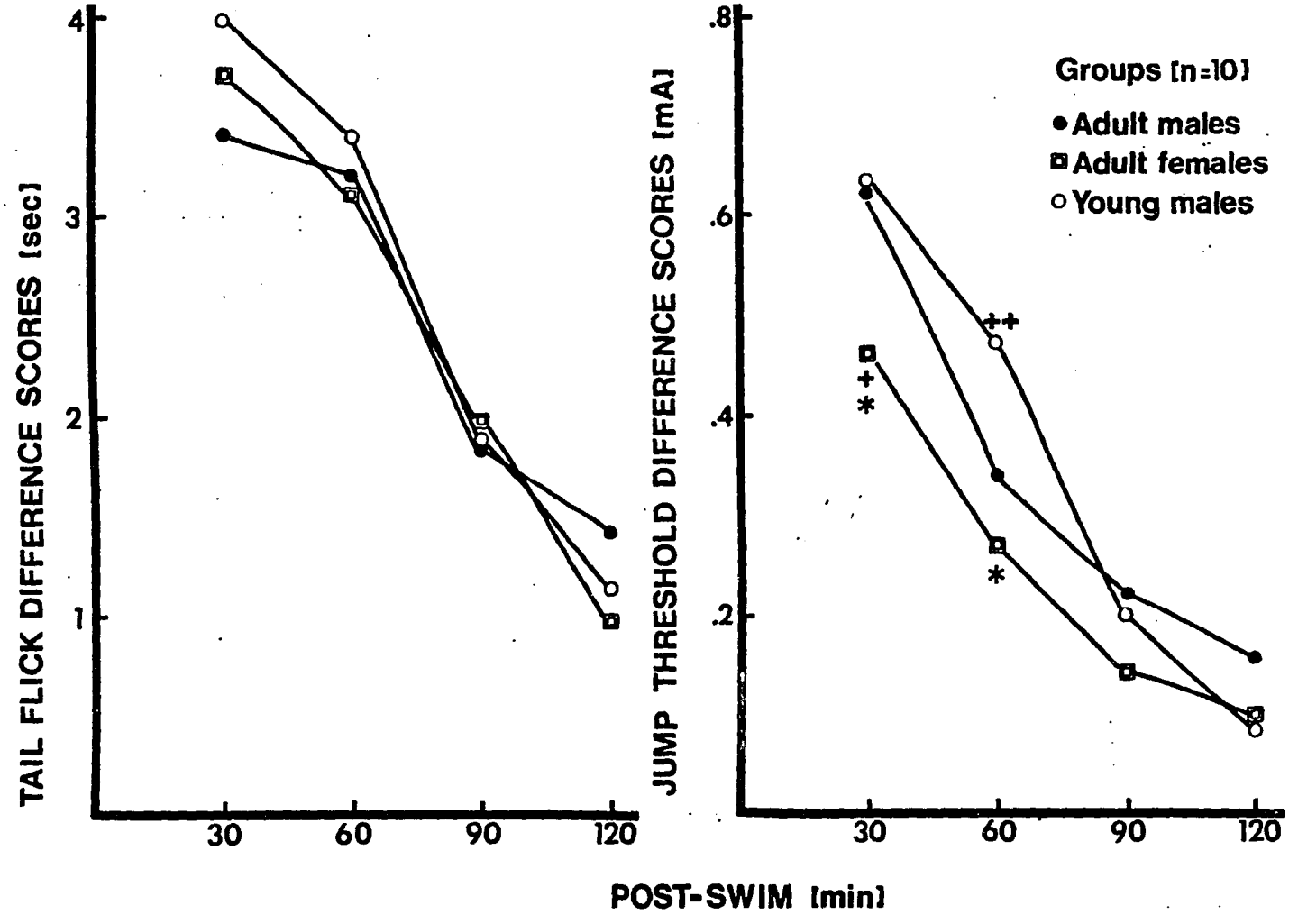
ICWS Analgesia (Tail-Flick Latencies): Significant differences were observed among baseline and post-swim time values ($F(4,108)= 176.43$, $p<.001$). ICWS significantly increased latencies across the post-swim

time course in all groups. The difference score analysis revealed significant differences in the magnitude of ICWS analgesia across test times ($F(3,81)= 95.33$, $p<.001$). The left panel of Figure 2 illustrates the absence of gender differences in the magnitude of ICWS analgesia on the tail-flick test.

ICWS Analgesia (Jump Test): Significant differences were observed among groups ($F(2,27)= 13.34$, $p<.001$), among baseline and post-swim time values ($F(4,108)= 189.64$, $p<.001$) and for the interaction between groups and test times ($F(8,108)= 3.75$, $p<.001$). ICWS significantly increased thresholds for up to 90 min in adult females and young males and across the post-swim time course in adult male rats. The difference score analysis revealed significant differences in the magnitude of ICWS analgesia among groups ($F(2,27)= 3.64$, $p<.04$), across test times ($F(3,81)= 149.82$, $p<.001$) and for the interaction between groups and test times ($F(6,81)= 3.72$, $p<.003$). The right panel of Figure 2 illustrates the significantly smaller magnitude of analgesia following ICWS in adult females relative to both young (30 and 60 min) and adult (30 min) male rats.

Morphine Analgesia (Tail-Flick Test): Significant differences were observed among groups ($F(2,27) =31.61$,

Figure 2. Magnitude of analgesia following ICWS in the tail-flick and jump tests in adult males, adult females and young males. +Significantly lower than adult males; *Significantly lower than young males; ++Signicantly higher than adult males; Dunn comparisons, $p<.05$.


TAIL FLICK DIFFERENCE SCORES [sec]
4
3
2
1
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES [mA]
.8
.6
.4
.2
++
+
*
*
Groups [n=10]
Adult males
Adult females
Young males
30
60
90
120
POST-SWIM [min]

$p<0.001$), among baseline and post-injection values ($F(6,162)=118.22$, $p<0.001$), and for the interaction between groups and test times ($F(12,162)=4.12$ $p<0.001$). Morphine significantly elevated latencies in adult males (3 h), adult females (2.5 h) and young male (1.5 h) rats; peak effects were observed in all groups 1 h following injection. The difference score analysis revealed significant differences in the magnitude of morphine analgesia among groups ($F(2,27)=11.69$ $p<0.001$), across test times ($F(5,135)=41.45$ $p<0.001$) and for the interaction between groups and test times ($F(10,135)=2.94$ $p<0.003$). The left panel of Figure 3 illustrates the significantly smaller magnitude of analgesia in females relative to adult males at 90 and 120 min following morphine administration. However, analgesia in young male rats was significantly smaller than that observed in either adult males (30, 90-180 min) or adult female rats (30, 120, 150 min).

Morphine Analgesia (Jump Test): Significant differences were observed among groups (($F(2,27)=50.10$ $p<0.001$), among baseline and post-injection values ($F(6,12)=59,93$ $p<0.001$) and for the interaction between groups and test times ($F(6,162)=3,70$ $p< 0.001$). Morphine significantly increased thresholds in adult male (3 h),

Figure 3. Magnitude of analgesia following systemic morphine (5 mg/kg) in the tail-flick and jump tests in adult males, adult females and young males. +Significantly lower than adult males; *Significantly lower than young males; **Signicantly lower than adult females; Dunn comparisons, $p<.05$.

TAIL-FLICK DIFFERENCE SCORES [sec]
0
1
2
3
4
30
60
90
120
150
180
JUMP THRESHOLD DIFFERENCE SCORES [mA]
0
.1
.2
30
60
90
120
150
180
POST-INJECTION [min]
Groups [n=10]
ADULT MALES
ADULT FEMALES
YOUNG MALES

in female (2 h), and in young (1.5 h) male rats; peak effects were observed in all groups 1 h following injection. Analgesic magnitude differed significantly among groups ($F(2,27)=11.69$ $p<0.001$), across test times ($F(5,135)=41.45$ $p<0.001$) and for the interaction between groups and test times ($F(10,135)=2.94$ $p<0.003$). The right panel of Figure 3 illustrates the significantly smaller magnitude of analgesia observed 60 and 90 min following morphine in females relative to both groups of males. In turn, the magnitude of analgesia in young males was significantly less than in adult males across the time course, except at 60 min following injection.

2DG analgesia (Tail-flick and Jump Tests): 2DG produced significant analgesia in all groups across the time course on both the tail-flick ($F(4,108)=33.42$, $p<.001$) and jump ($F(4,108)=86.56$, $p<.001$) tests. However, significant differenses failed to occur among groups in the magnitude of analgesia displayed on either test (data not shown).

Experiment IB

Method

Protocol: Six male and six female rats (90 days of age) received a guide cannula aimed at the lateral ventricle. Following determination of four days of baseline tail-flick latencies and jump thresholds, each rat then received four ICV injections: vehicle and 1, 5 and 10 ug of MOR in ascending order. Infusions were administered through a 28 gauge internal cannula (Plastic Products) in a volume of 5 ul of normal saline over a 100 sec interval. Tail-flick latencies and jump thresholds were assessed at 30 min intervals over 3 h following MOR administration. A one-week interval elapsed between each condition to prevent tolerance development (Yaksh et al., 1976).

Results

ICV Morphine Analgesia (Tail-Flick Test): Morphine significantly increased latencies ($F(12,120)=3.30$ $p<0.001$) following the 1 (2 h), 5 (3 h) and 10 (3 h) ug doses; peak effects were observed at 30 min. Difference

score analyses revealed significant differences across test times ($F(4,40)=9.66$, $p<.001$) and doses ($F(2,20)=10.12$, $p<.001$) but not between groups ($F(1,10)=.67$) or for any group interaction. Although the left panel of Figure 4 shows that female rats tended towards greater magnitude of analgesia than male rats following the 5 and 10 ug morphine doses, these effects failed to achieve statistical significance.

ICV Morphine Analgesia (Jump Test): Morphine significantly elevated ($F(3,30)=15.49$, $p<.001$) thresholds in both groups for 3 h following all three morphine doses; peak effects were observed after 30 min. Significant differences in the magnitude of analgesia were observed among doses ($F(2,20)=10.48$, $p<.001$) and test times ($F(4,40)=19.43$, $p<.001$), but not between groups ($F(1,10)=.50$) or any of the interactions. Again, female rats tended to display a greater magnitude of analgesia following the 5 and 10 ug morphine doses, but the differences failed to acheive statistical significance (Figure 4, right panel).

Figure 4. Magnitude of analgesia following ICV morphine administration (0, 1, 5 and 10 ug) in male and female rats. Significant differences failed to occurr between the two groups. The bars indicate SEM.

TAIL-FLICK DIFFERENCE SCORES [sec]
JUMP THRESHOLD DIFFERENCE SCORES [mA]
POST-INJECTION [min]
10µg
5µg
MALES (N=6)
FEMALES (N=6)

Experiment IC

Method

Protocol: Baseline latencies and jump thresholds were determined for 10 naive female rats (60 days of age) over seven days. Daily vaginal smears were taken from each animal to determine the phase of the estrous cycle on each experimental day. Tail-flick latencies and jump thresholds were determined immediately prior to and 30, 60, 90 and 120 min after CCWS. This procedure was repeated at weekly intervals until each rat was tested during the proestrous, estrous and diaestrous-metestrous phases of the estrous cycle. Each rat was tested only once during each phase, and the order of testing across phases was random across rats.

Results

CCWS Analgesia (Tail-Flick Test): CCWS significantly increased latencies ($F(4,36)= 79.30$, $p<.001$) for up to 60 min during the proestrous and metestrous-diestrous phases, and for up to 90 min during the estrous phase. The left panel of Figure 5 illustrates the failure to

observe differences among estrous phases in the magnitude of CCWS analgesia on the tail-flick test.

CCWS Analgesia (Jump Test): CCWS significantly increased thresholds ($F(4,36)= 60.26$, $p<.001$) across the post-swim time course during the proestrous and metestrous-diestrous phases, and for up to 90 min during the estrous phase. The right panel of Figure 5 illustrates the failure to observe differences among estrous phases in the magnitude of CCWS analgesia on the jump test.

Discussion

The first experiment demonstrated that: a) adult female rats display significantly less CCWS analgesia than either age- or weight-matched male rats on the jump test, and were less analgesic than age-matched males on the tail-flick test; b) adult female rats display significantly less ICWS analgesia than age- and weight-matched male rats on the jump test but not the tail-flick test; c) analgesia induced by systemic morphine was less in female rats than in age- and weight-matched male rats on the jump test; d) central morphine did not differentially affect male and female

Figure 5. Magnitude of analgesia following CCWS on the tail-flick and jump tests across the estrous cycle of adult female rats. Difference scores failed to differ as a function of estrous phase.

TAIL-FLICK DIFFERENCE SCORES [sec]
1
2
3
4
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES [mA]
.2
.4
.6
.8
POST-SWIM [min]
Groups [n=10]
● Proestrous
○ Metestrous-Diestrous
□ Estrous

rats; e) no gender differences were observed in 2DG analgesic effects; and f) CCWS analgesia failed to vary in females across the estrous cycle.

CCWS and ICWS analgesia: The present data indicate that adult female rats display significantly less CCWS and ICWS analgesia than age-matched adult male rats. Since the thickness of subcutaneous adipose tissue affect sensitivity to cold (Algeri et al., 1982), it is possible that the greater weight of male rats might account for this result. However, the gender differences could not be attributed to weight differences since adult female rats also displayed significantly less CCWS and ICWS analgesia on the jump test than younger, weight-matched male rats. In contrast to the gender differences found for the jump test, the tail-flick test failed to differentiate between males and females in ICWS analgesia, and only indicated a difference between age-matched males and females for CCWS analgesia. The different gender-specific patterns observed in the magnitude of CCWS and ICWS analgesia on the tail-flick and jump tests may be accounted for by several factors. The relative inability to observe consistent gender-related changes in CCWS and ICWS analgesia on the tail-flick test may be related to the use of a 6-sec cutoff criterion to avoid tissue damage.

The majority of the animals in each group, especially after ICWS, reached this ceiling criterion, and therefore any possible differences above this point could not be assessed. However, these data may also reflect the different underlying nature of each pain test. The tail-flick test is mediated by spinal and supraspinal mechanisms (D'Amour & Smith, 1941; Hayes et al, 1978; Grossman et al, 1982), whereas the jump test is supraspinally mediated (Evans, 1961). This implies than gender differences may be mediated by supraspinal structures.

Although baseline tail-flick latencies failed to differ among groups, adult female rats displayed significantly lower baseline jump thresholds than either adult or young male rats. This agrees with previous reports of hyperresponsiveness to electric shock in female rats (Pare, 1969; Beaty & Beaty, 1970; Marks et al., 1972). However, the gender-specific alterations in CCWS and ICWS analgesia occurred independently of gender differences in baseline pain thresholds since the latter effects were factored out of the difference-score analysis. Gender differences were observed in opioid-mediated ICWS analgesia and nonopioid-mediated CCWS analgesia, indicating that opioid mediation is not

fundamental for the expression of gender differences in analgesia.

Morphine analgesia: The gender differences observed following morphine administration are consistent with previous reports (Badillo-Martinez et al., 1984): female rats displayed a smaller magnitude of analgesia than age-matched or weight-matched male rats on the jump test. Badillo-Martinez and co-workers (1984) reported gender differences in morphine analgesia using the hot plate test; the present data extend these findings to the jump test. The gender-effect upon morphine analgesia is most clearly observed at morphine's peak effect (60 min) given the similar magnitudes of analgesia displayed by both young and adult males which were significantly higher than that displayed by adult females. The subsequent loss of analgesia in the young males may reflect differences in the absolute amount of drug administered to adult and young males, to differences in metabolism, or to differences in the pattern of utilization of the drug by the brain between groups (Cicero et al., 1986). The failure to find gender-related differences in morphine analgesia in the tail-flick test again may be due to the dichotomy between supraspinal and spinal characteristics

of the tests used since responsiveness on the hot plate test is also supraspinally-mediated. However, the data fron the tail-flick test may also suggest that the actual amount of administered drug is integral for the pattern of results, that is, adult males are more analgesic than young male rats because they receive more morphine. When comparing weight-matched male rats with female rats, female rats are significantly more analgesic than weight-matched male rats despite similar amounts of morphine administered. Thus, while morphine analgesia on the jump test is greater in male than in female rats, the opposite is true for tail-flick latencies. This prompted us to equalize morphine dosage in adult male and female rats by administering morphine centrally.

In this latter condition, gender differences were not found, although, consistent with the results in the tail flick test following systemic morphine administration, male rats tended to display less analgesia than female rats. The failure to observe gender effects following ICV morphine may be due to either increased variability in males or possible ceiling effects induced by the higher morphine doses. These opposing results may be explained by differential availability of the drug to relevant brain structures

influenced by factors not related to the direct effect of morphine. For instance, the pharmacokinetics may differ between male and female rats, thereby producing different amounts of available drug in the brain. Hence, although the number of brain opiate receptors may be similar in males and females, the actual number of activated sites may depend on drug bioavailability. In this regard, the liver is more efficient in male rats to induce N-demethylation of morphine, a reaction intimately involved in the pharmacological activity of opiates (Axelrod, 1956; Defrawy & Mannering, 1974). However, the opposite takes place in the brain, in which greater N-demethylation occurs in females (Hahn et al., 1977). Castration reduces enzymatic activity of the liver in males but not in the brain; ovariectomy fails to exert any effect. These data suggest that systemic opiate analgesic potency is mediated by liver activity, and that the observed gender differences mimic the outcome of this differential activity. A similar peripheral mechanism has been hypothezised for the potentiation of opiate effects by adrenalectomy: morphine potency is increased by adrenalectomy following systemic but not ICV administration (Holaday et al., 1977).

2DG Analgesia: The failure to find gender differences for 2DG analgesia is important because it demonstrates that gender-induced alterations in nociception are selective and depend upon the analgesic manipulation. Whereas gender differences are observed in nonopioid-mediated CCWS analgesia and opioid-mediated ICWS analgesia, 2DG analgesia, mediated by both opioid and nonopioid mechanisms (Bodnar et al., 1978; Spiaggia et al., 1979; Bodnar et al., 1980; Bodnar & Nicotera 1982) seems not to be influenced by gender. Therefore, it appears that the ability of a stressor to activate either opioid or nonopioid systems is not the only important factor, since it has been demonstrated that different endocrine and neurochemical systems in the brain respond specifically and in discrete patterns to different stressors (Mason, 1971). Cold exposure produces larger increases in prolactin levels and elevates levels of cyclic GMP in various brain areas as compared to forced immobilization (Lenox et al., 1980). These data, taken together with gender differences in adrenocortical response to stress (Dunn et al., 1972) suggest that gender differences occur at different functional levels of the organism. For example, crowding increases adrenal weights more in male rats than in female rats (Christian,

1963). Corticosteroid responses following ether stress also reveal gender differences: this response is initially more intense in females but then adapts quicker (Dunn et al., 1972; Kitay, 1963; Coyne & Kitay, 1969).

CCWS Analgesia and the estrous cycle: The failure to observe changes in CCWS analgesia on either the tail-flick or jump tests across the estrous cycle supports previous data from our laboratory (Bodnar & Komosaruk, 1984) and contrasts with the differential sensitivity of various estrous cycle phases to morphine analgesia (Banerjee et al., 1983) and shock analgesia (Bryan et al., 1985). Morphine analgesia was found to be most sensitive during the late diestrous phase. Analgesia induced by inescapable tail shocks was most pronounced during the estrous phase, and least pronounced during the metestrous phase. That the estrous cycle influences some, but not all forms of analgesia may be attributable to opioid mediation of morphine and shock analgesia (Banerjee et al., 1983; Akil et al; 1984) and the nonopioid nature of CCWS analgesia (Bodnar et al., 1978; Bodnar et al., 1980; Kirchgessner et al., 1982). The interaction between estrous influences and opioid forms of analgesia may be attributable to either the rises in

pituitary and plasma levels and/or decreases in hypothalamic levels of beta-endorphin during estrous (Lee et al., 1980). Further studies examining the role of estrous influences on other opioid-mediated analgesic responses such as ICWS are necessary to confirm this hypothesis. The failure to observe differences in either baseline tail-flick latencies or jump thresholds over the estrous cycle agrees with a previous report indicating estrous-induced fluctuations in flinch, but not jump thresholds (Drury & Gold, 1978).

In summary, gender differences in CCWS, ICWS and systemic morphine analgesia were observed, with females displaying a smaller magnitude of analgesia than either age- or weight-matched male controls. These effects are test specific since they are observed in the jump but not in the tail-flick test. Male and female rats failed to differ in the magnitude of ICV morphine or 2DG analgesia. CCWS analgesia failed to vary as a function of the estrous cycle.

The existence of gender differences in analgesic proceses poses the question of possible modulation of SIA by gonadal hormones. Since some evidence exists on the involvement of gonadal steroids in morphine analgesia, it is of interest to determine whether gonadal status of the

animal alters the gender differences found in CCWS and ICWS analgesia. Other factors are known to be influenced by gender and may in turn affect analgesic magnitude, such as temperature regulation, activity levels, etc. Thus, carefully monitoring of these factor is important. The second experiment had the purpose of investigating the relationship between gonadal status of the animals and analgesic magnitude in male and female rats, while controlling certain variables that could also influence analgesic magnitude.

Experiment II: Gonadal Modulation of Gender Differences in CCWS and ICWS Analgesia.

Method

Protocol: Baseline tail-flick latencies and jump thresholds were assessed over five days in 17 male and 17 female rats (90-100 days of age). The male rats were either castrated (n=10) or received sham surgery (n=7) and were matched for body weight. The female rats were either ovariectomized (n=10) or received sham surgery (n=7). The two groups were also matched for body weight before surgery. All rats were undisturbed for four weeks to allow for surgical recovery and development of weight differences (Marks & Hobbs, 1972). Post-operative baseline latencies and thresholds were redetermined over four days. All animals were exposed to five conditions: a) no swim, b) CCWS, c)ICWS, d) naloxone 14mg/kg SC)/CCWS, e) naloxone (14 mg/kg SC)/ICWS. The second and third conditions and the fourth and fifth conditions were counterbalanced within groups. While one to three days elapsed between the first two conditions, one week elapsed between subsequent conditions. Tail-flick latencies, jump thresholds and core body temperatures were assessed 30, 60, 90 and 120 min after each condition; core body temperature was also measured

immediately after each condition. Activity levels during swim conditions were also assessed for each animal by an independent observer who was unaware of the gonadal status of the animals. Naloxone was administered five minutes prior to CCWS or ICWS. Body weight was monitored throughout the entire experiment, and all testing occurred between 3 to 8 hours into the light cycle, at temperatures between 22 and 25 C. After completion of the experiment, all animals were killed with an overdose of sodium pentobarbital, and their accessory sex organs dissected and weighed.

Results

Body Weight: Ovariectomized rats (+38 g) gained significantly more weight ($F(1,15)=33.50$, $p<.001$) than female sham controls (+8 g). In contrast, although castrated male rats (+1 g) gained less weight than male sham controls (+13 g) these diferences were not stastistically significant ($F(1,15)=1.05$) (Table 1).

Accessory sex organs: Table 2 shows the significant reduction in the weight of in prostate and seminal vesicles in castrated males, and of uterus in ovariectomized females.

Baseline pain thresholds: Table 3 indicates that

Table 1. Body Weight (g, SEM) of Male and Female Rats Before and After Gonadectomy.

GROUP	Pre-Operative	Post-operative	Change
Sham-treated Males (7)	525.1 (11.4)	538.4 (14.7)	+13
Castrated Males (10)	529.0 (10.4)	530.1 (18.2)	+1
Sham-treated Females (7)	270.1 (10.4)	278.1 (8.0)	+8
Ovariectomized Females (10)	283.8 (7.9)	321.7 (9.9)	+38*

Note: The asterisk denotes a significant increase in body weight gain in ovariectomized relative to sham-treated females (Dunnett comparisons, $p<.05$).

Table 2. Alterations in Accesory Gonadal Tissue Weight (mg, SEM) Following Gonadectomy.

TISSUE	Sham-treated	Gonadectomized
A. Males		
Prostate	585 (70)	46 (7) *
Seminal Vesicles	815 (87)	148 (10) *
B. Females		
Uterus	546 (46)	124 (6) *

Note: Significant differences in accessory gonadal tissue between sham-treated and gonadectomized groups were observed for the prostate ($F(1,4)= 67.6$, $p<.001$) and the seminal vesicles ($F(1,15)= 73.6$, $p<.001$) in males and for the uterus ($F(1,14)= 106.6$ $p<.001$) in females.

Table 3. Pre- and Post-gonadectomy Base-line Tail-Flick Latencies (sec, SEM) and Jump Thresholds (mA, SEM) of Male and Female Rats.

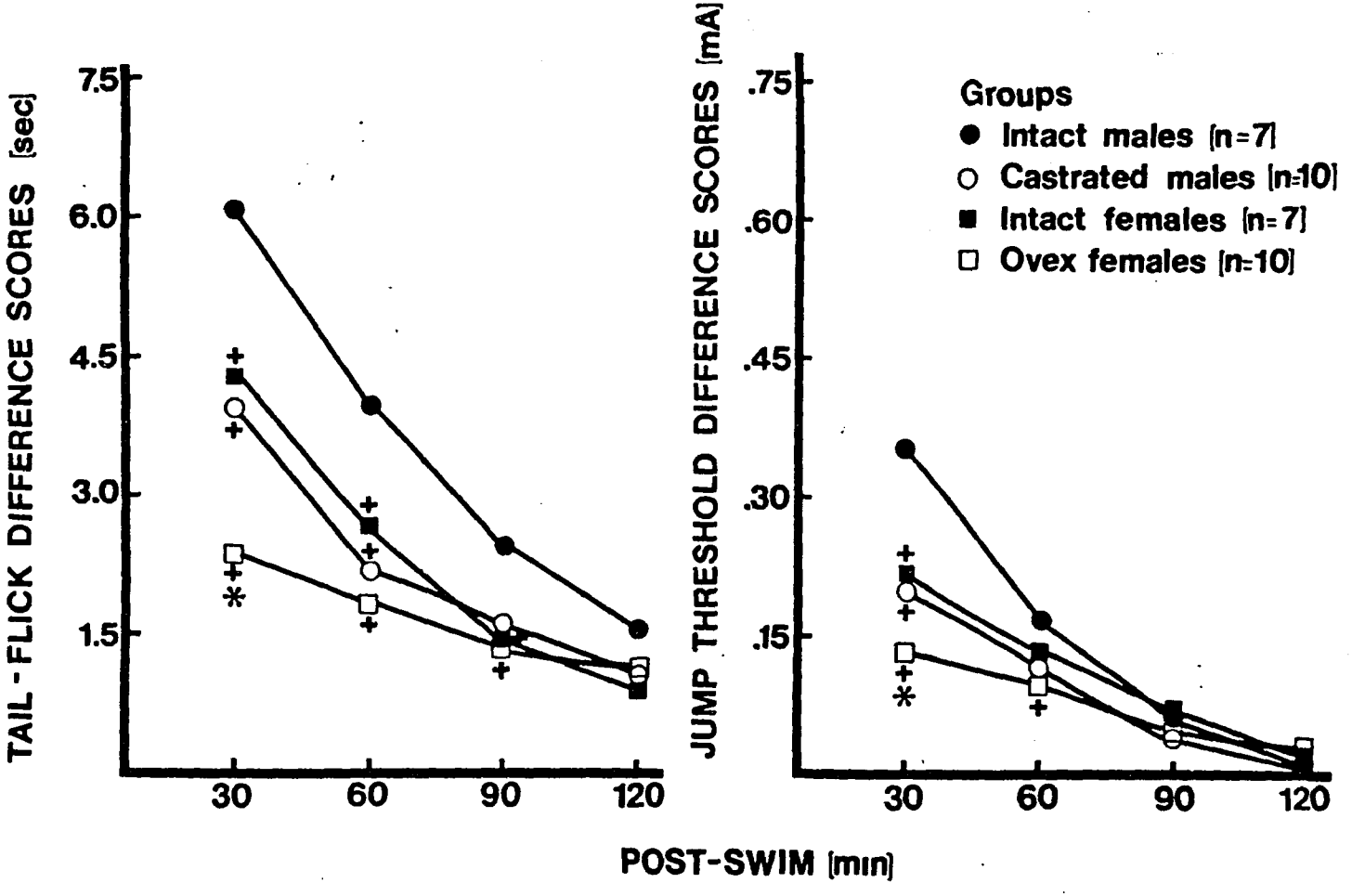
GROUP	Pre-Operative	Post-Operative	Change
A. Tail-Flick Latencies			
Sham-Treated Males	2.86 (.14)	2.73 (.14)	-.13
Castrated Males	2.87 (.12)	2.90 (.08)	+.03
Sham-Treated Females	3.39 (.10)+	3.41 (.10)+	+.02
Ovx Females	3.20 (.06)+	3.21 (.12)+	+.01
B. Jump Thresholds			
Sham-Treated Males	.405 (.008)	.374 (.020)	-.031
Castrated Males	.432 (.015)	.398 (.020)	-.034
Sham-Treated Females	.296 (.008)	.273 (.012)+	-.023
Ovx Females	.304 (.011)	.289 (.009)+	-.015

Note: The crosses denote the significantly greater baseline latencies in females than in males irrespective of surgical condition and the significantly lower baseline thresholds in females than in males irrespective of surgical condition (Dunnett comparisons, $p<.05$).

female rats had significantly higher baseline tail-flick latencies than male rats ($F(3,30)=12.63$, $p<.001$); gonadectomy failed to alter tail-flick latencies ($F(3,30)=.22$, $p>0.05$). The opposite pattern was observed for jump thresholds: females had significantly lower thresholds than males ($F(3,30)=29.06$, $p<.001$). Again, gonadectomy failed to alter jump thresholds irrespective of gender ($F(3,30)=.76$).

CCWS Analgesia (Tail-Flick Latencies): CCWS significantly increased latencies across the time course in all groups. Signficant differences were observed between groups ($F(3,30)=7.83$, $p<.001$), across the no-swim and post-swim time course, ($F(3,90)$ 233.98, $p<0.001$) and for the interaction between groups and time course ($F(6,180)=2.57$, $p<.001$). The difference score analysis revealed significant differences among groups ($F(3,30)=11.75$, $p<.001$), across test times ($F(3,90)=80,85$, $p<.001$) and for the interaction between groups and test times ($F(9,90)=4.29$, $p<.001$). The left panel of Figure 6 indicates the significantly greater magnitude of CCWS analgesia in intact male rats relative to intact females for up to 90 min following the swim. Both castrated male rats and ovariectomized female rats displayed significant decreases in analgesia relative to

Figure 6. Magnitude of CCWS analgesia in the tail-flick and jump tests in sham-operated male rats, castrated male rats, sham-operated female rats, and ovariectomized female rats. +Significantly different from sham-operated male rats; *Significantly different from sham-operated female rats; Dunn comparisons, $p<.05$.


TAIL - FLICK DIFFERENCE SCORES [sec]
7.5
6.0
4.5
3.0
1.5
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES [mA]
.75
.60
.45
.30
.15
Groups
Intact males [n=7]
Castrated males [n=10]
Intact females [n=7]
Ovex females [n=10]
POST-SWIM [min]

their respective sham controls at 30 min after CCWS. In fact, castrated males and intact females failed to differ from each other in the magnitude of CCWS analgesia.

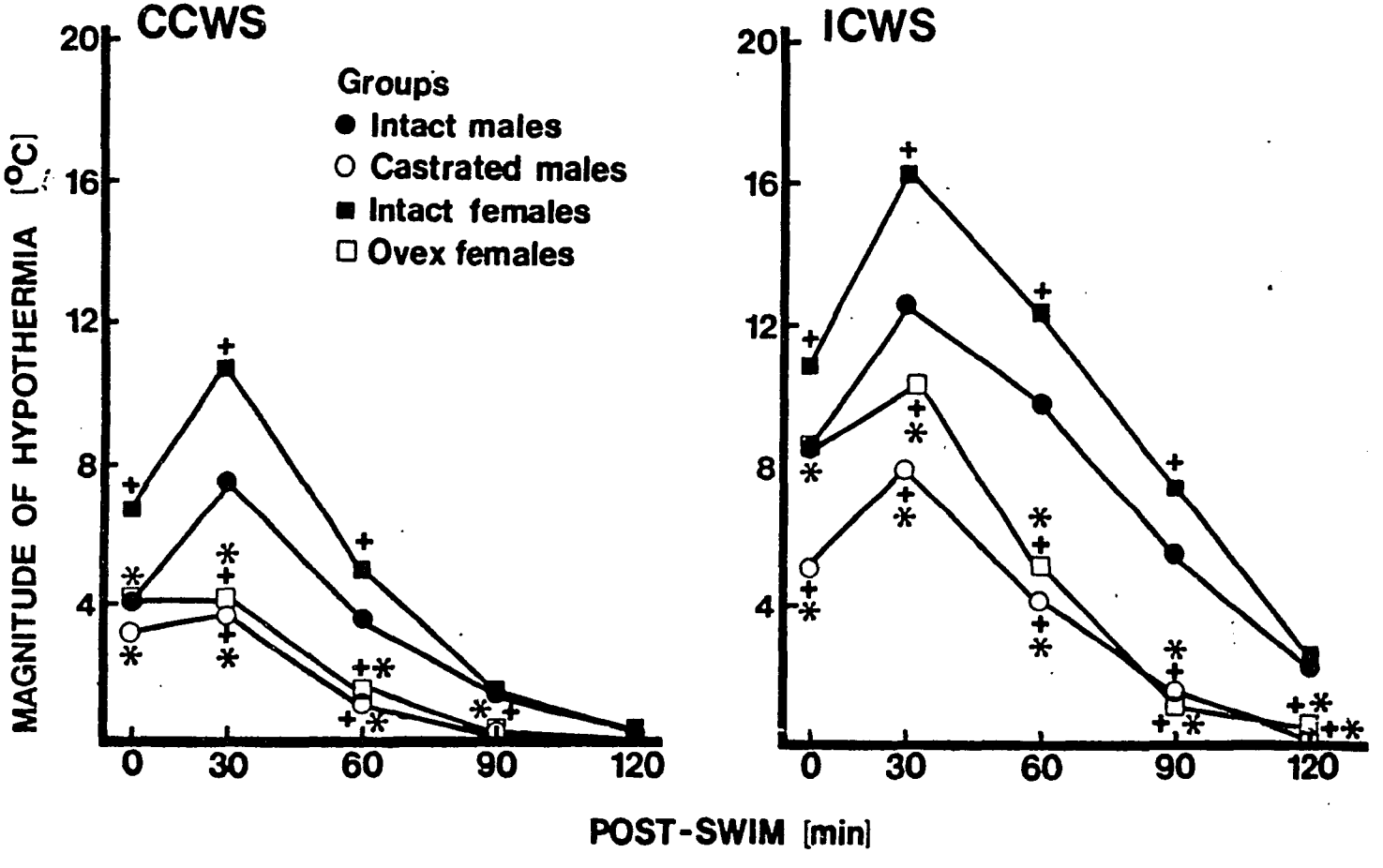
CCWS Analgesia (Jump Test): Significant differences were observed among groups ($F(3,30)= 10.37$, $p<0.001$), across the time course ($F(3,90)=67.43$, $p<0.001$) and for the interaction between groups and test times ($F(6,180)=46.57$, $p<0.001$). CCWS significantly increased jump thresholds in all groups. Analgesic magnitude differed significantly across test times ($F(3,90)=78.67$, $p<.001$), and for the interaction between groups and test times ($F(9,90)=4.66$, $p<.001$), but not among groups ($F(3,30)=2.67$). The right panel of Figure 6 indicates the significantly smaller magnitude of analgesia in intact females than in intact males 30 min after CCWS. Gonadectomy significantly decreased analgesic magnitude relative to respective intact controls at 30 min following CCWS. As was the case for the tail-flick test, castrated male rats and intact female rats failed to differ in the magnitude of CCWS analgesia.

CCWS Hypothermia: Significant differences were observed among groups ($F(3,30)=16,39$, $p<0.001$), among pre-swim and post-swim conditions ($F(10,300)=122.20$, $p<0.001$) and for the interaction between groups and time

course ($F(30,300)= 7.80$, $p<0.001$). Significant hypothermia persisted for up to 60 min in intact male and female rats, and for up to 30 min in gonadectomized rats. Thus, hypothermic duration was shorter than analgesic duration in all groups. Significant differences in the magnitude of hypothermia were observed among groups ($F(3,30)=13.11$, $p<.001$), across test times ($F(4,120)=258.41$, $p<0.001$), and for the interaction between groups and test times ($F(4,120)= 5.12$, $p<0.001$). The left panel of Figure 7 shows that peak hypothermia occurred 30 min following CCWS in all groups. Intact female rats were significantly more hypothermic than intact males, castrated males, and ovariectomized females for up to 60 min following CCWS. Gonadectomy significantly decreased CCWS hypothermia in that castrated males were less hypothermic than intact males at 30 and 60 min following CCWS and, ovariectomized females were less hypothermic than intact females for up to 90 min following CCWS.

ICWS analgesia (Tail-Flick Test): Significant analgesia was observed across the time course ($F(3,90)=118.11$, $p<.001$), and for the interaction between group and the time course ($F(9,90)=2.70$, $p<.008$) but not among groups ($F(3,30)=1.71$). Significant differences in

Figure 7. Magnitude of CCWS and ICWS hypothermia in sham-operated male rats, castrated male rats, sham-operated female rats, and ovariectomized female rats. +Significantly different from sham-operated male rats; *Significantly different from sham-operated female rats; Dunn comparisons, $p<.05$.


CCWS
ICWS
Groups
Intact males
Castrated males
Intact females
Ovex females
MAGNITUDE OF HYPOTHERMIA [°C]
20
16
12
8
4
0
30
60
90
120
POST-SWIM [min]

analgesic magnitude were observed across the time course ($F(3,90)=93.65$, $p<.001$) but not among groups ($F(3,30)=2.53$) or for the interaction between groups and time course ($F(9.90)=93.65$). The left panel of Figure 8 shows that ovariectomized females displayed significantly less analgesia than either intact males at 30 min following ICWS, or intact females at 60 min after ICWS.

ICWS analgesia (Jump Test): Significant differences were observed among groups, ($F(3,30)=15.17$, $p>0.001$), across test times ($F(3,90)= 188.88$, $p<0.001$) and for all interaction effects ($F(6,180)=77.24$, $p<0.001$). ICWS significantly increased jump thresholds in all groups across the time course. Significant differences in analgesic magnitude were observed among groups ($F(3,30)=11.18$, $p<0.001$), across test times ($F(3,90)= 200.55$, $p<0.001$) and for the interaction between groups and test times ($F(9,90)=7.04$, $p<0.001$). The right panel of Figure 8 indicates the significantly smaller analgesic effect in intact females relative to intact male rats for up to 90 min following ICWS. Gonadectomy decreased the magnitude of ICWS analgesia in castrated males for up to 60 min and in ovariectomized females for up to 90 min relative to intact controls. Again analgesic magnitude failed to differ between castrated males and intact

Figure 8. Magnitude of ICWS analgesia in the tail-flick and jump tests in sham-operated male rats, castrated male rats, sham-operated female rats, and ovariectomized female rats. +Significantly different from sham-operated male rats; *Significantly different from sham-operated female rats; Dunn comparisons, $p<.05$.

TAIL- FLICK DIFFERENCE SCORES [sec]
1.5
3.0
4.5
6.0
7.5
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES [mA]
.15
.30
.45
.60
.75
POST-SWIM [min]
Group
● Intact males [n=7]
○ Castrated males [n=10]
■ Intact females [n=7]
□ Ovex females [n=10]

females.

ICWS Hypothermia: Significant differences were observed among groups ($F(3,30)=21.45$, $p<0.001$), across test times ($F(10,300)=200.93$, $p<0.001$) and for the interaction between groups and test times ($F(30,300)=5.73$, $p<0.001$). Hypothermia was observed in intact male and female rats for up to 90 min and in gonadectomized rats for up to 60 min following ICWS. Significant differences in hypothermic magnitude were observed among groups ($F(3,30)=19.00$, $p<0.001$), across test times ($F(4,120)= 493.25$, $p<0,001$) and for the interaction between groups and time course ($F(12,120)=10.51$, $p<0.001$). The right panel of Figure 7 indicates the significantly greater hypothermia in intact females relative to either intact males (up to 90 min) or both gonadectomized groups (entire post-swim time course). Intact male rats were significantly more hypothermic than castrated males and ovariectomized females for up to 120 min following ICWS.

Activity: Table 4 shows that while activity levels during CCWS were not different in males and females ($F(3,30)= .63$), activity levels during ICWS were significantly higher in gonadectomized than in sham control rats regardless of gender ($F(3,30)=6.30$, $p<.002$).

Table 4. Activity Levels (sec, SEM) displayed by Male and Female Rats During CCWS and ICWS Following Gonadectomy or Sham Surgery.

	ACTIVITY (sec)	
GROUP	CCWS	ICWS
Sham-Treated Males	138.9 (14.1)	138.1 (5.6)
Castrated Males	137.1 (9.0)	159.1 (2.4)+
Sham-Treated Females	126.6 (9.5)	129.1 (6.3)
Ovariectomized Females	130.5 (10.4)	154.8 (4.7)+

Note: The crosses denote the gonadectomy induced increases on activity during ICWS (Dunn comparisons, $p<.05$)

CCWS analgesia (Naloxone Effects in Intact Rats): Figure 9 illustrates the effects of naloxone on the magnitude of CCWS analgesia 30 min following the swim on the tail-flick (second panel) and jump (fourth panel) test. Naloxone pretreatment failed to significantly reduce the magnitude of CCWS analgesia in either the tail-flick ($F(3,36)=.39$) or jump ($F(3,36)=1.14$) tests relative to control values across the time course. Naloxone reduced analgesic latencies by 19% and 11% in intact males and females respectively, and reduced analgesic jump thresholds by 21% and 11% in intact males and females respectively. However, naloxone pretreatment eliminated the significant gender differences observed for CCWS analgesia on the tail-flick and jump tests.

ICWS analgesia (Naloxone Effects in Intact Rats): The first and third panels of Figure 9 illustrate naloxone-induced alterations in ICWS analgesia 30 min after ICWS ($F(4,48)=347.4$, $p<.001$). Naloxone reduced ICWS analgesia by 45% on the tail-flick test and reduced ICWS analgesia by 37% on the jump test in intact males. In contrast, naloxone failed to affect the magnitude of ICWS analgesia in intact females on either the tail-flick (3% decrease) or jump (9% decrease) test. Thus, naloxone eliminated gender differences in the magnitude of ICWS

Figure 9. Naloxone (14 mg/kg) pretreatment effects on CCWS and ICWS analgesic magnitude in sham-operated male and female rats 30 min after the swim. *Significantly different from control; Dunn comparisons, $p<.05$.

TAIL-FLICK LATENCY DIFFERENCE SCORES (SEC)
0
1.5
3.0
4.5
6.0
7.5
9.0
ICWS
MALES
FEMALES
CCWS
MALES
FEMALES
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.15
.30
.45
.60
.75
.90
ICWS
MALES
FEMALES
CCWS
MALES
FEMALES
CONTROL
NAL 14 mg/kg

analgesia.

Naloxone Effects Upon Hypothermia and Activity (Intact Rats): Naloxone failed to alter the magnitude of CCWS or ICWS hypothermia (Figure 10). Naloxone also failed to affect activity during CCWS ($F(1,12)=.22$) or ICWS ($F(1,12)= .47$) (data not shown).

CCWS Analgesia (Naloxone Effects in Gonadectomized Rats): Naloxone pretreatment significantly altered analgesic magnitude on the tail-flick ($F(3,54)=4.51$, $p<.01$) and jump ($F(3,54)=7.17$, $p<.001$) tests. The second panel of Figure 11 illustrates the significant reductions in the magnitude of analgesia on the tail-flick test (26% decrease) in castrated males but not in ovariectomized females (20% increase) 30 min following CCWS. Naloxone reduced the magnitude of CCWS analgesia in castrated male rats (49% decrease) and ovariectomized females (57% decrease) on the jump test (see fourth panel of Figure 11).

ICWS analgesia (Naloxone Effects in Gonadectomized Rats): Naloxone pretreatment significantly altered analgesic magnitude on the tail-flick ($F(3,54)=5.53$, $p<.001$) and jump ($F(3,54)=8.32$, $p<.001$) tests. Naloxone produced a similar pattern of effects upon ICWS analgesia

Figure 10. Effects of naloxone (14 mg/kg) pretreatment on CCWS and ICWS hypothermic magnitude in sham-operated male and female rats. *Significantly different from intact males; Dunn comparisons, $p<.05$.

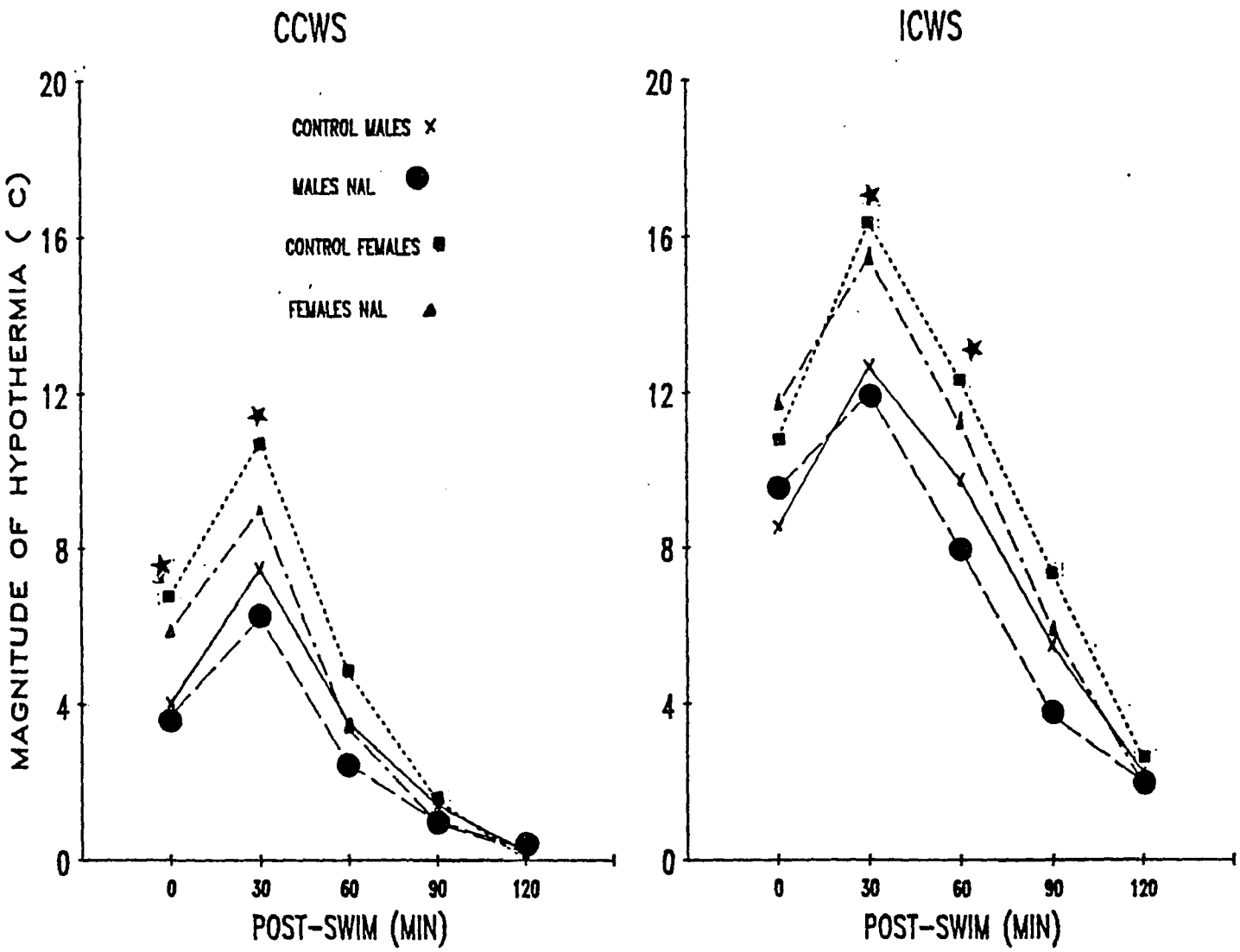
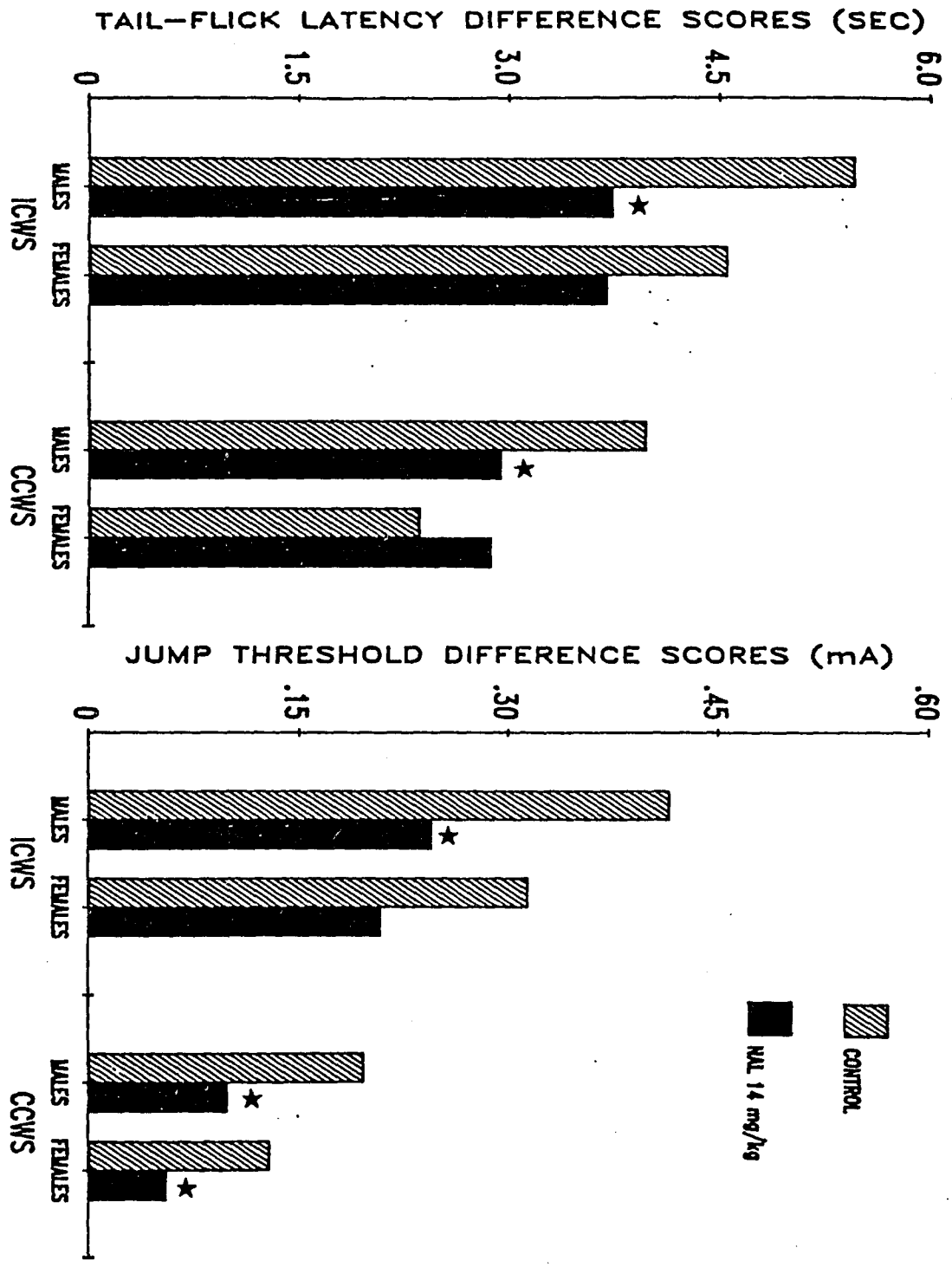

CCWS
ICWS
CONTROL MALES
MALES NAL
CONTROL FEMALES
FEMALES NAL
MAGNITUDE OF HYPOTHERMIA (C)
20
16
12
8
4
0
0
30
60
90
120
POST-SWIM (MIN)

Figure 11. Magnitude of CCWS and ICWS analgesic of gonadectomized male and female rats following naloxone pretreatment (14 mg/kg), 30 min after the swim. *Significantly different from control; Dunn comparisons, $p<.05$.


TAIL—FLICK LATENCY DIFFERENCE SCORES (SEC)
0
1.5
3.0
4.5
6.0
ICWS
MALES
FEMALES
CCWS
MALES
FEMALES
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.15
.30
.45
.60
ICWS
MALES
FEMALES
CCWS
MALES
FEMALES
NAL 14 mg/kg
CONTROL

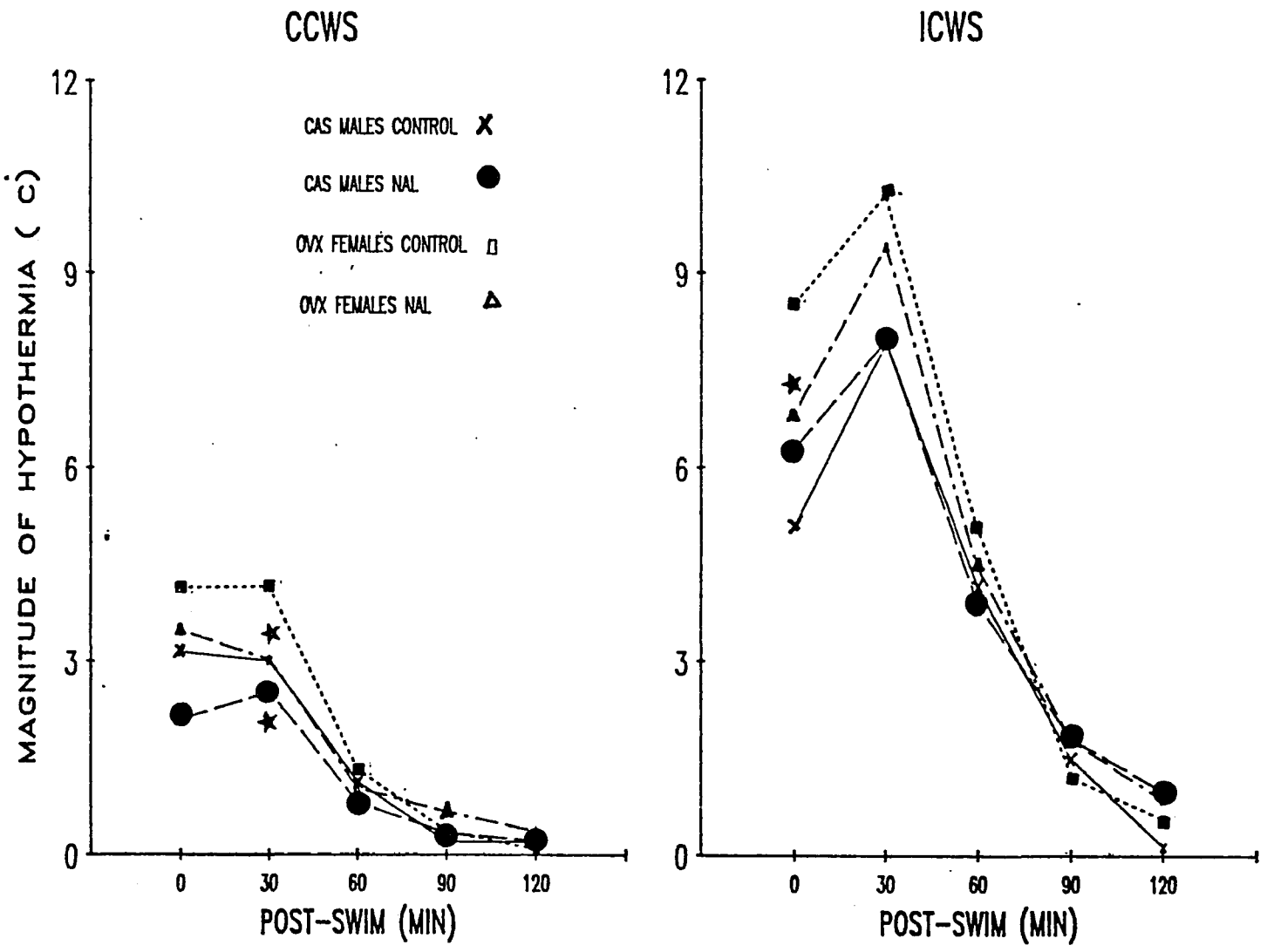
in gonadectomized as compared to intact rats. The first and third panel of Figure 11 illustrates the significant reductions in ICWS analgesia in castrated males on the tail-flick (31% decrease) and jump (41% decrease) tests. In contrast, naloxone failed to alter ICWS analgesic magnitude in ovariectomized females on the tail-flick (19% decrease) or jump (13% decrease) tests.

Naloxone Effects Upon Hypothermia and Activity (Gonadectomized rats): Figure 12 illustrates the significant naloxone induced reductions in the magnitude of CCWS hypothermia in gonadectomized male and female rats 30 min following CCWS ($F(4,72)=8.85$, $p<.001$). Following ICWS (0 min), only female rats displayed a significant reduction in ICWS hypothermia ($F(4,72)=3.51$, $p<.02$). Naloxone failed to alter activity during CCWS ($F(1,30)=.05$) or ICWS ($F(1,30)=.20$) (data not shown).

Discussion

The second experiment confirmed observations in the first experiment of gender differences in CCWS and ICWS analgesia in intact rats. Castration of male rats significantly reduced CCWS and ICWS analgesia to the

Figure 12. Hypothermic magnitude following CCWS and ICWS in gonadectomized male and female rats pretreated with naloxone (14 mg/kg). *Significantly different from control; Dunn comparisons, $p<.05$.


CCWS
ICWS
CAS MALES CONTROL
CAS MALES NAL
OVX FEMALES CONTROL
OVX FEMALES NAL
MAGNITUDE OF HYPOTHERMIA (C)
12
9
6
3
0
0
30
60
90
120
POST-SWIM (MIN)

level observed in intact female rats. Similarly, ovariectomy significantly decreased both forms of analgesia in female rats. These changes in analgesia could not be attributed to corresponding alterations in either body weight, basal reactivity, hypothermia or activity during the swim. The opiate receptor antagonist, naloxone, failed to alter the magnitude of nonopioid-mediated CCWS analgesia in either intact male or female rats. Naloxone significantly decreased ICWS analgesia in intact male, but not intact female rats. Naloxone eliminated the gender differences in CCWS and ICWS analgesia, which could not be attributed to corresponding changes in hypothermia or activity. CCWS and ICWS analgesia was significantly decreased by naloxone in castrated male rats but not in ovariectomized female rats. The completeness of the gonadectomy procedures was confirmed by significant reductions in prostate weight (92%) and seminal vesicle size (82%) in castrated males, and uterine weight (77%) in ovariectomized females.

Specificity of Gonadectomy Effects: Gonadectomy altered the magnitude of CCWS and ICWS analgesia relative to intact animals, and altered gender-specific relationships. Castration significantly decreased CCWS

analgesia on both the tail-flick and jump tests and decreased ICWS analgesia on the jump test to levels observed in intact females. Ovariectomy significantly decreased CCWS and ICWS analgesia on both nociceptive measures. Neither gender differences nor the gonadectomy-induced alterations in CCWS and ICWS analgesia were due to concomitant changes in either hypothermia or activity during the swim. Analyzing gender-specific effects in intact rats, the smaller magnitudes of CCWS and ICWS analgesia in intact females were accompanied by greater magnitudes of CCWS and ICWS hypothermia. Further, since intact and gonadectomized rats failed to display baseline core body temperature differences, any subsequent changes in CCWS or ICWS hypothermia in these groups cannot be attributed to pre-swim temperature differences. Gonadectomy does reduce basal core body temperature (Marrone, 1976), but the failure to observe such differences in the present experiment may be due to random assessment of body temperature in females. Estrous cyclicity affects rectal temperature (Yochim & Spencer, 1976; Brobeck et al., 1947); this was not systematically controlled because CCWS analgesia does not vary across the estrous cycle (Experiment 1; Bodnar & Komosaruk, 1984). In any case, the expected hypothermia following

gonadectomy, which presumably reflects depressed metabolism (Laudenslager et al., 1980), was not observed; indeed the present study showed decreased CCWS and ICWS hypothermia in gonadectomized animals. The observed dissociable effects of analgesia and hypothermia following swims have been reported previously for age-related differences following CCWS: older (18 mo) age cohorts of female rats display significant decreases in CCWS analgesia and significant increases in CCWS hypothermia (Kramer & Bodnar, 1986). Indeed, other relatonships have been observed: hypophysectomy, D-phenylananine and desipramine treatments (Bodnar et al., 1979; Bodnar et al., 1980; Bodnar et al., 1985).

Gonadectomy effects present a different pattern from gender differences: the decreased magnitude of CCWS and ICWS analgesia following gonadectomy were accompanied by decreased magnitudes of CCWS and ICWS hypothermia relative to same sex-controls. These results parallel the decreased magnitude of CCWS and ICWS hypothermia observed in rats neonatally treated with monosodium glutamate (Badillo- Martinez et al., 1984). However, the overall gonadectomy- induced changes in analgesia and hypothermia are not identical. Although castrated male and intact female rats displayed similar magnitudes of CCWS and ICWS

analgesia, intact female rats displayed more marked CCWS and ICWS hypothermia. Moreover, although castrated males displayed a greater magnitude of CCWS and ICWS analgesia than ovariectomized females, the latter group displayed a greater magnitude of CCWS and ICWS hypothermia. The different rank-orders of hypothermic potency (intact females >> intact males > ovariectomized females > castrated males) and analgesic potency (intact males > castrated males = intact females > ovariectomized females) suggest that mechanisms subserving gender-specific and gonadectomy-specific alterations in analgesic responses differ from those subserving hypothermia.

The measurement of activity levels during the swim was derived from studies of animal models of depression (Porsolt et al., 1977) and from studies identifying markers of adaptation to ICWS analgesia (Girardot & Holloway, 1985). Since gender differences in general activity have been reported (Grey, 1971) and since gonadal hormones seem to affect these differences (Gray et al., 1965, 1969), it was important to determine whether gender had any influence on activity during the swim, and whether analgesia could be related to this factor. Although activity levels during CCWS were

unaffected by gender or gonadectomy, gonadectomy increased activity levels during ICWS. This increased activity during ICWS in gonadectomized animals might account for decreased magnitudes of ICWS analgesia and ICWS hypothermia, yet the failure to observe parallel effects during CCWS questions the consistency of such effects. Moreover, gender-related activity effects failed to occur in the presence of decreased analgesia and increased hypothermia in females following both swim conditions. Further, while gonadectomized rats displayed similar increases in activity following ICWS, ovariectomized females showed less analgesia and more hypothermia than castrated males. Thus it appears that while activity during the swim might serve as a marker for subsequent analgesic and hypothermic responses under some circumstances, it is clear that all alterations in analgesia cannot be attributed to corresponding activity shifts, suggesting that the former responses and their alterations by gender and gonadectomy may reflect inherent changes in pain inhibition rather than changes in the sequelae of responses to the swim stressors.

Body weight changes following gonadectomy in male and female rats occurred in the expected direction (Marks & Hobbs, 1972): castrated males failed to gain weight at

the same rate as sham-controls, and ovariectomized females gained significantly more weight than intact females. However, body weight changes could not account for the obtained reductions in CCWS and ICWS analgesia following gonadectomy. Although castrated male rats were significanly heavier than intact females, both groups displayed comparable magnitudes of analgesia. The direction of change in body weight produced by gonadectomy (decrease in males, increase in females) did not parallel the alterations observed in analgesia (decreases in both groups).

The reduction of opioid-mediated ICWS analgesia by gonadectomy is consistent with the reported reduction of morphine analgesic potency (Chatterjee et al., 1982) and opiod receptor concentration in castrated males (Hahn, 1985). It also provides further evidence for the influence of endocrine status on analgesic proceses (Ryan et al., 1985; Crowley et al., 1976; Rothfeld et al., 1985), at least in those endocrine systems related to opioid systems. Chatterjee and co-workers (1982) established that testosterone replacement therapy administered to castrated males reinstated the normal analgesic potency of morphine. If analgesic magnitude following ICWS is also reinstated by testosterone

administration (see Experiment 3), this information would provide more evidence concerning the possible common mechanisms for the two types of analgesia. Moreover, since certain types of opioid mediated SIA have been correlated with changes in concentration or release of endogenous opiates (Lim et al., 1982; Millan et al., 1981a), it is possible that the gonadectomy-related changes in ICWS analgesia may be related to alterations in endogenous opioids affected by this procedure. Castrated males and ovariectomied females display significant increases in dynorphin, leu-enkephalin and B-endorphin concentrations in the anterior pituitary lobe (Molineaux et al., 1986). In turn, ovariectomy significantly decreases cortical dynorphin levels (Morley et al., 1984). Although the endogenous peptide system specifically involved in SIA has not been precisely determined, there is evidence for the role of B-endorphin (Lim et al.,1982), dynorphin (Millan et al., 1982) and enkephalin (Lewis et al., 1981). However, it seems premature to attempt to correlate these changes without more detailed information on the activity of endogenous opioids in SIA and particularly in ICWS analgesia. Further, since gonadectomy-related decreases in CCWS analgesia were also observed, and since CCWS activates

nonopioid pain inhibitory systems (Bodnar et al., 1979b; Kirchgessner et al., 1982), the probability that all changes are directly related to endogenous opioid levels becomes more remote.

Naloxone Effects: The insensitivity of CCWS analgesia to naloxone pretreatment in intact animals confirmed previous reports (Bodnar et al., 1978) in which various doses of naloxone failed to significantly reduce CCWS analgesia. It appears, therefore, that CCWS analgesia is mediated by nonopioid systems in both male and female rats, and that the gender differences observed in analgesic magnitude are not related to differential activation of opioid and nonopioid systems. The nonopioid mediation of CCWS has also been determined by the inability of the long acting opioid receptor antagonist naltrexone to reduce the response (Girardot & Holloway, 1984). Further, CCWS analgesia is potentiated by a the high affinity mu-1 receptor antagonist, naloxozone (Kirshgessner et al., 1982) and fails to develop crosstolerance with morphine (Bodnar et al., 1978). Differential effects upon CCWS and morphine analgesia are observed following hypophysectomy (Bodnar et al., 1979c). Further evidence of the role of gonadal steroids in the modulatiom of CCWS analgesia comes from the differential

effect of naloxone on intact and castrated males: CCWS analgesia was unaffected by naloxone in intact males, but was significantly reduced by naloxone in castrated males. This suggests that an nonopioid response may be transformed to an opioid response by gonadal manipulations. It is possible that castration suppresses the larger nonopioid component of CCWS analgesia and leaves a residual opioid component intact. Bodnar and Sikorsyky (1983) suggested this residual component by demonstrating that the analgesic magnitude following CCWS was positively correlated with partial naloxone reversal. However, determinations of any opioid-nonopioid characteristics of an analgesic process cannot be based exclusively on the ability of naloxone to block it. Therefore, it would be important to determine whether CCWS analgesia in castrated male rats meets other criteria of opioid mediated responses. The hypothesized shift from nonopioid to opioid mediation of CCWS analgesia in castrated males may also imply that male, but not female gonadal steroids, modulate the response. This would mean a further differentiation between CCWS analgesia and morphine analgesia, implying different mechanisms of action, since morphine analgesic potency appears to be influenced by both male and female gonadal

steroids (Chattejee et al., 1982; Banerjee et al., 1983).

Gender-related effects of naloxone were also observed in opioid mediated ICWS analgesia (Girardot & Holloway, 1984): analgesic magnitude in male but not in female rats was significantly reduced by naloxone pretreatment. Thus, it appears that ICWS activates both and opiate and nonopiate mechanism in males (Girardot & Holloway, 1984), whereas ICWS analgesia in females is nonopioid mediated. Therefore, gender, possibly via gonadal steroids, not only affects the magnitude of analgesia following a particular stressor, but also appears to determine the underlying mechanisms mediating a specific type of analgesia. Endogenous opioid levels in male and female rats differ in that females have lower levels of circulating B-endorphin (Mueller, 1980) and lower concentration of dynorphin in the hypophysis (Molineaux et al., 1986). Thus, it is possible that naloxone effects may be determined in part by these differences. Gonadectomy, however, failed to affect the pattern of naloxone effects observed in intact rats: the magnitude of ICWS analgesia was significantly reduced in castrated males but not in ovariectomized females.

In summary, following confirmation of gender differences in CCWS and ICWS analgesia (Experiment 1A),

it was found that both castration and ovariectomy significantly reduced CCWS and ICWS analgesia. Castrated males displayed similar magnitudes of analgesia as intact females. These gonadectomy effects could not be accounted for by alterations in CCWS and ICWS hypothermia, activity during the swim, or changes in body weight following gonadectomy. Naloxone significantly reduced opioid-mediated ICWS analgesia in male rats but not in female rats. Nonopioid- mediated CCWS analgesia was not reversed by naloxone.

Since morphine analgesia is reduced by castration and reinstated by hormonal replacement therapy (Banerjee et al., 1983), it was deemed important to determine whether the gonadectomy effects on CCWS and ICWS analgesia could similarly be reversed by steroid replacement therapy. The results would provide a more clear understanding of the modulatory function of gonadal steroids on CCWS and ICWS analgesic processes.

Experiment IIIA: Effects of handling on CCWS and ICWS Analgesia.

Method

Baseline tail-flick latencies and jump thresholds were determined over four days in 22 male and 22 female rats exposed to sham gonadal surgery. Following a four week recovery period animals were divided into nonhandled (seven males and seven females) and handled (15 males and 15 females) groups. "Daily handling" is defined in this experement as picking up the animal and giving it a subcutaneous injection of sesame oil. Handled animals, terefore, received sesame oil (4 ml/kg, SC) daily for 14 days; injections were made between 10-11 h into the light cycle. This procedure served as a control for subsequent gonadal replacement therapy (Experiment 3B). All rats received three conditions: no-swim, CCWS and ICWS; the latter two were counterbalanced in a crossover design. Tail-flick latencies, jump thresholds and core body temperature were determined 30, 60, 90 and 120 min after each condition.

Results

Basal Pain Thresholds: Table 5 indicates the failure of tail-flick latencies and jump thresholds of male and

Table 5. Baseline Tail-Flick Latencies (sec, SEM) and Jump Threshols (mA, SEM) Before Surgery, After Surgery and Following Vehicle Treatment in Male and Female Rats.

A. Tail-flick Latencies

GROUP	Pre-Surgery	Post-Surgery	Post-inj
Nonhandled Males	2.86 (0.14)	2.73 (0.14)	
Handled Males	3.08 (0.10)	3.27 (0.04)	3.14 (0.09)
Nonhandled Females	3.39 (0.10)	3.41 (0.10)	
Handled Females	3.38 (0.06)	3.39 (0.05)	3.40 (0.09)

B. Jump Thresholds

Nonhandled Males	.405 (.008)	.374 (.020)	
Handled Males	.388 (.009)	.400 (.007)	.395 (.008)
Nonhandled Females	.296 (.016)	.273 (.020)	
Handled Females	.305 (.009)	.282 (.007)	.290 (.008)

Note: Tail-flick and jump thresholds failed to differ significantly among conditions.

female rats to be altered as functions of surgical or injection manipulations.

CCWS Analgesia (Tail-Flick Latencies): Since alterations in pain thresholds following CCWS and ICWS are reported elsewhere (Experiment 2 and 3B), this and all subsequent analyses of Experiment 3A will be performed on difference scores. Significant differences were observed among groups ($F(1,40)=6.97$, $p<.02$), across test times ($F(3,120)=92.25$, $p<.001$) and for the interation among groups, test times and conditions ($F(3,120)=3.26$, $p<.03$) in the magnitude of CCWS analgesia. The left panel of Figure 13 indicates that nonhandled male rats displayed significantly greater analgesia than nonhandled female rats 30 min after CCWS. However, handled males, handled females and nonhandled females failed to differ from each other. The significant reduction in CCWS analgesia observed in handled males relative to nonhandled males appears to explain the failure to observe gender differences between handled males and females on this measure.

CCWS Analgesia (Jump Threshold): Significant differences were observed in the magnitude of CCWS analgesia among groups ($F(1,40)=22.35$, $p<.001$), across test times ($F(3,120)=102,49$, $<p.001$) and for the

Figure 13. Effects of daily handling and injection on CCWS analgesic magnitude in sham-operated male and female rats on the tail-flick and jump tests. *Significantly different from same sex handled group; +Significantly different from opposite sex nonhandled group; Dunn comparisons, $p<.05$.

TAIL—FLICK LATENCY DIFFERENCE SCORES (sec)
0
1
3
5
7
30
60
90
120
POST-SWIM (MIN)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.1
.3
.5
.7
30
60
90
120
POST-SWIM (MIN)
Nonhandled males
Handled males
Nonhandled females
Handled females

interaction between groups and conditions ($F(1,40)= 22.35$, $p<.001$). The right panel of Figure 13 shows that nonhandled female rats displayed significantly less analgesia relative to nonhandled males 30 min following CCWS. Both handled female and male rats displayed significantly greater analgesia relative to their nonhandled counterparts 30 and 60 min following CCWS. These latter increases in analgesic magnitude were greater in female rats which eliminated the gender differences between handled male and female rats.

CCWS Hypothermia: Significant differences were observed in hypothermic magnitude among groups ($F(1,40)= 18.35$, $p<.001$), across test times ($F(3,120)=477.76$, $p<.001$) and for the interaction between group and test times ($F(4,160)=25.88$, $p<.001$). Both handled and nonhandled female rats displayed significantly greater hypothermic magnitude relative to male groups for up to 90 min following CCWS. Handled and nonhandled groups failed to differ from each other (data not shown).

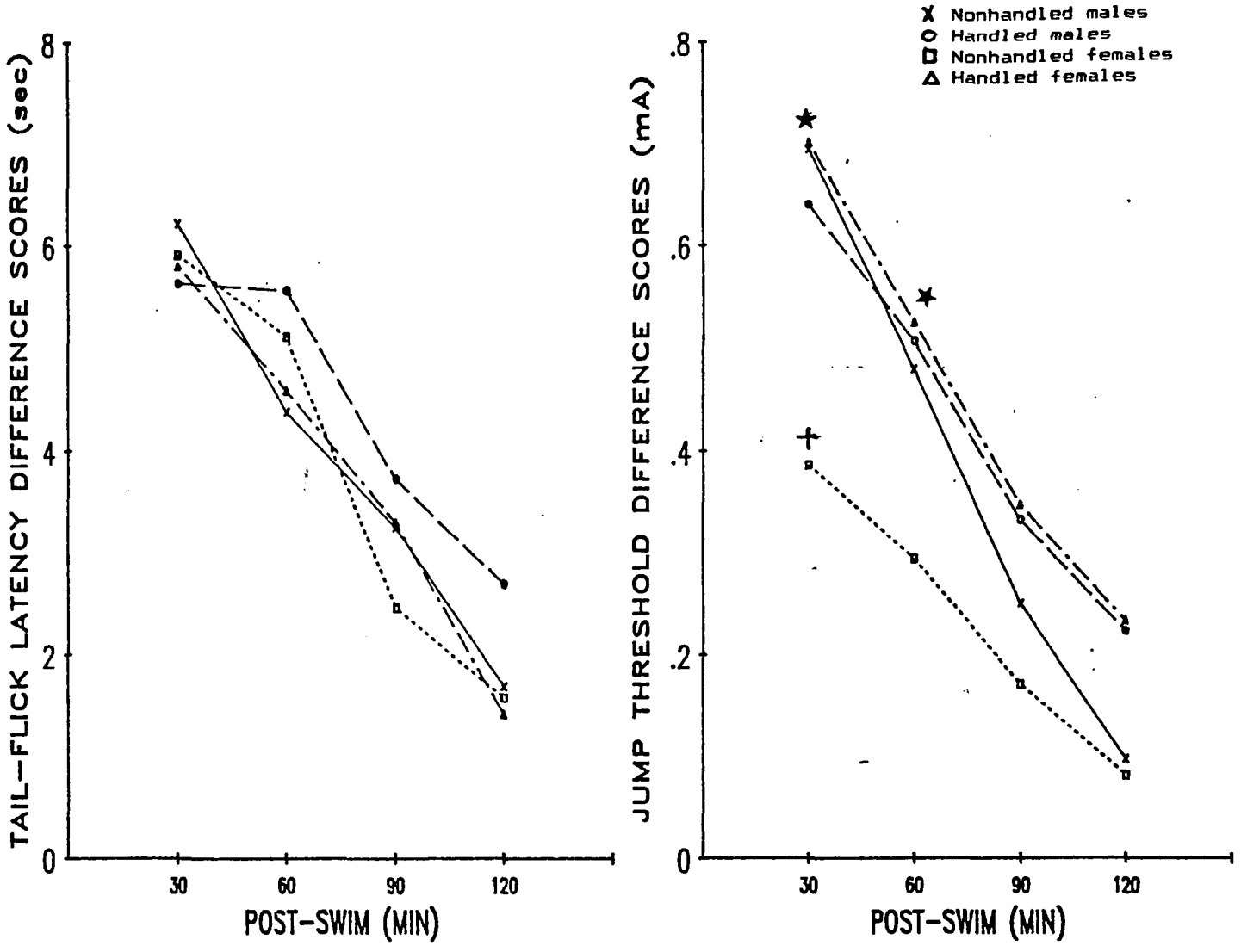
ICWS Analgesia (Tail-Flick Latencies): Significant differences in analgesic magnitude were observed across test times ($F(3,129)=91.00$, $p<.001$), but not among groups ($F(1,40)=1.09$), or for any of the interaction. The left panel of Figure 14 indicates the failure of male and

female rats, regardless of handling, to differ in analgesic magnitude.

ICWS Analgesia (Jump Threshold): Significant differences were observed in analgesic magnitude among groups ($F(1,40)=4.20$, $p<.05$), across test times ($F(3,120)=196.4$, $p<.001$) and for all interaction terms. The right panel of Figure 14 indicates that nonhandled female rats displayed significantly less analgesia than handled females, handled males and nonhandled males for up to 60 min after ICWS. The increase in analgesic magnitude in handled relative to nonhandled females appears to account for the lack of gender in the handled group.

ICWS hypothermia: Significant differences were observed in hypothermic magnitude among groups ($F(1,40)=12,72$, $p<.001$), across test times ($F(4,160)=369.24$, $p<.001$), for the interaction between groups and test times ($F(4,160)= 7.92$, $p<.001$) and groups and conditions ($F(4,160)=2.47$, $p<.05$). Hypothermic magnitude was significantly greater in female rats than in male rats regardless of handling conditions (data not shown).

Figure 14. Effects of daily handling and injection on ICWS analgesic magnitude in sham-operated male and female rats on the tail-flick and jump test. *Significantly different from same sex handled group; +Significantly different from opposite sex nonhandled group; Dunn comparisons, $p<.05$.


TAIL-FLICK LATENCY DIFFERENCE SCORES (sec)
8
6
4
2
0
30
60
90
120
POST-SWIM (MIN)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
.8
.6
.4
.2
0
30
60
90
120
POST-SWIM (MIN)
X Nonhandled males
O Handled males
D Nonhandled females
Δ Handled females

Experiment IIIB: Role of Gonadal Steroids in Gonadectomy Specific Effects upon CCWS and ICWS analgesia.

Protocol: Following four days of stable baseline tail-flick latencies and jump thresholds, either gonadectomy (30 males and 30 females) or sham-surgery (35 males and 35 females) was performed with groups matched for body weight. After four weeks of post-surgical recovery, baseline tail-flick latencies and jump thresholds were re-determined over four days. Twelve treatment groups were established: 1) sham males treated with vehicle (sesame oil, 4 ml/kg, SC: n=15); 2) sham males treated with TP (n=10); 3) sham males treated with EB (n=10); 4) castrated males treated with vehicle (n=8); 5) castrated males treated with TP (n=10); 6) castrated males treated with EB (n=10); 7) sham females treated with vehicle (n=15); 8) sham females treaed with TP (n=10); 9) sham females treated with EB (n=10); 10) ovariectomized females treated with vehicle (n=9); 11) ovariectomized females treated with TP (n=10); 12) ovariectomized females treated with EB (n=10). All injections were administered subcutaneously in sesame oil, between 10 to 11 hr into the light cycle to avoid interference with behavioral testing. TP (2 mg/kg) and

vehicle were given daily, for two weeks before the first condition and were continued through all conditions. EB (4 ug/kg) was administered daily for one week before the first condition and continued through all conditions. Three conditions were tested: no-swim, CCWS and ICWS; the latter two conditions were counterbalanced in a crossover design. Tail-flick latencies, jump thresholds and core body temperatures were assessed 30, 60, 90 and 120 min after each condition; three to seven days elapsed between conditions. Body weight was monitored throughout the experiment which was conducted in the same room at ambient temperatures between 22 and 25 C. After the last testing session, animals were killed and their accessory sex organs dissected and weighed.

Results

Body Weight: Significant differences ($F(1,123)=85.80$, $p<.001$) were found in body weight gain four weeks after surgery between male and female rats, and between gonadectomized and intact animals ($F(1,123)=85.80$, $p<.001$). Table 6 shows that ovariectomized rats gained significantly more weight (+65 g) relative to intact females (+10 g). Intact males gained significantly more weight (+26 g) than castrated

Table 6. Body Weight (g, SD) of Male and Female Rats Following Vehicle or Hormonal Treatment over 15 days (TP) or 8 days (EB).

GROUP	Post-Sur.	Post-Hor.
Sham-Operated Males		
Vehicle Treated (15 days)	487 (31)	525 (22)+
TP Treated (15 days)	472 (38)	482 (47)
Vehicle Treated (8 days)	487 (31)	518 (26)+
EB Treated (8 days)	498 (43)	473 (52)+
Castrated Males		
Vehicle Treated (15 days)	449 (57)	474 (64)+
TP Treated (15 days)	446 (50)	495 (64)+
Vehicle Treated (8 days)	449 (57)	468 (56)+
EB Treated (8 days)	473 (73)	460 (63)+
Sham-Operated Females		
Vehicle Treated (15 days)	287 (27)	303 (25)+
TP Treated (15 days)	273 (18)	335 (34)+
Vehicle Treated (8 days)	287 (27)	299 (26)
EB Treated (8 days)	282 (23)	296 (22)
Ovariectomized Females		
Vehicle Treated (15 days)	330 (32)	353 (33)+
TP Treated (15 days)	302 (47)	339 (34)+
Vehicle treated (8 days)	330 (32)	351 (33)+
EB Treated (8 days)	334 (48)	301 (40)+

Note: The asterisk denotes a significant difference from vehicle condition and the cross denotes a significant difference from pre-injection body weight (Dunn comparisons, $p<.05$).

males (-4 g). TP significantly retarded weight gain in intact males (+10 g) relative to intact vehicle-treated controls (+38 g). TP accelerated weight gain in: a) castrated males (+49 g) relative to castrated males receiving vehicle (+25 g); b) intact females (+62 g) relative to intact females receiving vehicle (+16 g) and c) ovariectomozed females (+37 g) relativ to ovariectomized females receiving vehicle (+23 g). EB significantly retarded body weight gain in intact (-25 g) and castrated (-13 g) males relative to intact (+38 g) and castrated (+19 g) males receiving vehicle. EB failed to affect body weight gain in intact females (+14 g) relative to vehicle-treated female rats (+12 g). However, EB treatment significantly retarded body weight gain in ovariectomized females (-33 g) relative to vehicle treatment (+21 g).

Baseline Pain Thresholds: Table 7 indicates small but significant differences in baseline levels as a function of surgery and treatments. Intact females displayed higher baseline tail-flick latencies (+.26 sec) than castrated males ($F(1,115)=6.47$, $p<.001$). Post-drug treatment latencies decreased significantly relative to post-surgery latencies ($F(2,230)=4.69$, $p<.001$) in intact, vehicle- treated males, castrated vehicle-treated males,

Table 7. Baseline Tail-Flick Latencies (sec, SEM) and Jump Thresholds (mA, SEM) Before Surgery, After Surgery, and After Vehicle or Hormonal Treatment in Male and Female Rats.

A. Tail-Flick Latencies

GROUP	Pre-Surgery	Post-Surgery	Post-Treatment
Sham-Operated Males			
Vehicle Treated	3.08	3.36	3.14
TP Treated	2.96 (.09)	3.12 (.13)	3.20 (.18)
EB Treated	3.04 (.11)	3.15 (.10)	3.18 (.16)
Castrated Males			
Vehicle Treated	3.09 (.12)	3.44 (.08)	3.05 (.14)
TP Treated	3.01 (.11)	3.29 (.11)	3.02 (.14)
EB Treated	2.99 (.12)	2.94 (.11)	2.88 (.11)
Sham-operated Females			
Vehicle Treated	3.38 (.18)	3.39 (.05)	3.40 (.09)
TP Treated	3.25 (.11)	3.20 (.07)	3.16 (.11)
EB Treated	3.25 (.07)	3.35 (.08)	3.49 (.10)
Ovariectomized Females			
Vehicle Treated	3.31 (.11)	3.27 (.10)	2.84 (.12)
TP Treated	3.22 (.09)	3.10 (.13)	2.88 (.08)
EB Treated	3.19 (.08)	3.12 (.11)	3.04 (.13)

Table 7 (cont.)

B.Jump Thresholds

Sham-operated Males			
Vehicle Treated	.388 (.009)	.400 (.007)	.395 (.007)
TP Treated	.384 (.014)	.373 (.009)	.384 (.007)
EB Treated	.378 (.012)	.395 (.010)	.383 (.012)
Castrated Males			
Vehicle Treated	.379 (.012)	.392 (.013)	.394 (.023)
TP Treated	.379 (.017)	.387 (.007)	.402 (.009)
EB Trated	.378 (.009)	.385 (.008)	.398 (.009)
Sham-Operated Females *			
Vehicle Traeated	.305 (.009)	.282 (.007)	.290 (.008)
TP Treated	.305 (.014)	.291 (.008)	.300 (.008)
EB Treated	.301 (.009)	.305 (.009)	.300 (.013)
Ovariectomized Females *			
Vehicle Treated	.296 (.023)	.308 (.008)	.316 (.012)
TP Treated	.302 (.006)	.306 (.007)	.290 (.005)
EB Treated	.285 (.009)	.309 (.007)	.295 (.009)

* Female rats displayed significantly lower jump thresholds than male rats.

castrated TP-treated males, ovariectomized vehicle-treated females and ovariectomized TP treated females. Jump threholds were significantly higher in male rats relative to female rats ($F(3,115)=110.55$, $p<.001$), but failed to differ among pre- surgery, post-surgery or post-drug treatment periods ($F(2,230)=1.18$).

CCWS analgesia (Tail-Flick Latencies): Significant differences were observed among groups ($F(3,115)=7.93$, $p<.001$), across no-swim values and post-swim time course ($F(1,115)=788.89$, $p<.001$) and for the interactions term ($F(6,354)=1.63$, p<.,02). CCWS significantly elevated latencies in all groups across the time course. Difference scores analyses revealed significant differences among groups ($F(3,115)=3.44$, $p<.02$), across test times ($F(3,345)=250.05$, $p<.001$), and for all interactions ($F(6,345)=2.62$, $p<.02$). The left panel of Figure 15 indicates that castrated males displayed less CCWS analgesia than intact males following vehicle treatment. TP significantly increased the magnitude of CCWS analgesia in intact and castrated males, reinstating the analgesic magnitude of the latter group to levels observed in intact male rats. In contrast, the left panel of Figure 16 indicates that EB failed to alter the magnitude of analgesia in intact or castrated males. The

Figure 15. CCWS analgesic magnitude in intact and castrated male rats following TP (2 mg/kg/14 days) treatment. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.

TAIL—FLICK LATENCY DIFFERENCE SCORES (sec)
0
2
4
6
30
60
90
120
POST-SWIM (MIN)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.2
.4
.6
30
60
90
120
POST-SWIM (MIN)
X Intact males + VEH
O Intact males + TP
□ Castrated males + VEH
Δ Castrated males + TP

Figure 16. CCWS analgesic magnitude in intact and castrated male rats following EB (5 ug/kg/7 days) treatment. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.

TAIL-FLICK LATENCY DIFFERENCE SCORES (sec)
0
2
4
6
30
60
90
120
POST-SWIM (MIN)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.2
.4
.6
30
60
90
120
POST-SWIM (MIN)
Intact males + VEH
Intact males + EB
Castrated males + VEH
Castrated males + EB

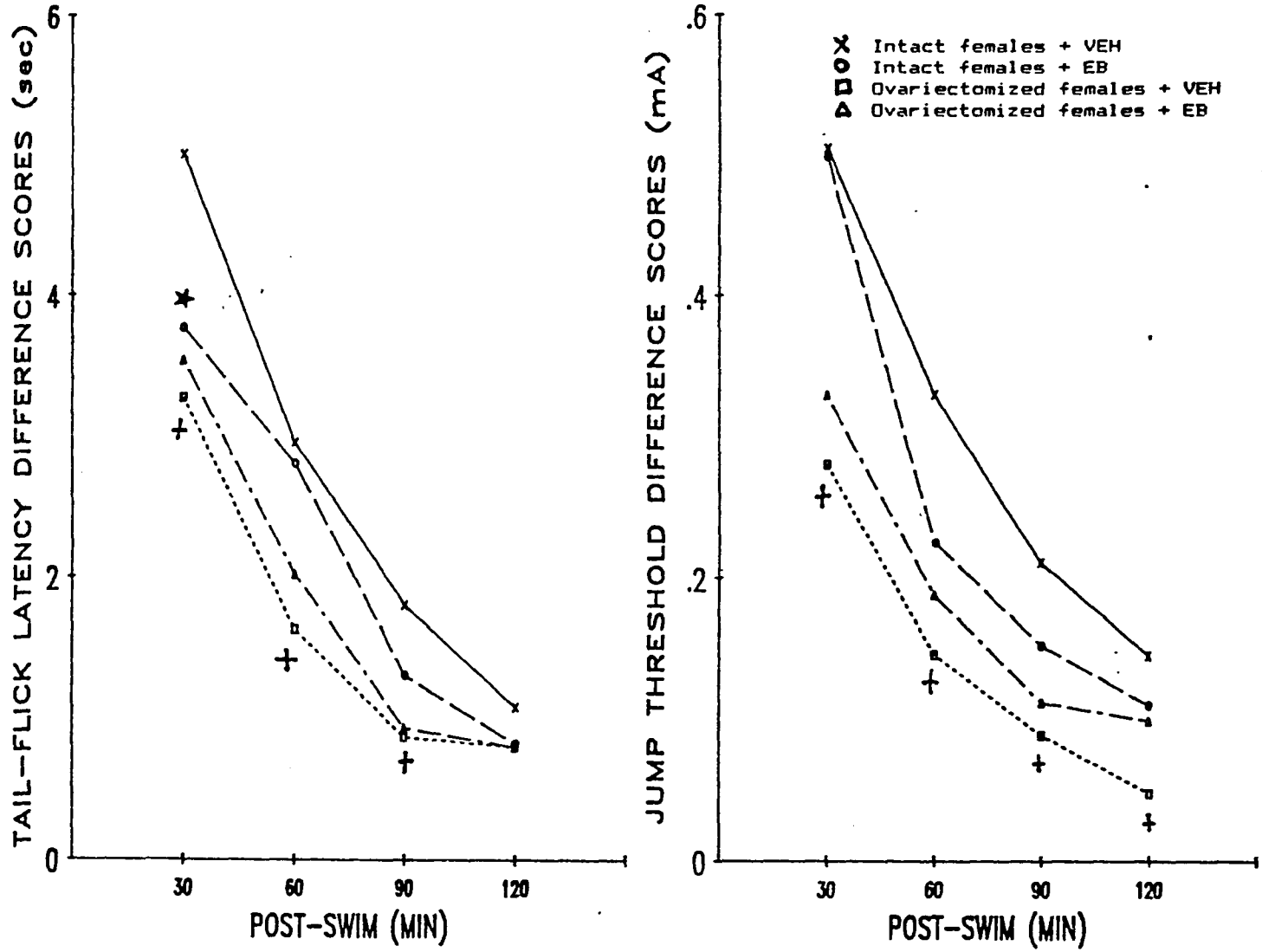
left panel of Figure 17 shows that the significantly smaller analgesia in ovariectomized females relative to intact females was reinstated by TP. In contrast, the left panel of Figure 18 indicates that EB significantly decreased the magnitude of analgesia in intact, but not ovariectomized females, and that the subsequent analgesic response was indistinguishable between the two EB-treated groups.

CCWS Analgesia (Jump Thresholds): Significant differences were observed among groups ($F(3.115)=33.62$, $p<.001$), across no-swim values and post-swim test times ($F(1,115)= 712.12$, $p<.001$) and for all interactions ($F(6,345)=4.07$, $p<.001$). CCWS significantly increased jump thresholds in all groups across the time course. The difference score analysis revealed significant differences among groups ($F(3,115)=2.97$, $p<.04$), across test times ($F(3,345)= 423.72$, $p<.001$) and for the interaction between groups and test times ($F(9,118)=3.53$, $p<.001$). The right panel of Figure 15 indicates that the smaller magnitude of analgesia observed in castrated relative to intact males following vehicle treatment was reinstated by TP; TP did not increase thresholds in intact males. The right panel of Figure 16 indicates that EB reinstated magnitude of analgesia of castrated males.

Figure 17. CCWS analgesic magnitude in intact and ovariectomized female rats following TP (2 mg/kg/14 days) treatment. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.

TAIL—FLICK LATENCY DIFFERENCE SCORES (sec)
0
2
4
6
POST—SWIM (MIN)
30
60
90
120
JUMP THRESHOLD DIFFERENCE SCORES (mA)
0
.2
.4
.6
POST—SWIM (MIN)
30
60
90
120
Intact females + VEH
Intact females + TP
Ovariectomized females + VEH
Ovariectomized females + TP

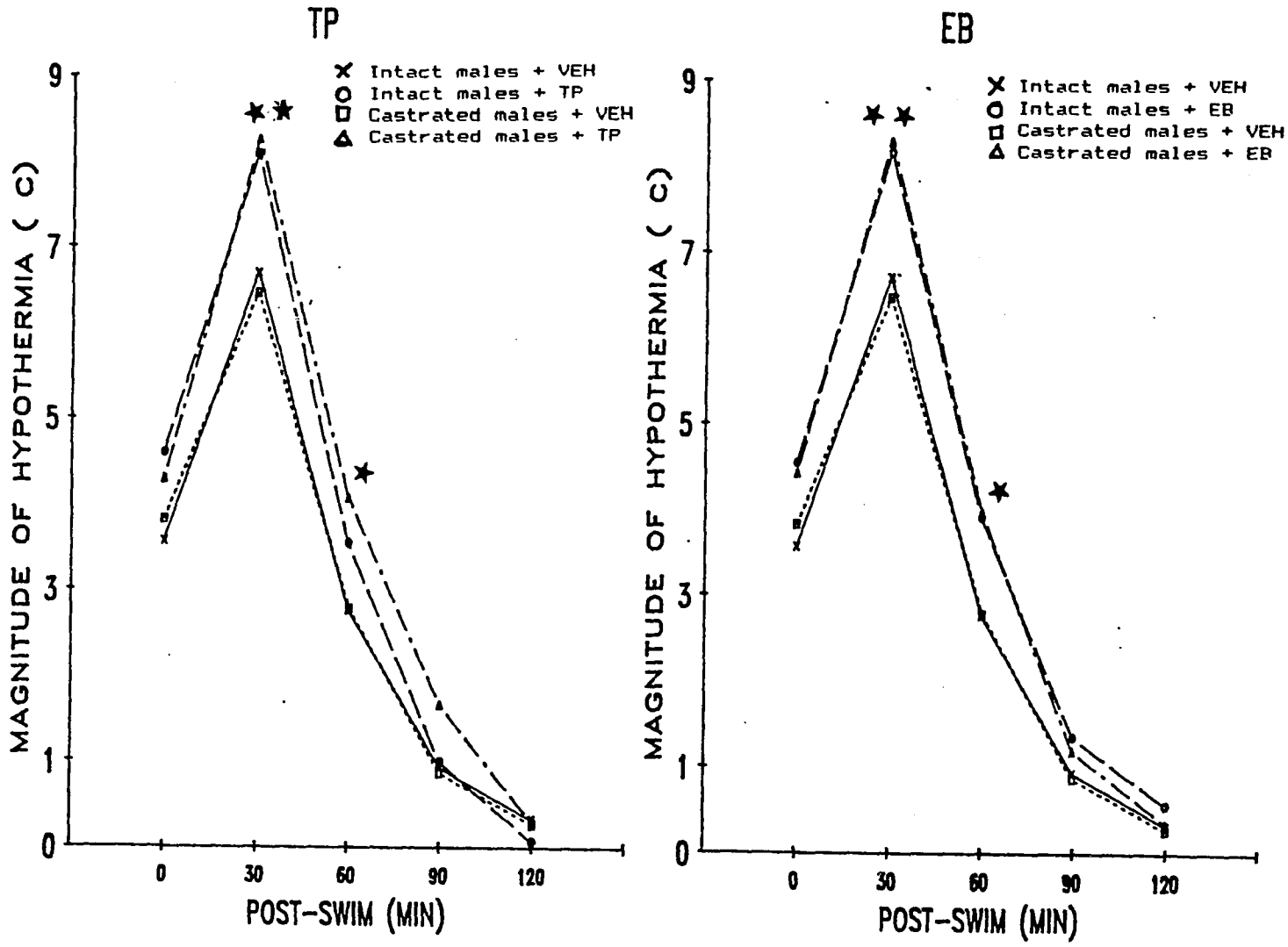
Figure 18. CCWS analgesic magnitude in intact and ovariectomized female rats following EB (4 ug/kg/7 days) treatment. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.


TAIL-FLICK LATENCY DIFFERENCE SCORES (sec)
6
4
2
0
30
60
90
120
POST-SWIM (MIN)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
.6
.4
.2
0
30
60
90
120
POST-SWIM (MIN)
Intact females + VEH
Intact females + EB
Ovariectomized females + VEH
Ovariectomized females + EB

The right panel of Figure 17 indicates that ovariectomized females displayed significantly less analgesia that intact females following vehicle treatment. TP reinstated analgesic magnitudes of ovarectomized females without altering analgesic magnitudes of intact females. The right panel of Figure 18 indicates the failure of EB to influence analgesic magnitudes of intact or ovariectomized females.

CCWS Hypothermia: Significant differences were not found in baseline core body temperatures of groups after vehicle or hormonal treatments. Significant differences in hypothermia were observed among groups ($F(3,115)=4.79$, $p<.004$), across test times ($F(5,575)=1203.95$, $p<.001$) and for the interaction between groups and test times ($F(15,575)=13.75$, $p<.001$). Significant hypothermia was observed up to 90 min in all groups following CCWS. The difference score analyses revealed significant differences among groups ($F(3,115)=8.87$, $p<.001$), across test times ($F(4,460)=1211.95$, $p<.001$) and for the interaction between groups and test times ($F(12,460)=15.27$, $p<.001$). Figure 19 illustrates that intact and castrated male rats failed to differ in hypothermic magnitude following vehicle treatment. Both TP (left panel) and EB (right panel) significantly

Figure 19. CCWS hypothermic magnitude in intact and castrated male rats following TP (2 mg/kg/14 days) or EB (4 ug/kg/7 days) treatments. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.


TP
Intact males + VEH
Intact males + TP
Castrated males + VEH
Castrated males + TP
MAGNITUDE OF HYPOTHERMIA (C)
POST-SWIM (MIN)
EB
Intact males + VEH
Intact males + EB
Castrated males + VEH
Castrated males + EB
MAGNITUDE OF HYPOTHERMIA (C)
POST-SWIM (MIN)

increased the magnitude of hypothermia 30 min after the swim in intact and castrated males. The previously observed (Experiment 1) gender differences in CCWS hypothermia were replicated in this experiment: intact females were significantly more hypothermic then intact males for up to 90 min following CCWS. The left panel of Figure 20 indicates that ovariectomized females were significantly less hypothermic than intact females following vehicle treatment. TP reinstated hypothermic magnitudes of ovariectomized females to levels observed in intact females without altering the hypothermia of the latter group. While the right panel of Figure 20 indicates that EB failed to affect the magnitude of hypothermia in intact females, it significantly increased the magnitude of hypothermia in ovariectomized females to a level which was still significantly less than that of intact females.

ICWS Analgesia (Tail-Flick Latencies): Significant differences were observed among groups ($F(3,115)=7.34$, $p<.001$), across no-swim and post-swim conditions ($F(1,115)= 1292.97$, $p<.001$) but not for any interactions ($F(18,345)=.84$). ICWS significantly increased latencies in all groups across test times. The difference score analysis revealed significant differences across the time

Figure 20. CCWS hypothermic magnitude in intact and ovariectomized female rats following TP (2 mg/kg/14 days) or EB (4 ug/kg/7 days) treatments. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.

MAGNITUDE OF HYPOTHERMIA (C)
0
3
6
9
12
POST-SWIM (MIN)
0
30
60
90
120
Intact females + VEH
Intact females + TP
Ovx females + VEH
Ovx females + TP
TP
MAGNITUDE OF HYPOTHERMIA (C)
0
3
6
9
12
POST-SWIM (MIN)
0
30
60
90
120
Intact females + VEH
Intact females + EB
Ovx females + VEH
Ovx females + EB
EB

course ($F(3,345)=237.30$, $p<.001$), but not among groups ($F(3,115)=2.10$) or for any interactions ($F(18,345)=.83$). The left panels of Figures 21 to 24 indicate that hormonal treatments failed to alter ICWS analgesia latencies in intact and gonadectomized rats relative to vehicle controls.

ICWS Analgesia (Jump Thresholds): Significant differences were observed among groups ($F(3,115)=33.56$, $p<.001$), across test times ($F(1,115)=2656.96$) and for the interaction between groups and test times ($F(3,115)=5.79$, $p<.001$). Jump thresholds were significantly increased in all groups over the time course following ICWS. The difference score analysis indicated significant differences among groups ($F(3,115)= 5.87$, $p<.001$), across post-swim test times ($F(3,345)= 724.72$, $p<.001$) and for the interaction between test times and conditions ($F(6,345)=3.64$, $p<.004$). The right panel of Figures 21 and 22 illustrate that castrated males displayed significantly less analgesia than intact males following vehicle treatment. Although neither TP nor EB affected ICWS analgesia in intact males, both reinstated ICWS analgesia in castrated males to levels observed in intact males. The right panels of Figures 23 and 24 show that ovariectomized females displayed significant reductions

Figure 21. ICWS analgesic magnitude in intact and castrated male rats following TP (2 mg/kg/14 days) treatment. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.

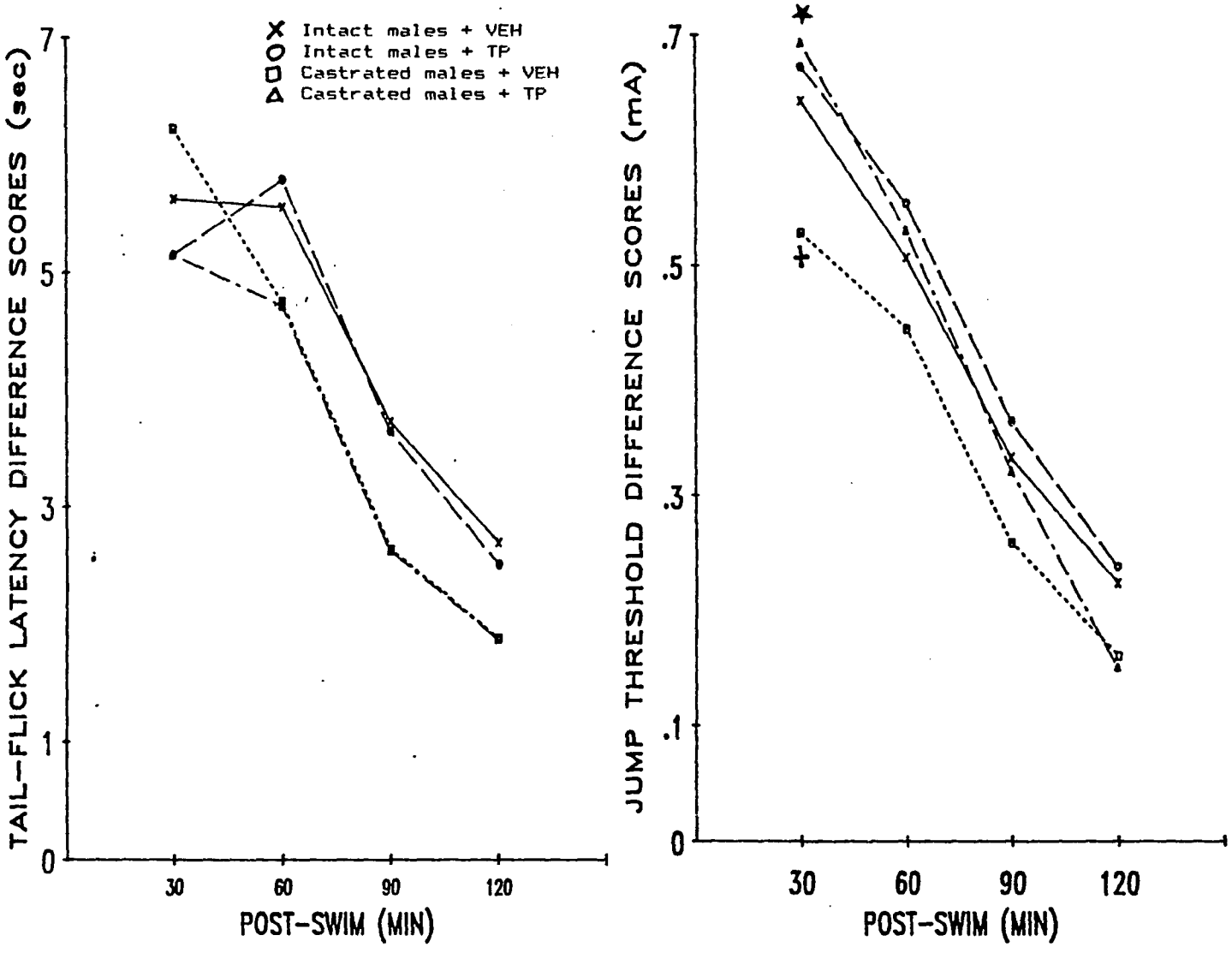

Intact males + VEH
Intact males + TP
Castrated males + VEH
Castrated males + TP
TAIL-FLICK LATENCY DIFFERENCE SCORES (sec)
JUMP THRESHOLD DIFFERENCE SCORES (mA)
POST-SWIM (MIN)
POST-SWIM (MIN)
0
1
3
5
7
.1
.3
.5
.7
30
60
90
120

Figure 22. ICWS analgesic magnitude in intact and castrated male rats following EB (4 ug/kg/7 days) treatment. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.

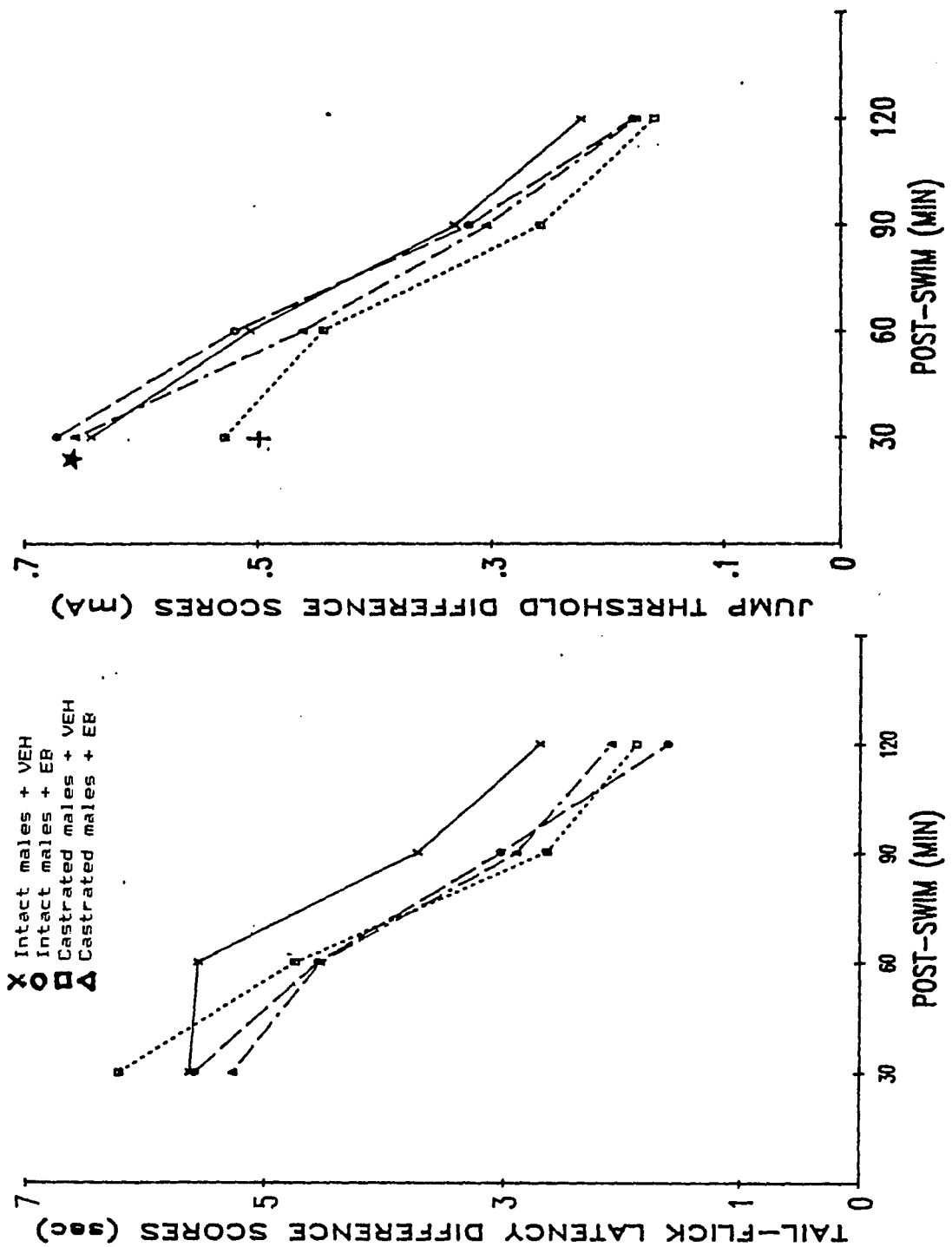

JUMP THRESHOLD DIFFERENCE SCORES (mA)
.7
.5
.3
.1
0
30
60
90
120
POST-SWIM (MIN)
TAIL-FLICK LATENCY DIFFERENCE SCORES (sec)
7
5
3
1
0
30
60
90
120
POST-SWIM (MIN)
X Intact males + VEH
O Intact males + EB
□ Castrated males + VEH
Δ Castrated males + EB

Figure 23. ICWS analgesic magnitude in intact and ovariectomized female rats following TP (2 mg/kg/14 days) treatment. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.

TAIL—FLICK LATENCY DIFFERENCE SCORES (sec)
0
1
3
5
7
POST-SWIM (MIN)
30
60
90
120
X Intact females + VEH
O Intact females + TP
□ Ovariectomized females + VEH
△ Ovariectomized females + TP
JUMP THRESHOLDS DIFFERENCE SCORES (mA)
0
.2
.4
.6
.8
POST-SWIM (MIN)
30
60
90
120

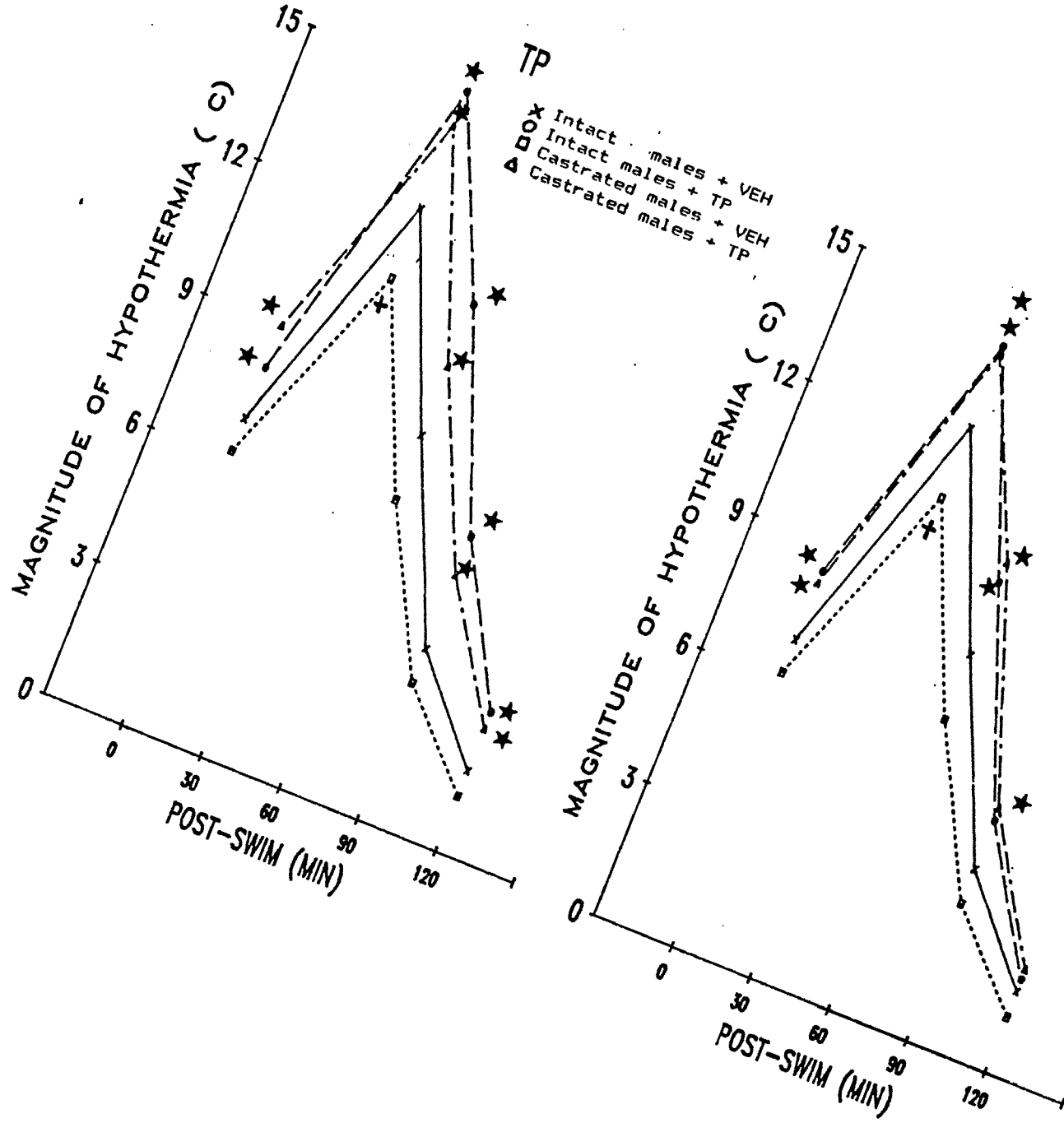
Figure 24. ICWS analgesic magnitude in intact and ovariectomized female rats following EB (4 ug/kg/7 days) treatment. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.

TAIL–FLICK LATENCY DIFFERENCE SCORES (sec)
0
2
4
6
8
POST–SWIM (MIN)
30
60
90
120
× Intact females + VEH
○ Intact females + EB
□ Ovariectomized females + VEH
△ Ovariectomized females + EB
JUMP THRESHOLDS DIFFERENCE SCORES (mA)
0
.2
.4
.6
.8
POST–SWIM (MIN)
30
60
90
120

in the magnitude of ICWS analgesia on the jump test. Both TP and EB significantly increased analgesic magnitude in ovariectomized females without altering analgesic magnitude in intact females. However, TP, but not EB, reinstated analgesic magnitude in ovariectomized females to levels observed in intact females.

ICWS Hypothermia: Significant differences were observed among groups ($F(3,115)=6.36$, $p<.001$), across test times ($F(5,575)=1616.38$, $p<.001$) and for the interaction between groups and test times ($F(15,575)=9.38$, $p<.001$). Significant hypothermia was observed in all groups following ICWS either across the time course or for up to 90 min (vehicle-treated castrated males, vehicle- and EB-treated ovariectomized females). The difference score analyses indicated significant differences among groups ($F(3,115)=8.93$, $p<.001$), across test times ($F(4,460)= 1420.60$, $p<.001$) and for the interaction between group and test times ($F(12,460)=9.35$, $p<.001$). Intact females displayed greater hypothermic magnitude than intact males for up to 60 min after ICWS. Figure 25 indicates that TP (left panel) and EB (right panel) significantly increased the magnitude of hypothermia in intact males for up to 2 h and 1 h,

Figure 25. ICWS hypothermic magnitude in intact and castrated male rats following TP (2 mg/kg/14 days) or EB (4 ug/kg/7 days) treatments. *Significantly different from VEH-treated males; +Significantly different from sham-operated males; Dunn comparisons, $p<.05$.


TP
Intact males + VEH
Intact males + TP
Castrated males + VEH
Castrated males + TP
MAGNITUDE OF HYPOTHERMIA (°C)
0
3
6
9
12
15
0
30
60
90
120
POST-SWIM (MIN)

EB
Intact males + VEH
Intact males + EB
Castrated males + VEH
Castrated males + EB
MAGNITUDE OF HYPOTHERMIA (°C)
0
3
6
9
12
15
0
30
60
90
120
POST-SWIM (MIN)

respectively. Castration significantly reduced hypothermic magnitude 30 min post-swim relative to intact males following vehicle treatment. Both TP and EB reinstated hypothermic magnitude in castrated males to levels observed in intact males. Figure 26 shows that ovariectomized females displayed significantly less hypothermia than intact females and that both TP and EB treatment reinstated hypothermic magnitude to control levels.

Accessory Sex Organs (Seminal Vesicles): Significant differences were observed among groups ($F(1,57)=106.82$, $p<.001$). Table 8 indicates that castration significantly reduced seminal vesicle weight relative to intact males following vehicle treatment. TP significantly increased seminal vesicle weights of both intact and castrated males. In contrast, EB significantly reduced seminal weights in intact males, but relative to vehicle, failed to affect seminal vesicle weight in castrated males.

Accessory Sex Organs (Uterus): Significant differences were observed among groups ($F(2,58)=60.64$, $p<.001$). Table 9 indicates that ovariectomy significantly reduced uterine weight relative to intact females following vehicle treatment. Both TP and EB significantly

Figure 26. ICWS hypothermic magnitude in intact and ovariectomized female rats following TP (2 mg/kg/14 days) or EB (4 ug/kg/7 days) treatments. *Significantly different from VEH-treated females; +Significantly different from sham-operated females; Dunn comparisons, $p<.05$.

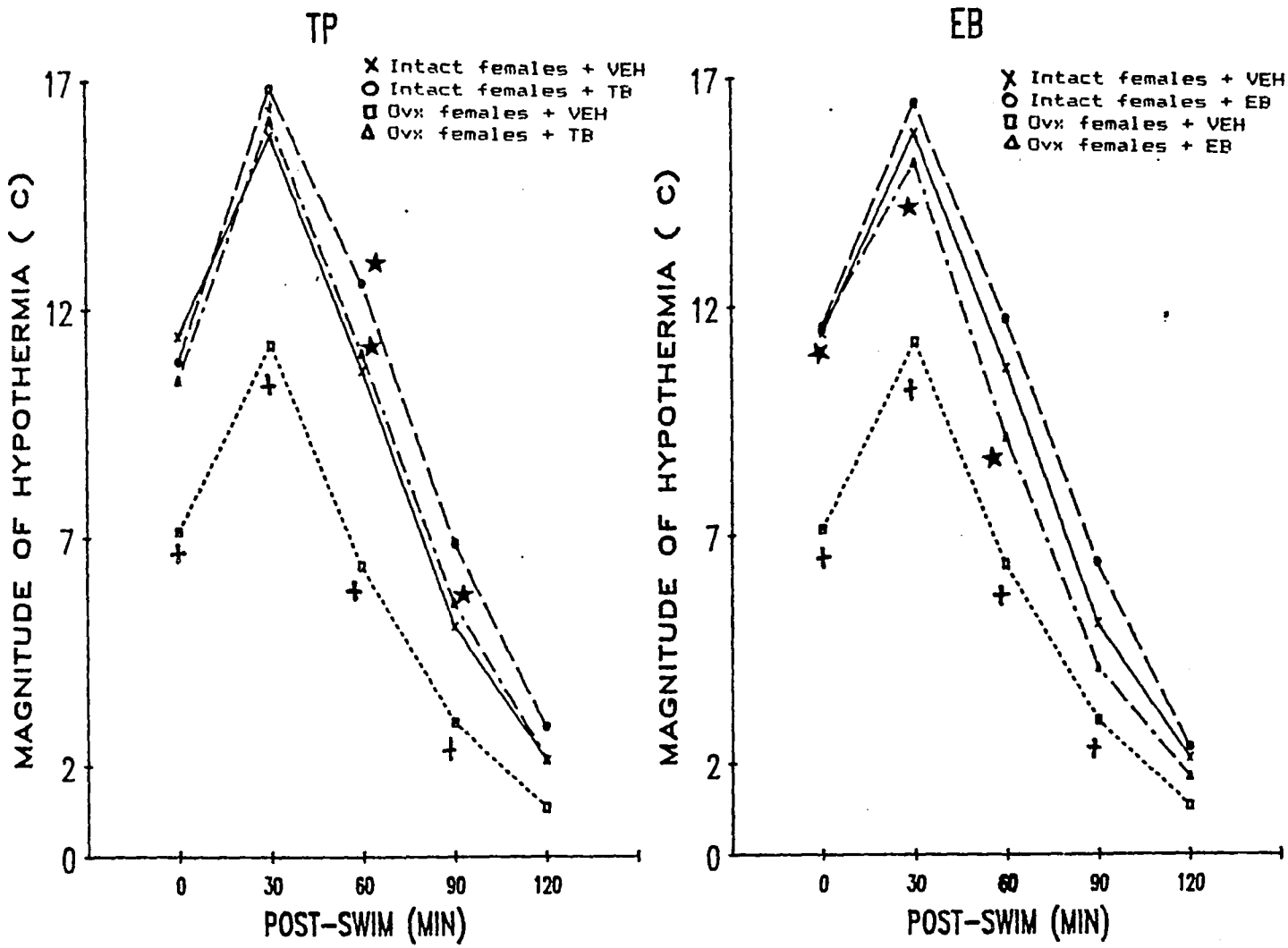

TP
EB
Intact females + VEH
Intact females + TB
Ovx females + VEH
Ovx females + TB
Intact females + EB
Ovx females + EB
MAGNITUDE OF HYPOTHERMIA (C)
POST-SWIM (MIN)
0
2
7
12
17
0
30
60
90
120

Table 8. Seminal Vesicle Weight (mg, SD) Following Vehicle or Hormonal Treatment in Intact and Castrated Males.

GROUP	TREATMENT		
	Vehicle	TP	EB
Sham-Operated	574 (71)	894 (115)*	367 (111)*
Castrated	127 (30)+	887 (113)*	126 (30)*

Note: The asterisk denotes a significant difference from vehicle treated controls. The cross denotes a significant difference from intact vehicle-treated animals.

Table 9. Alterations in Uterine Weight (mg, SD) Following Vehicle or Hormonal Treatment in Intact and ovariectomized Females.

GROUP	TREATMENT		
	Vehicle	TP	EB
Sham-Operated	512 (134)	837 (114)*	605 (135)
Ovariectomized	115 (30)+	514 (107)*	412 (45)*

Note: The asterisk denotes a significant difference from vehicle treated controls. The cross denotes a significant difference from intact vehicle-treated animals (Dunnet comparisons, $p<.05$).

increased uterine weight in intact females with the former producing significantly greater effects. Both TP and EB significantly elevated uterine weight in ovariectomized females to leves found in intact females.

Discussion

Seven main points emerged from this experiment. First, daily handling of male and female rats altered the gender differences in magnitude of CCWS and ICWS analgesia without any concommitant effects upon basal pain thresholds. This effect was specific: whereas the magnitude of CCWS analgesia on the tail-flick was decreased in handled male rats, analgesic magnitude of CCWS was increased in the jump test for handled female rats. The analgesic magnitude following ICWS in the jump test was also increased in handled males and females which resulted in the elimination of gender differences. Second, the effects of handling upon analgesia were not related to changes in hypothermia since handling failed to affect the magnitudes of CCWS or ICWS hypothermia. Third, gonadectomy-induced reductions in CCWS and ICWS analgesia were observed in handled rats, replicating the results obtained in Experiment 2 in relatively unhandled

rats. Fourth, hormonal supplements administered to intact and gonadectomized rats altered the gonadectomy-induced effects on CCWS analgesia. TP increased CCWS analgesia in intact males on the tail-flick test and reinstated CCWS analgesia to control values in castrated and ovariectomized rats on the tail-flick and jump tests. On the other hand, EB reduced CCWS analgesia magnitude to the levels observed for ovariectomized females on the tail-flick test, while failing to alter CCWS analgesia on the jump test in intact or gonadectomized males or females. Fifth, the magnitude of ICWS analgesia in the tail-flick test was not affected by either TP or EB in any group. However, TP reinstated ICWS analgesia on the jump test for castrated males and ovariectomized females; EB reinstated ICWS analgesia for castrated males. Sixth, the effects of TP and EB on CCWS and ICWS analgesia could not be explained by alterations in either hypothermic magnitudes or body weight in hormone-treated animals. Finally, the effectivness of hormonal replacement was confirmed by measured changes in accessory sex organ size.

Handling and Swim Analgesia: Handling has been shown to alter responsivness to a variety of measures in laboratory animals (Gray, 1971). However, it does not

appear to have been considered as an important variable that could affect SIA. Indeed, it is important to note that handling did not eliminate cold water swim analgesia in the present study, but rather changed the relationship of gender differences observed in relatively unhandled animals. Moreover, although our handling procedure altered the gender differences in CCWS and ICWS analgesia, it did not affect gonadectomy-induced effects. Further, when handling did eliminate gender differences in CCWS and ICWS analgesia, the way this variable was eliminated differed as a function of the nociceptive measure. Previous research focused upon the effects of early handling upon adult behaviors, and therefore generalizations to effects of repeated adult handling are difficult to make. Early handling elicits greater exploration, less defecation, faster learning acquisition and less vocalization in rats relative to nonhandled controls (Levine, 1967; Levine, 1956; Ader, 1975). In general, handled rats are described as less "emotional" than nonhandled animals. Concomitant with these behavioral changes, early handing alters adrenocortical activity in rats: under conditions of acute stress, handled animals respond more quickly to acute stress, displaying greater initial elevations and faster

subsequent rate of decrease of plasma corticosterone after stimulus termination (Ader, 1975; Ader et al., 1968; Levine et al., 1967; Grota, 1975; 1976). Moreover, handling effects are gender related in that corticoid responsiveness to stress is higher in handled male than in handled female rats (Levine, 1967; Grota, 1976). These physiological changes have been traced to alterations in the activity of tyrosine hydroxylase in the adrenal medulla (Rorundo, 1977; Pfeifer, 1972). Since CCWS analgesia is mediated in part by pituitary-adrenal activation (Bodnar et al, 1978c) and since the hormonal regimen required multiple injections of the experimental animals, it was deemed important to evaluate the handling effects upon analgesic magnitude in male and female rats. As stated previously, handling affected CCWS and ICWS analgesic magnitude in a gender-specific and test-specific manner. The analgesic magnitude of CCWS in the tail-flick test in handled rats lost gender specificity by specifically decreasing analgesic magnitude in handled males to levels observed in nonhandled females. This effect might be described as partial adaptation to the stressful properties of the stimulus in males but not in females following handling. In contrast, CCWS analgesia on the jump test was

increased in both male and female rats following handling, suggesting sensitization to stressful properties. The gender-related effect was lost because the sensitization was more pronounced in female rats. Although these gender-related effects of handling are consistent with reported effects of early handling in male and female rats (Levine, 1976), it is not clear why adaptation eliminates gender differences in one test and sensitization eliminates gender differences in the other. The magnitude of ICWS analgesia on the jump test was also affected by handling: the gender differences were eliminated because handled females were more analgesic than nonhandled controls. No effects of handling were observed in male rats. These changes in gender-related alterations in analgesic magnitude were not accompanied by changes in basal pain thresholds, indicating that such changes were specific to the response to stress, and were not shifts in the general responsivity of the animals to the testing situation. Further evidence of the specificity of the effect to the stress response is that neither CCWS nor ICWS hypothermia were influenced by handling. Since similar patterns of alterations in analgesic magnitude were observed in both handled and unhandled rats following gonadectomy, this suggests that

gonadal status does not play a role in adaptation/sensitization to stress.

Effects of Gonadal Steroids Administration on CCWS and ICWS analgesia: Since gender differences in CCWS and ICWS analgesia were abolished by handling, the effects of gonadal steriods administration was related to gonadectomy effects. Consequently, all comparisons were made with vehicle treated, same sex intact rats. In general, TP appears to be the more potent gonadal steroid capable of reversing the effects of gonadectomy on analgesic magnitude. CCWS analgesia was elevated to control levels in castrated males and ovariectomized females on both the tail-flick and jump tests. Similarly, the magnitude of ICWS analgesia was reinstated in castrated males and ovariectomized females on the jump test. TP also increased CCWS analgesia on the tail-flick in intact males relative to vehicle treated controls. The effects of EB were less consistent: it decreased CCWS analgesic magnitude in intact females on the tail-flick test, and reinstated CCWS and ICWS analgesic magnitude on the jump test in castrated males. It appears that TP reversal of gonadectomy effects on swim analgesia is manifested if TP is the only circulating gonadal hormone. Endogenous estrogen (or progesterone) may antagonize the

activity of TP, since TP administration to intact females failed to produce any alterations in analgesic magnitude. Although ovarian activity seems to be necessary for a normal analgesic response to stress, estrogen does not appear to be the critical gonadal steroid involved. These data are consistent with findings related to parenteral morphine potency to produce analgesia. Sensitivity to morphine varies during the estrous cycle and is significantly reduced in ovariectomized and post-partum female rats. However, estradiol and/or progesterone fail to reverse the effect of either condition, while TP reinstated morphine potency above control levels (Banerjee et al., 1983). This suggests that testosterone, which is also released by the ovary, might possess a physiological role of modulating CCWS and ICWS analgesic magnitudes, similar to that for morphine analgesia. Testosterone appears to have a similar function in the male, since castration reduces sensitivity to morphine (Chatterjee et al., 1982) and to both forms of cold swim analgesia. TP replacement therapy reverses the effects of castration. This modulatory action of TP is not dependent on the physiological or pharmacological profile of the analgesia elicited, since both nonopioid-mediated forms (CCWS) and opioid-mediated forms (ICWS and morphine) of

analgesia are similarly affected. It should be noted, that the doses of TP and EB employed in this study are those observed to reinstate sexual behavior in gonadectomized rats (Beyer et al., 1971; 1973), and probably produce circulating blood levels of the hormone higher than those observed in intact animals. This assertion is supported by finding of increase in the size of accessory sex organs in some TP and EB-treated rats. Physiological doses may produce a different pattern of results, since the feedback loops (hypothalamic-hypophyseal- gonadal axis) might not be totally disrupted.

There is evidence that endogenous opioids are involved in certain forms of SIA (Madden et al., 1976; Rossier et al., 1977). In addition, several lines of evidence suggest that gonadal steroids regulate endogenous opioid systems. Females have lower levels of circulating B-endorphin (Mueller, 1980), dynorphin (Molineaux et al., 1986) and met-enkephalin (Hong et al., 1982). Castration induces significant decreases in B-endorphin concentrations in the pituitary (Lee et al., 1980; Wardlaw et al., 1982), increases in leu-enkephalin (Molineaux et al., 1986), and decreases in met-enkephalin (Hong et al., 1980). Ovariectomy has mixed effects,

increasing pituitary met-enkephalin levels (Hong et al., 1982), or decreasing met-enkephalin levels in the intermediate lobe of the pituitary (Molineaux et al., 1986). Moreover, levels of B-endorphin and met-enkephalin vary during the estrous cycle (Hong et al., 1982). Administration of EB reduces the resting plasma and intermediate lobe levels of B-endorphin (Mueller, 1980) but increases met-enkephalin content in various brain areas including the periaqueductal grey (Dupont et al., 1980). EB administration also decreases the stress-induced release of B-endorphin (Mueller, 1980). In addition to gonadal steroid effects on the opioid system, endogenous opioid peptides modulate the release of pituitary gonadotropins and prolactin, subsequently producing alterations in gonadal steroids (Bruni et al., 1977). However, since stress appears to activate all opioid systems at central and peripheral levels, it is difficult to determine what system and at what level the interaction between gonadal steroids and opioids systems occur in analgesic resposes. Nevertheless, it is possible to hypothesize that the reversals observed in CCWS and ICWS analgesic magnitude following gonadal hormone administration are mediated in part by the influence of gonadal steroids on opioid systems. This is one of the

many ways in which modulation of analgesic responses may occur. Since both testosterone and estrogen produce alterations in endogenous opioid systems, there may be specific inhibitory and exitatory actions on the various systems. The surprising lack of effect of EB is difficult to explain in view of the multiple effects it produces on opioid systems.

The effects of gonadal steroid administration on the magnitude of CCWS and ICWS analgesia cannot be attributed to changes in hypothermic magnitude. Since handling failed to affect CCWS or ICWS hypothermia, gender differences were still observed on this measure. Intact females displayed more pronounced CCWS and ICWS hypothermia than intact males following vehicle treatment. However, analgesic magnitude failed to differ between intact males and females following vehicle. TP significantly increased CCWS hypothermia in both intact and castrated male rats and in ovariectomized females. While increased hypothermia corresponded with increased analgesic magnitude following CCWS in the tail-flick test, it failed to correlate with the increased analgesic magnitude in the jump test following CCWS and ICWS. EB increased hypothermic magnitude in intact and castrated male rats, but decreased it in intact and ovariectomized

female rats. Yet, EB generally failed to change analgesic magnitude among groups. Therefore, these data suggest that changes in analgesia are not a consequence of changes in hypothermia following hormone treatment. EB-related increases in CCWS and ICWS hypothermia may be the consequence of changes in metabolic rate due to supplemental administration of estrogen (Collett et al., 1966), which appear to reduce the animal's capacity to mantain body temperature when faced with cold ambient temperatures (Wilkinson et al., 1980). Alterations in cardiovascular responsivness to vasoactive substances have been reported in animals under estrogen treatment (Fregly & Thrasher, 1977), and this may be the factor responsible for the increased heat production and elevated dry heat loss observed in ovariectomized rats maintained with sustained plasma estradiol levels (Laudenslager et al., 1980). Similarly, EB increases heat intake in intact and castrated males, impairing their capacity to thermoregulate (Nieburgs & Greenblatt, 1948), which may very well explain the increased EB induced hypothermia found following CCWS and ICWS in intact and castrated males.

Body weight alterations following hormonal treatment could not explain analgesic changes, since TP decresed

body weight in intact male rats, but increased CCWS analgesia. EB retarded body weight gain in intact and castrated male rats, yet failed to alter CCWS analgesic magnitude.

The doses of TP and EB used in this study were effective in producing observable change in tissues sensitive to the particular hormone. In general, TP had an activating effect on both seminal vesicles and uteri, while EB enhanced growth of the uterus, but inhibited seminal vesicle development. Therefore, failure to detect changes in analgesic magnitude in certain groups was not due to a failure to achieve altered gonadal hormone status since tissue responsivity to the hormones was significantly altered in all groups. Finally, any baseline shifts in tail-flick latencies and jump thresholds could not account for the differential effects of gonadal steroids on CCWS and ICWS analgesia. Rather, it appears that the observed effects of TP and EB were a reflection of their action on analgesic mechanisms activated by swim stimuli.

General Discussion

The results of these experiments indicate: gender differences in CCWS, ICWS and morphine analgesia exist

with female rats displaying a smaller magnitude of analgesia than male rats. This difference is not secondary to differences in body weight since the difference persists when female rats are compared to weight-matched male rats. Test specificity of the effects was also found. Gonadal steroids were determined to be important in mediating the gender differences, since analgesic magnitude in castrated males was similar to that in intact females, and ovariectomized females displayed less analgesia than intact females. The reductions in swim analgesia observed in gonadectomized animals are related to gonadal hormones, in particular TP, since daily administration of the hormone reinstates analgesic magnitude in castrated and ovariectomized animals.

That gender affects the magnitude of CCWS and ICWS analgesia has both methodological and functional implications. In methodological terms, the obtained gender differences suggest that comparisons of the magnitude of analgesic responses following stress across studies must take gender into account. This adds gender to a growing list of variables that are capable of affecting the nature of the analgesic response following stress, including the parameters of the stressor (Bodnar

et al., 1978c; Girardot and Holloway, 1984; Kirchgesser et al., 1982; Terman et al., 1984), the age of the animal (Girardot and Holloway, 1985; Hamm and Knisely, 1986; Kramer and Bodnar, 1986), and the strain of the animal (Urca et al., 1985). The gender-related effects also appeared to depend upon the pain test employed: the jump test was far more effective in detecting gender differences in CCWS and ICWS analgesia than the tail-flick test. Speculation as to the functional significance of this finding in the absence of additional physiological data would be premature, but this demonstrates another instance in which the pain test is a critical determinant in indicating the direction and magnitude of given analgesic effects (e.g., Badillo-Martinez et al., 1984; Dennis et al., 1980; Ryan et al., 1985).

In functional terms, the data suggest that gonadal steroids are important in modulating the analgesic responses following CCWS and ICWS. Previous work (Badillo-Martinez et al., 1984) has shown that normal female rats display less morphine analgesia than age-matched male rats. Since ICWS analgesia is opioid-mediated (Girardot and Holloway, 1985) and CCWS analgesia is nonopioid-mediated (Bodnar et al., 1978c),

and since the magnitude of both forms of analgesia are less in adult females, it would appear that gonadal function is capable of modulating both opioid and nonopioid forms of analgesia. Evidence of this modulation is the finding that gonadectomy produces significant reductions in the magnitude of CCWS and ICWS analgesia in male and female rats. The suggestion that the male gonadal steroid, testosterone, is responsible for the increased analgesia in males is supported by the reduction in morphine analgesia following castration (Chatterjee et al., 1982; LaBella, 1975), the increase of opiate receptor number in castrated male rats (Hahn and Fishman, 1985) and the ability of testosterone to reinstate morphine's analgesic potency in both gonadectomized male and female rats (Banerjee et al., 1983). The function of estrogen is less clear, since it was found to be relatively ineffective in reversing the effects of gonadectomy. The apparent lack of involvement of estrogen in the regulation of swim analgesia is consistent with the inability of EB alone or in conjunction with progesterone to reverse the reduction in morphine potency in ovariectomized and post-partum female rats, when levels of estrogen are at their lowest (Banerjee et al; 1983). On the other hand, vaginal

stimulation-produced analgesia, characterized as both opiate and nonopiate mediated, is facilitated in animals given EB, but not in animals given EB plus progesterone (Rothfeld et al., 1985). Similar effects were reported in regard to opioid mediated tail-shock induced analgesia (Ryan et al., 1986). Moreover, evidence indicates that gonadal steroids, in particular estrogens, regulate endogenous opioid systems. B-endorphin, dynorphin, met-enkephalin levels differ in male and female rats (Mueller, 1980; Molineaux et al., 1986; Hong et al., 1982). Also, estrogens increase methionine-enkephalin-like levels in the striatum (Dupont et al., 1980) and levels of various opiate peptides change during the estrous cycle (Lee et al., 1980). Further, EB administration attenuates the stress induced-release of B-endorphin (Mueller, 1980). Similarly, in a feeding paradigm, EB decreases the sensitivity of females to naloxone, while ovariectomy increases their sensitivity. Similarly, ovariectomy decreases sensitivity to the hyperphagic effects of ketocyclazocine (Morley et al., 1984); in addition, it has also been shown that the opioid peptides modulate the release of pituitary gonadotropins and prolactin (Yen et al., 1985; Bicknell, 1985). It appears, therefore, that

opioid and gonadal steroid systems are closely interrelated, and that this relationship affects ingestive, pain-inhibitory and stress systems. Yet, the absence of an effect of EB, in the dose used in this study, on swim analgesia, especially in opioid mediated ICWS analgesia, is surprising and needs further exploration. It may be the case that steriod modulation of swim analgesia is not mediated by its influence on the opioid systems, a suggestion that is supported by the finding that ICWS analgesia was not reversed by naloxone in female rats. Other neurotransmitter and endocrine systems are also influenced by gonadal steroids such as norepinephine (Adler & Crowley, 1984), growth hormone and prolactin (Yen et al., 1985), and these systems may in turn modulate the analgesic response to stress.

The modulatory role of gonadal steroids is also apparent in thermoregulatory mechanisms: a) female rats display a greater magnitude of CCWS and ICWS hypothermia; b) gonadectomy reduces the magnitude of hypothermia, and c) TP and EB administration alters the magnitude of hypothermia following the swim. Despite the lack of complete correlation between TP and EB influence on swim analgesia and swim hypothermia, similarities exist in the direction of the alterations produced. However, it is

apparent that modulation of swim analgesia by gonadal steroids is not secondary to changes in swim hypothermia.

Regarding the locus of action of gonadal steroids, there is some indication of a peripheral effect, since the analgesic potency of systemic, but not centrally administered, morphine is dependent upon the sex of the rat. Yet, it is premature to eliminate the possibility of steroidal involvement at various levels of the nervous system. If we consider the abundant evidence on the existence of androgen- and estrogen-sensitive tissue in the central nervous system, mainly in the hypothalamic and hippocampal areas (McEwen, 1980), it is possible that pain modulation may occur at these levels. At a peripheral level, modulation can occur on the hypophysial-adrenal axis, since manipulations that alter this system also affect CCWS analgesia. Hypophysectomy attenuates CCWS analgesia while adrenalectomy potenciates it (Bodnar et al., 1979a). That influences exist between the hypophysial-adrenal and the hypophysial-gonadal axes has been determined by the inhibition of testosterone release in males subjected to stress (Bullock & New, 1971). Thus, it is possible that mutual modulation exists between the two systems, possibly at the spinal level. Further work needs to be done to verify the locus of

action.

It is clear that regardless of the underlying hormonal milieu, CCWS and ICWS are effective in reducing responsiveness to noxious stimuli in male and female rats, and in intact and gonadectomized animals. Thus, gonadal hormones appear to modulate these processes, possibly by facilitation, since reduction of gonadal steroids by gonadectomy attenuates the magnitude of these responses. A similar modulatory function has been described for adrenal hormones.

The results of these series of experiments may have long term clinical implicatios in the treatment of pain states in humans. There is some indication of differential responsivity to analgesic medication among women as compared to men (Classen & Netter, 1985) and of a higher occurrence of pain complaints among women. Thus an approach to pain management that takes gender into account can be invaluable in terms of efficacy. Moreover, the modulation of analgesic processes by gonadal steroids can, of itself, provide new methods of pain treatment.

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